WINTERKILLED COVER CROPS INTERSEEDED LATE INTO CORN AND SOYBEAN WITHIN DIFFERENT TILLAGE PRACTICES IN THE UPPER MIDWEST, U.S.A

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Dedication

Dedicated to the corn and soybean growers of Minnesota

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1 CHAPTER 1: INTRODUCTION

1.1 Background

A major consequence of the intensified corn (*Zea mays* L.)-soybean [*Glycine max* (L.) Merr.] agroecosystem, which dominates the U.S. Midwest region, is the high loads of N and P delivered to the U.S. Midwest watersheds. High N and P concentration in the watersheds eventually contribute to the formation of the hypoxic zones in the Gulf of Mexico (Alexander et al., 2008). Other consequences include deteriorated soil health, polluted groundwater, reduced biodiversity, impaired agroecosystem functions, and increased dis-services (Hanrahan et al., 2018; Kladivko et al., 2014; Tilman, 1999; Foley et al., 2011; Tiemann et al., 2015).

The use of cover crops to promote soil health is an old practice (Odland and Knoblauch, 1938), with renewed attention (Tully and McAskill, 2019). As such, researchers have studied the environmental and economic viability of cover crops in several environments. Cover crops are proven to provide ecosystem services such as the reduction in N losses via leaching (Strock et al., 2004; De Bruin et al., 2005; Meisinger and Ricigliano, 2017), improved soil quality and health (Lal, 2016), increased species diversity (Drinkwater and Snapp, 2007), increased functional diversity (Elhakeem et al., 2019), and weed suppression (Mirsky et al., 2011).

Because of the short growing season, overwintering cover crops are often the option to diversify corn and soybean production systems in the U.S. upper Midwest. Overwintering cover crops can be seeded early or late in the growing season either as a monoculture or frequently mixed with winterkilled species, and terminated before or soon after planting the next major crop the following spring (Rusch et al., 2020) As such, most research on cover crops in the region has been conducted on winter annual cover crops. While winter annuals provide ecosystem services in the spring and fit well in the cropping systems in warmer regions, they have the potential to delay the planting of primary crops in the colder regions such as the U.S. upper Midwest, mainly due to weather conditions associated to excess rainfall. This reality evidences the need for cover cropping options that can fit in the cropping systems of the region while providing ecosystem services. Winterkilled cover crops, planted either early or late in the growing season of major crops, might be an option. Winterkilled cover crops have the potential to produce biomass during the growing season and immobilize N in their plant tissue, which would otherwise be lost to the system.

Although there is abundant information in the literature regarding the biomass potential of cover crops (Ruffo et al., 2004; Poffenbarger et al., 2015; Finney et al., 2016; Thapa et al., 2018; Baraibar et al., 2018; Florence et al., 2019; Ruis et al., 2019; Rusch et al., 2020), there is a lack of information regarding the time at which the N tied up in the cover crop biomass is released back in the system. One of the main barriers to cover crop adoption has been the lack of knowledge on the synchrony of N release from cover crops and primary crop uptake (Nevins et al., 2020). Besides, no study has examined the effectiveness and potential of winterkilled cover crops in terms of biomass production, ground cover, N accumulation, and the N dynamics within winterkilled cover crops in the cold environment of the U.S. upper Midwest. Shedding light on these aspects can help weigh the ecosystem services provided by winterkilled cover crops and determine if such strategy is economically viable and environmentally sound.

This study was conducted with two main objectives: 1) assess the biomass and ground cover of winterkilled cover crops interseeded late in the corn and soybean growing season, and 2) elucidate if winterkilled cover cropping strategies affect corn and soybean production, provide ecosystem services, and affect nitrogen dynamics within corn and soybean production practices.

2 CHAPTER 2: POTENTIAL OF WINTERKILLED COVER CROPS INTERSEEDED LATE IN CORN AND SOYBEAN GROWING SEASON

Summary

The successful integration of cover crops in the conventional corn (*Zea mays* L.)-soybean [*Glycine max* (L.) Merr.] agroecosystem in the U.S. upper Midwest is challenging due to poor establishment, extensive use of fall tillage, and a short planting season. Most research conducted in the region has assessed the benefits of winter annual cover crops, but no research has assessed the benefits of summer annual cover crops seeded late in the growing season. Grower's interest in cover crops that winterkill lies in practical and economic reasons- save time and reduce costs associated with herbicide and labor at termination. The objectives of this study were to 1) assess the establishment and growth of winterkilled cover crops interseeded late into corn and soybean grown within different tillage practices, and 2) determine their effect in the productivity of major crops. The study was conducted within two long-term tillage trials located in Lamberton and Waseca, Minnesota. Cover crops were hand-broadcast at R5-R6 corn and R7-R8 soybean in fall 2017 and at R3-R4 corn and R5-R6 soybean in fall 2018. Tillage practices were conventional-, strip-, and no-till; and cover crop strategies included annual ryegrass (*Lolium multiflorum* Lam.) monoculture, denoted as AR; a two-way mixture of annual ryegrass and crimson clover (*Trifolium incarnatum* L.), denoted as ARCC; and a three-way mixture of annual ryegrass, crimson clover, and forage radish (*Raphanus sativus* L.), denoted as ARCCFR, with a no-cover as control denoted as NC.

The three-way mixture of ARCCFR produced the highest biomass (0.169 Mg DM ha^{-1}), followed by AR monoculture (0.154 Mg DM ha^{-1}) and ARCC (0.137 Mg DM ha^{-1}) when pooled across years, location, and primary crops. However, AR monoculture produced more biomass within corn in 2017 in both locations, suggesting that species richness does not always result in higher productivity. Annual ryegrass consistently produced more biomass than CC and FR when in mixtures, and CC had the lowest emergence and establishment. Cover crop average biomass pooled across location, year, tillage, and cover crop strategy yielded 0.254 Mg DM ha^{-1} in corn and 0.074 Mg DM ha^{-1} in soybean. Cover crop canopy cover averaged 24% in corn and 8% in soybean during the whole study. The yield of primary crops was affected by weather and year, rather than the cover crop strategy and tillage practice. The practicality of winterkilled, late interseeded cover crops lies in its potential to produce biomass and provide ground cover. Although these strategies can produce biomass within corn, it was unclear in this study if biomass in these small quantities can provide ecosystem services. The limited biomass production and ground cover of cover crops interseeded into soybean suggest that such cover cropping strategy may not add ecosystem services value within soybean production practices.

2.1 Introduction

The corn (*Zea mays* L.)-soybean [*Glycine max* (L.) Merr.] agroecosystem dominates the U.S. Midwest region (Russell et al., 2009), the largest producer of corn and soybean in the country (USDA NASS, 2019). Corn is highly responsive to nitrogen (N), and growers apply it in rates higher than recommended to maximize yield and minimize risk (Sela et al., 2016; Vetsch et al., 2019). Around 14% of total cropland in the U.S. and 31% of cropland in Minnesota is under the tile drainage system (USDA NASS, Census of Agriculture, 2017). The use of tile drainage in the Midwestern U.S. can maximize crop productivity and yields, especially in heavy, poorly drained soils. However, the combination of high rates of N fertilizer use and tile drainage causes increased nutrient leaching in the subsurface drainage. Consequently, the U.S. Midwest watersheds deliver the highest loads of N and P via leaching, contributing to the formation of the hypoxic zone in the Gulf of Mexico (Alexander et al., 2008). Other consequences include deteriorated soil health, polluted groundwater, reduced biodiversity, impaired agroecosystem functions, and increased dis-services (Hanrahan et al., 2018; Kladivko et al., 2014; Tilman, 1999; Foley et al., 2011; Tiemann et al., 2015). However, corn and soybean are likely to continue to be produced in the Midwest, which calls for improved farming practices.

Cover crop benefits have been realized since long ago (Odland and Knoblauch, 1938). Cover crops reduce N losses via leaching (Strock et al., 2004; De Bruin et al., 2005; Meisinger and Ricigliano, 2017), improve soil quality and health (Lal, 2016), increase species diversity (Drinkwater and Snapp, 2007), increase functional diversity

(Elhakeem et al., 2019), and suppress weeds (Mirsky et al., 2011). However, in the U.S. upper Midwest, the adoption of the practice is challenging due to weather conditions, combined with the short growing season (Rusch et al., 2020), leading to poor field establishment (Snapp et al., 2005; Noland et al., 2018; Rusch et al., 2020). Most cover crop studies in the U.S. upper Midwest involve cereal rye (*Secale cereale* L.), a cold-tolerant species that withstands the winter conditions in the region (Rusch et al., 2020, Snapp et al., 2005; Wilson et al., 2013), produces high biomass and uses residual soil nitrate (Strock et al., 2004; Feyereisen et al., 2006). Cereal rye, however, is also reported to reduce corn yield due to allelopathic effects when herbicide-terminated at corn planting (Johnson et al., 1998), and to promote changes in C:N ratio (Finney et al., 2016). Besides, cereal rye has the potential to attract polyphagous pests such as true armyworm (*Mythimna unipuncta* Haworth) and cutworms (*Agrotis ipsilon* Hufnagel), which can injury corn (Dunbar et al., 2016). These negative effects of cereal rye in corn could result in delayed planting in a region with an already short growing season. Cover crop strategies that do not need spring termination yet produce enough biomass in the fall and can provide agroecological benefits could be used.

In the past 50 years, the number of days with heavy rainfall has tripled in the Midwest U.S., particularly in the spring (Hatfield et al., 2013), as a result of surface temperature rise (Hess et al., 2020). This change, when coupled with poorly drained heavy soils, can result in wet conditions in the spring (Randall and Vetsch, 2005). Therefore, farmers tend to practice fall tillage after harvest so that the field dries up sooner in the spring to ensure timely planting. These factors evidence the significant challenge in adopting cover crops and conservation tillage practices in the region, particularly in southern Minnesota. Due to the limited window opportunity to get cover crops established, cover crops may be interseeded as early as V4-V6 leaf collar in corn, and as late as R5-R6 stages into corn and R7-R8 into soybean (Brooker et al., 2020; Rusch et al., 2020). Early-interseeded cover crops can compete with primary crops for resources such as water, nutrients, and light and can impact crop yields (Curran et al., 2018). However, interseeding overwintering or winterkilled cover crops in late-summer to early-fall can provide ecosystem services without interfering with crop yields. Late interseeded winterkilled cover crops can produce biomass in the fall comparable to overwintering cover crops with the potential to reduce N leaching and soil erosion in optimal weather conditions (Rusch et al., 2020). Additionally, late interseeded winterkilled cover crops can facilitate the timely planting of major crops with reduced herbicide and tillage cost in the spring (Grimmer and Masiunas, 2004), as compared to overwintering cover crops (Johnson et al., 1998; Grimmer and Masiunas, 2004).

The effect of overwintering cover crops on the productivity of major crops is abundant in the literature (Johnson et al., 1998; De Bruin et al., 2005; Mirsky et al., 2009; Wilson et al., 2013; Poffenbarger et al., 2015; Curran et al., 2018; Noland et al., 2018; Brooker et al., 2020; Rusch et al., 2020). However, little is known about the effect of winterkilled cover crops in crop productivity, their potential to provide agroecosystem services, and their performance in northern regions with a long history of tillage practices.

Major concerns with interseeding cover crops in the U.S. upper Midwest include establishment and growth, mainly due to a short growing season (Wilson et al., 2013). As such, studies have suggested the need for alternative cover crops (Rusch et al., 2020) along with innovative cropping systems and domestication of crops tailored to the conditions in the region (Liu et al., 2019; Ott et al., 2019). As of 2020, research on late interseeded winterkilled cover crops and their effects in corn-soybean production is minimal. The goal of this study was to assess the effect of tillage practices on the performance of winterkilled cover crops interseeded late in corn and soybean cropping systems. Specific objectives were to 1) determine the combined effect of crop and tillage practice on the establishment and biomass production of cover crops, and 2) assess the effect of cover crops on growth and yield of corn and soybean for the conditions typical of the upper Midwest U.S. The results of this study will increase our understanding of tradeoffs among environmental factors in corn-soybean production practices in northern climates.

2.2 Materials and Methods

2.2.1 Field Experiments

Field experiments were conducted from spring 2017 to fall 2019 at two sites that had existing long-term tillage trials. The first site was located at the University of Minnesota's Southern Research and Outreach Center at Waseca, MN (44°04'05.97" N 93°31'19.02" W) while the other site was located at the Southwest Research and Outreach Center near Lamberton, MN (44°14'02.20" N 95°18'6.87" W). The dominant soils were characterized as Webster clay loam (fine-loamy, mixed, superactive, mesic

Typic Endoaquolls) at Waseca, and Normania loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls) and Amiret loam (fine-loamy, mixed, superactive, mesic Calcic Hapludolls) at Lamberton (Soil Survey Staff, 2020). Both locations fall within the plant hardiness zone 4b (-31.7ºC to -28.9ºC) (USDA-ARS). Air temperature and precipitation data for the long-term and experimental years were obtained from weather stations located within 1.25 km and 0.5 km of the experiment site in Lamberton and Waseca, respectively. Solar radiation data for the long-term average were obtained from NASA POWER (https://power.larc.nasa.gov/), using R package nasapower for both locations (Sparks, 2018).

2.2.2 Experimental design

The experiment was designed as a split-split plot with four replications in each site-year. Main plot was primary crop (corn and soybean), sub-plot was tillage practice [conventional-till (CT), strip-till (ST), and no-till (NT)], and sub-sub-plot was cover crop strategy [annual ryegrass (*Lolium multiflorum* L.), AR; AR+ crimson clover (*Trifolium incarnatum* L., CC), ARCC; and AR + CC + forage radish (*Raphanus sativus* L., FR), ARCCFR; with a no-cover control, NC]. Sub-sub-plots were randomized within the sub-plots. Each experimental unit was 4.5 m wide (six 76-cm rows) and 16.5 m long in Waseca, and 3.75 m (five 76-cm rows) wide and 20 m long in Lamberton.

2.2.3 Agronomic Management

Strip-till was performed 15 d before planting corn and soybean to a depth of 15 cm and in 20 cm wide strips using an 8-row strip-tiller with 76 cm row spacing at both locations. At the Lamberton site, CT was performed a day before planting in corn and soybean plots to a depth of 15 cm using a chisel plow. At the Waseca site, however, CT was performed a day before planting soybean using a disc ripper and a field cultivator, and corn plots were only field cultivated to the depth of 10 cm. Tillage was performed only in the spring.

At Waseca, glyphosate [N-(phosphonomethyl)glycine] was applied before planting (1.26 kg a.e. ha^{-1}) and at V3-V4 leaf-collar stages of corn (0.86 kg a.e. ha^{-1}). At Lamberton, glyphosate was applied once (0.84 kg a.e. ha^{-1}) along with Fusilade (Fluazifop-P-butyl) (0.13 kg ae ha^{-1}) at planting. Weeds not controlled by herbicide treatment were removed by hand. Corn hybrid DKC49-72RIB (99-d RM RR2) was planted in both locations at the rate of 89,000 seeds ha^{-1}. Soybean variety AG2035 (2.0 RM RR2) was planted in both locations at the rate of 371,000 seeds ha^{-1}; in 2019, the variety AG20X9 (2.0 RM RR2) was used in Lamberton. Both crops were planted at a depth of 5 cm in 76-cm wide rows using a four-row John Deere 1700 MaxEmerge series planter at both locations.

Fertilizer amounts were estimated for highly productive corn, following the University of Minnesota guidelines (Kaiser et al., 2011). A total of 163 kg N ha^{-1} in the form of urea [NH_2-CO-NH_2] along with 17 kg S ha^{-1} in the form of gypsum [$CaSO_4$ $2H_2O$] were applied in Waseca; 63 kg N ha^{-1} applied at planting, and the remaining at the V6 corn stage. In Lamberton, a total of 200 kg N ha^{-1} were applied at planting along with 17 kg S ha^{-1} in the form of gypsum. At both sites, Agrotain®, [N-(n-butyl) thiophosphoric triamide, NBPT], a urease inhibitor, was applied each time urea was used.

Cover crop seeds were weighed separately for each species in the laboratory. Seeds were mixed in the field during seeding and hand broadcast below the canopy in both corn and soybean to avoid seeds landing in the canopy. In 2017, cover crops were hand broadcast at R5-R6 corn stage and R7-R8 soybean stage (early-Sept) at both locations. However, in 2018, cover crops were hand broadcasted 15 d earlier, at the R3-R4 stage corn and R6 stage soybean (mid-Aug) to increase growing degree days and subsequently cover crop growth. Cover crops were lightly raked only in 2017 at Waseca to increase seed to soil contact due to dry soil conditions.

2.2.4 Data collection

Growing degree days (GDD) were calculated each growing season from planting to physiological maturity for corn and soybean, and from seeding to two consecutive frost day for cover crops. Growing degree days was calculated based on the method as described in Mcmaster and Wilhelm (1997) for corn and soybean. The base temperature (T_{base}) of 8°C and the absolute maximum (T_x) of 33°C were used for corn. Absolute maximum (T_x) of 33°C and T_{base} of 10°C were used for soybean. For cover crops, T_{base} of 2°C, and T_x of 30°C were used for annual ryegrass, the most cold-tolerant cover crop in the study (Moot et al., 2000). GDD accumulation was calculated with the following formula:

$$GDD = \sum\left(\frac{T_{max} + T_{min}}{2} - T_{base}\right)$$

where, T_{max} = maximum daily temperature and T_{min} = minimum daily temperature. To eliminate the effect of air temperature below or above the absolute minimum and maximum temperatures, the following constraints were used:

If $T_{max} > T_x$ $then$ $T_{max} = T_x$

If $T_{max} < T_{base}$ $then$ $T_{max} = T_{base}$

If $T_{min} < T_{base}$ $then$ $T_{min} = T_{base}$

Photosynthetically active radiation (PAR) readings were taken from V6 leaf-collar corn stage and V3 soybean stage to R4-R5 corn and R6-R7 soybean with an LP-80 AccuPAR ceptometer (Meter Group, Inc. U.S.). Readings were taken between 1000 and 1400 h, when the PAR readings were higher than 600 µmol m^{-1} s^{-1}, in 10- to 15-d intervals. The ceptometer probe was placed perpendicularly in the middle of the two center rows (Meter Group, Inc. USA), where three readings were taken and averaged. An external sensor attached to a 3-m pole was used to measure above canopy PAR simultaneously with the below canopy PAR. The ratio of below canopy PAR and above canopy PAR (Tau), was then calculated.

Total above ground biomass was measured at the V6, VT/R1, and R6 stages of corn and V5/V6, R1, and R7/R8 stages of soybean. All plants within 1-m row length in 2017 and 2018 and within 0.5-m row length in 2019 were hand-harvested from the second row of each plot. Samples were then cut into small pieces using a chipper and collected in cloth bags to facilitate uniform drying. The samples were dried in a forced-air oven at 60°C until constant mass and then weighed. Grain and plant biomass samples were later ground separately using Thomas Wiley Mill Model 4 with a 2-mm screen (Thomas Scientific LLC), and subsamples taken in whirl-Pak bags to determine CNS with a vario MACRO cube (Elementar Americas Inc, NY).

Grain weight and moisture content of both crops were obtained at harvest using a two-row Kincaid 8-XP plot combine equipped with the weight and moisture measuring device HarvestMaster GrainGage (Juniper Systems, Inc).

Cover crop canopy was measured in the fall after the first frost day. Images were captured using a digital camera, and later uploaded into the mobile application Canopeo v 1.1.7 (Patrignani and Ochsner, 2015) to estimate fractional green canopy cover within a 0.3 x 0.3 m quadrat in Waseca and 0.5 x 0.2 m quadrat in Lamberton. All above ground cover crop biomass within the quadrat was collected using a pair of scissors. Weed pressure was negligible in this study because weeds not controlled by herbicides were manually uprooted. The biomass of each species was separated for treatments with cover crop mixes and placed in a brown paper bag. The biomass was later dried in a forced-air oven at 60°C until constant mass and then weighed.

2.2.5 Statistical Analyses

Data were analyzed using the statistical software R (version 3.6.2; R Core Team, 2019). Grain yield, cover crop biomass, PAR, and cover crop canopy cover were analyzed separately using linear mixed effects model ANOVA to determine significant main effects and interactive effects using package '*lmerTest*' (Kuznetsova et al., 2017). Analyses for each response variable were combined over time and space to address broad sense inference (Moore and Dixon, 2015). Location, year, tillage, cover crop strategies, and their interactions were considered fixed effects, and appropriate split-plot error terms were considered random effects. Visual representations were used to check the assumptions of normality and constant variance of the model residuals.

If the combined analysis resulted in significant interactions, separate ANOVA was conducted on the response variables of interest. Tillage and cover crop strategies were considered fixed effects, and split-plot error terms were considered random effects. Model residuals were used to diagnose for normality and the need for data transformation. Post hoc comparisons of all estimated marginal means were made on the response variables using a conservative Bonferroni's adjusted p values using the '*emmeans'* package (Lenth et al., 2019). Compact letter displays for significant differences were obtained using the *'multcomp'* package (Hothorn et al., 2008).

2.3 Results and Discussion

2.3.1 Weather Conditions

In 2017, monthly-average air temperature during the growing season (May-Oct) averaged within 2°C of the 30-yr long-term average (Figure 2.1 and Figure 2.2) in both locations. However, February was 7°C and 6°C warmer than the long-term average in Lamberton and Waseca, respectively. Overall, 2018 was cooler than the long-term average in both locations, notably April, which was 6°C and 7°C cooler than the long-term average in Lamberton and Waseca, respectively. The monthly-average air temperature in the 2019 growing season was within 2°C of the long-term average in both locations. February was 7°C cooler in Lamberton and 6°C cooler in Waseca in 2019. (Table 2.1 and Table 2.2).

Total rainfall during the growing season in 2017 was higher than the long-term average in Lamberton and lower than average in Waseca. Lamberton received a total of 720 mm rainfall during the growing season, and Waseca received 657 mm rainfall during the growing season. There was a high rainfall event of 99 mm on 3-Oct in Lamberton. Precipitation in 2018 was higher than long-term averages in both locations, with Lamberton receiving 807 mm, and Waseca receiving 862 mm total rainfall in the growing season. Waseca received 137 mm of rain in a single event on 5-Sept that year.

Similarly, 2019 precipitation totals were also higher than the long-term averages. Lamberton received 862 mm cumulative rainfall, and Waseca received 888 mm rainfall in 2019 (Table 2.1 and Table 2.2). Rainfall event occurred within a week of seeding cover crop in both sites in 2017 and 2018, with Waseca receiving the highest rainfall in 2018 as compared to other site years. At the Lamberton site, total weekly rainfall ranged from 0 to 15 mm after seeding cover crops in 2017 and 2018, respectively. At the Waseca site, however, the total weekly rainfall ranged from 5 to 41 mm in 2017 and 2018, respectively.

2.3.2 Growing Degree Days

Cumulative GDD for corn from planting to physiological maturity for the Lamberton site was 1562, 1550, and 1520 in 2017, 2018, and 2019, respectively. For soybean, cumulative GDD was 1310, 1318, and 1278 in 2017, 2018, and 2019, respectively. Similarly, cumulative GDD at the Waseca site was 1605, 1748, 1611 for corn and 1351, 1482, and 1348 for soybean in 2017, 2018, and 2019, respectively. Cover crops accumulated 474 and 570 GDDs in 2017 at Lamberton and Waseca sites, respectively. Cover crops accumulated 661 and 851 GDDs in 2018 at Lamberton and Waseca sites, respectively. The additional accumulation of ~200 GDDs in cover crops was due to ~15-d of earlier seeding in 2018 as compared to 2017 at both locations (Figure 2.3).

2.3.3 Effect of winterkilled cover crops on corn and soybean grain yield

Corn grain yield was affected by year, tillage, and the year x location, year x tillage, and year x location x tillage interactions, but not by location and cover crop strategies (Table 2.3). The 3-way interaction of year, location, and tillage can be explained by high corn grain yield observed at Waseca in 2019 within each tillage practices as compared to other site-years.

Soybean grain yield was affected by year, location, year x location, location x tillage, and year x location x tillage interactions, but not by tillage and cover crop strategies (Table 2.3). The 3-way interaction plot of year, location, and tillage revealed that soybean grain yield at Waseca in 2019 was significantly lower within the NT plots as compared to CT and ST (figure not shown).

Corn and soybean grain yield were not affected by cover crop strategies regardless of location, tillage, or year in this study. Researchers have found that cover crops can increase (Vyn et al., 1999), decrease (Kaspar et al., 2007; Pantoja et al., 2015) or have no effect (Strock et al., 2004; Curran et al., 2018; Noland et al., 2018; Brooker et al., 2020; Rusch et al., 2020) on corn yield. Cover crop effect on corn grain yield depends upon biomass production. Biomass production of legume cover crops has the potential to increase yields, mainly because of the N fixation contribution, and biomass production of grass cover crops can decrease yields, mainly because of N tied up in biomass (Christianson et al., 2017). Results of this study agree with research results from the upper Midwest U.S. reporting no effect of cover crop on the yield of corn mainly due to lower cover crop biomass production (Noland et al., 2018; Rusch et al., 2020). However, it should be stressed that Noland et al. (2018) used overwintering cover crops only while Rusch et al. (2020) compared both overwintering and winterkilled cover crops seeded early and late in the corn growing season.

Results of this study support previous research that report no effect of cover crops on the yield of soybean (Johnson et al., 1998; De Bruin et al., 2005; Caswell et al., 2019). However, these studies used overwintering annual cover crops. Other studies have reported penalization on soybean yield due to the direct or indirect use of cover crops. For example, Noland et al. (2018) reported soybean yield losses due to poor cover crop termination before planting soybean. Reddy (2001) reported decreased soybean yield in annual ryegrass plots for conditions in Mississippi, where AR biomass production was much higher as compared to the upper Midwest. Results from this research showed that soybean yield was not affected by late interseeded cover crops within tillage practices, which can be attributed to lower cover crop biomass production. This result is consistent with another study conducted under similar conditions with winterkilled cover crops (Johnson et al., 1998). Besides, cover crop in this research winterkill, so there are no yield losses related to poor cover crop termination in the spring.

2.3.4 Cover crop biomass

Cover crop biomass within corn was affected by location, tillage, year x location, and year x cover crop strategy interaction. Within soybean, cover crop biomass was affected by year, location, cover crop strategy, year x location, and year x cover crop strategy interaction (Table 2.3).

When pooled over corn, the Lamberton site produced 0.057 Mg DM ha^{-1} and 0.022 Mg DM ha^{-1} cover crop biomass on average in 2017 and 2018, respectively. Within soybean plots, Lamberton produced only 0.031 Mg DM ha^{-1} in 2017 and 0.007 Mg DM ha^{-1} in 2018. At Waseca, cover crops produced significantly higher biomass compared to Lamberton in both years, averaging 0.311 Mg DM ha^{-1} in 2017 and 0.555 Mg DM ha^{-1} in 2018 within corn plots. Pooled over soybean, cover crops at Waseca produced 0.179 Mg DM ha^{-1} and 0.062 Mg DM ha^{-1} in 2017 and 2018.

Higher cover crop biomass production in corn compared to soybean may be explained by a higher light interception in the latter compared to the former at the time of cover crop seeding (Youngerman et al., 2018). Photosynthetically active radiation (PAR) reaching the soil surface below corn canopy, expressed as Tau, increased consistently in each site year starting early August within corn. However, Tau did not increase until late August in soybean plots, evidencing less available light for cover crop growth (Figure 2.6). Field studies and crop models have suggested that intercepted light affects cover crop germination and growth (Feyereisen et al., 2006; Congreves et al., 2014). Also, soybean plots had high corn residue from the previous season, which could have further limited seed to soil contact. The combined effects of low germination due to poor soil-seed contact from increased residue and reduced growth due to high PAR interception of seeds that did germinate could explain poor cover crop establishment and biomass production. Previous studies have found rainfall within seven days after seeding to be a good predictor of biomass (Wilson et al., 2013). Although Waseca produced high biomass within corn plots in 2018 when rainfall within a week was higher as compared to other site years, the results of this study suggest rainfall 7 DAS was not a significant predictor of cover crop biomass. This result is because cover crop biomass is primarily a function of light, and soybean plots received comparatively less PAR than corn plots.

Cover crops were seeded ~15-20 d earlier in 2018 in both locations, resulting in increased GDDs accumulation, but limited cover crop growth and development in Lamberton. These results are consistent with results from studies conducted in similar spatial and temporal environments (Rusch et al., 2020). More than cover crop strategy and tillage practices, variation in cover crop biomass was observed to be weather-driven, where different abiotic factors such as light, rain, temperature, hailstone events affected cover crop biomass production.

When pooled over tillage practices, average cover crop biomass significantly varied within corn plots, with CT producing higher biomass than ST and NT. This difference may be the result of higher seed to soil contact in CT plots, which favors germination and emergence (Fisher et al., 2011). However, within soybean plots, no differences in cover crop biomass were observed among tillage practices. This result could be attributed to higher crop residue in soybean plots within each tillage practices, which may have decreased seed to soil contact. Additionally, the higher amount of intercepted light reaching the cover crops within the soybean plots and the use of moderately shade tolerant cover crops (Clark and Sustainable Agriculture Research &

Education (Program), 2007) may have resulted in no differences in the cover crop biomass among the tillage practices within soybean.

Within cover crop strategy with 2-way and 3-way mixes, AR growth outperformed other species within a mix (Figure 2.7 and Figure 2.8). In Lamberton, the overall pooled average of AR, ARCC, and ARCCFR biomass in 2017 was 0.041 Mg DM ha^{-1}, 0.03 Mg DM ha^{-1}, and 0.06 mg DM ha^{-1}, respectively. In 2018, AR, ARCC, and ARCCFR biomass was 0.012 Mg DM ha^{-1}, 0.001 Mg DM ha^{-1}, and 0.021 Mg DM ha^{-1}, respectively. Similarly, in Waseca, the overall pooled average of AR, ARCC, and ARCCFR biomass in 2017 was 0.242 Mg DM ha^{-1}, 0.211 Mg DM ha^{-1}, and 0.282 Mg DM ha^{-1}, respectively. In 2018, AR, ARCC, and ARCCFR biomass was 0.318 Mg DM ha^{-1},0.296 Mg DM ha^{-1}, and 0.312 Mg DM ha^{-1}, respectively.

Published literature suggests that ecosystem services of cover crops are driven by above ground biomass and total N content (Blanco-Canqui et al., 2015; Finney et al., 2016; Ruis and Blanco-Canqui, 2017; Thapa et al., 2018; Ruis et al., 2019). In this study, cover crop mixes did not consistently produce higher biomass than cover crop monoculture. This result is consistent with studies where mixtures designed to provide ecosystem services by "employing N acquisition strategy" did not produce more biomass than cover crop monocultures (Finney et al., 2016). These results can be attributed to certain cover crops in the mixture performing better than others. For example, studies conducted in Michigan have shown that FR is winterkilled while CC and AR survive the winter (Brooker et al., 2020). However, some studies report increased biomass, but not necessarily increased functionality, with higher species richness (Florence et al., 2019).

In this study, AR that did not germinate in the fall within the soybean plots but germinated in the next spring within corn plots at planting, suggesting that winterkilled late interseeded cover crops have weed potential in the next season if germination does not occur in the previous season. In contrary, this experiment suggests that winterkilled late interseeded cover crops into standing corn can produce biomass comparable to early seeded cover crop biomass when measured in the fall in certain years. For example, in 2018 in Lamberton and Waseca, cover crop biomass from this study was similar to the biomass produced by the same species that were interseeded early into corn and measured in the fall (Rusch et al., 2020). However, biomass production within soybean seemed to be challenging and minimal to provide enough ground cover due to factors such as light interception due to dense foliage of the major crop and corn residue from the previous growing season.

2.3.5 Cover crop canopy cover

Canopy cover of cover crops interseeded into corn was affected by year, location, cover crop strategy, the year x location, location x tillage, and year x cover crop strategy interactions. Within soybean, canopy cover varied by year, location, tillage, and interaction of year and cover crop strategy (Table 2.3).

At Lamberton site in 2017, cover crop canopy cover was not affected by strategy across tillage practice within corn. Within soybean, the three-way mix strategy (ARCCFR) was significantly different from AR within ST. However, in 2018, canopy cover was not affected by cover crop strategy within any tillage practices for both corn and soybean (Figure 2.9).

At Waseca site in 2017, canopy cover in corn plots was different between cover crop strategy such that the ARCCFR cover mixture resulted in greater canopy cover that AR in no-till and strip-till but not conventional tillage. However, for soybean plots, canopy cover did not vary among cover crop strategy regardless of tillage practice. In 2018, differences in canopy cover were not observed between cover crop strategy within tillage practices for both corn and soybean (Figure 2.10).

2.4 Conclusions

This research was conducted with the goal of assessing the effect of crops and tillage practices on the performance of winterkilled cover crops interseeded late in the corn and soybean growing season and determine their effect on the productivity of corn and soybean.

Yearly variations on establishment and growth of cover crops were explained by location, tillage practices, and crop, rather than cover crop strategy. Annual ryegrass had the highest establishment and growth and often outperformed CC and FR in mixtures. Although the 3-way mixture of ARCCFR did not always result in higher biomass production, it had more ground cover than 2-way mixes and AR monoculture. Forage radish showed winter injury earlier than AR and CC, but CC consistently had slower germination and growth.

Conventional tillage within corn produced the highest cover crop biomass as compared to other tillage practices, possibly due to higher seed to soil contact. No differences in cover crop biomass were observed due to tillage practices in soybean. Results from this study suggest that summer annual cover crops interseeded late in the season can establish successfully in the fall growth period within corn with no effect on growth and yield of major crops.

Although the cover crops used in this study do not overwinter and did not provide ecosystem benefits in the spring, seeding late in the season can produce biomass and provide ground cover in the fall, mainly after corn harvest. It is unclear if the benefits of these cover crop strategies can be economically sound and environmentally viable to corn growers. However, the cover crop biomass within soybean was marginal in this study, and the input costs may not outweigh the potential benefits provided by cover crops. Therefore, late interseeded cover crops within soybean systems are not feasible for the conditions in the upper Midwest.

Future studies in late interseeding cover crops should focus on direct ecosystem services such as the potential to reduce nitrate leaching, N uptake, C:N ratio, and N availability to next season crop and increase soil organic matter content. Also, comparing the performance of summer annual cover crops and winter annual cover crops in a study could help to better understand the effect of summer late seeded cover crops in the corn and soybean agroecosystems.

2.5 Tables and Figures

Table 2.1. Long-term average (LTA) of monthly total precipitation, average temperature, and solar radiation at SWROC, Lamberton, MN. Values followed by ± LTA are one standard deviation. Experimental years 2017, 2018, and 2019 are shown as departures from the LTA.

	Precipitation (mm)				Average Temperature (°C)				Solar Radiation (M J m^{-2})			
Month	**LTA**	**2017**	**2018**	**2019**	**LTA**	**2017**	**2018**	**2019**	**LTA**	**2017**	**2018**	**2019**
1	14 ± 11	-1	-2	-2	-10 ± 8	2	0	-2	6 ± 2	-1	1	1
2	16 ± 12	-15	0	29	-7 ± 8	7	-4	-7	10 ± 4	0	1	1
3	37 ± 22	-27	3	31	-1 ± 7	0	-2	-3	13 ± 5	-2	0	-1
4	75 ± 44	2	-30	83	7 ± 6	1	-6	0	16 ± 8	-3	2	-2
5	102 ± 53	50	13	13	14 ± 5	-1	4	-2	19 ± 8	0	0	-1
6	116 ± 63	-47	88	1	20 ± 4	0	1	0	21 ± 8	2	-3	0
7	92 ± 47	10	47	23	22 ± 3	0	0	0	22 ± 7	2	1	-2
8	92 ± 38	33	0	-36	21 ± 3	-2	1	-1	19 ± 7	-1	-2	0
9	85 ± 67	-31	83	71	17 ± 5	1	1	2	15 ± 6	-1	-2	-1
10	57 ± 43	93	13	42	9 ± 6	1	-3	-3	10 ± 5	-1	-1	-1
11	31 ± 32	-29	-5	-1	0 ± 7	-1	-4	-2	6 ± 3	0	-1	0
12	20 ± 13	-10	29	17	-7 ± 7	0	1	0	5 ± 2	0	0	0
Growing season §	563 ± 157	108	244	114	17 ± 5	-1	4	-4	17 ± 8	1	-8	-5

§ May to October.

Table 2.2. Long-term average (LTA) of monthly total precipitation, average temperature, and solar radiation at SROC, Waseca, MN. Values followed by ± LTA are one standard deviation. Experimental years 2017, 2018, and 2019 are shown as departures from the LTA.

Month	**Total precipitation (mm)**				**Average Temperature (°C)**				**Solar Radiation (M J m^{-2})**			
	LTA	**2017**	**2018**	**2019**	**LTA**	**2017**	**2018**	**2019**	**LTA**	**2017**	**2018**	**2019**
1	32 ± 21	5	15	1	-10 ± 7	3	-2	-1	6 ± 2	-1	0	-1
2	30 ± 17	9	-1	47	-8 ± 7	6	-4	-6	10 ± 4	0	1	-1
3	58 ± 25	-20	-28	-7	-1 ± 7	0	-1	-4	13 ± 5	-2	0	1
4	86 ± 38	-14	4	22	7 ± 6	2	-7	-1	16 ± 8	-3	3	-2
5	115 ± 43	14	19	46	15 ± 5	0	4	-3	18 ± 8	0	1	-2
6	139 ± 57	-34	8	-54	20 ± 4	1	1	0	21 ± 7	2	-4	-2
7	124 ± 60	42	-13	39	22 ± 3	1	0	1	22 ± 6	1	0	-3
8	105 ± 48	-6	16	30	21 ± 3	-2	0	-1	19 ± 6	-2	-2	0
9	102 ± 71	-51	165	68	17 ± 5	1	1	2	15 ± 6	1	-2	-3
10	70 ± 46	35	11	81	9 ± 6	1	-3	-2	10 ± 5	-2	-2	-2
11	47 ± 39	-43	-13	11	1 ± 6	-1	-5	-3	6 ± 3	0	-1	-1
12	40 ± 22	-17	14	1	-7 ± 7	-2	2	1	5 ± 2	0	3	0
Growing season §	678 ± 191	-21	184	210	14 ± 6	2	3	-4	17 ± 8	0	-9	-12

§May to October.

Table 2.3. Significance of fixed effects on corn and soybean grain, cover crop (CC) biomass and ground cover, and Tau (ratio of below- to above- PAR).

Source of variation	Response variable							
	Grain yield		CC biomass		CC ground cover		Tau	
	Corn	Soybean	Corn	Soybean	Corn	Soybean	Corn	Soybean
Year (Y)	0.000***	0.000***	0.733	0.000***	0.036*	0.000 ***	0.000***	0.000***
Location (L)	0.140	0.005**	0.001**	0.011*	0.001 **	0.004**	0.000***	0.004**
Tillage practice (T)	0.003**	0.239	0.019*	0.080.	0.103	0.034 *	0.310	0.157
Cover crop strategy (C)	0.697	0.763	0.086.	0.008**	0.000 ***	0.083.	0.982	0.892
Y x L	0.000***	0.007**	0.000***	0.017*	0.000 ***	0.15	0.527	0.000 ***
Y x T	0.000***	0.307	0.526	0.329	0.840	0.315	0.024*	0.817
L x T	0.694	0.000***	0.560	0.546	0.013 *	0.097.	0.039*	0.029 *
Y x C	0.437	0.939	0.032*	0.003**	0.034 *	0.013 *	0.996	0.968
L x C	0.831	0.599	0.543	0.290	0.172	0.304	0.956	0.996
T x C	0.265	0.729	0.893	0.924	0.639	0.892	0.994	0.999
Y x L x T	0.019*	0.017*	0.344	0.434	0.111	0.101	0.510	0.386
Y x L x C	0.504	0.836	0.713	0.380	0.678	0.227	0.981	0.998
Y x T x C	0.623	0.998	0.166	0.977	0.156	0.972	0.870	0.999
L x T x C	0.448	0.606	0.220	0.944	0.275	0.931	0.746	0.987
Y x L x T x C	0.581	0.994	0.767	0.962	0.935	0.942	0.971	0.999

Significance of fixed effects (P > F) on response: corn and soybean yield, cover crop biomass and ground cover, and relative tau at Lamberton and Waseca, MN in 2017-2019. Within each column, numbers followed by ***, **, *, and a single dot are significant at 0.001, 0.01, 0.05, and 0.1 level.

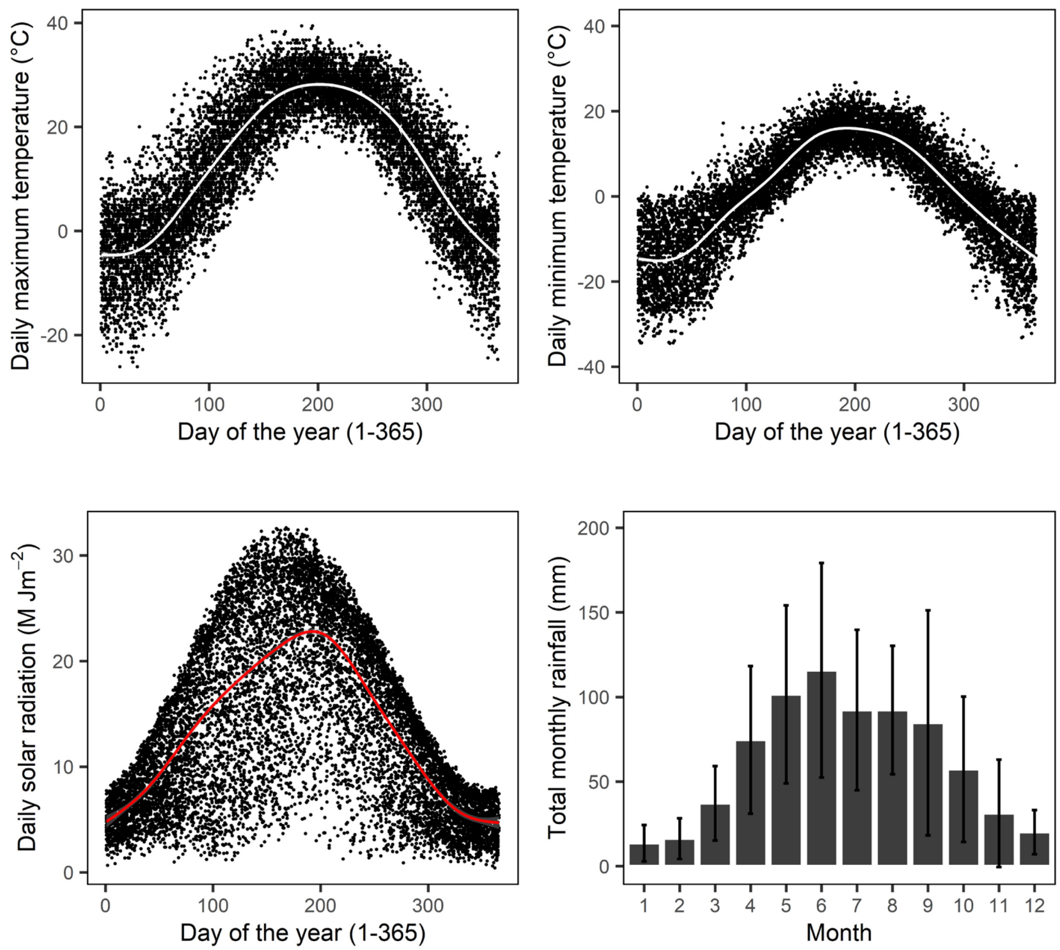

Figure 2.1. Long-term weather conditions at SWROC near Lamberton, MN, USA. Lines indicate long-term average, and points are daily values, DOY 1-365 corresponds to 1-Jan- 31-Dec, error bars represent one standard deviation for the 30-yr period.

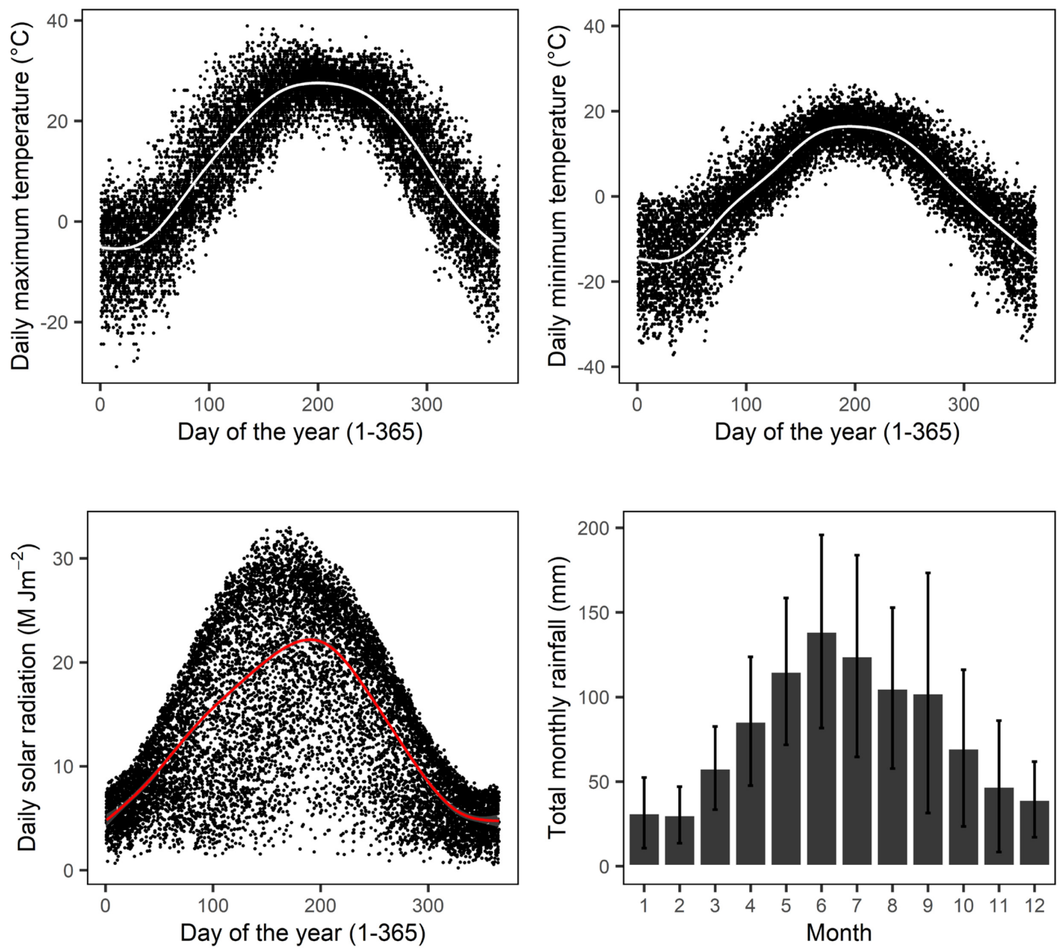

Figure 2.2. Long-term weather conditions at SROC Waseca, MN, USA. Lines indicate long-term average, and points are daily values, DOY 1-365 corresponds to 1-Jan- 31-Dec, error bars represent one standard deviation for the 30-yr period.

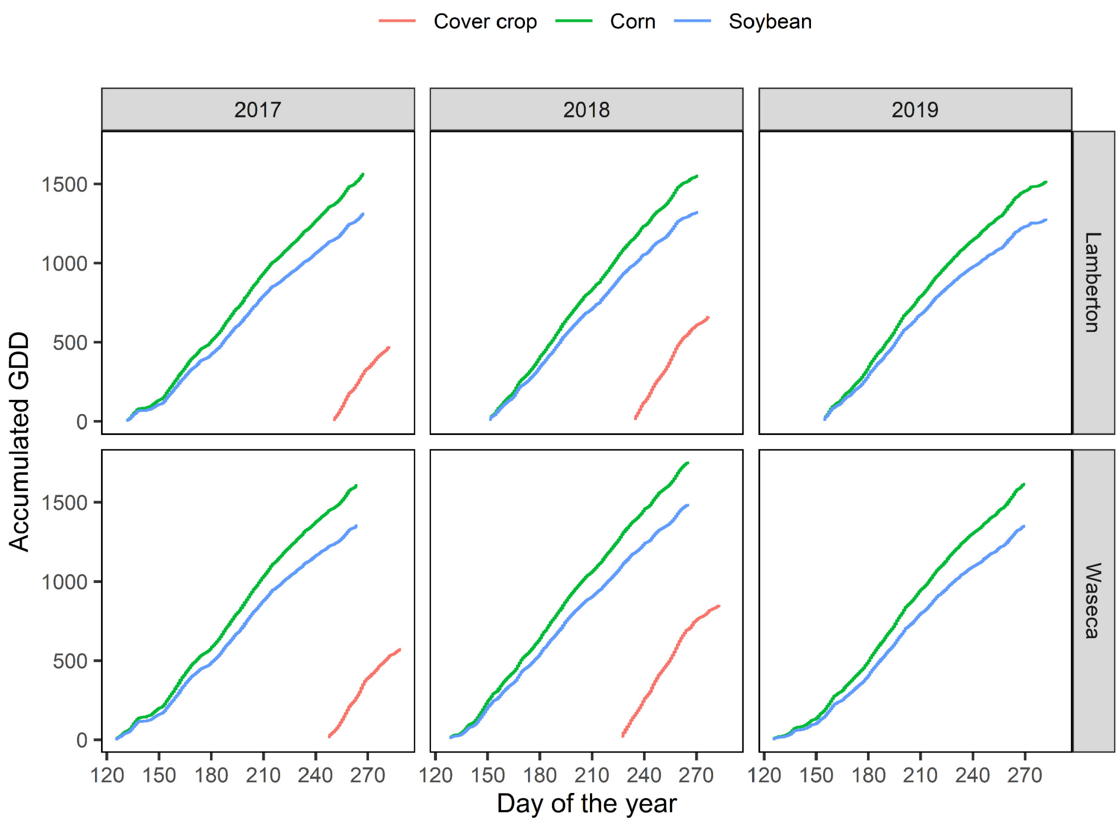

Figure 2.3. Growing Degree Day accumulation in corn and soybean and cover crops at SWROC near Lamberton, MN, USA; and SROC Waseca, MN, USA from 2017 to 2019.

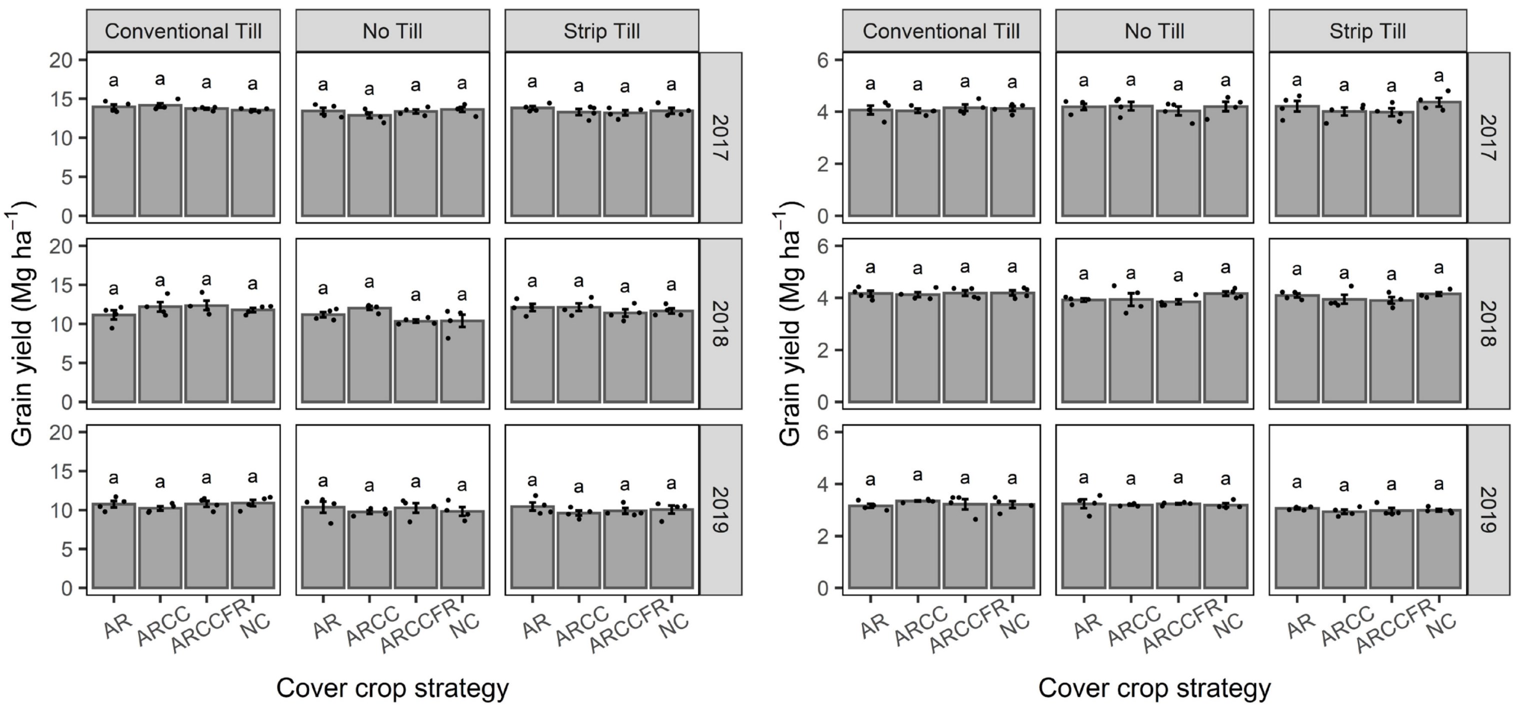

Figure 2.4. Corn (left) and soybean (right) grain yield at SWROC near Lamberton, MN, USA, from 2017 through 2019. Bars followed by the same letters are not significantly different at $P \leq 0.05$ within each tillage practice each year. Error bars represent SEM (n=4), and each dot represents data points. AR denotes annual ryegrass; ARCC denotes annual ryegrass + crimson clover; ARCCFR denotes annual ryegrass + crimson clover + forage radish; NC denotes no cover (control).

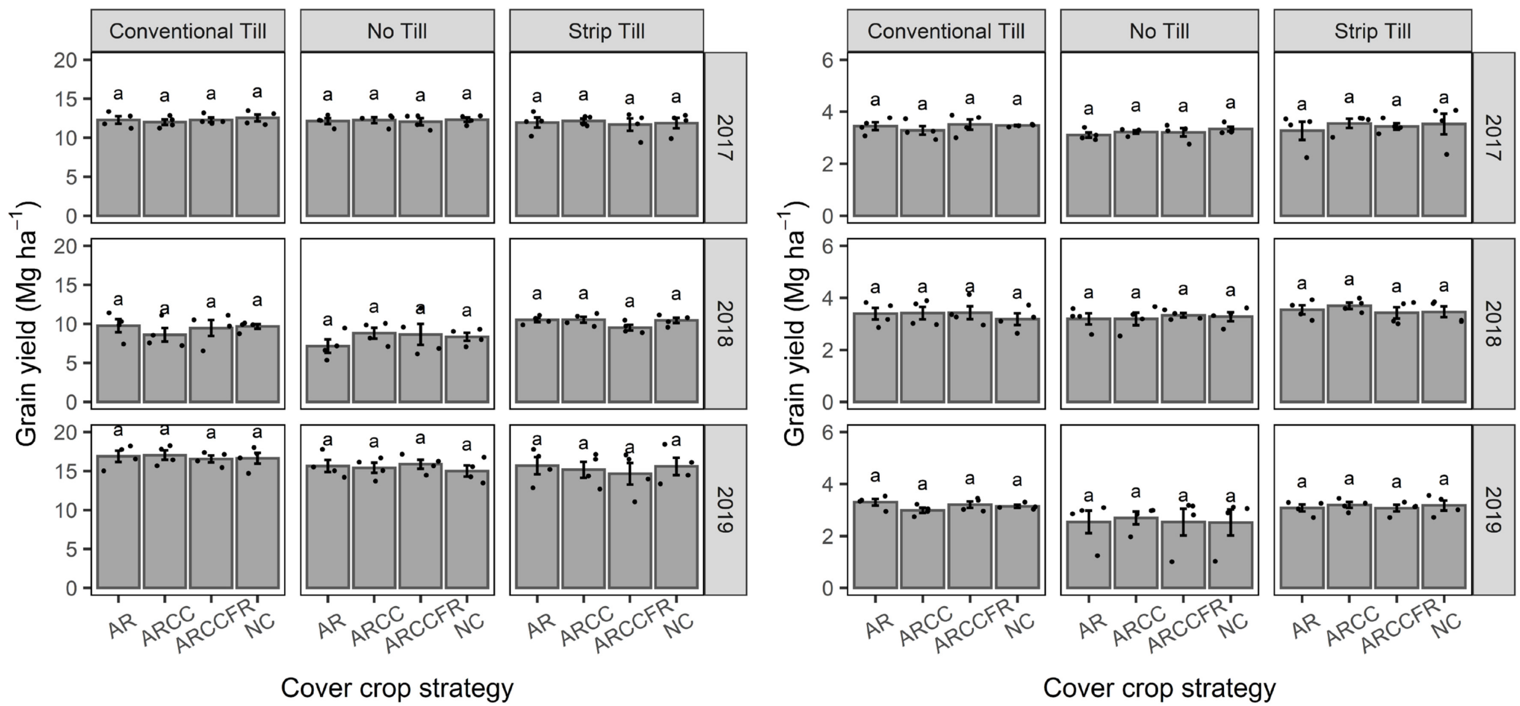

Figure 2.5. Corn (left) and soybean (right) grain yield at SROC, Waseca, MN, USA, from 2017 through 2019. Bars followed by the same letters are not significantly different at $P \leq 0.05$ within each tillage practice each year. Error bars represent SEM (n=4), and each dots represent data points. AR denotes annual ryegrass; ARCC denotes annual ryegrass + crimson clover; ARCCFR denotes annual ryegrass + crimson clover + forage radish; NC denotes no cover (control).

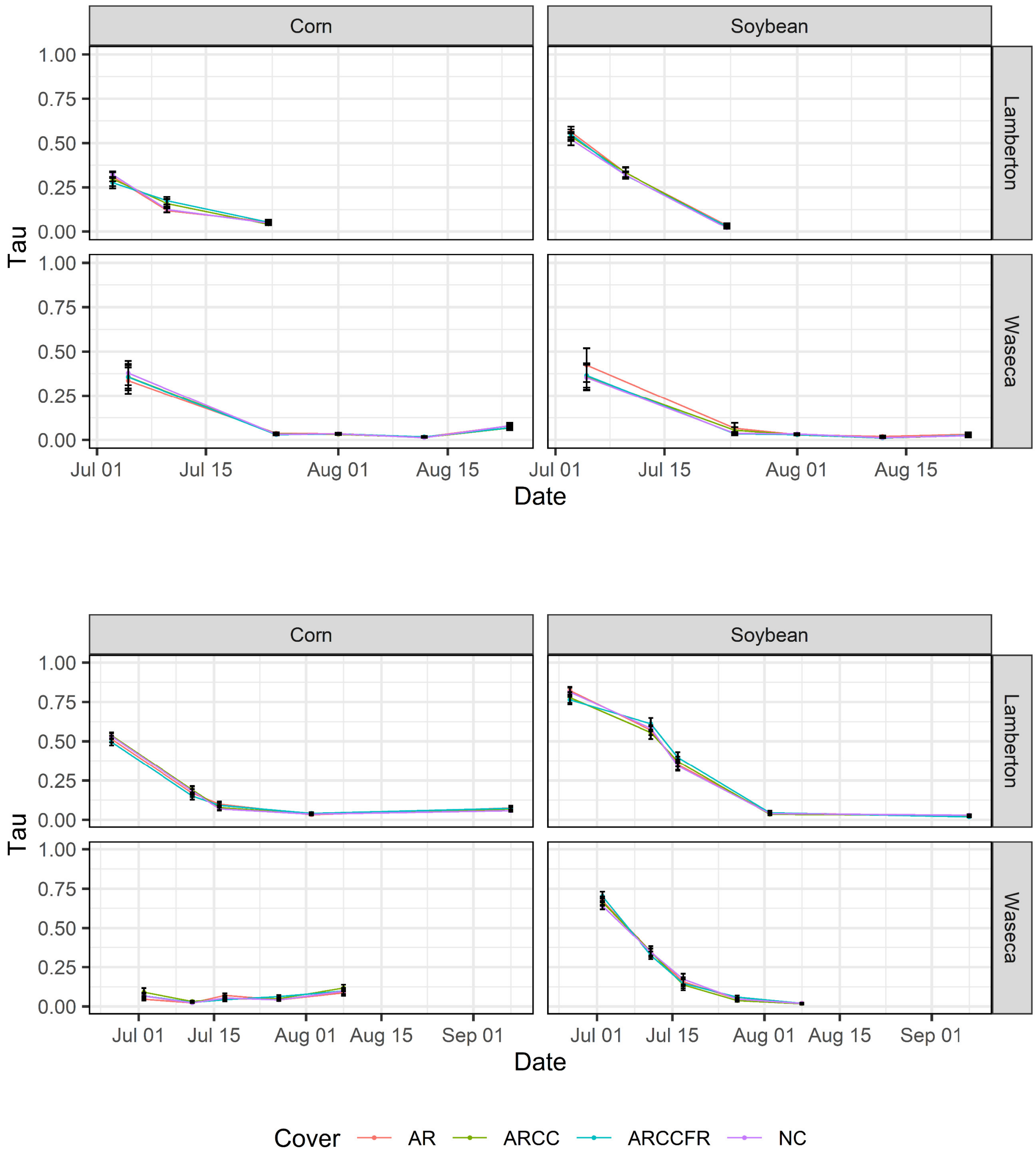

Figure 2.6. The ratio of below- to above-PAR (Tau) at SROC, Waseca, and SWROC near Lamberton, MN, USA, in 2017 (top) and 2018 (bottom). Error bars represent SEM (n=4). AR denotes annual ryegrass; ARCC denotes annual ryegrass + crimson clover; ARCCFR denotes annual ryegrass + crimson clover + forage radish.

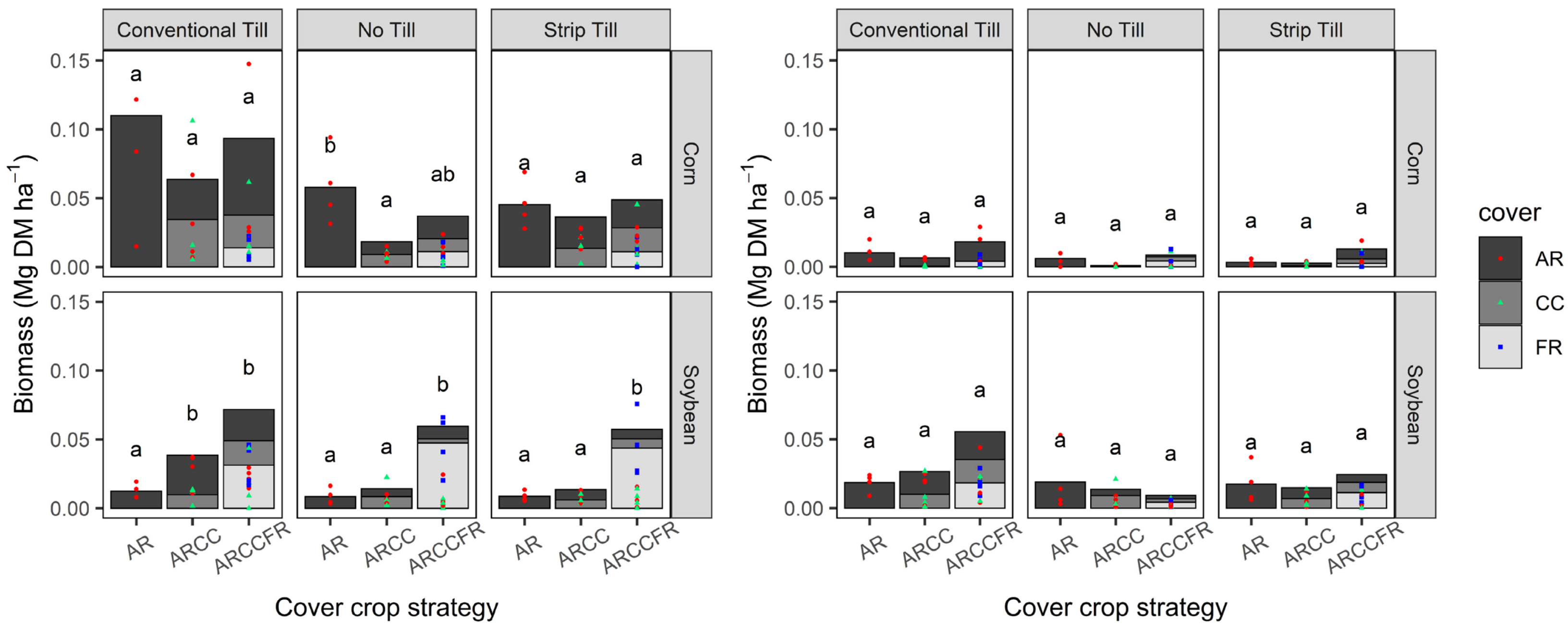

Figure 2.7. Cover crop dry biomass produced in fall 2017 (left) and fall 2018 (right) at SWROC near Lamberton, MN, USA. Different colors within a bar represent different species within a mix, and each dots represent data points (n=4 for each cover crop species within each cover crop strategy). AR denotes annual ryegrass; ARCC denotes annual ryegrass + crimson clover; ARCCFR denotes annual ryegrass + crimson clover + forage radish.

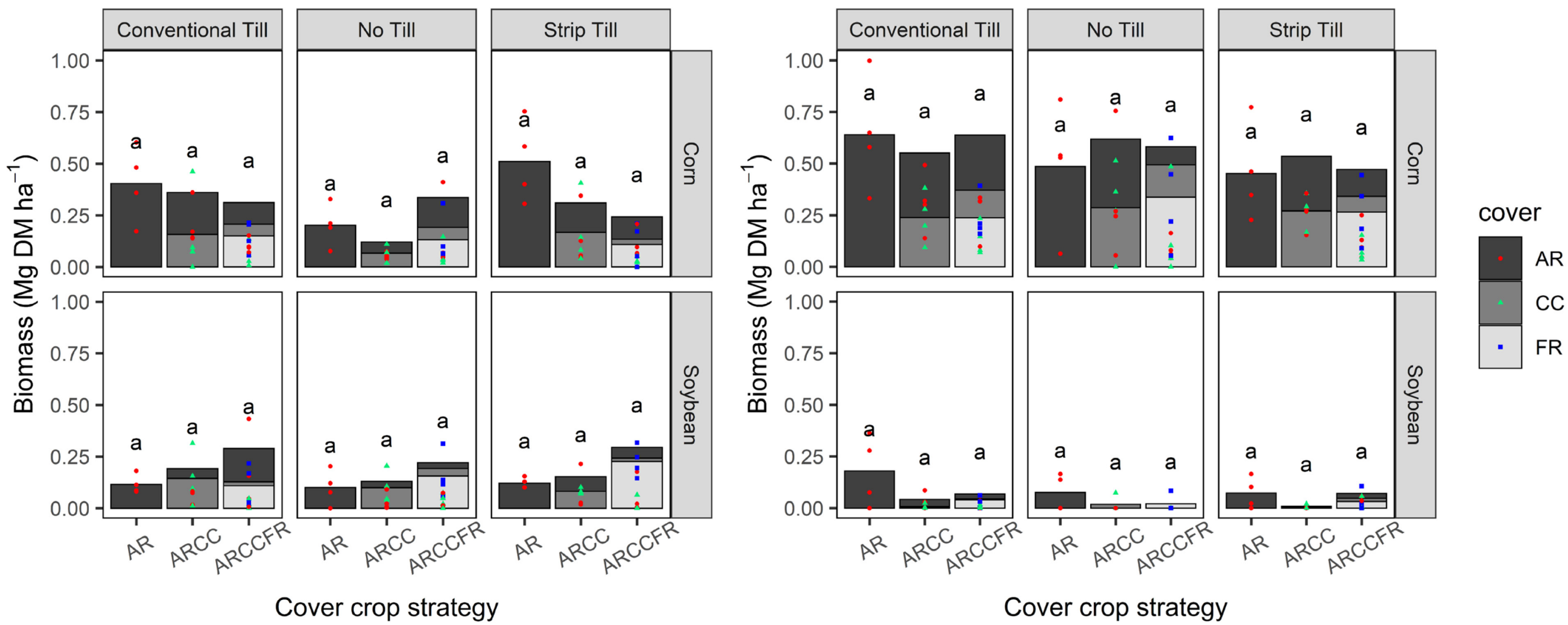

Figure 2.8. Cover crop dry biomass produced in fall 2017 (left) and fall 2018 (right) at SROC, Waseca, MN, USA. Different colors within a bar represent different species within a mix, and each dots represent data points (n=4 for each cover crop species within each cover crop strategy). AR denotes annual ryegrass; ARCC denotes annual ryegrass + crimson clover; ARCCFR denotes annual ryegrass + crimson clover + forage radish.

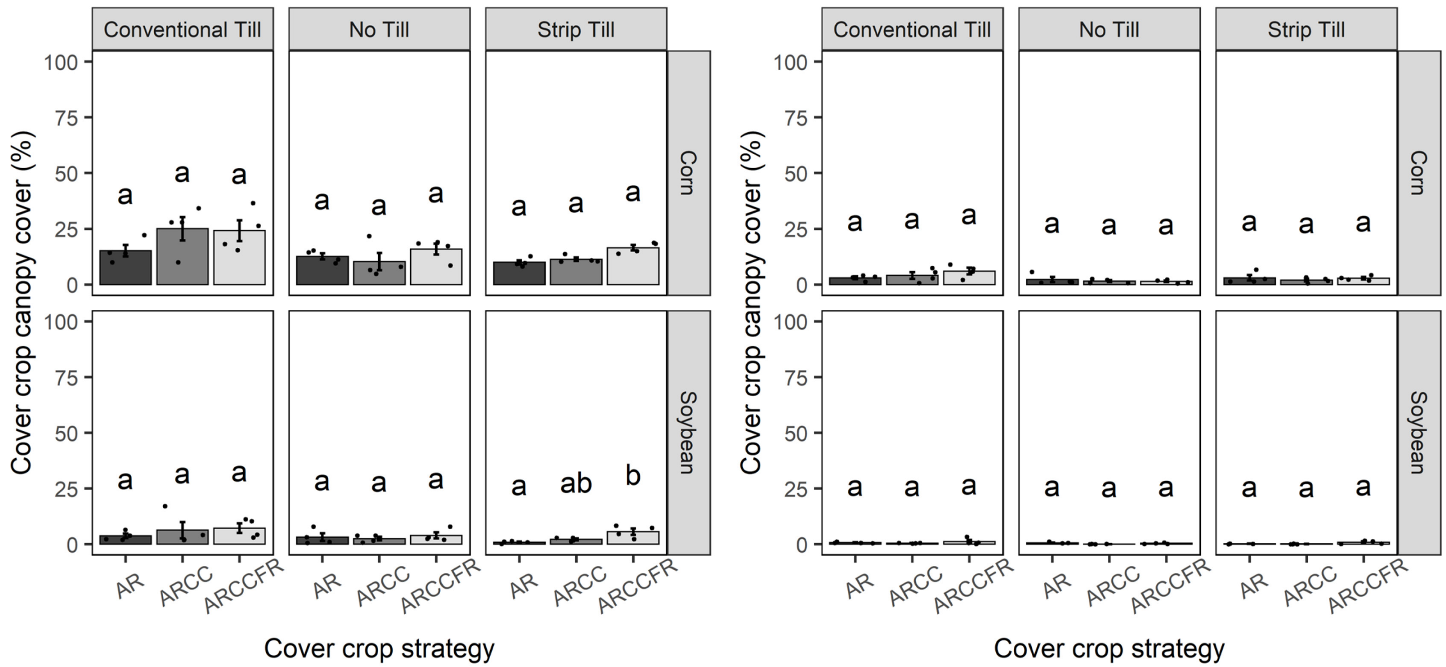

Figure 2.9. Cover crop canopy cover in fall 2017 (left) and fall 2018 (right) at SWROC near Lamberton, MN, USA. Bars followed by the same letters are not significantly different at $P \leq 0.05$ within each tillage practice each year. Error bars represent SEM (n=4), and each dots represent data points. AR denotes annual ryegrass; ARCC denotes annual ryegrass + crimson clover; ARCCFR denotes annual ryegrass + crimson clover + forage radish.

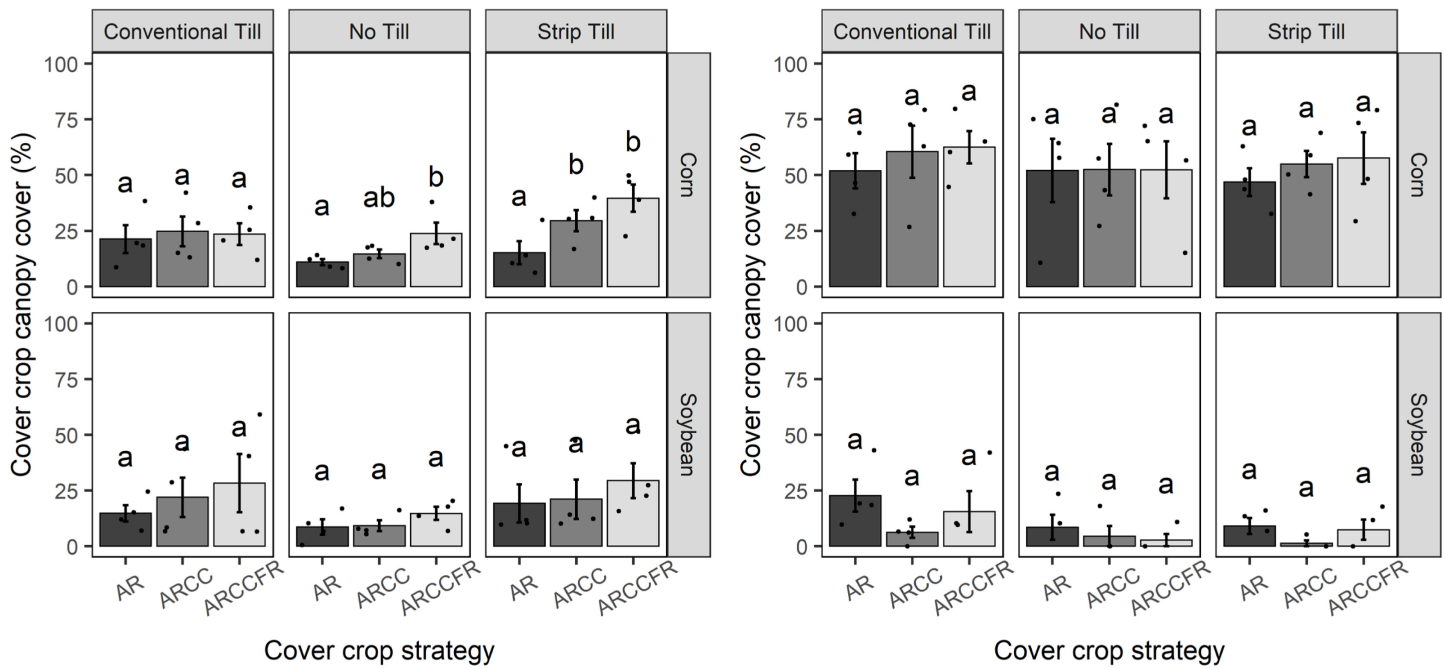

Figure 2.10. Cover crop ground canopy cover in 2017 (left) and 2018 (right) at SROC, Waseca, MN, USA. Bars followed by the same letters are not significantly different at P ≤ 0.05 within each tillage practice each year. Error bars represent SEM (n=4), and each dots represent data points. AR denotes annual ryegrass; ARCC denotes annual ryegrass + crimson clover; ARCCFR represents annual ryegrass + crimson clover + forage radish.

3 CHAPTER 3: NITROGEN DYNAMICS AFFECTED BY TILLAGE PRACTICES AND WINTERKILLED COVER CROPS WITHIN CORN-SOYBEAN ROTATION

Summary

The benefits of cover crops are known for a long time. Cover crop use as a tool to reduce the negative consequences of specialized farming practices is on the rise in the U.S. upper Midwest. However, most research conducted in the region has assessed the potential of overwintering annual cover crops, and very little is known about the potential benefits associated with the inclusion of winterkilled cover crops. Winterkilled cover crops are an attractive option to growers if they can provide ecosystem services similar to overwintering annual cover crops. The goal of this study was to advance our understanding of the fate of N as affected by late interseeded winterkilled cover crops in corn and soybean rotation within different tillage practices. Specific objectives were to 1) assess the effect of cover crops in the physicochemical properties of soil within three tillage practices, 2) estimate the N uptake of cover crops within three tillage practices, and their effect in corn and soybean N uptake, 3) assess the potential of cover crops to reduce residual soil NO_3-N, 4) determine the effect of cover crops on net N mineralization within three tillage practices.

Two experiments were conducted within two long-term tillage trials located in Lamberton and Waseca, Minnesota. In the first experiment, cover crops were hand-broadcast at R5-R6 corn and R7-R8 soybean in fall 2017 and R3-R4 corn and R5-R6 soybean in fall 2018. Tillage practices were conventional-, strip-, and no-till; and cover crop strategies included annual ryegrass (*Lolium multiflorum* Lam.) monoculture,

denoted as AR; a two-way mixture of annual ryegrass and crimson clover (*Trifolium incarnatum* L.), denoted as ARCC; and a three-way mixture of annual ryegrass, crimson clover, and forage radish (*Raphanus sativus* L.), denoted as ARCCFR, with a no-cover as control, denoted as NC.

Cover crop strategy and tillage practices significantly affected soil organic matter at 0-20 cm layer in spring after two seasons of cover cropping in Lamberton. The highest organic matter was present in the three-way mix of ARCCFR (4.3 mg kg^{-1}), followed by a two-way mix of ARCC (4.22 mg kg^{-1}), NC (4.2 mg kg^{-1}), and AR (4.1 mg kg^{-1}). Within tillage practices, NT had the highest soil organic matter (4.25 mg kg^{-1}), followed by CT (4.2 mg kg^{-1}) and ST (4.02 mg kg^{-1}). Nitrogen accumulation in corn-soybean stover and grain was not affected by cover crop strategy or tillage practices during the whole study. Nitrogen accumulation in cover crop biomass varied by cover crop strategy within corn and soybean, and by cover crop strategy and tillage in soybean. However, the N accumulation in cover crop biomass was relatively marginal. The three-way mix of ARCCFR accumulated more N (9.25 kg ha^{-1}) than ARCC (6.57 kg ha^{-1}) and AR monoculture (7.25 kg ha^{-1}) when pooled over years and location within corn. In soybean, N accumulation was 3.98, 1.56, and 2.28 kg ha^{-1} in ARCCFR, ARCC, and AR treatments. Pooled over the years, cover crop N accumulation was significantly higher in Waseca (9.08 kg ha^{-1}) than in Lamberton (1.08 kg ha^{-1}).

The two-way mixture of ARCC strategy consistently had a higher C:N ratio than the AR monoculture and the three-way mix of ARCCFR strategy, even though it produced lower biomass throughout the study. The pooled averages of the C:N ratio of ARCC was 12.1, and was 11.4 and 9.92 for AR and ARCCFR, respectively.

Cover crop strategy did not affect soil NO_3-N. Residual NO_3-N in the soil was always higher in the fall and the next spring than at the time of cover crop seeding. Results showed no consistent pattern in NO_3-N in the soil solution during the study. Net N mineralization during the growing season showed a decreasing trend as the season progressed, with higher net N mineralization within corn as compared to soybean.

Our results suggest that winterkilled cover crops interseeded late in the season did not provide ecosystem services within corn and soybean. Although these cover cropping strategies accumulated and immobilized some N in the fall, N mineralized before it was available to the next season corn, mainly due to low C:N ratio. We found in this study that winterkilled cover crops interseeded late in the season did not affect ecosystem services. Therefore, it may be beneficial to interseed winterkilled cover crops earlier in the season, rather than late, so that cover crops could produce enough biomass and provide ecosystem benefits during the same growing season. However, more research is needed to assess the potential of winterkilled cover crops to produce biomass and provide ecosystem services when planted earlier in the season for conditions in the U.S. upper Midwest.

3.1 Introduction

Nitrogen (N) is one of the most essential nutrients to corn (*Zea mays* L.) growth (Sinclair and Horie, 1989). The advent of the Haber Bosch process is attributed to increasing agricultural productivity (Erisman et al., 2008), generating synthetic N fertilizers that make the nutrient readily available to crops. Corn responds well to N, and growers tend to apply more than needed in their quest to reduce risk and maximize yield (Sela et al., 2016). Unused N, however, is lost via leaching and runoff, causing environmental impairment. As a consequence, the N use efficiency (NUE) of corn is estimated to be only 32% (Raun et al., 2002).

Cover crops use may help to mitigate the negative consequences of intensified farming in global agriculture. The practice is proven to provide ecological benefits, even in the cold and harsh climate like in the upper Midwest U.S. (Strock et al., 2004; Snapp et al., 2005; Kladivko et al., 2014; Blanco-Canqui et al., 2015). However, adoption of cover crops among farmers is not widespread in the region, with barriers such as weather conditions (Rusch et al., 2020), input costs (Roth et al., 2018), prolonged wet soil conditions in the spring, which delay planting of major crops in a region with a short growing season (Vetsch et al., 2019), and lack of knowledge on the potential benefits/drawbacks like synchrony of N release from cover crops and corn N demand (Nevins et al., 2020).

Several studies have shown that cover crops can produce enough biomass during the growing season to sequester and immobilize N from the soil (Finney et al., 2016; Thapa et al., 2018; Ruis et al., 2019). Most of these studies include winter annual cover crops, mainly cereal rye (*Secale cereale* L.), which withstand the winter conditions of the region. Cereal rye has shown to have consistent and high biomass production and thus effective in uptaking residual NO_3-N and reducing N losses to groundwater (Clark and Sustainable Agriculture Research & Education (Program), 2007). However, less is known about the efficacy of winterkilled cover crops in providing agroecological benefits. Similar to overwintering cover crops management in the region, winterkilled cover crops may be either early or late interseeded in the major crops growing season. Regardless of the cover crop used, questions remain about the synchrony of N release from their residue decomposition and uptake by the next major crop during the growing season (Nevins et al., 2020).

The C:N ratio of cover crops plays an essential role in determining N mineralization and immobilization and on the yield of the following crop (Kuo and Sainju, 1998; Poffenbarger et al., 2015; Finney et al., 2016). The C:N ratio is considered as a significant predictor of ecosystem services provided by cover crops (Finney et al., 2016). In northern climates like in the U.S. upper Midwest, where the effect of weather conditions challenge the diversification of crop production systems, C:N ratio could help to find opportunities for cover crops use. It is, therefore, important to determine cover crop C:N to assess their potential for ecosystem services and the fate of N in the growing season of major crops.

Nitrogen mineralization is the conversion of organic N into plant-available inorganic forms of ammonium (NH_4^+) and nitrate (NO_3^-); N immobilization is the opposite process (Hart et al., 1994). Cover crops affect the N cycle through mineralization and immobilization. Understanding the net mineralization of soil in the growing season can help to improve the prediction of N availability (Snapp and Borden, 2005) and fertilizer recommendations.

Understanding N dynamics in corn-soybean grown within different tillage practices following late interseeded, winterkilled cover crops may help to predict N availability in the system. To the best of our knowledge, the efficacy of late interseeded, winterkilled cover crops to provide ecosystem services through the uptake of residual N is not well documented in the literature, nor is their potential to affect physicochemical properties and soil NO_3-N dynamics during the growing season. Furthermore, the inclusion of cover crops within no-till corn systems can be challenging, both in terms of growth and termination (Eckert, 1988). Previous studies report contrasting results relating to the fate of N in cover crops within different tillage practices (Eckert, 1988; Kessavalou and Walters, 1997; Sainju et al., 2002, 2007), suggesting that further studies are needed.

The goal of this study was to advance our understanding of the fate of N as affected by late interseeded winterkilled cover crops in corn and soybean rotation within different tillage practices. We hypothesized that late interseeded winterkilled cover crops would affect the fate of N in corn and soybean agroecosystems. Specific objectives were to 1) assess the effect of cover crops on the physicochemical properties of soil within three tillage practices, 2) estimate the N uptake of cover crops within three tillage practices, and their effect in corn and soybean production, 3) assess the potential of cover crops to reduce residual soil NO_3-N, 4) determine the effect of cover crops on net N mineralization within three tillage practices.

3.2 Materials and Methods

3.2.1 Field Experiments

Field experiments were conducted at the University of Minnesota's Southern Research and Outreach Center at Waseca, MN (44°04'05.97" N 93°31'19.02" W) and Southwest Research and Outreach Center near Lamberton, MN (44°14'02.20" N 95°18'6.87" W). The soil type in the Waseca site is a Webster clay loam (fine-loamy, mixed, superactive, mesic Typic Endoaquolls), and the Lamberton site is dominated by Normania loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls) and Amiret loam (fine-loamy, mixed, superactive, mesic Calcic Hapludolls) soil (Soil Survey Staff, 2020).

The first experiment focused on estimating N used and exported by crops, namely in crop biomass and grain, along with cover crop C:N ratio and residual soil N. The experiment was conducted from spring 2017 to fall 2019. A second experiment (hereafter called buried bag experiment) was nested within the first experiment to determine the N mineralization potential throughout the 2018 and 2019 corn and soybean growing seasons. Cover crops were hand-interseeded in late 2017 at R5-R6 corn and R7-R8 soybean stage. In 2018, cover crops were hand-interseeded 15 d earlier than 2017 at the R3-R4 corn stage and R5-R6 soybean stage to provide the opportunity for an extended growth period.

3.2.2 Experimental design

The first experiment was designed as a split-split plot with four replications in each site-year. Primary crop (corn and soybean) was the main plot, tillage practice [conventional-till (CT), strip-till (ST), and no-till (NT)] was the sub-plot, and cover crop strategy [annual ryegrass (*Lolium multiflorum* L.), AR; AR+ crimson clover (*Trifolium incarnatum* L., CC), ARCC; and AR + CC + forage radish (*Raphanus sativus* L., FR), ARCCFR; with a no-cover control, NC] was the sub-sub-plot. Each experimental unit was 4.5 m wide (six 76-cm rows) and 16.5 m long in Waseca, and 3.75 m (five 76-cm rows) wide and 20 m long in Lamberton. The buried bag study, nested in the first experiment, was set as a classic split-plot in time with three replicates each site-year. The treatments in this experiment were the same from the first experiment. However, repeated measures were taken from the buried bags in a fixed interval of 15 d.

3.2.3 Agronomic Management

Strip-till was performed 15 d before planting corn and soybean to a depth of 15 cm and in 20 cm wide strips with an 8-row, 76 cm row spacing strip-tiller at both locations. At the Lamberton site, CT was performed 1 d before planting in corn and soybean plots to a depth of 15 cm using a chisel plow. At the Waseca site, however, CT was performed 1d before planting soybean using a disc ripper, and a field cultivator and corn plots were only field cultivated to the depth of 10 cm.

At Waseca, glyphosate [N-(phosphonomethyl)glycine] was applied before planting (1.26 kg a.e. ha^{-1}) and two additional applications at V3-V4 (0.86 kg a.e. ha^{-1}) corn stages. In Lamberton, glyphosate was applied once (0.84 kg a.e. ha^{-1}) along with Fusilade (Fluazifop-P-butyl) (0.13 kg ae ha^{-1}) at planting. Corn hybrid DKC49-72RIB (99-d RM RR2) was planted in both locations at the rate of 89,000 seeds ha^{-1}. Soybean variety AG2035 was planted in both locations at the rate of 371000 seeds ha^{-1}, except in

2019 at Lamberton, when the variety AG20X9 was used. Both crops were planted at a depth of 5 cm in 76-cm wide rows using a four-row John Deere 1700 MaxEmerge series planter at both locations.

The buried bag experiment was initiated before fertilizing the fields. First, soil samples were obtained at 0-15 cm soil layer at seven points within a plot with a regular soil auger (AMC Inc., ID), and then combined to make a composite sample. Polyethylene bags were then filled with ~0.5 kg soil and buried equidistant at 15-cm depth in the harvest row of each plot for in-situ incubation. Wire stake flags were used to poke holes to allow oxygen flow and pin each bag to the ground to determine the location of the bags later in the season. Within no-tilled plots, polyethylene bags were placed over the soil surface to mimic no-tilled conditions. Bags were recovered in 15-d intervals.

Fertilizer amounts were estimated for highly productive corn, according to the University of Minnesota guidelines (Kaiser et al., 2011). A total of 163 kg N ha^{-1} in the form of urea [NH_2-CO-NH_2] along with 17 kg S ha^{-1} in the form of gypsum [$CaSO_4$ $2H_2O$] were applied in Waseca; 63 kg N ha^{-1} at planting, and the remaining at the V6 corn stage. In Lamberton, a total of 200 kg N ha^{-1} was applied at planting along with 17 kg S ha^{-1} in the form of gypsum. At both sites, Agrotain®, [N-(n-butyl) thiophosphoric triamide, NBPT], a urease inhibitor, was applied each time urea was used.

Cover crop seeds were weighed separately for each species in the lab then mixed in the field at seeding. Cover crops were hand broadcast below the canopy in both corn and soybean to avoid seeds landing in the canopy. In 2017, cover crops were hand broadcast at the R5-R6 corn stage and R7-R8 soybean stage (early-Sept) at both locations. However, in 2018, cover crops were hand broadcast at the R4 stage corn and R6 stage soybean (mid-Aug) in our effort to increase growing degree days, and thereby extend the period of cover crop growth.

3.2.4 Data collection

Soil sampling for physicochemical analysis was conducted in the spring before planting primary crops, in the summer before seeding cover crops, and in the fall before frost at both locations. Soil samples were not collected in spring 2017 in both locations. In spring 2018, soil cores of 5 cm width and 40 cm depth were taken with a hydraulic core sampler (Giddings Machine Company Inc., Windsor, CO, USA) mounted to the rear of a tractor. Each soil core was divided into 0-20 and 20-40 layers and placed in separate paper bags. Soil samples were taken at 0-15 and 15-30 cm layers before seeding cover crops and at fall frost. Three soil sub-samples per plot were taken with a hand-operated soil core sampler to make a composite sample at the time of seeding cover crops. Soil samples were air-dried and ground using a Dynacrush soil crusher (Custom Laboratory Equipment Inc., Holden, MO, USA) and later used for physicochemical analysis. All physicochemical analyses, including extractable NO_3- N, pH, OM, Bray P, CEC, K^+, Ca^{+2}, and Mg^{+3} were conducted at the Minnesota Valley Testing Laboratory (www.mvtl.com), New Ulm, MN, USA.

The concentration of NO_3-N in the soil solution was obtained by collecting samples with ceramic suction cups of 0.1 MPa air-entry pressure (Soilmoisture Equipment Corp., Goleta, CA, USA). Ceramic suction cups were installed at 1 m depth in the harvest row of one plot per treatment only. A hand pump was used to create a 50 KPa vacuum 3-5 d before sample collection. Soil solution was sampled weekly. A total of 38, 48, and 42 samples were collected in 2017, 2018, and 2019, respectively. Soil solution was collected in 50 mL centrifuge tubes and frozen until laboratory analysis. The concentration of NO_3-N in the soil solution was determined by Vanadium (III) reduction via the manual spectrophotometric procedure (Doane and Horwáth, 2003).

In the buried bag experiment, soil from each bag was air-dried and ground. A 1g soil sample was transferred to 15ml centrifuge tubes followed by the addition of 10 mL of 2M KCl and vigorously agitated with a shaker. Next, samples were centrifuged for 5 min, and the extracts used to obtain NO_3-N and NH_4-N using the Vanadium (III) reduction method (Doane and Horwáth, 2003) and the salicylate microplate method (Bower and Holm-Hansen, 1980), respectively. The soil samples were stored at 4°C if they were not processed immediately after removal from the field. Net Nitrogen mineralization was calculated as the difference between the final and the initial total inorganic N ($NO_3^- + NH_4^+$) divided by the length of the in-situ incubation and was expressed on a gravimetric basis (μg N g^{-1} dry soil d^{-1}). Net N mineralization was calculated as described in (Snapp and Borden, 2005). The calculations are as follows:

$$Net\ Mineralization = \frac{\left[(NO_3^- + NH_4^+)_{T_n} - (NO_3^- + NH_4^+)_{T_0}\right]}{\Delta T}$$

where,

T_0 = time 0, when the bags were buried,

T_n = time n, in days, when the bags were removed, and

ΔT = difference between T_0 and T_n.

Above ground biomass of cover crop was sampled within a 0.1 m^2 quadrat in both locations after primary crop harvest to obtain N in tissue and C:N ratio. The biomass from cover crop mixes was separated by species and placed in brown paper bags. The biomass was later dried in a forced-air oven at 60°C until constant mass and then weighed. Cover crop biomass was ground using a 125 g electric spice grinder (Hamilton Beach Brands, Inc). Subsamples were transferred to whirl-Pak bags to determine the CNS content of the cover crops using a vario MACRO cube (Elementar Americas Inc, NY) via the combustion method.

Primary crops within 1 m length were cut at physiological maturity in 2017, 2018, and 2019 in Waseca; and in 2018 and 2019 in Lamberton. Ears were separated from the stover for corn, and grains were separated from soybean stover. Stover was shredded using a Kemp shredder (Kemp CO, Lititz, PA, USA) for both corn and soybean. Chipped corn and soybean stover were dried in a forced-air oven at 60°C until constant mass and weighed. Corn ears were shelled, and cobs were weighed separately. Individual samples were ground separately using Thomas Wiley Mill Model 4 with a 2-mm screen (Thomas Scientific LLC), and subsamples were transferred to whirl-Pak bags to determine CNS content with a vario MACRO cube (Elementar Americas Inc, NY) via the combustion method.

Corn and soybean grains were dried at 60°C, and a subsample was ground using Thomas Wiley Mill Model 4 with a 2-mm screen (Thomas Scientific LLC). Samples were then analyzed to determine CNS as described above, and subsequently to determine N content in corn and soybean grain and above ground biomass at maturity.

3.2.5 Statistical Analysis

Data were analyzed using the statistical computing software R (version 3.6.2; R Core Team, 2019). Nitrogen in primary crop grain and biomass, cover crop biomass, C:N ratio, soil physicochemical properties, residual soil NO_3-N, and soil NO_3-N dynamics were analyzed separately using linear mixed-effects model ANOVA to determine significant main effects and interactive effects using package '*lmerTest*' (Kuznetsova et al., 2017). Location, year, tillage, cover crop strategies, and their interactions were considered fixed effects, and appropriate split-plot error terms along with blocks within location within year were considered random effects. However, N mineralization potential within a growing season was analyzed separately for each season and year using functions from '*nlme*' package(Pinheiro et al., 2020). Visual representations were used to check the assumptions of normality and constant variance of the model residuals. Net N mineralization during each growing season was analyzed separately for each year, location, and crop for the sake of simplicity. Sampling date was modeled as a covariate to assess the trend of N mineralization over the season.

If the combined analysis resulted in significant interactions, separate ANOVA was then conducted on the response variables of interest. Tillage and cover crop strategies were considered fixed effects, and split-plot error terms were considered random effects. Block residuals were used to calculate the F statistic for tillage, and residuals from interactions between block and tillage were used to calculate F statistic for cover crop strategies. Post hoc comparisons of all estimated marginal means were made on the response variables using a conservative Bonferroni's adjusted p values using the '*emmeans'* package (Lenth et al., 2019). Compact letter displays for significant differences were obtained using the *'multcomp'* package (Hothorn et al., 2008).

3.3 Results and Discussion

3.3.1 Soil physicochemical properties

In the Lamberton site, two years of late interseeded winterkilled cover cropping did not affect pH, inorganic P, K^{+}, Ca^{+2}, Mg^{+3}, but influenced soil organic matter in spring 2019 (Table 3.1). When pooled over cover crop strategy, soil organic matter was highest in the ARCCFR strategy (4.3 mg kg^{-1}) followed by the 2-way mix of ARCC (4.22 mg kg^{-1}), NC control (4.2 mg kg^{-1}), and AR monoculture (4.09 mg kg^{-1}). Pooled over tillage practice, CT had slightly higher organic matter (4.3 mg kg^{-1}) than NT (4.27 mg kg^{-1}) and ST (4.05 mg kg^{-1}). This difference in organic matter in CT could be due to cooler and wetter conditions in ST and NT as compared to CT, which may have resulted in decreased mineralization of soil organic matter. Our results support those from Al-Kaisi and Yin (2005), a study conducted in Iowa, who also report no differences in organic matter content at depths below 10 cm among CT, ST, and NT. At the Waseca study site, however, no detectable effects of cover crops and tillage practices were observed in soil properties when measured in spring 2019 after two seasons of late interseeded cover cropping (Table 3.2).

3.3.2 Nitrogen accumulation in corn biomass and grain

We observed no significant differences between cover crop strategies in the total N in corn biomass and grain at both site years (Figure 3.1 and Figure 3.2). However, we found significant differences in grain N among tillage practice, where corn grain within

ST had more N than CT and NT when pooled over location and year (data not shown). Nitrogen in corn biomass varied between year and location (Table 3.3). When pooled over tillage and cover crop strategy, the highest N accumulation (148 kg ha^{-1}) in corn grain in 2019 was observed at Lamberton compared to 110 kg ha^{-1} in 2018 and 130 kg ha^{-1} in 2017. Nitrogen in corn grain (192 kg ha^{-1}) at Waseca was significantly higher in 2019 compared to 2018 (127 kg ha^{-1}) and 2017 (122 kg ha^{-1}). This difference is consistent with higher corn grain yield in 2019, which is reported in chapter 1. Pooled averages of N in corn grain across years was nearly identical among strategies—AR = 130 kg ha^{-1}, ARCC = 130 kg ha^{-1}, ARCCFR = 129 kg ha^{-1}, and NC = 128 kg ha^{-1} at Lamberton; and AR = 152 kg ha^{-1}, ARCC = 149 kg ha^{-1}, ARCCFR = 148 kg ha^{-1} and NC = 153 kg ha^{-1} at Waseca. These results indicate that cover crops and tillage did not affect N in corn biomass and grain. The difference of N in corn biomass and grain between years can be explained by the variation in weather conditions in each location. Since cover crops in this study are late interseeded and winterkilled, biomass produced was not substantial to affect N in corn grain and biomass.

Our results support those from studies conducted in the U.S. Midwest reporting that N accumulation by corn was near identical among cover cropped treatments (Basche et al., 2016). However, Basche et al. (2016) found differences in N accumulation in corn planted within different cover crop treatments earlier in the season, which could have been due to higher soil temperatures earlier in the season can affecting N uptake (Yoneyama et al., 1977) along with the N immobilization by the cover crops, which has also been reported by Nevins et al. (2020). However, a study on cereal rye cover crop in corn conducted in Michigan found no discernable effect of cover crop in corn N in biomass and grain compared to fallow corn (Snapp and Surapur, 2018), even though cover crops were seeded early in the growing season and produced a higher amount of biomass than our study. However, spring regrowth of winter annual cover crops like cereal rye can produce high spring biomass and immobilize N (Clark, 2007). In this study, cover crop strategy and tillage practice did not affect N in cover crops, which may be attributed to low biomass production.

3.3.3 Nitrogen accumulation in soybean biomass and grain

When pooled over tillage and cover crop strategy, at Lamberton N in accumulation in soybean grain in 2017 (234 kg ha^{-1}) was higher than in 2018 (223 kg ha^{-1}) and 2019 (145 kg ha^{-1}). Nitrogen accumulation in soybean grain was similar at Waseca among years, with 174 kg ha^{-1}, 170 kg ha^{-1}, and 162 kg ha^{-1} in 2017, 2018, and 2019, respectively. Pooled averages of N in soybean grain over years were similar across cover crop strategies, ranging from 195 to 202 kg ha^{-1} at Lamberton and 167 to 170 kg ha^{-1} at Waseca (Figure 3.3 and Figure 3.4). These results indicate that N in soybean grain was affected by years and location, rather than cover crop strategy and tillage practices. Similar to corn, the low biomass production of cover crops and early mineralization may explain no differences in N accumulation in soybean grain among treatments.

There is limited research information regarding N in soybean biomass and grain as affected by cover crops. A study conducted in Iowa, U.S. reports no effect of cover crops and tillage practices on soybean biomass (Karlen and Doran, 1991). More recently, another study in Iowa reported no effect of cover crops on N in soybean biomass throughout the growing season (Basche et al., 2016). It is crucial, however, to note that these studies included winter annual cover crops that produced an average of 1.63 Mg DM ha^{-1} in the spring. Our study included late interseeded winterkilled cover crops and produced an average of 0.073 Mg DM ha^{-1} of biomass. The limited biomass observed in this study can explain undiscernible differences among cover crop strategies on N in soybean biomass and grain.

3.3.4 Nitrogen in cover crop

Nitrogen in cover crops within corn was affected by location, cover crop strategy, year x location, and year x cover crop strategy interactions. Nitrogen in cover crops within soybean was affected by year, location, tillage, cover crop strategy, and the year x cover crop strategy interaction (Table 3.3).

When pooled over corn, N in cover crops at Lamberton was 2 kg ha^{-1} and 0.81 kg ha^{-1}on average in 2017 and 2018, respectively. Within soybean plots, cover crops in Lamberton accumulated 1.18 kg ha^{-1} in 2017 and 0.22 kg ha^{-1} in 2018. Nitrogen in cover crops within both corn and soybean at Waseca was significantly higher compared to Lamberton in both years; 9.22 kg ha^{-1} in 2017 and 18 kg ha^{-1} in 2018 following corn and 6.73 kg ha^{-1} and 1.83 kg ha^{-1} in 2017 and 2018 following soybean.

Studies conducted in the U.S. upper Midwest have reported comparatively higher N accumulation in cover crops interseeded within corn and soybean than the results reported in this study (Strock et al., 2004; De Bruin et al., 2005; Wilson et al., 2013; Noland et al., 2018). However, the cover crop used in those studies is mostly cereal rye seeded in the spring, except for Strock et al. (2004) and Wilson et al. (2013), who seeded in the fall after corn harvest. The former sampled cover crop biomass in the spring, and the latter sampled cover crop biomass in mid-Nov and early-Dec. These studies, therefore, report higher biomass and thereby higher cover crop N. Wilson et al. (2013) reported up to 45 kg N ha^{-1} aerial-fall seeded cereal rye when sampled in Nov-Dec, even though they reported cover crop N as low as 0.1 kg ha^{-1}. Cover crops in our study were late interseeded, winterkilled, and hand broadcast, which resulted in marginal biomass and N accumulation.

Nitrogen in cover crops varied among cover crop strategy in both corn and soybean in this study (Table 3.3). The three-way mix of ARCCFR accumulated more N (9.25 kg ha^{-1}) than ARCC (6.57 kg ha^{-1}) and AR monoculture (7.25 kg ha^{-1}) when pooled over years and location within corn. In soybean, N accumulation was 3.98, 1.56, and 2.28 kg ha^{-1} in ARCCFR, ARCC, and AR treatments. In Waseca in 2018, forage radish established well within corn, which could have contributed to more N than ARCC and AR monoculture. It is well known that FR is an excellent N scavenger, but is comparatively more sensitive to winter injury than AR and CC (Clark, 2007).

3.3.5 Carbon and Nitrogen in cover crops

The aboveground C and N content of each cover crop species was calculated by multiplying the tissue C and N content with the dry aboveground biomass. Cover crop C:N ratio within corn was affected by location and cover crop strategy and within soybean by year, location, cover crop strategy, year x tillage, year x cover crop strategy, and location x cover crop strategy interactions (Table 3.3).

At Lamberton site, within corn in 2017, the C:N ratio of AR monoculture, ARCC, and ARCCFR was 9.58, 10.3, and 9.02, respectively, while in 2018, the C:N ratio of AR, ARCC, and ARCCFR was 10, 10.2, and 8.9, respectively. Within soybean, C:N ratio in AR, ARCC, and ARCCFR was 10, 10.8, and 9.6, respectively, in 2017; and 12.3, 11.1, and 11.7, respectively, in 2018.

At Waseca, within corn in 2017, the pooled C:N ratios of cover crop strategies were 11.9, 13.0, and 10.8 for AR, ARCC, and ARCCFR, respectively, While in 2018, the C:N ratio of AR, ARCC and ARCCFR was 13.4, 13.1, and 9.9, respectively. Within soybean, C:N ratio in AR, ARCC, and ARCCFR was 11.1, 14.2, and 9.55, respectively, in 2017; and 13.5, 13.9, and 10.1, respectively, in 2018.

The C:N ratio of cover crops was not affected by tillage practices. At Lamberton, the only significant difference observed was between AR and ARCC strategies within ST in 2017 (Figure 3.7). However, at Waseca, differences in C:N of cover crops were observed within 2017 soybean and 2018 corn. The ARCC strategy, a mixture of grass and a legume species, had a significantly higher C:N ratio than AR and ARCCFR within the three tillage practices (Figure 3.8). The pooled averages of AR monoculture, ARCC, and ARCCFR over site-years was 11.4, 12.1, and 9.92, respectively. It is important to highlight that ARCC consistently had a higher C:N ratio than the other strategies, even though it produced lower biomass (0.137 Mg DM ha^{-1}) than ARCCFR (0.169 Mg DM ha^{-1}) and AR monoculture (0.154 Mg DM ha^{-1}) throughout the study.

In contrast to our results, Clark et al. (2007) report lower C:N ratio in cereal rye and hairy vetch mix compared to cereal rye monoculture; however, the biomass produced in their study was much higher than that reported in this study, mainly because their study was conducted at a warmer location, and cover crops were sampled in spring. Studies have reported that C:N ratio of cover crop mixtures is higher than that of monocultures. Finney et al., (2016) reported that the mixture of eight cover crops had a higher C:N ratio than most monocultures. However, Kuo and Jellum (2002) reported from a 4-yr study that a mixture of AR and hairy vetch had more C:N ratio than AR monoculture. Our results of a higher C:N ratio in ARCC as compared to AR monoculture corroborate the results of such study.

3.3.6 Residual nitrogen in soil within corn and soybean plots

Residual soil NO_3-N was affected by location and year at both 0-15 and 15-30 cm layers. Location x year interaction was observed during spring and fall seasons and depth, suggesting that NO_3-N in this study was affected by environmental conditions (Table 3.4). The analysis of residual soil NO_3-N at Lamberton revealed the effect of tillage x cover crop strategy interaction within corn plots at the time of seeding cover crops in 2017 at 0-15 cm layer (Table 3.5). However, this result is surprising and unusual since there were no cover crops seeded at the time of sampling. In another sampling event at Lamberton in the fall of 2018, tillage practice affected soil NO_3-N at 0-15 cm layer within soybean plots; within a tillage practice, the pooled average across cover crop strategies was 4.68 kg ha^{-1} in CT, 4.12 kg ha^{-1} in NT, and 4.76 kg ha^{-1} in ST (Table 3.5). However, no effect of tillage or cover crop strategy was found in any sampling event at 15-30 cm layer in Lamberton during the study (Table 3.6).

At Waseca site, residual soil NO_3-N at 0-15 cm layer was not affected by any treatment at any sampling event (Table 3.7). However, at the 15-30 cm layer, tillage significantly affected residual soil NO_3-N in spring 2018 within soybean plots, where corn was planted in 2017. Conventionally tilled plots had 10.7 kg ha^{-1} residual soil NO_3-N compared to 3.3 kg ha^{-1} in NT and 8.2 kg ha^{-1} in ST. In the same season, tillage and cover crop strategy effects were observed (Table 3.8). No other differences in the residual soil NO_3-N were observed throughout the study. Residual NO_3-N was significantly higher in 2018 as compared to 2017 at both depths in Waseca.

3.3.7 Soil Nitrogen dynamics in corn-soybean rotation with late interseeded winterkilled cover crops

3.3.7.1 Changes in NO_3-N from cover crop seeding to fall frost

At 0-15 cm layer, change in soil NO_3-N concentration from cover crop seeding to fall frost was significantly different between corn and soybean plots only (Table 3.9). Soil NO_3-N concentration was greater in the fall than at the time of seeding (Figure 3.9), which implies that the N uptake by cover crop in the period after seeding until fall frost was not enough to affect NO_3-N at 0-15 cm layer. However, soil NO_3-N movement downwards may have been possible because of mass flow as soil water is the carrier of NO_3-N, and due to concentration gradients (Zaporozec,1983). Since more precipitation occurred at the time of seeding than at fall frost, more NO_3-N could have leached in the soil profile. Similarly, NO_3-N at the 15-30 cm soil layer was higher in the fall as compared to the time of seeding (Figure 3.9).

3.3.7.2 Changes in NO_3-N from cover crops seeding to the next season spring

At the 0-15 cm layer, changes in soil NO_3-N from cover crop seeding in late summer to early spring differed only between corn and soybean plots (Table 3.9). Soil NO_3-N was greater at cover crop termination in the spring than at seeding (Figure 3.10). Similarly, at the 15-30 cm layer, soil NO_3-N was higher at cover crop termination in spring than at seeding in late summer (Figure 3.10). Differences in soil NO_3-N were larger in spring than in the fall, with greater concentration in the spring as compared to fall.

3.3.8 Nitrogen in soil solution within corn and soybean plots

The yearly average of NO_3-N concentration in the soil solution was higher at Lamberton than at Waseca for each study year. Average NO_3-N concentration in the soil solution at Lamberton during the growing season was 10.2, 12.36, and 9.69 mg kg^{-1} in 2017, 2018, and 2019, respectively, and at Waseca was 5.31, 5.69, and 7.52 mg kg^{-1} in 2017, 2018 and 2019, respectively. Data were not statistically analyzed due to the lack of replicates. Monthly averages of NO_3-N concentration in the soil solution at Lamberton and Waseca during the growing season of 2017, 2018, and 2019 are displayed as radar charts (Figures 3.11 to 3.16).

3.3.9 Net N mineralization during the corn and soybean growing season

At the Lamberton site, soil N mineralization varied with tillage practice within soybean plots in 2018. Tillage practice x sampling date interactions were significant in every analysis, except within soybean plots in 2019. A 3-way interaction of tillage x cover crop strategy x sampling date was observed in 2018 within soybean plots. In 2019, sampling date significantly affected net N mineralization within corn. At Waseca site, tillage practice significantly affected N mineralization during the growing season in 2018 only. Nitrogen mineralization differed between sampling dates in both study years at Waseca. Similar to Lamberton, tillage x sampling date interaction was observed in both 2018 and 2019 within corn and soybean plots. A 3-way interaction of tillage practice, cover crop strategy, and sampling date was observed only in the 2019 soybean plot (Table 3.10). These findings agree with our biomass results showing poor cover crop growth performance. Because of the extreme cold conditions during winter in the region, growth is marginal, so the little N accumulated by the cover crops along with the low C:N allows for rapid mineralization as early as conditions allow for it in the spring.

Net N mineralization in Lamberton in 2018 was negative mostly during the growing season within both corn and soybean plots, suggesting more N was immobilized than mineralized. However, in 2019 net N mineralization showed a decreasing trend in the growing season within corn plots. (Figure 3.17). No specific trend was observed within soybean plots, but net N mineralization overall was positive during the whole season (Figure 3.17). At the Waseca site, the net N mineralization was positive in both corn and soybean plots, except for the first date in late May 2018. A similar trend was observed during the 2019 growing season at Waseca, where net N mineralization decreased as the growing season progressed (Figure 3.18). Consistently, more N mineralization was observed in corn plots than soybean plots during the study period. These results suggest that cover crops interseeded late in the season did not affect net N mineralization in either primary crops grown in the next season of seeding cover crops.

3.4 Conclusions

This study was conducted to advance our understanding on the effect of late interseeded winterkilled cover crops on N dynamics in corn-soybean rotation under three tillage practices.

After two seasons of cover cropping, differences in soil organic matter were observed between cover crop strategies and tillage practice at the Lamberton site. Nitrogen in corn and soybean grain and stover was not affected by tillage practice or cover crop strategy. Nitrogen in crop grain and stover varied significantly by location, year, and location x year interactions, suggesting environmental effects more than tillage practice or cover crop strategy.

Nitrogen in cover crop biomass varied highly from year to year and was mostly marginal as compared to winter annual cover crop results reported in previous studies in the region. Nitrogen accumulation in the three-way mix of ARCCFR was consistently higher than the two-way mix of ARCC and AR monoculture throughout the study. This result is consistent with the amount of biomass produced among the cover crop strategies. On the other hand, C:N ratio differed significantly among cover crop strategy, with ARCC consistently having a higher C:N ratio as compared to ARCCFR and AR. This suggests that C:N ratio may not necessarily be affected by the amount of biomass produced by the cover crops at early vegetative growth stages.

Cover crop strategy and tillage practice seemed to have no effect on the residual NO_3-N at both locations and study years. Variations in residual NO_3-N were primarily driven by year, location, and their interactions. More soil NO_3-N concentration was observed in the fall and the next spring before planting the major crops than at the time of seeding cover crops late in the season, which could have been the result of residue from the major crop.

Inferences on the NO_3-N concentration in the soil solution were not made because of the lack of replicates and, consequently, the statistical analysis. However, a visual representation of monthly NO_3-N concentrations did not reveal any consistent patterns among cover crop strategies within tillage practice in either major crop.

The rate of net N mineralization decreased throughout the growing season in both locations and years, and the N mineralization potential within corn plots was higher than within soybean plots.

Although late interseeded, winterkilled cover crops have the potential to produce biomass in the fall within corn in the upper Midwest U.S., they did not produce the ecological benefits late- and early-interseeded overwintering or early interseeded winterkilled cover crops are known to provide. Therefore, the ecological benefits of late interseeded, winterkilled cover crops may not outweigh the input costs associated with such strategy.

Winterkilled cover crop research should be focused on early interseeding in the major crops growing season, rather than late interseeding. However, interseeding cover crops early in the season should be done cautiously, because it may compete with primary crops if seeded simultaneously with primary crops and may exhibit poor growth when planted after the primary crops close canopy. For late interseeding, overwintering cover crops may be a viable option in the cold upper Midwest U.S. conditions.

3.5 List of tables and figures

Table 3.1. Mean soil properties for 0-20 cm layer sampled in spring 2019 at SWROC near Lamberton, MN, USA. Values in parentheses are one standard deviation. ANOVA is reported by tillage and cover crop treatment in a corn-soybean rotation after two growing seasons of cover cropping.

Tillage	Cover crop strategy	pH	OM	Bray P	K^{+}	Ca^{+2}	Mg^{+3}	CEC
			mg kg^{-1}					meq 100 g^{-1}
Conventional Till	AR	5.9 (0.6)	4.1 (0.35)	23 (13.8)	118 (8.6)	2294 (498)	475 (52)	20 (1)
	ARCC	5.8 (0.3)	4.4 (0.34)	19 (6.2)	116 (21.7)	2241 (430)	487 (60)	20 (2)
	ARCCFR	5.7 (0.3)	4.4 (0.57)	21 (10.3)	140 (36.8)	2223 (397)	482 (61)	20 (1)
	NC	5.8 (0.6)	4.3 (0.33)	16 (6.8)	109 (12.1)	2189 (332)	457 (35)	20 (1)
No Till	AR	5.5 (0.2)	4.2 (0.39)	18 (6.2)	104 (12.4)	2063 (243)	436 (28)	21 (1)
	ARCC	5.5 (0.6)	4.3 (0.31)	22 (11.5)	112 (15.0)	2197 (369)	466 (60)	20 (1)
	ARCCFR	5.5 (0.2)	4.3 (0.34)	20 (7.8)	110 (15.2)	2117 (217)	456 (31)	21 (1)
	NC	5.7 (0.4)	4.2 (0.29)	19 (7.3)	113 (12.6)	2299 (423)	476 (41)	21 (1)
Strip Till	AR	6.1 (0.7)	4.0 (0.28)	17 (6.5)	121 (9.7)	2350 (355)	460 (28)	19 (2)
	ARCC	5.9 (0.7)	3.9 (0.23)	18 (13.3)	116 (13.8)	2273 (443)	459 (48)	20 (1)
	ARCCFR	5.7 (0.3)	4.1 (0.25)	14 (6.2)	110 (15.8)	2186 (384)	445 (43)	19 (1)
	NC	5.8 (0.3)	4.1 (0.33)	18 (7.4)	108 (15.6)	2242 (332)	463 (38)	19 (1)
Mean		5.75	4.2	19	115	2223	463	20
Tillage (T)		0.19	0.009**	0.37	0.13	0.72	0.22	0.003**
Cover crop strategy (C)		0.22	0.01*	0.75	0.17	0.66	0.55	0.91
T x C		0.26	0.2	0.47	0.004**	0.29	0.2	0.58

Significance of fixed effects (P > F) on soil properties. Within each column, numbers followed by ** and * are significant at 0.01 and 0.05 level. AR denotes annual ryegrass, ARCC denotes AR + crimson clover, ARCCFR denotes AR +CC + forage radish and NC. denotes no cover (control).

Table 3.2. Mean soil properties for 0-20 cm layer sampled in spring 2019 at SROC, Waseca, MN, USA. Values in parentheses are one standard deviation. ANOVA is reported by tillage and cover crop treatment in a corn-soybean rotation after two growing seasons of cover cropping.

Tillage	Cover crop strategy	pH	OM	Bray P	K^{+}	Ca^{+2}	Mg^{+3}	CEC
			----------	----------	mg kg^{-1}	----------	----------	meq $100g^{-1}$
Conventional Till	AR	7.1 (0.6)	6.3 (0.8)	15 (4.7)	163 (27.0)	4622 (605)	475 (52)	20 (1)
	ARCC	7.1 (0.5)	6.3 (0.9)	23 (11.5)	169 (35.2)	4542 (498)	487 (60)	20 (2)
	ARCCFR	6.9 (0.6)	6.2 (0.7)	21 (9.8)	156 (25.9)	4540 (721)	482 (61)	20 (1)
	NC	7.0 (0.6)	6.5 (0.8)	23 (7.5)	178 (52.8)	4387 (547)	457 (35)	20 (1)
No Till	AR	6.6 (0.1)	6.3 (0.6)	21 (17.0)	140 (20.3)	4599 (1116)	436 (28)	21 (1)
	ARCC	6.7 (0.8)	6.5 (0.5)	19 (9.2)	149 (11.7)	4298 (1025)	466 (60)	20 (1)
	ARCCFR	6.5 (0.8)	6.3 (0.8)	19 (11.2)	158 (50.1)	4324 (934)	456 (31)	21 (1)
	NC	6.6 (1)	6.5 (0.6)	21 (12.0)	147 (27.2)	4275 (959)	476 (41)	21 (1)
Strip Till	AR	6.5 (0.7)	6.6 (0.5)	25 (15.9)	165 (43.0)	4245 (795)	460 (28)	19 (2)
	ARCC	6.7 (0.6)	6.3 (1)	18 (13.3)	146 (21.4)	4276 (928)	459 (48)	20 (1)
	ARCCFR	6.9 (0.7)	6.7 (0.3)	20 (7.8)	156 (37.9)	4699 (986)	445 (43)	19 (1)
	NC	6.6 (0.8)	6.5 (0.6)	23 (15.6)	152 (27.7)	4409 (1019)	463 (38)	19 (1)
Mean		6.76	6.4	21	157	4435	471	28
Tillage (T)		0.42	0.69	0.93	0.16	0.91	0.99	0.08
Cover crop strategy (C)		0.72	0.77	0.82	0.96	0.41	0.82	0.1
T x C		0.33	0.44	0.43	0.43	0.26	0.63	0.65

Significance of fixed effects (P > F) on soil properties. AR denotes annual ryegrass, ARCC denotes AR + crimson clover, ARCCFR denotes AR +CC + forage radish, and NC. denotes no cover (control). Tillage, cover crop strategy and their interactions did not affect soil properties in Waseca.

Table 3.3. Significance of fixed effects on N accumulated in corn-soybean grain and biomass, cover crop biomass and cover crop C:N ratio.

Source of variation	Response variable							
	N in grain		N in stover		N in cover crop biomass		Cover crop C:N ratio	
	Corn	Soybean	Corn	Soybean	Corn	Soybean	Corn	Soybean
Year (Y)	0.000***	0.000***	0.000***	0.000***	0.253	0.000***	0.755	0.011*
Location (L)	0.000***	0.000***	0.000***	0.065	0.003**	0.005**	0.013*	0.054
Tillage practice (T)	0.067.	0.190	0.245	0.302	0.100	0.048*	0.374	0.410
Cover crop strategy (C)	0.938	0.473	0.110	0.089	0.002**	0.000***	0.000***	0.000***
Y x L	0.000***	0.000***	0.000***	0.013*	0.000***	0.022*	0.934	0.424
Y x T	0.007**	0.851	0.058	0.010*	0.808	0.222	0.240	0.008**
L x T	0.040*	0.144	0.151	0.243	0.599	0.430	0.057	0.313
Y x C	0.472	0.854	0.115	0.434	0.013*	0.001**	0.065	0.028*
L x C	0.787	0.298	0.207	0.116	0.206	0.394	0.084	0.000***
T x C	0.856	0.433	0.491	0.817	0.987	0.745	0.876	0.293
Y x L x T	0.189	0.342	0.156	0.363	0.193	0.538	0.097	0.304
Y x L x C	0.522	0.393	0.775	0.593	0.497	0.578	0.459	0.627
Y x T x C	0.817	0.933	0.575	0.808	0.197	0.982	0.224	0.191
L x T x C	0.057.	0.313	0.616	0.432	0.126	0.927	0.527	0.364
Y x L x T x C	0.481	0.539	0.120	0.119	0.447	0.995	0.927	0.356

Significance of fixed effects (P > F) on the response: N accumulated in corn-soybean grain and biomass, cover crop biomass, and cover crop C:N ratio at Lamberton and Waseca, MN in 2017-2019. Numbers followed by ***, **, *, and a single dot within a single column are significant at 0.001, 0.01, 0.05, and 0.1 level.

Table 3.4. Significance of fixed effects on residual soil NO_3-N in spring before planting corn and soybean in 2018, and 2019 at Lamberton and Waseca, MN, and before frost in the fall in 2017 and 2018. Data were analyzed separately for 0-15 and 15-30 cm layer.

Source of variation	Response variable							
	Spring 0-15 cm		Fall 0-15 cm		Spring 15-30 cm		Fall 15-30 cm	
	Corn	Soybean	Corn	Soybean	Corn	Soybean	Corn	Soybean
Year (Y)	0.000***	0.000***	0.000***	0.000***	0.007**	0.002**	0.002**	0.000***
Location (L)	0.970	0.563	0.000***	0.000***	0.000***	0.897	0.001**	0.167
Tillage practice (T)	0.623	0.092.	0.381	0.291	0.235	0.050.	0.916	0.190
Cover crop strategy (C)	0.492	0.720	0.360	0.843	0.889	0.778	0.113	0.657
Y x L	0.000***	0.000***	0.000***	0.012*	0.000***	0.000***	0.000***	0.000***
Y x T	0.527	0.002**	0.391	0.618	0.429	0.027*	0.908	0.958
L x T	0.186	0.045*	0.788	0.410	0.908	0.003	0.666	0.672
Y x C	0.709	0.855	0.515	0.496	0.178	0.824	0.095.	0.418
L x C	0.996	0.134	0.111	0.887	0.947	0.835	0.635	0.952
T x C	0.941	0.901	0.971	0.671	0.526	0.701	0.737	0.864
Y x L x T	0.021*	0.050.	0.725	0.197	0.469	0.306	0.906	0.774
Y x L x C	0.153	0.411	0.935	0.459	0.774	0.728	0.473	0.988
Y x T x C	0.892	0.505	0.566	0.350	0.253	0.875	0.705	0.142
L x T x C	0.423	0.557	0.075.	0.997	0.098.	0.565	0.709	0.860
Y x L x T x C	0.521	0.729	0.634	0.953	0.532	0.938	0.989	0.075.

Significance of fixed effects (P > F) on the response: residual soil NO_3-N in spring and fall at 0-15 and 15-30 cm at Lamberton and Waseca, MN. Numbers followed by ***, **, *, and a single dot within a single column are significant at 0.001, 0.01, 0.05, and 0.1 level.

Table 3.5. Residual soil NO_3-N (kg ha^{-1}) in 0-15 cm soil layer in spring before planting in 2018 and 2019, before late seeding cover crops in 2017 and 2018, and in the fall before frost in 2017, 2018, and 2019 in SWROC near Lamberton, MN, USA.

Tillage	**Cover crop strategy**	**2017**				**2018**						**2019**			
		Seeding		**Fall**		**Spring**		**Seeding**		**Fall**		**Spring**		**Fall** §	
		Corn	**Soy**	**Corn**	**Soy**	**Corn**	**Soy**	**Corn**	**Soy**	**Corn**	**Soy**	**Corn**	**Soy**	**Corn**	**Soy**
		------------------------------NO_3-N kg ha^{-1}------------------------------													
CT	AR	1.89	1.33	5.53	4.55	8.87	16.52	3.20	1.17	5.56	4.53	5.29	3.93	8.97	3.315
	ARCC	5.74	0.67	5.02	5.35	11.98	13.15	2.29	0.87	4.68	4.88	6.41	4.15	10.92	3.315
	ARCCFR	3.02	0.92	9.36	4.61	11.70	11.97	6.21	1.01	5.99	4.98	5.25	3.39	14.04	4.68
	NC	4.75	1.26	9.89	4.47	10.90	13.36	5.34	0.92	7.44	4.31	4.67	4.87	15.6	4.29
NT	AR	11.02**b**	0.78	7.48	6.00	13.12	15.04	2.45	0.70	4.78	2.78**a**	2.98	4.05	10.92	3.12
	ARCC	6.24**ab**	1.30	4.24	7.58	10.45	13.81	4.32	1.02	4.71	4.80**b**	4.39	5.03	9.75	2.145
	ARCCFR	2.2 **a**	0.66	5.07	6.16	13.48	8.67	1.58	1.00	6.61	4.12**ab**	3.94	4.09	9.75	2.925
	NC	8.33**ab**	0.85	6.38	5.28	16.14	10.08	4.74	0.79	4.79	3.42**ab**	3.29	4.05	6.63	2.145
ST	AR	10.87	2.91	4.82	4.64	17.08	13.05	3.09	1.21	3.90	5.26	4.91	4.77	21.255	2.73
	ARCC	4.9	1.20	5.91	5.61	12.29	15.10	4.95	0.89	5.03	4.36	4.59	3.98	17.94	3.12
	ARCCFR	9.04	1.12	4.34	5.17	17.71	12.58	2.28	0.87	6.59	4.66	4.97	3.94	10.14	3.9
	NC	5.06	1.80	5.29	5.59	16.34	13.05	4.38	0.83	6.10	4.76	4.47	3.79	26.91	4.875
mean		6.08	1.18	6.11	5.41	13.33	13.03	3.73	0.94	5.51	4.40	4.59	4.17	13.56	13.38
Tillage (T) ¥		0.316	0.122	0.243	0.231	0.231	0.409	0.536	0.916	0.602	0.026*	0.167	0.933		
Cover crop strategy (C)		0.483	0.233	0.275	0.611	0.611	0.104	0.179	0.878	0.08	0.346	0.398	0.874		
T x C		0.032*	0.309	0.113	0.983	0.983	0.661	0.106	0.797	0.602	0.804	0.175	0.907		

¥Significance of fixed effects (P > F) on residual soil NO_3-N. Numbers followed by ***, **, *, and a single dot within a single column are significant at 0.001, 0.01, 0.05, and 0.1 level. Within each column and tillage practice, values followed by different letters denote a significant difference between cover crop strategy. § Residual soil NO_3-N not analyzed for Fall, 2019, due to lack of replicates. AR denotes annual ryegrass, ARCC denotes AR + crimson clover, ARCCFR denotes AR +CC + forage radish, and NC. denotes no cover (control).

Table 3.6. Residual soil NO_3-N (kg ha^{-1}) in 15-30 cm soil layer in spring before planting in 2018 and 2019, before late seeding cover crops in 2017 and 2018, and in the fall before frost in 2017, 2018, and 2019 in SWROC near Lamberton, MN, USA.

Tillage	Cover crop strategy	2017				2018						2019			
		Seeding		Fall		Spring		Seeding		Fall		Spring		Fall §	
		Corn	Soy	Corn	Soy	Corn	Soy	Corn	Soy	Corn	Soy	Corn	Soy	Corn	Soy
		--NO_3-N kg ha^{-1}--													
CT.	AR	2.62	1.3	5.97	4.99	6.14	7.63	1.39	0.59	4.46	3.3	5.74	6.58	6.83	3.51
	ARCC	3.95	1.46	6.4	4.25	6.35	6.65	2.05	0.59	4.12	3.12	5.85	4.43	7.22	2.93
	ARCCFR	3.06	1.41	6.98	5.29	4.45	8.08	2.14	0.59	5.39	2.97	5.11	3.98	8.97	5.66
	NC	4.05	1.44	6.84	7.12	6.82	6.38	1.99	0.63	6.44	3.16	4.01	5.32	10.14	4.68
NT	AR	4.53	1.22	4.01	5.65	5.1	6.57	2.49	0.59	4.66	2.71	3.86	4.82	7.22	2.15
	ARCC	4.55	0.8	5.24	3.88	4.59	5.56	5.5	0.58	5.23	2.31	5.66	5.49	10.53	2.15
	ARCCFR	2.65	0.87	5.83	5.19	5.36	5.28	1.57	0.63	6.37	2.49	4.84	6.68	10.73	2.54
	NC	2.98	0.92	5.95	3.91	5.74	4.7	2.83	0.59	5.35	2.71	4.42	4.81	9.95	2.73
ST.	AR	8.29	0.96	5.4	4.03	6.37	7.9	2.24	0.71	3.73	4	5.04	3.34	15.41	2.93
	ARCC	4.27	2.53	4.38	5	5.31	8.28	1.3	0.7	4.17	2.54	4.41	3.94	17.94	1.95
	ARCCFR	5.67	1.06	5.21	4.18	4.66	7.04	1.12	0.77	6.83	2.98	6.12	4.16	9.75	2.54
	NC	3.79	1.7	5.17	3.63	5.33	6.78	1.27	0.59	4.25	3.31	6.35	4.46	13.65	3.12
mean		4.2	1.3	5.61	4.76	5.51	6.73	2.15	0.63	5.08	2.96	5.11	4.83	10.6	3.07
Tillage (T) ¥		0.455	0.219	0.207	0.355	0.515	0.233	0.349	0.322	0.80	0.146	0.39	0.285		
Cover crop strategy(C)		0.633	0.722	0.200	0.857	0.291	0.473	0.51	0.653	0.09	0.305	0.746	0.988		
T x C		0.36	0.506	0.445	0.210	0.662	0.948	0.357	0.610	0.711	0.905	0.146	0.534		

¥Significance of fixed effects (P > F) on residual soil NO_3-N. Numbers followed by ***, **, *, and a single dot within a single column are significant at 0.001, 0.01, 0.05, and 0.1 level. § Residual soil NO_3-N not analyzed for fall, 2019, due to lack of replicates. AR denotes annual ryegrass, ARCC denotes AR + crimson clover, ARCCFR denotes AR +CC + forage radish, and NC. denotes no cover (control).

Table 3.7. Residual soil NO_3-N (kg ha^{-1}) in 0-15 cm soil layer in spring before planting in 2018 and 2019, before late seeding cover crops in 2017 and 2018, and in the fall before frost in 2017, 2018, and 2019 in SROC, Waseca, MN, USA.

Tillage	**Cover crop strategy**	**2017**				**2018**						**2019**			
		Seeding		**Fall**		**Spring**		**Seeding**		**Fall**		**Spring**		**Fall** §	
		Corn	**Soy**	**Corn**	**Soy**	**Corn**	**Soy**	**Corn**	**Soy**	**Corn**	**Soy**	**Corn**	**Soy**	**Corn**	**Soy**
		---------- NO_3-N kg ha^{-1} ----------													
CT	AR	7.49	9.74	8.40	10.32	6.39	8.72	3.07	5.35	13.45	16.19	8.00	8.26	10.34	14.63
	ARCC	7.91	11.48	9.66	10.70	5.24	6.76	4.74	4.96	14.16	14.11	8.27	9.69	15.60	16.58
	ARCCFR	8.74	7.08	6.26	8.27	7.59	8.30	3.72	4.56	13.75	12.10	8.16	11.25	14.43	18.33
	NC	13.62	6.05	5.78	5.47	7.61	8.04	4.58	4.11	12.62	14.34	8.88	6.34	11.70	17.75
NT	AR	7.70	5.69	4.86	6.69	5.59	2.49	3.65	2.19	12.64	12.19	9.77	8.14	12.87	14.43
	ARCC	7.16	8.43	5.03	7.74	6.98	2.96	2.90	2.03	15.89	10.92	9.68	8.57	7.41	17.75
	ARCCFR	7.68	8.83	4.34	7.12	7.18	2.76	2.76	2.11	14.72	9.93	10.33	8.61	14.63	22.04
	NC	10.62	7.50	8.20	7.74	6.01	3.86	3.92	1.88	16.78	10.78	11.25	7.89	6.83	17.36
ST	AR	10.92	5.09	8.22	6.20	5.34	5.48	1.45	3.12	12.66	14.42	9.92	9.10	22.43	6.63
	ARCC	10.44	8.83	6.90	5.66	7.17	8.78	3.10	3.64	12.68	13.43	8.90	9.43	13.65	12.87
	ARCCFR	7.74	7.90	6.06	7.00	5.87	8.17	4.97	3.16	13.63	13.93	9.99	9.22	13.07	16.38
	NC	15.71	9.15	6.85	7.16	4.74	7.26	2.45	4.25	13.09	14.43	10.15	9.82	13.65	7.22
mean		9.64	7.98	6.71	7.51	6.31	6.13	3.44	3.45	13.83	13.06	9.44	8.86	13.05	15.16
Tillage (T) ¥		0.705	0.855	0.336	0.472	0.572	0.015	0.802	0.387	0.741	0.513	0.460	0.541		
Cover crop strategy (C)		0.133	0.482	0.614	0.929	0.640	0.518	0.148	0.997	0.666	0.24	0.618	0.393		
T x C		0.974	0.611	0.546	0.707	0.455	0.408	0.453	0.994	0.726	0.929	0.994	0.529		

¥Significance of fixed effects (P > F) on residual soil NO_3-N. Numbers followed by ***, **, *, and a single dot within a single column are significant at 0.001, 0.01, 0.05, and 0.1 level. § Residual soil NO_3-N not analyzed for Fall, 2019, due to lack of replicates. AR denotes annual ryegrass, ARCC denotes AR + crimson clover, ARCCFR denotes AR +CC + forage radish, and NC. denotes no cover (control).

Table 3.8. Residual soil NO_3-N (kg ha^{-1}) in 15-30 cm soil layer in spring before planting in 2018 and 2019, before late seeding cover crops in 2017 and 2018, and in the fall before frost in 2017, 2018, and 2019 in SROC, Waseca, MN, USA.

Tillage	Cover crop strategy	2017				2018						2019			
		Seeding		Fall		Spring		Seeding		Fall		Spring		Fall §	
		Corn	Soy	Corn	Soy	Corn	Soy	Corn	Soy	Corn	Soy	Corn	Soy	Corn	Soy
		---------- NO_3-N kg ha^{-1} ----------													
CT	AR	6.13	8.94	5.4	7.19	8.66	10.49	3.9	5.74	13.84	15.56	10.91	10.32	15.02	11.9
	ARCC	6.93	8.32	6.88	9.32	6.34	10.48	4.05	3.08	15.02	15.66	9.05	10.46	16.58	17.55
	ARCCFR	8.5	4.8	5.34	6.57	8.14	11.47	2.52	3.53	12.09	15.25	11.11	11.11	17.16	16.97
	NC	12.19	5.73	5.23	4.45	9.1	10.35	3.75	6.95	14.71	15.44	10.16	10.54	8.97	26.13
NT	AR	4.9	5.7	5.28	4.59	5.97	3.27	2.97	1.95	14.85	11.97	10.14	8.41	9.75	18.72
	ARCC	5.6	6.79	4.89	4.8	7.14	3.71	3.03	1.39	19.92	10.71	11.69	9.27	6.63	18.72
	ARCCFR	6.25	7.65	4.41	5.98	6.85	2.85	3.04	2.02	14.41	10.21	10.60	9.85	11.12	17.36
	NC	8.19	4.8	7.07	7.54	5.67	3.31	3.29	1.9	16.26	11.49	10.35	6.85	6.83	16.19
ST	AR	9.05	4.4	5.68	6.97	7.04**ab**	8.33	1.77	2.85	11.65	12.48	9.39	9.77	16.58	9.95
	ARCC	7.56	7.85	4.28	5.48	8.96**b**	9.57	2.51	3.35	15.33	13.11	11.61	10.18	14.43	14.43
	ARCCFR	6.97	5.57	6.49	7.19	5.67**a**	6.81	2.76	3.32	13.55	17.01	10.85	8.08	15.21	15.99
	NC	12.5	6.05	7.12	5.93	7.99**ab**	8.1	3.5	3.29	13.89	14.18	10	9.52	14.04	5.85
mean		7.89	6.38	5.67	6.33	7.29	7.39	3.09	3.28	14.62	13.58	10.48	9.53	12.69	15.81
Tillage (T) ¥		0.622	0.825	0.863	0.57	0.315	0.019*	0.819	0.36	0.491	0.285	0.927	0.218		
Cover crop strategy (C)		0.06	0.633	0.681	0.935	0.777	0.828	0.759	0.568	0.072.	0.845	0.887	0.522		
T x C		0.932	0.718	0.575	0.201	0.041*	0.977	0.856	0.791	0.856	0.299	0.768	0.103		

¥Significance of fixed effects (P > F) on residual soil NO_3-N. Numbers followed by ***, **, *, and a single dot within a single column are significant at 0.001, 0.01, 0.05, and 0.1 level. Within each column and tillage practice, only the values followed by different letters denote a significant difference between cover crop strategy. § Residual soil NO_3-N not analyzed for Fall, 2019, due to lack of replicates. AR denotes annual ryegrass, ARCC denotes AR + crimson clover, ARCCFR denotes AR +CC + forage radish, and NC denotes no cover (control).

Table 3.9. Significance of fixed effects on the difference in residual soil NO_3-N from seeding cover crops to fall and from seeding cover crops to spring before planting in the next season. Values are averaged over two seasons of cover cropping in Lamberton and Waseca, MN, USA.

Source of variation	Difference in residual soil NO_3-N (Δ soil NO_3-N)			
	Δ soil NO_3-N from seeding to fall		Δ soil NO_3-N from seeding to spring	
	0-15 cm	**15-30 cm**	**0-15 cm**	**15-30 cm**
Crop	0.000***	0.108	0.000***	0.003**
Tillage practice (T)	0.861	0.941	0.912	0.670
Cover crop strategy (C)	0.566	0.54	0.659	0.172
Crop x T	0.119	0.487	0.136	0.305
Crop x C	0.994	0.937	0.543	0.456
T x C	0.963	0.875	0.997	0.964
Crop x T x C	0.675	0.624	0.488	0.482

Significance of fixed effects (P > F) on the response: residual soil NO_3-N in spring and fall at 0-15 and 15-30 cm at Lamberton and Waseca, MN. Numbers followed by ***, **, *, and a single dot within a single column are significant at 0.001, 0.01, 0.05, and 0.1 level.

Table 3.10. Significance of fixed effects on net N mineralization in Lamberton and Waseca, MN, USA.

Source of variation	**Net N mineralization (µg g^{-1} d^{-1})**							
	Lamberton				**Waseca**			
	2018		**2019**		**2018**		**2019**	
	Corn	Soybean	Corn	Soybean	Corn	Soybean	Corn	Soybean
Tillage practice (T)	0.140	0.033*	0.135	0.342	0.000***	0.000***	0.338	0.237
Cover crop strategy (C)	0.715	0.108	0.932	0.127	0.127	0.137	0.356	0.329
Sampling date (D)	0.193	0.083.	0.000***	0.866	0.000***	0.049*	0.000***	0.000***
T x C	0.558	0.027*	0.725	0.179	0.417	0.311	0.500	0.397
T x D	0.000***	0.000***	0.000***	0.078.	0.004**	0.000***	0.008**	0.000***
C x D	0.071	0.120	0.660	0.522	0.003**	0.002**	0.070.	0.038*
T x C x D	0.424	0.005**	0.426	0.325	0.639	0.160	0.248	0.034*

Significance of fixed effects (P > F) on the response: net N mineralization in the growing season at Lamberton and Waseca, MN. Numbers followed by ***, **, *, and a single dot within a single column are significant at 0.001, 0.01, 0.05, and 0.1 level. Data were analyzed for each location, year, and crop separately for simplicity. Sampling date was analyzed as a covariate.

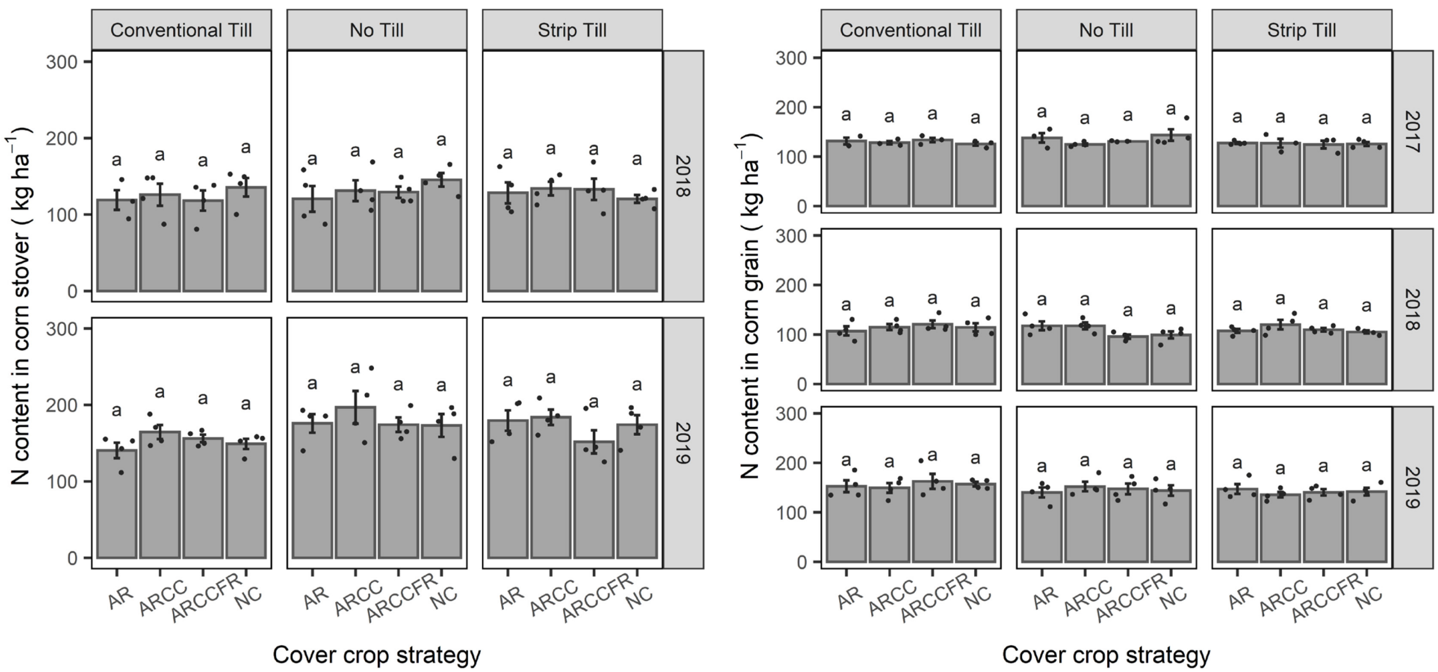

Figure 3.1. Nitrogen in corn stover (left) and corn grain (right) at SWROC near Lamberton, MN, USA, from 2017 through 2019. Data for N in corn stover was not available for 2017. Bars followed by the same letters are not significantly different at $P \leq 0.05$ within each tillage practice each year. Error bars represent SEM (n=4), and each dot represents data points. AR denotes annual ryegrass; ARCC denotes annual ryegrass + crimson clover; ARCCFR denotes annual ryegrass + crimson clover + forage radish; NC denotes no cover (control).

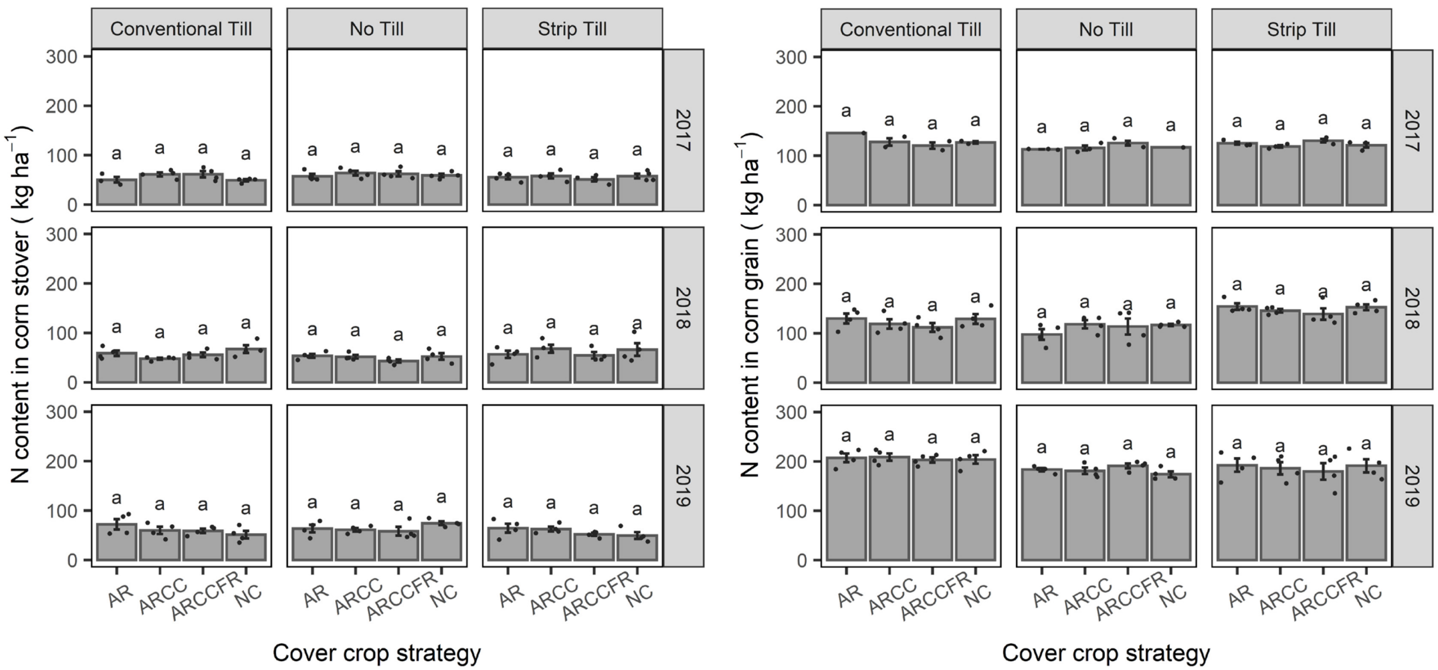

Figure 3.2. Nitrogen in corn stover (left) and corn grain (right) at SROC, Waseca, MN, USA from 2017 through 2019. Bars followed by the same letters are not significantly different at $P \leq 0.05$ within each tillage practice each year. Error bars represent SEM (n=4), and each dot represents data points. AR denotes annual ryegrass; ARCC denotes annual ryegrass + crimson clover; ARCCFR denotes annual ryegrass + crimson clover + forage radish; NC denotes no cover (control).

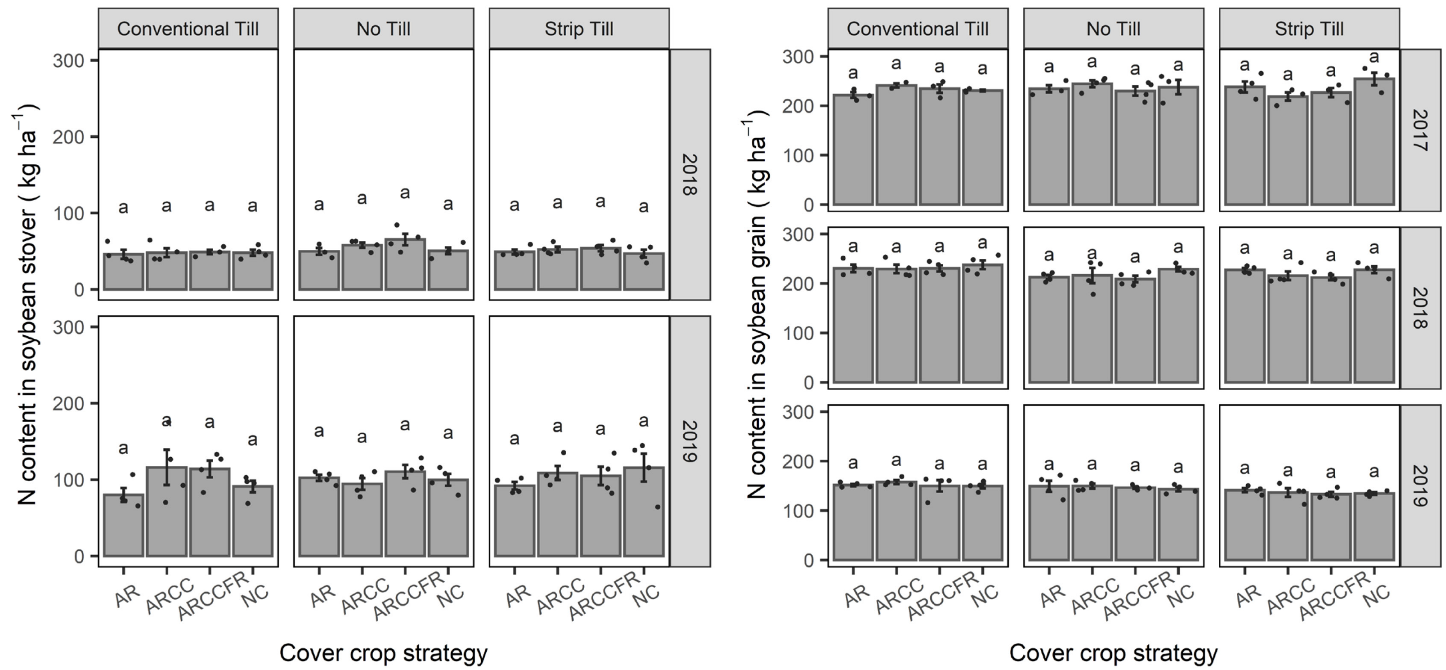

Figure 3.3. Nitrogen in soybean stover (left) and soybean grain (right) at SWROC near Lamberton, MN, USA, from 2017 through 2019. Data for N in soybean stover was not available for 2017. Bars followed by the same letters are not significantly different at $P \leq 0.05$ within each tillage practice each year. Error bars represent SEM (n=4), and each dot represents data points. AR denotes annual ryegrass; ARCC denotes annual ryegrass + crimson clover; ARCCFR denotes annual ryegrass + crimson clover + forage radish; NC denotes no cover (control).

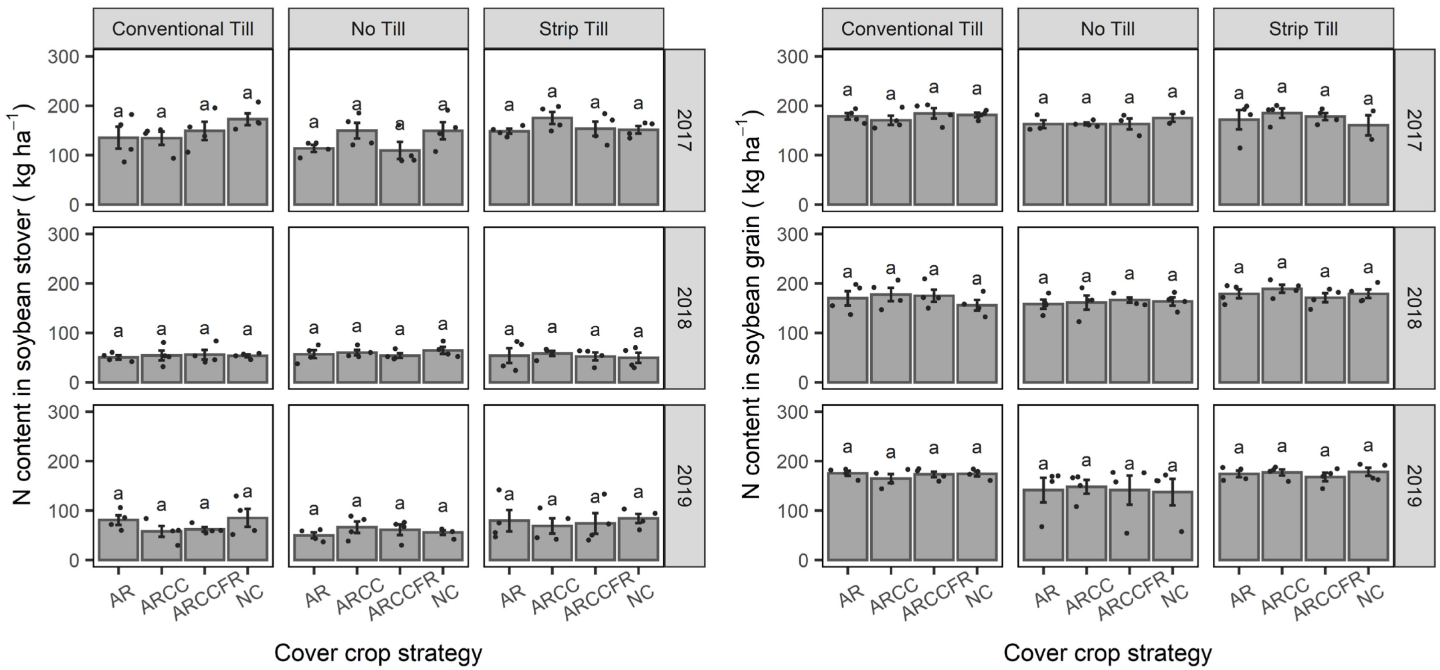

Figure 3.4. Nitrogen in soybean stover (left) and soybean grain (right) at SROC, Waseca, MN, USA from 2017 through 2019. Bars followed by the same letters are not significantly different at $P \leq 0.05$ within each tillage practice each year. Error bars represent SEM (n=4), and each dot represents data points. AR denotes annual ryegrass; ARCC denotes annual ryegrass + crimson clover; ARCCFR denotes annual ryegrass + crimson clover + forage radish; NC denotes no cover (control).

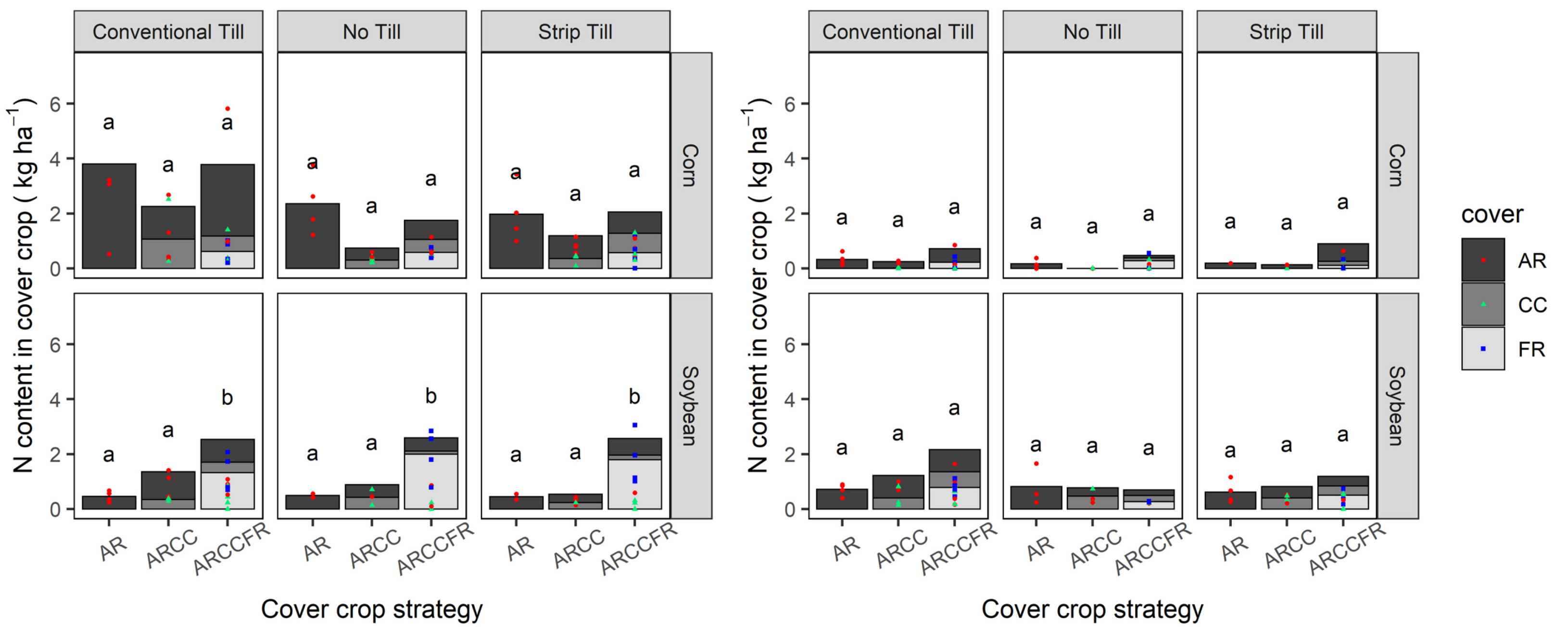

Figure 3.5. Nitrogen in cover crop biomass in fall 2017 (left) and fall 2018 (right) at SWROC near Lamberton, MN, USA. Different colors within a bar represent different species within a mix, and each dots represent data points (n=4 for each cover crop species within each cover crop strategy). Bars followed by the same letters are not significantly different at $P \leq 0.05$ within each tillage practice each year. AR denotes annual ryegrass; ARCC denotes annual ryegrass + crimson clover; ARCCFR denotes annual ryegrass + crimson clover + forage radish.

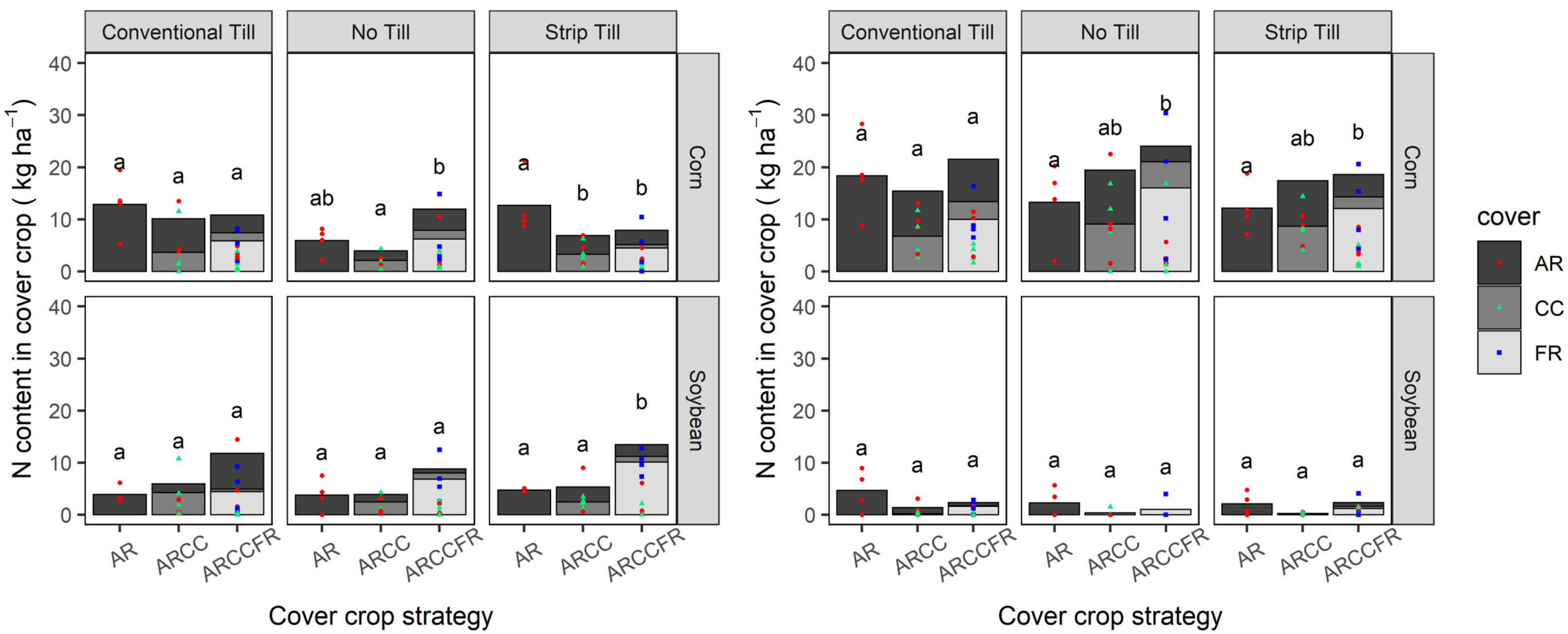

Figure 3.6. Nitrogen in cover crop biomass in fall 2017 (left) and fall 2018 (right) at SROC, Waseca, MN, USA. Different colors within a bar represent different species within a mix, and each dots represent data points (n=4 for each cover crop species within each cover crop strategy). Bars followed by the same letters are not significantly different at $P \leq 0.05$ within each tillage practice each year. AR denotes annual ryegrass; ARCC denotes annual ryegrass + crimson clover; ARCCFR denotes annual ryegrass + crimson clover + forage radish.

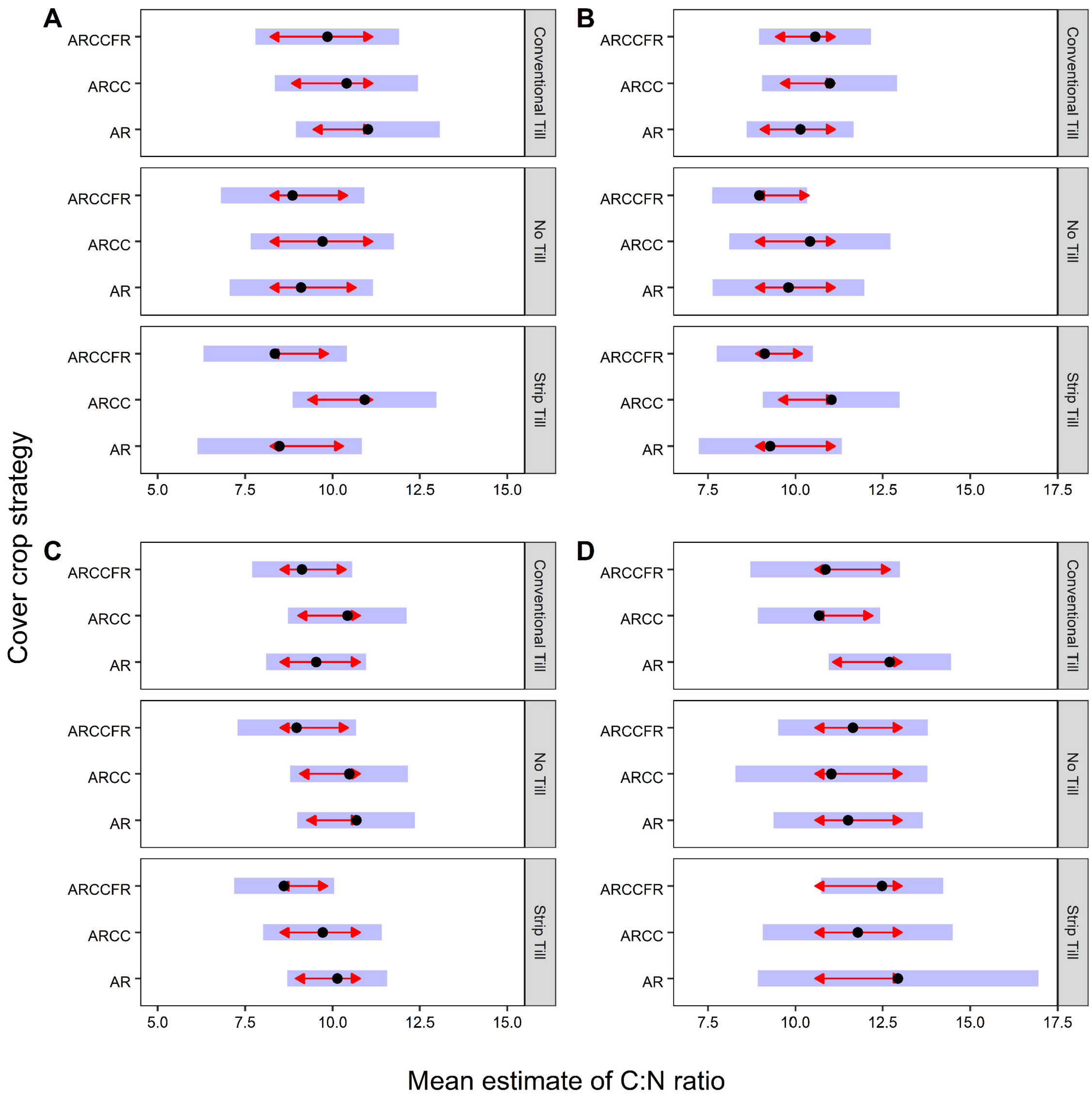

Figure 3.7. Means of C:N ratio among cover crop strategies within three tillage practices in corn (A) and soybean (B) in 2017; and in corn (C) and soybean (D) in 2018, in SWROC near Lamberton, MN, USA. Black dots represent estimated marginal means, and blue bars are 95% confidence intervals. Within each panel (tillage practice), red arrows overlapping among AR, ARCC, and ARCCFR are not significantly different at $p \leq 0.05$. AR denotes annual ryegrass; ARCC denotes annual ryegrass + crimson clover; ARCCFR denotes annual ryegrass + crimson clover + forage radish.

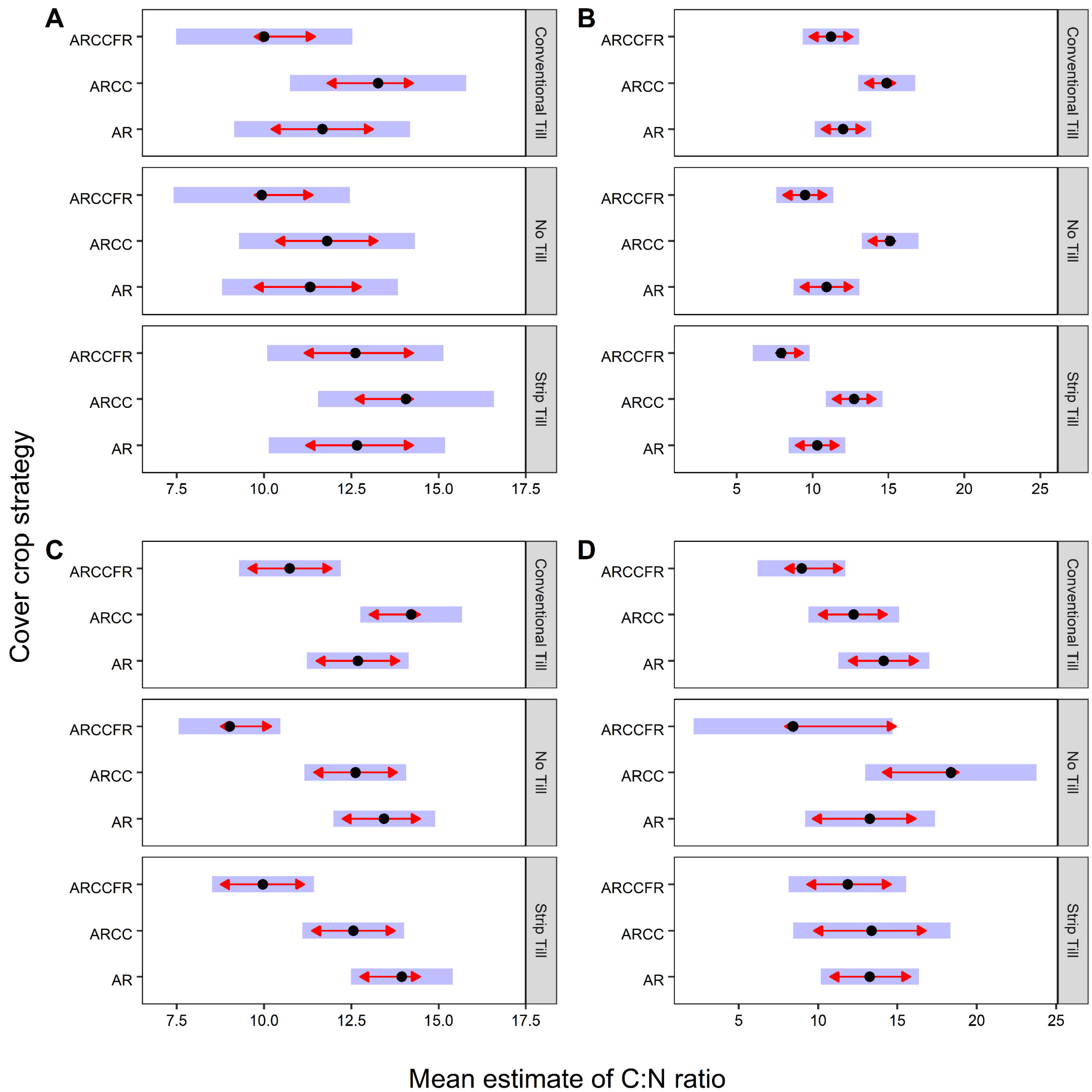

Figure 3.8. Means of C:N ratio among cover crop strategies within three tillage practices in corn (A) and soybean (B) in 2017; and in corn (C) and soybean (D) in 2018 in SROC, Waseca, MN, USA. Black dots represent estimated marginal means, and blue bars are 95% confidence intervals. Within each panel (tillage practice), red arrows overlapping among AR, ARCC, and ARCCFR are not significantly different at $p \leq 0.05$. AR denotes annual ryegrass; ARCC denotes annual ryegrass + crimson clover; ARCCFR denotes annual ryegrass + crimson clover + forage radish.

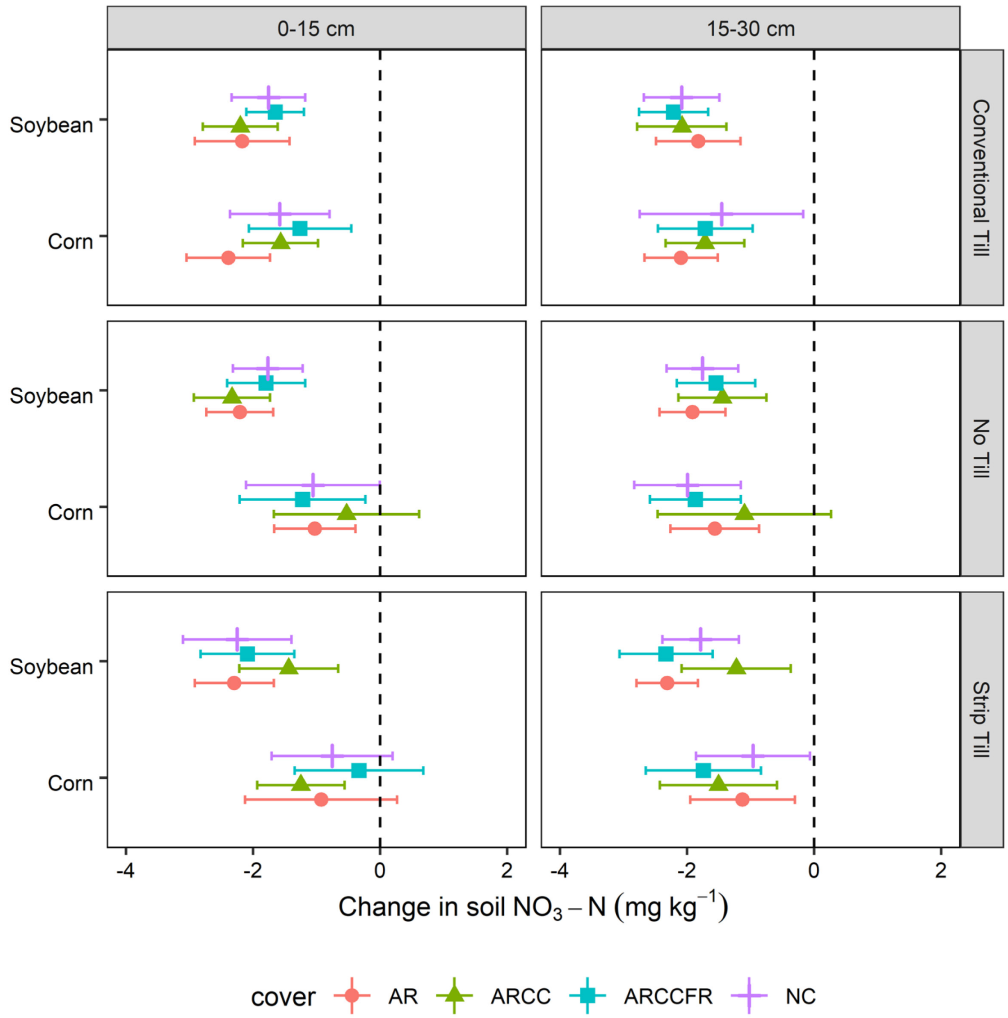

Figure 3.9. Change in NO_3-N concentration from seeding cover crops to fall before frost at two layers (0-15, 15-30 cm) in annual ryegrass (AR), AR+ crimson clover (ARCC), AR+CC+ forage radish (ARCCFR), and no-cover control (NC) within three tillage practices in the growing season of 2017 and 2018 in SWROC near Lamberton, and SROC, Waseca, MN, USA. Negative values (left to the vertical dashed line) denote higher NO_3-N concentration in the fall than at seeding time. Points represent mean values, and lines represent SEM (n=16). Lines that do not intersect the vertical dashed line are significantly different from 0.

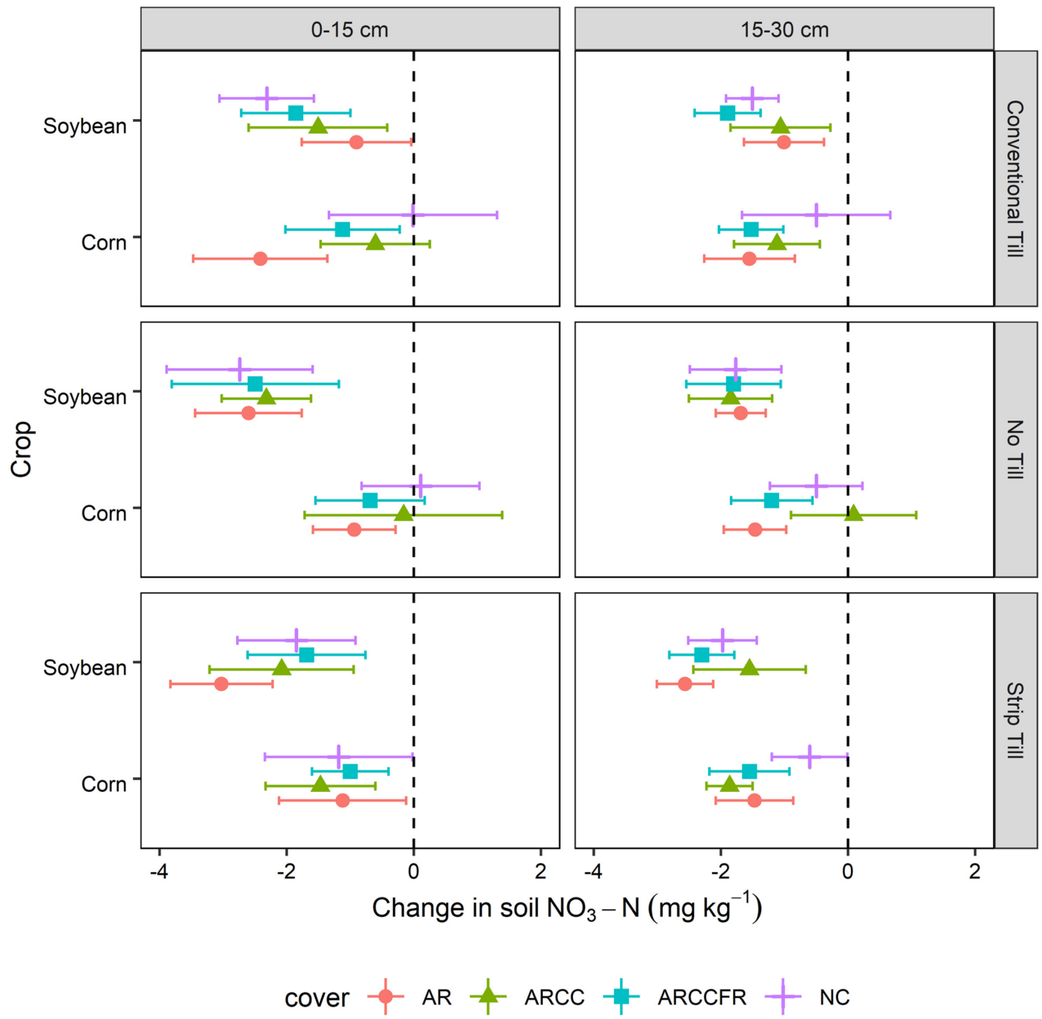

Figure 3.10. Change in NO_3-N concentration from seeding cover crops to next spring season at two layers (0-15, 0-30 cm) in annual ryegrass (AR), AR+ crimson clover (ARCC), AR+CC+ forage radish (ARCCFR), and no-cover control (NC) within three tillage practices in the growing season of 2017 and 2018 in SWROC near Lamberton, and SROC, Waseca, MN, USA. Negative values (left to the vertical dashed line) denote higher NO_3-N concentration in the spring than at seeding time. Points represent mean values, and lines represent SEM (n=16). Lines that do not intersect the vertical dashed line are significantly different from 0.

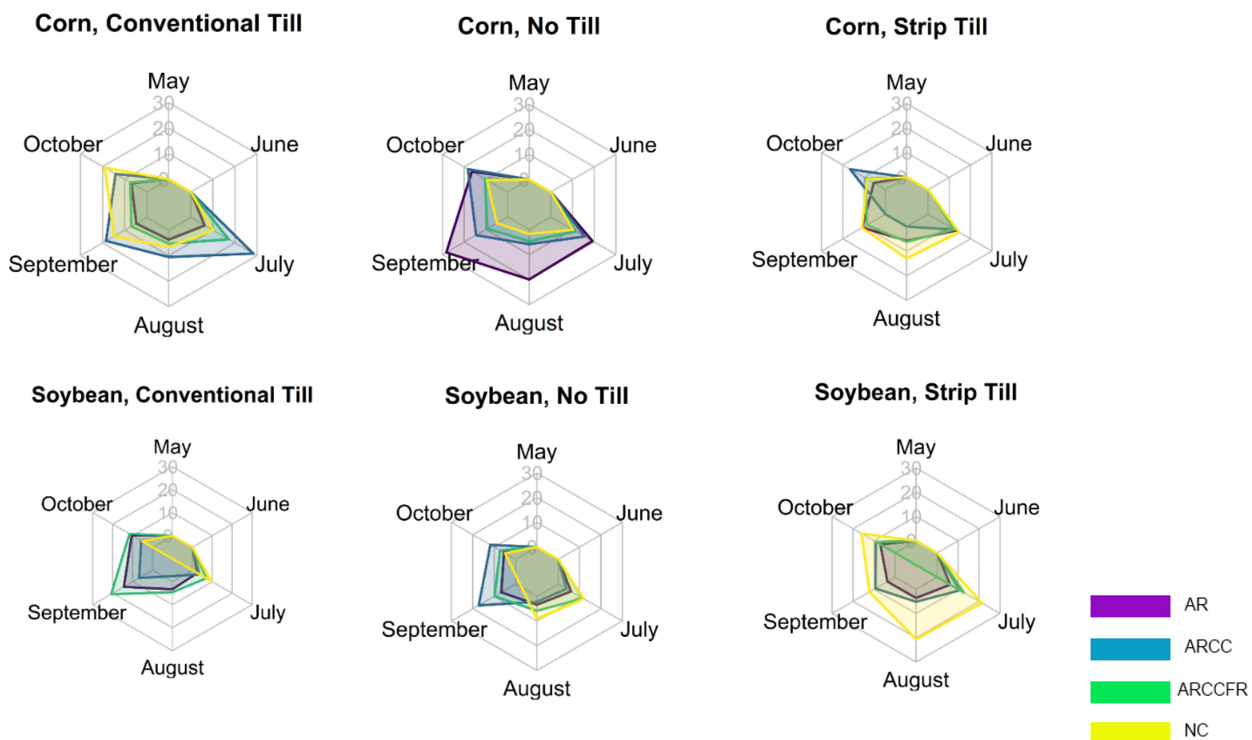

Figure 3.11. Radar charts showing the concentration of NO_3-N in the soil solution collected at 1 m depth during the growing season (May-Oct) of 2017 in SWROC near Lamberton, MN, USA. The axes are NO_3-N concentration (mg kg^{-1}) averaged over each month. AR denotes annual ryegrass; ARCC denotes AR+ crimson clover; ARCCFR denotes AR + CC + forage radish, NC denotes no cover (control).

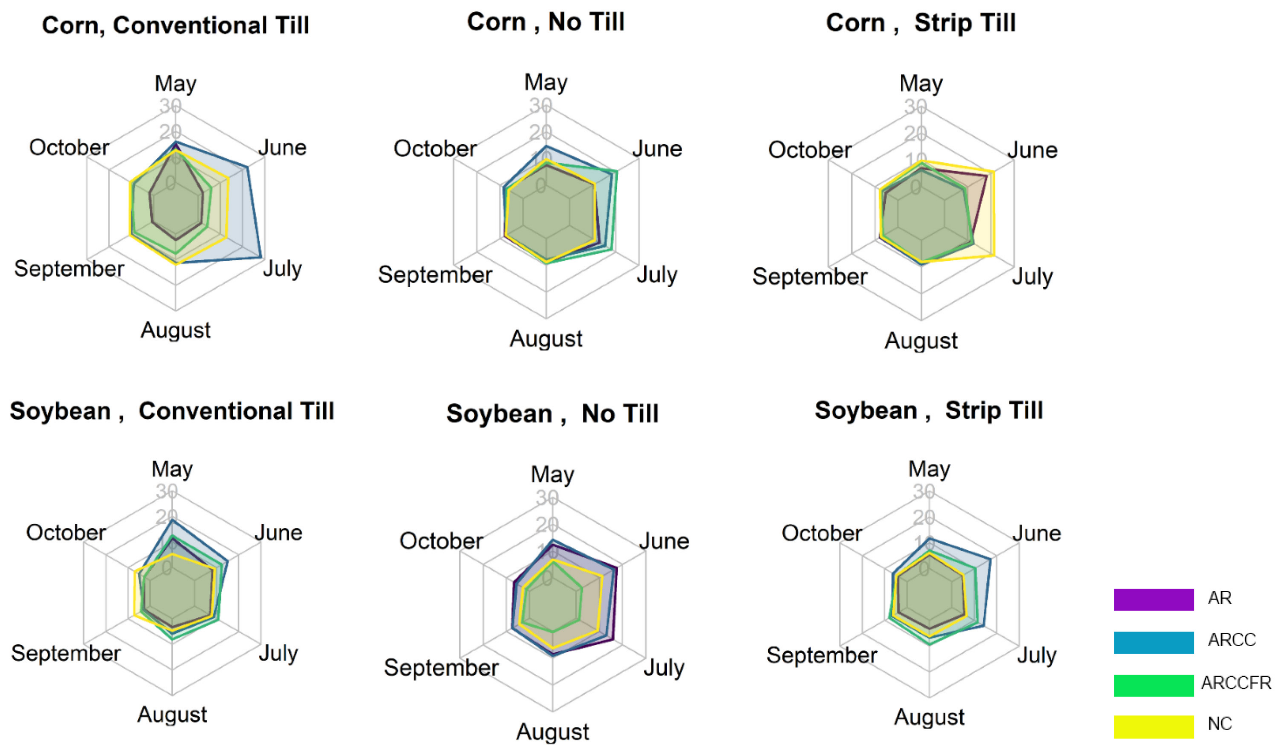

Figure 3.12. Radar charts showing the concentration of NO_3-N in the soil solution collected at 1 m depth during the growing season (May-Oct) of 2018 in SWROC near Lamberton, MN, USA. The axes are NO_3-N concentration (mg kg^{-1}) averaged over each month. AR denotes annual ryegrass; ARCC denotes AR+ crimson clover; ARCCFR denotes AR + CC + forage radish, NC denotes no cover (control).

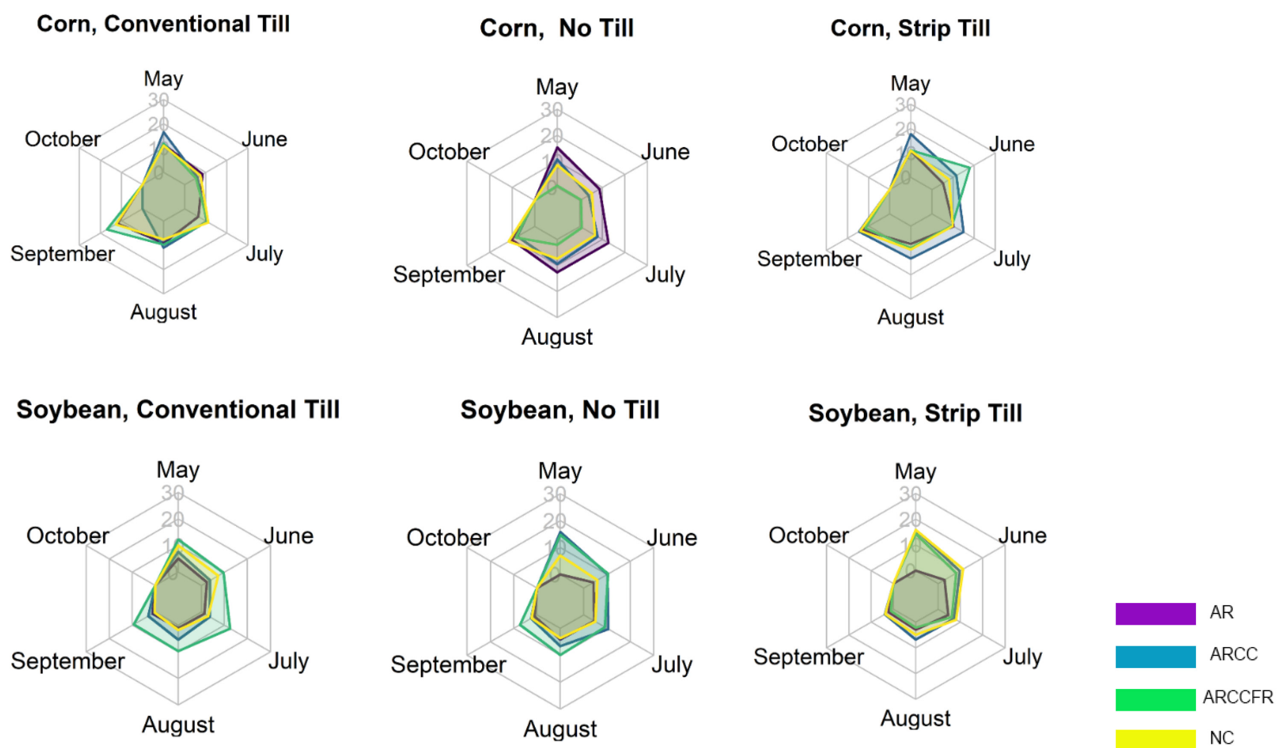

Figure 3.13. Radar charts showing the concentration of NO_3-N in the soil solution collected at 1 m depth during the growing season (May-Oct) of 2019 in SWROC near Lamberton, MN, USA. The axes are NO_3-N concentration (mg kg^{-1}) averaged over each month. AR denotes annual ryegrass; ARCC denotes AR+ crimson clover; ARCCFR denotes AR + CC + forage radish, NC denotes no cover (control).

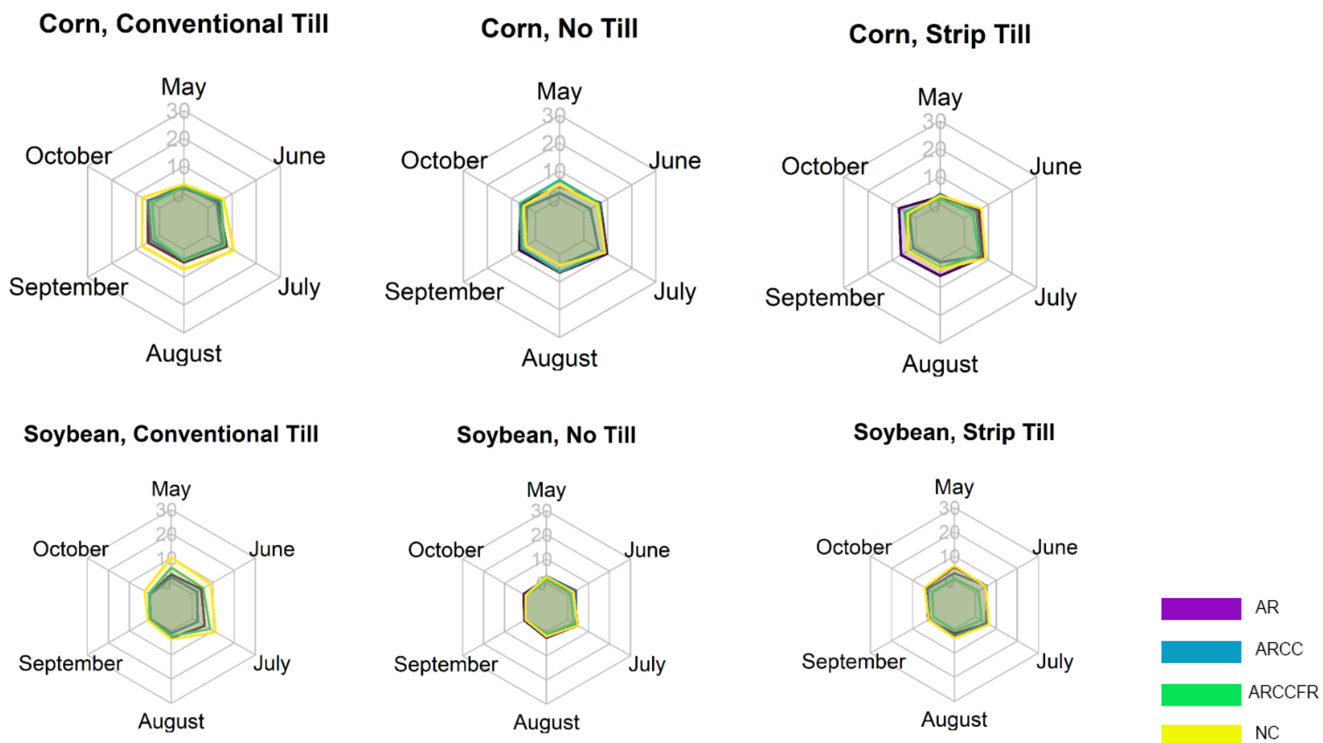

Figure 3.14. Radar charts showing the concentration of NO_3-N in the soil solution collected at 1 m depth during the growing season (May-Oct) of 2017 in SROC, Waseca, MN, USA. The axes are NO_3-N concentration (mg kg^{-1}) averaged over each month. AR denotes annual ryegrass; ARCC denotes AR+ crimson clover; ARCCFR denotes AR + CC + forage radish, NC denotes no cover (control).

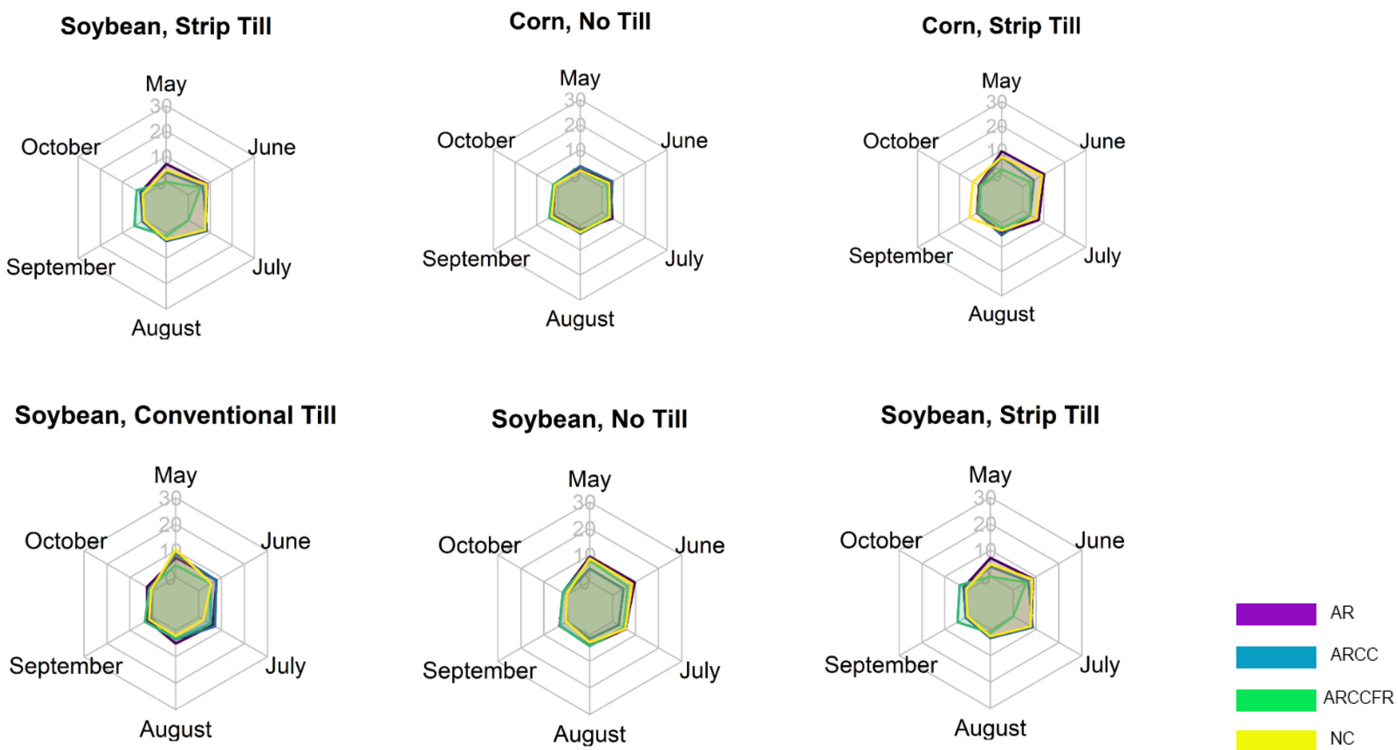

Figure 3.15. Radar charts showing the concentration of NO_3-N in the soil solution collected at 1 m depth during the growing season (May-Oct) of 2018 in SROC, Waseca, MN, USA. The axes are NO_3-N concentration (mg kg^{-1}) averaged over each month. AR denotes annual ryegrass; ARCC denotes AR+ crimson clover; ARCCFR denotes AR + CC + forage radish, NC denotes no cover (control).

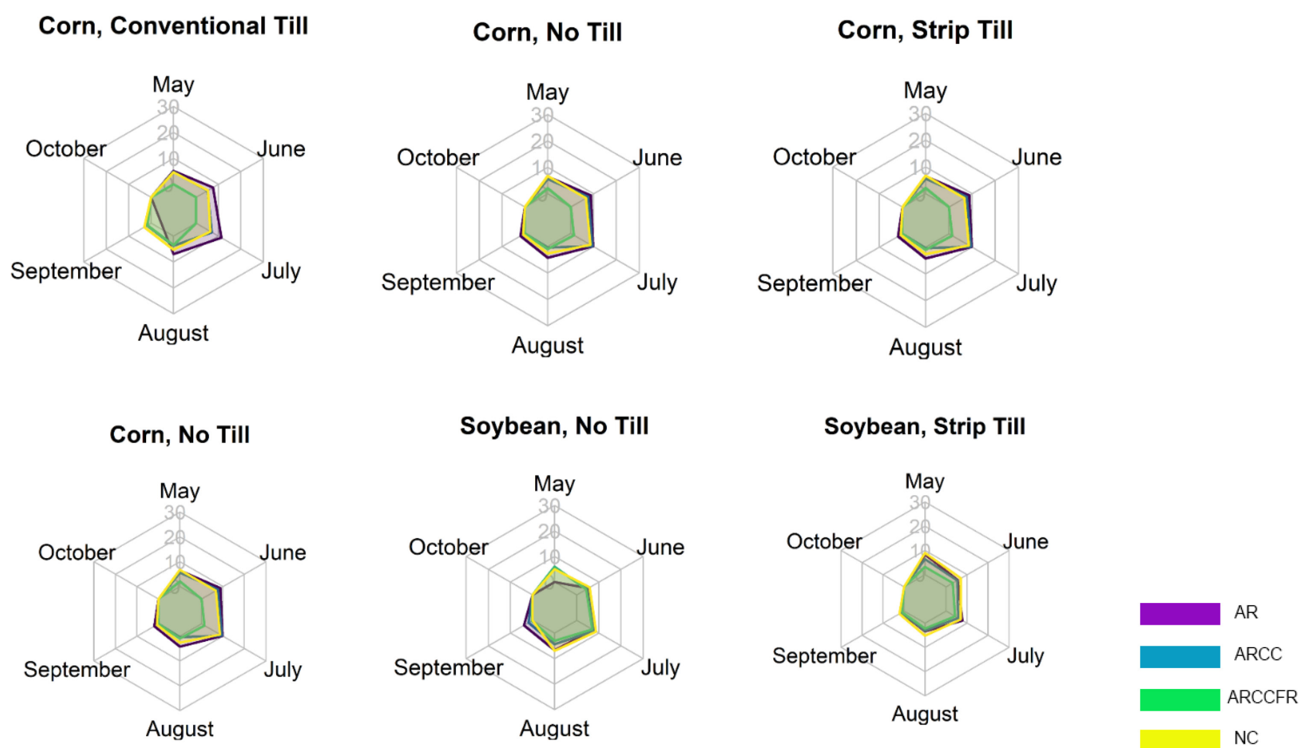

Figure 3.16. Radar charts showing the concentration of NO_3-N in the soil solution collected at 1 m depth during the growing season (May-Oct) of 2019 in SROC, Waseca, MN, USA. The axes are NO_3-N concentration (mg kg^{-1}) averaged over each month. AR denotes annual ryegrass; ARCC denotes AR+ crimson clover; ARCCFR denotes AR + CC + forage radish, NC denotes no cover (control).

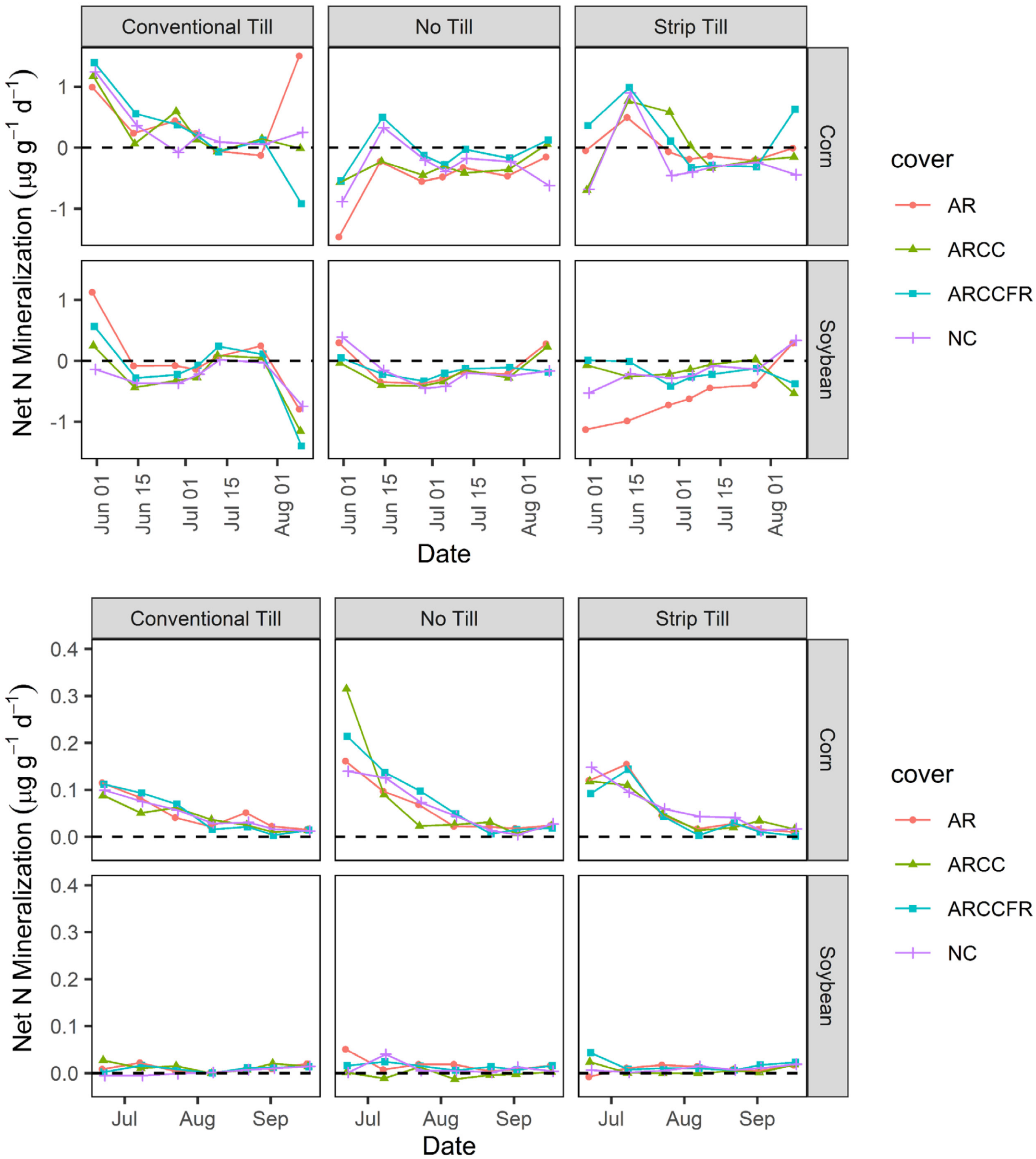

Figure 3.17. Net N mineralization during the growing season in 2018 (top) and 2019 (bottom) in SWROC near Lamberton, MN, USA. Negative values below the vertical dotted line represent N immobilization, and above the line represents N mineralization. AR denotes annual ryegrass; ARCC denotes AR+ crimson clover; ARCCFR denotes AR + CC + forage radish, NC denotes no cover (control).

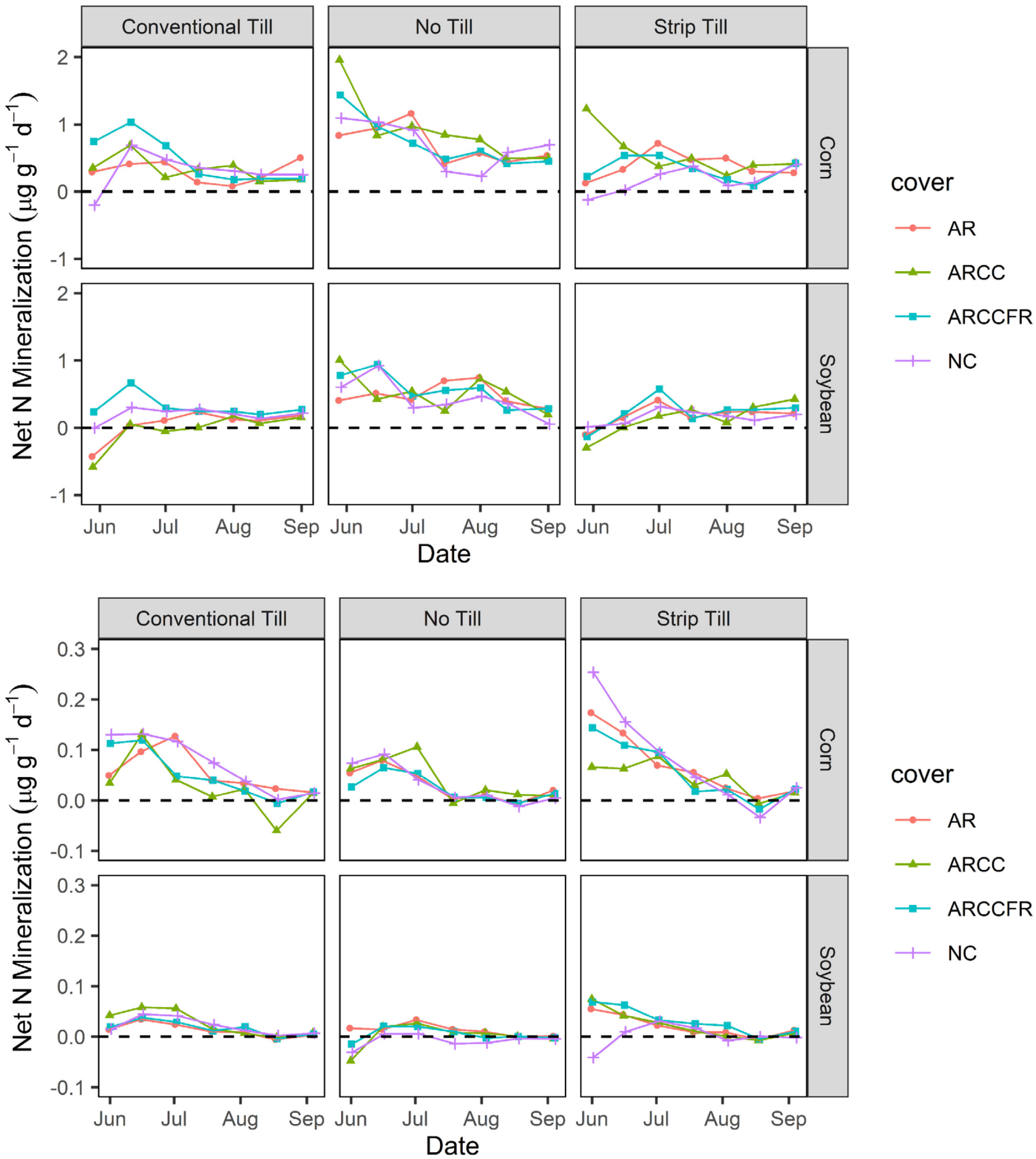

Figure 3.18. Net N mineralization during the growing season in 2018 (top) and 2019 (bottom) in SROC, Waseca, MN, USA. Negative values below the vertical dotted line represent N immobilization, and above the line represents N mineralization. AR denotes annual ryegrass; ARCC denotes AR+ crimson clover; ARCCFR denotes AR + CC + forage radish, NC denotes no cover (control).

4 CHAPTER 4: CONCLUSION

4.1 Overall summary

Cover crop usage has increased globally in the last decade, including in the U.S. upper Midwest. Winter annual cover crops are commonly being used in agroecosystems to increase functional diversity and improve soil health. In the U.S. upper Midwest, however, the need to explore cover crops other than overwintering annuals is felt due to long and cold winters, input costs and delayed planting, allelopathic, and pest effect on the crop following cover crops. Little information is available in the scientific literature and to growers regarding the synchrony of N available from cover crop biomass and crop N uptake. Hence, we addressed some of these major concerns in this study, which was conducted in two locations in southern Minnesota.

Chapter 1 briefly discusses the consequences of intensified corn-soybean production systems on the environment before introducing cover crops and their potential benefits with cover crop diversification in the U.S. upper Midwest. The need for cover crop options and strategies suitable to the short growing season in the region was our topic of research. Chapter 1 provides the rationale to study the effects of winterkilled cover crops in corn and soybean production, along with the potential to provide agroecosystem benefits.

Chapter 2 aimed to determine the biomass and ground cover produced by different late interseeded, winterkilled cover crop strategies within three tillage practices, and their effect in corn and soybean production. It was found that cover crop strategies and tillage practices had no effect on corn and soybean production, which was rather affected by weather and year. The three-way mixture of annual ryegrass, crimson clover, and forage radish (ARCCFR) produced the highest biomass compared to the two-way mixture of annual ryegrass and crimson clover (ARCC) and annual ryegrass (AR) monoculture when pooled over location and years. However, in 2017, AR dominated biomass production compared to ARCC and ARCCFR, suggesting that higher species richness did not always result in higher biomass. Biomass production within corn was higher than cover crops within soybean, with the latter generally producing marginal biomass and ground cover. It is concluded that although late interseeded, winterkilled cover crops have the potential to produce biomass within corn in the fall, the ecosystem services provided by such biomass may not outweigh the associated input costs. The relatively marginal biomass and ground cover produced within soybean suggest that such cover cropping strategy may not be a viable option for soybean growers.

Given such results, questions remain whether biomass produced by winterkilled cover crops have practical value regarding ecosystem services. To this end, Chapter 3 aimed to investigate the effect of late interseeded, winterkilled cover crops in the physicochemical properties of soils, determine the N uptake by cover crops and its effect in corn and soybean N use; C:N ratio of cover crops, residual soil NO_3-N dynamics, and net N mineralization in the season following cover crops.

Differences in soil organic matter were observed between cover crop strategies and tillage practice only at the Lamberton site in the 0-20 cm layer. Nitrogen accumulation in the cover crops was relatively marginal. The N accumulation in the three-way mixture ARCCFR was consistently higher than the two-way mix of ARCC and AR monoculture throughout the study, consistent with the higher biomass produced in the three-way mix of ARCCFR. Cover crop N accumulation did not affect the N uptake of the primary crops. The C:N ratio among cover crop strategy differed significantly, with ARCC consistently having more C:N ratio as compared to ARCCFR and AR, suggesting that the C:N ratio may not necessarily be affected by the amount of biomass produced by the cover crops at early vegetative growth stages. Cover crop strategies did not affect residual soil NO_3-N in the fall and spring. Residual NO_3-N was consistently higher in the fall and spring than at the time of seeding cover crops. Net N mineralization decreased throughout the season and was higher in corn plots as compared to soybean plots. Cover crop strategy did not affect N mineralization during the growing season within corn and soybean.

We conclude that winterkilled cover crops interseeded late into corn and soybean growing season did not provide ecosystem services due to marginal cover crop biomass and ground cover. Such cover cropping strategy is not viable for corn and soybean growers in the temperate region of the upper Midwest. Cover crops drill seeded at the early vegetative stage of corn and soybean may produce enough biomass and provide ecosystem services in such cold regions.

4.2 Limitations of this study

Although this study addressed previously unanswered questions about late interseeded winterkilled cover crops, few drawbacks were felt as the study progressed.

The primary weakness of this study was its inability to compare the effect of winterkilled cover crops with overwintering cover crops. A cereal rye control treatment could have helped to compare the differences between winterkilled and overwintering cover crops. Lack of replicates within the ceramic cups study hindered classical split-plot statistical analysis, and therefore, inferences were not made on the effect of late interseeded winterkilled cover crops in NO_3-N in the soil solution.

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