

POTENTIAL FOR INTERSEEDED COVER CROPS IN A MAIZE CROPPING
SYSTEM IN THE U.S. UPPER MIDWEST

A Thesis
SUBMITTED TO THE FACULTY OF
UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

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August 2019

Acknowledgements

I am grateful to Dr. Axel Garcia y Garcia and Dr. Jeffrey A. Coulter for co-advising me. Similarly, I extend my thanks to the members of my Advisory Committee: Dr. Julie M. Grossman - who also advised me in a Sustainable Agriculture minor - Dr. Gregg A. Johnson, and Dr. Paul M. Porter. I value your concern for my success. Also, my thanks to the faculty of the College of Food, Agricultural and Natural Resources Sciences for instructing me and pushing me to grow intellectually and professionally.

Thank you to the technical staff for carrying out this applied research project. Thank you, Nathan Dalman, Alexis Giangrande, Lee Klossner, Bruce Potter and Travis Vollmer at the Southwestern Research, and Outreach Center (SWROC); Kara Anderson and Matt Bickell at the Southern ROC (SROC), and Dan Braaten at the North Central ROC (NCROC) for all your support at field and in the lab. A special thank you to Thomas Donelan, and the summer help for collecting field data and processing samples, including: Jeremiah Timm, Davis Harder, Jared Schroepfer, Adam Hass, Matt Wordes, and Noah Stavnes, Jackie Otway, Colton Hudelson, and Julie Anderson.

Thank you to the Minnesota Corn Research & Promotion Council for providing the funding for this project and the MnDRIVE Global Food Ventures Fellowship program for supporting me in carrying it out.

Finally, I wish to thank my family and friends for providing emotional support and encouragement throughout this process. Thank you for your unconditional care and bringing joy to my life.

Abstract

The incorporation of cover crops into the maize (*Zea mays* L.)-soybean [*Glycine max* (L.) Merr.] rotation in the U.S. upper Midwest may improve economic and environmental sustainability. For example, cover crops grown during the fallow period between maize harvest and planting can reduce the loss of essential plant nutrients like nitrogen (N) to ground and surface waters thereby reducing surface and subsurface quality. However, long, cold winters in the upper Midwest region make selection of successful cover crop species and associated management practices a challenge. This study was conducted to evaluate the establishment and growth of a variety of cover crop monocultures and mixtures across multiple environments and their effect on maize growth and yield and N fate in the cropping system. Two experiments were conducted in Grand Rapids, Lamberton, and Waseca, MN from fall 2016 through spring 2019 to examine whether six cover crop strategies interseeded into maize at the four- to six-leaf collar stage (spring-interseeded) and at physiological maturity (fall-interseeded) compromised maize growth or yield. Annual ryegrass (*Lolium multiflorum* L., AR) and cereal rye (*Secale cereale* L., CR) were evaluated as monocultures and in mixtures with crimson clover (*Trifolium incarnatum* L., CC) and forage radish (*Raphanus sativus* L., FR).

Differences in cover crop canopy cover and biomass were observed at Waseca in 2018. Greater accumulated growing degree days resulting from an earlier interseeding that year did not translate into increased cover crop canopy coverage or biomass of fall-interseeded cover crops compared with 2017. Differences in cover crop canopy cover and

biomass among spring-interseeded cover crop strategies were observed at fall frost at all locations in 2017 and at Grand Rapids in 2018. Cover crop canopy cover and biomass at cover crop spring termination, soil moisture at maize planting, maize aboveground biomass and yield were unaffected by the regrowth of fall-interseeded cover crop strategies with CR. Similarly, maize aboveground biomass or yield were not affected by spring-interseeded cover crop strategies. These results highlight the potential for a variety of cover crop strategies to be interseeded into maize without negatively influencing maize production in the U.S. upper Midwest. Next steps might include exploring the influence of the cover crop strategies on soybean production to identify the suitability and optimal placement of cover crops within the maize-soybean rotation.

High variability characterized the effect of cover crops interseeded into maize on N fate. Interseeded cover crops had no effect on soil $\text{NO}_3\text{-N}$ in a well-drained loam soil but were found to reduce soil $\text{NO}_3\text{-N}$ relative to no cover in both the 0-20 cm and 20-40 cm layers on moderately well drained and somewhat poorly drained clay loam soils. Fall-interseeded cover crops with CR reduced $\text{NO}_3\text{-N}$ in the soil solution at all three study locations. However, at Grand Rapids, differences in $\text{NO}_3\text{-N}$ concentrations may be due to porous soils and there appear to be thresholds of cover crop growth at Lamberton and Waseca below which cover crops do not reduce $\text{NO}_3\text{-N}$ concentrations in soil solution.

Highly variable cover crop N accumulation results make it unclear which cover crop strategy poses the greatest potential for immobilizing N at each location. At Grand Rapids, greater N accumulation occurred in spring-interseeded cover crops than in fall-interseeded cover crops likely because more growing degree days were accumulated

when cover crops were interseeded at four- to six-leaf collar stage maize. Cover crops with AR at Grand Rapids accumulated more N than those with CR when spring-interseeded, and the AR monoculture accumulated more N than mixtures with AR. This suggests that spring-interseeding of AR into maize may hold the most promise for Grand Rapids. At Lamberton and Waseca, spring- and fall-interseeded mixtures with AR + CC + FR and CR + CC + FR accumulated more N than both monocultures and mixtures of AR + CC and CR + CC. However, they did not always accumulate significantly more N than other treatments. Thus, the 3-species mixtures may be as effective as or better than other cover crop treatments for N scavenging at Lamberton and Waseca. Future work could examine increasing the seeding rate and using a drill to interseed cover crops at the four- to six-leaf collar stage to enhance the capacity of cover crops to provide N loss reduction services to the maize cropping system.

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List of Abbreviations

AR, annual ryegrass

ARCC, annual ryegrass + crimson clover

ARCCFR, annual ryegrass + crimson clover + forage radish

ARNC, no cover crop control for annual ryegrass-based treatment group

CR, cereal rye

CRCC, cereal rye + crimson clover

CRCCFR, cereal rye + crimson clover + forage radish

CRNC, no cover crop control for cereal rye-based treatment group

RM, relative maturity

GDD, growing degree days

DM, dry matter

V4-V6, four- to six-leaf collar stage of maize development

R5-R6, dent stage to physiologically mature maize

LTARN, (University of Minnesota) Long-term Agricultural Research Network

Introduction

Cover crops extend the period of living green cover on agricultural landscapes planted to annual crops (Lenhart et al., 2017). Integrating cover crops into the maize (*Zea mays* L.)-soybean [*Glycine max* (L.) Merr.] rotation in the U.S. upper Midwest is one strategy to reduce nitrogen (N) contributions to surface waters (Kladivko et al., 2014). Fifty-two percent of the N from agricultural sources delivered to the Gulf of Mexico from the Mississippi River Basin have been linked back to maize and soybean production in the U.S. (Alexander et al., 2008). Maize production in the U.S. upper Midwest region (Iowa, Minnesota and Wisconsin) is characterized by high yield, high external inputs, and an extended fallow period. In 2018, 9.5 million hectares of maize grain were harvested in the U.S. upper Midwest, representing over \$15 billion in production (USDA NASS, 2018). The productivity of maize cropping systems is partially attributed to adequate plant nutrition supplied by fertilization. Nitrogen fertilizer is applied to 97% of all cropland planted to maize in the region (USDA ERS, 2018). Delivered in plant-usable forms, synthetic N fertilizer is taken up by plants and incorporated into plant tissue. However, maize's limited N use efficiency of 37% prevents the plant from making use of all the N available in fertilizer (Cassman and Walters, 2002). As a summer annual crop, maize completes its life cycle within a single growing season during which time it actively takes up water and nutrients to support plant growth. Cold temperatures in the U.S. upper Midwest limit the maize growing season to late-April or early-May (planting) through October or November (harvest). After harvest, land lies fallow until planting the

following spring. During this time, excess N is increasingly vulnerable to being lost from the cropping system. Agricultural management practices such as cover crops can be used to prevent these losses from occurring to protect water resources.

Able to grow at a lower air temperature than maize, some cover crops can provide ground cover and make use of water and nutrients in the soil after maize harvest and before planting maize. Cereal rye (*Secale cereale* L., CR) has often been used as a cover crop in the U.S. upper Midwest because it tolerates air temperature as low as 4.4°C (Mirsky et al., 2009). Studies have demonstrated that a CR cover crop seeded following maize harvest established and provided environmental services including nitrate (NO₃-N) reduction in drainage water (Strock et al., 2004; Feyereisen et al., 2006; Martinez-Feria et al., 2016). However, cover crop establishment after maize harvest is challenged by diminishing daylight hours, declining air temperature, and the onset of frost. Most cover crop species are not winter-hardy in the U.S. upper Midwest and are terminated by killing frost, which prevents spring coverage. While this represents a missed opportunity for reducing N losses in springtime it can also be viewed as a benefit in terms of eliminating the need for cover crop management in spring (Noland et al., 2018). In conventional crop production practices, winter-hardy cover crops that regrow in spring, such as CR, are terminated by chemical and mechanical means days to weeks before planting the next cash crop (e.g. maize) to safeguard against yield penalties (Acharya et al., 2016). Thus, weather conditions and species selection determine the extent to which cover crops extend the duration of living green on the landscape.

Interseeding cover crops into maize before harvest has been proposed to increase the likelihood of cover crop establishment and growth. Different timings of cover crop interseeding relative to maize development and cover crop species have been explored to achieve the dual objectives of increasing cover crop biomass production without hampering maize yield (Wilson et al., 2013; Curran et al., 2018; Noland et al., 2018; Liu et al., 2019). In southeastern Minnesota, CR was aerially interseeded into maize at advanced reproductive stages and produced up to 0.51 Mg DM ha⁻¹ (Wilson et al., 2013). In the Mid-Atlantic region, researchers experimented with drill-interseeding annual ryegrass and legume cover crops into maize at early vegetative stages and suggested interseeding cover crops into maize between the four- and six-leaf collar stage (Curran et al., 2018). Novel winter oilseed crops like field pennycress (*Thlaspi arvense* L.) and winter camelina [*Camelina sativa* (L.) Crantz] and species with potential as interseeded winter annual cover crops have been used in Minnesota (Noland et al., 2018; Liu et al., 2019). Results from these studies suggest that a range of options exists to establish and grow a variety of cover crops in temperate environments.

This study combines aerial interseeding with underrepresented cover crop species in the U.S. upper Midwest to expand the range of cover crop options for maize producers in the region. Chapter 1 focuses on the effect of cover crops interseeded into maize at the four- to six-leaf collar stage and at physiological maturity on the growth and yield of maize. Chapter 2 examines the potential of cover crop strategies to scavenge residual N and the effect of cover crop N accumulation on soil, water, and maize N content.

1. Chapter 1 – Interseeding cover crops did not reduce maize production in the U.S. upper Midwest

1.1 Introduction

The maize (*Zea mays* L.)-soybean [*Glycine max* (L.) Merr.] rotation dominates agricultural production in the U.S. upper Midwest. This system is characterized by high external inputs, and an extended fallow period. During the fallow period between harvest and planting, soils are vulnerable to erosion and essential plant nutrients can be lost to ground and surface waters. Integrating cover crops into the maize-soybean rotation can help to prevent these losses to increase nutrient use efficiency.

Cover crops deliver multiple ecosystem services (Tribouillois et al., 2015), such as reduced nutrient leaching (Kladivko et al., 2014; Hanrahan et al., 2018) through nutrient uptake (Ranells and Wagger, 1997), reduced soil erosion (Kaspar and Singer, 2011), enhanced soil fertility (Sullivan et al., 1991) and water dynamics (Basche et al., 2016a), weed suppression (Hayden et al., 2014; Baraibar et al., 2018), and forage production (Landry et al., 2019). They are promoted as a best management practice to avoid water quality impairment (Lenhart et al., 2017) and as a soil management tool (Kaspar and Singer, 2011), but their adoption remains low (Dunn et al., 2016). In northern temperate climates, the period for cover crop establishment after maize harvest in October or November is limited by available heat units and daylight hours. However, interseeding cover crops into maize before harvest may enhance cover crop establishment and function.

Little is known about the potential for integrating cover crops into the maize-soybean rotation. In Minnesota, cover crops interseeded into maize at the seven-leaf collar stage reduced soil nitrate, thus reducing the potential for nitrate leaching, without reducing maize yield (Noland et al., 2018). Cover crops did, however, reduce soil water content in a dry season and reduced soybean yield when they were not adequately terminated. Another study in Minnesota found that cereal rye (*Secale cereale* L.) aerially interseeded into maize or soybean in mid-August to mid-September produced more than 50 kg ha⁻¹ of biomass in 40% of the instances observed (Wilson et al., 2013). Until more is known about the consequences of interseeding cover crops, the practice is unlikely to be widely adopted by maize producers.

Additionally, more information is needed on the viability of alternative cover crops for the region. Until recently, research on cover crops in the U.S upper Midwest focused on a few species. Cereal rye (CR) is among the most popular cover crops in the United States (CTIC, NCSARE, 2016). The literature on CR provides insight into the best timing for planting to maximize CR biomass (Feyereisen et al., 2006) and the best timing for termination to avoid allelopathic effects (Krueger et al., 2011) and establishment options (Wilson et al., 2013; Curran et al., 2018; Noland et al., 2018). There is also work underway to identify alternative cover crops such as winter-hardy legumes like hairy vetch (*Vicia villosa* Roth) and emerging oilseed crops like field pennycress (*Thlaspi arvense* L.) and winter camelina [*Camelina sativa* (L.) Crantz], which could be used as double purpose crops; cash crops in sequence- or relay-cropping

with maize or soybean (Berti et al., 2017; Ott et al., 2019) or cover crops (Liu et al., 2019).

This study aims to increase the knowledge of cover crop interseeding options for the upper Midwest USA. To this end, six cover crop strategies including CR and underrepresented cover crop species were interseeded into maize at an early vegetative stage (spring-interseeded) and at an advanced reproductive stage (fall-interseeding), with the expected termination for all except CR occurring at the time of first killing frost. The objectives were to: 1) compare the establishment and growth of spring- and fall-interseeded cover crops across multiple environments, 2) evaluate the effect of fall-interseeded CR regrowth in the springtime on soil moisture at maize planting, and 3) assess whether interseeded cover crops impact maize yield. The results of this study provide insight into possible outcomes of alternative cover cropping practices for maize-based cropping systems and additional management options.

1.2 Materials and Methods

1.2.1 Experimental sites

Two field experiments were conducted from fall 2016 through spring 2019. Experiment 1 involved interseeding cover crops at maize physiological maturity and was conducted within the Minnesota Long-Term Agricultural Research Network. Experiment 2 consisted of interseeding cover crops at the four- to six-leaf collar stage of maize. Both studies were conducted at the University of Minnesota Research and Outreach Centers in Grand Rapids (47°18'N, -93°53'W), Lamberton (44°24'N, -95°31'W), and Waseca

(44°06'N, -93°53'W), Minnesota, USA. These three locations span a range of soil types, precipitation, and weather gradients. Soils were a well-drained Nashwauk loam (fine-loamy, mixed, superactive, frigid Oxyaquic Glossudalfs) at Grand Rapids, a moderately well drained Normania clay loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls) at Lamberton, and a somewhat poorly drained Nicollet clay loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls) at Waseca. Average annual cumulative precipitation for the 1990-2015 long-term average period was 700 mm in Grand Rapids, 708 mm in Lamberton, and 922 mm in Waseca. For the same period, the average annual maximum air temperature was 8°C in Grand Rapids and 13°C in Lamberton and Waseca. Average annual minimum air temperature for the same period was -1°C in Grand Rapids, 1°C in Lamberton, and 2°C in Waseca.

1.2.2 Experimental design

Both experiments were a randomized complete block design with four replications, except for Experiment 1 in Grand Rapids, which had three replications. Plots in Experiment 1 were 3.0 m wide by 6.1 m long at all locations. Plot size in Experiment 2 was 3.0 m wide by 9.1 m long at Grand Rapids, 3.0 m wide by 8.8 m long at Lamberton, and 4.6 m wide by 8.5 m long at Waseca.

Treatments included six cover crop strategies or a no cover crop control. Two grass species -- annual ryegrass (*Lolium multiflorum* L., AR) and cereal rye (CR) -- were used in a monoculture and in mixtures of two and three species. The two-species mixtures consisted of a grass plus crimson clover (*Trifolium incarnatum* L., CC) and are denoted as ARCC and CRCC. The three-species mixtures included a grass, CC, and

forage radish (*Raphanus sativus* L., FR) and are denoted as ARCCFR and CRCCFR. A no cover crop control treatment was assigned to each grass species and are denoted as ARNC and CRNC. Cover crop species were selected based on functional traits (i.e., potential for N uptake and soil fertility improvement), phenological niche (i.e., winter hardiness), suitability for interseeding (i.e., shade tolerance), and seed availability. Only CR overwintered to regrow in the spring and required termination; AR, CC, and FR winter-killed thereby eliminating the need for spring management. Thus, findings related to spring termination refer only to CR at the 100% seeding rate (monoculture) or the 50% seeding rate (CRCC and CRCCFR). Seeding rates used in this study vary by cover crop strategy and can be found in Table 1.1.

1.2.3 Agronomic Management

All plots were strip-tilled one to 15 d before planting maize. Maize was planted into tilled strips at 86,000 seeds ha⁻¹ at a depth of 5 cm in 76-cm wide rows (Table 1.2). For Experiment 1 and 2, spring CR regrowth was terminated using 6 L ha⁻¹ of glyphosate [N-(phosphonomethyl)glycine] applied one to seven days before maize planting. Maize in Experiment 1 was a 76 RM hybrid (Pioneer P7632AM) at Grand Rapids and a 103 RM hybrid (DEKALB DKC53-56RIB) at Lamberton and Waseca. Maize in Experiment 2 was a 76 RM hybrid (Pioneer P762AM1) at Grand Rapids, a 107 RM hybrid (Pioneer P0157AMX) at Lamberton, and a 99 RM hybrid (DEKALB DKC49-72RIB) at Waseca.

Nitrogen fertilizer in Experiment 1 was broadcast applied at 73 kg N ha⁻¹ as urea ([CO(NH₂)₂]) within one week of maize planting with an additional 70 kg N ha⁻¹ as urea sidedressed at the six-leaf collar stage of maize. In Experiment 2 at Grand Rapids and

Waseca, 63 kg N ha⁻¹ as urea and 17 kg S ha⁻¹ as gypsum (calcium sulfate dihydrate) within one week of maize planting, and an additional 101 kg N ha⁻¹ as urea was sidedressed at the six-leaf collar stage of maize. In Lamberton, no fertilizer was applied at planting and 135 kg N ha⁻¹ was sidedressed as urea at the six-leaf collar stage of maize due to wet field conditions.

Weeds were controlled with a post-emergence herbicide approximately six weeks after maize planting. Weeds in Experiment 1 were treated with glufosinate {(RS)-2-Amino-4-(hydroxy(methyl)phosphonyl)butanoic acid} while glyphosate was applied in Experiment 2.

Cover crop seed was weighed by species in the lab and mixed at the field. Cover crops were manually broadcast at the four- to six-leaf collar stage of maize (mid- to late June) in Experiment 2 and at the kernel dough stage to physiological maturity of maize (mid-August and mid-September) in Experiment 1. Cover crops seed for Experiment 1 was lightly incorporated with a rake at all locations in 2017 and at Lamberton in 2018.

1.2.4 Data Collection

Cover crop GDD accumulation was calculated using the minimum base air temperature for CR of 4.4°C (Mirsky et al., 2009). In each year, GDD accumulation was calculated for 1 March through the first day of 0°C air temperature in the fall. For maize, a minimum base air temperature of 10°C was used to calculate GDD. Maize GDD accumulation began at planting and ended at harvest. The maximum air temperature for GDD calculation was 30°C for CR and maize.

Cover crop canopy cover and biomass was measured in the fall when freezing air temperature remained consistent for three days (mid- to late-October to early-November) and in the spring prior to termination of CR regrowth. A digital image was captured using the Canopeo phone application version 1.1.7 for Android (Patrignani and Ochsner, 2015) to estimate the percentage of living green cover within a 0.1-m² quadrat. Subsequently, all biomass within the quadrat was collected, placed in a brown paper bag, dried in a forced-air oven at 60°C until constant mass, and weighed.

Soil moisture was collected on 7- to 10-d intervals in all cereal rye-based strategies in Experiment 1. A factory-calibrated PR2 soil moisture probe with an HH2 handheld readout device (Delta-T Devices, Cambridge, UK) was inserted into an access tube installed in the center of each plot to measure soil moisture as a percentage of volume. Three measurements per depth were taken in each plot, and the average was used as a single value for each plot. Results from soil moisture in the top 30 cm of soil are presented in this study.

Three maize plants per plot were collected at physiological maturity. Maize was cut at 5 cm above the soil surface and ears were separated from stover to determine harvest index. Stover was chipped in the field using a chipper. Maize stover and ears were dried in a forced-air oven at 60°C until constant mass and weighed. Maize grain weight and moisture content was measured after maize physiological maturity by harvesting the center two rows of each plot using a small-plot combine. Grain yield was calculated at 155 g kg⁻¹ moisture.

1.2.5 Statistical analysis

Data were analyzed at $P < 0.05$ by analysis of variance with a linear mixed effects model using the *lmer* package (Bates et al., 2014) in the R statistical software environment (R Core Team, 2013). Location, year, and cover crop strategy were considered fixed effects, and replication was considered a random effect. For analysis of soil moisture, depth was considered a fixed effect. Spring-interseeded cover crop canopy cover and biomass at spring termination were analyzed separately by year due to no CR regrowth at Grand Rapids or Lamberton in 2019. When fixed effects were significant, means were compared with Tukey's honestly significant difference test at $P < 0.05$ using the *lsmeans* package in R (Lenth, 2016).

1.3 Results

1.3.1 Weather conditions

During the study period, spring precipitation at Grand Rapids was less than the long-term average while fall precipitation was greater. Average maximum air temperature at Grand Rapids was above the long-term average except fall 2018 and spring 2019 when temperature was 2°C and 4°C cooler, respectively. Average minimum air temperature was 4°C below the long-term average in spring 2019.

At Lamberton, except for the winter of 2017 and spring of 2018, all seasons in the study period were wetter than the long-term average. Average maximum and minimum air temperature in spring 2018 and 2019 were colder than the long-term average. Average maximum and minimum air temperatures in the fall were warmer than the long-term

average in 2016 and 2017, but cooler in 2018. Winter at Lambertton was warmer than the long-term average in 2016, and cooler in 2017 and 2018.

Spring was drier than the long-term average at Waseca in 2017 and 2018 and colder in 2018. Significant precipitation events occurred at Waseca in 2016 (104 mm on 11 August and 194 mm on 22 September; the latter led to failure of cover crops in Experiment 2) and 2018 (137 mm on 5 September). Fall 2017 at Waseca was 42 mm drier than the long-term average. Fall maximum and minimum air temperatures were 4°C warmer than the long-term average in 2016, average in 2017, and below average in 2018.

Precipitation fell within one to two days of seeding the spring-interseeded cover crops at all locations. Spring 2018 was wetter than spring 2017. Fall-interseeded cover crops at all locations received precipitation within one to three days of interseeding in 2017 and 2018 except for Grand Rapids in 2018, which did not receive precipitation until 11 d after interseeding.

Cover crop GDD accumulation varied among locations and years. At Grand Rapids, the spring-interseeded cover crops accumulated 1300-1400 GDDs from seeding to fall harvest, whereas at Lambertton and Waseca 400-500 GDDs more were accumulated (Table 1.3). Similarly, the fall-interseeded cover crops at Grand Rapids accumulated fewer GDD compared with Lambertton and Waseca. Interseeding cover crops approximately two-weeks earlier in fall 2018 resulted in an additional accumulation of 181, 228, and 199 GDD before fall harvest at Grand Rapids, Lambertton, and Waseca, respectively.

1.3.2 Fall-interseeded cover crop canopy cover and biomass

Fall-interseeded cover crop canopy cover was significantly influenced by cover crop strategy in the fall (Table 1.4). Location, year, and their interactions also affected fall-interseeded cover crop canopy cover in the fall. Cover crop strategy did not influence cover crop biomass, soil moisture at maize planting, or maize aboveground biomass or yield. Location and soil depth affected soil moisture at maize planting, and location, year, and their interaction drove differences in maize biomass and yield.

Cover crops were interseeded into maize at an earlier date in 2018, but greater GDD accumulation in 2018 did not translate into greater cover crop canopy cover or biomass. At all locations and for all fall-interseeded cover crop strategies, cover crop canopy cover in the fall was 35% or less in 2017 and 2018 (Figure 1.1). Fall-interseeded cover crop canopy cover was greatest at Lamberton fall 2017, whereas in 2018 Waseca had the greatest canopy cover. In both years, fall cover crop canopy cover was least at Grand Rapids. Differences in cover crop canopy cover among cover crop strategies were not significant both years of the study, except for at Waseca in 2018 when ARCCFR produced more canopy cover than CR or CRCC.

Mean cover crop biomass in the fall at Grand Rapids in 2017 was 0.076 Mg DM ha⁻¹ and significantly less (0.010 Mg DM ha⁻¹) than that in 2018. At Lamberton, mean cover crop biomass in the fall was 0.149 and 0.076 Mg ha⁻¹ in 2017 and 2018, respectively. Waseca had the least year-to-year variation in cover crop biomass in the fall, with 0.158 and 0.134 Mg DM ha⁻¹ in 2017 and 2018, respectively.

Cover crop canopy cover and biomass at spring termination consisted of CR regrowth from fall-interseeding the previous year. No differences in canopy cover at spring termination were detected among cover crop strategies despite the greater seeding rate of the CR monoculture versus the CRCC and CRCCFR mixtures (Table 1.5). Canopy cover in the spring was significantly greater in 2017 than 2018 at Lamberton and Waseca for all cover crop strategies, but not at Grand Rapids. Similarly, cover crop biomass in the spring was not affected by cover crop strategy but was greater in 2017 than 2018 at all locations.

1.3.3 Spring-interseeded cover crop canopy cover and biomass

Location, year, cover crop strategy, and their interactions influenced cover crop canopy cover and biomass in the fall (Table 1.6). Maize biomass and yield were influenced by location and year and the interaction of location by year. Cover crop canopy cover and biomass at spring termination were affected by location, and cover crop biomass was also influenced by year.

Wide variation within a location and between years was observed in spring-interseeded cover crop canopy cover in the fall (Figure 1.2). At all locations, cover crop canopy cover was greater in 2017 than 2018, though the difference in cover crop canopy cover was not always significant between years. Annual ryegrass strategies at Grand Rapids had more canopy cover than CR strategies in 2017 and 2018. At Lamberton in 2017, all cover crop strategies produced similar canopy cover except ARCC, which had greater canopy cover than CR. Annual ryegrass-based strategies had greater canopy cover than Cereal rye and CRCC in 2017. No differences between cover crops were observed at

Lamberton and Waseca in 2018. Except for ARCC, strategies with AR produced greater canopy cover than strategies with CR in 2018 at Grand Rapids.

Spring-interseeded cover crop biomass in the fall ranged from a low of 0 Mg DM ha⁻¹ with CR at Waseca in 2018 to a high of 1.57 Mg DM ha⁻¹ with AR at Grand Rapids in 2017 (Figure 1.2). At Grand Rapids, Lamberton, and Waseca, AR, ARCC, and ARCCFR most frequently produce more cover crop biomass in the fall of 2017 compared with CR-based strategies, Cereal rye and CRCC produced the least biomass in the fall of 2017 at all locations. No significant differences in fall cover crop biomass were observed between any cover crop strategy at any location in 2018.

Cereal rye regrowth of spring-interseeded cover crops the following spring was low at all locations in 2018 and did not grow at Lamberton in spring 2019. Canopy cover was less than 2.5% and biomass did not exceed 0.035 Mg DM ha⁻¹ at any location in 2018 or 2019. No differences in CR canopy cover ($P = 0.661$) or biomass ($P = 0.418$) were observed between CR, CRCC, or CRCCFR at Grand Rapids in 2018. Differences in both CR canopy cover ($P = 0.013$) and biomass ($P < 0.01$) were observed at Lamberton in 2018. Year, location, and their interaction did not affect CR canopy cover or biomass at Waseca in the spring of 2018 or 2019 (Table 1.7).

1.3.4 Soil moisture at maize planting

Compared with no cover, cover crops did not affect soil moisture at the time of maize planting, which occurred on the same day as cover crop termination or up to 10 d later. Location, year and soil depth influenced soil moisture at maize planting at Grand Rapids, Lamberton, and Waseca (Figure 1.3). The 10-20 cm soil layer had less moisture

than the 20-30 cm layer, except at maize planting at Grand Rapids in 2017 and Lamberton in 2018. Significant differences among all three soil layers occurred at Lamberton and Waseca in 2017 and at Grand Rapids in 2018.

1.3.5 Maize biomass and yield

Maize aboveground biomass at physiological maturity and grain yield were not affected by cover crop strategy at any location in either year but were affected by location and year. At Grand Rapids in 2017, mean maize biomass ($19.0 \text{ Mg DM ha}^{-1}$) and grain yield (9.02 Mg ha^{-1}) were less than in 2018 ($22.1 \text{ Mg DM ha}^{-1}$ and 9.95 Mg ha^{-1} , respectively). Conversely, at Waseca in 2017, mean maize biomass ($24.5 \text{ Mg DM ha}^{-1}$) and grain yield (12.4 Mg ha^{-1}) were greater than in 2018 ($20.8 \text{ Mg DM ha}^{-1}$ and 10.2 Mg ha^{-1} , respectively). At Lamberton, biomass and grain yield decreased from $25.6 \text{ Mg DM ha}^{-1}$ and 11.1 Mg ha^{-1} in 2017, respectively, to $22.9 \text{ Mg DM ha}^{-1}$ and 13.6 Mg ha^{-1} in 2018, respectively.

Maize aboveground biomass at physiological maturity was not affected by spring-interseeded cover crop strategy across all years and locations. Maize biomass was not affected by year at Grand Rapids ($26.7 \text{ Mg DM ha}^{-1}$ in 2017 and $27.4 \text{ Mg DM ha}^{-1}$ in 2018) or Waseca ($24.7 \text{ Mg DM ha}^{-1}$ in 2017 and $22.7 \text{ DM Mg ha}^{-1}$ in 2018). However, At Lamberton maize biomass was significantly less productive in 2017 ($18.2 \text{ Mg DM ha}^{-1}$) than 2018 ($22.3 \text{ Mg DM ha}^{-1}$).

Maize grain yield was not affected by cover crop strategy but it was affected by year at each location. Maize grain yield at Grand Rapids was greater in 2017 (11.8 Mg ha^{-1}) than 2018 (11.1 Mg ha^{-1}). At Waseca, maize yield was 11.6 Mg ha^{-1} in 2017 and

9.19 Mg ha⁻¹ in 2018. In contrast, maize yield from spring-interseeded cover crops at Lamberton was greater in 2018 (13.6 Mg ha⁻¹) than in 2017 (11.1 Mg ha⁻¹).

1.4 Discussion

1.4.1 Factors affecting cover crop canopy cover and biomass

In rainfed agriculture in temperate regions, the timing and amount of precipitation is a key factor determining the success of cover crops. Lack of precipitation within seven days after seeding cover crops can limit their establishment (Wilson et al., 2013). At the northernmost location of this study (Grand Rapids) in 2018, no precipitation was received until 11 d after fall-interseeding of cover crops. This may explain why fall-interseeded cover crop canopy cover and biomass was low. In contrast, excess precipitation resulting in temporary ponding may partially explain the loss of spring-interseeded cover crops at the southernmost locations (Lamberton and Waseca) in 2018. Spring-interseeded forage radish at Lamberton emerged rapidly in 2018 but disappeared by mid-July after repeated ponding. In total, 285 mm of precipitation occurred at Lamberton between 25 June and 24 July in 2018, 40% of which fell within one week of spring-interseeding cover crops. On 22 September 2016 at Waseca, 94 mm of precipitation resulted in the failure of the spring-interseeded cover crops. Above-average precipitation at Waseca in the spring and summer of 2018 led to ponding and limited biomass and canopy cover of spring-interseeded cover crops in the fall of 2018 and spring of 2019.

The combined effects of precipitation and temperature may also influence cover crop establishment and growth. It is reported that in a 41-yr period at Lamberton, favorable conditions (i.e., warmer-than-average air temperature and near-average

precipitation) for CR growth occurs in 25% of the years (Strock et al., 2004). The fall of 2016 was the only time in our three-year study in which these favorable conditions were observed. The canopy cover and biomass of CR in the spring of 2017 was greater than in the same period of 2018, which followed a fall in 2017 with greater precipitation but near-average air temperature at Grand Rapids and Lamberton, and drier conditions with near-average air temperature at Waseca. Additionally, late-spring snowfall and below-average spring air temperature in 2018 and 2019 may have contributed to the limited canopy cover and biomass of cover crops in the spring. In 2018, snowfall occurred on 14 April and 15 April at Lamberton and Waseca, respectively. Air temperature increased sharply thereafter, and maize was planted a short time later. A late-spring snowfall also occurred on 11 April 2019 at Lamberton (26 mm) and Waseca (31 mm) and temperatures remained below average throughout the spring 2019, limiting CR regrowth.

The wide variation in cover crop biomass observed from year to year in the present study has also been reported from other studies (Strock et al., 2004; Feyereisen et al., 2006; Wilson et al., 2013; Noland et al., 2018). With aerially-interseeded CR into maize in late-August to mid-September, timing that coincides with that in the present study, CR biomass in southeastern Minnesota was reported fall at 0.027 Mg ha^{-1} in 2009 and 0.506 kg ha^{-1} in 2010, nearly a 20-fold difference (Wilson et al., 2013). In the present study, fall-interseeded CR biomass decreased from 2017 to 2018 by approximately one-half at Waseca, two-fold at Lamberton, and nine-fold at Grand Rapids. Spring biomass of fall-interseeded CR was less than 0.5 Mg ha^{-1} at all locations in 2017 and 2018, which is

within or below the ranges reported from other studies in the region (Strock et al., 2004; De Bruin et al., 2005).

In addition to the year-to-year variation due to weather conditions, management practices may also influence cover crop success. From fall 2016 to fall 2017, the date of fall-interseeding cover crops advanced approximately 15 d, from early- to mid-September to late-August to early-September. Similarly, from fall 2017 to fall 2018, the date of cover crop interseeding advanced approximately 15 d, to early-to mid-August. Although the period to establish cover crops in fall 2016 was less than in fall 2017 or 2018, subsequent cover crop canopy cover and biomass at spring termination in 2017 was greater than 2018 or 2019.

Possible explanations of why more GDD did not result in greater fall-interseeded cover crop canopy cover and biomass might include prolonged exposure of cover crop seedlings to warm, low light conditions. Cereal rye biomass penalties have been predicted in models of earlier fall aerial interseeding dates in maize systems in the central and upper U.S. Midwest (Baker and Griffis, 2009). The fall-interseeded cover crops in our study were shaded by the maize canopy initially and then gradually received more light as maize senesced. At the same time, the daily hours of sunlight declined, reducing the amount of photosynthetically active radiation reaching the cover crops, limiting their vegetative growth. Although the cover crop species used in our study were deemed shade tolerant (Humphreys et al., 2003; Baron et al., 2011), low canopy cover and biomass production and etiolated cover crop growth observed suggest that the maize canopy may have limited growth.

The spring-interseeded cover crops in our study accumulated more GDDs prior to termination and were exposed to shading from maize for a longer time but produced greater canopy cover and biomass than fall-interseeded cover crops. The maize canopy had not yet closed when cover crops were interseeded in the spring but shading from maize increased soon afterward. In a study of aerially interseeded cover crops in a maize-pea (*Pisum sativum* L.) rotation in Canada, cover crops broadcast into maize at the four to six and 10 to 12 leaf collar stages successfully germinated and established but then stagnated and died under the maize canopy (Belfry and Van Eerd, 2016). While the cover crops in our study did not die, there were signs of stress from reduced light such as etiolation.

Where differences cover crop canopy cover or biomass were observed between cover crop strategies, those with AR produced the most canopy cover and those with CR produced the least canopy cover. Among spring- and fall-interseeded cover crop strategies at all locations and both years, the AR monoculture most often produced the greatest canopy cover and biomass, followed by ARCCFR and ARCC, in that order. However, differences between cover crop strategies with CR were not always significant but the CR monoculture was most often the lowest producing cover crop strategy for both spring- and fall-interseeded strategies. The latter suggests that a higher CR seeding rate did not result in greater cover crop canopy cover or biomass than other strategies. Similarly, other research has shown that a higher CR seeding rate did not reduce N leaching any more than mixtures with lower seeding rates (Kaye et al., 2019).

Cover crop strategies with CR did not result in differences in soil moisture at maize planting. This coincides with findings that increasingly diverse cover crop mixtures did not reduce soil moisture (Wortman et al., 2012). Despite below-average precipitation at all locations in spring 2017 and 2018 (except Lamberton in 2017), cover crop strategies did not affect soil moisture in the 0-30 cm soil layer at maize planting compared with the no cover crop treatment. This may be due to low springtime CR regrowth. It may also suggest that cover crops in the region can be used without negatively influencing soil moisture. In a Minnesota study of soil moisture in a forage maize system with a CR cover crop showed that soil moisture after CR terminated between 25 to 28 April was similar to the control (Krueger et al., 2011).

1.4.2 Cover crop effects on maize production

Spring- and fall-interseeded cover crops were not detrimental to maize biomass or grain yield. While the effect of cover crops on maize yield reported in the literature is variable, studies have found that cover crops may reduce maize yield. When yield penalties have been observed due to interseeded cover crops, weather (Abdin et al., 1998; Curran et al., 2018) and management (Belfry and Van Eerd, 2016; Noland et al., 2018) have been cited to explain yield decreases. The low cover crop productivity observed in the present study may have resulted in little to no competition between plant species and therefore had no effect on maize yield.

1.5 Conclusion

This study provides new insight into the potential of cover crop monocultures and mixtures and their effect on maize productivity in the U.S. upper Midwest. It highlights

the opportunity for broadcast interseeding cover crops in the spring at the four to six maize leaf collar stage. Spring-interseeded AR either as monoculture or mixtures (ARCC and ARCCFR) and fall-interseeded cover crop strategies with AR produced greater total cover crop canopy cover and biomass by fall frost than CR strategies in most cases. These findings suggest that AR may be an equally good or better option compared with CR in terms of producing canopy cover and biomass as a cover crop. However, AR winter kills, eliminating spring cover crop management before planting maize but also the opportunity to provide environmental services in the springtime.

Increased GDD due to early planting of fall-interseeded cover crops did not translate into greater cover crop establishment or growth in 2018. Conversely, spring-interseeded cover crops naturally accumulated more GDD thereby producing greater canopy cover and biomass than fall-planted cover crops in most cases. Additional research on the timing and method of cover crop interseeding, along with detailed information on corresponding field conditions, may lead to the identification of optimal interseeding times and potential tradeoffs of interseeding at different times during the growing season.

Our results show that interseeding cover crops into maize at the four to six leaf collar stage produced highly variable results but was not detrimental to maize production. Regrowth of fall-interseeded CR did not reduce soil moisture at maize planting or subsequent maize biomass and grain yield. Extending the study period or creating a controlled environment to observe the effects of the soil moisture level on cover crop

biomass on maize yield would garner additional insight into the impact of spring CR regrowth on maize productivity.

Future research may seek to understand the impact of the cover crop strategies explored herein on soybean production to provide valuable information about their suitability and optimal placement within the maize-soybean rotation. Enhanced knowledge of how and when to best manage interseeded cover crops in maize cropping systems may lead to greater soil cover and associated environmental, ecological, and management benefits during traditional fallow periods in the U.S. upper Midwest.

1.6 Tables and Figures

Table 1.1. Cover crop seeding rates in Experiments 1 and 2.

Cover crop	Monoculture		2-species mixture		3-species mixture	
	AR	CR	ARCC	CRCC	ARCCFR	CRCCFR
	Seeding rate (kg ha ⁻¹)					
Annual ryegrass	28	-	14	-	14	-
Cereal rye	-	67		33.5		33.5
Crimson clover	-	-	22	22	16.5	16.5
Forage radish	-	-	-	-	10	10

Table 1.2. Maize planting and harvest dates.

Experiment	Year	Maize planting			Maize harvest		
		Grand Rapids	Lamberton	Waseca	Grand Rapids	Lamberton	Waseca
1: Fall-interseeded cover crops	2016	15-May	30-Apr	29-Apr	25-Oct	28-Sep	16-Oct
	2017	10-May	8-May	24-Apr	9-Nov	30-Oct	1-Nov
	2018	22-May	16-May	7-May	5-Nov	20-Oct	27-Oct
2: Spring-interseeded cover crops	2016	-	19-May	-	-	21-Oct	-
	2017	10-May	12-May	5-May	9-Nov	24-Oct	29-Oct
	2018	22-May	19-May	7-May	5-Nov	18-Oct	28-Sep

Table 1.3. Cover crop seeding dates and accumulated growing degree days (GDD) from March 1 through spring harvest and from cover crop seeding to fall harvest.

Location	Year	Spring harvest	GDD	Seeding date	Fall harvest	GDD
Spring-interseeded cover crops						
Grand Rapids	2017	-	-	27-Jun	26-Oct	1376
	2018	15-May	205	26-Jun	13-Oct	1332
	2019	8-May	130	-	-	-
Lamberton	2017	28-Apr	204	15-Jun	25-Oct	1714
	2018	7-May	133	15-Jun	26-Oct	1802
	2019	15-May	241	-	-	-
Waseca	2017	-	-	14-Jun	30-Oct	1872
	2018	14-May	170	14-Jun	16-Oct	1781
	2019	26-Apr	147	-	-	-
Fall-interseeded cover crops						
Grand Rapids	2017	7-May	199	3-Sep	26-Oct	446
	2018	15-May	205	10-Aug	13-Oct	627
	2019	11-May	145	-	-	-
Lamberton	2017	21-Apr	171	31-Aug	25-Oct	504
	2018	7-May	133	14-Aug	27-Oct	732
	2019	15-May	241	-	-	-
Waseca	2017	21-Apr	229	4-Sep	30-Oct	560
	2018	14-May	170	13-Aug	16-Oct	759
	2019	28-May	368	-	-	-

Table 1.4. Significance of fixed effects of fall-interseeded cover crops in fall.

Source of fixed variation†	Fall frost		Spring termination		Soil moisture at maize planting	Maize above-ground biomass	Maize yield at 15.5% moisture
	Cover crop canopy cover	Cover crop biomass	Cover crop canopy cover	Cover crop biomass			
L	<0.01	<0.01	<0.01	0.63	<0.01	<0.01	<0.01
Y	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	0.03
C	<0.01	<0.01	0.23	0.15	0.14	0.76	0.63
D	-	-	-	-	<0.01	-	-
L x Y	<0.01	0.24	<0.01	0.26	<0.01	<0.01	<0.01
L x C	0.05	0.11	0.954	0.26	0.12	0.37	0.94
Y x C	0.79	0.68	0.27	0.24	0.87	0.26	0.97
L x D	-	-	-	-	<0.01	-	-
Y x D	-	-	-	-	0.97	-	-
C x D	-	-	-	-	0.52	-	-
L x Y x C	0.59	0.82	0.93	0.16	0.90	0.84	0.29
L x Y x D	-	-	-	-	0.01	-	-
L x C x D	-	-	-	-	0.49	-	-
Y x C x D	-	-	-	-	0.90	-	-
L x Y x C x D	-	-	-	-	0.33	-	-

Significance of fixed effects ($P > F$) for fall-interseeded cover crop canopy cover and biomass at fall frost and spring termination, soil moisture at maize planting, and maize aboveground biomass and yield response to six cover crop strategies interseeded into maize at Grand Rapids, Lamberton, and Waseca, MN in 2016-2018.

†L, location; Y, year; C, cover crop strategy; D, soil depth.

Table 1.5. Fall-interseeded cereal rye canopy cover and biomass at spring termination.

	Grand Rapids		Lamberton		Waseca	
	2017	2018	2017	2018	2017	2018
Living Canopy Cover (%)	4.43A	1.67A	26.30A [†]	6.09B	18.20A	17.10B
Cover Crop Biomass (Mg DM ha ⁻¹)	0.34A	0.03B	0.49A	0.01B	0.43A	0.03B

Table 1.6. Significance of fixed effects of spring-interseeded cover crops in fall.

Source of fixed variation [†]	Fall frost		Maize biomass	Maize grain yield
	Cover crop canopy cover	Cover crop biomass		
L	<0.01	<0.01	<0.01	<0.01
Y	<0.01	<0.01	0.0334	<0.01
C	<0.01	<0.01	0.977	0.198
L x Y	<0.01	0.195	<0.01	<0.01
L x C	<0.01	<0.01	0.702	0.351
Y x C	<0.01	<0.01	0.542	0.726
L x Y x C	<0.01	<0.01	0.439	0.0960

Significance of fixed effects for spring-interseeded cover crop canopy cover and biomass in the fall and maize aboveground biomass and grain yield at Grand Rapids, Lamberton, and Waseca, MN in 2016, 2017, and 2018

[†]L, location; Y, year; C, cover crop strategy

Table 1.7. Significance of spring-interseeded cover crops in spring.

Sources of variation [†]	Cover crop canopy cover	Cover crop biomass
Y	0.054	0.065
C	0.214	0.600
Y x C	0.270	0.708

Significance of fixed effects for spring-interseeded cover crop canopy cover and biomass at spring termination in response to six cover crop strategies interseeded into maize at Grand Rapids and Lambertton in 2018 and at Waseca in 2018 and 2019.

[†] Y, year; C, cover crop strategy

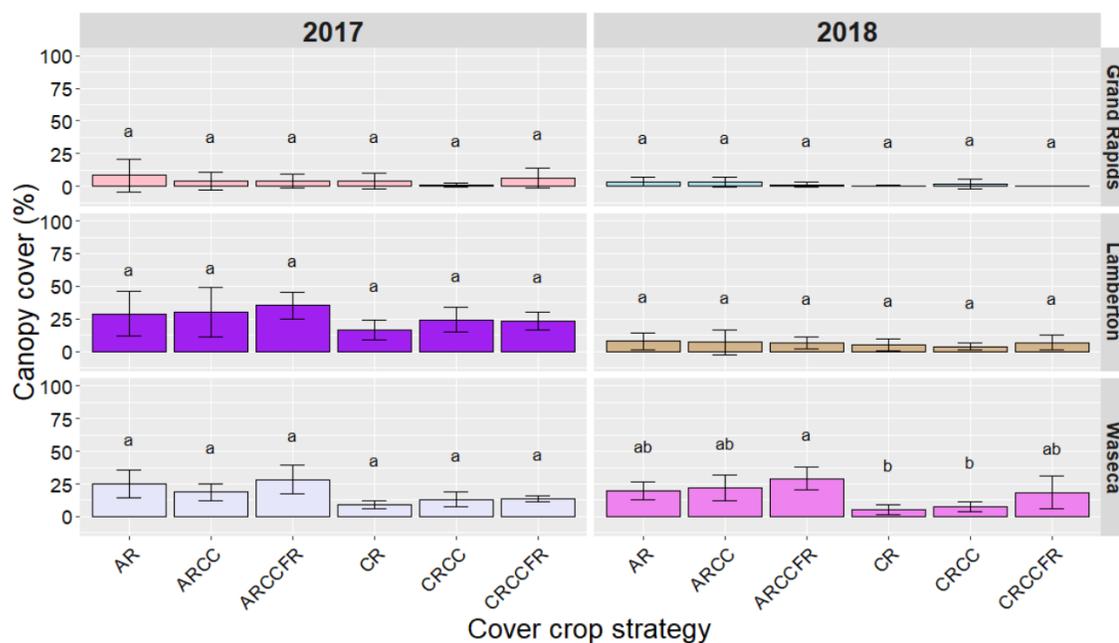


Figure 1.1. Fall-interseeded cover crop canopy cover in the fall. Different lowercase letters indicate means that are significantly different at $P < 0.05$. Error bars are standard errors of the mean.

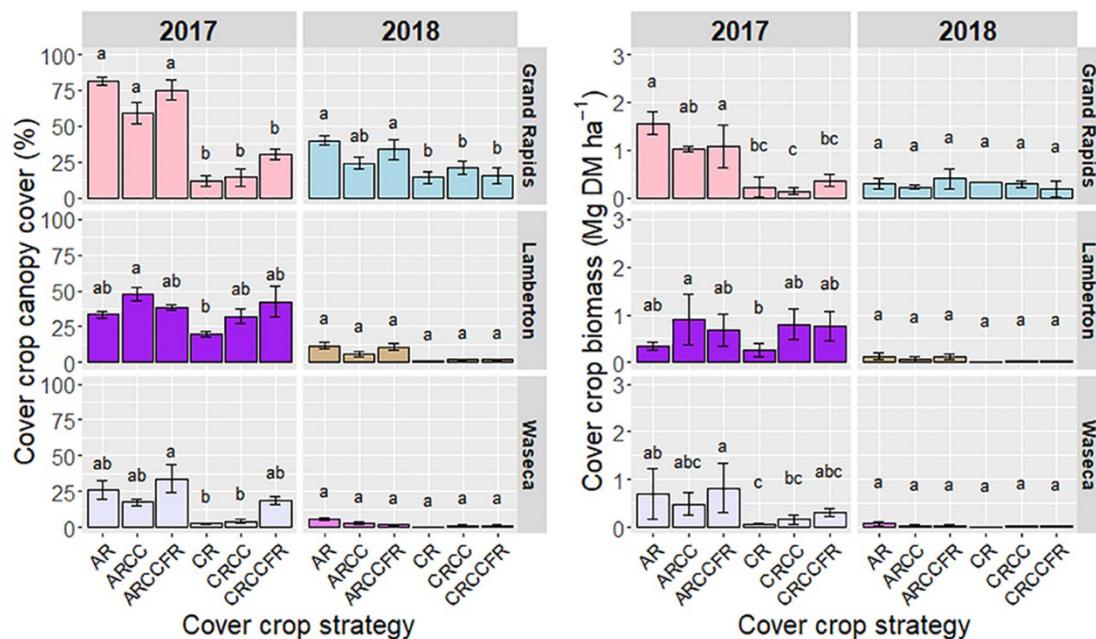


Figure 1.2. Spring-interseeded cover crop canopy cover and biomass in the fall. Spring-interseeded cover crop canopy cover (left) and biomass (right) at frost. Different lowercase letters over bars indicate significant difference at $P < 0.05$. Error bars represent two standard errors.

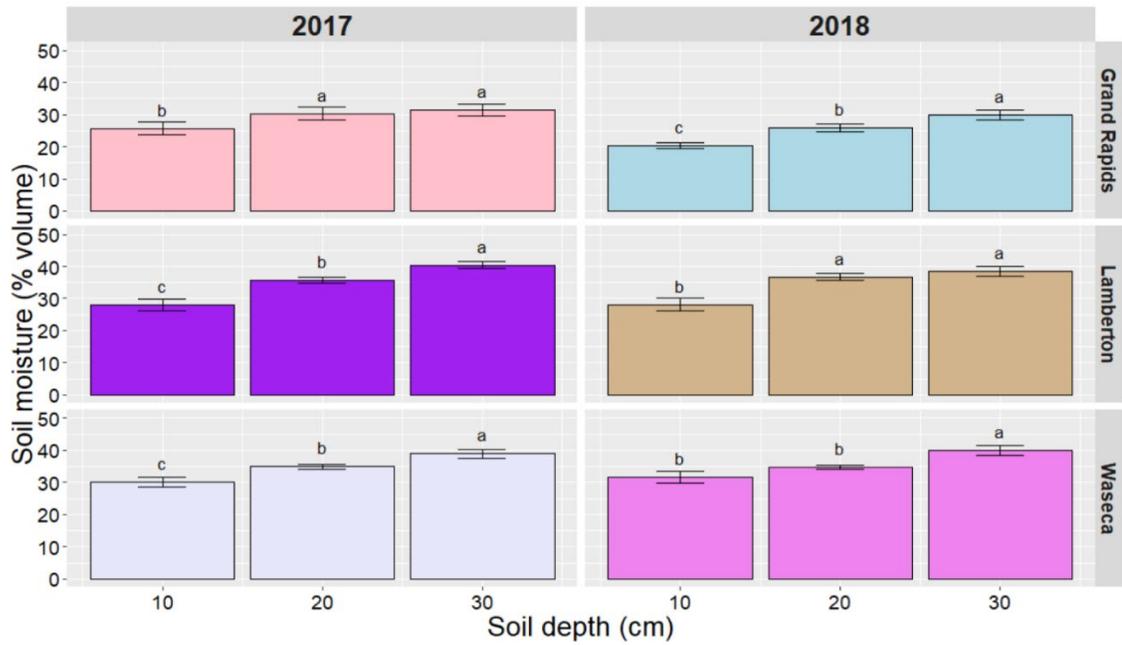


Figure 1.3. Soil moisture at maize planting. Mean soil moisture in the 0-10, 10-20, and 20-30 cm soil layers at maize planting after cereal rye cover crop termination in 2017 and 2018 at Grand Rapids, Lamberton, and Waseca. Different lowercase letters indicate means that are significantly different at $P < 0.05$. Error bars are standard errors of the mean.

2. Chapter 2 – Interseeded cover crops to manage nitrogen in a maize cropping system

2.1 Introduction

Surface and groundwater contamination have been linked to the accumulation of excess N from agricultural runoff (David et al., 2010; Kladvko et al., 2014). Estimates attribute 52% of the N from agricultural sources contributing to the Gulf of Mexico Hypoxic Zone to maize and soybean [*Glycine max* (L.) Merr.] grown in the United States (Alexander et al., 2008). Consequently, there is mounting pressure to improve nitrogen (N) use efficiency in maize (*Zea mays* L.) production systems. Nitrogen, an essential nutrient for plant growth that is often limited in nature, is supplemented with synthetic sources in conventional production to meet the high N requirement of maize. At the same time, maize has a limited N-fertilizer recovery efficiency estimated at 37% (Cassman and Walters, 2002). Residual N is vulnerable to loss through multiple pathways. Thus, while synthetic N fertilizer has resulted in an increase in agricultural productivity it has also been accompanied by a decline in water and air quality (Donner et al., 2004) and related social costs (Keeler et al., 2016).

Managing the balance between N supply and demand in maize presents several challenges. Although N is abundant in the atmosphere, it must be converted from its inert form (N_2) to a plant-usable form such as ammonium (NH_4^+) or nitrate (NO_3^-). Secondly, the rate of conversion is regulated by uncontrollable factors like precipitation and temperature. Thirdly, residual soil NO_3^- is soluble in water and can be assimilated by plants through uptake by the roots (Rhezali and Lahlali, 2017). In the absence of plant

roots, NO_3^- is vulnerable to surface runoff, denitrification in saturated soils, and leaching. Additionally, field conditions (e.g. soil temperature, adequate soil moisture, crop height) dictate the application timing, which does not align necessarily with maize demand. Despite the challenges, opportunities to improve N use efficiency of maize cropping systems, while maintaining yield, are being pursued.

Cover crops represent one approach to improving nitrogen use efficiency of maize production (Kaye et al., 2019). In annual cropping systems like maize, cover crops can extend the period of living green cover on the landscape through the bare fallow period. Nitrogen from the soil is immobilized by incorporation into cover crop tissue thereby reducing the supply of N in the soil profile that might otherwise be lost. Multiple services may be derived from cover crops such as reducing NO_3 -N leaching (Hanrahan et al., 2018) and soil erosion (Kaspar and Singer, 2011), producing forage (Drinkwater and Mcisaac, 2010), providing wildlife habitat (Wilcoxon et al., 2018), and recycling nutrients within the cropping system (Ranells and Wagger, 1996).

Among cover crops, cereal rye (*Secale cereale* L.; CR) is commonly employed to capture excess N in the soil (CTIC, NCSARE, 2016). It tolerates air temperature as low as 4.4°C (Mirsky et al., 2009), making it a viable candidate for both pre-planting and post-harvest growth in cool regions. Much cover crop research has focused on CR and established it as an effective N scavenger capable of reducing N losses through drainage water (Feyereisen et al., 2006; Kaspar et al., 2012; Malone et al., 2014). It has been shown to have a stabilizing effect during extreme weather events (Daigh et al., 2014; Basche et al., 2016b). Multiple potential tradeoffs deserve consideration when deciding to

adopt CR. For example, there remains some uncertainty regarding the outcomes of CR on maize yield (Krueger et al., 2012; Marcillo and Miguez, 2017; Snapp and Surapur, 2018), the potential effect of allelopathy (Raimbault et al., 1990), seedling disease (Acharya et al., 2016), and costs in terms of economic, time, and labor (Roesch-McNally et al., 2018).

Several strategies have been suggested to address these concerns. One way to reduce management costs is to select cover crop species that do not overwinter. These species provide fall plant cover while eliminating the need for springtime management, namely termination of a live cover crop, before planting maize. Experience with cover crop alternatives to CR is increasing along with interest in cover crop mixtures, yet experience with these systems remains limited in cool and wet regions (Kaye et al., 2019). Concerns about time management of field activities may be addressed by interseeding cover crops into maize. Some interseeding experience has been documented including aerially broadcasting CR into mature maize (Wilson et al., 2013), drilling different cover crop monocultures and mixtures into maize at the two- to four-leaf collar stage (Curran et al., 2018), and experimenting with different levels of soil disturbance at the seven-leaf collar stage (Noland et al., 2018). Drill interseeding cover crops at the two- to three-leaf collar stage was found to reduce maize yield in one study (Curran et al., 2018), while this and other studies showed that interseeding at the four- and seven-leaf collar stages did not reduce corn yield (Curran et al., 2018; Noland et al., 2018).

The present research sought to build on the existing knowledge of cover crop options for cool climates with rainfed agriculture. Four cover crop species, including CR and three winter-kill cover crop species - annual ryegrass (*Lolium multiflorum* L.; AR),

crimson clover (*Trifolium incarnatum* L.; CC), and forage radish (*Raphanus sativus* L.; FR) alone or in combination - were broadcast interseeded at the four to six-leaf collar (V4-V6) and at dent to physiological maturity (R5-R6) stages of maize development. This research attempts to address four main objectives: 1) assess if interseeded cover crops influence soil NO₃-N levels in the 0-20 and/or 20-40 cm soil layers, 2) evaluate if interseeded cover crops reduce NO₃-N in soil solution, 3) measure how much N interseeded cover crops use, and 4) determine if interseeded cover crops influence maize biomass and/or grain N content. To answer these questions, field data was collected at three locations in the U.S. upper Midwest spanning a range of soil types and weather gradients and analyzed to evaluate the ability of cover crops to contribute towards increasing the N use efficiency of maize cropping systems.

2.2 *Materials and Methods*

2.2.1 **Experimental sites**

Two cover crop interseeding experiments were conducted during 2016-2018 at the University of Minnesota Research and Outreach Centers in Grand Rapids (47°18'N, -93°53'W), Lamberton (44°24'N, -95°31'W), and Waseca (44°06'N, -93°53'W), Minnesota, USA. The first experiment was focused on fall-seeded cover cropping systems and conducted from fall 2016 through fall 2018 in a maize-soybean rotation. The experimental sites had been maintained since 2014 as part of the University of Minnesota's Long-Term Agricultural Research Network (LTARN). Cover crops were broadcast interseeded into maize at R5-R6 stages of development. Hereafter experiment 1 will be referred to as the fall-interseeded experiment. Given low cover crop growth in

2017, in 2018 fall-interseeded cover crops were seeded approximately 15 d earlier to increase the number of growing degree days available for cover crop growth. This resulted in interseeding into maize at milk to dough stages (R3-R4). The second experiment focused on spring interseeded cover crop systems and was conducted from fall 2016 at Lamberton and Waseca and spring 2017 at Grand Rapids to fall 2018. This experiment was conducted at a different field site than the first experiment at all three locations. Cover crops were broadcast interseeded into maize at the V4-V6 stages of development. Hereafter experiment 2 will be referred to as the spring-interseeded experiment. Table 2.1 provides a summary of soil types and weather conditions by location.

2.2.2 Experimental design

A randomized complete block design was used for both experiments. Each experiment at each site was replicated four times, except for the fall-interseeded experiment in the LTARN at Grand Rapids that had three replications. Fall-interseeded plots measured 3.0 m wide by 6.1 m long. Spring-interseeded plots varied from 3.0-m to 4.6-m wide by 8.5-m to 9.1-m long.

Four species were used as cover crops in different combinations. A monoculture of AR, a mixture of AR and CC (ARCC), and mixture of AR, CC, and FR (ARCCFR) provided fall coverage only as the three species winter-killed. A monoculture of CR, a mixture of CR and CC (CRCC), and a mixture of CR, CC, and FR (CRCCFR) provided fall and spring cover as a winter-hardy species that regrew the following spring, therefore requiring spring termination. Additionally, there were two no cover crop controls (ARNC

and CRNC). The AR monoculture seeding rate was 28 kg ha⁻¹ that was reduced to 14 kg ha⁻¹ in ARCC and ARCCFR. The CR monoculture seeding rate was 67 kg ha⁻¹ that was reduced to 33.5 kg ha⁻¹ in CRCC and CRCCFR. The CC seeding rate was 22 kg ha⁻¹ for ARCC and CRCC and reduced to 16.5 kg ha⁻¹ for ARCCFR and CRCCFR. The FR seeding rate was 10 kg ha⁻¹ in ARCCFR and CRCCFR. A hand rake was used to incorporate fall-interseeded cover crops into the soil at all locations in 2017 and at Lamberton in 2018.

2.2.3 Agronomic management

Strip tillage was applied to all plots 15 d before planting maize in 76-cm wide rows at 86,000 seeds ha⁻¹ and at a depth of 5 cm using a 4-row planter. Springtime CR regrowth was terminated using glyphosate [N-(phosphonomethyl)glycine] 1 to 7 d before maize planting (Table 2.2). Maize used in the fall-interseeded experiment was 76 RM hybrid (Pioneer P7632AM) at Grand Rapids and a 103 RM hybrid (DEKALB DKC53-56RIB) at Lamberton and Waseca. Maize used in the spring-interseeded experiment was a 76 RM hybrid (Pioneer P762AM1) at Grand Rapids, a 107 RM hybrid (Pioneer P0157AMX) at Lamberton, and a 99 RM hybrid (DEKALB DKC49-72RIB) at Waseca.

Plots in the LTARN received 73 kg N ha⁻¹ as urea ([CO(NH₂)₂]) broadcasted within one week of maize planting. At V6 maize, 70 kg N ha⁻¹ as urea were sidedressed. Spring-interseeded plots at Grand Rapids and Waseca were fertilized with 63 kg N ha⁻¹ as urea and 17 kg S ha⁻¹ as gypsum (calcium sulfate dihydrate) within one week of maize planting. An additional 101 kg N ha⁻¹ as urea was sidedressed at V6 maize. In Lamberton,

wet field conditions prevented fertilizer application at planting. However, 135 kg N ha⁻¹ were sidedressed as urea at V6 maize.

Weed control with a post-emergence herbicide occurred approximately six weeks after maize planting. Glufosinate {(RS)-2-Amino-4-(hydroxy(methyl)phosphonyl)butanoic acid} was used in the fall-interseeded experiment while glyphosate was applied in the spring-interseeded experiment.

2.2.4 Data collection

Before planting maize, a single 5 cm wide by 40 cm depth soil core was extracted from the center of each experimental plot using a Giddings probe (Giddings Machine Company Inc., Windsor, CO, USA) to determine soil NO₃-N content in the spring. Each soil core was divided into 0-20 cm and 20-40 cm layers. Soil cores were also collected in the fall to a depth of 30 cm using either a Giddings probe or three samples per plot using a hand probe before ground freezing except at Grand Rapids in 2017 where the ground froze before soil samples could be collected. Soil cores were divided into 0-15 cm and 15-30 cm layers and analyzed for NO₃-N content in the fall. All soil cores were air dried and ground with a soil crusher before chemical analysis was performed in the laboratory to determine residual soil NO₃-N content. For data analysis, 0-15 cm and 15-30 layers considered as representing 0-20 and 20-40 cm layers.

Weekly soil solution samples were collected from a ceramic suction cups (Soilmoisture Equipment Corp., Goleta, CA, USA) of outside diameter 48 mm and height 50 mm installed at 1-m depth in the middle of each plot receiving a treatment with CR in LTARN. Soil solution was extracted by applying 50-60 cbars vacuum 3-5 d prior to

sample collection. Approximately 1 mL of soil solution was collected in a plastic vial and frozen until laboratory testing. The vanadium reduction nitrate microplate procedure was used to determine NO₃-N in the soil solution (Doane and Horwath, 2003).

Cover crop biomass samples were collected after maize harvest and before killing frost (mid- to late-October to early-November) and in the spring prior to termination of CR regrowth. A single biomass sample from a random spot within the middle three rows of the experimental plot was collected using 0.1-m² quadrat. Biomass was dried in a forced-air oven at 60°C until constant mass and weighed. Cover crop biomass was ground using a CT193 Cyclotec Sample Mill (FOSS, Denmark). The N content of the cover crops was determined using an Elementar vario MACRO cube (Elementar-Straße, Langenselbold, Germany) in CNS mode.

Three maize plants per plot were collected at V4-V6 and R5-R6 stages of development. Maize samples at V4-V6 were cut at the soil and R5-R6 maize was cut 5 cm above the soil surface and ears were separated from stover. Stover was chipped with a Kemper chipper. Maize stover and ears were each dried in a forced-air dryer at 60°C until constant mass and weighed. Maize ears were shelled and cobs were weighed separately. Cobs and stover were each ground separately using a Model 4 Wiley laboratory mill with a 2-mm screen (Thomas Scientific, Swedesboro, NJ, USA), a subsample was combined and analyzed for CNS as described previously.

Maize grain N content was obtained after physiological maturity by harvesting the center two rows of each plot using a small-plot combine. Grain was air dried for 5-7 d. Then, two cups of grain were measured and placed in a forced-air dryer at 60°C for 24

hours. Once dry, 300 seeds were weighed and a subsample was ground using a 2-mm screen and analyzed for CNS as described previously.

2.2.5 Statistical analysis

Data were analyzed using R statistical software (R Core Team, 2013). A linear mixed effects model [*lmer* package, (Bates et al., 2014)] was used to compare cover crop treatments using analysis of variance at $P < 0.05$. For all analyses, cover crop treatment was considered as a fixed effect and replication was considered as a random effect. The analysis of soil $\text{NO}_3\text{-N}$ was conducted separately by depth such that the effect of cover crop treatment in the 0-20 cm soil layer was independent of any effects in the 20-40 cm soil layer. For the analysis of $\text{NO}_3\text{-N}$ concentration in soil solution, data was analyzed independently by season (spring, summer, and fall); location, year, and cover crop treatment were considered fixed effects. All other dependent variables were analyzed separately by location and year due to missing data and marginal cover crop growth, which varied by location and year. When fixed effects were significant, means were compared with Tukey's honestly significant difference test at $P < 0.05$ using the *lsmeans* package in R (Lenth, 2016).

2.3 Results

2.3.1 Residual soil $\text{NO}_3\text{-N}$

Significant differences in residual soil $\text{NO}_3\text{-N}$ among fall-interseeded cover crop treatments were observed at Lamberton and Waseca (Table 2.3). In spring 2017 at Lamberton in the 0-20 cm soil layer, less $\text{NO}_3\text{-N}$ was found in CR than ARCC and ARCCFR - which did not have any spring cover crop growth - and CRNC. In fall 2017 in

the 0-20 cm soil layer, ARCC had lower residual soil NO₃-N levels than CRNC, and in the 20-40 cm soil layer ARCCFR had lower levels than CRCC and CRNC. At Waseca in spring 2017, residual soil NO₃-N in the 20-40 cm was reduced in CR compared with ARNC. Similarly, in spring 2018 in the 20-40 cm soil layer lower soil NO₃-N levels were observed in CR and CRCC than in ARNC and CRCCFR. In spring 2018, CR had significantly less soil NO₃-N than all other treatments, except CRCC. Cover crops did not influence residual soil NO₃-N at Grand Rapids, likely because of marginal growth. At all locations and within a soil depth, residual soil NO₃-N was not affected by spring-interseeded cover crops (Table 2.4). A dramatic increase in residual soil NO₃-N was observed at Waseca from spring to fall 2018. From 8.08 kg N ha⁻¹ in the 0-20 cm soil layer in spring 2018, residual soil NO₃-N increased to 34.07 kg N ha⁻¹ in fall 2018. Similarly, residual soil NO₃-N in the 20-40 cm soil layer increased from 10.09 kg N ha⁻¹ in spring 2018 to 38.76 kg N ha in fall 2018. This large increase may be partially due to accelerated mineralization of cover crop and maize residue prompted by lodging and ponding in 2018.

2.3.2 Seasonal precipitation and NO₃-N in soil solution

Precipitation patterns varied by location and year. Spring (March-May) precipitation during the study period was below the long-term average for the period from 1990-2015 at Grand Rapids but fall (September-November) precipitation was above it. At Lamberton, there was more seasonal precipitation than the long-term average except in winter 2017 (December 2016-February 2017) and spring 2018. In 2017 and 2018 at Waseca, precipitation in spring was below the long-term average. In fall 2016

and 2018 at Waseca, 194 mm were recorded on 22 September and 137 mm were recorded on 5 September, respectively. These precipitation events appeared to have substantial effects on cover crops growth.

Seasonal NO₃-N concentration in the soil solution was collected only for fall-interseeded CR, CRCC, CRCCFR, and CRNC. The seasonal NO₃-N concentration in soil solution was affected by location, year, cover crop strategy and their interactions (Table 2.5). At Grand Rapids in fall 2017, CR had greater NO₃-N concentration in soil solution than CRCC, whereas in fall 2018 CRNC had the greatest concentration and CR and CRCC were significantly less (Table 2.6). Year-to-year variation was only significant in fall at Grand Rapids. At Lamberton, NO₃-N concentration in soil solution was significantly greater in CRNC than in CR, CRCC, and CRCCFR in spring and summer 2017. Comparing across years, lower NO₃-N concentration levels were reported in 2017. At Waseca, significantly lower NO₃-N concentration was observed in CR as compared to CRNC in spring 2017. Lower NO₃-N concentration levels were seen in summer and fall 2018 as compared to 2016 and 2017.

2.3.3 Cover crop N accumulation

Nitrogen accumulation in fall-interseeded cover crop biomass at the time of fall sampling was less than 20 kg ha⁻¹ in 2017 and 2018 at all locations (Table 2.7). Cover crop N accumulation at Grand Rapids was marginal both years, mainly because of little to no biomass either year. Averaged across treatments, cover crops at Grand Rapids accumulated 2.62 kg ha⁻¹ in 2017 and 0.61 kg ha⁻¹ in 2018. The greatest average fall-interseeded cover crop N accumulation was observed in fall of 2017 at Lamberton

(CRCCFR 18.15 kg ha⁻¹). In 2018, however, cover crop N accumulation at Lamberton did not exceed 4.76 kg ha⁻¹ (CRCCFR). Both CRCCFR and ARCCFR accumulated more N at Lamberton both years, though in 2018 N accumulation was similar among cover crop treatments. At Waseca, cover crop biomass N accumulation data was unavailable for analysis in fall 2017 due to misplaced samples nor in fall of 2018 due to poor cover crop establishment, limiting the collection of cover crops biomass and thus, N use was not determined. The ARCCFR treatment accumulated more N than other treatments in fall 2018 at Waseca.

The N accumulation of fall-interseeded CR-based cover crops was not affected by cover crop treatment at spring termination, before planting maize. At Grand Rapids in spring 2017, no differences among cover crop treatments were observed. Averaged across treatments, cover crop N accumulation at Grand Rapids in 2017 was 14.19 kg ha⁻¹. No data was available for analysis of cover crop N accumulation for spring 2018 at Grand Rapids or spring 2017 at Lamberton due to lost samples. In spring 2018 at Lamberton, no differences in the N accumulation of fall-interseeded cover crops at spring termination were observed; the pooled average of N accumulated among cover crops was rather marginal (0.54 kg ha⁻¹). Similarly, no differences in cover crops N accumulation were observed at Waseca in 2017 or 2018. With an average of 17.92 kg ha⁻¹ across all treatments in 2017, CR-based cover crops accumulated more N; however, this amount was reduced to 2.70 kg ha⁻¹ in spring 2018.

Spring-interseeded cover crop biomass at fall frost exhibited differences in N accumulation among cover crop treatments, except for fall 2018 at Grand Rapids and

Waseca (Figure 2.1). In fall 2017 at Grand Rapids, AR accumulated significantly more N than all other cover crop treatments except ARCC. At Lambertton in 2017, ARCCFR and CRCCFR accumulated more N than did monocultures, and in 2018, ARCCFR accumulated more N than all other cover crop treatments except for AR. At Waseca in 2017, ARCCFR accumulated significantly more N than CR and CRCC. Spring-interseeded cover crop N accumulation at fall frost in 2018 did not exceed 2.33 kg ha⁻¹.

Cereal rye regrowth of spring-interseeded cover crops was available only for Lambertton in spring 2017 as cover crops at Waseca did not survive in 2016. Spring-interseeding at Grand Rapids began in 2017, thus CR regrowth was only available in spring 2018 for this location. Nitrogen accumulation of CR regrowth was not affected by cover crops except at Lambertton in spring 2017 where CR accumulated 51.41 kg ha⁻¹, which was significantly more than CRCC (25.59 kg ha⁻¹) and CRCCFR (28.55 kg ha⁻¹). In spring 2018, however, N accumulation was marginal, averaging 2.01 kg ha⁻¹ (CR) or less at Lambertton, 0.45 kg ha⁻¹ (AR) or less at Waseca, and 0.57 kg ha⁻¹ (CRCC) or less at Grand Rapids.

2.3.4 N accumulation in maize biomass and grain

It was hypothesized that N accumulation at V4-V6 leaf collar stage of maize would be affected by CR-based cover crops due to CR regrowth in the spring. No differences between spring-interseeded cover crop were observed at any location either year. At Lambertton or Waseca in 2017 and 2018, fall-interseeded had no effect; however, at Grand Rapids in 2017, fall-interseeded ARCC had a significantly greater N concentration (4.61%) compared with ARNC (4.32%) and CRCCFR (4.10%).

The N content in maize R5-R6 biomass was not affected by fall-interseeded cover crop treatment at Grand Rapids but differences were observed at Lamberton and Waseca in 2017 and 2018 (Table 2.8). At Lamberton in 2017, ARCCFR followed by CRCC accumulated more N than CRCCFR and CRNC. In 2018, however, CRNC accumulated more N than CRCC. At Waseca, CRCC accumulated more N than all other treatments. Cereal rye and CRNC accumulated more than ARCC and ARCCFR, and CRCCFR accumulated more than ARNC.

Nitrogen accumulation in maize grain was unaffected by fall-interseeded cover crop strategies except at Grand Rapids in 2017 (Table 2.8). The CRNC treatment accumulated the most N while AR accumulated the least N. At Lamberton, mean separation test found no differences among cover crop treatments. Maize grain N accumulation across all cover crop strategies averaged 116 kg ha⁻¹ in 2017 and 128 kg ha⁻¹ in 2018. Data for 2017 maize grain was unavailable for Waseca due to misplaced samples; in 2018, no differences in maize grain N were observed among cover crop treatments, which averaged 96.48 kg ha⁻¹.

Spring-interseeded maize biomass and grain N content were not influenced by cover crop treatment (Table 2.9). Year-to-year variation was observed in mature maize biomass N content at Lamberton, with a tripling of mature maize biomass N content occurring from 2017 to 2018. Similarly, maize grain N content in 2018 was nearly twice the 2017 level.

2.4 Discussion

2.4.1 Residual soil NO₃-N

Cereal rye reduced soil NO₃-N in spring 2017 at Lamberton and at Waseca in 2018. The effectiveness of CR in scavenging NO₃-N is well documented (Feyereisen et al., 2006; Jewett, M.R. and Thelen, 2008; Kaspar et al., 2012; Snapp and Surapur, 2018). Lower reduction of soil NO₃-N reported in the present study may indicate low CR growth as compared to other studies (Krueger et al., 2011; Pantoja et al., 2015, 2016).

In spring 2017, ARCCFR followed by ARCC and CRNC – none of which exhibited any springtime growth – had significantly greater residual soil NO₃-N levels than CR at Lamberton in the 0-20 cm soil layer. Greater residual soil NO₃-N levels observed in ARCCFR and ARCC may be the result of rapid decomposition of winter-killed CC and FR. Crimson clover has been found to release more than 50% of its N contents within two to four weeks after termination (Parr et al., 2014). Similarly, FR was found to uptake more than 30 kg N ha⁻¹ of residual soil NO₃-N in the fall but winter decay made this NO₃-N vulnerable to spring leaching (Weyers et al., 2019). The effectiveness of FR in fall may explain why in 2017 at Lamberton in the 20-40 cm soil layer, ARCCFR reduced residual soil NO₃-N relative to CRCC.

2.4.2 NO₃-N concentration in soil solution

Lower levels of spring 2017 NO₃-N concentration in soil solution following fall-interseeded CR treatments at Lamberton and Waseca may be attributed to greater cover crop growth in spring 2017 as compared to spring 2018 (Rusch et al., unpublished). A late spring in 2018 prevented cover crop growth leaving NO₃-N in the soil vulnerable to leaching. Additionally, heavy snowfall in winter 2018 led to substantial snow melt and

soil moisture. Variable year-to-year reductions can be expected. Weather conditions favorable to CR and related NO₃-N leaching reductions were found to be one of four years in southwestern Minnesota (Strock et al., 2004).

A carry over effect from spring 2017 CR regrowth at Lamberton may possibly help to explain differences in NO₃-N concentration in the soil solution in summer 2017. The effectiveness of CR as an N scavenger paired with its slow breakdown may have prevented NO₃-N from cycling more quickly through the cropping system. Cereal rye retained 59% of its initial N 16 weeks after desiccation (Wagger, 1989). While this slow release of N back into the systems may prevent N loss, it may not synchronize with maize demand for N making an N input into the system necessary.

At Grand Rapids, reductions in NO₃-N concentration in the soil solution attributed to a cover crop with CR was only observed in fall 2018. Although cover crop N accumulation in fall 2018 was negligible, the NO₃-N concentration in the soil solution was at least 10 mg L⁻¹ less among cover crop treatments than the no cover crop control. This suggests that even a slight amount or a history of cover crop may be adequate to derive environmental benefits. However, cover crop treatments that produced greater biomass did not reduce NO₃-N concentrations in soil solution suggesting that differences may be due to coarse soils that produce highly variable soil NO₃-N results.

2.4.3 Cover crop N accumulation

Nitrogen accumulation of fall- and spring-interseeded cover crops at fall frost varied widely. Fall-interseeded cover crop N accumulation at fall frost ranged between 0.21 kg ha⁻¹ (ARCCFR at Grand Rapids in 2018) and 18.15 kg ha⁻¹ (CRCCFR at

Lamberton in 2017), which falls within the lower end of the 0.1-45 kg ha⁻¹ range observed by Wilson et al. (2013) at fall sampling of CR aerially broadcast at a seed rate of 112 kg ha⁻¹ into mature maize. None of the values for fall-interseeded cover crop biomass N accumulation observed in the present study, however, approached the 46.3 kg N ha⁻¹ or 32.5 kg N ha⁻¹ reported for a seed rate of 79-94 kg CR ha⁻¹ drill into maize residue after harvest at fall frost in mid-November in 2007 and 2008, respectively, in west central Minnesota (Krueger et al., 2011). The higher seeding rates in these studies may have played a role in the higher reported CR N accumulation.

Spring-interseeded cover crop biomass N accumulation by the time of fall frost ranged from 0.32 kg ha⁻¹ (CR at Lamberton in 2018) to 73.76 kg ha⁻¹ (CRNC at Lamberton in 2017). The lower end of this range coincides with the 0.3-2.6 kg ha⁻¹ range of N accumulation by fall for several cover crop treatments and three different seeding methods interseeded into maize at the seven-leaf collar stage in southern and southwestern Minnesota (Noland et al., 2018)). The difference in cover crop N accumulation at fall frost may be due to limited initial residual soil NO₃-N (Pantoja et al., 2016), cover crop establishment and growth, and cover crop species mixture. At Grand Rapids, cover crop establishment may have been limited by predation. Signs that birds may have been eating cover crop seed, such as feces on the soil between rows and up to 50% yield damage in some maize plots, were observed. A cooler and wetter than average fall in 2018 may also have limited cover crop growth.

Variable CR regrowth N accumulation was observed for both fall- and spring-interseeded cover crops at the time of spring termination. In 2017, fall-interseeded CR

regrowth at Grand Rapids and Waseca and spring-interseeded CR regrowth at Lamberton averaged between 14.19 to 35.18 kg N ha⁻¹, whereas CR regrowth did not surpass 1.56 kg N ha⁻¹ in 2018 for fall- or spring-interseeded cover crops. Year-to-year variation was also observed from 2002 to 2003 at Waseca and Rosemount, MN where drill-seeded CR regrowth seeded after the previous maize harvest averaged 14.2 kg N ha⁻¹ and 16.2 kg N ha⁻¹ in 2002, respectively, and decreased to 5.0 kg N ha⁻¹ and 5.7 kg N ha⁻¹ in 2003, respectively (De Bruin et al., 2005). Additionally, the fall-interseeded cover crop N accumulation at spring termination reported here for 2017 overlap with those for CR reported by others (De Bruin et al., 2005). Cereal rye drilled into maize residue in 2008 was found to accumulate 11 kg N ha⁻¹ in CR regrowth the following spring (Pantoja et al., 2015). Spring-interseeded cover crop N accumulation at Lamberton in 2017 exceeded these levels; however, in 2018 fall- and spring-interseeded cover crop N accumulation at all locations fell below these levels.

In the present study greater N accumulation in the three-species mixtures of both fall- and spring-interseeded cover crops at fall frost was observed. When grouped by grass-base, fall-interseeded ARCCFR accumulated more N than AR or ARCC at Lamberton in fall 2017 and at Waseca in 2018. Similarly, fall-interseeded CRCCFR accumulated more N than CR or CRCC at Grand Rapids and Lamberton in fall 2017 and at Waseca in 2018. The same was true for spring-interseeded ARCCFR and CRCCFR, except at Grand Rapids in 2017, when AR accumulated more than ARCCFR and at Waseca in fall 2018, where AR and CR accumulated the most. It must be noted that no spring-interseeded FR was found at Lamberton or Waseca at the time of biomass

sampling in fall 2018. Forage radish emerged shortly after interseeding at the four to six-leaf collar stage and was drowned by ponding from frequent and heavy precipitation events that occurred in spring and early summer 2018. The fact that mixtures with FR accumulated the most N suggests an important contribution of FR biomass. The productivity and stability of FR has been reported elsewhere (Wortman et al., 2012), and combined with the N scavenging ability of FR, it is perhaps not surprising that the three-species mixtures accumulate the most N when FR was present.

2.4.4 Maize biomass and grain N content

In most cases, no differences in maize biomass or grain N accumulation were observed between cover crop treatments and the no cover crop control. In an eight-year study of a CR cover crop in a corn-corn-soybean rotation in southwest Michigan, no differences in maize N were reported (Snapp and Surapur, 2018). Inconsistent differences were observed between fall-interseeded cover crop treatments in N accumulation of maize biomass and grain. In four to six-leaf collar stage maize at Grand Rapids in 2017, the significantly greater biomass N concentration of ARCC (4.61%) as compared to ARNC (4.32%) suggests strong AR and CC growth. The superior N concentration of ARCC as compared to CRCCFR (4.10%) may also suggest poor CR and especially FR growth. However, the lack of differences among most cover crop treatments makes it difficult to derive any clear understanding of the causes of the differences in four to six-leaf collar stage maize biomass N accumulation.

Few differences in maize biomass and grain N accumulation were observed among spring-interseeded cover crop treatments. Similar N concentration levels of maize

at V4-V6 across all cover crops suggests that spring regrowth of CR did not influence early maize growth. The difference in mature maize biomass N concentration between ARCC (0.68%) and CRCCFR (0.87%) observed at Grand Rapids in 2017 suggests poor CC growth in ARCC. A mixture containing CC, an N-fixing legume, would be expected to accumulate N in its tissue. The low N concentration of ARCC may be attributed to the fact that it contained half the amount of AR seed as the AR monoculture, and did not contain FR. The combination of a reduced grass seeding rate and the lack of a third species to compensate for the poor growth of CC may help explain this difference.

Year-to-year variation in crop growth is expected, thus the differences in spring-interseeded mature maize biomass and maize grain N content from 2017 to 2018 are not very surprising. However, the large differences observed in mature maize biomass and maize grain N content at Lamberton deserve some explanation. At Lamberton, mean aboveground maize biomass and grain yield were significantly less in 2017 than in 2018. Aboveground biomass was 18.2 Mg DM ha⁻¹ in 2017 versus 22.3 Mg DM ha⁻¹ in 2018. Maize grain yield was 11.1 Mg ha⁻¹ in 2017 and 13.6 Mg ha⁻¹ in 2018. Thus, the magnitude of plant material, not N concentration, primarily influenced differences in N content of mature maize biomass and grain N content.

2.5 Conclusion

Variability existed in the effect of fall- and spring-interseed cover crops on NO₃-N in the soil and soil solution, as well as in N accumulation by cover crops across three U.S. upper Midwest locations included in this study. Evidence of the ability of

cover crops to reduce the potential for N losses was observed, suggesting that cover crops may be a tool to improve N management in maize cropping systems. Interseeded cover crops had no effect on soil NO₃-N in a well-drained loam soil but were found to reduce soil NO₃-N relative to no cover in both the 0-20 cm and 20-40 cm layers on moderately well drained and somewhat poorly drained clay loam soils. Cereal rye-based fall-interseeded covers were effective in reducing NO₃-N in the soil solution at all three study locations. However, at Grand Rapids differences in NO₃-N concentrations may be due to coarse soils and thresholds of cover crop growth exist at Lamberton and Waseca below which cover crops do not reduce NO₃-N concentrations in soil solution.

Highly variable cover crop N accumulation results make it unclear which cover crop treatments pose the greatest potential for each location. At Grand Rapids, the northernmost location, greater N accumulation occurred in spring-interseeded cover crops than in fall-interseeded. This is likely due to the greater number of GDD available to cover crops established at V4-V6 maize as compared with R5-R6. Annual ryegrass-based cover crops at Grand Rapids accumulated more N than CR-based cover crops when interseeded at V4-V6, and AR accumulated more than mixtures. Thus, deriving from the results of this study, interseeding AR into V4-V6 maize at Grand Rapids may be the best option for improving the N use efficiency of maize cropping systems. At Lamberton and Waseca, spring- and fall-interseeded ARCCFR and CRCCFR accumulated more N than monocultures and 2-species mixtures of cover crops. However, they did not always accumulate significantly more N than other treatments and when differences did arise they were inconsistent making it challenging to derive any clear trends from the data.

Spring-interseeded cover crops did not affect mature maize biomass and grain N content. This may encourage additional experimentation with V4-V6 interseeding. Fall-interseeded cover crops, however, were associated with differences in maize biomass N maize grain N.

Given the high variability reported here and the short duration of the project, more research is needed to adequately address the questions posed here. To ensure greater cover crop success, future work could examine increasing the seeding rate and drill-interseeding cover crops. We conclude that interseeding could occur at V4-V6 leaf collar stage to enhance the capacity of cover crops to provide N loss reduction services to the maize cropping system.

2.6 Tables and Figures

Table 2.1. Soil description and weather conditions based on the long-term average for the 1990-2015 period for each of the three experiment locations in Minnesota, USA.

Town	Soil				Weather		
	Taxonomic class	Series	OM (%)	Drainage	Annual cumulative precipitation (mm)	Maximum temperature (°C)	Minimum temperature (°C)
Grand Rapids	fine-loamy, mixed, superactive, frigid Oxyaquic Glossudalfs	Nashwauk loam	2.3	Well drained	700	8	-1
Lamberton	fine-loamy, mixed, superactive, mesic Aquic Hapludolls	Normania clay loam	4.5	Moderately well drained	708	13	1
Waseca	fine-loamy, mixed, superactive, mesic Aquic Hapludolls	Nicollet clay loam	5.6	Somewhat poorly drained	922	13	2

Table 2.2. Schedule of cover crop and maize seeding and harvest dates for fall- and spring-interseeding experiments.

<i>Year</i>	<i>Activity</i>	<i>Fall-interseeded</i>			<i>Spring-interseeded</i>		
		Grand Rapids	Lamberton	Waseca	Grand Rapids	Lamberton	Waseca
2017	Cover crop harvest	7-May	21-Apr	21-Apr	-	28-Apr	-
	Cover crop termination	10-May	29-Apr	23-Apr	-	4-May	-
	Maize planting	10-May	8-May	24-Apr	10-May	12-May	5-May
	Cover crop interseeding	3-Sep	31-Aug	4-Sep	27-Jun	15-Jun	14-Jun
	Maize harvest	26-Oct	25-Oct	30-Oct	9-Nov	24-Oct	29-Oct
	Cover crop harvest	9-Nov	30-Oct	1-Nov	26-Oct	26-Oct	30-Oct
2018	Cover crop harvest	22-May	16-May	7-May	15-May	7-May	14-May
	Cover crop termination	22-May	8-May	10-May	22-May	8-May	17-May
	Maize planting	22-May	16-May	7-May	22-May	19-May	7-May
	Cover crop interseeding	10-Aug	14-Aug	13-Aug	26-Jun	15-Jun	14-Jun
	Maize harvest	13-Oct	26-Oct	16-Oct	5-Nov	18-Oct	29-Sep
	Cover crop harvest	5-Nov	20-Oct	27-Oct	13-Oct	26-Oct	16-Oct

Table 2.3. Soil NO₃-N in the 0-20 cm and 20-40 cm soil layers for fall-interseeded cover crop plots at spring soil sampling in 2018 after ground thaw and in fall of 2017 and 2018 before ground freezing. Significant differences were determined at $P < 0.05$.

Soil depth (cm)	2017				2018			
	Spring		Fall		Spring		Fall	
	0-20	20-40	0-20	20-40	0-20	20-40	0-20	20-40
	<i>Soil NO₃-N (kg ha⁻¹)</i>							
<i>Grand Rapids</i>								
AR [§]	11.18	13.80	-	-	8.69	5.26	9.2	8.18
ARCC	8.34	13.04	-	-	13.33	9.93	7.22	9.25
ARCCFR	8.43	14.02	-	-	13.93	5.45	6.88	11.9
ARNC	9.89	15.28	-	-	23.91	18.59	6.36	8.57
CR	13.58	11.10	-	-	16.17	5.74	6.02	9.93
CRCC	12.13	13.82	-	-	15.31	11.49	6.97	9.54
CRCCFR	9.63	9.25	-	-	10.92	13.33	7.31	9.15
CRNC	10.06	14.89	-	-	14.45	6.03	7.74	9.44
<i>Lamberton</i>								
AR	15.27ab [‡]	8.22	5.43ab	5.43ab	6.96	6.57	8.13	3.64
ARCC	15.73a	7.13	3.88b	4.11ab	8.45	7.28	9.62	4.03
ARCCFR	16.9a	6.67	5.43ab	3.1b	7.28	7.41	10.7	4.1
ARNC	15.19ab	8.91	7.36ab	4.88ab	7.93	8.13	12.00	4.03
CR	8.68b	3.95	8.06ab	5.12ab	6.31	7.74	10.7	5.07
CRCC	8.22b	4.88	10.4ab	7.98a	8.71	7.02	12	4.75
CRCCFR	10.77ab	5.27	8.6ab	5.35ab	5.72	7.15	11.1	4.81
CRNC	15.42a	8.68	11.5a	11.1a	6.70	5.98	10.1	4.29
<i>Waseca</i>								
AR	7.09	7.74ab	5.98	7.15	3.58	7.49ab	10.94	11.09
ARCC	8.19	6.76ab	6.31	7.74	4.25	7.13ab	11	10.73
ARCCFR	7.61	7.41ab	5.85	5.85	4.79	6.84ab	11.07	12.31
ARNC	6.44	11.2a	8.97	6.96	4.46	8.78a	13.3	14.04
CR	5.59	4.88b	9.23	7.22	3.51	5.9c	14.31	13.39
CRCC	6.44	6.57ab	8.71	6.24	3.51	6.12bc	13.84	11.81
CRCCFR	8.13	8.51ab	7.21	6.31	4.25	9.14a	13.97	17.06
CRNC	8.58	6.76ab	7.74	5.98	4.86	8.42ab	14.78	16.13

[§] Annual ryegrass, AR; crimson clover, CC; forage radish, FR; no cover crop control, NC; cereal rye, CR

[‡] Within a location and year, values followed by a different lowercase letter are significantly different at $P < 0.05$.

Table 2.4. Mean residual soil NO₃-N in the 0-20 cm and 20-40 cm soil layers for spring-interseeded cover crops. Values after the ± are standard deviations.

Soil depth (cm)	2017		2018			
	Fall		Spring		Fall	
	0-20	20-40	0-20	20-40	0-20	20-40
	<i>Soil NO₃-N (kg ha⁻¹)</i>					
Grand Rapids	-	-	11.99 ± 5.83	10.88 ± 10.39	10.38 ± 2.52	9.79 ± 3.76
Lamberton	8.55 ± 4.05	3.93 ± 1.72	13.04 ± 4.50	5.75 ± 2.05	9.18 ± 2.89	10.37 ± 3.74
Waseca	6.29 ± 3.17	6.23 ± 3.17	8.08 ± 2.77	10.09 ± 10.09	34.07 ± 20.32	38.76 ± 29.09

Table 2.5. Significance of fixed effects for NO₃-N in soil solution in response to six cover crop strategies fall-interseeded into maize at Grand Rapids, Lamberton, and Waseca, MN in 2016, 2017, and 2018.

Source of variation [§]	Spring	Summer	Fall
Location (L)	<0.01	<0.01	<0.01
Year (Y)	<0.01	0.08	<0.01
Cover Crop (C)	<0.01	<0.01	<0.01
L x Y	<0.01	<0.01	<0.01
L x C	<0.01	<0.01	0.6
Y x C	<0.01	<0.01	<0.01
L x Y x C	<0.01	<0.01	<0.01

Table 2.6. The concentration of NO₃-N in soil solution in fall-interseeded cover crop plots receiving a treatment with cereal rye for spring (March-May), summer (June-August), and fall (September-November). Values after the ± are standard deviations.

Strategy [§]	Grand Rapids			Lamberton			Waseca		
	2016	2017	2018	2016	2017	2018	2016	2017	2018
<i>NO₃-N concentration (mg L⁻¹) in soil solution</i>									
<i>Spring</i>									
CR	-	10.5±6.4	8.2±4.1	-	1.8b [†] B±2.3	5.3A±1.9	-	1.5bB ±1.6	5.4A±0.5
CRCC	-	5.5±1.6	8.0±4.5	-	3.2bB ±3.8	6.9A±2.1	-	2.4abA±2.5	4.0A±0.2
CRCCFR	-	14.8±4.6	8.8±4.3	-	2.1bB ±2.1	6.5A±2.7	-	2.8abA±2.2	4.8A±0.4
CRNC	-	12.7±4.1	12.4±8.2	-	8.9aA ±3.6	7.1A±1.6	-	3.6aA ±1.9	3.9A±1.0
<i>Summer</i>									
CR	-	10.6±3.9	9.8 ± 8.6	-	3.7bB±3.5	10.2A±6.6	-	3.7A±2.8	4.2A±3.8
CRCC	-	6.6±3.8	9.8 ± 6.5	-	7.1bA±5.8	11.0A±5.9	-	4.2A±3.1	3.9A±3.2
CRCCFR	-	9.4±5.2	11.8 ± 7.7	-	3.3bB±3.3	10.3A±5.3	-	5.3A±3.8	3.0B±2.5
CRNC	-	9.3±4.1	15.9 ±12.1	-	16.8aA±8.4	11.5A±5.2	-	6.3A±3.8	3.2B±3.3
<i>Fall</i>									
CR	10.1aB [†] ± 4.8	10.9aA ±4.4	13.4bA ±12.6	7.9A±3.6	0.6B±0.9	6.0A±5.0	1.7AB±1.9	2.1A±1.6	0.3B±2.9
CRCC	12.9aA ± 6.0	5.6bB ±3.8	9.4bAB ±10.8	8.9A±3.5	0.4B±0.8	5.5A±4.6	0.9AB±0.9	1.7A±1.5	0.1B±3.3
CRCCFR	12.2aA ± 6.5	9.5abA±5.8	13.9abA± 8.5	6.3A±4.9	1.3B±2.1	8.7A±2.7	1.6AB±2.2	3.2A±2.3	0.3B±2.8
CRNC	13.6aAB±10.6	7.9abB±4.6	23.1aA ±15.2	7.4A±4.1	2.5B±1.6	9.4A±5.4	1.8AB±1.3	3.7A±2.2	0.7B±2.6

[§] Cereal rye, CR; crimson clover, CC; forage radish, FR; no cover crop, NC

[†] Between years within a season and cover crop treatment, values followed by different uppercase letters are significantly different between years at P < 0.05.

[‡] For a given season within a location and a year, values followed by a different lowercase letter are significantly different at P<0.05

Table 2.7. Mean N content (kg N ha⁻¹) of fall-interseeded cover crop biomass at fall frost collection and coefficient of variation (CV). Mean values following ± are the standard deviation of the mean.

Location	Cover crop [§]	2017		2018	
		Mean	CV	Mean	CV
Grand Rapids	AR	5.56 ± 4.51	0.81	0.64	-
	ARCC	2.25 ± 0.88	0.39	1.37	-
	ARCCFR	0.95 ± 0.44	0.46	0.21	-
	CR	1.14 ± 0.57	0.50	0.23	-
	CRCC	0.34	-	-	-
	CRCCFR	3.35 ± 0.54	0.16	-	-
Lamberton	AR	4.68b ± 1.38	0.29	3.79 ± 4.49	1.18
	ARCC	10.66ab ± 4.88	0.46	3.17 ± 4.20	1.32
	ARCCFR	16.11a ± 5.61	0.35	4.11 ± 3.09	0.75
	CR	4.65b ± 2.23	0.48	2.80 ± 2.16	0.77
	CRCC	9.05ab ± 3.96	0.44	2.07 ± 1.30	0.63
	CRCCFR	18.15a ± 9.6	0.53	4.76 ± 4.32	0.91
Waseca	AR	-	-	4.37b	-
	ARCC	-	-	5.63b ± 0.30	0.05
	ARCCFR	-	-	11.13a ± 3.38	0.30
	CR	-	-	2.78bc	-
	CRCC	-	-	2.57c ± 0.80	0.31
	CRCCFR	-	-	5.61bc	0.63

[§] Annual ryegrass, AR; Crimson clover, CC; Forage radish, FR; Cereal rye, CR

[†] Within a location and a year, mean values with the same lowercase letter are significantly different at $P < 0.05$.

Table 2.8. Nitrogen accumulation in the biomass of physiologically mature maize biomass and grain following fall-interseeded cover crops.

Cover crop [§]	Grand Rapids		Lamberton		Waseca	
	2017	2018	2017	2018	2017	2018
	<i>Total maize N (kg ha⁻¹) in R5-R6 biomass</i>					
AR	76	80	58ab	1120ab	57bcd	55ab
ARCC	90	108	59ab	115ab	52cd	45b
ARCCFR	84	116	79a	107ab	49cd	51ab
ARNC	84	121	62ab	96b	45d	66a
CR	78	117	66ab	102ab	89b	51ab
CRCC	77	103	77a	93b	99a	62ab
CRCCFR	84	107	52b	127ab	73bc	50ab
CRNC	90	108	48b	130a	87b	54ab
	<i>N exported (kg ha⁻¹) in grain</i>					
AR	78b	102	109	123	-	105
ARCC	87ab	97	105	121	-	96
ARCCFR	88ab	100	109	120	-	103
ARNC	84ab	95	113	124	-	99
CR	85ab	87	112	129	-	89
CRCC	93ab	100	134	135	-	87
CRCCFR	94ab	93	121	135	-	90
CRNC	96a	102	125	136	-	103

[§] Annual ryegrass, AR; Crimson clover, CC; Forage radish, FR; No cover, NC; Cereal rye, CR

[†] Within a location and year, mean values followed by a different lowercase letter are significantly different at $P < 0.05$.

Table 2.9. Nitrogen content accumulated in the biomass of spring-interseeded maize at physiological maturity and exported in maize grain.

	Grand Rapids		Lamberton		Waseca	
	2017	2018	2017	2018	2017	2018
	<i>(kg N ha⁻¹)</i>					
Mature maize biomass	102	101	46	150	-	65
Maize grain	135	150	93	169	110	113

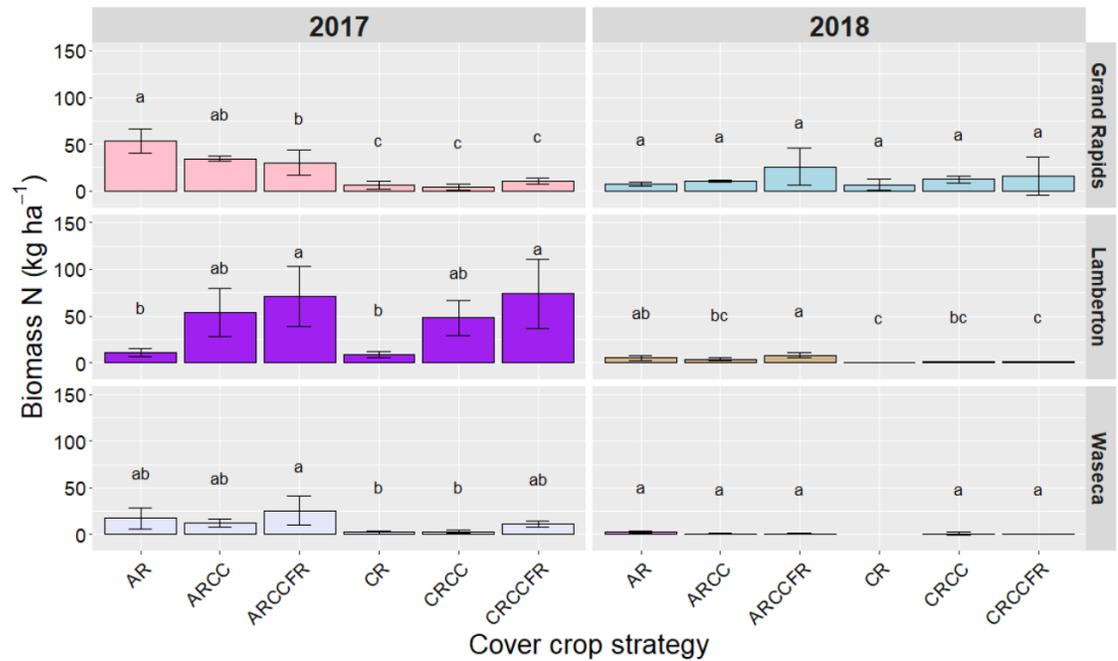


Figure 2.1. Accumulation of N in spring-interseeded cover crop biomass at fall frost. Within a location and year, means with different lowercase letters indicate significant difference at $P < 0.05$. Bars are standard errors of the mean.

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