

8-7-2020

Monoculture to Biculture: Cover Cropping Effects on Biomass, Nitrogen Dynamics, and Yield in a Strip-Tilled Corn Production System

Eduardo Samuel Garay Lagos

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Monoculture to biculture: Cover cropping effects on biomass, nitrogen dynamics, and yield in a
strip-tilled corn production system

By

Eduardo Samuel Garay Lagos

Approved by:

Jac J. Varco (Major Professor)

Brien Henry

Rocky Lemus

Michael Cox (Committee Member/Graduate Coordinator)

George M. Hopper (Dean, College of Agriculture and Life Sciences)

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Plant and Soil Sciences
in the Department of Plant and Soil Sciences

Mississippi State, Mississippi

August 2020

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Eduardo Samuel Garay Lagos
2020

Name: Eduardo Samuel Garay Lagos

Date of Degree: August 7, 2020

Institution: Mississippi State University

Major Field: Plant and Soil Sciences with a Concentration in Agronomy

Major Professor: Jac J. Varco

Title of Study: Monoculture to biculture: Cover cropping effects on biomass, nitrogen dynamics, and yield in a strip-tilled corn production system

Pages in Study: 78

Candidate for Degree of Master of Science

Improved N management is required to enhance crop productivity, while reducing concomitant losses. Research was conducted for 4-y studying winter cover crops. Three legume species grown alone or with either cereal rye (*Secale cereale*) or ‘Tillage Radish[®]’ (*Raphanus sativus L.*) were used to quantify cover crop biomass and N content plus their effects on corn grain yield and N recovery. The effects of these cropping systems on selected soil health indicators was also determined. Rye bicultures enhanced biomass production, but antagonistically affected corn performance. Radish inclusion resulted in equal or greater cover crop N than rye. In year 3, the addition of radish across legume species increased corn N content (10.6 kg ha⁻¹) and grain yield (1050 kg ha⁻¹). Although cover crops did not affect soil bulk density, both bicultures increased soil C/N. The legume-radish association offers a novel practice towards improving crop performance and soil quality.

DEDICATION

I would like to dedicate this important milestone in my life to God almighty, my family, and the people who believe in me and motivate me to keep dreaming high. I also want to acknowledge that this project would have not been possible without the help of many people that will have my eternal gratitude. First and foremost, I want to thank my major professor Dr. Jac J. Varco for taking a giant leap of faith with me by taking me as his student. After the past 2 years I cannot express how grateful and proud I am of having the opportunity to work with such a disciplined, passionate, and noble hearted human being as he is. Also, I want to thank the Varco's soil fertility crew: Andrea Elvir, Andrew Dygert, Derek Hilfiker, Bennie Sanders, Zac Sumner, and Jake Sims. This crew gave me their selfless commitment every time I needed it and made this project possible. A special thanks to my committee members: Drs. Brien Henry, Rocky Lemus, and Michael Cox which through their experience and expertise have made this project better.

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CHAPTER I

INTRODUCTION

Demand for food production and supply are fueled by current and predicted world population growth. High quality food production is necessary as well incremental increases in quantity. The per capita demand for crops, measured as caloric or protein content of all crops combined, has been increasing since 1960. This relationship forecasts a 100–110% increase in global food demand towards 2050 (Tilman et al., 2011). This upward trend suggests that more output must be produced with equal or lower inputs, while also considering the environmental impact of these practices.

Worldwide grain production limitations may occur because of technical constraints that prevent producers from increasing their productivity. For instance, the lack of financial access and technical knowledge to improve irrigation, fertilization, efficient machinery use, crop-protection and soil conservation practices (Charles et al., 2010). Consequently, the implementation of a strategy that enhances soil fertility and crop nutrient use efficiency worldwide, while minimizing the environmental impact may provide a promising path towards sustainable agricultural intensification and an equitable global food supply (Tilman et al., 2011).

Nitrogen supply and role of cover crops in crop production

Nitrogen in the soil commonly exists as NO_3^- -N, NO_2^- -N, and NH_4^+ -N, while the greatest fraction is stored in soil organic matter. The most abundant source on Earth is in the air we breathe, with close to 79% of the air's volume composed of N_2 . In living organisms, N is a principal constituent of amino acids, DNA and RNA, while it is also involved at every level of biological functions. Fixation of atmospheric N_2 occurs through chemical and biological conversion to NH_3 , with subsequent deposition in soil and availability for plant uptake (Leigh, 2004). N is central to living organisms. Its addition to cropping systems is an essential facet of modern crop management and one of the major reasons that crop production has kept pace with human population growth (Robertson and Vitousek, 2009).

Synthetic N use has increased proportionally with agricultural intensification. For instance, a recent study authored by Lu and Tian (2017), demonstrated that fertilizer N consumption increased from 11 Tg N yr^{-1} in 1961 to 108 Tg N yr^{-1} in 2013. Regardless of the increasing trend in the use of this macronutrient, N availability is still considered one of the major limitations to plant growth and yield in modern intensive farming. Increases in global fertilizer N use is not only because of cropland expansion, but also increasing fertilizer application rates per unit of cropland area. It is important to consider that fertilizer N application rates are also influenced by a market that dictates grain or crop prices and inputs of variable costs such as fertilizers (Pannell, 2016).

Legume cover crops are capable of replacing or at least partially reducing fertilizer N requirements in crop production systems (Varco et al., 1999; Seo et al., 2000; Muramoto et al., 2011; Zablotowicz et al., 2011). Choosing the ideal family and species of cover crop requires an understanding of the intended use and need. Additionally, a successful selection should consider

the average temperature, precipitation, soil condition, potential pests, plant morphological structure and growth rate. Species selection includes the legume, grass, and brassica families depending on the intended role or desired or perceived benefit.

Cover Crops

Agriculturists recognized the fundamental role that soil management and conservation practices play towards enhancing the sustainability of food production. Interest in cover crops as an element of soil improvement practices has expanded across the years, but adoption by farmers is still not globally widespread. Cover crops are defined as any living ground cover that are planted into or after the primary cash crop to provide soil cover and protection and then terminated prior to planting the next crop. Cover crops include legume, grass, and brassica species. Benefits of cover crops include erosion control, water runoff reduction, organic matter addition, soil structure improvement, weed suppression, pest reduction, atmospheric N₂ fixation, and N scavenging and recycling (Hartwig and Ammon, 2002).

The Conservation Technology Information Center (CTIC, 2017), recently surveyed 2012 farmers across the U.S. with respect to cover cropping implementation. Results of the study indicated that gaining insight towards the level of expertise of this practice is very important to extension agents, crop advisors, seed dealers, and other technical service providers. Increased familiarity with cover cropping by practitioners and the industry could result in better management and greater willingness by producers to investigate them and potentially implement them in routine crop production practices.

Soil Quality Improvement

A study by D'Hose et al. (2014), demonstrated crop production was directly proportional to soil quality with respect to physical, biological and chemical properties. Cover crops may improve soil quality; therefore, shifting towards greater sustainability and production efficiency. Consequently, by improving soil quality, variable costs may decrease causing an increase in the financial return of the crop investment.

In agricultural systems, optimization of C and N cycling can improve soil fertility and yields, while reducing negative environmental impact (Drinkwater et al., 1998). Previous studies have demonstrated that it is possible to achieve crop yields similar to those using commercial fertilizers with the incorporation of cover crops (Sullivan et al., 1991; Seo et al., 2000). As an example, Zablotowicz et al. (2011) demonstrated that leguminous cover crops were able to provide up to 150 kg N ha⁻¹ to cotton (*Gossypium hirsutum L.*) in the Mississippi Delta region. More importantly, the study reported no treatment differences between legume cover crops and the greatest fertilizer rate of 134 kg N ha⁻¹. Furthermore, Varco et al. (1999) evaluated fertilizer N rate optimization associated with winter cover crops in no-tillage cotton. The results indicated that compared to winter fallow, implementing cover crops resulted in greater cotton lint yield and lowered fertilizer N requirements in the case of legumes, which in turn increased the profitability.

Cover crops are critical in soil conservation because they provide soil protection by dissipating the kinetic energy associated to highly erosive rain periods (Dabney et al., 2001). Therefore, cover crops are a mitigation practice for reducing annual soil losses. Additionally, this benefit suggests an intrinsic ability of water conservation within the production model.

Cover crops also improves soil structure, resulting in enhanced soil water infiltration and percolation. Previous research has shown that mechanical tillage loosens compacted soil, while Calonego et al. (2017) concluded that although tillage resulted in a better immediate improvement in soil structure and soybean yield, the benefits were not observed during the second year of the study. The beneficial effects of cover crops were observed from the medium to long-term, where yields were equal to or greater than occasional chiseling. Additionally, long-term use of cover crops improved soil structure within deeper depths compared to chisel plowing.

Weed suppression and pest reduction

Inclusion of cover crops in year-round production systems may reduce the economic impact from current weed control management practices. This occurs through competition, allelopathy, soil environmental changes, enhancement of weed seed decay, and by maintaining surface residues (Conklin et al., 2002). Generally, living cover crops are more likely to suppress weeds throughout their life cycle and possibly more than cover crop residue alone. In their living state, cover crops absorb incoming red light sufficiently to inhibit phytochrome mediated seed germination. Nevertheless, cover crop residue impairs weed germination in soils through effects on incoming solar radiation and the chemical environment for the seed by releasing phytotoxins that inhibit growth (Teasdale et al., 2007).

The literature also refers to cover crops breaking disease and pest cycles which consequently reduces the need for fumigation and pesticides. Reduced pesticide use can lower production costs and may offer environmental benefits both internal and external to the farm (Snapp et al., 2005). In a study by Hartwig and Ammon (2002) the effects of a cereal rye cover crop on corn production was evaluated under different management systems. They reported that

the population of aphids (*Aphis fabae*) was reduced compared to a traditional no cover crop treatment. They also observed that more predators were present on living mulches, thereby limiting aphid populations.

Leguminous, grass, and brassica cover crops

Cover crop choice depends strongly on the objectives of the producer. Whether the objective is to enhance soil fertility, prevent erosion or suppress weeds weighs into the specific species selection decision. The C:N ratio of plant residues has frequently been used as a tool to predict decomposition rates. A wider cover crop C:N ratio tends to result in a slower decomposition rate which provides an extended soil surface cover during the cash crop season (Odhiambo and Bomke, 2001). Therefore, prolonged weed control is provided whilst reducing the evaporative water losses which aids in ameliorating moisture stress during periods of no or low rainfall (Fageria et al., 2005). However, the narrower the C:N ratio of the cover crop selected, the greater the N availability potential after termination (Drinkwater et al., 1998).

Legume cover crops have a low C:N ratio (i.e. less than 20) and are highly efficient in fixing atmospheric N₂ which can then be cycled through the plant system (Seo et al., 2000). Low C:N ratios result in faster decomposition and greater net release of N in better synchrony of the demand for N by the consecutive crop (Balkcom et al., 2015). Two bacterial species: *Rhizobium* and *Bradyrhizobium* are responsible for this symbiotic N₂ fixation. Legume cover crops explore and capture essential nutrients through their extensive rooting system from subsoil nutrient pools. Once their tissues begin to decompose any absorbed nutrients begin to cycle back into the soil system (Poffenbarger et al., 2015a).

Additionally, the consequent decomposition of a legume cover crop after incorporation or herbicide desiccation results in the mineralization of a portion of N into a usable form. Thus,

legume cover crops can be used as a partial substitute for fertilizer N; the level of substitution depends upon the dry matter yield potential, N₂ fixation efficiency, and environmental conditions during the cover crop growing and decomposition periods (Varco et al., 1991). Legumes are often touted for supplying a more sustainable source of N input to succeeding crops, as opposed to grass cover crops which may require exogenous fertilizer N for adequate plant residue biomass accumulation and production of a subsequent grain crop (Zablotowicz et al., 2011).

The C:N ratio of grass cover crops is generally greater than 20 and usually do not provide a net N benefit and immobilize soil N with C:N ratios greater than 30. Therefore, systems including grass cover crops require greater fertilizer N rates applied to the cash crop than legume and no cover crop systems to maintain yields. Despite this, an early spring termination of cereal grains while they are still succulent and not entirely mature have a lower C:N ratio which allows for a faster decomposition and release of N to the following crop (Parr et al., 2011).

Cereal cover crops generally produce greater biomass especially when significant residual soil N is present and should be considered when the goal is to reduce soil erosion and rapidly build soil organic matter. Some members of the grass family of cover crops have a greater scavenging capacity than legumes for mobile nutrients such as NO₃⁻ (Shipley et al., 1992). This performs a key role in reducing N lost by leaching, especially in fertilized systems. For example, previous studies have demonstrated the effect of winter rye on reducing N losses and increasing N recovery as compared to winter fallow and legume species cover cropping systems (McCracken et al., 1994; Ranells and Wagger, 1997; Adeli et al., 2011). From an agronomic perspective, high NO₃⁻ levels in groundwaters derived from leaching out of the rootzone represents a loss of a resource required for efficient crop production as well as a

potential contaminant. Consequently, recommended fertilizer N rates for corn production are based on utilization efficiencies of approximately 60% ; However, this percentage might decrease when suboptimal growing conditions are present (Hartwig and Ammon, 2002).

Brassicas and their implementation in cover crop management are gaining popularity and are considered a more recent option compared to legumes and grasses (CTIC, 2017). The C:N ratios representative of this family are considered intermediate to high depending on the species (Ruark et al., 2012). Their attributes include rapid growth, high biomass production, well-developed taproot elongation, excellent nutrient-scavenging ability, and high responsiveness to residual soil available N. The residue from brassicas decomposes very quickly which minimizes immobilization of N when compared to cereal cover crops and can often result in net N mineralization (Furay and Zimmerman, 2009). For instance, the well-developed taproot from radishes serves to improve soil structure resulting in rooting improvement by a consecutive crop potentially increasing water and nutrient absorption.

An option such as the ‘Tillage Radish[®]’ (*Raphanus sativus* L. niger (Mill) S. Kerner) has an aggressive 2.5 to 5 cm diameter taproot growing 15- to 30-cm deep and under favorable conditions may reach up to 80 cm deep. Not only their cylindrical shape and fleshy composition causes a “bio-tillage” effect, but also the proliferation of branch and lateral roots in compacted layers serve to help loosen soil (Williams and Weil, 2004). Moreover, this winter cover crop recovers residual soil N following crop harvest, and recycle it to the next crop once it is terminated (Groff, 2008). Radish cover crops also scavenge N with equal or greater efficiency than grass species such as rye, which combined with a quicker rate of decomposition suggest a possible higher N delivery to the subsequent crop (Dean and Weil, 2009). Additionally, brassicas

produce glucosinate-containing residues which suppress plant-parasitic nematodes (Snapp et al., 2005).

Strip Tillage and Cover Crop Influence on Soil C and N

As the intensification of agriculture has increased with time, soil conservation practices have become fundamental to maintain the quality of this nonrenewable resource. In fact, the paramount struggle to achieve food security should be conducted considering the soil where the crops are grown and the environmental impact of agricultural practices (Busari et al., 2015). Conservation tillage practices enhance soil conditions, while maintaining or improving crop yields (Blanco-Canqui and Lal, 2008; Jabro et al., 2011; Marshall et al., 2016). These practices are associated with soil erosion control and compaction amelioration, moisture conservation, and a reduction in production costs.

Further, reducing the intensity of soil disturbance through reduced tillage practices lowers fuel and energy consumption whilst increasing C sequestration through increasing soil organic matter (Holland, 2004). Within the last 30-y strip till (ST) has emerged as an alternative conservation practice. In this system, tillage or soil disturbance is focalized in the crop row while no disturbance occurs within the inter-row position (Fernández et al., 2015). Hence, ST creates an increased soil temperature plus improved seedbed conditions within the planted row, while decreasing the risk of erosion losses (Licht and Al-Kaisi, 2005). More importantly, the combination of a cover crop – ST production system suggests a complementary response due to the desirable individual characteristics that each component offers.

From an input perspective, cover crops represent a substantially greater residue addition in comparison to a winter fallow system. Depending on the specie's C:N and dry matter accumulation potential, cover crops may result in greater C or N availability for the subsequent

crop (Sainju et al., 2008). There is a link between the C input through plant residue and the C and N sequestration potential of the soil (Mazzoncini et al., 2011). Moreover, in the absence of an inorganic N fertilization, cover crops that provide a greater N availability will have a positive effect on the biomass production of the subsequent crop (Carneiro et al., 2006). Overall, cover crops may increase soil C and N stocks by increasing the C and N inputs into the system before and after the main crop. Thus, cover crops offer a great soil building option by enhancing sequestration of atmospheric CO₂ and recovery of leachable soil NO₃⁻ which in turn increases plant growth while adding soil organic matter (Halvorson et al., 1999). The literature suggests cover crop effects of increasing soil C and N stocks under conservation tillage systems (Hu et al., 1997; Zhou et al., 2012; Cong et al., 2014; Moore et al., 2014).

Cover Crop Mixtures

Considering the wide range of benefits that each family and species offers, recent research explores synergistic or antagonistic results of multi-species cover crop plantings. Findings demonstrate strong evidence that usage of multi-species mixtures may be a viable solution to increase the ecological stability and resilience for row crops (Holmes et al., 2017). For instance, Wortman et al. (2012) compared 8 sole species of brassicas and legumes with 4 mixtures, demonstrating that the combination of species had a potential for over-yielding based on the Land Equivalent Ratio (i.e. LER values > 1.0). The LER may be an indicator used to compare the productivity of sole cover crop species to those in a mixture by representing the relative amount of land required when a species is grown alone to achieve the productivity of a mixture.

The complementarity of mixtures can benefit ecosystem services via N retention, N supply, and yield enhancement. Tradeoffs in plant competition, growth habit, residue quantity,

quality, crop yield, and biological N₂ fixation rates must be considered for a mixture selection. In many instances, these trade-offs can be predicted from the characteristics and proportions of individual species (Seman-Varner et al., 2017). In addition, the C:N ratio is a promising predictor of both, how the mixtures will perform and their effects on the subsequent crops (Finney et al., 2016).

Biculture mixtures that include a legume component tend to have a lower C:N ratio than nonlegume monocultures, while also providing the potential synergy of the N acquisition and management benefits of each species (Ranells and Waggoner, 1997). Further, the legume proportion with respect to the nonlegume species should be at least 40% to ensure a significant beneficial effect on the N content and productivity of the consecutive crop (Kuo and Sainju, 1998). Thus, the implicit cost associated with biculture adoption may be compensated for by increasing the cash crop yield and decreasing inputs such as fertilizer N or herbicide applications (Reddy, 2001). The legume – rye association has been thoroughly studied indicating cover crop production benefits (Daniel et al., 1999; Reddy, 2001; Thapa et al., 2018), but variable effects on the N balance affecting the consecutive crop (Muramoto et al., 2011; Snapp and Surapur, 2018). In contrast, research evaluating the legume - radish association as an alternative cover crop management strategy is limited and needs to be further explored.

Summary

The relevance of efficient nutrient management is predicated on an ever-increasing demand for nutrients. There is a need for greater output while ensuring that implemented practices are sustainable and environmentally sound. Nitrogen plays a key-role as it affects crop development and yield directly. Cover crops have been identified as relevant due to their wide range of attributes. Benefits include soil property improvement, soil conservation, organic matter

enhancement, non-chemical weed control, and pest management as well as an alternative source of N.

Cereal rye leads the overall species selection for cover crop implementation followed by a variety of legumes and most recently brassicas are gaining popularity. The preference in rye selection is generally related to the need of cold-tolerant species for a narrower cover crop season for certain locations, such as the North Central USA region (Snapp and Surapur, 2018). Radish species lead the selection within the brassica family (CTIC, 2017). Each crop family offers convenience for a certain N related fate approach. Legumes are biologically N₂ fixers, whereas grasses and brassicas are considered N scavengers. Nevertheless, each species characteristics and C:N ratio varies and may offer a different available N supply rate to the next crop depending on the crop or mixture used. The C:N ratio also influences the decomposition rate of cover crop residues implying that low C:N residues would not be as desirable for soil erosion prevention or weed control due to rapid decomposition.

Therefore, there is a need for exploring bicultures composed of legumes coupled to the top-choice nonlegumes used by growers to understand, document, and quantify the complementary, synergistic, or antagonistic effects produced and the resulting expression of their specific known attributes. Additionally, research evaluating the effects of long-term biculture cover cropping systems are particularly limited in the southern United States, an area representing 40% of all farms and 30% of the total farmland in the country (O'Connell et al., 2014). Overall, more research is required to acknowledge adequate cover crop management techniques that will enhance the N benefit whereby less reliance on inorganic fertilizer N inputs can be achieved.

The objectives of this study are to compare the performance of three legume cover crops grown as a monoculture or in combination with either cereal rye or 'Tillage Radish[®]' on biomass dry matter and C:N content. Consequently, to quantify the effect of these monocultures versus bicultures on corn N content and grain yield in a ST production system. Additionally, the effects of these cover cropping systems on soil health parameters such as bulk density and total soil C and N were determined. It is hypothesized that the inclusion of a second cover crop species will enhance cover crop performance and soil health parameters compared to monocultures which consequently will increase corn N content and grain yield.

CHAPTER II

MATERIALS AND METHODS

This study was located at the W.B. Andrews Agricultural Research Systems Farm at Mississippi State, MS (33°28' N, 88°45' W) and was conducted from October 2015 to October 2019. The dominant soil series mapped at the experimental site indicated was the alluvial derived Marietta fine sandy loam (fine-loamy, siliceous, active, thermic Fluvaquentic Eutrudept) soil. The local weather is characterized by cool winters (January daily average high 11.9°C and low -0.7°C) and hot summers (July average high 33.1°C and low 21.5°C) with average rainfall of 1402 mm distributed somewhat evenly throughout the year (NOAA, 2020). Temperatures during the experiment tended to be slightly warmer than normal. Similarly, annual rainfall was greater than normal with exception of 2016 (Tables 3.6 and 3.9).

Prior to the establishment of the study plots, composited soil samples were collected at a depth of 0- to 15-cm. Soil samples were air-dried and then ground to pass a 2 mm sieve. The soil samples were analyzed for pH using a 1:2 soil:d.i. H₂O. The soil pH ranged from 6.23 to 7.01 (Slightly acid to neutral) across the plot area. Furthermore, the initial macronutrient content was quantified using the Mississippi soil test extraction method (Rasberry and Lancaster, 1977), which provided relevant insight of the soil fertility *in situ* (table 2.1).

Table 2.1 Average soil testing results prior to establishment for extractable macro-nutrients, CEC = 8.8 cmol_c kg⁻¹.

Macronutrient	Concentration mg kg ⁻¹	SD	Test Range
P	95.8	16.1	Very high
K	121.8	28.0	High
Ca	1449.2	413.9	NR
Mg	232.2	30.0	Very high

SD: Standard deviation; NR: Not rated

Study allocation and design

Treatments were arranged as a randomized complete block design (RCB). There were 10 experimental plots per block with 4 replications. Plots within blocks were 9.1 m long and 3.9 m wide comprised of 4 rows with 0.96 m spacing between them. Experimental plots were blocked based on an initial determination of variability in soil test results for CEC across the study site. The spatial design and assignment of treatments was maintained each year across the entire duration of the study.

A one-year cycle of cover crop – ST corn production of this study consisted of three main stages. First, cover crops were planted in early October as a winter-spring cover cropping system. Cover crop seed was drill seeded into minimally tilled beds on 9 Oct. 2015 and into untilled corn residue on 6 Oct. 2016 and 2017. During the last cycle of the study, cover crop seed were broadcast on 9 Oct. 2018. Prior to planting each year, legume cover crop seed was inoculated with rhizobacteria (La Crosse Seed, WI, USA). Most of the cover crop growth and development occurred during the spring season with warming temperatures. Consequently, cover crops were terminated early April (Table 2.2) with glyphosate [N-phosphonomethyl] glycine] as the potassium salt at 1.54 kg a.i. ha⁻¹. Following herbicide application and approximately 2

weeks, plots were prepared for corn production by strip-tilling and using a bed roller to adequately firm and shape beds.

Table 2.2 Important dates for cultural practices performed for the cover crop – strip tilled production system from October 2015 – September 2019.

Cultural Practice	2016	2017	2018	2019
CC planting	9 Oct. 2015	6 Oct. 2016	6 Oct. 2017	9 Oct. 2018
CC termination	6 Apr. 2016	6 Apr. 2017	3 Apr. 2018	3 Apr. 2019
Corn planting	18 Apr. 2016	13 Apr. 2017	19 Apr. 2018	19 Apr. 2019
1-m harvest	8 Aug. 2016	15 Aug. 2017	15 Aug. 2018	13 Aug. 2019
Corn harvest	30 Aug. 2016	24 Aug. 2017	18 Sept. 2018	6 Sep. 2019

CC = Cover Crop

Corn Dekalb[®] hybrids (DKC Monsanto, St. Louis, MO) 66-97 in 2016, 67-72 in 2017, and 67-44 in 2018 and 2019 were planted using a vacuum planter at a rate of 74,000 kernels ha⁻¹ (See Table 2.2 for planting and harvest dates). Weeds during the corn season were controlled with a pre-emergence tank mix application following planting of atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) at 4.02 kg a.i. ha⁻¹, mesotrione (2-[4-methylsulfonyl]-2-nitrobenzoyl]cyclohexane-1,3-dione) at 0.16 kg a.i. ha⁻¹, S-metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(1-methoxypropan-2-yl)acetamide] at 1.22 kg a.i. ha⁻¹, and glyphosate at 1.54 kg a.i. ha⁻¹. A post emergence application of glyphosate at 1.54 kg a.i. ha⁻¹ was made each year approximately at a V6 corn stage. Corresponding data for specific years of this continuous winter-spring cover crop and ST corn system will be referred to when applicable as its corn harvest year (i.e., first cover crop-ST corn experimental cycle from 2015 to 2016, referred as 2016). During the entire duration of this study, strip tillage was the main cultural practice implemented for soil preparation prior to corn establishment. Nevertheless, prior to

cover crop establishment in fall of 2017, the study area was re-hipped, and a bed conditioner was used to knock down the beds and smooth.

Cover crop treatments were assigned *ad random* to the plots within each block. Each monoculture or biculture was assigned to the same plot each year, although certain legume species were dropped and substituted for as indicated in Table 2.3. Each year, legume species used in a biculture mixture were seeded at approximately 80% of the original monoculture seeding rate (Table 2.3). Seeding rate calculations were corrected for any seed coating and percent live seed.

Table 2.3 Cover crop species and seeding rates used for treatment assignment on fall of 2015-2018.

Year	Family	Specie	Monoculture	Biculture
			----- kg ha ⁻¹ -----	
2015		Hairy Vetch (<i>Vicia Villosa</i>)	33.5	28.0
2015-2016		Red Clover (<i>Trifolium pratense</i>)	13.5	11.2
2015-2018	Legumes	Crimson Clover (<i>Trifolium incarnatum</i>)	13.5	11.2
2016-2018		Persian clover (<i>Trifolium resupinatum</i>)	7.0	5.5
2017-2018		Berseem Clover (<i>Trifolium alexandrinum</i>)	22.0	18.0
2015-2018	Brassica	‘Tillage Radish’ [®] (<i>Raphanus sativus</i> L.)	NA	4.5
2015-2018	Grass	Cereal rye (<i>Secale Cereale</i> L.)	NA	28.0

NA: Non-applicable, Cereal rye and ‘Tillage Radish’[®] were not used as a sole specie monoculture treatment.

Each block or replicate consisted of 9 cover crop treatments comprised of 3 legume monocultures factorially combined with either cereal rye or ‘Tillage Radish’[®], and referred to as bicultures (Table 2.3). Additionally, a winter fallow control was included to quantify the natural occurring dynamics (i.e., N mineralization-corn accumulation) the soil provided without the

influence of cover crops. During the entirety of all production cycles, no synthetic fertilizer N was used in an effort to isolate the cover crop N and associated benefits to ST corn.

Parameters evaluated

Soil samples were collected each spring prior to corn planting at depths of 0- to 5- and 5- to 15-cm. These samples were used to monitor Mississippi Soil Test extractable macronutrients and to determine pH 1:2 soil:d.i. H₂O. The extracted nutrients were quantified using an Avio 200 ICP-OES (Perkin Elmer, Waltham, MA). Monitoring of extracted soil elements provided nutrient cycling insight for this annual production system. Consequently, to minimize the impact of a macronutrient deficit in the system, applications of P, K, Mg, and S were made each year after corn planting. Fertilizer sources used were concentrated superphosphate [Ca(H₂PO₄), 0-46-0], muriate of potash (KCl, 0-0-60) and K-Mag (K₂SO₄ 2MgSO₄, 0-0-22-11-22) at a rate of 11 kg P ha⁻¹, 66 kg K ha⁻¹, 6 kg Mg ha⁻¹ and 12 kg S ha⁻¹.

Each year, prior to cover crop termination, four 0.25 m² quadrats of cover crop aboveground biomass were randomly sampled from areas in the plots outside of the middle two harvest corn rows to minimize removal of cover crop residues from the primary study area. ‘Tillage Radish[®]’ was hand harvested by pulling the whole plant, due to its elongated fleshy taproot composition. The samples were then oven-dried at 65°C and ground in a Wiley mill to pass a 0.42 mm sieve and subsequently analyzed to determine C and N concentration (%) using a NC 1500 dry combustion analyzer (Carlo Erba, Milan, Italy). The aboveground biomass dry matter accumulation (Mg ha⁻¹) was determined following oven drying at 65 °C with resulting yield used to calculate along with tissue N% total cover crop N content (kg ha⁻¹).

As corn reached physiological maturity (i.e. black layer), 1-m of one of the two center rows was harvested (See Table 2.2 for sampling dates) from each plot. Grain and stover (i.e.

cobs, stalks, and leaves) were separated prior to drying at 65°C in a forced air dryer. The ground 1-m grain and respective stover were analyzed for total N content using the same methodology as the cover crop samples previously described. The 1-m grain plus its respective stover were used to determine the total aboveground corn N content (kg ha^{-1}). Lastly, corn grain from machine harvesting of the full length of the center two rows of each plot using an automated plot combine, was dried, ground and analyzed for total N. Grain collected from the 1-m sampling at physiological maturity was added to harvest data for total plot calculation of grain yield (kg ha^{-1}). Grain yield was adjusted to a 15.5% moisture content.

Following the last corn harvest on 18 October 2019 soil samples were collected to assess possible soil health (bulk density and total C and N) differences from the long-term use of cover crops. The soil sampling was chosen during this post-harvest time based on an expected stable condition in the field (i.e. cool temperatures plus no recent fresh biomass addition) to successfully complete the long-term assessment (Jokela et al., 2009). Using a soil probe and the soil approximately at field capacity, 6 cores were randomly collected from the ST corn row area and 6 from the un-trafficked and undisturbed inter-row positions, totaling 12 cores per plot. Row spacing was comprised of 0.38 and 0.58 m from the ST corn row and the undisturbed inter-row positions, respectively. Each soil core was separated by depths of 0- to 5- and 5- to 15-cm and composited accordingly in tin cans. Subsequently, samples were oven dried at 105 C° to determine the oven-dry weight. The oven-dried weight and the measured soil probe volume (16 cm^3 for 0- to 5-cm samples and 32 cm^3 for 5- to 15-cm) were used to calculate bulk density.

All soil samples were ground to pass a 0.25 mm sieve to determine total C and N concentration ($\mu\text{g g}^{-1}$) using a Vario Max Cube (Elementar, Hesse, Germany) dry combustion analyzer. Bulk density samples and C and N concentrations were used to calculate the

treatment's total C and N contents on a mass basis (Mg ha^{-1}). Soil samples collected prior to the establishment of the study were prepared and analyzed for C and N concentration ($\mu\text{g g}^{-1}$) using the same methodology previously described. These last samples represent a point of reference towards the assessment of long-term cover cropping effects on soil health.

Statistical Analyses

The distribution of all the data collected was first assessed using residual plots and a Shapiro-Wilk test for normality. Once the normality assumption was met, the General Linear Model package procedure was used to conduct an ANOVA using SAS 9.4 software (SAS Institute, Cary, NC). For each year, legume (three levels corresponding to the legumes used each cycle; Table 2.3) and biculture (three levels corresponding to legume only, legume + rye and legume + 'Tillage Radish[®]') were tested as fixed effect factors, while block was considered a random effect. Hereafter, levels of the biculture main effect will be referred as legume monoculture (average of three legumes), rye biculture, and 'Tillage Radish[®]' biculture. When significant treatment differences were found, a Fisher's protected LSD was used to separate treatment means at $\alpha = 0.05$. This process was used to analyze the biomass and cover crop N content among the nine cover crop treatments each year of the study. Corn yield and total corn N content (stover plus grain from 1-m samples) are reported as a net effect in relation to the cover crop treatment and corrected for by subtracting the winter fallow effect by corresponding blocks from these variables. All corn variables were compared for all cover crop treatments and years.

The 2019 soil bulk density was assessed as a split-plot arrangement considering cover crop treatments (including winter fallow) and row position as main effects and soil depth as split-plot using SAS PROC GLIMMIX. Block was considered a random effect for all the soil variables analyzed. Further, total C and N by mass basis were analyzed at each depth separately

while using the treatment and row position as fixed factors. A single-factor ANOVA was performed to examine treatment differences at each depth after properly calculating a weighted average across row positions. Lastly, C and N variables were examined between the initial and final year of the experiment using a weighted treatment average of the 2019 C and N concentration across row position and depth since no bulk density, depth or row position data were collected prior to establishment. A Fisher's protected LSD was used to separate means of the soil variables at $\alpha = 0.05$ if significant main effects were found. For all the variables presented in this study if a main effect interaction was found, means were separated using an LSMEANS in SAS PROC GLM. The LSD was then computed by multiplying the appropriate t value by the standard error of the difference of means provided in the output from the PDIFF option in the LSMEANS statement (Fernández et al., 2015).

CHAPTER III
RESULTS AND DISCUSSION

Cover Crop Biomass Dry Matter

Significant differences were found across all years for the biculture main effect ($P < 0.05$; Table 3.1) on cover crop dry matter yield. In 2016 and 2018, no difference was found between the legume monoculture (averaged across 3 legume species) versus the average effect when they were grown with the ‘Tillage Radish[®]’ as a biculture. However, dry matter accumulation was greater than either of these two average effects when grown with rye (Table 3.1). More importantly, the inclusion of rye into a biculture mixture caused an average increase in dry matter accumulation of 33% and 39% or 0.59 and 1.34 Mg ha⁻¹ across the three legumes in 2016 and 2018, respectively. By the last year of the study, the pattern persisted with the rye biculture averaging the greatest dry matter accumulation of 3.78 Mg ha⁻¹ across the three legumes (Table 3.1). Overall, results indicated that the grass-legume association offered a complementary improvement in the functional trait of ‘biomass production’ compared to legume monocultures (Hayden et al., 2014).

Table 3.1 Average cover crop dry matter accumulation across legume and non-legume bicultures for 2016 to 2019 experimental years and Analysis of Variance of main and interactive effects.

Treatment	Dry Matter			
	2016	2017	2018	2019
	----- Mg ha ⁻¹ -----			
Legume main effect				
Hairy Vetch / Persian clover	1.72	1.80	2.16	2.42
Crimson clover	2.88	3.90	4.74	3.38
Red clover / Berseem clover	1.39	1.60	4.67	3.40
Biculture main effect				
Legume only	1.71	1.77	3.42	2.29
Legume + 'Tillage Radish [®]	1.89	2.88	3.40	3.13
Legume + rye	2.39	2.66	4.76	3.78
LSD (0.05)	0.31	¶	0.41	0.48
ANOVA				
	----- P > F -----			
Legume	<0.0001	<0.0001	<0.0001	0.0003
Biculture	0.0004	0.0002	<0.0001	<0.0001
Legume × Biculture	0.454	0.0249	0.4247	0.9057

Hairy Vetch (2016), Persian clover (2017-2019), Red clover (2016-2017), Berseem clover (2018-2019).

¶ LSD non-applicable due to an interactive effect of legume × biculture according to ANOVA.

Due to the potential for rapid growth and dry matter production of rye, the inclusion of this cover crop in a biculture mixture creates greater biomass benefiting the consecutive crop. This complementary effect agrees with previous research assessing aboveground biomass accumulation of legume-rye biculture cover crop mixtures (Sainju et al., 2005; Poffenbarger et al., 2015b). For instance, Thapa et al., (2018) reported through a meta-analysis of 55 site-years that on average, hairy vetch-rye bicultures outperformed the hairy vetch monocultures by accumulating 39% greater aboveground biomass on coarse textured soil sites. Following termination of legume-rye mixtures, the greater biomass at sampling indicates greater residue cover for the subsequent corn production system and associated benefits. Inclusion of rye with

legumes may enhance agroecosystem services namely water conservation, erosion control, weed suppression, and N retention (Finney et al., 2016).

Moreover, significant differences were found across all years when analyzing the legume main effect ($P < 0.0001$; Table 3.1). Across the biculture main effect, crimson clover resulted in the greatest dry matter accumulation at 1.32 Mg ha^{-1} more than the average of the other two legumes in 2016 (Table 3.1). This effect is primarily attributed to the slower growth and lower yield at harvest for red clover. Red clover grows at a slower rate and matures later in the season, but nonetheless is well adapted to this area (Queen et al., 2009). The low productivity potential within the time frame necessary for optimum corn planting however appears to limit its utility in this system.

In 2016, due to the wet and cold conditions *Phytophthora* sp. damage to hairy vetch was prevalent and its susceptibility to this disease under the conditions that prevailed on an alluvial soil was exposed. This incident led to a species rotation of hairy vetch with Persian clover due to its tolerance towards these conditions and adaptation to silty clay loam soils for the subsequent years of the study. During 2018 and 2019, crimson clover consistently showed superior performance by clearly outperforming Persian clover. Nonetheless, these same years in which berseem clover was introduced into the study, there were no differences found with crimson clover averaged across the biculture main effect (Table 3.1). This indicated that both crimson and berseem clovers, are equally high productive legumes suitable for a biculture specie selection.

The expected synergistic effect of rye coupled with a legume that results in the highest dry matter accumulation was not observed in 2017 and there was no difference when compared to 'Tillage Radish[®]' biculture (Figure 3.1). However, there was a significant interaction between legume and biculture main effects in 2017 ($P = 0.0249$; Table 3.1) due to the Persian clover-rye

biomass lower yield as compared to the other legume species. The dissimilar biomass response of this particular mixture contradicts Sainju et al. (2005) who concluded that legume-cereal bicultures had greater biomass yield than either monocultures of legumes or cereals. The lower dry matter production is attributable to an apparent slower growth and development of Persian clover further reduced by competition from the rye companion crop. Decades of cover cropping research has demonstrated that cereals are usually the dominant component in cereal-legume mixtures, suppressing the growth of the legume and being the principal contributors of biomass to the total mixture yield (Hayden et al., 2014). Nevertheless, there was a positive response of increasing biomass production when coupling rye with crimson and red clovers, accumulating more dry matter than their respective legumes alone. Additionally, crimson clover and the average of its biculture mixtures resulted in greater biomass (3.23 and 4.22 Mg ha⁻¹ respectively) than the other legume species and their mixtures for 2017 (Figure 3.1).

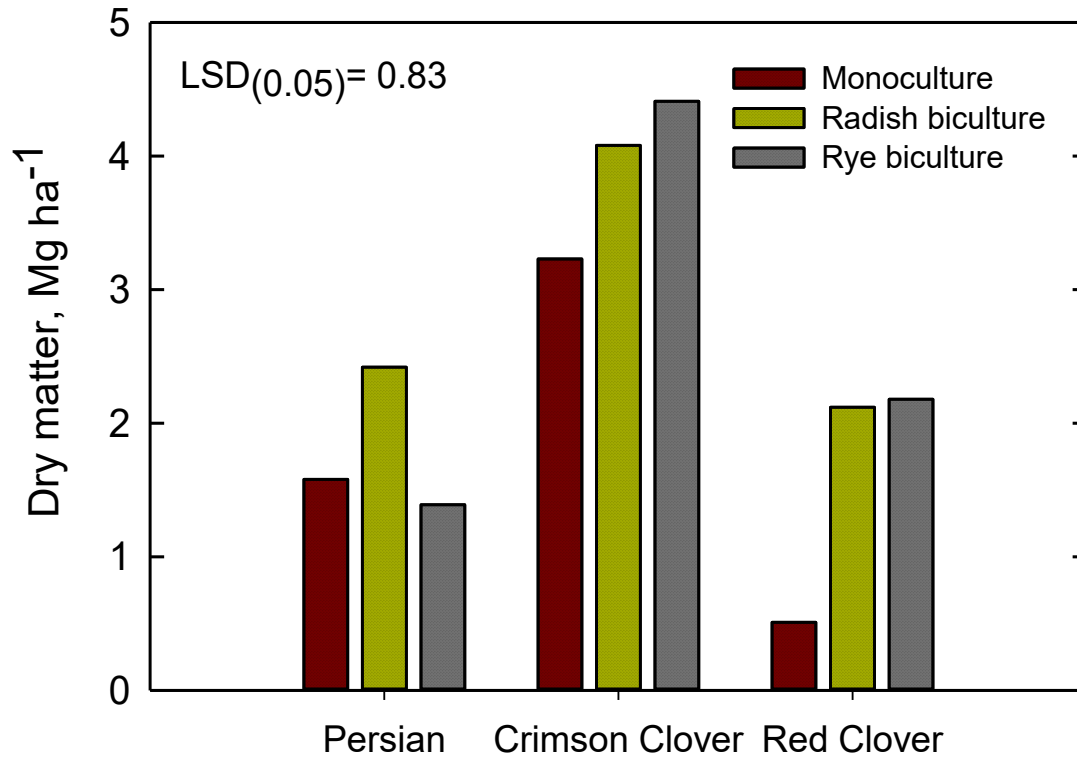


Figure 3.1 Interaction of legume monoculture × non-legume biculture treatment combinations (Tillage Radish[®] or Rye) on biomass dry matter accumulation in 2017.

With exception of a single negative response in 2017 (Persian clover-rye biculture; Figure 3.1), there was a constant tendency throughout the study of greater biomass yield with the inclusion of rye in the biculture mixture. Therefore, combining species of different phenology and N acquisition strategy might result in mixtures that produce more biomass than monocultures (Finney et al., 2016). Nevertheless, it is important to keep in mind that biomass production is strongly affected by environmental conditions as evidenced by the year to year variations in cover crop yields (Table 3.1 and Figure 3.1) as well as soil fertility and crop management practices as has been reported previously (Fageria et al., 2005).

Cover Crop N Content

Sustainable cover crop biculture practices should offer a substitute for the need for synthetic fertilizer N for subsequent crops, whilst providing a tangible economic benefit as well as a potential for soil management improvement. Throughout the 4-y of this study, significant differences were found by the biculture main effect except for 2016 (Table 3.2). The first year's pattern agrees with the results of Thapa et al. (2018), where the legume-rye mixture accumulated as much N as that of corresponding legume monoculture irrespective of the soil type or previous crop. This effect is attributable to the 'Tillage Radish[®]' bicultures not producing biomass accumulation differences when included and in addition to a similar C:N resulted in fairly equal cover crop N contents (Table 3.3). Furthermore, cover crop biculture mixtures might not show immediate differences to monoculture N contents the first year of establishment. Daniel et al. (1999) reported differences between biculture mixtures and legume monocultures N contents did not occur until the third and final year of the study. Differences found from 2017 to 2019 in this experiment also suggest a possible long-term benefit when implementing cover crops in the regular production cycle.

Table 3.2 Average cover crop N content across legume and non-legume bicultures for 2016 to 2019 experimental years and Analysis of Variance for main and interactive effects.

Treatment	Cover Crop N			
	2016	2017	2018	2019
	----- kg ha ⁻¹ -----			
Legume main effect				
Hairy Vetch / Persian clover	39.0	53.9	43.0	50.3
Crimson clover	69.9	104.2	97.3	60.7
Red clover / Berseem clover	37.0	35.4	90.9	67.3
Biculture main effect				
Legume only	46.6	53.3	76.6	50.3
Legume + 'Tillage Radish [®] '	47.7	76.6	87.1	64.3
Legume + rye	51.5	63.6	67.6	63.8
LSD (0.05)	9.4	¶	10.5	10.1
	ANOVA			
	----- P > F -----			
Legume	<0.0001	<0.0001	<0.0001	0.0069
Biculture	0.5383	0.0052	0.0032	0.0129
Legume × Biculture	0.7896	0.0378	0.3923	0.8787

Hairy Vetch (2016), Persian clover (2017-2019), Red clover (2016-2017), Berseem clover (2018-2019).

¶ LSD non-applicable due to an interactive effect of legume × biculture according to ANOVA.

In 2017, there was an interaction between legume × biculture main effects (P = 0.0378; Table 3.2). Moreover, this same year no differences were found between biculture mixtures coupled to crimson and red clovers at $\alpha = 0.05$. However, adding 'Tillage Radish[®]' to these legumes resulted in greater N contents compared to their monocultures with an increase of 23.4 and 31.9 kg N ha⁻¹, respectively (Figure 3.2). The N content effect and performance when non-legumes were blended with the legumes is likely a result of the N scavenging ability that either 'Tillage Radish[®]' or rye offer (Furay and Zimmerman, 2009; Adeli et al., 2011).

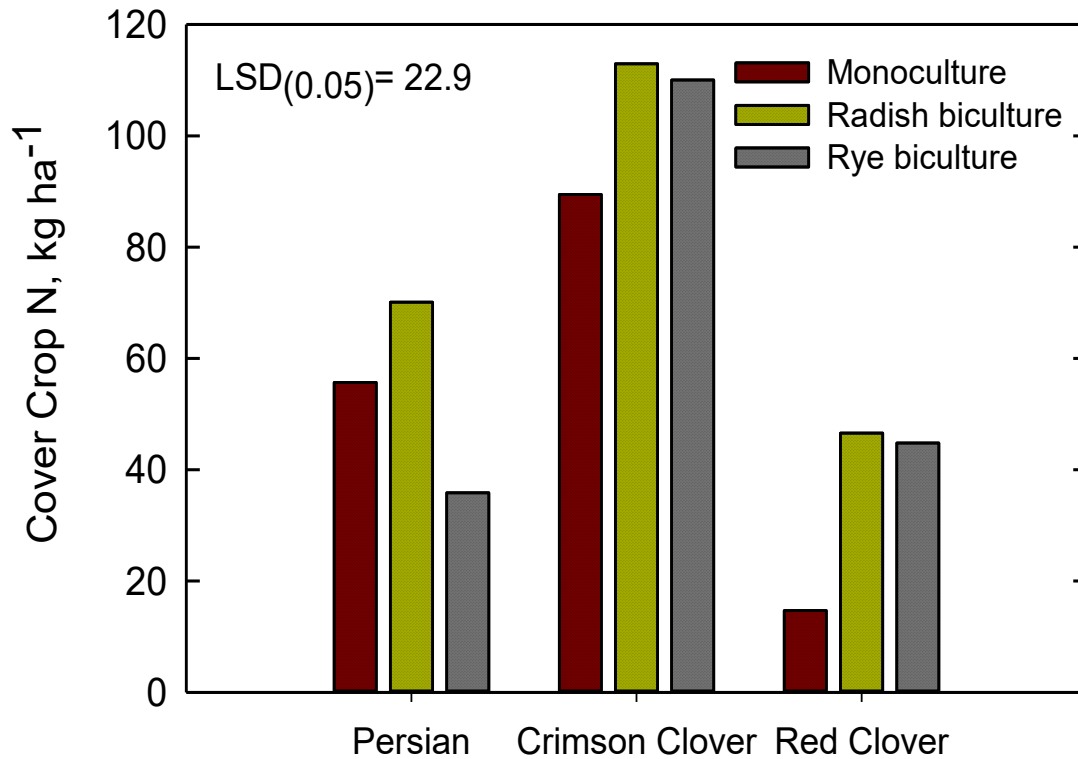


Figure 3.2 Interaction of legume monoculture × non-legume biculture treatment combinations (Tillage Radish[®] or Rye) on cover crop N in 2017.

This study did not utilize any source of N inorganic fertilization for either the cover crop or the corn production seasons. Therefore, the productivity of these non-legume cover crops is indicative of low residual soil N conditions following the harvest of a non-fertilized corn crop. More importantly, any residual N availability is likely determined by any subsequent soil N mineralization and N mineralized from decomposition of previous crop residues (Huggaard-Nielsen and Jensen, 2001). In addition, the negative biomass effect from rye on Persian clover development in 2017 resulted in an interaction that also decreased cover crop N content (Figures 3.1 and 3.2). It is also likely that precipitation which was below the 30-y average during the

spring of 2017 influenced lesser cover crop development; therefore, causing similar biomass and N content accumulations between crimson and red clovers regardless of the non-legume biculture added (Table 3.4).

In 2018, ‘Tillage Radish[®]’ bicultures resulted in the greatest N content as indicated by an increase across legumes of 21% or 15.1 kg ha⁻¹ compared to the average of rye bicultures plus legume monocultures (Table 3.2). This confirms the potential of this species for capturing residual N from the soil with equal or greater effectiveness than rye. For example, Dean and Weil (2009) demonstrated in a 3 site-y study that on average ‘Tillage Radish[®]’ had a greater N shoot content in comparison to rye cover crop systems.

The fall (Oct to Dec) of 2017 was colder than usual. There was a total of 26 d (16 d in Dec) that reached temperatures under 0 C°, mostly during nocturnal hours. Within the time frame of the study, this temperature behavior represented 17 d more than 2015 and 10 d more than in 2016 and 2018. This caused a winter kill of the ‘Tillage Radish[®]’, which released the N scavenged which was then potentially assimilated by the surviving companion legume cover crop. It is likely that the combination of specific low temperature events plus the N acquisition and aggressive growth phenology of the ‘Tillage Radish[®]’ created the superior synergistic effect observed this year even though 100% of the ‘Tillage Radish[®]’ was winter killed. Interestingly, it was visually observed that the ‘Tillage Radish[®]’ was the best it ever looked in terms of growth during the fall of 2017. Consequently, legume - ‘Tillage Radish[®]’ mixture effects on C:N ratios are shown in Table 3.3. Legume species combined with the ‘Tillage Radish[®]’ tended to result in the lowest C:N ratio in 2018 across the individual years when compared to their corresponding legumes alone. These N dynamics are supported by the work of Dean and Weil (2009) where

they concluded that winter killed 'Tillage Radish[®]' in coarse textured soils resulted in greater pore water NO₃⁻ concentrations than rape (*Brassica napus*), rye, and the weedy control fallow.

Table 3.3 Average C:N ratio of legume × non-legume biculture treatment combinations for 2016-2019.

Treatment	2016	2017	2018	2019
	----- C:N -----			
Hairy Vetch / Persian clover	20	12	14	17
Crimson clover	17	16	19	22
Red clover / Berseem clover	14	15	19	19
Vetch / Persian + 'Tillage Radish [®] '	20	15	14	18
Crimson + 'Tillage Radish [®] '	17	15	16	22
Red / Berseem + 'Tillage Radish [®] '	15	19	17	20
Vetch / Persian + rye	21	17	37	26
Crimson + rye	21	18	27	27
Red / Berseem + rye	22	22	29	25

Hairy Vetch (2016), Persian clover (2017-2019), Red clover (2016-2017), Berseem clover (2018-2019).

For the last year of the study, differences were found between the average effects of the legume monocultures which had lower N contents than average effects of both bicultures, but no differences ($\alpha = 0.05$) were found between the two biculture mixtures (Table 3.2). Moreover, this consistent pattern confirms a potential advantage of increasing cover crop N accumulation using biculture mixtures. The legume monoculture accumulation of N is directly influenced by biological N₂ fixation. Therefore, equal or greater N contents observed when using a biculture mixture indicate the possibility of greater residual soil N scavenging by the non-legume companion cover crop (Adeli et al., 2011).

Throughout the 4-y of the experiment, the cover crop periods of Oct to Dec in 2015 and 2018 reported approximately a 2-fold difference in precipitation (466 and 499 mm respectively) compared to the averages of 2016 and 2017 (Table 3.4). Thus, there was a possibility of losing a

fraction of the residual soil N through NO_3^- leaching, especially prior to rapid cover crop growth (Huggaard-Nielsen and Jensen, 2001). Additionally, excessive rainfall and wet soil likely caused a diminishing effect on the cover crop biomass production, which combined with a lower N pool for scavenging was reflected as a similar N accumulation irrespective of the non-legume biculture for 2019.

Table 3.4 The 30-y normal monthly mean air temperature and total precipitation and deviation from the mean for the duration of the winter-spring cover crop growing cycles (Oct to Mar) for the 4-y experiment duration. Source: NOAA, 2020.

Month	Temperature						Precipitation					
	30-y [†]	2015	2016	2017	2018	2019	30-y [†]	2015	2016	2017	2018	2019
	C°						mm					
Oct.	17	2	4	2	3	2	104	-41	-103	-49	-29	201
Nov.	12	2	2	1	-2		118	94	-30	-89	73	
Dec.	7	6	2	1	1		132	59	-15	7	92	
Jan.	6		0	5	-2	2	137		-23	-3	-85	62
Feb.	8		2	4	5	4	145		67	-58	118	78
Mar.	12		2	2	1	0	123		73	-3	19	-15

[†] 30-y normal period average of 1981-2010.

For instance, Daniel et al. (1999), reported from a 3-y study the greatest cover crop biomass and N content response occurred with near average rainfall during the winter and with adequate rainfall during the spring when the majority of biomass accumulation occurs. Comparatively, from an input perspective, the best cover crop performance was in 2018 possibly as a result of minimal residual soil N loss and greater potential for N scavenging when close to average precipitation occurred during the 2017 fall months (particularly in Dec). Adequate rainfall during the spring months including above average in Feb, prompted an increase in growth and development (Table 3.4). This rainfall pattern also led to a greater potential

phenotypical expression from using ‘Tillage Radish[®]’ before the winter-kill event, which then benefited the surviving companion legume cover crop.

Overall, cover crop N was significantly influenced by the legume main effect across the 4-y of the study ($P < 0.0001$ for 2016-2018 and $P = 0.0069$ in 2019; Table 3.2). During the first year crimson clover had the greatest N content averaged across biculture. However, no differences were observed compared to berseem clover in 2018 and 2019 at $\alpha = 0.05$ due to the specie’s equally high biomass production. More importantly, both legumes offer a potentially greater N input into the system than Persian clover, depicting their complementary benefits in a biculture mixture from solely an aboveground input perspective. This observed species performance coincides with Parr et al. (2011), where no differences were found for total N contents between 4 cultivars of crimson and berseem clovers in cover crop monocultures, as part of a no-till organic corn production system.

Conservation tillage combined with cover cropping practices can help maintain and supplement existing surface residue to offset degraded soil conditions such as depleted organic matter pools (Balkcom et al., 2015). The synergy of combining species not only minimizes soil degradation, but as evidenced in this study, may also represent a sustainable source of the partial N requirements for the subsequent crop. However, producers need to consider other factors that will also influence the input efficiency of this practice. The N supplying capacity of cover crop systems certainly is influenced by factors such as timing of cover crop termination, C:N ratio and the biomass produced under a given set of environmental conditions (Balkcom et al., 2015).

Net Corn N Content

The N content of the corn cash crop indicates the sustainable efficiency of the cover cropping practice to supply it with N and provide a basis for concomitantly reducing purchased fertilizer N inputs. Throughout the 4-y of this study, a significant interaction was only found in 2016 between legume \times biculture main effects ($P = 0.0311$; Table 3.5). During the first year, rye coupled with crimson and red clovers had a significantly lower corn N content than the corresponding legume species alone, resulting in a net negative response. In contrast, the inclusion of ‘Tillage Radish[®]’ resulted in the greatest corn N content between bicultures when coupled to hairy vetch and crimson clover, although these radish mixtures were not significantly different from their legumes alone at $\alpha = 0.05$ (Figure 3.3). However, a negative effect was observed when ‘Tillage Radish[®]’ was grown with red clover, which contributed to the interaction (Figure 3.3).

Table 3.5 Average net corn N content across legume and non-legume bicultures for 2016 to 2019 experimental years and Analysis of Variance of main and interactive effects.

Treatment	Net§ Corn N Content			
	2016	2017	2018	2019
	----- kg ha ⁻¹ -----			
Legume main effect				
Hairy Vetch / Persian clover	8.0	40	22.1†	8.2
Crimson clover	8.4	31.9	24.8†	9.6
Red clover / Berseem clover	-4.1	13	18.0†	12
Biculture main effect				
Legume only	6.8	35.1	28.8	9.2
Legume + 'Tillage Radish' [®]	7.2	35.1	40.2	13.3
Legume + rye	-1.6	13.8	-4.1	7.3
LSD (0.05)	¶	10.5	9.5	NS
ANOVA				
	----- P > F -----			
Legume	0.0001	<0.0001	0.3462	0.4255
Biculture	0.0053	0.0003	<0.0001	0.1332
Legume × Biculture	0.0311	0.159	0.6004	0.7186

Hairy Vetch (2016), Persian clover (2017-2019), Red clover (2016-2017), Berseem clover (2018-2019).

- ¶ LSD non-applicable due to an interactive effect of Legume × Biculture according to ANOVA.
- † Within columns, means of the main effect are not significantly different according to ANOVA.
- NS Means from neither fixed effect factors are significantly different according to ANOVA.
- § Cover crop net effect, corrected by subtracting winter fallow effect by corresponding blocks

As previously mentioned, during the first experimental year, a *phytophthora* sp. infection affected the normal growth and development of hairy vetch. Thus, it is likely that a healthy scenario for this species could have caused a greater corn N response from the monoculture. As a result, a significant decrease in corn N content across the three legumes would have been observed from the inclusion of rye. Disregarding this phyto-pathological effect, it is possible to attain similar N supply efficiencies between a hairy vetch – rye biculture and the corresponding

monoculture. Nonetheless, the N supplying efficiency will be strongly influenced by the Growing Degree Days (GDD) of a specific location (Thapa et al., 2018).

Conversely, in 2016 no differences were found in cover crop N contents between biculture mixtures and their respective legumes alone. Hence, similar C:N ratios with the ‘Tillage Radish[®]’ biculture resulted in a similar output effect on the corn N content (Tables 3.2 and 3.3). The particular net negative corn N response when combining ‘Tillage Radish[®]’ with red clover may be a result of the legume high levels of spatial variation in growth and development, and therefore, resulting in spatially variable N credits across whole production fields (Queen et al., 2009). The slower and more variable development from red clover might have led to outperformance by Brassica species which likely reduced biological N₂ fixation within the mixture. Thus, potentially greater residual soil N scavenging by the ‘Tillage Radish[®]’ biculture allowed for delivery of a similar N input to the corn compared to the legume alone (Table 3.2). Therefore, it is speculated that a decrease in biological N₂ fixation as a result of reduced growth of the companion legume, in this case red clover, due to competition caused a slightly lower N pool during advanced corn physiological stages later in the season resulting in a net negative response (Figure 3.3). For example, lower soil N mineralization or availability may affect grain development due to the rapid uptake and N partitioning that occurs after pollination (Gentry et al., 2013).

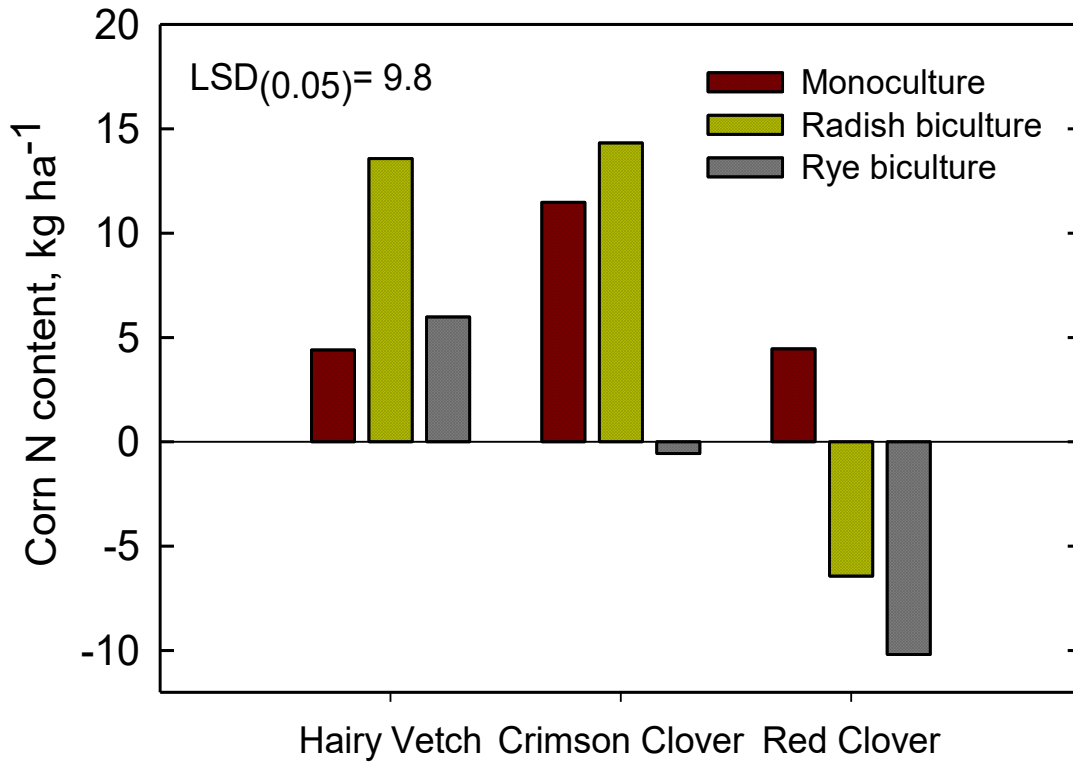


Figure 3.3 Interaction of legume monoculture × non-legume biculture treatment combinations (Tillage Radish[®] or Rye) on net corn N content at physiological maturity in 2016.

For the duration of this study, 2017 was the only year significant differences were found for the legume main effect ($P < 0.0001$; Table 3.5). Corn N content was not different between Persian and crimson clover systems at $\alpha = 0.05$, but they both had better performance than red clover which accumulated almost 3 times less corn N (Table 3.5). From a cover crop biomass and N content perspective, Persian clover was consistently outperformed by crimson clover during the 3 years the species was used. This counterintuitive response suggests that Persian clover possibly compensates by having lower aboveground biomass and N accumulation with greater belowground N storage in its root system. This result agrees with Askegaard and Eriksen

(2007), where they compared the N supply performance of 6 legumes and 4 nonlegume cover crops in conventionally tilled barley (*Hordeum vulgare*). They indicated Persian clover resulted in the greatest soil NO₃⁻ content and claimed an inconsistency between the N fertilizer replacement capacity observed and the N content in the Persian clover shoots and recommended further cover crop rhizodeposition research. However, research quantifying the belowground N contribution in roots, nodules and rhizodeposition in cover crops is limited. Anglade et al. (2015) indicated through a meta data analysis that this particular lack of belowground research might be related to the challenge in physically recovering N rhizodeposits which do not have a well-defined structure or due to thin roots that are not collected from the soil. Nonetheless, the author indicated that belowground N contributions may range from 19 to 75 % of total plant derived N from clover type legumes. Further, Persian clover monocultures had the lowest C:N ratios for all years included (2017 to 2019) which suggests faster decomposition and N release from its residues and or greater N recycling efficiency (Drinkwater et al., 1998; Table 3.3).

During the 2017-2018 experimental years, the corn N content was significantly affected by the biculture main effect ($P = 0.003$ for 2017 and $P < 0.0001$ for 2018; Table 3.5). Results from 2017 showed no differences between the legume monoculture and ‘Tillage Radish[®]’ biculture ($\alpha = 0.05$), with similar net corn N content of 35 kg ha⁻¹ (Table 3.5). However, adding rye into a biculture mixture created an antagonistic effect which reduced the N accumulation in corn by 61% compared to the legume monoculture and the ‘Tillage Radish[®]’ biculture, averaged across the legume main effect (Table 3.8).

For the third year of this study, the inclusion of ‘Tillage Radish[®]’ resulted in the greatest N content accumulation compared to the legume monoculture and the rye biculture. The addition of ‘Tillage Radish[®]’ contributed to an average corn N increase of 40% or 11.4 kg ha⁻¹ across the

three legumes species. In contrast, an antagonistic effect from rye was observed, resulting in the lowest N content for each year and causing a corn N credit of -4.1 kg ha^{-1} as compared to the non-fertilized winter fallow check treatment (Table 3.5).

Regardless of the resulting similar effects between the ‘Tillage Radish[®]’ biculture and the legume monoculture in 2017, there was an individual tendency of increasing corn N content when adding the brassica cover crop to crimson clover (Data not shown). It is possible that a non-quantified belowground N input from Persian clover plus the variant growth and development from red clover mixtures, led to the same average effect between the ‘Tillage Radish[®]’ bicultures and corresponding legume monocultures. However, the response in 2017 concurs with Samarappuli et al. (2014), where similar N delivery values were found between forage radish (*Raphanus Sativus* L. niger) and legumes as input sources preceding the production of energy forage crops.

In 2018, legume species selections resulted in a more consistent performance of the ‘Tillage Radish[®]’ N supply effect across the three species. From an input perspective, a cover crop N increase across legumes resulted when coupled with ‘Tillage Radish[®]’. Precipitation close to the 30-y average for the summer months minimized N losses and may have resulted in a more consistent N supply (Askegaard and Eriksen, 2007; Table 3.6). A corn N response due to ‘Tillage Radish[®]’ bicultures suggest that achieving higher levels of N supply with cover crop mixtures is possible with the selection of nonlegume species that substantially reduce NO_3^- leaching while synchronously delivering the nutrients to the consecutive crop (White et al., 2017). The response noted in 2018 of the ‘Tillage Radish[®]’ bicultures to supply more N from decomposing cover crop residues is also attributable to the particularly lower C:N compared to their respective legume monocultures this year. Thus, addition of the radish component resulted

in a more efficient decomposition and net N mineralization (Rosecrance et al., 2000; Table 3.3). Additionally, the relevance of the legume complementarity increases when winter kill occurs in a companion species as it did with the ‘Tillage Radish[®]’ during fall of 2017. The ability of the ‘Tillage Radish[®]’ to scavenge residual soil N may be negated following winter kill and winter precipitation events, especially on sandier highly permeable soils due to NO₃⁻ leaching (Dean and Weil, 2009). More importantly, the ‘Tillage Radish[®]’-legume synergy implies a greater reduction potential in N fertilization necessary to maintain highly productive cash crop yields compared to legume monocultures (Drinkwater et al., 1998).

Adding rye into a biculture mixture resulted in an overall antagonistic corn N accumulation response during the first 3-y of the study (Table 3.5). This diminishing tendency agrees with previous cover crop research evaluating the N supply efficiency of legume-rye bicultures on subsequent cash crops such as corn (Ranells and Wagger, 1997; Balkcom et al., 2015; Seman-Varner et al., 2017, 2019), cotton (Daniel et al., 1999; Varco et al., 1999), barley (Askegaard and Eriksen, 2007), and broccoli (*Brassica oleracea L.*) (Muramoto et al., 2011). Conversely, from an input perspective rye bicultures provided an equal or greater cover crop N than the legumes alone throughout the study. Thus, this behavior confirms the species potential to scavenge residual N before and after the crop season (Fageria et al., 2005). Nonetheless, including rye in a cover crop mixture resulted in the greatest C:N every individual year compared to the radish bicultures and legume monocultures (Table 3.3). Greater C:N is usually associated with a greater dry matter accumulation potential from the grass species that perhaps results in greater N dilution (Odhiambo and Bomke, 2001). Similarly, an increasing GDD in the cover crop season induces rye development to maturity with a resulting decline in tissue N concentration (White et al., 2017). Hence, an increase in C:N of the rye-biculture

residues probably resulted in greater N immobilization which reduced cover crop residue N release to the corn N content. This was particularly evident in 2018, where rye bicultures had the greatest C:N ratio average across legumes (31:1) whilst causing the lowest average net N content throughout the study of 4.1 kg ha^{-1} less than the non-fertilized winter fallow check (Table 3.5).

Lastly, in 2019 neither the legume nor biculture main effects significantly affected corn N content ($P > 0.05$; Table 3.5). Compared to the previous three years of the experiment, only 2019 resulted in the same average N accumulation by the corn irrespective of the cover crop treatment. This response contradicted expected results based on differences found between legume monocultures and the bicultures cover crop N contents (Table 3.2). Nevertheless, the single instance of this effect is mainly attributable to a substantial increase in precipitation during the corn production season compared to the 30-y average and previous three experimental years. More specifically, the sum of precipitation from April through May resulted in 549 mm which is a 2.5-fold increase compared to the 2016 to 2018 average for these same months or a 2.3-fold (308 mm) increase from the 30-y average (Table 3.6).

Table 3.6 The 30-y normal monthly mean air temperature and total precipitation and deviation from the mean for the duration of the summer corn growing cycles (Apr to Sep) for the 4-y experiment duration. Source: NOAA, 2020.

Month	Mean temperature					Precipitation				
	30-y [†]	2016	2017	2018	2019	30-y [†]	2016	2017	2018	2019
	----- C° -----					----- mm -----				
Apr.	17	1	3	-2	0	125	-15	-18	25	232
May	21	0	0	3	2	116	-35	38	-68	76
Jun.	26	2	-1	2	1	106	-7	128	4	106
Jul.	27	1	1	1	0	105	-15	-9	21	166
Aug.	27	2	0	1	1	104	-16	92	-32	37
Sep.	23	4	1	4	4	87	-17	37	195	-86

[†] 30-y normal period average of 1981-2010

It is likely that this precipitation pattern increased cover crop N residue degradation and release of N which may have been susceptible to losses; thus, reducing or minimizing N available for corn uptake resulting in a more uniform response across cover crop systems. Accordingly, Poffenbarger et al. (2015a) indicated using a mesh bag approach that cover crop residue from mixtures containing a greater legume proportion released most of their accumulated N within 4 weeks (>50 % and >75% on year 1 and 2 of the experiment, respectively). Consequently, the study also suggested a greater susceptibility to possible denitrification and leaching losses prior to the period of rapid N uptake by corn. Within continuous cover crop-corn production cycles, the period of greatest N demand from corn generally occurs 7 to 8 weeks following cover crop desiccation or termination (Rosecrance et al., 2000). Therefore, the coinciding 2.5-fold increase in precipitation following cover crop termination possibly resulted in diminished N availability for the corn crop. The 2019 corn season had the greatest precipitation throughout the study with a total of 1173 mm which is a 1.8-fold deviation from the 30-y average (Table 3.6). Despite the sub-optimal environmental conditions, in terms of ranking

the ‘Tillage Radish[®]’ bicultures coupled with Persian and crimson clovers tended to result in greater N credit differences compared to other treatments and the non-fertilized winter fallow check (Figure 3.4).

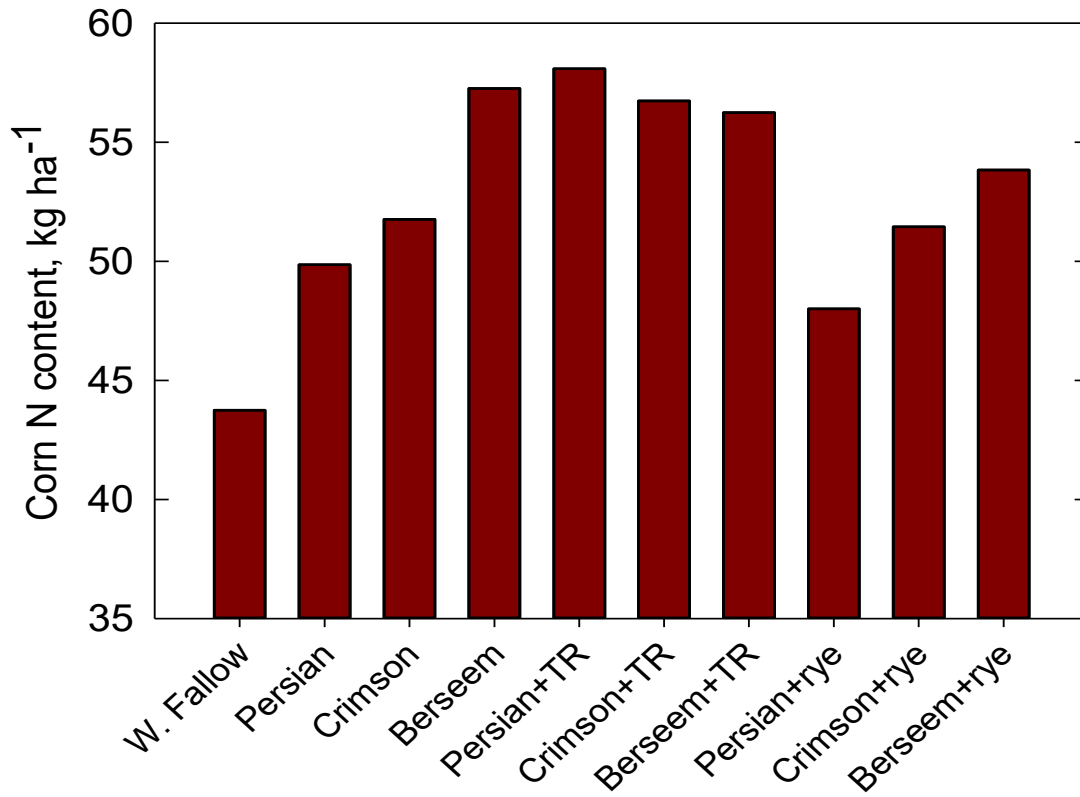


Figure 3.4 Corn N content at physiological maturity for all cover crop treatments and winter fallow check in the 2019 production cycle.

Net Grain Yield

The N benefits ascribed to cover cropping practices can influence sustainability while non-N benefits perceived can aid by incentivizing a greater adoption rate by producers. This 4-y study showed that the grain yield was significantly influenced by the legume main effect only in 2016 ($P = 0.0318$; Table 3.7). Also, in 2016 there were no differences between hairy vetch and crimson clover at $\alpha = 0.05$, averaged across biculture main effects. However, red clover grain yield resulted in a value 3 times lower than the average effect of these two legumes (Table 3.7).

This difference resulting from legume species selection on corn performance may primarily be attributable to the lower N input that red clover provided grown alone or in a biculture mixture. Similar negligible or variable corn responses have been reported, such as by Gentry et al. (2013) who indicated differences in corn yield between red clover and the winter fallow check year 1 but not year 2 of the experiment across 4 management systems. Additionally, despite *Phytophthora* sp. damage to hairy vetch, an equal yield response to crimson clover confirms the hairy vetch's known ability for supplying N and non-N benefits to a subsequent crop (Hayden et al., 2014).

Table 3.7 Average net grain yield across legume and non-legume bicultures for 2016 to 2019 experimental years and Analysis of Variance of main and interactive effects.

Treatment	Net§ Grain Yield			
	2016	2017	2018	2019
	----- kg ha ⁻¹ -----			
Legume main effect				
Hairy Vetch / Persian clover	635	2211	1540†	903
Crimson clover	538	2083	1810†	863
Red clover / Berseem clover	192	637	1346†	1266
Biculture main effect				
Legume only	668	2171	2141	971
Legume + 'Tillage Radish [®] '	753	2121	3192	1159
Legume + rye	-57	640	-637	902
LSD (0.05)	340	¶	559	NS
ANOVA				
	----- P > F -----			
Legume	0.0318	<0.0001	0.2475	0.1269
Biculture	<0.0001	<0.0001	<0.0001	0.4565
Legume × Biculture	0.3783	0.0243	0.2341	0.6964

Hairy Vetch (2016), Persian Clover (2017-2019), Red Clover (2016-2017), Berseem Clover (2018-2019).

¶ LSD non-applicable due to an interactive effect of Legume × Biculture according to ANOVA.

† Within columns, means of the fixed effect are not significantly different according to ANOVA.

NS Means from neither fixed effect factors are significantly different according to ANOVA.

§ Cover crop net effect, corrected by subtracting winter fallow effect by corresponding blocks

Moreover, a significant interaction was found in 2017 between legume × biculture main effects ($P = 0.0243$; Table 3.7). Further, 'Tillage Radish[®]' bicultures had similar yields with their respective legume alone. On the contrary, rye tended to cause an antagonistic grain yield effect across legume species, although a nonsignificant effect was found when rye was combined with crimson clover at $\alpha = 0.05$ (Figure 3.5). The results observed in 2017 were expected when considering the corn N content for this year. Moreover, an equal N accumulation was observed from the legume monocultures and radish bicultures. On the contrary, rye bicultures caused a

penalty in the net corn N content which produced a similar depression in grain yield. Despite no statistical differences, there was still a tendency for a yield decrease for the grass-crimson clover biculture. Nevertheless, the lack of significance agrees with Ranells and Waggoner (1997) where this specific biculture was studied and the results suggested a possible equal N supply towards a subsequent crop compared to crimson clover alone. Moreover, crimson clover had the lowest increase in C:N when mixed with rye (from 16:1 to 18:1 respectively; Table 3.3) compared to the other legume species and corresponding bicultures, possibly due to its earlier progression to maturity (Parr et al., 2011). This lower shift was equally reflected in the corn N content which resulted in a statistically negligible yield difference.

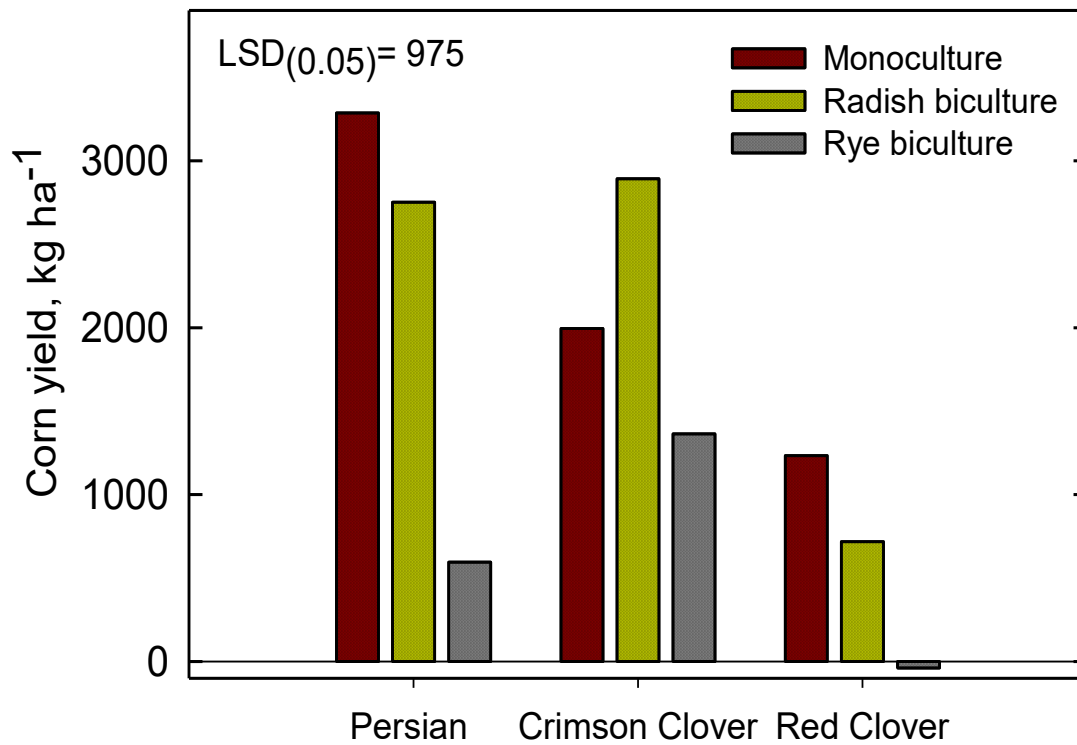


Figure 3.5 Interaction of legume monoculture × non-legume biculture treatment combinations (Tillage Radish[®] or Rye) on net grain yield in 2017.

Throughout the study, significant differences were found for the biculture main effect for net grain yield in 2016 and 2018 ($P < 0.0001$; Table 3.7). For 2016, the addition of ‘Tillage Radish[®]’ resulted in 753 kg ha⁻¹ of net grain yield which was the highest output averaged across the legume main effect. However, no statistical difference was found with the legume monoculture. In 2016, an antagonistic effect by rye was observed indicating 57 kg ha⁻¹ less grain yield than the non-fertilized winter fallow check (Table 3.7). Long-term, differences in 2018 were found between the ‘Tillage Radish[®]’ biculture and the legume monoculture ($P < 0.05$), indicating a positive response by increasing in net grain yield 49% or 1051 kg ha⁻¹ across the three legumes. Likewise, the tendency observed in previous years for rye prevailed where grain yield decreased by 2780 kg ha⁻¹ compared to the legume monocultures and 637 kg ha⁻¹ than winter fallow (Table 3.7).

The equal yield response found between the radish bicultures and monocultures during 2016 may be attributable to the interaction found for corn N content where a reduced N accumulation effect was observed for the red clover-radish mixtures (Figure 3.3). Consequently, a decline in grain yield for the red clover-radish mixtures may be related to the lower N content of this cover crop system which may have caused a non-significant average effect when ‘Tillage Radish[®]’ was included across legumes. In addition, because this study involved a low N input system, there was likely a greater sensitivity towards N stress causing yield losses that may have been reflected as equal treatment responses (Scharf et al., 2002).

Furthermore, in 2018 the addition of ‘Tillage Radish[®]’ produced a substantial grain yield increase across the legumes which is attributable to the greater N acquisition and delivery efficiency from this biculture. As mentioned previously, the environmental conditions in this production cycle plus the legume selection allowed the brassica specie to phenotypically express

a greater N input reflected in greater grain yield. Previous research has also acknowledged subsequent cash crop improvements with radishes when non-confounded by other factors (i.e. management and environment). For instance, Finney et al. (2016) demonstrated yield service benefits to corn when including forage radish in a 4-specie cover crop mixture in year 1, and negative in year 2 due to greater rye biomass composition. Also, Wortman et al. (2012) remarked on the particularly high competitiveness and suitability of oilseed radish (*Raphanus Sativus* L.) as an input resource towards production in the western Corn Belt.

Interestingly, research reporting the specific benefits on corn N and grain yield of legume cover crop species grown in combination with the ‘Tillage Radish®’ is limited to nonexistent. Similar to corn N content, the lower C:N ratio in 2018 for radish bicultures compared to legume monocultures proved to be highly related with corn yield performance (Finney et al., 2016; White et al., 2017). In addition, ‘Tillage Radish®’ has also proven to penetrate compacted soil profiles better than fibrous rooted species, due to the diameter growth of a cylindrical-fleshy taproot with additional thick branch roots providing possible “biological tillage” (Chen and Weil, 2010). Due to the nature of this species it is likely that the corn development and resource acquisition was facilitated by possible soil physical property improvements which contributed to greater nutrient exposure leading to a yield improvement (Scharf et al., 2002).

The yield penalty effect from rye bicultures that was observed in the first 3 experimental years is mainly attributable to the decreased N corn content due to a greater soil N immobilization rate. Previous research results also vary according to location, demonstrating a rye preference in the U.S.-northern region due to its winter hardiness plus other known benefits (i.e. weed suppression, erosion control, and water conservation) and sometimes negligible effects in corn yield (Snapp and Surapur, 2018). In addition, the legume-rye association should provide

intermediate N-mineralization rates and lower C:N in comparison to rye monocultures (Rosecrance et al., 2000). Nevertheless, there are studies where the results coincide with the corn yield penalty caused by the inclusion of rye in the present study (Sainju et al., 2005; Muramoto et al., 2011; Chu et al., 2017; White et al., 2017). Although not quantified in this study, literature also indicates that rye can create an allelopathic effect on corn that reduces grain yield (Fageria et al., 2005). Thus, the response observed when implementing rye bicultures suggest the requirement for greater fertilizer N rates in order to attain an optimum corn grain production level.

For the last year of the experiment, no significant differences were found for either legume or biculture main effects (Table 3.7). These results may have been influenced by weather conditions which prevailed during the corn production cycle of 2019 which created greater crop N stress resulting in nonsignificant grain yield differences. Despite these observations, for rainfed production systems it certainly is expected that yearly variability in weather conditions interact with soil moisture availability, N losses, and other soil bio-physical properties that might affect N mineralization and nutrient availability for the corn (Snapp and Surapur, 2018). Due to the lack of significance in 2019, the data was re-analyzed by including the non-fertilized winter fallow check response and doing an overall cover crop treatment comparison. Consequently, Figure 3.6 shows that despite the sub-optimal weather conditions, seven out of nine cover crop treatments caused a significantly greater grain yield response ($P < 0.05$) by producing at least 715 kg ha^{-1} more than the winter fallow treatment. More importantly, the data suggests the overall cover cropping importance plus risk and environmental impact amelioration compared to a fall-spring bare soil fallow and a main crop inorganic fertilizer N dependency.

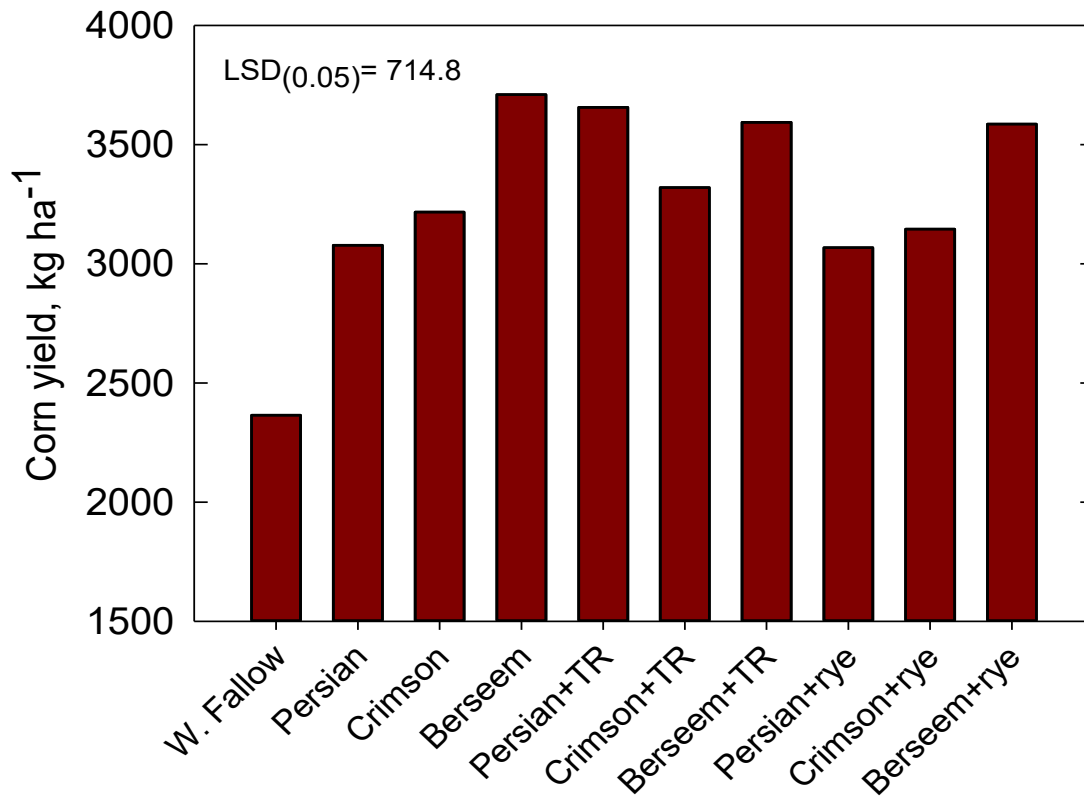


Figure 3.6 Corn grain yield for all cover crop treatments and winter fallow in the 2019 production cycle.

Soil Bulk Density

After 4-y of continuously implementing a cover cropping – ST corn production system, a counterintuitive response was observed showing that cover crop treatments did not significantly influenced soil bulk density ($P > 0.05$; Table 3.8). This lack of effect was not expected due to the increase in soil physical disturbance from cover crops during their growing season (Oct to Apr) compared to the bare soil winter fallow. In addition, the greater input effect of cover crops on corn lead to speculate on a quantifiable bulk density decrease. However, the only relevant statistical significance was found when considering the row position \times soil depth interaction effect on soil bulk density ($P < 0.0001$; Table 3.8).

Table 3.8 Analysis of Variance of cover crop treatment, row position, soil depth and interactive effects on soil bulk density after corn harvest in fall 2019.

Effect	Bulk Density
	----- P > F -----
Treatment	0.4546
Row position	<0.0001
Depth	<0.0001
Treatment \times Position	0.2167
Treatment \times Depth	0.9965
Position \times Depth	<0.0001
Treatment \times Position \times Depth	0.8728

The 4-y of cover cropping practices used in this study did not result in a quantifiable bulk density improvement in the surface 15 cm of the soil profile. This specific result agrees with Chen and Weil (2011), where they indicated that two brassica and a grass cover crop specie did not significantly influence bulk density on two experimental sites of a winter cover crop – no till corn production system. Furthermore, Sainju et al. (2007) also demonstrated that soil bulk density was not significantly affected by cover crop monocultures and mixtures of three legumes

and rye as part of a ST cotton production system. Although an overall tangible improvement was expected from cover crops, it is still possible that significant differences could have been detected at greater soil profile depths which were not evaluated (i.e., 15- to 30 and 30- to 55-cm). For example, Marshall et al. (2016) demonstrated that using rye in a no-till cotton system resulted in a reduction of soil cone index compaction in the E horizon (20- to 30-cm) of three soil series with different textures.

The lack of differences despite of the ‘Tillage Radish[®]’ traits may also be attributable to the specie’s greater “bio-drilling” impact at soil profile depths below 15 cm and an increasing growth responsiveness to soil compaction. Induced stress due to soil compaction elevates ethylene production by ‘Tillage Radish[®]’ roots, thus increasing tap-root aggressiveness and branch root proliferation (Sharp and LeNoble, 2002). In agreement, Chen and Weil (2010) evaluated the penetration of compacted soils with forage radish, indicating that only this species from the three evaluated had a positive relationship between root count and soil strength at a 15- to 50-cm depth. Further, under no compaction the authors observed negligible differences in root vertical penetration among the three cover crops compared.

Moreover, bulk density may not always reflect other physical property improvements such as potential increases in soil macroporosity or changes in pore size distribution (Calonego et al., 2017). In this study, ‘Tillage Radish[®]’ was grown at a reduced seeding rate as a companion crop, thus its plant density was not as great as if it were planted alone, which implies the soil physical property benefits may not be taken full advantage of at reduced seeding rates. However, with increasing years of including ‘Tillage Radish[®]’, the benefits of the deep taproot creating root channels and hence reflecting a quantifiable decrease in soil bulk density may be realized (Chen and Weil, 2011). In addition, evidence from this study also suggests that cover crops

might need more than 4-y for some soil quality indicators such as bulk density to detect significant changes in the surface 15 cm layer (Jokela et al., 2009). The work of Calonego et al. (2017), indicated that significant decreases in soil bulk density at depths of 10- to 20-cm from the use of cover crops did not occur until the eighth year of the experiment, where it was reported that the effectiveness of the practice was equal to or better than occasional chiseling.

As observed in Figure 3.7, results from the row position \times soil depth interaction indicated that both sampled row positions (ST corn row versus undisturbed inter-row) significantly increased in bulk density as a function of soil depth. More specifically, the corn row and the inter-row area demonstrated increasing bulk density by 0.07 and 0.21 g cm⁻³ or 4.8 and 16.5 % respectively, from the 0- to 5-cm to the 5- to 15-cm sampling depth. Conversely, results also indicated that at both depths, soil bulk density was significantly lower for the undisturbed inter-row area in comparison to ST corn row by 0.20 and 0.06 g cm⁻³ or 15.7 and 4.1% from 0- to 5-cm to 5- to 15-cm, respectively.

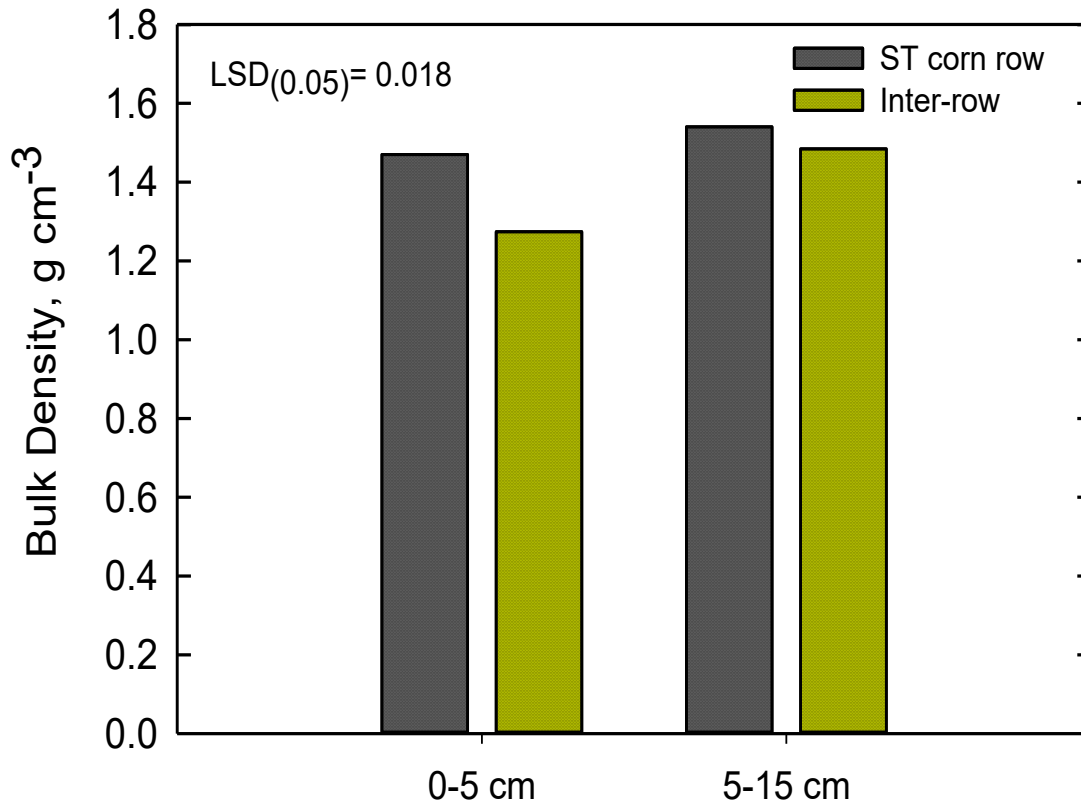


Figure 3.7 Interaction effect of row position \times soil depth on soil bulk density after corn harvest in fall 2019.

The row position \times soil depth interaction observed was mainly attributable to the difference in soil disturbance between sites plus the expected increase in soil bulk density related to the profile depth. The ST practice provides alternating strips of tilled and untilled soil that combined with continuous usage might create patterns of variable soil physical properties within relatively close areas across a field (Jabro et al., 2011). As a result, the practice also creates a constant movement of organic residues into the inter-row position, which may progressively cause differences in soil organic matter that will affect bulk density (Fernández et al., 2015).

Hence, it is possible that the 4-y ST practice implementation caused the lower bulk density observed at the inter-row position. In accordance, Boupai, (2017) evaluated bulk density in an experiment located adjacent to the field used in this study and indicated a higher value in the corn row position while a reversely lower value was found at the inter-row position from year 1 to 2, respectively. Additionally, Fernández et al. (2015) evaluated different soil properties in a field after five years of ST and observed significantly greater soil organic matter content in the inter-row versus the corn row position that could consequently influence soil bulk density. Furthermore, previous research also refers to the direct relationship between bulk density and depth attributable to lower organic matter, root density, and tillage impacts which serve to support increasing bulk density values for each row position at the 5- to 15-cm depth (Licht and Al-Kaisi, 2005; Jabro et al., 2011; Raphael et al., 2016).

Soil Total Carbon and Nitrogen

The organic input management approach used in this study permitted an assessment of the long-term effects of cover crops on the soil C and N stocks, which will consequently enhance soil quality and benefit the overall production system. There was a tendency from cover crop treatments to accumulate more soil C and N on a mass basis for the inter-row position at the 0- to 5-cm depth (Table 3.10). However, no significant differences were found by row position at $\alpha = 0.05$. Likewise, this lack of statistical difference between row positions was also observed for the 5- to 15-cm depth (Table 3.10). Consequently, a weighted average between row positions at each depth was calculated for both response variables and re-analyzed with a one-way ANOVA to properly determine treatment differences and respective mean separation with an LSD. Further, it was found that total soil C and N were significantly influenced by cover crop treatments only for the surface 0- to 5-cm depth ($P = 0.0441$ for total C and 0.0192 for total N, respectively; Table 3.9). In addition, no significant interactions were found between main effects for both sampling position and respective depths.

Table 3.9 Analysis of Variance of main and interactive effects on soil total C and N by mass basis at 0- to 5-cm and 5- to 15-cm after corn harvest in fall 2019.

Effect	Total C	Total N
	----- P > F -----	
	<u>0- to 5-cm</u>	
Treatment	0.0012	0.0005
Row position	0.1755	0.8874
Treatment × Row position	0.5626	0.5659
By depth†	0.0441	0.0192
	<u>5- to 15-cm</u>	
Treatment	0.7282	0.6616
Position	0.3829	0.1005
Treatment × Position	0.9752	0.9696
By depth†	0.9320	0.9099

† One-side ANOVA to determine treatment mean separation of row positions weighted average at a given depth.

For the 0- to 5-cm depth and averaged across row positions, the inclusion of rye tended to result in the greatest soil total C accumulation when coupled with a legume. More specifically, coupling rye with either crimson or berseem clovers resulted in a soil C increase of 20 and 17% or 1.17 and 1.12 Mg ha⁻¹ more than the winter fallow, respectively (Table 3.10). Nonetheless, Persian clover was the only legume that did not result in greater total C accumulation when mixed with this companion crop and did not differ from winter fallow at $\alpha = 0.05$. Despite the greater C stocks with crimson and berseem clovers when mixed with a rye non-legume, only the berseem biculture was significantly greater compared to its respective legume monoculture. More importantly, of the three legume monocultures only crimson clover resulted in significantly greater soil C compared to winter fallow, by accumulating 15% or 0.87 Mg C ha⁻¹ more (Table 3.10). Similar to rye, it was found that ‘Tillage Radish[®]’ tended to increase soil total C, although no differences were found with their respective legume monocultures or rye bicultures.

Moreover, legumes coupled with ‘Tillage Radish[®]’ resulted in significantly greater soil total C when compared to the winter fallow, except for berseem clover (Table 3.10).

Cover crop treatments influenced total soil N in a similar pattern to the results observed for total soil C for the 0- to 5-cm depth. The addition of rye into a mixture tended to cause the greatest increase in total soil N, except when coupled with Persian clover. Crimson and berseem clovers combined with this non-legume companion crop caused an average increase of 16% or 0.09 Mg N ha⁻¹ compared to the winter fallow check. Nonetheless, only the berseem clover biculture was different from its respective legume monoculture (Table 3.10). In relation to soil C, crimson clover was the only legume monoculture that resulted in a greater soil N accumulation when compared to the winter fallow check. More importantly, there were no differences between this legume monoculture and the highest yielding rye bicultures with respect to soil N (Table 3.10). Further, pairing ‘Tillage Radish[®]’ with a legume also resulted in greater total soil N when compared to the winter fallow, except for berseem clover. However, radish bicultures had a negligible difference with the rye bicultures and their greater soil N accumulation (Table 3.10).

As evidenced in this study, the individual and aggressive growth production trait of cereal rye tends to increase dry matter cover crop accumulation of bicultures. This specific characteristic in addition to a greater C:N results in this species having great potential for increasing soil organic matter in comparison to a conventional production system merely dependent on inorganic fertilizers (Hartwig and Ammon, 2002). Furthermore, greater rates of N immobilization for rye based cover crop systems as compared to other cover cropping systems suggests greater N sequestration in the soil rather than being rapidly available to a corn crop (Melkonian et al., 2017).

Table 3.10 Long-term assessment of soil total C and N by mass basis at 0- to 5-cm and 5- to 15-cm as influenced by cover crop treatment combinations in fall 2019 after corn harvest.

Treatment	Soil Total Carbon			Soil Total Nitrogen		
	Corn row	Inter-row	Avg [†]	Corn row	Inter-row	Avg [†]
	----- Mg ha ⁻¹ -----					
	<u>0- to 5-cm</u>					
Winter Fallow	6.16	5.80	5.95	0.59	0.56	0.57
Persian	6.16	6.68	6.48	0.61	0.64	0.63
Crimson	6.49	7.03	6.82	0.63	0.66	0.65
Berseem	6.09	6.25	6.19	0.58	0.59	0.59
Persian + Radish	6.78	6.77	6.77	0.66	0.63	0.65
Persian + Rye	6.01	6.15	6.10	0.58	0.58	0.58
Crimson + Radish	6.32	6.92	6.68	0.61	0.66	0.64
Crimson + Rye	6.80	7.32	7.12	0.65	0.69	0.67
Berseem + Radish	6.77	6.51	6.61	0.65	0.62	0.63
Berseem + Rye	7.07	6.90	6.96	0.68	0.64	0.66
LSD (0.05)			0.73			0.06
	<u>5- to 15-cm</u>					
Winter Fallow	9.51	9.27	9.36	0.95	0.93	0.94
Persian	9.24	9.30	9.27	0.95	0.94	0.94
Crimson	9.77	9.12	9.37	1.01	0.91	0.95
Berseem	8.76	9.03	8.93	0.90	0.89	0.90
Persian + Radish	9.86	9.51	9.65	1.00	0.95	0.97
Persian + Rye	9.25	9.14	9.19	0.93	0.91	0.92
Crimson + Radish	9.22	9.54	9.41	0.94	0.96	0.95
Crimson + Rye	9.68	9.61	9.64	0.98	0.96	0.97
Berseem + Radish	9.57	9.03	9.25	0.96	0.90	0.93
Berseem + Rye	9.87	9.51	9.65	1.00	0.95	0.97
LSD (0.05)			NS			NS

Avg[†] weighted average considering area covered by corn row and inter-row positions in a plot. NS means from main effect is not significantly different according to ANOVA.

Generally there has been reported that a strong positive relationship exists between the C input that a production system provides through its residue and long-term C and N soil sequestration (Mazzoncini et al., 2011). Therefore, the consistently greater residue added to the

soil with the addition of rye into a mixture can help to explain the greater soil C and N stocks for the surface 0- to 5-cm sampled depth following 4-y of cover cropping. Rye as a companion crop did not result in a synergistic response except when grown with Persian clover. This particular observation is attributable to the clear underperformance of Persian clover in terms of dry matter production throughout this study. More specifically, there was also an intrinsic effect during 2016 where *phytophthora sp.* infestation reduced hairy vetch dry matter accumulation prior to rotating to Persian clover from 2017 to 2019. Moreover, every year that Persian clover was included in the study it produced significantly less dry matter yield when compared to the average effect of the other two legumes. In fact, during 2017 an interaction between main effects was found showing a lower dry matter accumulation for Persian clover-rye bicultures. Thus, it is likely that in spite of the individual high dry matter input trait of rye, the lower dry matter complementarity offered from Persian clover resulted in lower soil total C and N accumulation compared to the other rye bicultures (Moore et al., 2014).

Among the legumes used each year; crimson clover was the only species evaluated during the entire duration of this study. Interestingly, this species demonstrated a consistently high dry matter accumulation either as a monoculture or as part of mixture/biculture every year. Hence, this may help to explain the non-significant difference for soil C and N between the crimson clover monoculture and the greatest yielding rye bicultures for the 0- to 5-cm soil depth. In agreement with these results, Carneiro et al. (2006) reported a greater soil organic C accumulation of $0.43 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ when using tropical legume cover crops compared to rye in a no-till corn production system. They related this quantifiable increase to the greater inputs of C and N from aboveground biomass and roots from the tropical legumes. Moreover, it is important to consider that red clover was grown during the first two experimental years and was later

substituted for by berseem clover. Hence, it is likely that the slower growth and development rate offered by red clover within this cover crop rotation resulted in less accumulation of soil total C and N as compared to its respective rye biculture, where the rye was productive each year of this study.

The enhanced effect on soil C and N sequestration observed when rye was coupled with crimson and berseem clovers is supported by the observations of Raphael et al. (2016) who noted the greatest total soil C and N stocks measured after a 10-y cover crop - soybean production system when grass cover crops were utilized. Moreover, Sainju et al. (2007) also indicated that adding rye as a companion crop to a three-specie legume blend increased soil organic C following 3-y of implementing the practice in a dryland cotton production system in central Georgia, U.S. It is important to consider that the successful storage of soil C and N will not entirely depend on the residue input, but also on the net C and N balance affected by mineralization-immobilization rates which in turn affect runoff, erosion, and leaching losses (Mazzoncini et al., 2011). Nonetheless, in the long-term it is likely that the increase in C and N stocks in the soil infers greater soil organic matter accumulation and enhancement of soil quality and possibly long-term crop productivity. For instance, Moore et al. (2014) showed following a 9-y assessment of a cereal rye cover crop in a corn silage - soybean rotation, that the species resulted in an increase of 15% in soil organic matter and 38% greater potentially mineralizable N compared to a winter fallow check.

Even though adding rye into a mixture resulted in a greater soil total C and N content, there were no significant differences between the rye and ‘Tillage Radish[®]’ bicultures. Moreover, the addition of radish appears to cause a consistent positive effect across legumes. However, coupling this non-legume with berseem clover did not significantly result in a

difference from winter fallow. This response may also be related to the lower dry matter and C input from having red clover in the cover crop rotation during the first two years of the study. It is possible that due to the growth nature of each nonlegume species and its competitiveness, there was a greater influence of the legume component towards biomass production within the radish bicultures than the rye bicultures (Samarappuli et al., 2014). This may help to explain the enhanced performance of berseem clover only when coupled with the rye companion crop. Nonetheless, this upward trend suggests that with time, the use of ‘Tillage Radish[®]’ may result in a significant increase in C and N sequestration across legume species (Jokela et al., 2009).

More importantly, this lack of differences suggests that despite lower dry matter and C input from radish as compared to the rye biculture, there was an equal soil C and N accumulation potential. This response is likely caused by the greater N availability from the ‘Tillage Radish[®]’ bicultures plus a residue input equal or even greater than the legume monocultures (Table 3.1). In the absence of inorganic fertilizers, soil organic matter may be increased by using species that accumulate more N. This increase may be observed in less labile fractions such as total C and N (Raphael et al., 2016).

Corn yield has been shown to be positively correlated to residue quantity produced by the crop following harvest (Fernández et al., 2015). Throughout the study, ‘Tillage Radish[®]’ resulted in greater corn grain yield compared to the antagonistic effect observed with the legume-rye bicultures. Hence, radish bicultures may have compensated their lower cover crop dry matter accumulation with the potential for greater corn residue inputs from greater N availability as compared to the legume-rye bicultures, although a lack of significant differences in total soil C and N was observed. In agreement, Carneiro et al. (2006) indicated that the greater C accumulation rates observed from topical legumes were attributable to the greater C and N inputs

plus the soil N availability improvement which caused an increase in corn biomass residue. However, Abdollahi and Munkholm, (2014) demonstrated that soil organic C and total N were not affected by 5-y of using fodder radish (*Raphanus Sativus* L.) as a cover crop monoculture within a barley production system. In fact, from production and soil health perspectives this study supports the synergistic advantage of utilizing a legume-radish biculture cover cropping system rather than their respective monocultures.

For the 0- to 5-cm soil depth, there was a trend showing greater soil C and N accumulation for the undisturbed inter-row sampling location in comparison to the corn row position across most treatments (Table 3.10). However, the trend had an opposite pattern for the 5- to 15-cm soil profile depth. This pattern was likely related to the lack of soil incorporation of the cover crop residues (Sainju, 2013). Thus, it is expected that the undisturbed inter-row position for the 0- to 5-cm depth would result in lower C and N mineralization compared to the ST corn-row, consequently causing a greater soil total C and N storage (Mazzoncini et al., 2011). It is important to consider that despite these tendencies, no significant differences were found between the row positions at both depths. Nonetheless, the pattern supports the significant differences observed for soil bulk density especially for the 0- to 5-cm depth where the undisturbed inter-row site was 15.7% lower in bulk density compared to the ST corn row. It is also likely that more than 4-y of this production system are required in order to confidently determine the long-term row position effect on total soil C and N accumulation at different depths. For example, Blanco-Canqui and Lal, (2008) did a long-term evaluation (i.e. data over 4-y) of 11 Major Land Resource Areas distributed among three U.S. States. The authors indicated an overall significant increase in soil organic C in undisturbed row sites for the surface 10-cm, but a lack of differences for 10- to 60-cm depth.

More importantly, the lack of a significant cover crop effect for the 5- to 15- cm depth may be attributable to residue accumulation at the surface combined with the lack of soil mixing where non-tilled systems tend to stratify soil organic matter (Halvorson et al., 1999). In accordance, other studies show that the greatest influence of cover crops on total soil C and N was within the 0- to 5-cm sampled depth (Blanco-Canqui and Lal, 2008; Hubbard et al., 2013; Sainju, 2013; Moore et al., 2014). In fact, wide enough differences may not be detected when the soil is sampled at a 0- to 15-cm depth and not divided and analyzed in smaller depth increments. Chu et al. (2017) indicated a nonsignificant effect from 4-y of continuous usage of cover crops on soil organic C when collecting samples for a single depth of 0- to 15-cm. In addition, the authors claimed a possible dilution of the differences in C levels of the surface layers by collecting samples at a single larger depth increment.

Soil total C and N concentrations prior initiation of the study were compared with data available from the soil assessment of 2019. Although there was a lack of statistical robustness, treatment differences were found in the soil C shift between years at $\alpha = 0.1$. Every treatment showed an overall trend of increasing total soil C concentration with an evident exception from the Persian-rye biculture and the unfertilized winter fallow check (Table 3.11). Nonetheless, an LSD treatment mean separation was not performed for the soil C shift between years. In contrast, a significant effect of cover crop treatments was found when evaluating the soil total N change between years ($P = 0.0484$; Table 3.11). Further, only both crimson clover bicultures and the berseem-rye mixture had greater total N concentration than winter fallow. These three treatments were not different between each other, accumulating on average 52% or 0.09 g kg^{-1} more N concentration than the unfertilized winter fallow (Table 3.11). No significant differences were found each individual year in either of the variables at $\alpha = 0.05$.

Table 3.11 Long-term assessment of soil total C and N concentrations for the 0- to 15-cm depth as influenced by cover crop treatments.

Treatment	Soil Total C			Soil Total N		
	2015	2019	Δ	2015	2019	Δ
	----- g kg ⁻¹ -----					
Winter Fallow	7.53	7.01	-0.52	0.53	0.69	0.16
Persian	7.16	7.25	0.09	0.53	0.72	0.20
Crimson	7.04	7.58	0.53	0.53	0.74	0.21
Berseem	7.02	7.01	-0.02	0.50	0.69	0.19
Persian + Radish	7.50	7.67	0.16	0.54	0.75	0.21
Persian + Rye	7.64	7.14	-0.50	0.54	0.70	0.16
Crimson + Radish	6.70	7.48	0.78	0.47	0.74	0.27
Crimson + Rye	7.02	7.72	0.70	0.50	0.76	0.25
Berseem + Radish	7.27	7.48	0.21	0.52	0.73	0.21
Berseem + Rye	7.16	7.78	0.63	0.53	0.76	0.23
LSD (0.05)	NS	NS	NS	NS	NS	0.06
	ANOVA					
	----- P > F -----					
Treatment	0.8286	0.4514	0.0794	0.6848	0.4263	0.0484

NS means from main effect is not significantly different according to ANOVA.

Results for total C and N concentration comparison between years are related to the differences observed when including the soil mass factor. Following 4-y of these cover crop systems, the increase in soil total C and N was analogous to the increase in C and N by mass quantified in the 2019 sampling (Table 3.10). Similarly, cover crop treatments that resulted in greater soil total N compared to winter fallow also show more accumulated N mass in 2019. It is also important to consider that concentration comparisons between different periods of time do not factor in possible changes in soil bulk density that might affect total C and N mass changes (Halvorson et al., 1999). For example, Sainju et al. (2008) showed that the inclusion of a rye cover crop did not influence soil total C and N concentration levels in a 10-y evaluation of intensively cropping systems. In addition, Chu et al. (2017) also found a similar effect by

reporting similar soil C concentrations of 10.7 and 10.9 g kg⁻¹ between 4-y of cover crop usage and the initial year baseline values, respectively.

The lack of detection of treatment differences ($P = 0.05$) for total soil C is likely related to the implied variability between row positions which was not considered in the 2015 sampling (Licht and Al-Kaisi, 2005; Jabro et al., 2011). As previously discussed, a single sampling layer depth may also decrease the probability of finding a significant cover crop effect in total C and N, especially in no-till, strip till, or other forms of reduced tillage systems. For example, Hubbard et al. (2013) indicated that 4-y of cover cropping practices under no-till increased C and N levels only in the top 2.5 cm of soil. In relation, there was no influence of cover crops on soil total C and N concentrations for 2019 (Table 3.11), while evident differences were found by depth when bulk density was factored in to calculate soil C and N mass (Table 3.10).

CHAPTER IV

CONCLUSIONS

Results from this study reveal valuable insight with respect to cover cropping utilizing individual legume species as well as grown with a companion non-legume species. These results offer valuable information to growers when making decisions on improving sustainability and enhancing soil fertility with an overall goal of improving main crop productivity. Tradeoffs observed from an input perspective showed that the inclusion of rye into a mixture resulted in the highest aboveground dry matter accumulation or 'residue biomass'. Nonetheless, 'Tillage Radish[®]' bicultures demonstrated an ability to scavenge residual soil N with an equal or superior effectiveness than rye bicultures, as reflected as a greater cover crop N content. Additionally, the 'Tillage Radish[®]'-legume association offers a greater N acquisition potential compared to legume monocultures which rely mostly on atmospheric N₂ fixation. This specific biculture was able to accumulate up to 87 kg ha⁻¹ of cover crop N in a production system without any supplemental inorganic N fertilizers.

Throughout the study, the inclusion of a rye companion crop resulted in a wider C:N ratio of the residues which likely increased the N immobilization rate. Thus, rye bicultures decreased corn N content and grain yield compared to the other cover cropping systems. In fact, during 2018 the dry matter and cover crop N from the mixture peaked, but conversely resulted in a lower corn N content and grain yield compared to the unfertilized winter fallow. However, the slower decomposition rate offered a prolonged soil surface cover during the corn season. In

contrast, ‘Tillage Radish[®]’- legume mixtures may synergistically deliver more N and increase corn grain yield compared to legume monocultures. The superior performance from this biculture occurred particularly in 2018, when prolonged cold temperatures resulted in winter kill of the radish component. Consequently, ‘Tillage Radish[®]’ bicultures caused a 10.6 and 1051 kg ha⁻¹ increase when compared to legume monocultures with respect to corn N content and grain yield.

With respect to the soil health assessment, bulk density was not significantly influenced by cover crops. More importantly, ‘Tillage Radish[®]’ inclusion resulted in equal soil total C and N stock within the 0- to 5-cm depth as compared to the rye bicultures which had a greater residue input. In comparison to rye bicultures, greater N availability from ‘Tillage Radish[®]’ increased corn biomass, thus compensating for lower cover crop residue input. Although biculture performance was also affected by the legume selection each year, crimson and berseem clover offer the best options from a cover crop and soil C and N stock perspective on alluvial soils that are prone to occasional flooding. The legume selection of 2018 which included Persian, crimson and berseem clovers appears to offer an equal complementarity component in relation to corn performance.

Variable environmental conditions affected yearly performances of the specific production systems, particularly due to substantial increases in rainfall both winter and early spring. Interestingly, these results suggest a legume - ‘Tillage Radish[®]’ biculture synergy can offer benefits to a high N demanding crop such as corn, while reports of this effect are limited to nonexistent. This species association offers a novel practice towards reducing N inputs and concomitant losses while improving soil quality. Future cover crop studies should evaluate the long-term effect (i.e. over 4-y) of this biculture to confidently assess performance in relation to

environmental effects including a wider range in soil properties. Also, deeper soil layers (i.e. 15- to 50-cm) must be examined to properly evaluate possible improvements in soil physical properties from winter crops as well as deep rooted ones.

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