COVER CROP MIXTURES AS PARTIAL SUMMERFALLOW REPLACEMENT IN THE SEMI-ARID NORTHERN GREAT PLAINS

by

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DEDICATION

This thesis is dedicated to my husband, Brett, for his constant love and support. And to my daughter Grace, who inspires me to improve the world she will someday inherit. I am incredibly fortunate to have them both in my life.

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ABSTRACT

Farmers in the semi-arid northern Great Plains are currently experimenting with multi-species cover crops, or cover crop mixtures (CCMs), as a partial summerfallow replacement and conservation practice, in response to the anecdotal claim that CCMs provide more ecosystem services than their single-species legume green manure (LGM) counterparts. This is in the absence of any published data. We conducted a 2-yr plot scale study from 2012 to 2013 at four on-farm locations in Montana to compare fallow with a pea LGM and nine CCM treatments comprised of four plant functional groups, including nitrogen fixers, fibrous roots, tap roots, and brassicas. Agronomic factors reported include cover crop biomass yield, biomass N yield, soil water and nitrate-N use, and subsequent wheat yield and quality. In addition, soil biological factors measured included; microbial respiration rate, soil enzyme activity, potentially mineralizable nitrogen, and mycorrhizal colonization. Mean cover crop biomass by site ranged from 0.4 Mg ha^{-1} in a record dry year, to 3.7 Mg ha^{-1} in a record wet year. Cover crop C:N differed between single-species Pea and an eight-species Full mix only at one site-year with 13.4 and 16.7 measured for each treatment, respectively. Soil water after cover crop treatments was less than Fallow at the time of cover crop termination at three site-years, and was strongly correlated with decreased subsequent wheat yield at three N fertility levels. Surface soils were 5 to 10 °C cooler with Pea and Full cover crops than Fallow from the time of cover crop canopy closure until six to eight weeks later. Soils following cover crops had increased microbial respiration rate at one site, however, no differences in six measured soil enzymes activities were found. Mycorrhizal colonization of wheat increased at one site from 11 to 22% following cover crops when compared with Fallow. Very few differences were observed between the pea LGM and the CCM treatments in all measured factors, indicating little advantage of CCMs over LGMs after one cover crop cycle. However, field observations indicate CCMs may have potential to provide biological pest control and this topic is recommended for further study.

CHAPTER ONE

INTRODUCTION

Single-species legume green manure (LGM) cover crops have shown promise in mitigating the negative effects of summerfallow and improving biological soil quality indicators in the semiarid Northern Great Plains (NGP). Soil quality parameters such as microbial biomass, fungal populations, soil enzyme activity, and potentially mineralizable nitrogen have demonstrated improvement with the use of LGM crops (Bandick and Dick, 1999; Biederbeck et al., 2005; Liebig et al., 2006; O'Dea, 2011).

Recently, farmers in the NGP have been experimenting with multi-species cover crops, or cover crop mixtures (CCMs), in no-till wheat (*Triticum aestivum* L.)-fallow rotations in response to anecdotal claims that the species diversity of a CCM stimulates greater soil biological activity than a single-species LGM crop. However, to date, few scientific studies have been conducted to investigate this claim. As a result, a knowledge gap exists on how multi-species cover crops affect select soil biological parameters compared with single-species cover crops in no-till wheat rotations in the low rainfall areas of the NGP.

The Summerfallow Challenge

The practice of alternating a year of a small grain crop with a year of no crop, otherwise known as summerfallow, has been common in the dryland small grain producing regions of Montana since the 1930s (Ford and Krall, 1979; Janzen, 2001). Summerfallow began as a means to increase soil moisture storage for the following

year's grain crop and to stabilize grain yields. However, research has shown that most water stored during summerfallow is lost to evaporation (Tanaka and Aase, 1987), with at most 40% of the precipitation collected during the fallow period stored for future crop use (Peterson, et al., 1996). In addition, summerfallow has serious soil quality disadvantages, including greater soil erosion potential (Campbell et al., 1991), increased potential for saline seeps (Daniels, 1987) and nitrate leaching (Bauder et al., 1993), decreased soil organic matter (Campbell et al., 2000), and decreased soil biological activity (Acosta-Martinez et al., 2007).

Summerfallow acreage has declined in the NGP, decreasing from 17 M ha in 1971 to fewer than 4 M ha today (Tanaka et al., 2010). No-till farming practices have contributed to this decline by conserving more water than conventional or mulch-tillage practices, allowing for greater crop intensification (Grant et al., 2002) such as partial or full season crops in the summerfallow period (Farahani et al., 1998).

One exception to the decreased summerfallow trend is in the NRCS Major Land Resource Area 52, which contains 6 M ha and represents the largest wheat-growing region in Montana (Fig.1.1; USDA-NRCS, 2006). In this region, approximately 1 M ha are in a summerfallow rotation (USDA-NASS, 2007), representing 25% of the summerfallow area of the entire NGP. As a result, the MLRA 52 is an important target region for research into management practices that decrease total summerfallow area.

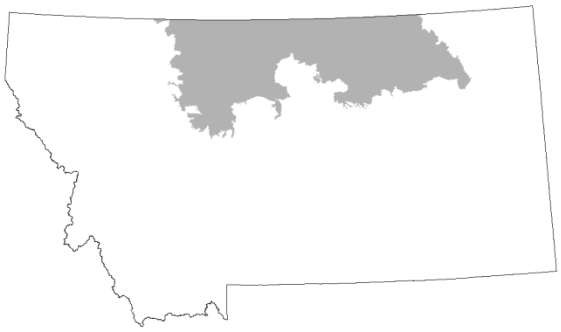

Fig.1.1. NRCS MLRA 52

Agronomic Practices and Soil Biology

The soil microbial community is responsible for the decomposition of plant and animal materials, the immobilization and mineralization of plant nutrients, and the maintenance of soil structure (Schloter et al., 2006). Changes in the soil biota may change important soil functions such as nutrient cycling and availability, energy flow, soil aggregate formation and stability, and water infiltration (Liebig et al., 2006). Agronomic practices that manipulate the soil biological community and its processes may improve soil function and help overcome the negative effects of summerfallow. Biological parameters can respond more rapidly than most chemical and physical parameters to changes in land use (Nannipieri et al., 2001; Nannipieri and Badalucco, 2002; Gil-Sotres et al., 2005), making them good candidates for indicators of the early effects of agronomic changes.

Both no-till practices and crop intensification positively affect soil biological activity (Lupwayi et al., 1998; Frey et al., 1999; Liebig et al., 2004). No-till increases the

amount of soil macro-aggregates, which serve as important habitats for microbial activity (Gupta and Germida, 1988), and crop intensification increases the soil C inputs necessary for microbial growth and function (Doran et al., 1988). No-till systems can have greater microbial biomass and AMF colonization than tilled systems (Drijber et al., 2000), and increased crop intensity can increase microbial biomass, soil enzyme activity, and PMN (Acosta-Martinez et al., 2007; O'Dea, 2011).

Cover crops can intensify crop production and provide a source of labile organic C and N, which in turn provides a substrate for microbial growth and activity (Roper and Gupta, 1995). One type of cover crop for summerfallow replacement in the NGP is a partial-season legume green manure crop (LGM), grown in the peak precipitation window from April to mid-June and terminated at early to mid-bloom (Tanaka et al., 2010). Specifically, pea (*Pisum sativum* L.) and lentil (*Lens culinaris* Medik.) have shown promise as partial fallow replacement LGM crops in Montana (Miller et al., 2003; Miller et al., 2011). Early termination of LGMs is an important management practice for soil water conservation, as crops grown past early bloom can use too much water and depress subsequent wheat yields (Zentner et al., 2004). LGMs can increase bacteria, fungi, microbial biomass C and N, C mineralization, and enzyme activity compared with soils in a wheat-fallow rotation (Biederbeck et al., 2005). Importantly, improved biological activity resulting from increased crop diversity and intensification may also correlate with improved crop yields (Acosta-Martinez et al., 2011).

Plant Diversity Effects on Soil Biota

Crop diversity may also affect soil biological function, as different plant species can influence the below-ground biome via various parameters, including: biomass production, biomass quality, root exudates, and mutualistic associations. However these interactions are highly complex and the effect on soil biota can be positive, negative, or neutral (Bardgett and Wardle, 2010).

Biomass Production

Increased plant diversity may increase biomass production, thereby providing more labile carbon and nitrogen available for microbial growth and activity. Multiple plant species grown together as an intercrop may capture light resources more efficiently than single species, by providing contrasting leaf architectures and densities to capture solar radiation (Keating and Carberry, 1993). Likewise, the various root morphologies of multiple plant species may exploit distinct spatial regions of the soil profile, allowing for more efficient use of soil nutrients (Li et al., 2003; Fang et al., 2011; Postma and Lynch, 2012). Potential maximization of these resources could allow multi-species cover crops to yield more biomass than their single species counterparts (Fukai and Trenbath, 1993; Malezieux et al., 2009).

Studies of multiple plant-species effects on biomass within annual plant communities have shown mixed results. Carr et al. (2004) discovered that the biomass production of a no-till forage intercrop is dependent on plant species in southwestern North Dakota. Biomass yields of oat (*Avena sativa* L.)-pea and barley (*Hordeum vulgare* L.)-pea intercrops were 20% greater than monoculture oat and barley forage crops, but

25% less than monoculture pea. Researchers in Nebraska compared cover crop mixtures containing two to eight species of brassicas and legumes and found the biomass production of all the mixed-species cover crops was greater than the single-species cover crops (Wortman et al., 2012). Park et al. (2002) reported that varying the seeding rates of maize (*Zea mays* L.) and bean (*Phaseolus vulgaris* L.) intercrops greatly influenced biomass production and grain yield, with intercrops producing greater yields than monocrops in only one-third of all possible seeding ratios.

Biomass Quality

Plant diversity may also influence belowground soil processes through plant biomass quality. Plant litter with low C:N can be associated with greater populations of bacteria and nematodes and may encourage greater rates of decomposition and net N mineralization (Parmelee et al., 1989). In contrast, plant litter with a higher C:N may promote a fungal-based decomposition pathway with slower nutrient mineralization (Wardle et al., 2002), reduced N leaching (de Vries and Bardgett, 2012), and increased ability to retain nutrients during drying and wetting cycles (Gordon et al., 2008). Previous studies have shown mixed cover crops of legumes and grasses can produce a higher C:N ratio than single species legume cover crops alone (Hauggaard-Nielsen et al., 2003; Sainju et al., 2005), with the potential result of increased soil organic matter (Kuo et al., 1997) and reduced N leaching (McCracken et al., 1994).

Plant C:N ratios were correlated with plant growth rates in perennial pasture systems in northern Europe (Orwin et al., 2010). Fast-growing plants produced low C:N ratios and slow-growing plants produced high C:N ratios. Agricultural crops tend to be

fast growing, short-lived, and with lower C:N ratios than native systems. As a result, agricultural soils favor a bacterial-based decomposition pathway and have lower organic matter levels than adjacent, native perennial system soils (Hedlund et al., 2004; van der Wal et al., 2006).

Root Exudates

Plant root exudates are an important mechanism by which plant species influence the environment, as each plant species has a specific root exudate quantity and composition, which promotes the growth of various microbial communities (Somers et al., 2004). Root exudates are a complex chemical mixture of ions, carbon-based metabolites, amino acids, enzymes, oxygen, water, and other substances that influence the rhizosphere (Berg and Smalla, 2009). Some of these exudates are not a passive loss of carbon, but rather are actively excreted (Broeckling et al., 2008), and include a significant amount of the photosynthetically fixed carbon produced in the aboveground parts of the plant (Bais et al., 2006). This carbon source directly influences microbial communities by providing a growth substrate. Exudates may also indirectly influence microbial communities by altering the pH of the rhizosphere as part of the root nutrient acquisition process (Dakora and Phillips, 2001). Soil pH can vary across micro-niches in the soil environment, and plays a major role in the selection of microbial communities, by functioning as one of the main determinants of which bacterial species will grow in a given soil environment (Fierer and Jackson, 2006).

Several studies have shown that changes in plant species can change the bacterial community in the rhizosphere and while some bacterial species are ubiquitous, many

bacteria show a high degree of plant host-specificity (Berg and Smalla, 2009). Germida and Siciliano (2001) discovered that old wheat cultivars were colonized by a diverse bacterial community, while modern wheat cultivars were dominated by fast-growing *Proteobacteria*. Likewise, Graner et al., (2003) documented fungal specificity for cultivars of oilseed rape (*Brassica napus* L). In another study, strawberry [*Fragaria* x *ananassa* (Duchense) Decaisne & Naudin (family: Rosaceae) cv. Elsanta], and oilseed rape, both hosts of the fungal pathogen *Verticillium dahlia* Kleb., were grown under field conditions at different sites. Results showed that plant species affected the structure and function of the fungal community, but the effect was less pronounced than for the bacterial community (Berg et al., 2005). Likewise, Broeckling et al., (2008) reported that plant root exudates regulate the soil fungal community of the annual plant species mouseear cress (*Arabidopsis thaliana* L.) and barrel medick (*Medicago truncatula* Gaertn.).

While these studies serve to illustrate the influence of plant species via root exudates, the more important question is: Do these changes in microbial community composition affect ecosystem function within an agricultural context? Evidence suggests that some soil processes can change as a result of root exudates from various plant species. Briones et al., (2002) reported that a new rice (*Oryza sativa* L.) cultivar had greater ammonia-oxidizing bacteria in its root zone than two traditional varieties, resulting in higher nitrification rates due to increased O_2 availability in the root zone. Bremer et al. (2007; 2009) reported that the presence of individual plant species affected grassland denitrification by affecting the amount and composition of organic carbon in the root exudates.

While plant species play a role in microbial community structure, abiotic factors can also play a major part. Marschner et al. (2001) compared the bacterial communities of three plant species [chickpea (*Cicer arietinum* L.), canola (*Brassica napus* L.), and Sudangrass {*Sorghum bicolor* (L.) Moench subsp. *Drummondii* (Nees ex Steud.) de Wet & Harlan}] in three soil types and reported that the bacterial composition in the rhizosphere is affected by complex interactions between plant species, soil type, and root zone location, not just plant species alone. Likewise, soil type played a greater role than maize variety in bacterial community composition in a study by da Silva et al. (2003). As a result, one cannot assume the homogeneity of the soil microbial community within a monoculture of plant species. Rather, variability should be expected with changes in soil type, root location, root age, herbivory, organic matter distribution, and more. The specific nature and degree of any effect of root exudates on soil function will be impossible to estimate without empirical field studies involving the plant species, soil type, and environment of interest.

Mutualistic Relationships

Mutualistic relationships are another way in which individual plant species can influence belowground ecology, particularly via arbuscular mycorrhizal fungi (AMF) and rhizobial bacterial associations. Individual plant species can select for particular taxa of AMF or rhizobia, which can directly or indirectly alter ecosystem functioning. In a classic study, Johnson (1992) reported the importance of AMF associations in agricultural systems by describing how mycorrhizae might partially explain the rotation effect in a maize and soybean (*Glycine max* L.) system. Maize and soybean had different

associated AMF taxa with each plant species, and continuous monoculture of either crop selected mycorrhizal species that were less beneficial to their hosts over time. Crop rotation decreased the abundance of less mutualistic AMF associated with each crop and increased both the biomass and grain yield of the corn and soybean.

Rhizobial associations are important due to their capacity to fix nitrogen. Much has been written on the nature and degree of legume N-fixation. However, fewer studies have focused on the importance of legumes within a mixed species plant community. Three recent studies demonstrated that the presence of a dominant legume species in a mixed plant community can alter soil processes such as nitrate leaching, carbon storage, and the resilience of the microbial community to drought, more than other species (Scherer-Lorenzen et al., 2003; Orwin and Wardle, 2005; De Deyn et al., 2009). In all studies, the presence of a dominant legume within the community was the driver of treatment differences leading to increased ecosystem stability.

Multi-species Cover Crops

Since 2001, farmers in central North Dakota have been experimenting with multi-species cover crops, or CCMs, and claim that these mixtures greatly increase soil biological activity, increase N availability, suppress weed growth, and allow for decreased use of synthetic N fertilizers and herbicides (Jay Fuhrer, NRCS, personal comm., 2009). These CCMs typically contain six to twelve plant species and include multiple plant functional groups, such as legumes, grasses, tap roots, and brassicas. Farmers in Montana have noticed these claims and begun experimenting with CCMs (Fig. 1.2), especially under ‘prevented planting’ scenarios (USDA – Risk Management

Agency). The USDA Natural Resource Conservation Service (NRCS) is promoting the use of CCMs as a conservation practice by providing payments to farmers to offset the cost of experimenting with this new practice. Most of this enthusiasm is fueled by anecdotal claims that the plant diversity provided by a CCM stimulates greater soil biological activity than a single-species cover crop. This is in the absence of any published peer-reviewed data to support the claim that CCMs provide more soil quality benefits than single-species cover crops.

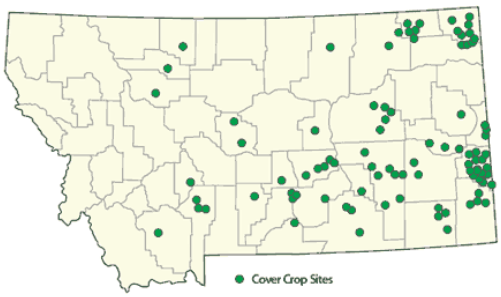

Fig. 1.2. Cover crop mixture farm trials sponsored by NRCS, 2011

Project Objectives

The goal of this thesis was to compare the short-term effects on plant growth, soil quality, and soil biological parameters of a single-species legume cover crop (pea) with mixed-species cover crops. The first study compared cover crop biomass, biomass quality, soil water and nitrate use, and subsequent wheat yield and quality among fallow, a single-species pea cover crop, and nine mixed-species cover crops (Chapter 2). The second study measured treatment differences in soil microbial respiration rate, soil enzyme activity, potentially mineralizable nitrogen, and mycorrhizal colonization in the

wheat response crop among fallow, a single-species pea cover crop, and one mixed-species cover crop (Chapter 3).

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CHAPTER TWO

MULTI-SPECIES COVER CROPS: EFFECTS ON SOIL NITRATE, WATER AND WHEAT YIELD IN THE NORTHERN GREAT PLAINS

Contribution of Authors and Co-Authors

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Multi-species cover crops: Effects on soil nitrate, water and wheat yield in the northern Great Plains

Abstract: Farmers in the semi-arid northern Great Plains are currently experimenting with multi-species cover crops, or cover crop mixtures (CCMs), as a partial summerfallow replacement and conservation practice, in response to the anecdotal claim that CCMs provide more ecosystem services than their single-species legume green manure (LGM) counterparts. This is in the absence of any published peer-reviewed data. We conducted a 2-yr plot scale study from 2012 to 2013 at four on-farm locations in Montana to compare fallow with a pea LGM and nine CCM treatments comprised of four plant functional groups, including nitrogen fixers, fibrous roots, tap roots, and brassicas. Agronomic factors reported include cover crop biomass yield, biomass N yield, soil water and nitrate-N use, and subsequent wheat yield and quality. Mean cover crop biomass by site ranged from 0.4 Mg ha^{-1} in a record dry year, to 3.7 Mg ha^{-1} in a record wet year. Cover crop C:N differed between single-species Pea and an eight-species Full mix only at one site-year with 13.4 and 16.7 measured for each treatment, respectively. Soil water after cover crop treatments was less than Fallow at the time of cover crop termination at three site-years, and was strongly correlated with decreased subsequent wheat yield at three N fertility levels. Soil nitrate was less than Fallow after all measured cover crop treatments. Very few differences were observed between the pea LGM and the CCM treatments in all measured factors, indicating little advantage of CCMs over LGMs after one cover crop cycle.

Key Words: dryland wheat–northern Great Plains–green fallow–no-till–multi-species cover crop–legume green manure

The practice of alternating a year of small grains and a year of no crop, otherwise known as summerfallow, has been common in the dryland small grain producing regions of Montana since the 1930s (Ford and Krall 1979; Janzen 2001). The main purpose of summerfallow is to increase soil moisture storage for the following year's grain crop, thus maximizing grain yields. However, research has shown that most water stored during the summerfallow period is lost to evaporation (Peterson et al. 1996), with at most only 30% of the precipitation collected during the fallow period stored for future crop use. In addition, summerfallow has demonstrated serious soil quality disadvantages, including greater soil erosion potential (Campbell et al. 1991), increased potential for saline seeps and nitrate leaching (Daniels 1987; Bauder et al. 1993), decreased soil organic matter (Campbell et al. 2000), and decreased soil biological activity (Acosta-Martinez et al. 2007).

Summerfallow acreage has declined substantially in the northern Great Plains (NGP), decreasing from 17 M ha in 1971 to fewer than 4 M ha today (Tanaka et al. 2010). No-till farming practices have contributed to this decline, as the technique conserves more water than conventional or mulch-tillage practices, allowing for greater crop intensification (Grant et al. 2002). One region that hasn't experienced decreased summerfallow is the NRCS Major Land Resource Area (MLRA) 52, which contains 6 M ha and represents the largest wheat-growing region in Montana (USDA-NRCS 2006). In this region, approximately 42% of cropland is in summerfallow at any given time (USDA-NASS 2007), representing 25% of the summerfallow area of the entire NGP. As

a result, the MLRA 52 is an important target region for research into management practices that decrease total summerfallow area.

Single-species legume green manure (LGM) cover crops show promise in mitigating the negative effects of summerfallow in the semiarid northern Great Plains (NGP), by increasing the soil N benefit, organic matter C, and wet aggregate stability relative to wheat-fallow rotations (Biederbeck et al. 1993, 1998; Zentner et al. 2004). Likewise, LGMs may increase annualized precipitation use efficiency (Tanaka et al. 2005), and reduce N leaching and development of saline seeps by capturing surplus soil water percolation (Biederbeck and Bouman 1994).

Proper soil water management is essential for LGM cover crop success in the MLRA 52, as adoption of LGMs will be lower if they result in decreased yield in the following crop. Terminating at early bloom is essential for conserving soil water storage for the subsequent wheat crop (Zentner et al. 2004), but results remain mixed on the economic viability of this technique. Miller et al. (2006) reported few differences between wheat-fallow and early terminated-wheat-LGM rotations in a no-till plot scale study across three locations in Montana. Wheat yields and protein, plant available soil water (PASW), and soil nitrate-N levels were comparable between the two rotations. In contrast, O'Dea et al. (2013) reported an average 6% wheat yield decrease compared with fallow after one-year of LGMs across five farm-scale sites in Montana during a relatively wet growing season. Available N, and not soil water, was the chief limiting factor.

Recently, farmers in the NGP have been trying multi-species cover crops, or cover crop mixtures of two or more plant species as a partial summerfallow replacement and/or supplemental livestock forage, in response to anecdotal claims of improved soil

quality and decreased input costs. In addition, the USDA-NRCS has been promoting cover crop mixtures as a conservation practice, along with cost incentive payments, yet there is a dearth of published peer-reviewed data on the effects of cover crop mixtures in the NGP region.

Cover crop mixtures have the potential produce more biomass than their single-species LGM counterparts due to functional complementarity (Fukai and Trenbath 1993; Malezieux et al. 2009). Mixed plant species may capture light resources more efficiently than single species by providing various leaf architectures and densities to capture solar radiation (Keating and Carberry 1993). Likewise, the contrasting root morphologies of different plant species may exploit distinct spatial regions of the soil profile, allowing for more efficient uptake of soil nutrients (Li et al. 2003; Fang et al. 2011; Postma and Lynch 2012). In addition, utilizing multiple plant species may produce plant residues with increased C:N ratio compared with a single-species LGM, resulting in slower nutrient mineralization (Wardle and van der Putten 2002) and reduced N leaching (de Vries and Bardgett 2012). However, a possible disadvantage of multi-species cover crops is the over-extraction of water and nutrients from the soil profile (Hauggaard-Nielsen and Jensen 2005) due to varied rooting depths (Aase and Pikul 2000; Thorup-Kristiansen 2001; Miller and Holmes 2012) resulting in decreased subsequent crop yields.

In the absence of any published data in the region, it is unknown how cover crop mixtures compare with single-species LGMs. The objectives of this study were to compare effects of early terminated cover crop mixtures with both fallow and a single-species LGM control in a no-till wheat-fallow system on biomass production, residue quality, soil water and nitrate-N use, and subsequent wheat yield and protein content.

Materials and Methods

The study began with two sites (Amsterdam and Conrad, MT) seeded to cover crops in April 2012 and then seeded to spring wheat in April 2013. Two more sites (Bozeman and Dutton, MT) were seeded to cover crops in May 2013 and then seeded to winter wheat in Sept 2013 (Table 2.1). In each year, one site was located in the MLRA 52 (Conrad, Dutton) 350 to 400 km from Bozeman, and one site was located in the Gallatin Valley of southwestern Montana (Amsterdam, Bozeman) comparatively near Montana State University. All locations, except Bozeman, were sited on commercial farms that had been under no-till management for a minimum of 3 yr. The Bozeman site is a university research property previously under tilled management that provided a much wetter environment and greater soil organic matter, in contrast with the other three sites. Growing season air temperature and precipitation data were collected on-site with automated gauges (HOBO® data loggers, Onset®, Bourne, MA) at each location. Annual temperature and precipitation data were also collected from nearby meteorological stations within 24 km of each site (Table 2.2).

Each site was established as a randomized complete block design with a split plot arrangement with four replicates. Year 1 cover crop treatments comprised each main plot (8 x 12 m; Figure 2.1), and in Year 2, three N rates comprised each subplot (8 x 4 m). Year 1 cover crop treatments consisted of four plant functional groups, which included; *legumes* for N-fixation, *grasses* for fibrous root systems and carbon addition, *brassicas* for rapid ground cover (Lawley et al. 2011) and possible bioactivity (Larkin and Griffin 2007), and *tap root* crops to reduce soil compaction (Chen and Weil 2010) and nitrate

leaching (Dunbabin et al. 2003). Two plant species were selected from each plant functional group to provide redundancy, with plant species selection varying slightly from 2012 to 2013 (Tables 2.3 and 2.4). Study treatments (Table 2.5) were designed to measure the specific contributions of each plant functional group by growing them separately, jointly, and absent from the mixture. Treatments 1 to 3 constituted the core treatments of the study, and included both a summerfallow and a single-species pea as control treatments, along with the eight-species Full mix treatment. Treatments 4 to 7 consisted of each plant functional group grown separately. Treatments 8 to 11 consisted of the full eight-species cover crop *minus* one plant functional group to compare the specific contributions of each functional group. The study design was modeled after an unpublished study at USDA-Agricultural Research Station (ARS) Mandan, ND that similarly examined cover crop mixtures with respect to presence and absence of selected plant species (Mark Liebig, pers. comm., Oct. 2010).

Agronomic management details are presented in Table 2.6. In 2012, Year 1 cover crop sites were seeded with a low-disturbance drill into standing wheat stubble in early April. Seeding rates were calculated by dividing the recommended seeding rate for each species if planted individually (or as a monoculture) by the number of species in the mixture (Table 2.7). In 2012, the row spacing was offset, with approximately 15 cm between paired rows of large-seeded and small-seeded species within each functional group (Table 2.3), with the large seeded species planted at a 4-cm depth, and the small seeded species planted at a 2-cm depth. All rows in the single species pea treatment were planted at a depth of 4 cm and all legume-containing treatments were inoculated with

rhizobia (*Rhizobium leguminosarium*; Cell-Tech peat, Novozymes BioAg, Brookfield, WI). No other seed treatments were used.

The Conrad site experienced severe downy brome (*Bromus tectorum* L.) pressure due to inadequate herbicide application in early April. This resulted in a loss of cover crop treatments with a fibrous root component, due to lack of any selective herbicide to control downy brome in those treatments. Cover crop treatments were terminated with glyphosate [N-(phosphonomethyl)glycine] application on or near 15 June to meet the USDA-Risk Management Agency (RMA) summerfallow cutoff deadline (USDA-FCIC 2012). Termination timing occurred just prior to first bloom of field pea (growth stage 51 in the BBCH scale; Lancashire et al. 1991) with a flower bud present on one or more nodes. Both Amsterdam and Conrad were sprayed again in mid-July with a glyphosate and 2,4-D (2,4-Dichlorophenoxyacetic acid) mixture, due to failure of pea and especially common vetch (*Vicia sativa* L.) to terminate effectively with glyphosate alone.

In 2013, Year 1 cover crop sites were seeded in early May to allow for more effective pre-plant herbicide application, to fit a practical work-window after normal spring planting is complete, and to add a warm-season species to the mix. Assessment of the observed plant densities in 2012 caused us to revisit our rationale for determining seeding rates. We recognized that there was no published data available to determine optimal seeding rates for these species in a cover crop scenario, and so decided to allow all species equal densities to better understand how they interact. Thus, seeding rates were calculated by dividing a constant target plant population of 120 plants m^{-2} by the number of species in each mixture (Table 2.7). All seeds were seeded to a depth of 1 to 2 cm with 26-cm row spacing. The Dutton site was seeded into standing wheat stubble and

the Bozeman site was seeded into standing barley (*Hordeum vulgare* L.) stubble. The Bozeman site experienced moderate volunteer wheat pressure in all plots. As a result, biomass from the fallow treatment was included in the 2013 biomass analysis. All treatments were terminated with a mixture of glyphosate and dicamba (diglycolamine salt of 3,6-dichloro-o-anisic acid) application on 5 July at Bozeman, and glyphosate on 10 July at Dutton. Termination timing occurred approximately at first bloom of field pea development, with an open flower present on one or more nodes on at least 50% of the plants (growth stage 60 in the BBCH scale; Lancashire et al. 1991)

In April 2013, the Amsterdam and Conrad sites were seeded to spring wheat (cv. Duclair). Wheat was seeded with a low-disturbance drill at 75 kg ha^{-1} at a depth of 2.5 cm, with 26-cm row spacing. Three urea fertilizer treatments (0, 44, 88 kg N ha^{-1}) were banded >5 cm below and to the side of the seed row at wheat seeding to create sub-plots (Figure 2.1). No fungicide was applied to the wheat seed to not confound subsequent soil biological analysis. The Amsterdam site was not harvested due to complete hail damage on 1 Aug, just days before it was ready to harvest.

Sampling Methods. To characterize soils at each site, soils were sampled in the spring, 2 to 4 wk prior to cover crop seeding. Six soil cores were taken from 12 plots at each site to a depth of 15 cm and composited by plot. Samples were analyzed for nitrate-N, Olsen P, exchangeable K, organic matter, pH, electrical conductivity, and texture (Table 2.1; AgVise Laboratories, Northwood, ND).

Plant population stand counts were conducted for all cover crop treatments 4 to 6 wk after seeding. Four quadrats (0.5 m^2) were systematically placed in each plot, and all plants within each quadrat were identified and counted. Aboveground plant biomass was

sampled for all cover crop treatments within 5 d of termination. Four systematically placed quadrats (0.25 m^2) were placed in each plot; all plants were severed at the root/shoot interface and sorted by species in the field.

Soil water and nitrate-N were quantified at cover crop termination and at spring wheat seeding by extracting two soil cores from each plot to a depth of 0.9 m using a truck-mounted hydraulic probe with a 3-cm diameter sample tube. Samples were partitioned into 0.3-m segments in the field and the two subsamples mixed. Samples were packaged in plastic-lined bags and transported in coolers to the laboratory. Soil water at Conrad was only reported for treatments without a Fibrous Root component, and results of soil water and nitrate at Dutton were only reported to a depth of 0.6 m, due to widely variable results at the lowest 0.3 m increment, most likely due to inconsistent growth and water extraction of the 2012 crop due to chemical injury.

All soils for nitrate-N were collected at the time of cover crop termination, except for those at Amsterdam which were collected in spring 2013, due to unreliably high values from the summer of 2012. The treatments sampled for nitrate-N at Amsterdam were Fallow, Pea, Minus Nitrogen Fixers, and Fibrous Roots. The three treatments sampled at Conrad, Bozeman, and Dutton were Fallow, Pea, and Full. Soils were collected to a depth of 0.9 m, and values were reported at three 0.3 m increments, except for Dutton, which was reported to a depth of 0.6 m, due to widely variable results in the lowest 0.3 m.

Laboratory Procedures. Each soil sample was weighed wet from the field, and then re-weighed after 14 d at 50°C to determine gravimetric water content (GWC). Volumetric water content was determined by multiplying the GWC by the average soil

bulk density of each site for each depth increment. Plant samples were oven dried 4 to 7 d at 50°C and weighed directly from the oven. Plant biomass was ground with a Wiley Mill (Thomas Scientific, Swedesboro, NJ) and then finely ground (< 0.5 mm) with an Udy Cyclone Mill (Udy Corporation, Ft. Collins, CO). Biomass C and N content were analyzed from a 0.1-g subsample of all Pea and Full treatments for all sites, except Conrad, where the Minus Fibrous Root treatment replaced the Full treatment due to excess downy brome. C:N ratios were determined for the Full and Minus Fibrous Root treatments by analyzing each plant species separately by plot; then composited by multiplying each species by its respective biomass ratio. Analysis was conducted using a LECO CNS combustion analyzer (LECO Corp., St. Joseph, MI). Biomass N yield was calculated by multiplying the plant N concentration by the cover crop biomass yield. Wheat protein and moisture were measured with an Infrared 1241 Grain Analyzer (Foss of North America, Eden Prairie, MN) at a 12% moisture standard. Seed N yield was calculated by dividing protein concentration by a factor of 5.7 (Jones, 1941) and multiplying by grain yield.

Statistical Analysis. Statistical analysis was performed with R statistical software (The R Foundation for Statistical Computing, Vienna, Austria 2013). Linear models were constructed with treatment and block as independent variables and analyzed with ANOVA. Assumptions of normality, independence, and equal variance were evaluated with residual plots and Q-Q normal plots. Sites were analyzed independently in 2012 because uncontrolled downy brome at the Conrad site resulted in the loss of all treatments with a fibrous root component. When sites were analyzed together in 2013, blocks were nested within site. Years were analyzed independently due to change in plant species,

seeding rate, and growth window from 2012 to 2013. Specific treatment comparisons were reported using Fisher's Protected Least Significant Difference (LSD) procedure with an associated α value of 0.05, using the *agricolae* package (de Mendiburu 2013) with no *p*-adjustment. Correlation analysis was conducted in Excel with the CORREL function. Correlation significance was calculated using an online p-value calculator for correlation coefficients.

Results and Discussion

Precipitation varied between cover crop growing years, with 2012 drier than average and 2013 wetter than average (Table 2.2). Winter precipitation from 2011 to 2012 was drier than average at both Amsterdam and Conrad, with total 2012 precipitation drier than average at Amsterdam. In 2013, the Bozeman site experienced drier than average precipitation for the year, but wetter than average precipitation during the month of June, the month of greatest cover crop growth. In contrast, Dutton received greater than average total precipitation in 2013, with wetter than average precipitation during both May and June.

Seedling Stand Counts. Seedling stand counts varied from their target densities in both 2012 and 2013. Variation in seedling densities indicates the difficulty of attaining specific seeding goals with eight plant species, all with different seeding depth and growth requirements.

At Amsterdam, five treatments were within 20% of the target seeding rate (Pea, Full, Fibrous Root, Minus Nitrogen Fixer, and Minus Fibrous Root), with the remaining five treatments ranging from 47% less to 41% greater than the target seeding rate (Table

2.8). At Conrad, five treatments were also within 20% of the target seeding rate (Fibrous Root, Tap Root, Minus Nitrogen Fixer, and Minus Fibrous Root) with the remaining five treatments ranging from 35% less to 60% greater than the target seeding rate (Table 2.9). Oat (*Avena sativa* L.) and common vetch (*Vicia sativa* L.) were higher than expected at both sites, possibly due to cold tolerance and early vigor. Turnip (*Brassica rapa* L.), radish (*Raphanus. sativus* L. var. *longipinnatus*), and camelina (*Camelina sativa* L. Crantz cv. SO-02) seedling densities at Conrad were lower than expected, likely due to two consecutive nights of < -6 °C in early May, resulting in freezing injury and death. Likewise, turnip, safflower (*Carthamus tinctorius* L. cv. MonDak), and camelina seedling densities were lower than expected at Amsterdam, for unknown reasons.

At Bozeman, all stand counts either met or exceeded their targets (Table 2.10). Seven treatments were within 20% of the target rate (Pea, Fibrous Root, Tap Root, Brassica, Minus Nitrogen Fixer, Minus Tap Root, and Minus Brassica) while the remaining three treatments ranged from 13 to 55% greater than the target rate. In contrast, at Dutton , all stand counts either met or were less than their targets (Table 2.11). Six treatments were within 20% of the target rate (Pea, Full, Nitrogen Fixer, Minus Fibrous Root, Minus Tap Root, and Minus Brassica), with the remaining four treatments ranging from 22 to 36% less than the target rate. It is not clear why the Bozeman and Dutton sites differed, as timely rains and warmer temperatures associated with the May seeding date occurred at both sites. It is important to note that some plant species competed less vigorously than others, with lentil (*Lens culinaris* Medik.) and millet (*Panicum miliaceum* L. sp.) almost non-existent in most final 2013 biomass measures.

Cover Crop Shoot Biomass. Cover crop biomass was low at both 2012 sites, with mean biomass production of 0.9 Mg ha^{-1} at Amsterdam and 0.4 Mg ha^{-1} at Conrad (Tables 2.12 and 2.13). Cover crop biomass was four to six times greater in 2013 than 2012, with mean biomass production of 3.7 and 2.7 Mg ha^{-1} at Bozeman and Dutton, respectively (Table 2.14). Correlation analysis found no relationship between plant species number and cover crop biomass yield at Conrad, but a weak positive relationship at the remaining three site-years (Table 2.15).

Increased heat units combined with timely spring precipitation was the most likely explanation for the difference in cover crop biomass between 2012 and 2013 (Table 2.2). The shift in growth window from 2012 to 2013 (Apr -June 2012 to May-July 2013) increased the cover crop growing degree days (GDD), allowing more heat units for crop growth. GDD increased from 2012 to 2013 by 30% at the two MLRA 52 sites (716 to 931) and by 8% at the two Gallatin Valley sites, (832 to 899). Likewise, cover crop growing season precipitation increased by 8% at the MLRA 52 sites (from 154 to 166 mm), and by 33% at the Gallatin Valley sites (147 to 196 mm) from 2012 to 2013. Even though both 2012 sites had greater than average April precipitation and close to average May precipitation, cover crop biomass was not high, suggesting that an early April seeding may have delayed emergence due to colder soil temperatures. Also, by following a strict calendar deadline of June 15 (imposed by USDA-Risk Management Agency for 'fallow' level crop insurance), the plant growth stage was not as advanced at the point of termination in 2012 as it was in 2013.

In 2012, cover crop biomass differed among treatments at both sites. At Amsterdam (Table 2.12), the Fibrous Root treatment yielded the greatest biomass, while

the Tap Root treatment yielded the least (1.13 and 0.41 Mg ha^{-1}, respectively). In addition, the Full treatment yielded more than the Pea treatment (1.01 and 0.76 Mg ha^{-1}). At Conrad (Table 2.13), the Pea, Nitrogen Fixers, and Minus Fibrous Root treatments yielded the most biomass (0.61, 0.51, and 0.43 Mg ha^{-1}), while the Tap Root and Brassica treatments yielded the least (0.19 and 0.21 Mg ha^{-1}).

In 2013, cover crop biomass differed among treatments only at Bozeman (Table 2.16), with the Minus Brassica, Fibrous Root, and Full treatments yielding the most biomass (4.4, 4.3, and 4.3 Mg ha^{-1}), and the Tap Root treatment yielding the least (2.9 Mg ha^{-1}). In contrast, no cover crop treatment differences existed at Dutton (Table 2.17), with all treatments yielding between 2.4 and 3.5 Mg ha^{-1}. Lack of treatment differences at Dutton suggests that most species had very similar above-ground growth rates.

Biomass production differed between Bozeman and Dutton in 2013 (Table 2.14) with no measured site by treatment interaction. Site differences in 2013 may have been due to greater June precipitation, nitrate, and organic matter at Bozeman, resulting in greater water and nutrient availability than at Dutton. Fallow biomass at Bozeman was high due to poor pre-emergent herbicide control, primarily of volunteer wheat.

The relatively high cover crop yield of 2013 was an anomaly, as other studies conducted across the state have reported lower biomass yields, ranging from 0.4 to 2.8 Mg ha^{-1} for single-species pea cover crops. Burgess (2012) reported mean early-terminated pea LGM biomass of 2.8 Mg ha^{-1} across three years at no-till farm field locations near Amsterdam, MT. In all three years, May and June precipitation was at or near the 30-year average. In contrast, O'Dea (2013) reported mean early-terminated pea LGM biomass of 1.0 Mg ha^{-1} across four no-till farm field sites in the MLRA 52 in a low

precipitation year. Likewise, Miller et al. (2006) reported early-terminated pea LGM shoot biomass values ranging from 0.4 to 1.7 Mg ha^{-1} across three no-till field sites in Montana in a year with lower than average precipitation. In most of these cases, growing season precipitation accounted for differences in cover crop biomass yield.

Cover Crop C:N Ratios and Biomass N. Our results indicate no clear difference between pea and mixed species cover crops on C:N and biomass N. The C:N of the cover crop biomass differed between the two measured treatments only at Amsterdam, and even then the difference was minimal, with 13.4 for Pea and 16.7 for Full (Table 2.18). Mixed cover crops of legumes and grasses can produce a higher C:N than single species legume cover crops alone, resulting in a potential increase in soil organic matter (Kuo et al. 1997) and reduced N leaching (McCracken et al. 1994). The C:N of a winter cover crop of hairy vetch (*Vicia villosa* Roth) increased from 12 to 32 with the addition of cereal rye (*Secale cereal* L.). However, this increase occurred in only one of three study years due to poor rye establishment (Sainju et al. 2005). Timing of C:N measurement is important, as differences in residue quality can be greater when crops are grown to full maturity and plant tissues have senesced; for example, the C:N of a crop residue of pea after grain harvest increased from 54 to 84 with the addition of barley (Hauggaard-Nielsen et al. 2003). It is possible that the Full mixture of eight-species in our study diluted any increased C contribution from the Fibrous Root functional group, as fibrous species constituted only 9, 22 and 30% of the total biomass at Dutton, Bozeman, and Amsterdam, respectively (including grassy weeds). Notably, there was no fibrous root component in any of the two measured treatments at Conrad. It may be that a greater

proportion of Fibrous Root is required in a cover crop mixture for any noticeable difference in C:N to be detected.

No treatment differences in cover crop biomass N yield were detected for any site-year (Table 2.18). Results varied widely, from a low of 9 kg N ha^{-1} in the 2012 Minus Fibrous Root treatment at Conrad to a high of 131 kg N ha^{-1} in the 2013 Full treatment at Bozeman. In 2012, mean biomass N yields were 26 kg N ha^{-1} at Amsterdam and 12 kg N ha^{-1} at Conrad. In comparison, mean 2013 biomass N yields were 129 kg N ha^{-1} at Bozeman, and 65 kg N ha^{-1} at Conrad (Table 2.19).

Overall, biomass N values from this study had a wider range than other values reported from the region, due to low precipitation and biomass production at both 2012 sites and high residual nitrate and moisture at Bozeman. Previous Montana studies have found biomass N values of 32 kg ha^{-1} (O'Dea 2013), 83 to 116 kg N ha^{-1} (Burgess 2012), and 15 to 54 kg ha^{-1} (Miller 2006).

Soil Water. In 2012, soil water differed among treatments at Conrad, but not at Amsterdam (Table 2.21). Mean total soil water was 162 mm across all eleven treatments at Amsterdam. Although cover crop biomass production was relatively low, we expected there would have been less soil water after cover crop treatments than after Fallow. It is notable, however, that 2012 was the driest year in the Gallatin Valley in the last 50 yr. Between these unusually dry conditions, and a relatively sandy soil, precipitation likely evaporated from Fallow faster than it could be accumulated as stored water. In contrast, at Conrad, soil water varied by treatment at each depth with Fallow having the most total soil water (242 mm). Water use in the 0.6 to 0.9 m depth was unexpected since previous cover crop studies in this region showed water extraction usually occurred in the 0 to 0.6

m depth (Miller et al. 2006, Burgess 2012). The Nitrogen Fixer group had the greatest total soil water difference when compared with Fallow (174 mm, or 28% less), indicating the greatest soil water use. Common vetch proved difficult to kill with glyphosate herbicide alone at both 2012 sites, and did not completely terminate until after additional herbicide application, about 30 d after soil water samples were taken. Therefore, total soil water use in 2012 treatments with a nitrogen fixer functional group is likely greater than measured. Correlation analysis found a strong negative relationship between cover crop biomass production and total soil water at Conrad (Table 2.21), with 35 mm of soil water used for every 0.5 Mg ha^{-1} of cover crop biomass produced.

In 2013, soil water differed among treatments at both Bozeman and Dutton above 0.6 m (Table 2.22). Notably, at both sites, soil water following Pea was the most different from soil water for Fallow, indicating it used more soil water than all other cover crop treatments. At Bozeman, Pea had 49 mm (20%) less total soil water than Fallow, and at Dutton, Pea had 51 mm (27%) less total soil water than Fallow. At Bozeman, the Fibrous Root treatment was not different than Fallow, and at the Dutton site, the Fibrous Root, Nitrogen Fixer, and Minus Fibrous Root treatments were not different than Fallow. All other cover crop treatments at both sites had measurably less soil water than Fallow, and it is not clear why the Fibrous Root treatment resulted in a similar response as Fallow at both sites, even with substantial biomass production (4.33 and 2.85 Mg ha^{-1} in the Fibrous Root treatment at Bozeman and Dutton, respectively). At Bozeman, no treatments differed from Fallow at the 0.6 to 0.9 m depth, suggesting that the greatest water use during the cover crop growing season occurred in the top 0.6 m of the soil profile at this site-year. This agrees with earlier studies, in which early-terminated pea LGMs in

Montana no-till systems had a pattern of preferential water use in the top 0.6 m (Miller et al. 2006; Burgess et al. 2012; O'Dea et al. 2013). Correlation analysis did not indicate cover crop biomass to be associated with total soil water at either 2013 site (Table 2.21). Reasons for this lack of correlation are unclear, although timely precipitation close to the time of cover crop termination may have recharged the soil profile enough to mask any treatment effects.

Soil Nitrogen. At all four site-years, Fallow had more nitrate-N than each cover crop treatment. In 2012, total soil nitrate-N values at Amsterdam were greater in Fallow than in all three measured cover crop treatments (Table 2.23). Overall, the combined cover crop treatments had 31 kg NO_3-N ha^{-1} (31%) less mean total soil nitrate-N than Fallow at Amsterdam. Nitrate-N treatment differences were only detectable in the top 0 to 0.3 m increment at the Amsterdam site. Likewise, the combined cover crop treatments had 18 kg NO_3-N ha^{-1} (50%) less mean total soil nitrate-N than Fallow at Conrad. Notably, treatment differences were detected only at the 0 to 0.3-m depth at both sites, and there were no treatment differences among the cover crop treatments, suggesting similar N uptake mechanisms in a low-precipitation year.

In 2013, Fallow averaged 109 kg NO_3-N ha^{-1} (45%) more nitrate-N at the time of cover crop termination than either Pea or Full at the Bozeman site (Table 2.24). Differences in soil nitrate-N were only detected in the top 0 to 0.3 and 0.3 to 0.6 m depths. Notably, the Bozeman site-year was the only one of the four in which there was a measurable total soil nitrate-N difference between the measured cover crop treatments, and it was judged substantial at 64 kg NO_3-N ha^{-1}. Given the lack of difference in the C:N ratios and biomass N yields of the Pea and Full above-ground plant tissues at this site

(Table 2.18), we assume that the difference in soil nitrate-N values is due to greater biological N fixation by the Pea cover crop. At the Dutton site, total soil nitrate-N averaged 42 kg NO_3-N ha^{-1} (66%) greater in the Fallow than in the Pea and Full treatments to a depth of 0.6 m. A difference in cover crop treatments detected only at the 0.3 to 0.6 m depth, where the Full treatment had 9 and 22 kg NO_3-N ha^{-1} less soil nitrate-N than Pea and Fallow.

The Dutton site was more N limited than Bozeman based on mean soil nitrate pools at cover crop termination (Tables 2.24), with 122 kg NO_3-N ha^{-1} at Bozeman and 35 kg NO_3-N ha^{-1} at Dutton to a depth of 0.6 m. Although N fixation was not measured, roots were inspected at each site, with N-fixing nodules clearly present and active, indicating some of the biomass N in the legume-containing treatments was provided from biological N fixation, in addition to available soil N.

Results from all site-years show that cover crops depleted soil nitrate-N compared with Fallow. These results are not surprising, and agree with other studies in the region. Pikul et al. (1997) reported 15 kg NO_3-N ha^{-1} (35%) less soil nitrate-N after one year of lentil green manure when compared with fallow in a no-till rotation at the time of spring wheat seeding. Likewise, O'Dea (2013) reported 16 kg NO_3-N ha^{-1} (44%) less mean soil nitrate-N values after termination of single-species LGMs when compared with fallow across five no-till sites in Montana. These results were measured after only one year of LGMs in the rotation, similar to this study. Although legumes have the capacity to fix biological nitrogen, it has been shown that they first use substantial available soil N before initiating N fixation (van Kessel and Hartney 2000), and would be expected to deplete soil nitrate-N when compared with fallow in the short term. What is not known is

how long term N mineralization will compare between single species and multi-species cover crops.

Greater soil nitrate-N treatment differences were anticipated among the cover crop treatments, due to the presence of deep rooted Tap Root and Brassica functional groups within the mixtures, and it was hypothesized that mixtures containing these functional groups would have less soil nitrate-N than the Pea treatment. However, this was not the case, as only the Full treatment had less total soil nitrate-N than the Pea treatment in only one of four site-years. Rooting depth of different plant species has been shown to play a part in soil water use in the region (Miller and Holmes 2012), with both sunflower and safflower having deeper soil water depletion than pea or lentil at maturity, and we assumed the soil nitrate-N dynamics would be similar. Likewise, Thorup-Kristensen (2001) explored the root growth of brassica, legume, and monocot grass winter cover crops, and reported that brassica cover crops have faster and deeper root development than monocot cover crops, resulting in greater soil nitrate-N uptake.

The one site-year where cover crop treatment differences existed was at the Bozeman site, with the highest soil organic carbon (Table 2.1), and in a wetter than normal precipitation year, where the four Tap Root and Brassica plant species (turnip, safflower, radish, and winter canola) comprised 67% of the total biomass of the Full treatment. In contrast, the Dutton site had no soil nitrate-N differences between the Pea and Full treatments, even though the Full treatment was comprised of 72% of the Tap Root and Brassica plant species. Inherent differences in soils, and overall biomass yield likely have more to do with this than slight variation in composition of mixtures.

Wheat Yield, Protein, and Seed N Yield. Spring wheat productivity was only measured for the Conrad site in 2013, as the Amsterdam wheat crop was destroyed by hail prior to harvest. Only values for treatments not compromised by downy brome in the cover crop year were reported. These included Fallow, Pea, Nitrogen Fixers, Tap Root, Brassica, and Minus Fibrous Root. No treatment by fertilizer interaction was detected, and treatment means were reported across all three N fertility levels (Table 2.25). Wheat following Fallow had the greatest yield (3.27 Mg ha^{-1}) while the wheat following the Nitrogen Fixers treatment had the least yield (2.49 Mg ha^{-1}, or 24% less). Correlation analysis (Table 2.26) indicates that both lower soil water and decreased soil nitrate-N after cover crop treatments may explain the wheat yield decrease after cover crop treatments. Notably, decreased soil water was significantly correlated with decreased wheat yield at all three N fertility levels, while decreased soil nitrate-N was only significantly correlated with decreased wheat yields at the low N fertility level. Our results showed that decreased soil water after a cover crop was a much greater problem than decreased soil nitrate-N.

Decreased wheat yields following the first year of a cover crop agree with results reported by O'Dea (2013), who showed that grain yield was lower after one year of a single-species LGM than Fallow by 0.24 Mg ha^{-1}, or 6%. However, O'Dea (2013) showed that N immobilization, and not water use, was the reason for reduced wheat yield in a similar 'dry year' – 'wet year' sequence in the same region of Montana. Our study showed that a single-species Pea treatment decreased subsequent wheat yield by a mean value of 0.62 Mg ha^{-1} (19%) across all N fertility levels. In contrast, Miller et al. (2006)

reported no negative effect on wheat yield after single-species pea green manure compared with fallow.

Not surprisingly, fertilizer had an effect on grain yield in our study, with a mean yield increase of 0.25 Mg ha^{-1}, or 9%, when both the high and medium fertilizer treatments were compared with the low fertilizer treatment. Interestingly, the high fertilizer treatment yielded no better than the medium fertilizer treatment level across all cover crop treatments, likely due to water limitation

Neither wheat grain protein nor seed N yield responded to cover crop treatments (Table 2.25). This is similar to results from Miller et al. (2006) who reported no differences between Fallow and early-terminated LGM treatments in subsequent wheat protein and seed N yield. However, O'Dea (2013) showed that LGM treatments depressed mean seed N yield by 7 kg ha^{-1} compared with Fallow.

Fertilizer N treatments increased both wheat protein and seed N yield markedly at all three treatment levels. In contrast, fertilizer N treatments resulted in a wheat yield increase only between the low and medium fertilizer treatments (Table 2.25). Correlation analysis found a strong positive relationship between total soil nitrate-N at the time of cover crop termination and wheat yield only at the low fertilizer N treatment (Table 2.26). Likewise, a strong positive relationship was found between total soil nitrate-N and seed N yield at the low and medium fertilizer N treatments levels. Total soil nitrate-N at the time of cover crop termination had little effect on subsequent wheat protein, even when measured in the low fertility sub-plot.

Aase et al. (1996) reported that no more than 50 mm of soil water should be used by a cover crop compared with fallow to not diminish subsequent grain yields in the

NGP. Correlation analysis of the effect of total soil water on subsequent wheat yield in our study supports this idea (Table 2.26), with low soil water strongly correlated with wheat yield at all three N fertility levels. In 2012, only the Pea and Nitrogen Fixer treatments used at least 50 mm more soil water than Fallow (55 and 68 mm, respectively) at the Conrad site. Not surprisingly, in 2013 the Conrad wheat yield was lowest following the Pea and Nitrogen Fixer treatments (Table 2.25). Total soil water after cover crop termination was strongly negatively correlated with wheat protein only at the high fertilizer N treatment, with less soil water associated with increased wheat protein (Table 2.26).

Summary and Conclusions

Results from this study indicate few notable differences between mixed cover crops and a single-species pea cover crop after only one crop rotation. Only minor differences were measured in the above-ground parameters of biomass, C:N, and biomass N yield. Cover crop mixtures produced substantially more biomass than Pea only at one of four sites, Likewise, the Full treatment had a slightly greater C:N ratio than Pea only at one of four sites, and this difference did not translate into a biomass N yield difference.

Few differences were detected between mixed cover crop and a single-species pea in the below-ground parameters of soil water and nitrate-N after one year. At three of four sites-years, soil water after mixed cover crops was similar to Pea. Only at the one site-year were differences in soil nitrate-N detected between mixed-species and Pea treatments.

We had hypothesized that different plant species in a cover crop mixture would provide different ecosystem services when compared with a single species cover crop; but after one year this does not seem to be the case. This study will continue for two more years, with repetition of each cover crop planting at each site. It is possible that after completion of this 4-yr study, more pronounced differences will emerge. Research from the region suggests that six to eight years of rotation may be necessary prior to seeing any cover crop benefits (Zentner et al. 2004; Allen et al. 2011; Miller et al. 2014), and it is likely that a similar time-frame is needed to see any differences between single and mixed species cover crops.

Acknowledgements

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Table 2.1. Site characteristics of four cover crop study sites in Montana. Soil analyses conducted on soils collected within 30 days of cover crop seeding.

Property	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
Elevation (m)	1446	1039	1486	1050
Soil classification	frigid Typic Calciustoll	frigid Aridic Argiustoll	frigid, Typic Argiustoll	frigid, Aridic Argiustoll
Texture†	Silt loam	Clay loam	Silt loam	Clay loam
pH	8.2	6.5	7.0	6.7
Soil Organic Carbon (g kg^{-1})	1.4	1.4	3.3	1.9
NO_3-N (mg kg^{-1})	6.0	8.5	7.3	7.5
Olsen P (mg kg^{-1})	13	28	32	43
Extractable K (mg kg^{-1})	359	498	346	595
Sampling date	26 Mar 2012	11 Apr 2012	12 Apr 2013	10 Apr 2013

All samples analyzed by AgVise Laboratories, Northwood, ND of samples from the 0 to 15 cm depth.

†Laboratory methods used include; texture, Buouyocos hydrometer; pH and salinity, 1:1 soil-to-water method; soil organic matter, loss of weight on ignition (LOI); NO_3-N, KCl extraction, Cd reduction, spectrophotometer determination; Olsen P, $NaHCO_3$ extraction, colorimetric determination; extractable K, NH_4OAc extraction, atomic absorption spectrometry.

Table 2.2. Monthly precipitation and cover crop growing season GDD (0 °C) at four cover crop mixture plot study sites in Montana. Long-term average (LTA) calculated from 1981-2010, Western Regional Climate Center, Reno, NV. Nearest WRCC station code and distance given for each site.

	Amsterdam			Conrad			Bozeman†		Dutton	
	240622 (19 km)			241974 (24 km)			241044 (6 km)		241974 (26 km)	
	LTA	2012	2013	LTA	2012	2013	LTA	2013	LTA	2013
	Precipitation (mm)									
Sept. – Mar.	137	83	76	98	69	92	212	134	98	92
April	39	68	18	26	60	25	57	24	26	25
May	61	53	89	50	48	81	80	85	50	71
June	62	34	59	64	83	84	79	102	64	80
July	30	27	16	35	24	12	38	10	35	26
August	26	13	13	32	10	42	35	30	32	42
TOTAL	358	278	271	303	319	336	501	385	303	356
Cover crop growing season	--	147	--	--	154	--	--	196	--	166
	Average Temperatures (°C)									
April	7.3	7.5	4.2	5.9	8.1	4.3	6.3	4.6	5.9	4.3
May	12.0	9.1	11.2	11.1	10.8	10.9	10.9	11.2	11.1	10.9
June	16.4	15.6	15.6	15.2	15.5	15.3	15.2	15.7	15.2	15.3
July	20.8	21.1	20.6	18.8	19.6	20.0	19.5	20.5	18.8	20.0
August	20.1	18.9	19.9	18.1	18.0	20.0	18.9	19.8	18.1	20.0
LTA	7.4	7.3	6.3	6.2	7.0	6.8	7.0	6.9	6.2	6.8
GDD (0 °C)‡	--	832	--	--	716	--	--	899	--	931§

†Bozeman precipitation from WRCC station, not from on-site weather monitor.

‡GDD calculated from day after seeding to day of herbicide termination.

§Dutton GDD calculated from Conrad, MT NRCS SCAN site data, not from on-site weather station.

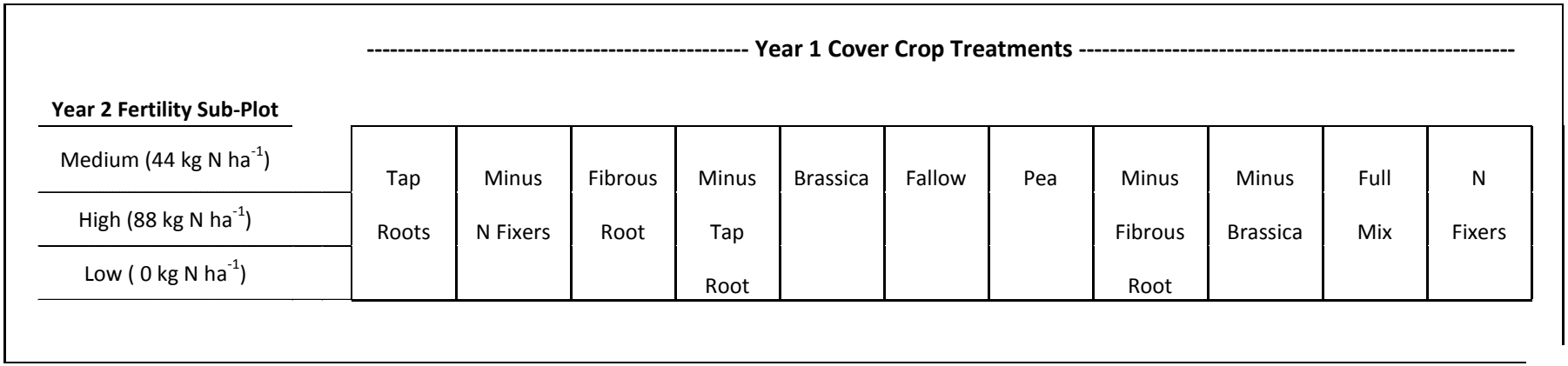

Figure 2.1. Layout of one field block with all Year 1 cover crop treatments. Left margin notes Year 2 wheat fertilization sub-plot layout. All cover crop and fertility treatments randomized within four blocks at all four sites.

Table 2.3. Plant functional groups and species used to compose cover crop treatments at two sites in Montana, 2012. Large and small-seeded species seeded in offset rows at 4 and 2 cm depth, respectively.

Functional Group		Species		Seed Size
Nitrogen Fixers	NF	Pea	*Pisum sativum* L. cv. Arvika	Large
		Common Vetch	*Vicia sativa* L.	Small
Fibrous Roots	FR	Oat	*Avena sativa* L. cv. Monico	Large
		Italian Ryegrass	*Lolium perenne* L. ssp. *multiflorum* Lam. Husnot cv. Tetila	Small
Tap Roots	TR	Safflower	*Carthamus tinctorius* L. cv. MonDak	Large
		Turnip	*Brassica rapa* L.	Small
Brassicas	BC	Radish	*Raphanus. sativus* L. var. *longipinnatus*	Large
		Camelina	*Camelina sativa* L. Crantz cv. SO-02	Small

Table 2.4. Plant functional groups and species used to compose cover crop treatments at two sites in Montana, 2013. All species seeded together at 1 cm depth.

Functional Group		Species	
Nitrogen Fixers	NF	Pea	*Pisum sativum* L. cv. Arvika
		Lentil	*Lens culinaris* Medik. cv. Indianhead
Fibrous Roots	FR	Oat	*Avena sativa* L. cv. Oatana
		Millet	*Panicum miliaceum* L. sp.
Tap Roots	TR	Safflower	*Carthamus tinctorius* L. cv. MonDak
		Turnip	*Brassica rapa* L.
Brassicas	BC	Radish	*Raphanus. sativus* L. var. *longipinnatus*
		Winter Canola	*Brassica napus* L. var. *napus* cv. Dwarf Essex

Table 2.5. Cover crop treatments and functional group composition at four plot study sites in Montana, 2012-2013.

	Treatment	Abbreviation	Functional Group Composition
1	Fallow	SF	___
2	Pea	PEA	Pea
3	Full	FULL	Nitrogen Fixers, Fibrous Roots, Tap Roots, Brassicas
4	Nitrogen Fixer	NF	Nitrogen Fixers
5	Fibrous Root	FR	Fibrous Roots
6	Tap Root	TR	Tap Roots
7	Brassica	BC	Brassicas
8	Minus Nitrogen Fixer	MNF	Fibrous Roots, Tap Roots, Brassicas
9	Minus Fibrous Root	MFR	Nitrogen Fixers, Tap Roots, Brassicas
10	Minus Tap Root	MTR	Nitrogen Fixers, Fibrous Roots, Brassicas
11	Minus Brassica	MBC	Nitrogen Fixers, Fibrous Roots, Safflower†

† *Turnip excluded from MBC treatment to avoid confounding the treatment effects, as turnip is also a brassica.*

Table 2.6. Agronomic field management for cover crop mixture study at Amsterdam, Conrad, Bozeman, and Dutton, 2012-2013.

Event	Amsterdam		Conrad		Bozeman	Dutton
	2012	2013	2012	2013	2013	2013
Soil sample date	26 Mar	1 Apr	11 Apr	4 Apr	12 Apr	10 Apr
Cover crop PRE herbicide	26 Mar[†]	--	--	--	3 May[‡]	--
Cover crop seeding date	3 Apr	--	4 Apr	--	2 May	5 May
Urea application (kg N ha^{-1})	16 Apr (34)	3 Apr (0, 44, 88)	--	27 Apr (0, 44, 88)	--	--
Cover crop herbicide	16 Apr.[§]		--		--	--
Cover crop insecticide	--		--		6 June[¶]	--
Cover crop stand counts	7 May	--	14 May		5 June	10 June
Cover crop herbicide	14 May[#]	17 May[#]			--	--
Cover crop termination	13 June	--	16 June		5 July[††]	10 July[‡‡]
Cover crop biomass harvest	14 June	--	20 June		8 July	11 July
Soil sample date	14 June		27 June		16 July	11 July
Cover crop POST herbicide	17 July[††]					
Wheat cultivar		Duclair sw		Duclair sw	Warhorse ww	Warhorse ww
Wheat seeding date		3 Apr		27 Apr	21 Sept	12 Sept
Wheat PRE herbicide		11 Apr[†]		6 Apr[§§]	16 Sept[‡‡]	17 Sept [†]
Wheat herbicide		--		7 May [†]		
Wheat harvest date		NA		20 Aug		

[†] 0.68 kg ha^{-1} of N-(phosphonomethyl)glycine in the form of isopropylamine salt

[‡] 0.68 kg ha^{-1} of N-(phosphonomethyl)glycine in the form of isopropylamine salt, plus 0.04 kg ha^{-1} aminated phosphoric and carboxylic acids, sulphurated amides, and spray deposition aids as an adjuvant.

[§] 0.06 kg ha^{-1} of Quizalofop-P-ethyl {ethyl(R)-2-[4-(6-chloroquinoxalin-2-yloxy)-phenoxy]propionate}

[¶] 0.03 kg ha^{-1} Lambda-cyhalothrin on all plots, except SF and FR.

[#] 0.22 kg ha^{-1} (E)-2[1-[[(3-chloro-2-propenyl)oxy]limino]propyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one on all plots without a fibrous root component.

[††] 0.68 kg ha^{-1} of N-(phosphonomethyl)glycine in the form of isopropylamine salt, plus 0.16 kg ha^{-1} diglycolamine salt of 3,6-dichloro-o-anisic acid

[‡‡] 0.92 kg ha^{-1} N-(phosphonomethyl)glycine in the form of isopropylamine salt

[§§] 0.68 kg ha^{-1} N-(phosphonomethyl)glycine in the form of potassium salt

Table 2.7. Seeding rate monoculture equivalents for cover crop mixture study at four Montana locations.

Species	2012	2013
	----- Target Plant Population m^{-2} -----	
Pea	80	120
Common Vetch	80	--
Lentil	--	120
Oat	160	120
Italian Ryegrass	240	120
Millet	--	120
Turnip	60	120
Safflower	50	120
Radish	60	120
Camelina	200	--
Winter Canola	--	120

Table 2.8. Cover crop stand count 34 d after seeding, 7 May 2012, Amsterdam, MT. Percentages represent variation of the actual stand count from the target seeding rate.

Treatment	Target	Total	Pea	Common Vetch	Oat	Italian Ryegrass	Turnip	Safflower	Radish	Camelina
		Plants m^{-2}								
Pea	80	77 (-4%)	77	--	--	--	--	--	--	--
Full	121	121 (0%)	17	11	39	23	5	5	9	13
Nitrogen Fixer	80	113 (+41%)	41	73	--	--	--	--	--	--
Fibrous Root	200	189 (+6%)	--	--	109	81	--	--	--	--
Tap Root	55	29 (-47%)	--	--	--	--	12	17	--	--
Brassica	130	95 (-27%)	--	--	--	--	--	--	41	54
Minus Nitrogen Fixer	128	103 (-20%)	--	--	44	23	5	7	9	14
Minus Fibrous Root	88	80 (-9%)	18	15			6	7	10	23
Minus Tap Root	137	107 (-22%)	16	12	28	22	--	--	10	21
Minus Brassica	122	153 (+25%)	22	30	40	55	--	6	--	--

Table 2.9. Cover crop stand count 39 d after seeding, 14 May 2012, Conrad, MT. Percentages represent variation of the actual stand count from the target seeding rate.

Treatment	Target	Total	Pea	Common Vetch	Oat	Italian Ryegrass	Turnip	Safflower	Radish	Camelina
		---------- Plants m^{-2} ----------								
Pea	80	128 (+60%)	128	--	--	--	--	--	--	--
Full	121	171 (+41%)	22	20	46	35	5	10	9	23
Nitrogen Fixer	80	155 (+94%)	50	105	--	--	--	--	--	--
Fibrous Root	200	224 (+12%)	--	--	122	102	--	--	--	--
Tap Root	55	57 (+4%)	--	--	--	--	18	39	--	--
Brassica	130	84 (-35%)	--	--	--	--	--	--	28	56
Minus Nitrogen Fixer	128	150 (+17%)	--	--	56	41	8	10	5	30
Minus Fibrous Root	88	91 (+3%)	17	14	--	--	7	14	13	27
Minus Tap Root	137	153 (+12%)	18	24	37	34	--	--	9	31
Minus Brassica	122	146 (+20%)	23	24	52	31	--	15	--	--

Table 2.10. Cover crop stand count 34 d after seeding, 5 June 2013, Bozeman, MT. 120 plants m^{-2} target rate for all treatments. Percentages represent variation of the actual stand count from the target seeding rate.

Treatment	Total	Pea	Lentil	Oat	Millet	Turnip	Safflower	Radish	Winter Canola
	Plants m^{-2}								
Pea	119 (-1%)	119	--	--	--	--	--	--	--
Full†	186 (+55%)	18	18	21	12	61	16	23	17
Nitrogen Fixer	152 (+27%)	78	74	--	--	--	--	--	--
Fibrous Root	113 (-6%)	--	--	75	38	--	--	--	--
Tap Root	111 (-8%)	--	--	--	--	63	48	--	--
Brassica	124 (+3%)	--	--	--	--	--	--	72	52
Minus Nitrogen Fixer	126 (+5%)	--	--	31	13	18	23	20	21
Minus Fibrous Root	145 (+21%)	25	23	--	--	28	21	27	21
Minus Tap Root	135 (+13%)	23	29	27	13	--	--	25	18
Minus Brassica	126 (+5%)	29	28	33	15	--	21	--	--

†Turnip rates exceeded target due to seed weighing error.

Table 2.11. Cover crop stand count 34 d after seeding, 10 June 2013, Dutton, MT. 120 plants m^{-2} target rate for all treatments. Percentages represent variation of the actual stand count from the target seeding rate.

Treatment	Total	Pea	Lentil	Oat	Millet	Turnip	Safflower	Radish	Winter Canola
	Plants m^{-2}								
Pea	106 (-12%)	106	--	--	--	--	--	--	--
Full†	127 (+6%)	16	15	17	2	35	11	19	12
Nitrogen Fixer	121 (+1%)	58	63	--	--	--	--	--	--
Fibrous Root	77 (-36%)	--	--	63	14	--	--	--	--
Tap Root	83 (-31%)	--	--	--	--	35	48	--	--
Brassica	94 (-22%)	--	--	--	--	--	--	60	34
Minus Nitrogen Fixer	90 (-30%)	--	--	24	5	12	16	21	12
Minus Fibrous Root	107 (-11%)	23	19	--	--	15	17	20	13
Minus Tap Root	98 (-18%)	23	14	20	3	--	--	21	17
Minus Brassica	100 (-17%)	24	20	31	7	--	18	--	--

†Turnip rates exceeded target due to seed weighing error.

Table 2.12. Cover crop biomass by plant species 71 d after seeding, 13 June 2012, Amsterdam, MT. Treatments with different letters were significantly different from each other ($p < 0.05$, LSD).

Treatment	Total		Pea	Common Vetch	Oat	Italian Ryegrass	Turnip	Safflower	Radish	Camelina	Weeds
	Mg ha^{-1}										
Pea	0.76	de	0.74	--	--	--	--	--	--	--	0.02
Full	1.01	ab	0.16	0.05	0.30	0.04	0.07	0.02	0.13	0.10	0.14
Nitrogen Fixer	0.70	e	0.39	0.30	--	--	--	--	--	--	0.00
Fibrous Root	1.13	a	--	--	0.91	0.14	--	--	--	--	0.08
Tap Root	0.41	f	--	--	--	--	0.29	0.12	--	--	0.00
Brassica	0.83	cde	--	--	--	--	--	--	0.51	0.31	0.01
Minus Nitrogen Fixer	0.86	bcd	--	--	0.35	0.06	0.08	0.02	0.08	0.11	0.17
Minus Fibrous Root	0.83	cde	0.23	0.06	--	--	0.13	0.04	0.19	0.17	0.01
Minus Tap Root	1.03	ab	0.16	0.06	0.32	0.06	--	--	0.15	0.15	0.12
Minus Brassica	0.98	abc	0.23	0.06	0.44	0.05	--	0.03	--	--	0.16
	p-value										
	<0.001										
LSD (p<0.05)	0.17										
SE	0.06										

Fallow biomass not measured in 2012.

Table 2.13. Cover crop biomass by plant species 73 d after seeding, 16 June 2012, Conrad, MT. Treatments with different letters were significantly different from each other ($p < 0.05$, LSD).

Treatment	Total		Pea	Common Vetch	Oat	Italian Ryegrass	Turnip	Safflower	Radish	Camelina	Weeds
	Mg ha^{-1}										
Pea	0.61	a	0.60	--	--	--	--	--	--	--	0.0
Nitrogen Fixer	0.51	a	0.35	0.16	--	--	--	--	--	--	0.0
Tap Root	0.19	b	--	--	--	--	0.07	0.13	--	--	0.0
Brassica	0.21	b	--	--	--	--	--	--	0.03	0.18	0.0
Minus Fibrous Root	0.43	a	0.19	0.06	--	--	0.04	0.02	0.01	0.12	0.0
	p-value										
	<0.001										
LSD (p<0.05)	0.18										
SE	0.06										

Fallow biomass not measured in 2012.

Table 2.14. Cover crop biomass including weeds at Bozeman and Dutton, MT, 2013.

	----- Mg ha^{-1} -----
Bozeman	3.68
Dutton	2.65
	------ *p*-values ------
Site	<0.001
Treatment x Site	0.69

Table 2.15. Pearson's correlation coefficients (r) for relationship between cover crop biomass and plant species number at four sites in Montana, 2012-2013. Cover crop biomass includes weed biomass. All Fallow treatments excluded. (n=40).

Amsterdam 2012	Conrad† 2012	Bozeman 2013	Dutton 2013
0.41**	NS	0.39*	NS

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

†Conrad treatments only include Pea, MFR, NF, TR, and BC (n=20).

Table 2.16. Cover crop biomass by species 64 d after seeding, 5 July 2013, Bozeman, MT. Treatments with different letters were significantly different from each other ($p < 0.05$, LSD).

Treatment	Total		Pea	Lentil	Oat	Millet	Turnip	Safflower	Radish	Winter Canola	Weeds†
	---------- Mg ha^{-1} ----------										
Pea	3.85	ab	3.55	--	--	--	--	--	--	--	0.29
Full	4.30	a	0.41	0.07	0.57	0.0	0.67	0.32	1.58	0.32	0.36
Nitrogen Fixer	3.44	bc	2.76	0.37	--	--	--	--	--	--	0.31
Fibrous Root	4.33	a	--	--	3.60	0.03	--	--	--	--	0.70
Tap Root	2.89	c	--	--	--	--	1.35	1.33	--	--	0.21
Brassica	3.73	ab	--	--	--	--	--	--	2.78	0.69	0.26
Minus Nitrogen Fixer	4.21	a	--	--	1.30	0.0	0.15	0.43	1.55	0.45	0.32
Minus Fibrous Root	4.09	ab	0.75	0.09	--	--	0.36	0.41	1.71	0.40	0.36
Minus Tap Root	3.93	ab	0.70	0.10	0.94	0.0	--	--	1.55	0.45	0.27
Minus Brassica	4.36	a	1.14	0.18	1.76	0.0	--	0.71	--	--	0.57

	p-value
	<0.001
LSD (p<0.05)	0.71
SE	0.25

†Volunteer wheat constituted the majority of weeds.

Fallow biomass was 1.30 Mg ha^{-1} and was comprised mainly of volunteer wheat.

Table 2.17. Cover crop biomass by species 65 d after seeding, 10 July 2013, Dutton, MT. Treatments with different letters were significantly different from each other ($p < 0.05$, LSD).

Treatment	Total		Pea	Lentil	Oat	Millet	Turnip	Safflower	Radish	Winter Canola	Weeds
	---------- Mg ha^{-1} ----------										
Pea	2.66	a	2.66	--	--	--	--	--	--	--	0.00
Full	3.48	a	0.63	0.05	0.30	0.00	1.12	0.42	0.75	0.21	0.01
Nitrogen Fixer	2.39	a	2.01	0.37	--	--	--	--	--	--	0.01
Fibrous Root	2.85	a	--	--	2.78	0.03	--	--	--	--	0.04
Tap Root	2.83	a	--	--	--	--	0.84	1.94	--	--	0.05
Brassica	2.93	a	--	--	--	--	--	--	2.21	0.68	0.04
Minus Nitrogen Fixer	2.73	a	--	--	0.57	0.00	0.25	0.52	1.02	0.35	0.03
Minus Fibrous	3.23	a	0.56	0.11	--	--	0.54	0.46	1.14	0.37	0.05
Minus Tap Root	2.81	a	0.58	0.18	0.46	0.00	--	--	1.10	0.46	0.03
Minus Brassica	3.05	a	0.82	0.17	1.04	0.01	--	0.93	--	--	0.08
	p-value										
	<0.001										
LSD (p<0.05)	1.16										
SE	0.40										

Fallow biomass was 0.13 Mg ha^{-1}.

Table 2.18. Cover crop C:N and biomass N yield by treatment for four sites, MT, 2012-2013.

Treatment	Amsterdam		Conrad		Bozeman		Dutton	
	C:N	Biomass N	C:N	Biomass N	C:N	Biomass N	C:N	Biomass N
		kg ha^{-1}		kg ha^{-1}		kg ha^{-1}		kg ha^{-1}
Pea	13.4	25	18.7	14	13.2	127	18.0	67
Full	16.7	26	---	---	14.2	131	25.9	63
Minus Fibrous Root	---	---	20.2	9	--	--	--	--
	ANOVA *p*-values							
	0.03	0.61	0.80	0.36	0.15	0.84	0.10	0.81

Table 2.19. C:N and biomass N yield by treatment for combined Bozeman and Dutton sites, 2013.

	2013	
	C:N	Biomass N
		kg ha^{-1}
Bozeman	13.7	129
Dutton	21.9	65
	ANOVA *p*-values	
Site	0.005	<0.001
Treatment x Site	0.08	0.74

Table 2.20. Soil water measured to a depth of 0.9 m at termination of cover crop treatments at Conrad and Amsterdam, MT 2012. Treatments with different letters were significantly different from each other ($p < 0.05$, LSD).

Treatment	Amsterdam 18 June 2012				Conrad† 27 June 2012			
	0 to 0.3 m	0.3 to 0.6 m	0.6 to 0.9 m	TOTAL	0 to 0.3 m	0.3 to 0.6 m	0.6 to 0.9 m	TOTAL
	Soil Water (mm)							
Fallow	63	55	49	168	94 a	86 a	61 a	242 a
Pea	56	52	49	157	83 c	55 c	48 bc	187 cd
Full	63	56	50	169	--	--	--	--
Nitrogen Fixer	59	52	48	160	81 c	51 c	43 cd	174 d
Fibrous Root	57	50	46	153	--	--	--	--
Tap Root	60	54	49	162	85 bc	66 b	51 abc	201 bc
Brassica	59	53	47	159	92 ab	69 b	51 abc	212 b
Minus Nitrogen Fixer	62	55	48	165	--	--	--	--
Minus Fibrous Root	59	55	47	160	83 c	60 bc	54 ab	195 bc
Minus Tap Root	59	52	46	157	--	--	--	--
Minus Brassica	67	55	49	172	--	--	--	--
	ANOVA *p*-values							
	0.30	0.40	0.65	0.41	0.02	<0.001	0.008	<0.001
LSD (p<0.05)	NS	NS	NS	NS	9	11	8	20
SE					3	4	3	7

†Conrad treatments with substantial downy brome pressure not included in analysis.

Table 2.21. Pearson's correlation coefficients (r) for relationship between cover crop biomass and total soil water to 0.9 m at cover crop termination at four sites in Montana, 2012-2013. Cover crop biomass includes weed biomass. Fallow treatments excluded (n=40).

Amsterdam 2012	Conrad† 2012	Bozeman 2013	Dutton‡ 2013
NS	-0.48*	NS	NS

* Significant at the 0.05 probability level.

†Conrad treatments only include Pea, MFR, NF, TR, and BC (n=20).

‡Total soil water measured to 0.6 m at Dutton.

Table 2.22. Soil water measured to a depth of 0.9 m at Bozeman, MT and to 0.6 m at Dutton, MT at termination of cover crop treatments. Treatments with different letters were significantly different from each other ($p < 0.05$, LSD).

Treatment	Bozeman, MT 16 July 2013				Dutton, MT 12 July 2013		
	0 to 0.3 m	0.3 to 0.6 m	0.6 to 0.9 m	TOTAL	0 to 0.3 m	0.3 to 0.6 m	TOTAL
	Soil Water (mm)						
Fallow	74 a	81 a	86	241 a	93 a	92 abc	186 a
Pea	51 d	63 b	78	192 c	65 e	70 de	135 e
Full	56 cd	61 b	77	194 bc	72 de	71 de	143 de
Nitrogen Fixer	51 d	68 b	87	206 bc	85 b	96 ab	181 a
Fibrous Root	66 abc	91 a	87	244 a	86 ab	87 abcd	172 abc
Tap Root	59 cd	64 b	86	209 bc	80 bc	79 bcde	160 bcd
Brassica	65 abc	68 b	71	203 bc	80 bcd	74 de	153 cde
Minus Nitrogen Fixer	62 bc	63 b	79	204 bc	80 bc	67 e	147 de
Minus Fibrous Root	70 ab	67 b	77	214 b	83 b	96 a	179 ab
Minus Tap Root	58 cd	69 b	79	206 bc	79 bcd	79 cde	156 cd
Minus Brassica	56 cd	63 b	79	198 bc	75 cd	78 de	150 de
	ANOVA p-values						
	<0.001	<0.001	0.15	<0.001	<0.001	0.004	<0.001
LSD (p<0.05)	10	10	NS	22	8	17	21
SE	4	3		7	3	6	7

Table 2.23. Soil nitrate-N measured to a depth of 0.9 m at Amsterdam (prior to spring wheat seeding) and Conrad (after cover crop termination), 2012-2013. Treatments with different letters were significantly different from each other ($p < 0.05$, LSD).

Treatment	Amsterdam 27 Feb 2013				Conrad 27 June 2012			
	0 to 0.3 m	0.3 to 0.6 m	0.6 to 0.9 m	TOTAL	0 to 0.3 m	0.3 to 0.6 m	0.6 to 0.9 m	TOTAL
	kg NO_3-N ha^{-1}							
Fallow	73 a	14	14	101 a	20 a	8	8	36 a
Pea	47 b	19	14	80 ab	9 b	3	4	16 b
Minus Fibrous Root	--	--	--	--	10 b	6	5	20 b
Minus Nitrogen Fixer	37 b	12	15	65 b	--	--	--	--
Fibrous Root	33 b	14	18	65 b	--	--	--	--
	ANOVA *p*-values							
	0.005	0.171	0.646	0.02	0.01	0.09	0.45	0.005
LSD (p<0.05)	19	NS	NS	22	7	NS	NS	10
SE	6			7	2			3

Table 2.24. Soil nitrate-N measured to a depth of 0.9 m at Bozeman, MT and to 0.6 m at Dutton, MT at cover crop termination, 2013. Treatments with different letters were significantly different from each other ($p < 0.05$, LSD).

Treatment	Bozeman 16 July 2013				Dutton 12 July 2013		
	0 to 0.3 m	0.3 to 0.6 m	0.6 to 0.9 m	TOTAL	0 to 0.3 m	0.3 to 0.6 m	TOTAL
	kg NO_3-N ha^{-1}						
Fallow	74 a	70 a	105	249 a	37 a	26 a	63 a
Pea	21 b	55 a	101	177 b	14 b	13 b	27 b
Full	30 b	15 b	68	113 c	11 b	4 c	16 b
	ANOVA *p*-values						
	0.007	0.017	0.063	0.002	0.049	0.003	0.01
LSD (p<0.05)	28	33	NS	54	21	9	28
SE	8	10		15	6	3	8

Table 2.25. Means for cover crop treatment effects at three N fertilizer rates on spring wheat yield, protein and N yield at Conrad, MT, 2013. Treatments with different letters were significantly different from each other ($p < 0.05$, LSD).

Treatment	Wheat Yield	Wheat Seed Protein	Seed N yield
	--- Mg ha^{-1} ---	---- g kg^{-1} ----	---- kg ha^{-1} ----
Fallow	3.27 a	134	77
Pea	2.65 cd	134	62
Nitrogen Fixers	2.49 d	147	64
Tap Root	3.08 ab	139	75
Brassica	2.81 bcd	134	66
Minus Fibrous Root	2.98 abc	137	72
	---------- ANOVA *p*-values ----------		
Source of variation			
Treatment	0.005	0.24	0.09
Treatment x Fertilizer	0.58	0.66	0.93
LSD ($p < 0.05$)	0.37	NS	NS
SE	0.12		
Fertilizer N			
0 kg N ha^{-1}	2.72 b	120 c	57 c
44 kg N ha^{-1}	2.99 a	132 b	69 b
88 kg N ha^{-1}	2.94 a	160 a	82 a
	---------- ANOVA ----------		
	<0.001	<0.001	<0.001
LSD ($p < 0.05$)	0.13	11	5
SE	0.05	4	2

Table 2.26. Pearson's correlation coefficients (r) for relationships between total soil water and nitrate to 0.9 m at cover crop termination and subsequent wheat yield and quality.

Fertility Sub-plot	n	Wheat Yield	Wheat Protein	Seed N Yield
	----------Total Soil Water (mm) ----------			
Low	23	0.58**	NS	0.46*
Medium	23	0.70***	NS	NS
High	23	0.77***	-0.59**	NS
	----------Total Soil N (kg NO_3-N ha^{-1}) ----------			
Low	12	0.72**	NS	0.76**
Medium	12	NS	NS	0.62*
High	12	NS	NS	NS

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

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CHAPTER 3

MULTI-SPECIES COVER CROPS EFFECTS ON SOIL BIOLOGY AFTER ONE YEAR IN THE SEMI-ARID NORTHERN GREAT PLAINS

Introduction

Wheat (*Triticum aestivm* L.) is the predominant cash crop in the northern Great Plains (NGP) of Montana. The NGP is marked by low and erratic rainfall, and as a result, producers have historically alternated wheat production with a fallow year to conserve soil water and nitrate. The main grain production region of the state (USDA-NRCS Major Land Resource Area 52) consists of 6 M ha, in which a wheat-fallow rotation remains the majority practice, with 42% of arable land in fallow in any given year (USDA-NASS, 2007). This is in contrast with neighboring regions of the NGP which have intensified crop production for economic reasons, and with the advent of no-till systems, which have greater conservation of soil water than tilled systems. There are many problems with fallow, including decreased soil organic matter (Campbell et al., 2000), decreased soil biological activity (Acosta-Martinez et al., 2007), increased saline seep and nitrate leaching (Daniels, 1987; Bauder et al., 1993), and greater soil erosion potential (Campbell et al., 1991).

Agronomic practices that enhance the soil microbial community and its processes may improve soil function and help overcome the negative effects of fallow. The soil microbial community is responsible for the decomposition of plant and animal materials, the immobilization and mineralization of plant nutrients, and the maintenance of soil

structure (Schloter et al., 2006). Therefore, changes in the soil biota may affect important soil functions such as nutrient cycling and availability, soil aggregate formation and stability, and water infiltration (Liebig et al., 2006). Biological parameters can respond more rapidly than most chemical and physical parameters to changes in land use (Nannipieri et al., 2001; Nannipieri and Badalucco, 2002; Gil-Sotres et al., 2005), making them good candidates as indicators of the early effects of agronomic management changes.

Soil communities are composed of multiple trophic levels that form a create a soil food web, with bacteria and fungi as the primary food source for higher organisms such as protozoa, nematodes, earthworms and other organisms. This food web cycles carbon and nitrogen through the soil system (Kladivko and Clapperton, 2011). Because of the complexity of soil food webs, there is no single indicator to measure soil biological activity. Rather, a minimum data set of several parameters is often used, based on land use, soil type, and climate (Karlen et al., 2001).

Microbial biomass is the living component of soil organic matter, excluding macro- and mesofauna and plant roots (Jenkinson and Ladd, 1981). Microbial biomass is a small percentage of the total organic matter content, with microbial biomass C (MBC) comprising 1-5% of the total soil organic C, and microbial biomass N (MBN) comprising 1-6% of total soil organic N. Microbial biomass has a much faster turnover rate than total soil organic matter and can therefore be a sensitive indicator of changes in soil management (Sparling, 1997).

Soil enzymes are exuded by bacteria and fungi and can catalyze the cycling of carbon, nitrogen, and other essential elements in the soil. Enzyme concentrations can characterize a soil's metabolic potential, quality, and fertility, as enzymes are sensitive to land management changes and are often the first measureable biological parameter to change in response to treatment (Bandick and Dick, 1999; Ndiaye et al., 2000; Shaw and Burns, 2006). Enzymes remain stable in the soil due to their ability to bind with humus and clay. As a result, management that promotes the formation and stability of organic matter also promotes the formation and stability of soil enzymes (Dick, 2011), and soils with greater microbiological activity would be expected to have greater enzyme production and buildup over time. However, results cannot be compared among different sites as soil type has a greater effect on enzyme activity than management practices (Dick, 2011). As a result, sites with different soil types can only be compared by relative treatment differences.

Potentially mineralizable nitrogen (PMN) is the amount of organic N that can be converted to mineral N, given specific climactic conditions (USDA-NRCS, 2014). This process is mediated by microorganisms and is a measure of N available for crop growth throughout the growing season (Canali and Benedetti, 2006), making it a relevant indicator of soil fertility.

Arbuscular mycorrhizal fungi (AMF) live in symbiosis with plant roots and aid in the uptake of mineral nutrients to the plant, especially phosphorus (Bloem et al., 2006). They are an essential part of nutrient cycling, plant nutrition, and soil structure (Johnson et al., 1999) making them an indicator of potential nutrient uptake and soil aggregation.

Mycorrhizal colonization measures the percent of mycorrhizal hyphae present in a given quantity of fine root samples.

No-till management and crop intensification positively affect soil biological activity in the NGP (Lupwayi et al., 1998; Frey et al., 1999; Liebig et al., 2004). Soil macro-aggregates increase with no-till management, and those aggregates serve as important habitats for microbial activity (Gupta and Germida, 1988). Crop intensification increases the soil C inputs necessary for microbial growth and function (Doran et al., 1998). No-till systems have greater microbial biomass and AMF colonization than tilled systems (Drijber et al., 2000), and increased crop intensity results in increased microbial biomass, soil enzyme activity, and PMN (O'Dea, 2011; Acosta-Martinez et al., 2007).

Cover crops are a form of crop intensification and provide a source of labile organic C and N, which in turn provides a substrate for microbial growth and activity (Roper and Gupta, 1995). The most promising cover crop for summerfallow replacement in the NGP is a partial-season legume green manure crop (LGM), grown in the peak precipitation window from April to mid-June and terminated at early to mid-bloom (Tanaka et al., 2010). Specifically, pea (*Pisum sativum* L.) and lentil (*Lens culinaris* Medik.) have shown promise as partial fallow replacement LGM crops in Montana (Miller et al., 2003; Miller et al., 2011). Early termination of LGMs is an important management practice for soil water conservation, as crops grown past early bloom can use too much water, which results in lower subsequent wheat yields (Zentner et al., 2004).

Soils in long-term LGM rotations have increased bacteria, fungi, microbial biomass C and N, C mineralization, and enzyme activity when compared with soils in a wheat-fallow rotation (Biederbeck et al., 2005). Importantly, improved biological activity resulting from increased crop diversity and intensification may improve crop yields (Acosta-Martinez et al., 2011).

Crop diversity can affect soil biological function, as plant species influence the below-ground biome via root exudates, litter quality, and mutualistic relationships. Different plant root exudates can change the soil bacterial community composition (Berg and Smalla, 2009), which can affect ecosystem processes such as nitrification (Briones et al., 2002) and denitrification (Bremer et al., 2007; 2009). Likewise, plant residue with a low C:N may promote greater bacterial populations, resulting in greater rates of decomposition and net N mineralization (Parmelee et al., 1989), while plant residue with a high C:N may promote a fungal-based decomposition pathway with slower nutrient mineralization (Wardle and Van der Putten, 2002), reduced N leaching (de Vries and Bardgett, 2012), and increased ability to retain nutrients during drying and wetting cycles (Gordon et al., 2008). Finally, individual plant species may have preferential mutualistic relationships with different taxa of AMF (Johnson et al., 1992) and nitrogen-fixing rhizobia (De Deyn et al., 2009), which in turn influence plant P acquisition and N dynamics.

Recently, farmers in the NGP have been experimenting with multi-species cover crops, or cover crop mixtures (CCMs), of six to twelve species as a partial summerfallow replacement or livestock forage. This is in response to anecdotal claims that the plant

diversity of a mixed species cover crop increases soil biological activity more than a single species cover crop. However, to date, there are no scientific studies to support this claim. The objectives of this study were to assess the initial affects an early terminated CCM with both a single-species LGM and a fallow treatment in a no-till wheat-fallow system by quantifying treatment effects on microbial biomass, enzyme activity, potentially mineralizable nitrogen (PMN), and mycorrhizal colonization after one year of cover crop growth.

Materials and Methods

A 2-yr plot-scale cover crop study was initiated at Amsterdam and Conrad, MT in 2012 (Table 3.1). The Conrad site was located in the NRCS-MLRA 52, 390 km north of Bozeman, and the Amsterdam site was located 32 km west of Bozeman. Both locations were on commercial farms which had been under no-till management for a minimum of 3 yr. Growing season air temperature and precipitation data were collected on-site with automated gauges (HOBO® data loggers, Onset®, Bourne, MA) at each location. Non-growing season temperature and precipitation data were also collected from nearby meteorological stations within 24 km of each site (Table 3.2).

Each site was established as a randomized complete block design with a split-plot arrangement with four replicates. In 2012, cover crop and fallow treatments comprised each main plot (8 x 12 m), and in 2013, three N rates comprised each subplot (8 x 4 m) in the wheat response crop. For this study we examined three treatments: 1) fallow 2) single-species pea, and 3) a mixed species treatment. The Amsterdam mixed species

cover crop treatment was an eight-species ‘Full’ mixture, consisting of four plant functional groups, which included; *legumes* for N-fixation, *grasses* for fibrous root systems and carbon addition, *brassicas* for rapid ground cover (Lawley et al., 2011) and possible bioactivity (Larkin and Griffin, 2007), and *tap root* crops to reduce soil compaction (Chen and Weil, 2010) and improve water infiltration. Two plant species comprised each plant functional group to provide redundancy (Table 3.3 and 3.4). The Conrad mixed species cover crop treatment was a six-species ‘Minus Fibrous Root’ mixture, consisting of legumes, brassicas, and tap root crops. The ‘Full’ mixture at Conrad was heavily infested with downy brome (*Bromus tectorum* L.) and was not considered in this study (Chapter 2).

Agronomic management details are presented in Table 3.5. In 2012, cover crop treatments were seeded with a low-disturbance drill into standing wheat stubble in early April. Seeding rates were calculated by dividing proportionally a recommended monoculture rate for each species by the number of species in the mixture (Table 3.6). Row spacing was offset, with approximately 15 cm between paired rows. Paired rows alternated between large-seeded and small-seeded species within each functional group (Table 3.3), with the large seeded species planted at a 4-cm depth, and the small seeded species planted at a 2-cm depth. All rows in the single species Pea treatment were planted at a depth of 4 cm and all treatments were inoculated with rhizobia (*Rhizobium leguminosarium*; Cell-Tech® peat, Novozymes BioAg, Brookfield, WI). No other seed treatments were used. The Minus Fibrous Root treatment All treatments were terminated with glyphosate [N-(phosphonomethyl)glycine] application on or near 15 June to meet

the USDA-Risk Management Agency (RMA) summerfallow cutoff deadline (USDA-FCIC, 2012). Termination timing occurred just prior to first bloom of field pea (growth stage 51 in the BBCH scale, Lancashire et al., 1991) with a flower bud present on one or more nodes. Both sites were sprayed again in mid-July with a glyphosate and 2,4-D [2,4-Dichlorophenoxyacetic acid] mixture, due to failure of pea and especially common vetch (*Vicia sativa* L.) to terminate effectively with glyphosate alone.

In 2013, both sites were seeded to spring wheat (cv. Duclair) in April. Wheat was seeded with a low-disturbance drill at 75 kg ha^{-1} at a depth of 2.5 cm, with 26 cm row spacing. Three urea fertilizer treatments (0, 44, 88 kg N ha^{-1}) were banded >5 cm below and to the side of the seed row at wheat seeding to create sub-plots. No fungicide was applied to the wheat seed in order to not confound subsequent soil biological analysis. The Conrad site was harvested 20 Aug. The Amsterdam site grew to full maturity, but was not harvested due to complete hail damage 1 Aug.

Sampling Methods

To characterize soils at each site, samples were collected in the spring 2012, 2-4 wk prior to cover crop seeding. Six soil cores were taken from 12 plots at each site to a depth of 15 cm and composited by plot. Samples were analyzed for NO_3-N, Olsen P, exchangeable K, organic matter, pH, electrical conductivity, and texture (Table 3.1; AgVise Laboratories, Northwood, ND).

In April 2013, soils were sampled for microbial biomass, soil enzymes, and PMN, two and 23 d prior to wheat seeding at Amsterdam and Conrad, respectively. This timing was chosen to measure the possible effects of cover crops on the subsequent wheat crop. Four soil

cores were taken from each plot to a depth of 10 cm and composited in the field. All cores were taken using a truck-mounted hydraulic probe with a 3-cm diameter sample tube. The soil probe was carefully rinsed with water to remove soil particles and wiped with a disposable bleach (1% sodium hypochlorite) towel between each treatment to prevent cross-contamination. Samples were placed in temporary cold storage for transport and then stored at 4 °C until processed.

Wheat roots were harvested for mycorrhizal colonization analysis in July 2013 during grain fill (Zadoks stage 75; Zadoks et al, 1974). Four plants were pulled by hand from each treatment from the medium rate N fertilizer sub-plot. Root balls of each individual plant were wrapped in a sealed plastic bag and placed in temporary cold storage for transport and then stored at 4 °C until processed.

Laboratory Procedures

All soils were sieved (≤ 2 mm) and stored at 4 °C until analysis. A 5-g subsample from each soil sample was weighed and dried to determine moisture content. Microbial biomass was analyzed by substrate-induced respiration using a yeast solution, with a modified protocol from West and Sparling (1986). From each soil sample, two lab replicates were analyzed. 5 g of soil was placed into a 50-mL centrifuge tube equipped with a gas-tight lid and rubber septa. 10-ml of yeast solution was added to each sample tube. Tubes were capped and shaken at 20 °C for 4 h. Time-zero (T0) was measured 10 to 20 min after initially sealing the tubes. Headspace CO_2 concentration was measured with a gas chromatograph at 0, 2, and 4 h. Final measurements were reported as *microbial respiration rate*, rather than converting to mass units of microbial biomass, due to the lack of a reliable regression equation for soils in our region (Noah Fierer, personal communication, Jan 2014).

A suite of six enzymes were measured, including β-glucosidase (EC 3.2.1.21) and β-glucosaminidase (EC 3.1.2.52), important for C and N mineralization; acid phosphatase (EC 3.1.3.2), alkaline phosphatase (EC 3.1.3.1), and phosphodiesterase (EC 3.1.4.1), associated with P mineralization; and arylsulfatase (3.1.6.1), associated with S mineralization. Enzymes were assayed using 1-g field-moist soil with a p-nitrophenol-labelled substrate for each enzyme, incubated for 1 h at 37 °C, and then analyzed for p-nitrophenol using a spectrophotometer (Tabatabai, 1994; Parham and Deng, 2000). Two lab replicates and one control were assayed for each soil sample and compared with calibrated standards for each enzyme.

PMN was analyzed by anaerobic 14-d incubation (Keeney and Nelson, 1982). 5 g of soil from each sample was placed into six flasks. Three flasks were analyzed immediately for NH_4^+, using 1 M KCl extraction and a cadmium reduction analyzer (Lachat Instruments, Loveland, CO; Robertson et al., 1999). 12.5 mL of DDI H_2O was added to each of the remaining three flasks. N_2 gas was added to fill the headspace of each flask and create an anaerobic environment, and flasks were incubated at 30 °C for 14 d. After incubation, flasks were analyzed for NH_4^+ using 2 M KCl extraction. PMN was reported as the amount of NH_4^+ after the incubation minus the amount measured prior to incubation.

Mycorrhizal colonization was measured by placing root fragments of approximately 5 cm in length from the harvested wheat plants in KOH for 24 h, acidifying them in 3% HCl for 12 h, and then staining them in Trypan blue dye for 12 h. Root fragments were then placed on slides and colonization levels counted using the magnified intersection method (McGonigle et al., 1990).

Statistical Analysis

Statistical analysis was performed with R statistical software (The R Foundation for Statistical Computing, Vienna, Austria, 2013). Linear models were constructed with treatment and block as independent variables and analyzed with ANOVA. Assumptions of normality, independence, and equal variance were evaluated with residual plots and Q-Q normal plots. Specific comparisons were reported using Fisher's Protected Least Significant Difference (LSD) procedure using the *agricolae* package (de Mendiburu, 2013) with no *p*-adjustment. An associated α value of 0.1 was selected, as minor changes in response variables were expected after only one cover crop year. Sites were analyzed independently, due to different cover crop treatments at each site. Pre-planned orthogonal contrasts were used to compare the combined cover crop (CC) treatments with Fallow (F) for microbial biomass and mycorrhizal colonization factors.

Results and Discussion

Cover Crop Biomass

Winter precipitation from 2011-2012 was drier than average at both Amsterdam and Conrad, with precipitation in the cover crop growth season of Apr-Jun drier than average at Amsterdam (Table 3.2). Low precipitation combined with cold spring temperatures resulted in low cover crop biomass production at both sites, while downy brome competition and subsequent herbicide application may have further decreased cover crop yields at Conrad. Cover crop biomass yield was 0.76 and 1.01 Mg ha^{-1} in the Pea and Full treatments at Amsterdam (Table 3.7), and 0.61 and 0.43 Mg ha^{-1} in the Pea

and Minus Fibrous Root treatments at Conrad (Table 3.8). There was no difference between the single-species and the mixed species in either C:N or biomass N yield (Table 3.9) at both sites.

2013 was a normal to slightly wetter precipitation year, resulting in mean wheat yield of 2.97 Mg ha^{-1} at Conrad (Table 3.10). Wheat yields following both cover crop treatments were less than Fallow, mainly due to soil water use (Chapter 2). Wheat yield after fallow was 3.27 Mg ha^{-1}, while wheat yields after Pea and minus Fibrous Root cover crops were 2.65 and 2.98 Mg ha^{-1}, respectively. Cover crops had no effect on subsequent wheat protein, with mean protein across all three treatments of 135 g kg^{-1}. The Pea treatment decreased wheat seed N yield compared with Fallow (62 and 77 kg ha^{-1}, respectively) due to the associated decreased wheat yield. No wheat crop was harvested at the Amsterdam site, due to destruction of the wheat crop by hail prior to harvest.

Microbial Respiration Rate

No differences in microbial respiration rates were detected among all three treatments at either site (Table 3.11). Mean CO_2 production across all treatments was 255 and 212 µl CO_2 g^{-1} soil h^{-1} at Amsterdam and Conrad, respectively. However, when cover crop treatments were compared together against Fallow in an orthogonal contrast, the Fallow treatment had 13% less microbial respiration than the cover crop treatments at the Amsterdam site (234 and 268 µl CO_2 g^{-1} soil h^{-1}). This same effect was not detected at the Conrad site. Our results indicate cover crop treatment effects on microbial respiration rate can be detected in the spring following only one cycle of cover crop growth.

Similar studies in the NGP have reported increased microbial biomass after plant biomass addition during the fallow period. O'Dea (2011) measured microbial biomass C levels 27% greater in wheat-legume rotations than in a wheat-fallow rotation after 8-yr in a no-till system. Likewise, Biederbeck et al. (2005) reported a 170% increase in MBC after a 6-yr rotation of wheat-legume green manure when compared with wheat-fallow in a tilled system.

Liebig et al. (2006) compared microbial biomass C measurements from eight different long-term sites in the Great Plains of the U.S. and Canada and reported that four sites had greater microbial biomass in alternative crop systems (greater crop intensity, less tillage, greater crop diversity) than in conventional crop systems. All of these locations had been in production for 9 to 32 y. All sites with greater MBC also had a greater MBC to soil organic carbon (SOC) ratio. This study also reported that frequency of fallow and mean annual temperature were the two-factors most negatively correlated with MBC. This is not surprising, as fallow decreases the amount of soil carbon available for microbial growth by decreasing organic inputs (Doran et al., 1998), and increases the rate of organic carbon decomposition due to higher surface soil temperatures (Parton et al., 1987).

Early temperature analysis of a similar set of cover crop treatments at a different site in 2013 suggests that soils at a depth of 5 cm are warmer in Fallow than cover crops from the time of canopy closure until 6 to 8 w after termination (Figure 3.1; Appendix C), with daily 4 p.m. Fallow soil temperatures often 5 to 10 °C above both Pea and Full cover crop treatments. From these preliminary results, we would expect increased microbial

biomass in soils with cover crops compared with fallow, due to the decreased rate of organic carbon decomposition via cooler soils.

Soil Enzymes

No treatment differences were detected for any of the six enzymes at both sites after one cover crop cycle (Table 3.12 and 3.13). Lack of treatment differences was not surprising, given the short management period and the low cover crop biomass production. Other reports from the region report enzyme activity (EA) differences after at least two cover crop cycles (Biederbeck et al., 2005; Acosta-Martinez et al., 2011). Acosta-Martinez et al. (2011) found treatment differences in six EAs after two winter rye cover crops in a sorghum-cotton rotation in the Southern Great Plains of Texas. Notably, there was no EA differences between tilled and no-till sub-plots, indicating that crop intensity, and not tillage system was responsible for the difference. Biederbeck et al. (2005) reported EA differences after 6 yr when a wheat-legume green manure rotation was compared with a wheat-fallow rotation in a tilled system in the Northern Great Plains of Saskatchewan. In that study, the mean legume green manure (LGM) biomass production was 1.8 Mg ha^{-1}, during alternating years. Soil enzyme activity of dehydrogenase, phosphatase, and arylsulfatase increased by 202%, 171%, and 287%, respectively, in the LGM treatments when compared with fallow. Notably, both of these studies came from relatively low rainfall areas with 470 mm and 377 mm of annual precipitation at the TX and SK sites, respectively.

In contrast, Ndaiye et al. (2000) found differences in arylsulfatase and β-glucosidase enzyme activity after only one winter cover crop cycle in a summer

vegetable system in the Willamette Valley of Oregon. However, the mean annual precipitation at the study sites was 1075 mm, most likely resulting in greater cover crop biomass production than at drier sites.

EA was strongly correlated with soil organic C and total N content in a long-term, tilled, semi-arid wheat-fallow rotation in the Pacific Northwest (Dick et al., 1988). Notably, a wheat-fallow rotation with the addition of 2.24 Mg ha^{-1} of dry pea vine in each fallow year had 64% greater β-glucosidase activity and 52% greater arylsulfatase activity than a wheat-fallow rotation with no amendments. Acid and alkaline phosphatases showed no difference between the two treatments. These values were reported after 55 years of crop rotation.

Potentially Mineralizable Nitrogen

Soils following cover crop treatments had greater potentially mineralizable nitrogen (PMN) than Fallow at both sites (Table 3.14). Pea and Full treatments had 95 and 134% more PMN than Fallow at Amsterdam (17.1 and 20.5, compared with 8.7 kg NH_4 ha^{-1}), while the Pea treatment had 53% more PMN than Fallow at Conrad (18.3 compared with 12.0 kg NH_4 ha^{-1}). Notably, the six-species Minus Fibrous Root treatment (9.9 kg NH_4 ha^{-1}) was not different than Fallow at the Conrad site. Because PMN is closely correlated with biomass N yield and negatively correlated with C:N ratios of any added plant material (Lupwayi et al., 1999; Lupwayi et al., 2006), we expected that the residue quality and quantity of the cover crops would affect soil PMN levels. It is interesting to note that the biomass N yield did not differ between cover crop treatments at either site (Table 3.9). Lack of biomass N yield differences explains the lack of PMN

treatment differences between the Pea and Full treatments at Amsterdam, but it is unclear why the same pattern was not observed at the Conrad site between the Pea and Minus Fibrous Root treatments.

In comparison, O'Dea (2011) also reported greater PMN values after continuous wheat, pea-wheat, and LGM-wheat rotations than fallow-wheat after a14-d incubation, indicating that increased crop intensity results in higher PMN values. Fallow had 10.5 mg PMN kg^{-1} soil, while the other three treatments had a mean 13.2 mg PMN kg^{-1} soil, or 26% more than Fallow. After a 16-d incubation, Beiderbeck et al. (1998) reported PMN values 19% greater in soils following four different LGM species than following fallow after 6 yr in a tilled system. Fallow had 120 mg PMN kg^{-1} soil, while the four LGM treatments had a mean 143 mg PMN kg^{-1} soil. Interestingly, there was no difference between wheat-fallow and continuous wheat PMN values.

Mycorrhizal Colonization

No differences in wheat mycorrhizal colonization levels were detected among treatments at either site (Table 3.15). Mean colonization was 53% and 18% at Amsterdam and Conrad, respectively. The relatively higher mean colonization rate at the Amsterdam site was likely due to low soil P availability at the Amsterdam site (13 mg P kg soil^{-1}) compared to the critical level of 16 mg P kg-soil-1. Treatment differences were detected only at the Conrad site when cover crop treatments were combined and compared with Fallow in an orthogonal contrast (p=0.04). The colonization level was almost double in wheat following cover crops than wheat following Fallow, with 21 and 11%, respectively.

Few comparable AMF studies exist from the NGP region. Lehman et al. (2012) reported that after one rotation of winter cover crops, AMF propagules increased at two South Dakota locations. At one site, only an oat cover crop and an oat-pea cover crop mixture resulted in increased AMF propagules, while no change was detected after winter canola (*Brassica napus* L.), crimson clover-alsike clover (*Trifolium incarnatum* L. and *Trifolium hybridum* L.), pea-timothy (*Phleum pretense* L.), and radish-pea mixtures, highlighting the importance of cover crop composition for increased AMF infectivity potential.

Turmel et al. (2011) reported no effect from a continuous black medic (*Medicago lupulina* L.) cover crop on AMF colonization of flax (*Linum usitatissimum* L.) in a no-till wheat-oat-flax rotation with no fallow period. Total mean colonization levels were 75% and 51% at two different sites in Manitoba and Saskatchewan. Lack of differences may have been due to the lack of fallow period in the rotation: Crop intensity was already close to maximum in this study.

The practical significance of increased mycorrhizal colonization in Montana agricultural soils has yet to be understood, and it is not clear how subsequent agricultural productivity is affected by a change in colonization levels following a cover crop. Johnson et al. (1997) argued that mycorrhizal associations in agricultural systems can be beneficial, neutral, or parasitic and depend on the interaction of plant species, soil type, and AMF taxa. One study from Pennsylvania reported greater nutrient uptake and plant productivity of maize following one winter wheat cover crop due to increased AMF colonization (Boswell et al., 1988). In contrast, research from the grain producing regions

of Australia suggests that within certain soil and climatic regions, increased AMF colonization may decrease subsequent wheat yield (Ryan et al., 2005; Ryan and Kirkegaard, 2012). Regardless of AMF's effect on grain crop yield, there may be other ecosystem benefits to increased presence of AMF in an agricultural system (Rillig, 2004a), such as maintenance of soil structure through increased soil macro-aggregation (Rillig, 2004b), protection against root pathogens (Newsham et al., 1995), and increased resilience against drought (Augé, 2001).

Summary

Results from this study indicate few soil biological differences between cover crops and fallow after one year, and almost no differences between single-species and multi-species cover crop treatments. Microbial respiration increased by 14% at one site after cover crop treatments compared with Fallow, but no difference in six enzyme activity levels were detected. PMN was greater after both cover crop treatments at the Amsterdam site and after only the Pea treatment at the Conrad site compared with Fallow, and AMF colonization increased after cover crops at only the Conrad site.

Lack of large differences may simply be due to the short time frame of the study and low cover crop biomass production at both sites, and we hypothesize that greater differences in biological function would be detected after more years of cover crops in the rotation. It is unknown whether differences between the single and mixed-species cover crops would emerge after a longer time period. However, it is clear from previous studies that cover crops increase soil biological activity in the NGP region and may be a practical management tool for improved soil function.

Table 3.1. Site characteristics of two on-farm cover crop sites in Montana.

	Amsterdam	Conrad
Location	45°43''6.74''N 111°21'52.37"W	48°12'47.55''N 111°29'41.09''W
Elevation (m)	1446	1039
Soil classification	frigid Typic Calciustoll	frigid Aridic Argiustoll
Texture†	Silt loam	Clay loam
pH	8.2	6.5
Soil Organic Carbon (g kg^{-1})	1.4	1.4
NO_3-N (mg kg^{-1})	6.0	8.5
Olsen P (mg kg^{-1})	13	28
Extractable K (mg kg^{-1})	359	498
Sampling date	26 Mar 2012	11 Apr 2012

All samples (0 to 15 cm) analyzed by AgVise Laboratories, Northwood, ND.

†Laboratory methods used include; texture, Bouyoucos hydrometer; pH and salinity, 1:1 soil-to-water method; soil organic matter, loss of weight on ignition (LOI); NO_3-N, KCl extraction, Cd reduction, spectrophotometer determination; Olsen P, $NaHCO_3$ extraction, colorimetric determination; extractable K, NH_4OAc extraction, atomic absorption spectrometry.

Table 3.2. Monthly precipitation and cover crop growing season GDD (0 °C) at two cover crop mixture plot study sites in Montana. Long-term average (LTA) calculated from 1981-2010, Western Regional Climate Center, Reno, NV. Nearest WRCC station code and distance given for each site.

	Amsterdam			Conrad		
	240622 (19 km)			241974 (24 km)		
	LTA	2012	2013	LTA	2012	2013
Month	----------Precipitation (mm) ----------					
Sept – Mar	137	83	76	98	69	92
April	39	68	18	26	60	25
May	61	53	89	50	48	81
June	62	34	59	64	83	84
July	30	27	16	35	24	12
August	26	13	13	32	10	42
TOTAL	358	278	271	303	319	336
	----------Average Temperatures (°C) ----------					
April	7.3	7.5	4.2	5.9	8.1	4.3
May	12.0	9.1	11.2	11.1	10.8	10.9
June	16.4	15.6	15.6	15.2	15.5	15.3
July	20.8	21.1	20.6	18.8	19.6	20.0
August	20.1	18.9	19.9	18.1	18.0	20.0
LTA	7.4	7.3	6.3	6.2	7.0	6.8
GDD (0 °C)†	--	832	--	--	716	--

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

Table 3.3. Plant functional groups and species used to compose cover crop treatments at two sites in Montana, 2012. Large and small-seeded species seeded in offset rows at 4 and 2 cm depth, respectively.

Functional Group		Plant Species		Seed Size
Nitrogen Fixers	(NF)	Pea	*Pisum sativum* L. cv. Arvika	Large
		Common Vetch	*Vicia sativa* L.	Small
Fibrous Roots	(FR)	Oat	*Avena sativa* L. cv. Monico	Large
		Italian Ryegrass	*Lolium perenne* L. ssp. *multiflorum* Lam. Husnot cv. Tetila	Small
Tap Roots	(TR)	Safflower	*Carthamus tinctorius* L. cv. MonDak	Large
		Turnip	*Brassica rapa* L.	Small
Brassicas	(BC)	Radish	*Raphanus. sativus* L. var. *longipinnatus*	Large
		Camelina	*Camelina sativa* L. Crantz cv. SO-02	Small

Table 3.4. Cover crop treatments and functional group composition at two plot study sites in Montana, 2012.

Treatment	Abbreviation	Functional Group Composition
Fallow	SF	——
Pea	PEA	Pea
Full	FULL	Nitrogen Fixers, Fibrous Roots, Tap Roots, Brassicas
Minus Fibrous Root	MFR	Nitrogen Fixers, Tap Roots, Brassicas

Table 3.5. Agronomic field management for cover crop mixture study at Amsterdam and Conrad, MT, 2012-2013.

Event	Amsterdam		Conrad	
	2012	2013	2012	2013
Soil sample date	26 Mar	1 Apr	11 Apr	4 Apr
Cover crop PRE herbicide	26 Mar†	--	--	--
Cover crop seeding date	3 Apr	--	4 Apr	--
Urea application (kg N ha^{-1})	16 Apr (34)	3 Apr (0, 44, 88)	--	27 Apr (0, 44, 88)
Cover crop herbicide	16 Apr.‡		--	
Cover crop insecticide	--		--	
Cover crop stand counts	7 May	--	14 May	
Cover crop herbicide	14 May§	17 May§		
Cover crop termination	13 June	--	16 June	
Cover crop biomass harvest	14 June	--	20 June	
Soil sample date	14 June		27 June	
Cover crop POST herbicide	17 July¶			
Wheat cultivar		Duclair sw		Duclair sw
Wheat seeding date		3 Apr		27 Apr
Wheat PRE herbicide		11 Apr†		6 Apr#
Wheat herbicide		--		7 May †
Wheat harvest date		NA		20 Aug

† 0.68 kg ha^{-1} of N-(phosphonomethyl)glycine in the form of isopropylamine salt
‡ 0.06 kg ha^{-1} of Quizalofop-P-ethyl {ethyl(R)-2-[4-(6-chloroquinoxalin-2-yloxy)-phenoxy]propionate}
§ 0.22 kg ha^{-1} (E)-2[1-[[(3-chloro-2-propenyl)oxy]limino]propyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one on all plots without a fibrous root component.
¶ 0.92 kg ha^{-1} N-(phosphonomethyl)glycine in the form of isopropylamine salt
# 0.68 kg ha^{-1} N-(phosphonomethyl)glycine in the form of potassium salt

Table 3.6. Seeding rate monoculture equivalents for cover crop mixture study at two Montana locations, 2012.

	Target Plant Population m^{-2}
Pea	80
Common Vetch	80
Oat	160
Italian Ryegrass	240
Turnip	60
Safflower	50
Radish	60
Camelina	200

Table 3.7. Cover crop biomass by plant species 71 d after seeding, 13 June 2012, Amsterdam, MT.

Treatment	Total	Pea	Common Vetch	Oat	Italian Ryegrass	Turnip	Safflower	Radish	Camelina	Weeds
	---------- Mg ha^{-1} ----------									
Pea	0.76	0.74	--	--	--	--	--	--	--	0.02
Full	1.01	0.16	0.05	0.30	0.04	0.07	0.02	0.13	0.10	0.14
	p-value									
	0.16									

Table 3.8. Cover crop biomass by plant species 73 d after seeding, 16 June 2012, Conrad, MT.

Treatment	Total	Pea	Common Vetch	Turnip	Safflower	Radish	Camelina	Weeds
	Mg ha^{-1}							
Pea	0.61	0.60	--	--	--	--	--	0.0
Minus Fibrous Root	0.43	0.19	0.06	0.04	0.02	0.01	0.12	0.0
	p-value							
	0.11							

Table 3.9. Cover crop C:N ratio and biomass N yield by treatment for Amsterdam and Conrad, MT, 2012

	Amsterdam		Conrad	
	C:N ratio	Biomass N (kg ha^{-1})	C:N ratio	Biomass N (kg ha^{-1})
Pea	13.4	25	18.7	14
Full	16.7	26	---	---
Minus Fibrous Root	---	---	20.2	9
	ANOVA *p*-values			
Treatment	0.03	0.61	0.80	0.36

Table 3.10. Means for cover crop treatment effects at three N fertilizer rates on spring wheat yield, protein and N yield at Conrad, MT, 2013. Treatments with different letters were significantly different from each other (p<0.05, LSD).

Treatment	Wheat Yield	Wheat Protein	Seed N yield
	--- Mg ha^{-1} ---	---- g kg^{-1} ----	---- kg ha^{-1} ----
Fallow	3.27 a	134	77 a
Pea	2.65 b	134	62 b
Minus Fibrous Root	2.98 ab	137	72 ab
	---------- ANOVA p-values ----------		
	0.03	0.85	0.04
Treatment x Fertilizer	0.76	0.97	0.98
LSD (p<0.05)	0.41	NS	11
SE	0.12		3
Fertilizer			
0 kg N ha^{-1}	2.81 b	118 b	57 c
44 kg N ha^{-1}	3.06 a	128 b	69 b
88 kg N ha^{-1}	3.02 ab	158 a	82 a
	---------- ANOVA p-values ----------		
	0.05	<0.001	<0.001
LSD (p<0.05)	0.21	15	7
SE	0.07	5	3

Table 3.11. Microbial respiration rate after cover crop treatments at two Montana sites, April 2013.

Treatment	Amsterdam	Conrad
	---------- µl CO_2 g^{-1} soil h^{-1} --------	
Fallow	234 (16)	223 (18)
Pea	255 (14)	232 (32)
Full	276 (10)	--
Minus Fibrous Root	--	180(20)
	---------- ANOVA p-values ----------	
	0.19	0.11
Orthogonal Contrasts		
	--------- µl CO_2 g^{-1} soil h^{-1} ---------	
Fallow	234 (16)	223 (18)
Cover Crops	266 (9)	206 (20)
	---------- ANOVA p-values --------	
	0.09	0.58

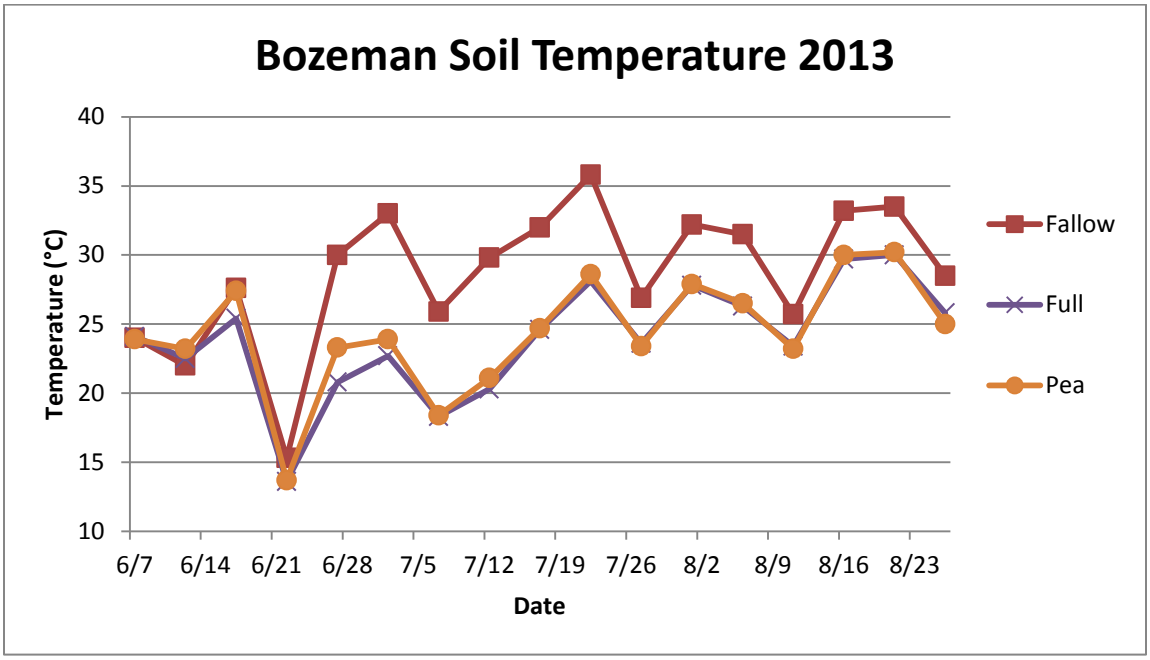

Figure 3.1. Soil temperatures of Fallow, single-species Pea, and eight-species Full cover crop mixture at 5-d intervals at a depth of 5 cm at 4 pm, Bozeman, MT, 2013. Cover crops terminated on 5 July 2013 with herbicide and plant residue left on field.

Table 3.12. Soil enzyme activity at Amsterdam, MT April 2013. Mean treatment values represent mg of *p*-nitrophenol (PN) produced per kg soil per hour. Values in parentheses represent treatment standard errors.

Treatment	Arylsulfatase	Phosphodiesterase	Acid Phosphatase	Alkaline Phosphatase	β-Glucosidase	β-Glucosaminidase
	mg PN kg^{-1} soil h^{-1}					
Fallow	48.9 (2.0)	40.7 (2.8)	82.3 (8.3)	218.1 (28.9)	196.4 (14.0)	16.3 (0.6)
Pea	49.7 (5.9)	44.8 (1.2)	77.0 (6.2)	237.4 (8.2)	195.3 (12.1)	15.9 (0.3)
Full	40.7 (7.6)	45.7 (1.2)	81.6 (9.6)	240.1 (13.8)	174.9 (5.6)	18.1 (1.0)
	ANOVA *p*-values					
	0.43	0.30	0.87	0.72	0.41	0.17

Table 3.13. Soil enzyme activity at Conrad, MT April 2013. Mean treatment values represent mg of *p*-nitrophenol (PN) produced per kg soil per hour. Values in parentheses represent treatment standard errors.

Treatment	Arylsulfatase	Phosphodiesterase	Acid Phosphatase	Alkaline Phosphatase	β-Glucosidase	β-Glucosaminidase
	mg PN kg^{-1} soil h^{-1}					
Fallow	13.7 (2.8)	19.8 (5.2)	242.6 (11.7)	64.3 (32.4)	216.5 (31.8)	23.8 (1.5)
Pea	22.0 (6.9)	27.2 (8.2)	236.2 (50.7)	112.3 (45.4)	217.3 (32.3)	28.1 (5.6)
Minus Fibrous Root	8.8 (3.1)	12.2 (3.4)	227.1 (12.2)	42.2 (2.4)	146.8 (30.5)	20.2 (4.5)
	ANOVA *p*-values					
	0.16	0.28	0.94	0.38	0.32	0.33

Table 3.14. PMN after cover crop treatments at two Montana sites, April 2013. Values in parentheses represent treatment standard errors. Treatments with different letters were significantly different from each other (p<0.1, LSD).

Treatment	Amsterdam		Conrad	
	----------kg NH_4 ha^{-1} ----------			
Fallow	8.7 (2.2)	b	12.0 (0.6)	b
Pea	17.1 (1.7)	a	18.3 (4.0)	a
Full	20.5 (2.0)	a	--	
Minus Fibrous Root	--		9.9 (1.8)	b
	---------- ANOVA p-values ----------			
	0.03		0.08	
LSD (p<0.1)	6.5		6.0	

Table 3.15. Mycorrhizal colonization of wheat plants after cover crop treatments at two sites in Montana, July 2013. Values in parentheses represent treatment standard errors.

Treatment	Amsterdam	Conrad
	-------- Percent colonization -------	
Fallow	46 (7)	11 (2)
Pea	52 (3)	22 (2)
Full	60 (4)	--
Minus Fibrous Root	--	20 (5)
	-------ANOVA p-values --------	
Treatment	0.14	0.15
Orthogonal Contrast		
	-------- Percent colonization -------	
Fallow	46 (7)	11 (2)
Cover Crops	56 (3)	21 (3)
	-------- ANOVA p-values ---------	
	0.14	0.04

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CHAPTER FOUR

EPILOGUE

Background

The impetus for this research came from a trip I made to North Dakota in 2009 as the staff agronomist for the National Center for Appropriate Technology (NCAT) in the National Sustainable Agriculture Information Service (or ATTRA) project. I had heard of the work being done by the Burleigh County Soil Conservation District (www.bcscd.com), and was eager to see it in person. While there, I visited several farms and wrote a series of four in-depth case studies based on their management practices (www.attra.org). Most of the producers used multi-species cover crops in the late summer shoulder season (July – October) and terminated the cover crop with frost kill and livestock grazing. Every farmer had a compelling story to tell about soil health and could talk at length about soil food webs, mycorrhizae, compost tea, and more. It was exciting to see how these farmers had incorporated plant diversity, merged crop and livestock production, decreased their purchased inputs, and improved their bottom line. Particularly impressive was that they were doing it within the context of a large-scale, modern farm system which would be recognizable by other no-till farmers in the region. This was not a romantic, fringe, agricultural movement, but rather a thoughtful and progressive group of farmers willing to try new things to improve their soil. I was inspired. Specifically, I wanted to know if these multi-species cover crops could be a viable practice in my home state of Montana.

Conclusions

This study had two stated objectives; 1) compare the agronomic response variables of fallow, a single-species pea green manure, and nine multi-species cover crops, and 2) compare select soil biology parameters of fallow, a pea green manure, and one multi-species crop. After two years of study, in both a record dry year and a record wet year, very few differences were measured between the pea and the multi-species cover crop treatments. Few differences in cover crop biomass and quality were measured, and few differences were measured in soil water and nitrate at the time of cover crop termination. At one site, during a record wet year, soil nitrate was greater following the pea green manure than the eight-species cover crop mixture, probably due to greater biological N fixation in the pea crop. Wheat yields following cover crops were less than fallow, due to water use during the cover crop year. Slight differences in biological parameters were detected at the time of wheat seeding, but only when both cover crop treatments were combined in an orthogonal contrast.

There was also a third unstated objective, which was to discover how to conduct field research on multi-species cover crops. Quite a bit of effort went into designing the study, and adjustments were made from 2012 to 2103 as we learned what worked and what did not work. As a result, the study design is more robust as it enters the 2014 growing season.

It is important to bear in mind that this thesis reports differences at the early stages of the study. The study will continue for two more years, and possibly more, with each cover crop treatment grown in the same plot location to accelerate any possible soil

biological response. It is expected that treatment differences will be greater over the long-term than the short-term.

Future Considerations

Over the course of the first phase of this study, several questions have emerged that may guide future research. Of particular interest is the difference in soil temperature in cover crops vs fallow. To my knowledge, no study of this kind has been published, and it would be of great interest to pursue this research further. Specifically, how do cooler soils under cover crops effect soil biota in our semi-arid agricultural systems compared with warmer fallow soils?

Possible work could also be done by using the Soil Management Assessment Framework (SMAF) tool as an index to compare the relative changes in soil management. Stott et al. (2010) recommended using the ratio of β -glucosidase and soil organic carbon within the SMAF as a reliable indicator of C sequestration trends. Applying a standard soil quality index would assist in measuring and comparing land management practices. This was an area of interest that I did not have the time to explore.

At one site-year we did observe differences between the Pea and Full treatments in soil nitrate levels after cover crop termination, probably due to increased biological N fixation by the Pea treatment. While biological N fixation has been measured in single-species legume green manures in Montana (McCauley et al., 2012) we have little understanding of how the biological N fixation of a multi-species cover crop compares. This may also be of interest for future study.

In a like matter, we observed that each plant species C:N changed depending on the treatment it was measured in. However, these differences were not detectable when species were combined to report the total C:N of a cover crop mixture, as the composited value masked the individual species differences. Our preliminary results indicate that plant species competition affects individual plant C:N, which may be of interest for further study. Specifically, it would be of interest to measure the C:N of each functional group treatment (Nitrogen Fixer, Fibrous Root, Tap Root, and Brassica).

Another area of future exploration is cover crop termination via grazing. Two producers I have spoken with have mentioned that mixed cover crops only work economically if used for livestock forage to offset the decreased subsequent grain yield. It may be useful to talk with a grazing specialist on the value of grazing cover crops for termination and include a simple "back of the envelope" economic analysis in future reports to USDA-WSARE and at presentations to producers. Likewise, it may be of value to measure the forage quality of the cover crop treatments with a standard lab analysis, including; crude protein, acid detergent fiber, neutral detergent fiber, total digestible nutrients, and Ca and P concentrations (Carr et al., 2004). The challenge in grazing these cover crops will be two-fold; 1) given our spring growth window, cover crops will not completely terminate if grazed in early July. They will simply regrow and will require post-grazing herbicide application for a complete kill, and 2) forage quality in early July may be too high in nitrates, making it potentially lethal to livestock (Larry Holzworth, pers. comm., April 2014).

Another topic for future study is the effects of mycorrhizae on wheat production in the MLRA 52. Of particular interest are the recent studies from Australia which report increased wheat yield associated with reduced mycorrhizal colonization (Ryan et al., 2005; Ryan and Kirkegaard, 2012). We currently do not understand the practical significance of mycorrhizal colonization in Montana agricultural soils: Specifically, are mycorrhizae beneficial, neutral, or parasitic when associated with wheat in Montana's main grain producing regions? This is a large question that requires further study to adequately answer.

Finally, and perhaps most importantly, our field observations indicate that multi-species cover crops may have value in the biological control of wheat stem sawfly (*Cephus cinctus* Norton), a predominant insect pest in Montana. Specifically, oat (*Avena sativa* L.) functions as a trap crop when planted in early April (Weaver et al., 2004). In addition, the radish (*Raphanus. sativus* L. var. *longipinnatus*) flowers present in a cover crop mixture provide a source of nectar for pollinators and predators of sawfly (David Weaver, pers. comm., June 2012). During cover crop biomass harvest, our mixtures were practically humming with pollinator and parasitoid wasp activity due to the presence of the radish flowers. (It should be noted that radish only flowers when planted in the spring and will not flower when planted later in the summer.) It would be of great benefit to the wheat farmers of the state to investigate this topic further, as the wheat stem sawfly causes $250 million in annual damages to Montana's farm incomes, and the only possible methods of control are cultural practices (Fulbright et al., 2011).

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APPENDICES

APPENDIX A

LAND EQUIVALENCE RATIO (LER) CALCULATIONS OF COVER CROP BIOMASS

Land Equivalent Ratio

A Land Equivalent Ratio (LER; Mead and Willey, 1980) was calculated for each cover crop mixture of six or more species (Table A.1), using the following equation:

$$\text{LER} = \frac{Y_A}{S_A} + \frac{Y_B}{S_B} + \cdots + \frac{Y_X}{S_X}$$

where Y_A and Y_B are the yield of each functional group in the mixed-species treatments (Full, Minus Fibrous Root, Minus Tap Root, and Minus Nitrogen Fixer) and S_A and S_B are the yield of each functional group when grown alone (FR, TR, NF, and BC). No LER calculation was made for the Minus Brassica treatment, as turnip was not included in the treatment and no reasonable yield estimate of a sole crop of safflower could be made. Study design and data are reported in Chapter 2. All mixtures had an LER ≥ 1, except for the Minus Nitrogen Fixer treatment at Amsterdam, indicating overall increased biomass production when multiple functional groups were combined. Care must be taken when interpreting our LER values, as we used only one target seeding rate for each treatment in a replacement series design (Jolliffe, 2000).

Table A.1. Land Equivalent Ratio (LER) values of cover crop mixtures at four sites in Montana, 2012-2013.

Treatment	Amsterdam 2012	Conrad 2012	Bozeman 2013	Dutton 2013
Full	1.10	--	1.23	1.28
Minus Nitrogen Fixer	0.86	--	1.16	1.01
Minus Fibrous Root	1.43	1.28	1.17	1.16
Minus Tap Root	1.00	--	1.19	1.02

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APPENDIX B

DUTTON SOIL WATER AND NITRATE

AT 0.6 TO 0.9 m DEPTH

Dutton Soil Water and Nitrate, 0.6 to 0.9 m

Soil water and nitrate-N values were measured to a depth of 0.9 m at all four site-years (Chapter 2).. However, at the Dutton site, variation was noticed in the lowest 0. 3 m increment (0.6 – 0.9 m). We theorize these differences were due to herbicide injury in the previous year's crop (2012) resulting in uneven water and nitrate uptake. As a result, these values were not included in the main thesis. However, they are included here in case there is a need for future reference.

Table B.1. Soil water measured to a depth of 0.9 m at termination of cover crop treatments at Dutton, MT, 12 July 2013. Treatments with different letters were significantly different from each other ($p < 0.05$, LSD).

Treatment	0 - 0.3 m		0.3 - 0.6 m		0.6 - 0.9 m		TOTAL	
	---------- Soil Water (mm) ----------							
Fallow	94	a	95	a	91	ab	279	a
Pea	65	e	71	c	78	bc	214	b
Full	72	de	70	c	73	c	215	b
Nitrogen Fixers	85	b	94	a	103	a	281	a
Fibrous Roots	86	ab	87	ab	98	a	272	a
Tap Roots	81	bc	80	abc	72	c	233	b
Brassica	80	bcd	76	bc	72	c	227	b
Minus N Fixer	81	bc	67	c	72	c	220	b
Minus Fibrous Root	83	b	93	a	104	a	281	a
Minus Tap Root	79	bcd	80	abc	78	bc	237	b
Minus Brassica	75	cd	76	bc	80	bc	231	b
	---------- *p*-values ----------							
	<0.001		0.002		<0.001		<0.001	
LSD (p<0.05)	8		15		18		34	
SE	3		5		6		12	

Table B.2. Soil nitrate measured after cover crop termination to a depth of 0.9 m at Dutton, MT, 12 July 2013. Treatments with different letters were significantly different from each other ($p < 0.05$, LSD).

Treatment	0 to 0.3 m		0.3 to 0.6 m		0.6 to 0.9 m		TOTAL	
	kg NO_3-N ha^{-1}							
Fallow	37	a	26	a	24	a	87	a
Pea	14	b	13	b	21	a	47	b
Full	11	b	4	c	8	b	24	b
	P-values							
	0.049		0.003		0.001		0.006	
LSD (p<0.05)	21		9		6		30	
SE	6		3		2		9	

APPENDIX C

SOIL TEMPERATURE DURING COVER CROP GROWTH AT TWO MONTANA SITES, 2013

Introduction

Cover crops may indirectly influence soil biology through abiotic mechanisms. Because microbial growth and enzyme activity is sensitive to temperature and water availability, changes in these parameters may affect soil biota. Teasdale and Mohler (1993) reported that a hairy vetch (*Vicia villosa* (Roth) and winter rye (*Secale cereal* L.) cover crop reduced the maximum soil temperature at a depth of 5 cm by 3°C and moderated temperature fluctuations over a 4-wk period following the addition of cover crop biomass.

Materials and Methods

Study design and field activities are given in detail in Chapter 2. Soil temperature was recorded in the cover crop phase of the study with iButton temperature data loggers (Thermochron®; Maxim Integrated, San Jose, CA). Immediately after cover crop seeding in 2012, and at the time of stand counts in 2013, two iButtons were installed in each of the following seven treatment plots: Fallow, Pea, Full, Nitrogen Fixers, Fibrous Root, Tap Root, and Brassica. Soil temperatures were not measured in the minus treatments (Minus Nitrogen Fixers, Minus Fibrous Root, Minus Tap Root, and Minus Brassica), as I hypothesized there would not be a significant temperature difference among these treatments. The iButtons were systematically installed at a depth of 5 cm at two locations in each plot, with a road hair installed 30 cm away from each button to aid in future button location. Locations of the 2012 iButtons are given in Fig. C.1. The 2013 iButtons were placed in similar locations as the 2012 iButtons, but locations were determined with

respect to the stand count sampling flags. The iButtons were programmed to record temperature every 4 h and were removed from the 2012 sites in mid-September, and from the 2013 sites in early to mid-August. In 2012, iButtons were placed in the ground with no protective cover and most of the data was corrupted, possibly due to water damage. In 2013, all iButtons were wrapped in clear Parafilm (Sigma-Aldrich, St; Louis, MO) and all data was recovered.

At both 2013 sites, cover crop biomass was decreased in the front half of Reps 1 and 2. At the Bozeman site, we theorize that an old road bed was present, causing decreased biomass production in the southern half of all treatments in Reps 1 and 2 (Fig. C.2.). At the Dutton site, we theorize that residual herbicide caused decreased biomass production in the eastern half of all treatments in Reps 1 and 2 (Fig. C.3). As a result, data from only one iButton from Reps 1 and 2 were used. Only the northern iButton data was used from Reps 1 and 2 at Bozeman, and only the western iButton data was used from Reps 1 and 2 at Dutton. Both iButtons from Reps 3 and 4 were used from both sites.

Statistical Analysis

Data was imported into Excel and data points were reported at 5-d intervals from the 4 pm temperature measurement. When data from two iButtons per plot was available, the two points were averaged. No statistical analysis was performed.

Results and Discussion

No data was reported for 2012. However, all data was recovered from the Bozeman and Dutton sites in 2013. All treatments at the Bozeman site are reported

graphically in Fig. C.4. Three treatments from the Bozeman site (Fallow, Pea, and Full) are reported in Fig. C.5. All treatments at the Dutton site are reported graphically in Fig. C.6. Three treatments from the Dutton site (Fallow, Pea, and Full) are reported in Fig. C.7. Treatment differences were not noticed until about 6 to 8 w after seeding, at approximately the time of canopy closure. Fallow was about 5 to 10 °C warmer than most cover crop treatments at both sites at 4 pm. The cooling effect of most cover crop treatments continued for 4 to 6 wk after termination. The one exception was the Fibrous Root treatment, which was about 3 °C cooler than Fallow, and cooled the soil for a shorter time length than the other cover crop treatments. We theorize that the upright leaf architecture of the Fibrous Root functional group allowed for more solar radiation to reach the soil surface than the other cover crop treatments.

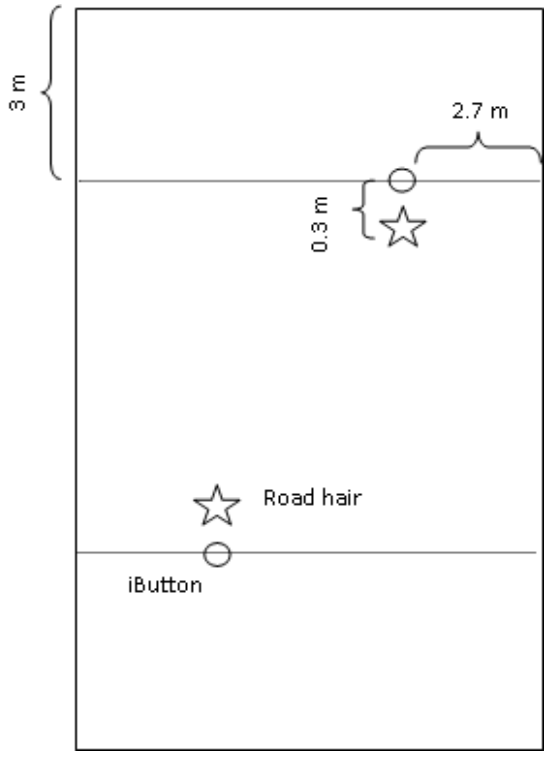

Figure C.1. Location of iButtons within each plot, 2012

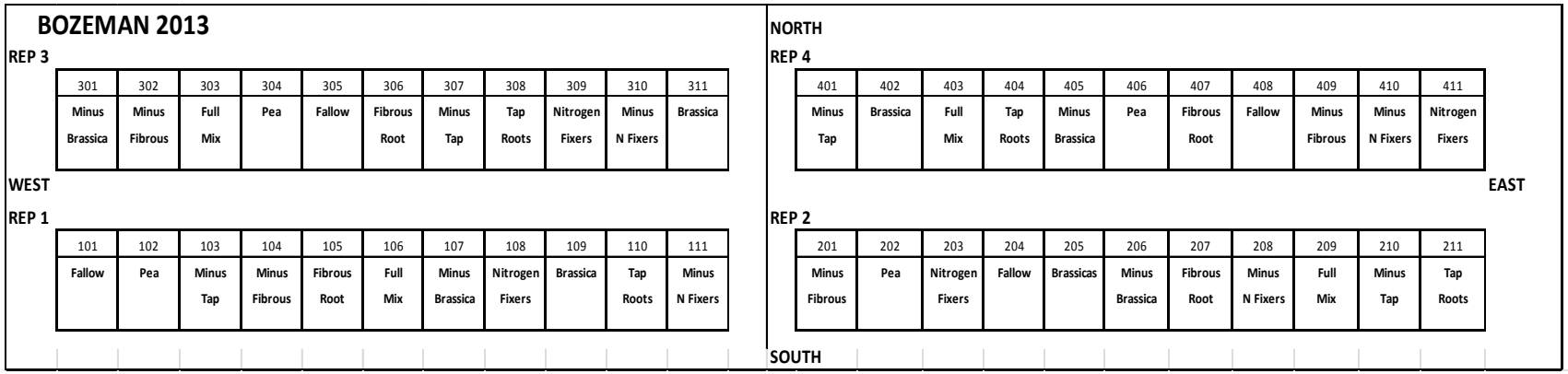

Figure C.2. Replicated complete block design with cover crop treatments, Bozeman, MT, 2013. Rep 1 and 2 had compromised biomass in the southern half of each plot.

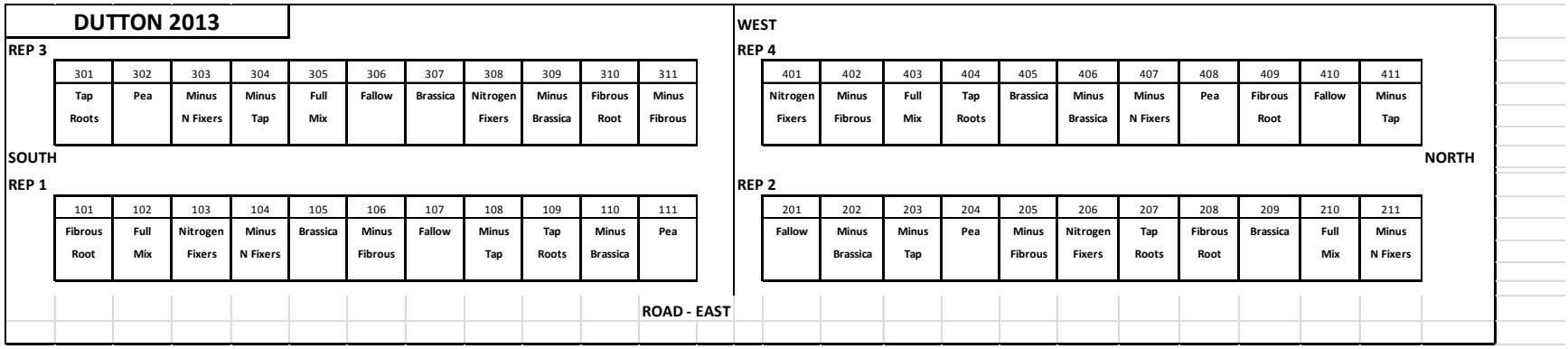

Figure C.3. Replicated complete block design with cover crop treatments, Dutton, MT, 2013. Rep 1 and 2 had compromised biomass in the eastern half of each plot.

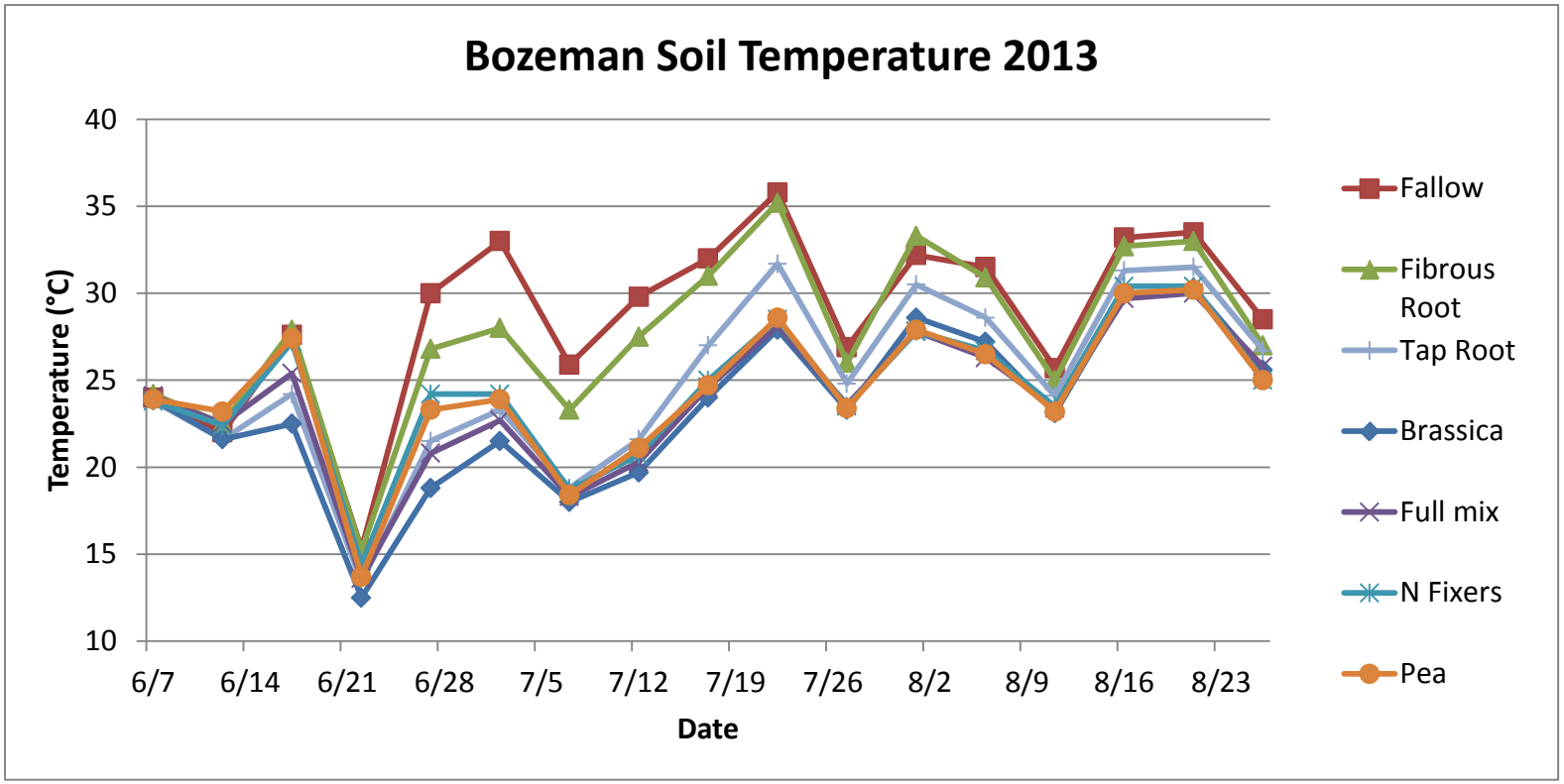

Fig. C.4. Bozeman soil temperature at 5 cm depth for seven treatments. Data points reported at 5-d intervals at 4 pm. Cover cop terminated on 5 July 2013 via herbicide.

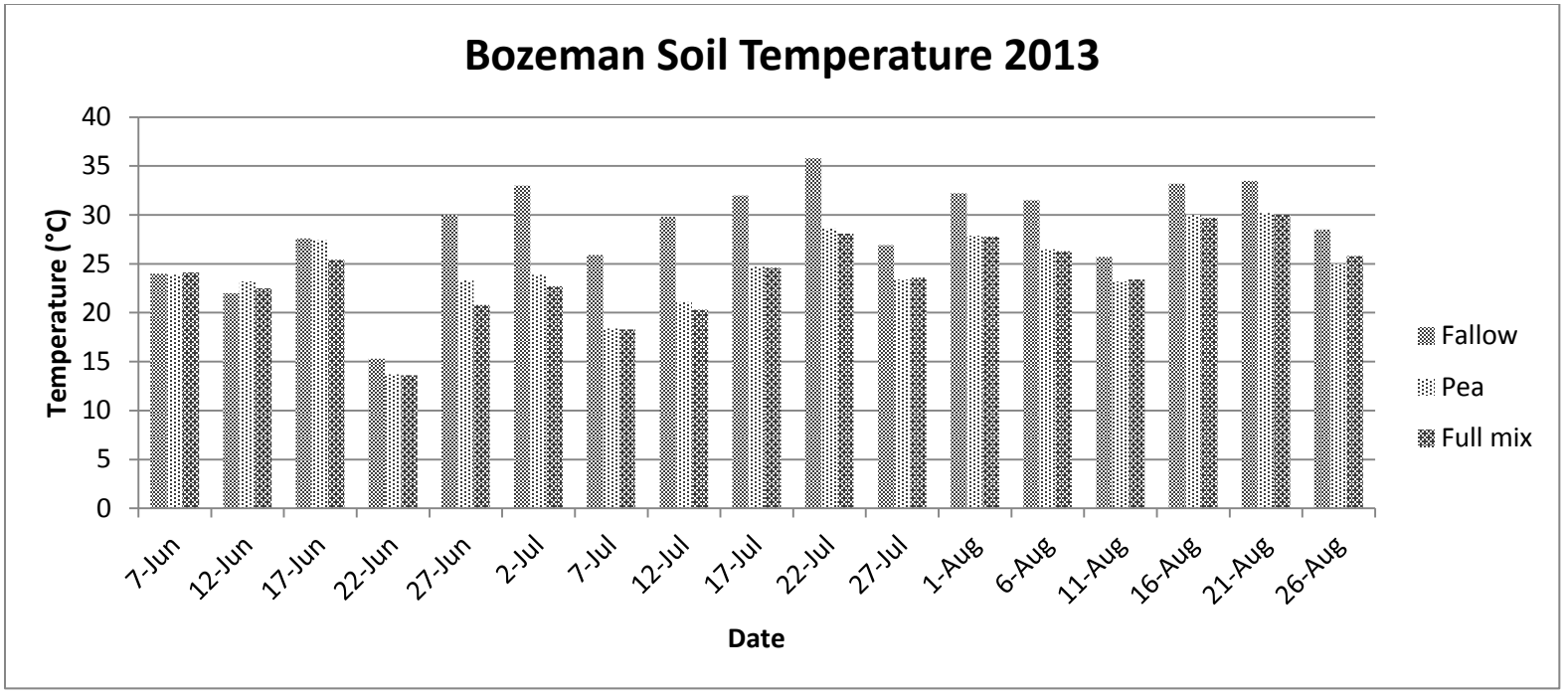

Fig. C.5. Bozeman soil temperature at 5 cm depth for three treatments. Data points reported at 5-d intervals at 4 pm. Cover cop terminated on 5 July 2013 via herbicide.

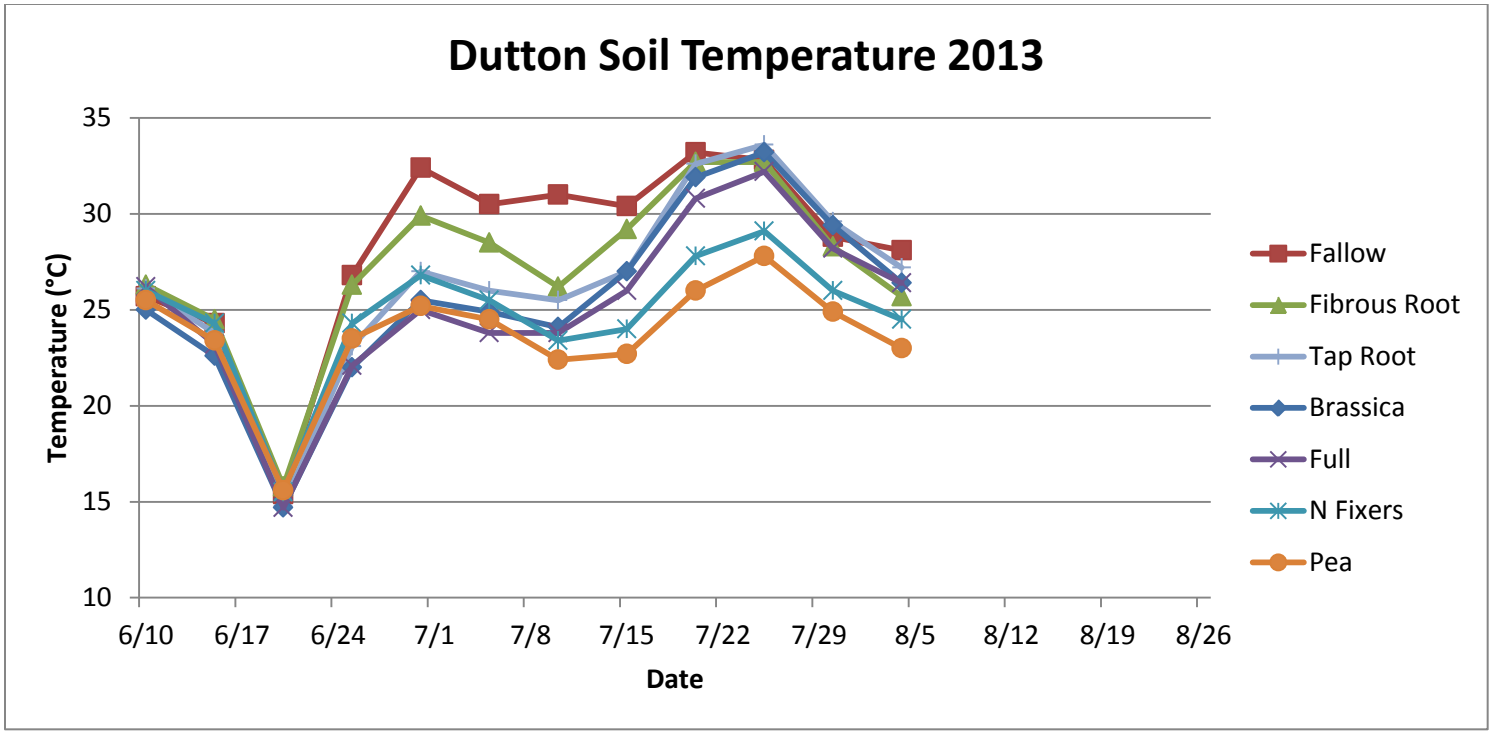

Fig. C.6. Dutton soil temperature at 5 cm depth for seven treatments. Data points reported at 5-d intervals at 4 pm. Cover cop terminated on 10 July 2013 via herbicide. iButtons removed on 9 Aug 2013.

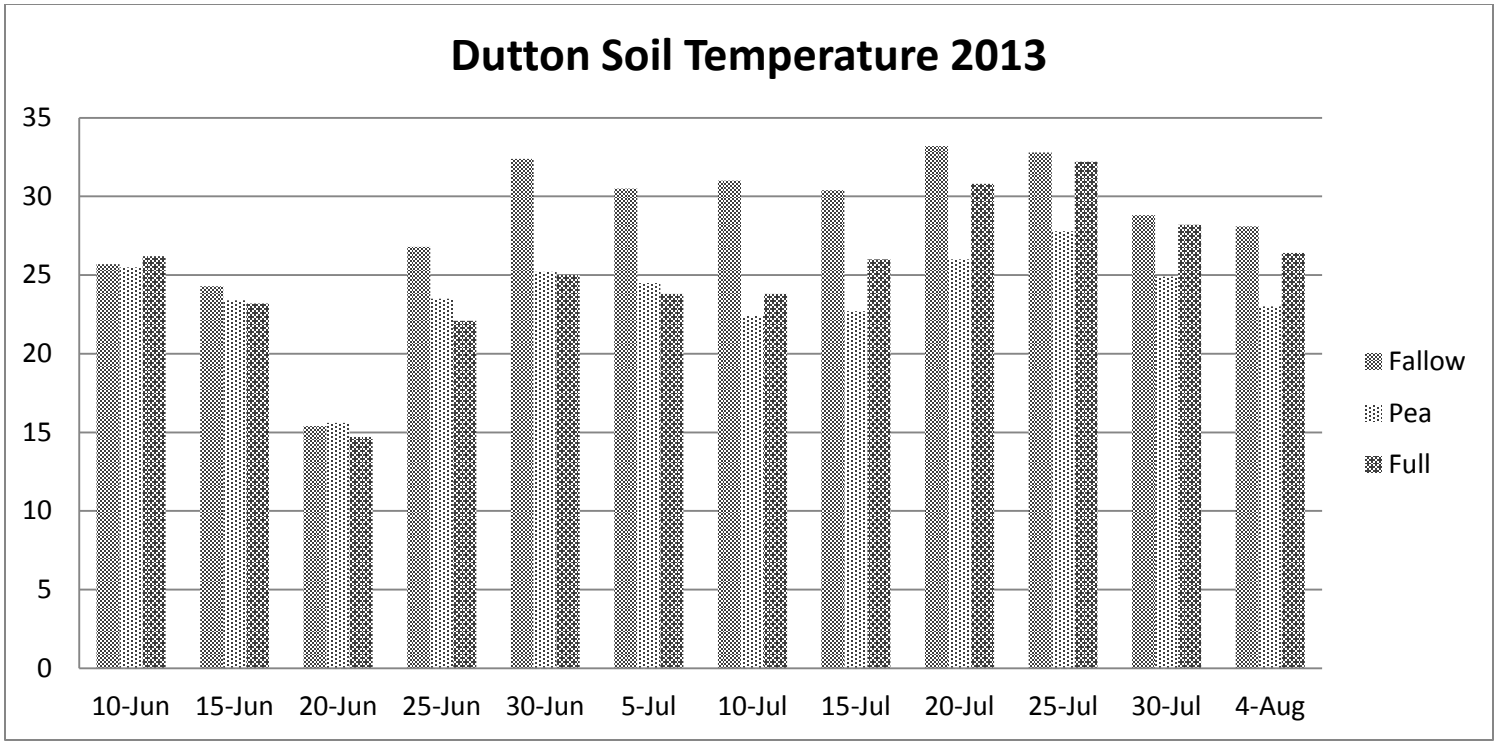

Fig. C.7. Dutton soil temperature at 5 cm depth for three treatments. Data points reported at 5-d intervals at 4 pm. Cover cop terminated on 10 July 2013 via herbicide. iButtons removed on 9 Aug 2013.

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