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Evaluating Drill Interseeded Cover Crop Establishment and Nitrogen Impact in Irrigated Corn

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EVALUATING DRILL INTERSEEDED COVER CROP ESTABLISHMENT AND NITROGEN IMPACT IN IRRIGATED CORN

by

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Cover crops are increasingly being used as a strategy to promote soil health and farming system sustainability in the United States. Preemergence (PRE) herbicides with soil residual activity are widely applied in corn (*Zea mays* L.) production systems to prevent early season weed emergence, crop-weed competition, and yield loss. When PRE herbicides are applied in the field, the active ingredients remain in the soil rhizosphere for a period of time, depending on soil moisture, composition, temperature and chemistry. However, PRE herbicides can also impact the establishment of interseeded cover crops (CC). Greenhouse bioassay and on-farm herbicide plot experiments were conducted to evaluate the PRE herbicide carry-over potential to interseeded CCs. Greenhouse study findings showed that saflufenacil + dimethenamid-P (Verdict) resulted in less CC biomass reduction (≤ 30%) for winter wheat (*Triticum aestivum*), hairy vetch (*Vicia villosa*) and radish (*Raphanus sativus*) within 20-30 days after application (DAA) and also control ≥ 90% of giant foxtail and Palmer amaranth up to 48 DAA. Atrazine + bicyclopyrone + mesotrione + s-metolachlor (Acuron) and acetochlor + mesotrione + clopyralid (Resicore) showed above 30% biomass reduction within 20-30 DAA for all CCs species tested and weed control above 90% for giant foxtail and Palmer amaranth. On-farm plot experiment also confirmed the safety of saflufenacil + dimethenamid-P (Verdict) being

applied as PRE emergence herbicide whereas atrazine + bicyclopyrone + mesotrione + s-metolachlor (Acuron) resulted in biomass reduction above 30%.

On-farm research was conducted to evaluate nitrogen (N) uptake by interseeded CCs that could potentially decrease N losses. The objective of this study was to evaluate the effect of CCs on N pools in soil including data collection of corn-N, cover crop-N, and soil nitrate at different depths up to 183 cm. In 2022 CC and no-cover crop (NCC) treatments showed significant difference (p = 0.0092) on upper soil layer only (0 – 30 cm). Corn total N in 2022 also showed significant difference (p = 0.0492) between CC and NCC. Yield in NCC treatment in 2022 was greater than CC treatment. The research findings from both studies can improve the understanding of how early interseeded CCs can impact positively soil-N pool by decreasing nitrate leaching into groundwater in Nebraska and also how CCs can be impacted by applying PRE emergence herbicides.

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CHAPTER 1: AN OVERVIEW OF NITROGEN DYNAMICS, COVER CROPS ADOPTION, AND THEIR CHALLENGES IN THE MIDWEST OF UNITED STATES

Nitrogen (N) is the nutrient with the greatest impact on corn (*Zea mays L.*) grain yield (Li et al., 2007), and for other agricultural crops that require proportionally more N than other nutrients to achieve maximum yield (St. Luce et al., 2011). Moreover, N is one of the most essential nutrients for life, highlighting plants by being a major component of chlorophyll, a compound that plants use to capture sunlight and convert it into energy (photosynthesis). Nitrogen is also a major component of amino acids, which combine to produce proteins. Nitrogen exists in numerous forms in the environment such as organic and inorganic N. Moreover, N undergoes many transformations, such as nitrification, volatilization, denitrification, immobilization and mineralization that will be explained hereafter. Most N transformation processes are mediated by microorganisms in the soil. When these microorganisms are in low concentrations, N processes are affected, impacting directly plant development and net productivity, resulting in a decrease in yield. Nitrogen exists in the environment in various forms including Nitrate (NO^{3-}), Nitrite (NO^{2-}), Ammonium (NH_{4+}), Organic N, and Nitrogen Dioxide (NO^{2}) dissolved in water, and where some of those N forms are dependent on soil bacteria to convert to other forms and become available for plant uptake. Organic N, for example, constitutes up to 90% of the total N in the plow soil layer (Olk, 2008) and only about 1–4% is mineralized for plant-available N (NH_{4+} and NO^{3-}) each year (Tisdale et al., 1985). The mineralization of organic N compounds into inorganic N is considered the major source of plant-available N and generates the soil N supply and it is also an important source for potential losses of N resulting in adverse environmental impacts (Myrold and Bottomley, 2008). Mineralization occurs largely through biological activities that are temperature and moisture dependent (Agehara and Warncke, 2005; Stevenson, 1986) and the process consists of sequential aminization and ammonification reactions. Aminization requires extracellular enzymes of bacterial and fungal origin such as proteinases and proteases that break down complex proteins into simpler amino acids and amino sugars (Myrold, 2005; Whalen and Sampedro, 2010). These organic N compounds are further hydrolyzed within microbial cells by intracellular enzymes such as arylamidase and amidohydrolase during ammonification to yield NH_{4+} (St. Luce et al., 2011), on which the nitrification process occurs. The nitrification process occurs by chemoautotrophic microorganisms that oxidize ammonia, resulting in the production of nitrate (NO^{3-}) readily available for plant uptake, however nitrification involves three key reactions. Ammonium is first oxidized to hydroxylamine and then to nitrite (NO^{2-}) by ammonia-oxidizing bacteria and archaea (AOA), and finally to NO^{3-} by nitrite-oxidizing bacteria (Whalen and Sampedro, 2010). Nitrate (NO^{3-}) and ammonium (NH_{4+}) are anions and very mobile in soil (Myrold and Bottomley, 2008). Ammonia-oxidizing bacteria is also responsible to denitrify the NO^{2-} to NO, N_2O, and N_2 through the nitrifier-denitrification pathway (Wrage et al., 2001), and therefore cause gaseous N loss from the soil–plant system. Nitrite does not accumulate in soil because it is rapidly transformed to NO^{3-} by nitrifiers that possess nitrite oxidoreductase or other oxidizing enzymes (Whalen and Sampedro, 2010). Moreover, other microbes can also produce NO^{2-} and NO^{3-} by enzymatic oxidations that are not linked to microbial growth (Myrold, 2005; Sahrawat, 2008; Whalen and Sampedro, 2010). Conversely, immobilization occurs when microorganisms assimilate recently mineralized N and inorganic N from the soil solution (St. Luce et al., 2011). Crop residues with a low C/N ratio are expected to decompose rapidly and cause little immobilization, thereby potentially increasing soil N supply during the early part of the growing season (Willson et al., 2001). Biological Nitrogen fixation (BNF) is a process restricted to prokaryotes and occurs when N_2 is transformed to organic N being mediated by an enzyme complex called nitrogenase, which is composed of two proteins, dinitrogenase and nitrogenase reductase (Myrold and Bottomley, 2008). It is the dominant natural process by which N enters soil biological pools. (Robertson et al., 2006). N_2 fixation contributes about 40–60% of the N requirements of leguminous crops (Herridge et al., 2008). Dinitrogen gas (N_2) comprises 79% of our atmosphere and is by far the most abundant form of N in the biosphere and it is used in the BNF process. Furthermore, dinitrogen gas can be an output of the denitrification process (conversion of nitrates to NO, N_2O and N_2) and is an important process under anaerobic conditions (Drury et al., 1992; Richardson et al., 2009), Denitrification process is also a major part of the nitrogen cycle in soils below certain depths (Rodriguez et al., 2005), depending on NO^{3-} and C availability, and soil pH (Davis et al., 2008; Miller et al., 2008). The anerobic denitrification process involves the utilization of NO^{3-} as an alternative electron acceptor for O_2 by facultative anaerobes resulting in the reduction of NO^{3-} to NO^{2-}, which is then subsequently reduced to NO by nitric oxide reductase, N_2O by nitrous oxide reductase and release dinitrogen gas (N_2) as the end product (Whalen and Sampedro, 2010). Denitrification rates are highly variable (Burton and Beauchamp, 1984) and depend on environmental, soil, and agricultural management factors (Beauchamp, 1997). Moreover, split applications of N fertilizer can reduce denitrification (Burton et al., 2008) depending on climatic conditions (St. Luce et al., 2011).

In many cropping systems inorganic N is often applied as a fertilizer. Nitrogen fertilizer application, as organic or mineral forms, for corn s is a practice that farmers across United States rely on to achieve higher crop yields. Urea for example, one of the most common N fertilizers for corn, is converted into ammonium (NH_{4+}) via hydrolysis by urease enzymes. Annual inputs of N fertilizer as an inorganic form for agricultural production can lead to a surplus N in soils, also known as legacy N, which can eventually leach into groundwater (Weitzman et al. 2022) or lost to the atmosphere by volatilization process. However, plant N use can be altered by different management practices and interactions between soil management practices, N rate, and N-application timing (Al-Kaisi, M., and Kwaw-Mensah, D., 2007), and the efficient use of N fertilizers in crop production has major importance (Rathke et al., 2006). Applying N to meet N crop requirement (Sharifi et al., 2007a; Zebarth and Rosen, 2007) by synchronizing the application of plant-available N with crop N uptake in space and time (Ma et al., 1999) would potentially decrease N loss. However, this is difficult to achieve due to substantial variation in both crop N demand and in soil N supply across years and within and between fields (Zebarth et al., 2009). Because nitrate is a mobile nutrient in soil, and because of water percolation caused by high irrigation rates, which is common, especially in sandy soils, excessive amounts of N in soil move downward, reaching the water table (Gholamhoseini et al., 2013). Excessive N application for cash crop production, such as corn, has the potential to contribute to N losses to the system, with 20% to 70% of N-fertilizer lost from soil-crop systems in some circumstances (Dawson et al., 2008). In some soil types, especially light-textured soils, over application of N fertilizer may lead to high N loss rate (Gholamhoseini et al., 2013). Whatever is not captured by the crop might possibly be lost to the atmosphere by volatilization, as nitrate below the crop rootzone which can potentially leach into groundwater, loss through denitrification, N loss as a result to soil erosion. Leaching of nitrate-N is one of the biggest concerns from the N loss pathways from cropping systems because it can contaminate groundwater, negatively affecting drinking water quality that can cause human health issues, especially in young children (Robertson and Vitousek, 2009; Fowler et al., 2013). Several studies in the United States have been evaluating strategies to decrease N losses to the environment. Owens et al., (2000) conducted a study evaluating the corn-soybean rotation evaluating whether nitrate leaching could potentially decrease by allowing N credit from soybean, thus decreasing N fertilizer applied to corn. Another study conducted by Toth and Fox (1998) evaluated corn-alfalfa rotation as a strategy to decrease N loss from fertilizer applied on corn year by N credit from alfalfa (*Medicago sativa* L.). Both studies found evidence that legumes, alfalfa and soybean, tied up N that could potentially leach into the groundwater and also could decrease further N fertilization for corn. Staver and Brinsfield (1998) conducted a study planting cereal rye (*Secale cereale* L.) cover crops (CC) after corn to evaluate nitrate concentration in soil and findings showed a 60% reduction of groundwater nitrate concentration in nine years of study. Thus, adding CCs might have an opportunity to reduce N leaching.

Cover crops are defined as grasses, legumes, and forbs, used for seasonal cover and other conservation purposes that may be terminated by natural causes such as frost or intentionally terminated through chemical application, crimping, rolling, tillage, or cutting (USDA-NRCS, 2014c; USDA-NRCS, 2014e). Cover crops have many benefits such as capturing surplus N that could potentially be lost (Delgado, 1998) increasing the nutrient use efficiency of farming systems, N fixation (Chatterjee and Clay, 2016), soil aggregation improvement (McVay et al. 1989; Drury et al. 1991; Roberson et al. 1991) and water quality improvement. CCs can also scavenge residual soil nitrate after crops have matured, converting scavenged nitrogen into forage protein (Dabney et al., 2001), which has also been used as a strategy to reduce nitrate leaching into groundwater. CCs include a wide range of species which provide unique ecological services and can be divided into functional groups, such as grasses (N scavenger and weed suppression), legumes (N fixation and erosion prevention) and brassicas (weed suppression and nutrient scavenging). Despite all the benefits mentioned above, CC adoption is low (about 5% of crop land in the US), primarily due to high seed prices, extra labor, and lack of access to planting equipment (drill or broadcast). One of the main challenges for growing CCs in the Upper Midwest of United States is the short growing season. As a result, fall drilled cover crops may not produce enough biomass to provide a significant reduction of soil N loss (Singer, 2008; Belfry and Van Eerd, 2016), and may reduce the number of farmers willing to adopt CCs within their cropping systems. Interseeding cover crops into corn during the early vegetative growth stage gives farmers an option to establish cover crops and produce more biomass in an otherwise short growing season following a corn rotation (CTIC 2017). Previous studies have shown that consistent establishment is achieved by drilling CCs at the V4 to V6 corn growth stage, approximately 35 to 56 d after planting (Roth et al. 2015). Farmers reported that grasses are currently the best cover crop choice for interseeding (51%), followed by clovers

(14%) and radish (10%) (CTIC 2017). Interseeding cover crops before harvest also provides more time for CC establishment and biomass accumulation compared with a fall seeding (Wilson et al., 2013; Blanco-Canqui et al., 2015; Belfry and Van Eerd, 2016), thus more N surplus in the soil profile would be captured diminishing leaching potential. In the following spring, the interseeded cover crops would be terminated before cash crop planting, releasing N back into soil from biomass decomposition.

Corn PRE emergence herbicide residual herbicide activity can impact interseeded CCs emergence and performance (Wallace et al., 2017). Ribeiro et al. (2021) conducted a study evaluating the efficacy of preemergence soybean herbicide in the greenhouse to evaluate the impact of PRE emergence herbicides on CCs and results showed great impact of chlorimuron-ethyl and metribuzin on cereal rye and radish. The potential for herbicide carryover injury is a significant concern for those high-value crop producers when applying residual herbicides, such as mesotrione (Felix et al., 2007), saflufenacil (Robinson and McNaughton, 2012), S-metolachlor and acetochlor (Pridie et al., 2020) for both corn and soybean rotations. Furthermore, in order to be active and effective at weed control, these active ingredients rely on soil properties. Soil pH and moisture, for example, play an important role in herbicide effectiveness and alter the availability of the chemical, which can remain for days after application, suppressing weeds but potentially limiting CCs development when interseeding early Spring. Active ingredients such as bicyclopyrone, S-metolachlor and acetochlor, for example, remain in soil for long period of time if soil pH and moisture levels are adequate, otherwise would be ineffective on weed control. Usually, interseeding cover crops in corn typically occur between 35 to 56 d after application of soil-applied preemergence (PRE) herbicides (Wallace et al., 2017).

In previous research, residual herbicides (atrazine, fluridone and pyrithiobac) reduced fall-seeded crimson clover and rapeseed biomass (Palhano et al. 2018) and growth when some legumes and grasses species were seeded in late summer or fall (Brooker et al., 2019). However, little is known about the effects of PRE herbicides on early season interseeded CCs species commonly used by farmers. Research on cover crop tolerance to herbicides is limited to a few cover crop species, soil types, and climatic regions, and very little research has been conducted for cover crops interseeded within zero to five weeks following a residual herbicide application in corn (Brooker et al., 2019).

Research Justification and Goals

Although N cycling occurs naturally in the environment, applying N fertilizer to maximize grain yield may result in excessive soil-N resulting in N leaching. To reduce potential losses, strategies such as irrigation management, N application management, and cover cropping have been implemented. Cover crops can be sown after harvest, early corn development stage, and before harvesting. However, because of the limited growth window for CCs in Nebraska, which results in lower biomass production, CCs could be interseeded early in the season to achieve higher biomass production. However, with early interseeding PRE emergence herbicides applied at corn planting may have a detrimental impact on CCs establishment, resulting in decreased biomass production throughout the crop season. Therefore, this study evaluated the impact of interseeded CCs into corn at V3 growth stage on N dynamics with an on-farm research trial in Nebraska. Furthermore, potential herbicide residual carry-over impacts on CCs establishment were tested in a greenhouse bioassay.

The objectives of these studies were: (1) to elucidate the impact of corn PRE herbicides on CC establishment; (2) to identify the ideal time to interseed cover crops following herbicide application; and (3) to evaluate the effect of cover crops on nitrogen uptake, including corn-N, cover crop-N, and soil nitrate in different depths.

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CHAPTER 2: EVALUATING GREENHOUSE CORN PREEMERGENCE HERBICIDE CARRY-OVER POTENTIAL TO INTERSEEDED COVER CROPS

Abstract

The adoption of cover crops (CC) as a strategy to improve soil health and cropping systems sustainability is increasing in the United States. The application of PRE herbicides with soil residual activity is commonly used in corn production systems to reduce early season weed establishment and minimize crop-weed competition and yield loss. Active ingredients from preemergence herbicides, when sprayed in the field, remain in the soil rhizosphere for a period killing weed seedlings as they emerge. However, PRE herbicides can also impact the establishment of interseeded cover crops. To evaluate the impact of commonly used corn PRE herbicides on interseeded cover crops, a series of greenhouse dose response and planting time bioassays were conducted in Lincoln, NE in 2021 and 2022, and on-farm experiment in Creighton, NE, both in a randomized complete block design. PRE herbicide treatments consisted of atrazine + S-metolachlor + bicycloprione + mesotrione (Acuron), acetochlor + mesotrione + clopyralid (Resicore), and saflufenacil + dimethenamid-P (Verdict), at six rates for a dose response, and full label rate across six planting times. Six cover crops species and two weed species were used in this study. Bioassay results showed that to increase potential for biomass production of interseeded CC at 20-30 days after applying (DAA), saflufenacil + dimethenamid-P would less result in biomass reduction below 30% for winter wheat, hairy vetch and radish. The on-farm experiment also confirmed saflufenacil + dimethenamid-P had less impact on CC establishment than atrazine + S-metolachlor +

bicycloprione + mesotrione. Moreover, saflufenacil + dimethenamid-P could be safely applied at corn planting while safely interseeding winter wheat, hairy vetch or radish at 20-30 DAA, and also would provide weed control above 90% for giant foxtail and Palmer amaranth. This study provides important information regarding potential herbicide carry-over on interseeded CC that can negatively impact its establishment. Our findings suggest that there is a window between applying PRE herbicides and planting CC that can maximize CC's establishment while also providing satisfactory weed control.

Keywords: Cover crops, PRE emergence herbicides, interseeding cover crops, herbicide carry-over

Introduction

The complexity of weed control due to increasing herbicide-resistant weeds is driving growers' interest in using cover crops (CCs) to diversify weed control strategies (Dorn et al. 2015). Cover crops provide important ecosystem services such as soil erosion and nutrient loss reduction, increased nutrient use efficiency, fixation of atmospheric N_2 through symbiosis with legume CCs, improved soil quality (Chatterjee and Clay, 2017; Kaye and Quemada, 2017) and weed control from residue cover (Sabbagh et al., 2020). The weed-suppressing potential of a CC is species specific and related to characteristics that include uniform emergence, creating a dense soil cover, rapid growth rate or biomass production per unit area, and ability to establish and thrive under variable weather conditions (Buchanan et al. 2016; Campiglia et al. 2012; Dorn et al. 2015; Teasdale and Mohler 2000). Several CCs have been used to suppress weeds in corn, with the aim of reducing dependence on herbicides (Burgos and Talbert 1996; Hoffman et al. 1993; Teasdale et al. 1991), but also for soybean where use of PRE herbicides has substantially increased from 2006 through 2017, particularly with sulfentrazone (21%), metribuzin (16%), S-metolachlor (15%), and flumioxazin (10%; USDA 2020). According to a national survey, cover crop adoption rates have been rising due to the multitude of benefits CCs offer to the soil, crops, and the environment (SARE, 2017). In addition, interseeding CCs before harvest provides time for the better establishment and biomass accumulation compared with post-harvest seeding, providing more protection from wind erosion (Wilson et al., 2013; Blanco-Canqui et al., 2015; Belfry and Van Eerd, 2016), increased water infiltration (Baumhardt et al. 2015), nutrient management (Bergtold et al. 2012; Finney et al. 2016; Syswerda et al. 2012), weed suppression (Leavitt et al. 2011), and scavenging residual inorganic N (Meisinger et al., 1991; Shipley et al., 1992). To produce more CC biomass, planting CCs earlier in the season is becoming more common practice among farmers in the Midwest United States, given the short window to grow the CCs before winter season following cash crop harvest. In the upper Midwest region, typically the average first light frost (<0°C) and first killing frost (<−6°C) occurs by mid-September and mid- to late-October, respectively, thus limiting cover crop establishment (National Weather Service, 2019). Farmers may encounter challenges such as residual herbicide carry-over from PRE emergence herbicides, planting timing decisions, seeding rate and species selection when planting early season interseeding cover crops in corn. Cover crops interseeded before canopy closure must be planted early enough to establish and optimize solar radiation reaching the soil surface, yet late enough to avoid direct competition with the main crop for water, nutrients, and solar radiation (Grubinger, 2014;

Noland et al., 2018). In corn, several studies have investigated interseeding cover crops at corn stages up to V7. For example, Brooker and Renner (2021) conducted research analyzing different interseeding timing in corn, from V1 to V7 corn stage, and found that farmers may interseed CCs at V2-V4 corn stage to boost cover crop biomass production. Interseeded CC establishment is a challenge, as limited information is available on location-specific planting time, optimum mixture of cover crop species, proper seed rate, inadequate soil moisture at seeding, and injury due to herbicide carryover (Cornelius and Bradley 2017; Keeling et al. 1996; Rogers et al. 1986).

The selection of PRE herbicides is often based on weed species previously identified in the field. Therefore, informed selection of PRE herbicides can reduce early season weed interference and give growers more flexibility for timely POST applications (Knezevic et al. 2019) and is a good strategy to reduce the selection pressure when postemergence (POST) herbicide options are limited (Norsworthy et al., 2012). However, residual herbicides from group 2 (ALS), group 5 (triazine), group 14 (PPO), or group 27 (HPPD) can interfere with the establishment of some of the broadleaf cover crop species, whereas residual herbicides that have activity on grass weeds species such as Poaceae family can interfere with the establishment of some grass CC species, especially the smaller seeded annual ryegrass species (Zimmer and Johnson, 2020). Moreover, these PRE active ingredients can potentially reduce establishment of post-harvest seeded CCs (Cornelius and Bradley, 2016; Palhano et al., 2018; Rector et al., 2020).

Interseeding CCs at corn growth stage V3 to V6, or approximately 28 to 49 days after PRE emergence herbicide applications, significantly increases the likelihood of increased cover crop establishment, thus biomass production, as the concentration of PRE herbicides in the soil degrades over time. However, successful cover crop establishment in corn-soybean rotations where PRE herbicides are used remains a concern (Cornelius and Bradley, 2017; Oliveira et al., 2019; Whalen et al., 2019). When interseeding multiple species that include grasses, legumes, and brassicas, then residual herbicide options are fewer due to different degree of sensitivity of CCs species to different PRE herbicides. Thus, single CC species (e.g. grass or legume) can allow greater herbicide options (Roth et al., 2015). Herbicide application timing greatly influences the risk of carryover interfering with cover crop establishment and, in general, herbicides applied at planting have a lower risk of interfering with fall seeded cover crop establishment than herbicides applied postemergence later in the year (Zimmer and Johnson, 2020). Due to a lack of research in Nebraska evaluating corn PRE emergence herbicide carry-over potential to interseeded CCs, farmers remain concerned whether to adopt CCs or not. Therefore, the objectives of these studies are to elucidate the impact of corn PRE emergence herbicides on CC establishment across different interseeding time, to evaluate PRE emergence herbicide dose response in the greenhouse and to evaluate biomass reduction of interseeded CCs from residual herbicides under field conditions.

Materials and Methods

Greenhouse Bioassays

Studies were conducted in the Fall of 2021 and 2022 at East Campus greenhouses, located at University of Nebraska-Lincoln (UNL), Lincoln, NE (40.50°N, 96.39°W). The treatments consisted of three corn PRE herbicides commonly used by farmers (Table 2-2), eight bioindicator species and an untreated control (Table 2-3) for both dose response and planting time studies. Eight bioindicator species were planted in individual potting cells and included: six cover crops, radish (*Raphanus sativus*), cereal rye (*Secale cereale*), annual rye (*Lolium multiflorum*), winter wheat (*Triticum aestivum*), red clover (*Trifolium pratense*) and hairy vetch (*Vicia villosa*); and two small-seeded weed species, Palmer amaranth (*Amaranthus palmeri*) and giant foxtail (*Setaria faberi*). These CCs and weed species were selected based on the frequency and commonality they are used as CCs and also common of weeds across the Midwest United States (Oliveira et al. 2019; WSSA 2020). To ensure constant planting rates, the quantity of seeds per pot was determined based on seed size, field seeding rate, and pot size for both CCs and weeds (Table 2-3). The experiments were arranged in a randomized complete block design with six replications. The bioassay experiment unit consisted of a 262 cm^3 (7.8 cm width x 5.8 cm length x 5.8 cm depth; 1200 Series T.O Plastics Inc., Clearwater, MN) potting cell in a twenty-four-tray cell filled with field soil (Table 2-1). Herbicides were sprayed using a single nozzle sprayer chamber (DeVries Manufacturing Inc., Precision Research Sprayer, Hollandale, MN 56045). Herbicides were applied using TP 8001EVS even flat fan nozzle (TeeJet Technologies, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60187) calibrated to deliver 140 L ha^{-1} at a constant speed of 3.7 km h^{-1}. The temperature at the greenhouse was set for 27-30°C (Day) and 18-21°C (Night), and lights were set for 14 hours a day. Plant biomass was collected at 28 days after planting (DAP) for both studies. Above ground biomass samples were cut at the soil surface, placed in paper bags, and dried (60°C) until a constant weight was achieved. After plant biomass dried, it was weighted, Biomass results were expressed as percent biomass reduction compared with the untreated control.

Table 2-1. Greenhouse soil description for 2021 and 2022.

Year	Soil type	Organic matter	pH
		%	
Fall 2021	Silt loam (11% sand, 64% silt, 25% clay)	2.2	7.7
Fall 2022	Silt loam (13% sand, 70% silt, 17% clay)	1.8	7.2

Table 2-2. Trade names, active ingredients, companies, site of action group, herbicide families and half-lives.

Trade name	Active ingredient	Company	Group (SOA#)	Herbicide family	Half-life
					Avg days
Acuron	Atrazine	Syngenta	PSII (5)	Triazine	60
	Bicyclopyrone		HPPD (27)	Triketone	213
	Mesotrione		HPPD (27)	Triketone	5-15
	S-metolachlor		VLCFA (15)	Chloroacetamide	112–124
Resicore	Acetochlor	Corteva	VLCFA (15)	Chloroacetamide	90
	Mesotrione		HPPD (27)	Triketone	5-15
	Clopyralid		SAH (4)	Carboxylic acid	14-56
Verdict	Saflufenacil	BASF	PPO (14)	Pyrimidinedione	15–29
	Dimethenamid-P		VLCFA (15)	Chloroacetamide	35–42

Abbreviations: HPPD, p-hydroxyphenylpyruvate dioxygenase inhibitor; PPO, protoporphyrinogen oxidase; PSII, photosystem II; SAH, synthetic auxin; VLCFA, very long chain fatty acid; SOA#, site of action.

[a]Half-life values were obtained from the WSSA Herbicide Handbook (8th ed.; Vencill, 2002). Acetochlor and saflufenacil half-life were obtained from Jablonkai (2000) and Camargo et al. (2013), respectively.

Planting Time

The planting time study was conducted over two independent runs, the first run in October 2021 and second run in August 2022. All pots were filled with soil and sprayed on the same day. Plants were sown every seven days, starting the same day herbicide treatments were applied up to 70 days after application (DAA). Herbicides were applied at the full label recommended rate for corn. The treatments consisted of three PRE herbicides sprayed at recommended full label rate, six species of CCs, two species of weeds, and an untreated control (Table 2-3). To estimate corn stage V3 window and correlate with planting timing results accumulated growing degree days (GDD) units was calculated using daily minimum and maximum temperature for corn based on 2022 weather information obtained by weather stations across Nebraska (High Plains Regional Climate Center - HPRCC).

Dose Response

The dose response study was conducted over two separate runs in the Fall of 2021, first run on 10/21/2021 and second run on 10/28/2021. Pots were seeded and sprayed on the same day. The herbicide rates were determined relative to the full label rate (Table 2-3) and included 1.0x, 0.8x, 0.6x, 0.4x, 0.2x and 0x (untreated control). For Acuron® and Resicore® the rates were 5.8, 4.7, 3.5, 2.3 and 1.2 L ha^{-1} respectively. The rates for Verdict® were 0.6, 0.5, 0.4, 0.2 and 0.1 L ha^{-1} respectively. Thus, treatments consisted of three PRE herbicides sprayed at six rates applied to six CC species and two weed species (Table 2-3).

Table 2-3. Seeds per pot by species and herbicides recommended label rates applied for planting time study.

Bioindicator Species		Acuron®	Resicore®	Verdict®
	Seeds pot^{-1}	Rate L ha^{-1}		
Annual rye	10	5.8	5.8	0.6
Cereal rye	8			
Winter wheat	8			
Hairy vetch	12			
Red clover	18			
Radish	8			
Palmer amaranth	60			
Giant foxtail	30			

On-farm Herbicide Plot Experiment

To evaluate the impact of PRE emergence herbicides on CCs, small herbicide plots were placed in a farmer's field located near Creighton, NE (42.26°N, 97.53°W), in 2022. The field was planted to corn (*Zea mays* L.) on May 19th, at 79,074 seeds ha^{-1}, 76 cm row spacing following strip-tillage. 200 kg ha^{-1} of Nitrogen was split applied, and irrigation managed by the farmer. A preemergence herbicide mix of saflufenacil + dimethenamid-p (Verdict®, BASF; 0.6 L ha^{-1}) and dicamba (DiFlexx®, Bayer; 0.2 L ha^{-1}) were applied at 140 L ha^{-1} at planting to the whole field. Cover crops were drill interseeded with a modified air seeder (Hiniker® 7020) in 0.2 m wide field length strips replicated five times at the V3 corn stage (June 15th) with a mix of winter wheat (62 kg ha^{-1}) and hairy vetch (50 kg ha^{-1}). The experimental design for the on-farm herbicide plot experiment was randomized complete block design (RCBD) with ten replications. Three small herbicide plots (76 cm width x 152.4 cm length) were randomly placed within each CC strip. Two of the three small plots were covered with plastic prior to the whole-field PRE herbicide application. Following the whole-field application, plastic covers were removed, and plots were either treated with atrazine + bicyclopyrone + mesotrione + s-metolachlor (Acuron®, Syngenta; 5.8 L ha^{-1}) at 140 L ha^{-1} rate and the untreated plot as a control.

Each small plot was divided in three subplots (30.5 cm width x 30.5 cm length) for above ground CC biomass sampling at corn stage V8 (July 19th), before corn harvest (October 25th), and before first frost killing (November 28th). The CC biomass was cut at the soil surface, separated by species and placed in paper bags (15.2 cm width x 10.2 cm length x 30.5 cm depth; Uline Inc., Pleasant Prairie, WI) dried at 60°C for four days or until constant weight and weighed.

Statistical Analyses

Greenhouse bioassay experiments biomass data over the two runs from both planting time and dose response studies were analyzed for nonlinear regression using the *DRC* package (Ritz et al., 2015) in R (R Core Team 2022). For planting time experiment, analysis of biomass reduction was performed to evaluate species response to herbicides across 70 DAT. For the dose response experiment, analysis biomass reduction was performed to evaluate response of each species to an herbicide rate applied. For planting time and dose response experiment, a three-parameter and four-parameter log-logistic models were used respectively and were chosen based on the lowest Akaike information criterion (AIC) value in a model comparison and the Lack-of-fit test ($P \geq 0.05$) was performed to confirm nonlinear regression model fits.

For planting time experiment, dry biomass as a percent of the control was plotted against the planting timing, in days, following herbicide application and fit with three-parameter log-logistic model, (equation [1]). The three-parameter log-logistic model [1] is parameterized, where coefficient *b* is the slope of the dose response curve, coefficient *d* is the upper asymptote or limits of response, the lower limit is set to 0 and *e* the effective dose ED50 (Ritz et al., 2015).

$$f(x) = \frac{d}{1 + \exp(b(\log(x) - \log(e)))} \qquad [1]$$

For dose response experiment, dry biomass as a percentage of the untreated control was plotted against the herbicide dose and data were fit with four-parameter log-logistic model LL.4 (equation [2]). The four-parameter log logistic model [2] is parameterized in a modified structure, where coefficient *b* is the slope of the dose response curve, coefficients *c* and *d*, the lower and upper asymptotes or limits of response, respectively, and *e* the effective dose resulting in 50% biomass reduction (ED50) or the inflection point (Ritz et al., 2015).

$$f(x) = c + \frac{d - c}{(1 + \exp\,(b(\log(x) - \log\,(e))))} \qquad [2]$$

For the on-farm herbicide plot experiment, biomass data were subjected to Analysis of Variance (ANOVA) performed with a generalized linear mixed model using *glmmTMB* package in RStudio (Brooks et al., 2017). Levene's test was performed using *leveneTest* function of *CAR* package to test the homogeneity of residual variance. Cover crop species and herbicides were considered fixed effects; replication was considered a random effect. Mean separation was conducted with Tukey's Honest Significant Difference test at $\alpha = 0.05$ using *multcomp* package in RStudio (Bretz et al., 2016).

Results and Discussion

Planting time and dose response studies were designed to estimate the effect of different PRE herbicides on interseeded CC biomass production and weed suppression at the V3 corn growth stage for Nebraska. Using GDD calculated for Nebraska, corn was estimated to reach the V3 growth stage, 20 to 30 days after application (DAA). This range was used to interpret data obtained from the planting time bioassay. For interseeded CCs ≤ 30% biomass reduction (BR_{30}) has been considered an acceptable or moderate level of herbicide injury (Curran, 2017). For weeds species ≥ 90% of biomass reduction (BR_{90}) is typically considered minimum acceptable weed control level by applying effective herbicide dose (Nurse et al., 2007).

Greenhouse Bioassay

For the Acuron treatment, annual rye resulted in 100% biomass reduction between 20 and 30 DAA (Table 2-4, Figure 2-1). To achieve BR_{30} it was estimated CC planting would need to occur 90 DAA, however this falls outside the range of planting timings tested and around R4 corn stage which is not ideal for drill interseeding CCs. The estimated Acuron dose resulting in 30% of biomass reduction was 0.7 L ha^{-1} (Table 2-5, Figure 2-1). The ED_{30} was below 20% of full labeled dose for Acuron (5.8 L ha^{-1}) showing annual rye is sensitive to Acuron at low doses and not recommended if interseeding at the V3 corn stage.

Interseeding at 20 DAA results in 71% biomass reduction, whereas interseeding at 30 DAA results in 52% biomass reduction (Table 2-4, Figure 2-1). To achieve BR_{30}, CC interseeding timing was estimated at 46 DAA, which would be around the V9 corn stage in Nebraska. The ED_{30} with Acuron on cereal rye was estimated at 2.2 L ha^{-1} (Table 2-5, Figure 2-1), which is approximately 38% of the full labeled dose for Acuron. Among the grasses tested on this study, cereal rye is the least sensitive to Acuron and produced 52% of biomass compared to the untreated under greenhouse conditions.

Interseeding winter wheat 20 DAA results in a 71% biomass reduction, whereas interseeding at 30 DAA results in a 63% biomass reduction (Table 2-4). Winter wheat would need to be interseeded at R2 corn stage to result in 30% threshold biomass reduction. The ED_{30} for winter wheat was estimated at 2.0 L ha^{-1} which is 34% of the full labeled dose (Table 2-5, Figure 2-1). Based on results from this greenhouse study, interseeding winter wheat at V3 corn stage could impact establishment and lead to biomass reduction above 63%.

Hairy vetch interseeded between 20 to 30 DAA for corn would result in biomass reduction above the 30% threshold, at 20 DAA would result in 92% biomass reduction, whereas at 30 DAA biomass reduction was estimated approximately 86% (Table 2-4). To achieve BR_{30} was estimated at 155 DAA, which is outside the 70 days tested with the regression the model and after black layer corn stage. Dose response for hairy vetch showed sensitivity at 20% of the full labeled dose with ED_{30} estimated at 0.1 L ha^{-1} (Table 2-5, Figure 2-1). Interseeding hairy vetch into corn would result in biomass loss above 30% if Acuron were used as PRE emergence herbicide.

Biomass reduction for red clover was 100% for 30 DAA, whereas for 20 DAA estimated reduction was 100% (Table 2-4, Figure 2-1). The model estimated 30% of biomass reduction for red clover interseeded at 131 DAA, which falls outside the planting time range of planting timings tested in this study. The ED_{30} was estimated at 0.5 L ha^{-1}, which is less than 10% of the full labeled dose (Table 2-5, Figure 2-1). Therefore, red clover would not be recommended for interseeding if Acuron was applied as a PRE emergence herbicide.

Radish showed biomass reduction of 77% at 20 DAA, whereas at 30 DAA it was estimated at 64%, (Table 2-4 and Figure 2-1). BR_{30} threshold was estimated if radish was interseeded at 69 DAA, when corn is at the VT growth stage. The ED_{30} was estimated at 0.3 L ha^{-1} (Table 2-5, Figure 2-1), indicating sensitivity of radish with Acuron applied at rates lower than 20% of full labeled dose. Therefore, radish would not be recommended to be interseeded at V3 corn stage due biomass reduction ≥ 30% threshold.

For giant foxtail the non-linear regression model did not converge as biomass reduction was ≥ 90% up to the 70 DAA tested for this study (Table 2-6, Figure 2-1). However, the ED_{90} was estimated at 0.6 L ha^{-1}, representing 10% of full labeled dose (Table 2-6). Even as concentration in the soil decreases over time, Acuron would provide effective control giant foxtail for at least 70 DAA.

Palmer amaranth was estimated to be 90% controlled up to 104 DAA (Table 2-6). However, this model estimate was outside the planting range of 70 days tested, at R5 corn stage which would not impact on corn establishment. The ED_{90} for Palmer amaranth was estimated at 1.4 L ha^{-1}, 24% of full labeled dose (Table 2-6, Figure 2-1), suggesting Acuron would provide desired control of Palmer amaranth up to 70 DAA.

According to our results, none of CCs species achieved ≤ 30% threshold biomass reduction within 20-30 DAA range (Table 2-4). Moreover, all CCs species had BR_{30} with less than 50% of the full labeled rate (Table 2-5). Among grasses species tested on this study, BR_{30} for cereal rye within 20 to 30 DAA of Acuron was lower than annual rye and winter wheat. Annual rye showed high response to Acuron with 100% biomass reduction. Similar sensitivity of annual rye was reported by Mallory-Smith and Retzinger (2003) in a PRE herbicide field experiment where annual rye stands were reduced by more than

60% after application of Group 15 herbicides, validating greenhouse bioassay conducted with Acuron containing S-metolachlor (Group 15). Moreover, S-metolachlor resulted in biomass reduction > 75% in annual rye noted by Wallace et al. (2017). Acuron contains S-metolachlor, a group 15 herbicide, as one of the active ingredients which might be responsible for the high biomass reduction seen in this study on grasses. Legumes resulted in higher biomass reduction than grasses within the window of 20-30 DAA, with 100% biomass reduction for red clover and 86% biomass reduction for hairy vetch at 30 DAA. Herbicide injury symptoms on legumes for this study were noted as leaf bleaching followed by necrosis, a common symptom for Group 27 (HPPD) herbicides. Radish had lower herbicide injury from Acuron compared with the legume species tested in this study, but similar biomass reduction as cereal rye and winter wheat. Brooker et al. (2019) reported that when oilseed radish interseeded into corn at the V3 stage, applications of mesotrione, pyroxasulfone, and acetochlor also resulted in reduced stands. Therefore, interseeding grasses, legumes or brassica species tested on this study at V3 corn stage, Acuron would result in biomass reduction between 30% and 100%. Regarding weed control, giant foxtail resulted in > 90% control within the 70 days range tested. Response to Acuron was noted in the dose response study, where 10% of the full label rate resulted in 90% control. The ability of S-metolachlor to suppress giant foxtail growth gives support to the efficacy and selectivity of VLFCA-inhibitor herbicides on small-seeded annual weed grass species (Parker et al., 2005; Yamaji et al. 2014). Palmer amaranth also resulted in 100% control up to 70 DAA. In a field experiment testing application of atrazine + bicyclopyrone + mesotrione + S-metolachlor as PRE emergence herbicide in corn, Sarangi and Jhala (2018b) reported no Palmer amaranth plants at 14 d and 6 plants

m^{-2} 42 d after treatment, showing results different than observed in the greenhouse, however the study conducted by Sarangi and Jhala (2018b) evaluated Palmer amaranth in field conditions. Therefore, Acuron would be recommended to be applied to control giant foxtail and Palmer amaranth (Table 2-6), however CCs biomass reduction would be > 30%. While these results noted from previous studies mentioned in this section are similar to the current study, they suggest that field conditions may influence degradation rates of Acuron differently than under greenhouse conditions.

For the Resicore treatment, annual rye resulted in 99% biomass reduction at 20 DAA, whereas at 30 DAA biomass reduction was estimated 94% (Table 2-4, Figure 2-2). However, 30% or less of biomass reduction would be achieved 64 DAA, which would be around V16 corn stage. The estimated Resicore dose resulting in 30% of biomass reduction was 1.3 L ha^{-1} (Table 2-5, Figure 2-2), 22% below the full labeled dose at 5.8 L ha^{-1}. Therefore, interseeding annual rye into V3 corn stage would not result in biomass production ≥ 30%.

Interseeding cereal rye at 20 DAA estimated 77% biomass loss, whereas 55% biomass loss was estimated at 30 DAA (Table 2-4, Figure 2-2). To target BR_{30}, interseeding would occur at 44 DAA, which is around V9 corn stage. The ED_{30} for cereal rye was estimated at 3.2 L ha^{-1} (Table 2-5, Figure 2-2), corresponding 55% of the full labeled dose. Thus, interseeding cereal rye between 20-30 DAA would impact on ≥ 30% biomass reduction.

Among grasses tested on this study, winter wheat was the species that had more tolerance to Resicore within 20 to 30 DAA (Figure 2-2). Interseeding winter wheat at 20 DAA estimated 55% of biomass reduction, whereas at 30 DAA 45% of biomass reduction was estimated (Table 2-4, Figure 2-2). To target ≤ 30% threshold biomass reduction, interseeding would occur around 44 DAA, which is around V9 corn stage. ED_{30} for winter wheat was estimated at 3.4 L ha^{-1} (Table 2-5, Figure 2-2), corresponding 58% of the full labeled dose.

Interseeding hairy vetch within the range of 20 to 30 DAA estimated total biomass reduction (Table 2-4, Figure 2-2). To target BR_{30}, hairy vetch would need to be interseeded 75 DAA, falling at R2 corn stage. ED_{30} for hairy vetch was estimated at 0.1 L ha^{-1} (Table 2-5, Figure 2-2). Therefore, interseeding hairy vetch at V3 corn stage would result in biomass loss above 30%.

Red clover resulted in total biomass reduction within 20 to 30 DAA range (Table 2-4, Figure 2-2). Hairy vetch would need to be interseeded 89 DAA to achieve 30% threshold biomass reduction, however it falls outside the planting dates range tested on this study at R3 corn stage. ED_{30} was estimated at 0.8 L ha^{-1} (Table 2-5, Figure 2-2), which corresponds 13% of full labeled dose. Thus, interseeding red clover into V3 corn stage would not be recommended due to high total biomass loss potential.

Interseeding radish within 20 to 30 DAA resulted in biomass reduction above 30% threshold. A 75% biomass reduction was estimated 20 DAA, whereas 66% biomass reduction was estimated if radish interseeded 30 DAA (Table 2-4, Figure 2-2). However, to achieve ≤ 30%, radish would need to be interseeded 119 DAA, corresponding around R5 corn stage. ED_{30} was estimated at 0.7 L ha^{-1} (Table 2-5, Figure 2-2). Therefore, radish would not be recommended to be interseeded if Resicore applied as PRE emergence herbicide.

Giant foxtail was estimated to be 90% controlled 48 DAA with Resicore (Table 2-6), suggesting good control as PRE emergence herbicide. Estimated dose to control 90% was estimated at 0.2 L ha^{-1} (Table 2-6, Figure 2-2), which corresponds to 3% of the full labeled dose. Palmer amaranth would be 90% controlled if germination occurs 68 DAA (Table 2-6, Figure 2-2). ED_{90} was estimated at 1.5 L ha^{-1} (Table 2-6), which is 26% of the full labeled dose. Therefore, Resicore resulted in control ≥ 90% for both Palmer amaranth and giant foxtail across 70 DAA, suggesting slow degradation in soil over time.

According to our results, Resicore had slightly less impact on grasses than Acuron. These observed results are similar to previous studies (Whalen et al., 2019) which included application of acetochlor with 90 d half-life average (Group 15), active ingredient found in Resicore, resulted to reduce ground cover including annual rye and radish. Hairy vetch and red clover resulted in total loss 20 to 30 DAA, against results obtained by Whalen et al. (2019) and Cornelius and Bradley (2017) that described hairy vetch as least susceptible species to biomass reduction. The grasses tested on this study showed biomass reduction between 45% to 99% if interseeding occurs at V3 corn stage, corresponding to 23 DAA. Legumes showed higher biomass reduction of 99% with Resicore than Acuron within 20 to 30 DAA window. Red clover for example resulted in total loss, and are similar to previous studies (Wallace et al., 2017) where mesotrione were sprayed resulting in 80% biomass reduction. Therefore, interseeding CC species tested on this study at V3 corn stage would result in biomass reduction above 30% threshold. Resicore resulted in 90% control for both giant foxtail and Palmer amaranth up to 48 DAA, corresponding to V12 corn stage.

For Verdict, interseeding annual rye at V3 corn stage would result in biomass reduction ≥ 30% within 20 to 30 DAA with 85% biomass reduction estimated at 20 DAA, and 78% of biomass reduction estimated at 30 DAA (Table 2-4, Figure 2-3). To target 30% of biomass reduction, annual rye would need to be interseeded around 179 DAA, which falls outside the planting dates range tested on this study, potentially after corn harvest. The ED_{30} of Verdict was estimated at 0.2 L ha^{-1} (Table 2-5, Figure 2-3), which is 33% of the full label dose at 0.6 L ha^{-1}. Thus, interseeding annual rye at V3 corn stage would result ≥ 30% biomass reduction.

Interseeding cereal rye at 20 DAA resulted in 48% biomass reduction, whereas at 30 DAA BR_{30} was estimated at 39% (Table 2-4, Figure 2-3). A 30% biomass reduction would be achieved if interseeding cereal rye occurs around 41 DAA, which would be at V7 corn stage. ED_{30} was estimated for cereal rye at 0.3 L ha^{-1} (Table 2-5, Figure 2-3). Therefore, interseeding cereal rye at V3 corn stage would result in biomass reduction ≥ 30%.

Winter wheat interseeded at 20 DAA was estimated 37% biomass reduction, whereas at 30 DAA was estimated 22% biomass reduction (Table 2-4, Figure 2-3). ED_{30} was estimated at 0.6 L ha^{-1} (Table 2-5, Figure 2-3). Therefore, winter wheat could be interseeded at V3 corn stage following Verdict application.

Among legumes, hairy vetch was the only species that resulted in biomass reduction ≤ 30%. Interseeding hairy vetch 20 DAA estimated 37% biomass reduction, whereas 30 DAA estimated 27% biomass reduction (Table 2-4, Figure 2-3), which is below 30% threshold for biomass reduction. ED_{30} was estimated at 0.3 L ha^{-1} (Table 2-5, Figure 2-3), suggesting half labeled dose would result 30% biomass reduction. Thus, hairy vetch could be safely interseeded at V3 corn stage following Verdict application would result in ≤ 30% biomass reduction.

Interseeding red clover 20 DAA estimated 46% of biomass reduction, whereas at 30 DAA estimated 38% of biomass reduction (Table 2-4, Figure 2-3). However, interseeding red clover 41 DAA would achieve 30% threshold of biomass reduction, which falls at V7-V8 corn stage. ED_{30} was estimated at 0.2 L ha^{-1} (Table 2-5, Figure 2-3) corresponding 33% of the full labeled dose. Therefore, interseeding red clover at V3 corn stage would not be recommended.

A 35% biomass reduction was estimated for radish interseeded at 20 DAA, whereas at 30 DAA estimated 26% biomass reduction (Table 2-4, Figure 2-3), which is ≤ 30% threshold of biomass reduction. ED_{30} was estimated at 0.4 L ha^{-1} (Table 2-5, Figure 2-3), corresponding 66% of full labeled dose.

Giant foxtail estimated 90% control up to 54 DAA (Table 2-6). Moreover, ED_{90} was estimated at 0.1 L ha^{-1} (Table 2-6, Figure 2-3), suggesting 16% of the full labeled dose. Therefore, giant foxtail could be controlled at 90% by applying full Verdict dose.

Palmer amaranth was estimated to be 90% controlled up to 48 DAA (Table 2-6, Figure 2-3). Moreover, ED_{90} was estimated at 0.2 L ha^{-1} (Table 2-6, Figure 2-3), which suggests 90% control if 33% of full labeled dose sprayed. Therefore, between the two weeds tested on this study Palmer amaranth would show less tolerance for low dose than giant foxtail, but 90% control potential would remain for longer days after planting corn, compared with giant foxtail.

According to our findings, winter wheat, hairy vetch and radish showed a biomass reduction below 30% threshold tested on planting time range of 20-30 DAA. However, among all CC species tested, annual rye resulted in major biomass reduction by applying saflufenacil + dimethenamid-P (Verdict) as PRE emergence herbicide. A similar study supports our findings, which saflufenacil and saflufenacil + dimethenamid-P applied at full labeled rate and at 0.5x of full rate respectively reduced red clover biomass (Wallace et al., 2017). Among grasses, winter wheat resulted in lowest biomass reduction of 22% at 30 DAA, which also suggests application of saflufenacil + dimethenamid-P would pose minimal risk of injury to grass and broadleaf CCs (Yu et al. 2015). Moreover, dose response for winter wheat suggested tolerance for Verdict resulting in 30% biomass reduction even at the full labeled dose of 0.6 L ha^{-1}. Among legumes, hairy vetch presented the lowest biomass reduction at 30 DAA with 27% biomass reduction. A similar study applied 735 g ha^{-1} of saflufenacil + dimethenamid-P three months before seeding hairy vetch and results showed no biomass reduction (Yu et al. 2015), suggesting that both active ingredients break down faster than bicyclopyrone, S-metolachlor and acetochlor. Moreover, radish showed biomass reduction of 26% at 30 DAA, below 30% threshold. Therefore, interseeding winter wheat, hairy vetch and radish as monoculture or mix at V3 corn stage would result in ≤ 30% biomass reduction, which could be well established throughout the crop season and meeting grower’s objectives. Giant foxtail and Palmer amaranth had 90% control at 54 and 48 DAA respectively. Thus, we assume interseeded CC species would compete with both weed species emerged later and enforce great control combined with herbicide effect.

Table 2-4. Cover crop (CC) biomass reduction (%) at 20 and 30 days after application (DAA) for each PRE emergence herbicide.

	Acuron		Resicore		Verdict	
Species	20 DAA (± SE)	30 DAA (± SE)	20 DAA (± SE)	30 DAA (± SE)	20 DAA (± SE)	30 DAA (± SE)
	%					
Annual rye	-	99 (1)	99 (1)	94 (1)	85 (3)	78 (3)
Cereal rye	71 (1)	52 (1)	77 (2)	55 (2)	48 (2)	39 (2)
Winter wheat	72 (2)	63 (2)	55 (2)	45 (2)	37 (2)	22 (1)
Hairy vetch	92 (3)	86 (3)	-	99 (1)	37 (2)	27 (2)
Red clover	-	99 (1)	-	99 (1)	46 (8)	38 (8)
Radish	77 (2)	64 (2)	74 (2)	66 (2)	35 (2)	26 (1)

Abbreviations: SE, standard error of the mean.

Table 2-5. Estimated herbicide dose (ED_{30}) to achieve 30% biomass reduction for cover crops (CCs).

	Acuron	Resicore	Verdict
Species	ED_{30} (± SE)	ED_{30} (± SE)	ED_{30} (± SE)
		L ha^{-1}	
Annual rye	0.7 (0.06)	1.3 (0.04)	0.2 (0.01)
Cereal rye	2.2 (0.32)	3.2 (0.11)	0.3 (0.01)
Winter wheat	2.0 (0.17)	3.4 (0.09)	0.6 (0.02)
Hairy vetch	0.1 (0.07)	0.1 (0.10)	0.3 (0.01)
Red clover	0.5 (0.32)	0.8 (0.14)	0.2 (0.01)
Radish	0.3 (0.11)	0.7 (0.06)	0.4 (0.01)

Abbreviations: SE, standard error of the mean.

Table 2-6. Estimated days of weed control with 90% biomass reduction (BR_{90}) and herbicide estimated dose (ED_{90}) to achieve 90% control of weed species.

	Biomass Reduction			Dose Response		
	Acuron	Resicore	Verdict	Acuron	Resicore	Verdict
Species	BR_{90} (± SE)	BR_{90} (± SE)	BR_{90} (± SE)	ED_{90} (± SE)	ED_{90} (± SE)	ED_{90} (± SE)
	Days			L ha^{-1}		
Giant foxtail	-	48 (2)	54 (1)	0.6 (0.18)	0.2 (0.13)	0.1 (0.01)
Palmer amaranth	104 (16)	68 (1)	48 (1)	1.4 (0.61)	1.5 (0.20)	0.2 (0.01)

Abbreviations: SE, standard error of the mean.

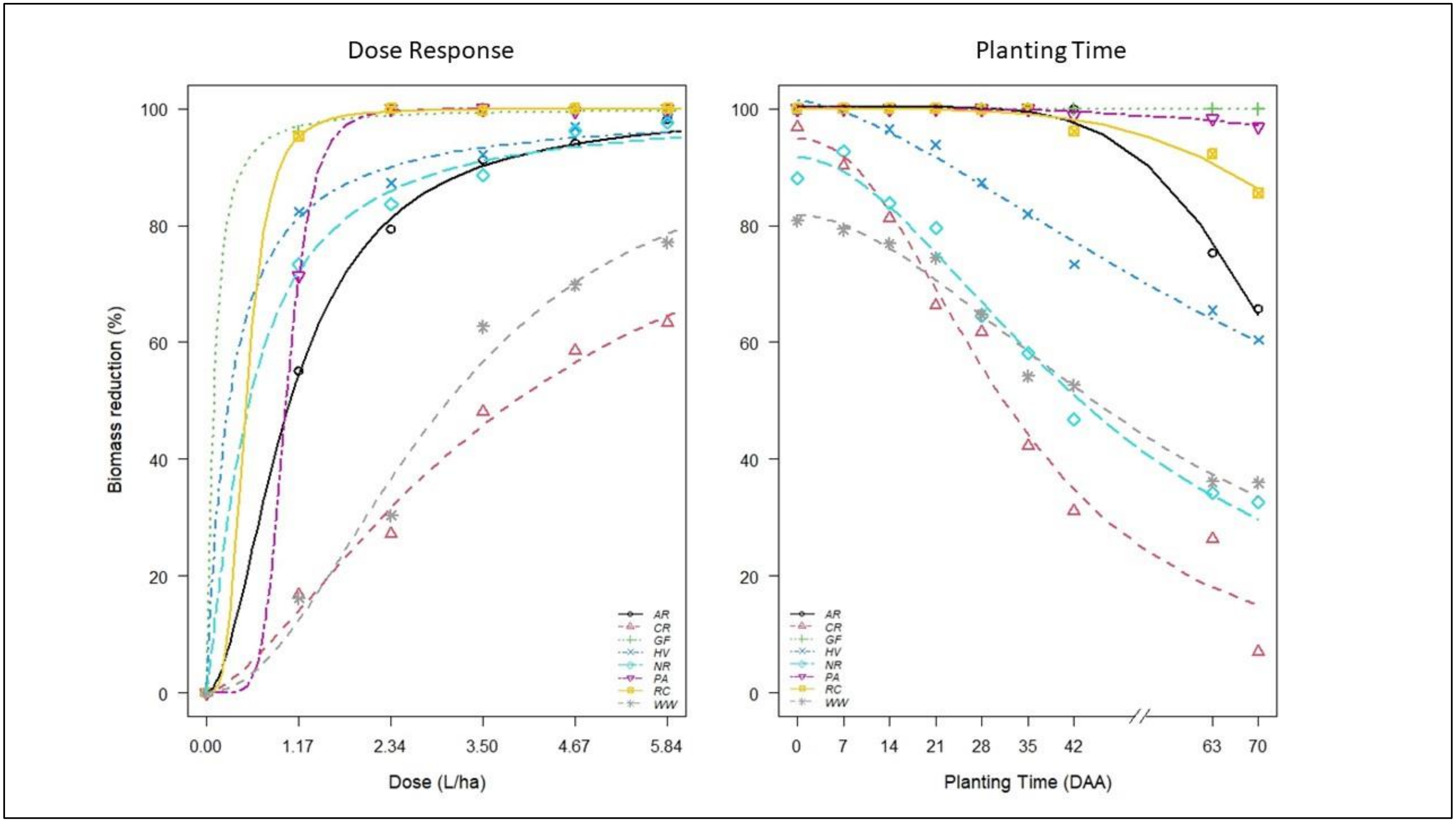

Figure 2-1. Biomass reduction of cover crop and weed species over the dose range applied of Acuron and for planting time on seven days cycle up to 70 days after application of with planting time break between 42 and 63 days. Abbreviations: AR, annual rye; CR, cereal rye; GF, giant foxtail; HV, hairy vetch; NR, radish; PA, Palmer amaranth; RC, red clover; WW, winter wheat.

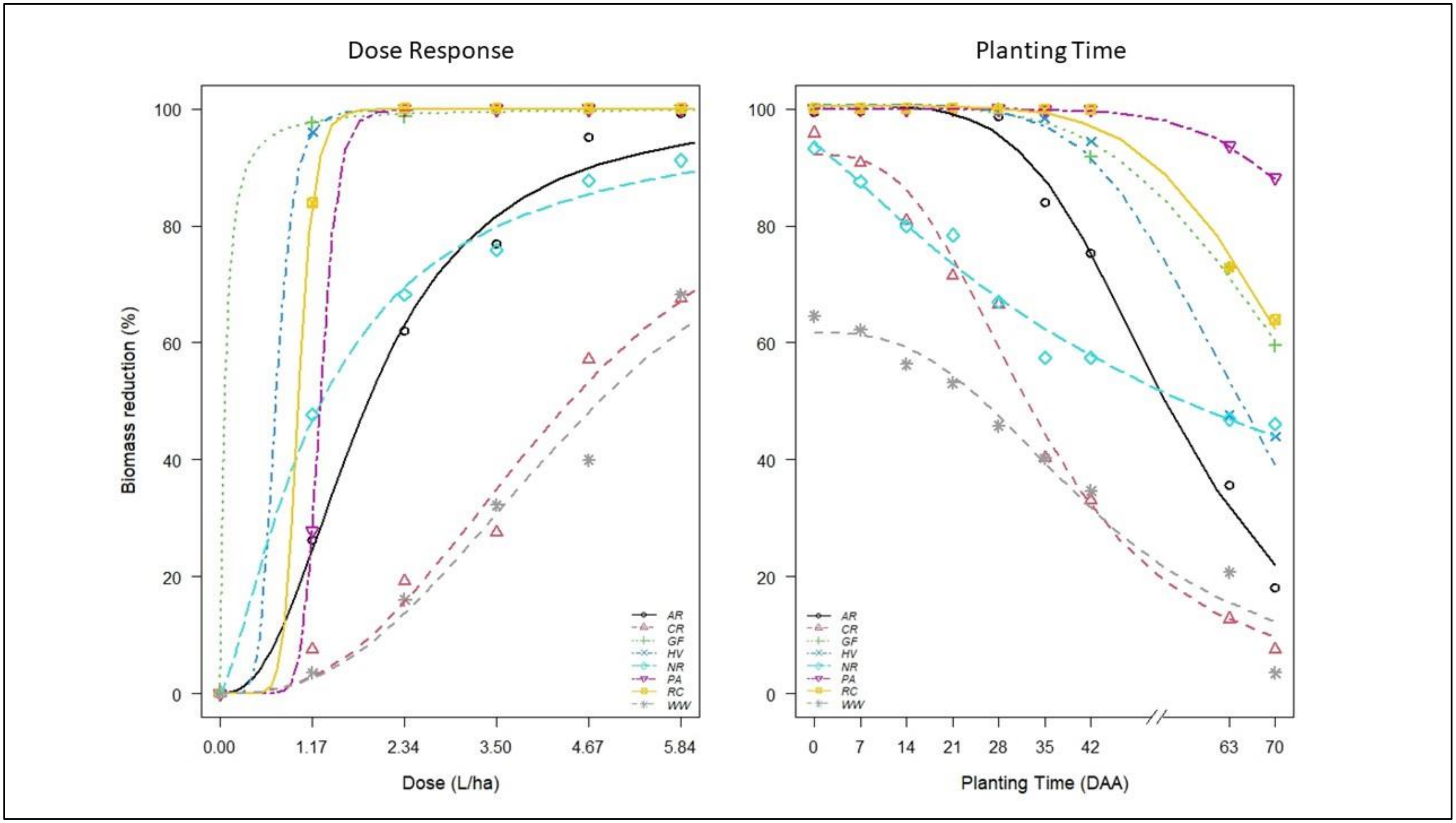

Figure 2-2. Biomass reduction of cover crop and weed species over the dose range applied of Resicore and for planting time on seven days cycle up to 70 days after application with planting time break between 42 and 63 days. Abbreviations: AR, annual rye; CR, cereal rye; GF, giant foxtail; HV, hairy vetch; NR, radish; PA, Palmer amaranth; RC, red clover; WW, winter wheat.

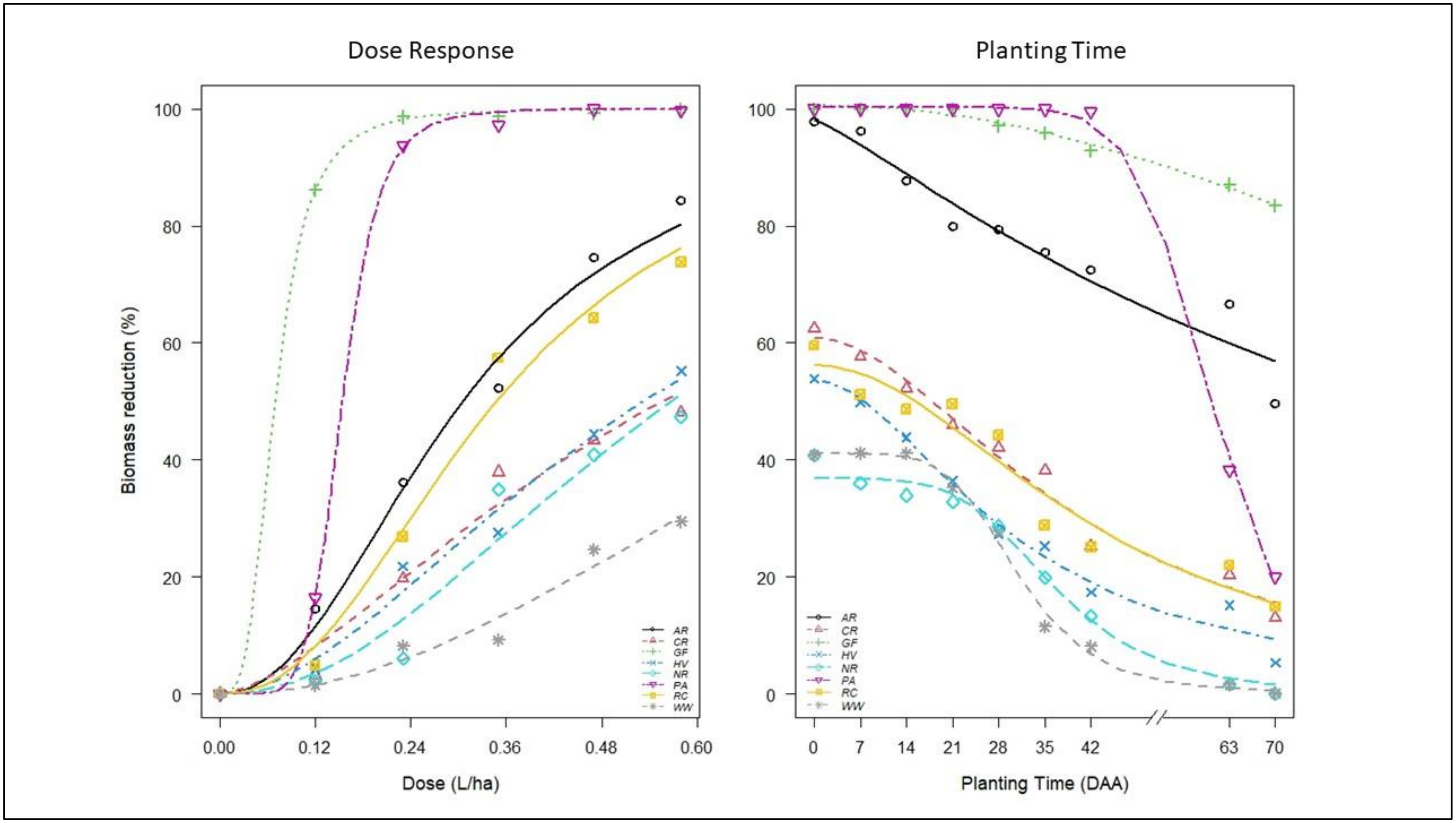

Figure 2-3. Biomass reduction of cover crop and weed species over the dose range applied of Verdict and for planting time on seven days cycle up to 70 days after application with planting time break between 42 and 63 days. Abbreviations: AR, annual rye; CR, cereal rye; GF, giant foxtail; HV, hairy vetch; NR, radish; PA, Palmer amaranth; RC, red clover; WW, winter wheat.

Field Experiment

The on-farm experiment tested the impact of Acuron and Verdict on CC biomass reduction under field conditions. There were differences in biomass reduction of both species (hairy vetch and winter wheat) between Acuron and Verdict ($p < .0001$, Table 2-7 and Table 2-8 respectively), Figure 2-4.

At V8 corn stage, Acuron resulted in on 97% of biomass reduction of hairy vetch ($p < .0001$, Table 2-7), while Verdict resulted in 19% biomass reduction ($p = 0.0209$), Figure 2-4. For winter wheat, Acuron reduced biomass 62% ($p < .0001$) and Verdict reduced biomass 7% ($p = 0.1378$, Table 2-8). Before harvest, Acuron resulted in 82% ($p < .0001$) biomass reduction for hairy vetch, whereas Verdict resulted in biomass reduction of 15% ($p = 0.2233$, Table 2-7). Winter wheat resulted in 80% ($p < .0001$) biomass reduction by applying Acuron, whereas Verdict impacted in 13% ($p = 0.3729$, Table 2-8) biomass reduction. Moreover, before first frost killing, Acuron reduced biomass at 98% ($p < .0001$) of hairy vetch, whereas Verdict reduced biomass at 22% ($p = 0.0396$, Table 2-7). For winter wheat, Acuron reduced biomass at 88% ($p < .0001$) and Verdict at 14% ($p = 0.1318$, Table 2-8). However, biomass reduction might be influenced also for canopy closure as corn growth advanced over the time, and not only herbicide impact.

Hairy vetch showed greater biomass reduction response of Acuron compared with response from Verdict throughout the season, confirming the high sensitivity noticed from the greenhouse bioassay experiment. Biomass reduction for hairy vetch was below 30% threshold the whole season and confirmed Verdict as an option to apply as PRE emergence herbicide.

Likewise, winter wheat had greater biomass reduction by Acuron than Verdict during the crop season, with biomass reduction above 62%. Verdict resulted in biomass reduction below 20% (Table 2-8, Figure 2-4), confirming the tolerance of winter wheat to Verdict. Our findings from the greenhouse bioassay planting time confirmed our bioassay experiment regarding such tolerance of winter wheat to Verdict (saflufenacil + dimethenamid-P) and suggesting not applying Acuron as PRE emergence herbicide when interseeding hairy vetch or winter wheat.

Considering interseeding mix of hairy vetch and winter wheat under field conditions, there was interaction between Acuron and Verdict ($p < .0001$, Table 2-9, Figure 2-5). At V8 corn stage, the biomass of the CC mixture (1184.4 kg ha^{-1}) following Verdict was 82% greater than the Acuron treatment (212.4 kg ha^{-1}, Table 2-9, Figure 2-5). The difference of biomass between Verdict and Acuron across the sampling dates remained greater at 75%. Therefore, the field experiment supported the results obtained from the greenhouse bioassay.

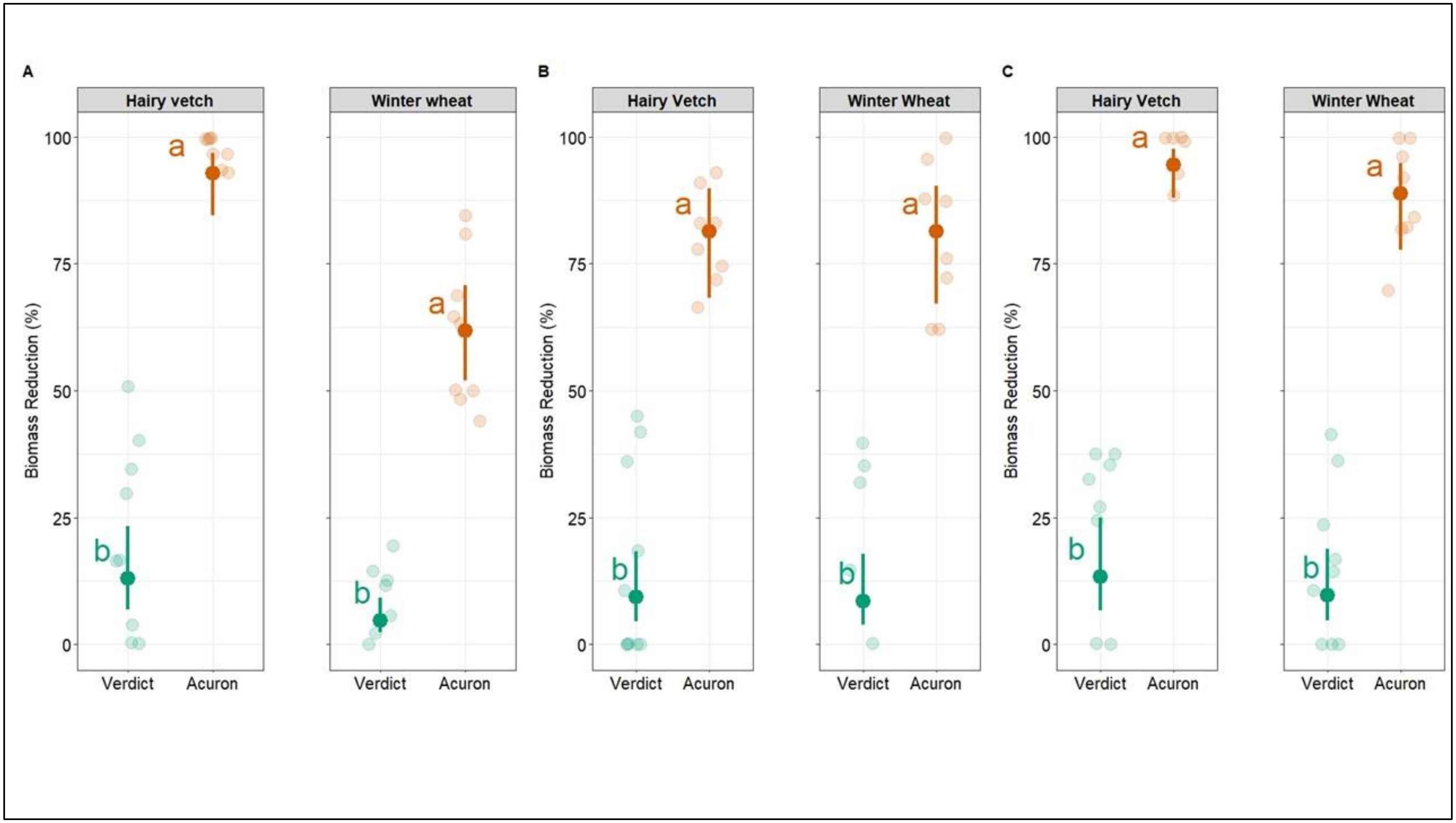

Figure 2-4. Biomass reduction of hairy vetch and winter wheat per treatment. Panel A, sampling time V8 corn stage; Panel B, before harvest; Panel C, before first killing frost.

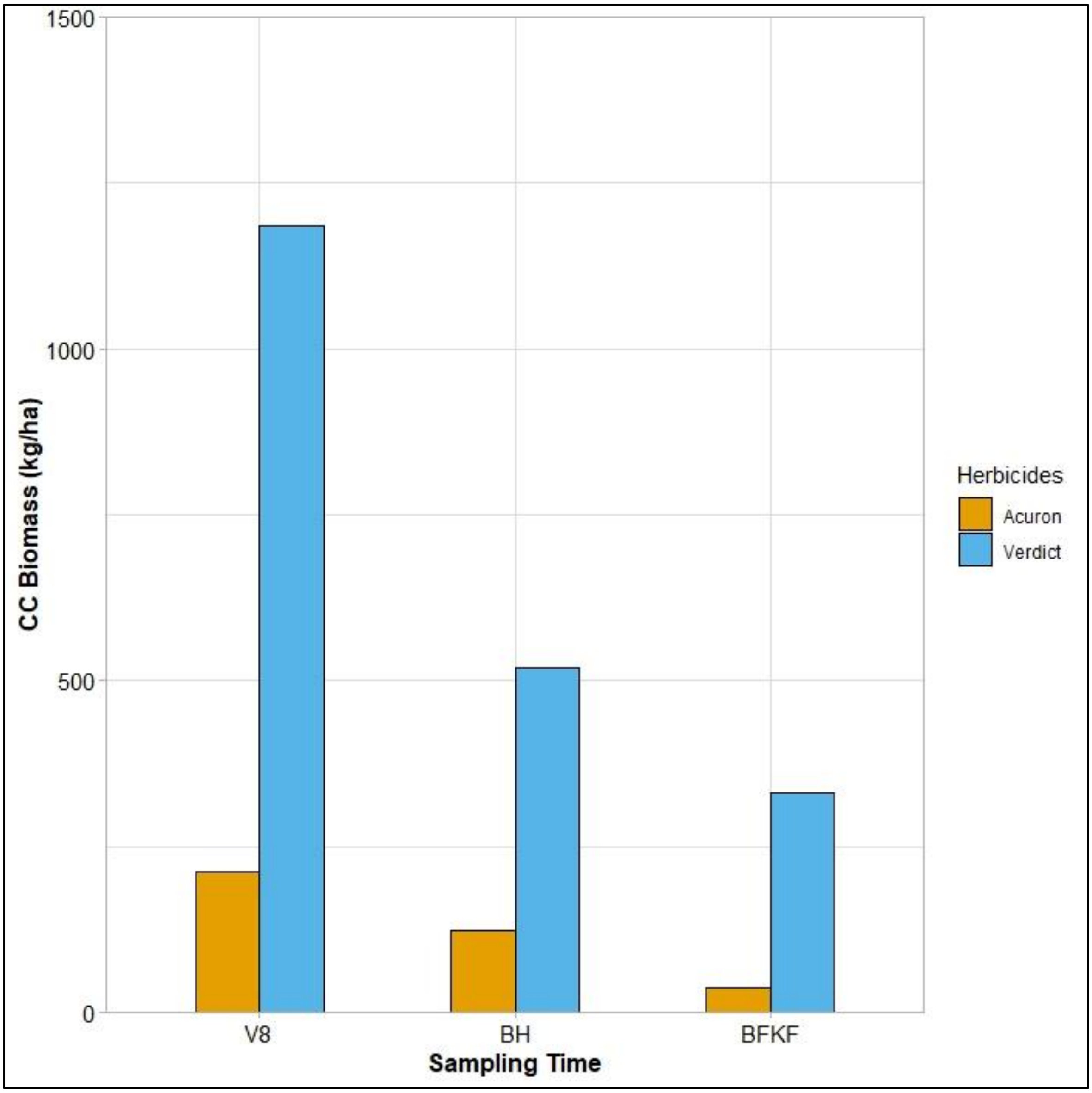

Figure 2-5. Mix total biomass at each sampling timing for both herbicide treatments. Abbreviations: V8, sampling time at V8 corn stage; BH, before harvest; BFKF, before first killing frost.

Table 2-7. Hairy vetch biomass reduction at V8 corn stage, before harvest, and before first killing frost sampling timing. Replication was considered random effect in the ANOVA model. Numbers followed by different letters represent significant differences with Tukey adjustment at the $p \leq 0.05$.

	V8		Before Harvest		Before First Killing Frost	
	Biomass Reduction %					
Treatments	Mean (± SE)		Mean (± SE)		Mean (± SE)	
Acuron	97 (3)	A	82 (11)	A	98 (2)	A
Verdict	19 (17)	B	15 (11)	B	22 (15)	B
	p-values					
Acuron x Verdict	<.0001		<.0001		<.0001	

Abbreviations: SE, standard error of the mean.

Table 2-8. Winter wheat biomass reduction at V8 corn stage, before harvest, and before first killing frost sampling timings. Replication was considered random effect in the ANOVA model. Numbers followed by different letters represent significant differences with Tukey adjustment at the $p \leq 0.05$.

	V8		Before Harvest		Before First Killing Frost	
	Biomass Reduction %					
Treatments	Mean (± SE)		Mean (± SE)		Mean (± SE)	
Acuron	62 (15)	A	80 (14)	A	88 (10)	A
Verdict	7 (6)	B	13 (11)	B	14 (12)	B
	p-values					
Acuron x Verdict	<.0001		<.0001		<.0001	

Abbreviations: SE, standard error of the mean.

Table 2-9. Total biomass of the mix of hairy vetch and winter wheat at V8 corn stage, before harvest, and before first killing frost sampling timings. Replication was considered random effect in the ANOVA model. Numbers followed by different letters represent significant differences with Tukey adjustment at the $p \leq 0.05$.

	V8		Before Harvest		Before First Killing Frost	
	kg ha^{-1}					
Treatments	Mean (± SE)		Mean (± SE)		Mean (± SE)	
Acuron	212.4 (92.0)	A	123.5 (10.8)	A	37.9 (34.8)	A
Verdict	1184.4 (73.0)	B	518.7 (26.7)	B	330.2 (10.4)	B
			p-values			
Acuron x Verdict	<.0001		<.0001		<.0001	

Abbreviations: SE, standard error of the mean.

Conclusions

The outcomes of this study emphasized the importance of evaluating the impact of PRE herbicide selection to maximize interseeded CCs biomass production. Overall, our findings showed biomass reduction for all species from the herbicides tested ranked as follows: Acuron > Resicore > Verdict. PRE-emergence herbicides may have active ingredients that remain in soil for an extended period, improving weed control but also limiting CC establishment. Previous study supported our findings, which indicated that mesotrione resulted in significant injury to interseeded red clover, whereas saflufenacil impacted in minimal biomass reduction at full labeled rate (Wallace et al., 2017). The greenhouse bioassay study addressed the objective of elucidating the impact of corn PRE emergence herbicide on CCs species and identifying optimal interseeding timing for CCs in corn. Our findings indicated that Verdict was the best option for use as a PRE herbicide if interseeding winter wheat, hairy vetch, or radish occur within the 20-30 DAA window resulting in less than 30% biomass loss. The field trial confirmed tolerance of both hairy vetch and winter wheat to Verdict, with less than 25% biomass reduction throughout the crop season.

Acuron and Resicore resulted in biomass losses over the 30% threshold for V3 corn interseeding. Nevertheless, if Acuron used as a PRE emergence herbicide, cereal rye and radish might be interseeded 100 DAA, and if Resicore is used, annual rye, cereal rye, and winter wheat could also be interseeded 100 DAA. As a result, these CC species may be interseeded safely later on corn growing season around R5 growth stage or after harvest, with a potential biomass reduction below 30% threshold. However, sowing CCs after harvest might have short window to establish and to produce expected biomass before next Spring termination. Based on our results, it is important to note that to increase potential for interseeded CC biomass production at V3 corn stage, Verdict would less result in biomass reduction for winter wheat, hairy vetch and radish. Moreover, we observed that mesotrione impacted negatively both hairy vetch and red clover tested on this study 20-30 DAA with biomass reduction greater than 86%. Further research could investigate the impact of increased CC seeding rate to counterbalance the biomass loss from PRE herbicides. According to a 2019 SARE report, CC seeds can cost between $10 and $50 per acre. However, raising the seeding rate would raise the CC seed cost, potentially increasing farming costs. Lastly, further research could assess applying PRE herbicides earlier, before sowing corn, to assess the potential impact on CC establishment but also weed control.

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CHAPTER 3: EVALUATING NITROGEN UPTAKE BY USING COVER CROPS IN IRRIGATED CORN

Abstract

Cover crops (CCs) are being used by farmers as a tool to capture surplus Nitrogen (N) in the soil, as well as to improve soil health, reducing nutrient losses and other ecological benefits CCs provide cropping systems. A two-year on-farm study located near Creighton, NE, was conducted to assess biomass production of a mix of CCs interseeded into corn (*Zea mays* L.) under irrigation. Therefore, the objective of this study was to evaluate the effect of drill-interseeded CCs on nitrogen pools within a midwest row-crop system. Nitrogen uptake by CCs that could decrease N loss potential into groundwater and N captured by corn were also assessed on this study. The CC mix consisted of hairy vetch (*Vicia villosa*) and winter wheat (*Triticum aestivum*), which were interseeded early Spring in 2021 and 2022, at corn stage V4/V5 and V3 respectively. Across years, CCs were interseeded at different rates, and herbicide management differed, which impacted CCs establishment. In 2021, due to low seeding rate, planting timing, and corn preemergence herbicide injury, CC aboveground biomass were collected only at corn stage V8 for hairy vetch 46.6 kg ha^{-1} and winter wheat 284.7 kg ha^{-1} as it did not survive later in the season. However, in 2022, preemergence herbicide and CC seeding rate, and planting timing were adjusted, thus CC aboveground biomass was collected three times during crop season for both species. Hairy vetch biomass ranged from 100.2 kg ha^{-1} to 449.4 kg ha^{-1} and winter wheat biomass from 229.9 kg ha^{-1} to 735.0 kg ha^{-1}. Two years results for soil sampling showed difference only at 0-30 cm depth for soil sampled in November 2022. Less N content in corn under CCs than no-CC was observed in 2022 and yield was negatively impacted by CCs.

Keywords: Cover crops, nitrogen uptake, interseeding cover crops

Introduction

Cover crops (CC) have many benefits and provide important ecosystem services such as reducing nutrient losses, N fixation (Chatterjee and Clay, 2016), and protection from wind erosion (Wilson et al., 2013; Blanco-Canqui et al., 2015; Belfry & Van Eerd, 2016). Regarding soil health, CCs can enhance soil properties such as aggregate stability, beneficial microbial activity, and increase soil organic matter (Snapp et al., 2005). The improvement of soil health with CCs has been one of the focuses of farmers, and in some cases supported by Federal and State conservation programs. According to a 2017 SARE (Sustainable Agriculture Research and Education) survey, the adoption of CC increased 50% from 10.3 million acres in 2012 to 15.4 million acres in 2017, and Nebraska was ranked 5th, with 747,903 acres of CCs. Sown to conserve soil and maintain ground coverage, cover crops may be grown during fallow periods or concurrently with crop production (Liebman and Dyck, 1993; Moyer et al., 2000). However, due to the short growing season in the Upper Midwest, fall seeded CCs may not produce enough biomass to provide a significant reduction soil N loss (Singer, 2008; Belfry and Van Eerd, 2016). To overcome the short window for growing cover crops following harvest, farmers may seed cover crops into corn at early vegetative stages with a drill interseeder or when corn is around the R5 growth stage with a high-clearance seeder or aerial application.

Interseeding cover crops before harvest provides more time for establishment and biomass accumulation compared with fall seeding. Cover crops interseeded before the canopy closure must be planted early enough to establish roots and aboveground biomass while solar radiation reaches the soil surface, yet late enough to avoid direct competition with the main crop for water, nutrients, and solar radiation (Grubinger, 2014; Noland et al., 2018).

Cover crop species selection is important as it may influence farmers desired goals, such as to catch surplus of nitrogen (N), suppress weeds, erosion control, and among other benefits that CC's have. For example, legume cover crops are most often selected to biologically fix N thus reduce N inputs required for the succeeding crop (Ebelhar et al., 1984). Small grain cover crops, such as cereal rye (*Secale cereale*), grow rapidly and produce substantial biomass, making them excellent at scavenging residual inorganic N (Meisinger et al., 1991; Shipley et al., 1992). Among grasses, legumes, and brassicas, the last one is not utilized extensively by farmers; nonetheless, they show promise in a range of management systems as nitrogen trap and green manure crops, and they may also influence on soil porosity, disease control, and weed populations (Mojtahedi et al., 1991; Smolinska et al. 1997; Francis et al., 1998; Isse et al., 1999; Thorup-Kristensen, 2003; Haramoto and Gallandt, 2004; Williams and Wiel, 2004; Snapp et al., 2005; Collins et al., 2007). Cover crops have traditionally been planted as single-species monocultures or simple grass–legume bicultures (Snapp et al. 2005). Sowing a mixture of several cover crop species have the potential to exploit more than one intended attribute (Belfry & Van Eerd, 2016). For example, a mixture of rye and hairy vetch (*Vicia villosa*), the rye provides protection for the vetch during establishment as well as physical support for growth the following spring (Curran et al., 2006), while hairy vetch has benefit to fix atmospheric N. When considering a cover crop, the entire system should be evaluated because the benefits and weaknesses can extend beyond the duration the cover crop is actively growing (Chatterjee and Clay, 2017).

Intensification practices such as crop fertilization and CC can modify nutrient cycling in agricultural systems (Crespo et al., 2021). Cover crops and tillage practices influence C and N pools in soil, which can affect dissolved organic C (DOC) and N leaching from agricultural fields (Singh et al. 2021). Fertilization for cash crops such as corn may result in excess N that the corn crop does not utilize and may then be lost into the vadose zone and ultimately leach into the groundwater. Among all CC species, only a few CCs species capture more N than others, catching surplus of N in soil and releasing it for the next crop and include cereal rye, annual rye (*Lolium multiflorum*) and oats (*Avena sativa*). Cover crops are recognized as an effective tool for reducing NO_3 leaching from agroecosystems (Staver and Brinsfield, 1998; Dinnes et al., 2002; Yeo et al., 2014; Thapa et al., 2018). Winter cover crops use soil residual N that may otherwise leach into groundwater after crop harvest in the fall and, depending on species, can sequester atmospheric C and/or N, thereby reducing the amount of N fertilizer required for subsequent cash crops (Hargrove 1986; Meisinger et al. 1991; Kuo et al. 1997a, b). Multiple studies have shown that cereal rye (*Secale cereale* L.) reduces nitrate leaching to groundwater and surface waters by scavenging residual N from the soil after harvest of cash crop (Brandi-Dohrn et al., 1997; Meisinger & Ricigliano, 2017; Staver & Brinsfield, 1998). The novelty of this study is that it evaluates early season interseeded mix of CCs species in corn that might potentially reduce nitrate leaching while taking into account both species' winter hardiness in Nebraska.

Therefore, the objective of this study was to evaluate the effect of drill-interseeded CCs on nitrogen pools within a midwest row-crop system. The N pools evaluated include corn-N, cover crop-N, and soil nitrate. We hypothesized that interseeding CCs in irrigated corn at the V3 growth stage will impact the soil nitrate pool and lead to decreased nitrate leaching potential into groundwater.

Materials and Methods

Experiment Location and Experimental Design

This study was conducted in 2021 and 2022 in a farmer's field located near Creighton, NE (42.26°N, 97.53°W). The experiment was established in an irrigated field planted in a continuous corn rotation with strip-till soil management. The corn hybrid P1366Q (Pioneer®) was planted in 2021 and hybrid P1278Q (Pioneer®) was planted in 2022, both at seeding rate of 79,074 seeds ha^{-1} and row spacing of 76 cm. The experiment was conducted in a randomized complete block design with five replications. The treatments consisted of an interseeded CC mix (CC) and non-cover crop (NCC) as control, with plots measuring 24 m (width) x 30 m (length). The CC mix consisted of winter wheat (*Triticum aestivum*) and hairy vetch respectively and sown at a 20:80 ratio of by weight (Table 3-1). CCs were planted in a double-row spacing of 25 cm between each corn row, with seeding rates and planting dates as shown in Table 3-1.

Table 3-1. Herbicide information, corn planting date and hybrid information, and fertilizer information. Irrigation information considering water applied when fertigation. Cover crops were interseeded at different corn growth stages in 2021 and 2022 using different seeding rates. Fertilizer use was selected based commercial lab recommendation and product chosen according to farmer's choice. POST-emergence applied to control weeds only in 2021 before interseeding CCs, and no application in 2022 due to no weed in the field.

Year	Herbicide (time, product and rate)		Corn planting date	Corn hybrid and seeding rate (seeds ha)	Fertilizer (time, source, rate)	Irrigation (mm)	CC planting date (corn stage)	CC species and seeding rate	Corn harvest date
2021	5/13/2021	Acuron Flexi (5.8 L ha^{-1}) RoundUp Power Max (0.5 L ha^{-1}) 2,4-D LV6 (0.2 L ha^{-1})	5/13/2021	Pioneer P1366Q (113 days maturity); 79074	Corn planting, starter (13.3-20-0), 25.8 kg ha^{-1}; sidedress, Urea (46-0-0), 170.2 kg ha^{-1}; UAN (28-0-0), 67.3 kg ha^{-1}	200 mm	6/16/2021 (V4/V5)	Hairy vetch (16 kg ha^{-1}) Winter wheat (20 kg ha^{-1})	11/2/2021
2022	5/25/2022	Verdict (0.6 L ha^{-1}) Dicamba (0.2 L ha^{-1})	5/19/2022	Pioneer P1278Q (112 days maturity); 79074	Corn pre-planting (4/18/2022), ammonium monophosphate (11-52-0) & Urea (46-0-0), 79.4 kg ha^{-1}; at corn planting, starter (13.3-20-0), 25.8 kg ha^{-1}; at UAN (28-0-0), 94.8 kg ha^{-1}	315 mm	6/15/2022 (V3)	Hairy vetch (44.8 kg ha^{-1}) Winter wheat (55.2 kg ha^{-1})	11/1/2022

Abbreviations: CC, cover crop; UAN, urea ammonium nitrate.

Data Collection

Cover Crop Biomass

Cover crop aboveground biomass were collected in 2021 and 2022, at V8 corn stage, and in 2022 before corn harvest (BH), and before first killing frost (BFKF). To collect the aboveground biomass, a 30.5 cm (width) by 30.5 cm (length) PVC frame was randomly tossed twice within each plot. Clippers were used to cut aboveground plant biomass at soil level. Biomass were separated by species in paper bags (15.2 cm width x 10.2 cm length x 30.5 cm depth; Uline Inc., Pleasant Prairie, WI) placed in a forced-air oven at 60°C for six days and dry biomass weighed. The biomass was ground and a subsample of 15 mg was used to perform a C:N ratio analysis in the University of Nebraska-Lincoln soils lab. C:N ratio analysis were performed by flash combustion of the samples introduced in tin capsules using Flash 2000 Thermo Scientific™ Analyzer. C:N ratio was calculated using equation [1]:

$$C{:}N\ Ratio = \frac{\%C}{\%N} \qquad [1]$$

where %C is the percentage of carbon and %N is the percentage of nitrogen obtained. The %N content was converted in kg ha^{-1} using the equation [2] to determine amount of N in the CC biomass at each sampling time, thus, to estimate nitrogen content:

$$N = D \times \left(\frac{N\%}{100}\right) \qquad [2]$$

where D is the dry weight of the biomass collected and %N is the percentage of N obtained previously from flash combustion of samples.

Corn Biomass

Aboveground corn biomass was collected in 2021 and 2022 at the end of season, before harvest. Six corn plants were selected randomly on adjacent central plot rows from each treatment were collected from all five replications, by cutting at soil level. Corn grain from the six corn plants was separated by hand from the cob, weighed, and tested for moisture content (Model Dickey John GAC 2100 Agri Bench Grain Moisture Tester, Dickey-John Corporation, Auburn, IL). Aboveground corn biomass including cob and grain samples were sent to a commercial lab (Ward Laboratories, Inc. Kearney, NE) to analyze the nitrogen content and results were described as %N, dry matter and biomass weight.

Corn Yield

Yield was collected using a commercial combine, which was measured using a yield monitor (AgLeader® Integra) and calibrated using a grain cart scale and truck load net weight. Yield from 2021 and 2022 were analyzed comparing treatments with CCs and without CCs (control). Four central rows of each plot were harvested. Data was post processed to exclude the plot buffer areas and 12 m plot length was used to calculate yield on an area of 9.3 m^2.

Soil-N Content

Soil samples were collected at depths of 0-30, 30-60, 60-91, 91-122, 122-152 and 152-183 cm on April of 2021, 2022 and 2023 and November of 2021 and 2022, and sampling was performed across the five replications for each treatment, only one core per depth per plot was taken. The soil-N content was analyzed by soil depth for each sampling timing to identify N movement throughout the crop season (CC and NCC treatments). For samples collected from 0 cm up to 122 cm, hand probe (30 cm depth, 1.9 cm diameter; JMC Soil Samplers Inc., Newton, IA) was used to collect 90 cm^3 of soil core. Soil from 152 cm and 183 cm depths were collected using hydraulic soil probe (Giddings Probe 10-SCS, Model GSPS; Giddings Machine Co., Windsor, CO) to achieve and collect deep soil samples. Each core corresponds to each depth. The soil samples were placed in appropriate soil samples bags and delivered on the same day of collection for analysis at a commercial lab (Ward Laboratories, Inc. Kearney, NE). Nitrate analyses were performed by the commercial lab by the KCl solution extract. The soil N content was analyzed by soil depth for each sampling timing to identify N movement throughout the crop season (CC and NCC treatments).

Partial Nitrogen Balance

Partial N balance was estimated using a simple balance calculation at the end of both crop years to estimate N distribution during the season and to identify potential N loss. To calculate N balance (equation [3], Pieri et al., 2011; Ross et al., 2008) for this study, N outputs and soil total N are subtracted from N inputs:

$$N\ balance = N\ unavailable - N\ available \quad [3]$$

N available includes N applied in an inorganic formulation (fertilizer) and soil-N between 0-122 cm depth (root zone). N unavailable includes N removed by grain harvested, corn-N, soil-N below 122 cm up to 183 cm and cover crop-N. The balance equation results, either surplus or deficit, is a measure of enrichment (unavailable < available) or of the net depletion (unavailable > available) of the system (Watson and Atkinson, 1999; Oenema et al., 2003; Oenema and Heinen, 1999).

Statistical Analysis

Due to differences in interseeding timing and seeding rate of CCs between 2021 and 2022, data were analyzed separately by year. All data obtained from corn (stover, cob and grain), cover crop aboveground biomass (weight, C:N ratio, N content) and soil sampling (nitrate across depths between treatments) were subjected to analysis of variance (ANOVA) using the *lm* function in RStudio (R Core Team 2022). Levene test was performed using *leveneTest* function of *CAR* package to test the homogeneity of residual variance, all pair-wise comparisons of treatment means were conducted using Tukey's HSD method at a significance level $\alpha = 0.05$ using *multcomp* package (Bretz et al., 2016) in RStudio. For soil sampling, corn plant contents and corn yield analyses, CC and no-CC treatments were considered fixed factors in the model, and the replication nested within year were considered as random factor in the model. For CC C:N ratio and N content, each species was analyzed separately, and sampling timing was considered a fixed factor and replication was considered random factor in the model.

Results and Discussions

In 2021, the mix of CCs did not establish well and CC biomass was collected only at V8 corn stage. The low seeding rate and V5 interseeding timing, corn preemergence herbicide residual carry-over might have contributed to CCs injury and death. However, in 2022, the CCs were interseeded at V3 corn stage with higher seeding rate than 2021 and also a low residual corn preemergence herbicide was sprayed in the field, allowing them to establish well and produce biomass throughout the crop season.

Cover Crop Biomass, C:N Ratio and N Content

In 2021, CC biomass of the mix of winter wheat and hairy had a total of 331.3 kg ha^{-1} sampled at V8 corn stage (Figure 3-1). Winter wheat produced 284.7 kg ha^{-1} biomass, whereas hairy vetch had 46.6 kg ha^{-1} biomass. Total N content for the mix was 16.9 kg N ha^{-1}, whereas winter wheat and hairy vetch resulted in 14.4 kg N ha^{-1} and 2.5 kg N ha^{-1}, respectively. Moreover, C:N ratio for winter wheat and hairy vetch were 6.6 and 6.7 respectively. Due to the early growth stage of both species, heights averaging 28 cm for both species, C:N ratio is lower than C:N ratio often seen in mature plants. A CC termination date that is early results in a lower cover crop biomass yield (Clark et al., 1997a), and results in lower N uptake (Kramberger et al., 2014).

In 2022, at V8 corn stage, mix had total biomass of 1,184.4 kg ha^{-1} , winter wheat and hairy vetch biomass 735.0 kg ha^{-1} (27.6 kg N ha^{-1}) and 449.4 kg ha^{-1} (18.8 kg N ha^{-1}) biomass respectively (Figure 3-2). Before harvest total biomass was measured 518.7 kg ha^{-1}, 372.1 kg ha^{-1} (9.5 kg N ha^{-1}) and 146.5 kg ha^{-1} (4.7 kg N ha^{-1}) for both winter wheat and hairy vetch respectively (Figure 3-2). Before the first killing frost, total biomass of the mix was measured of 330.2 kg ha^{-1}, biomass for winter wheat and hairy vetch were measured 229.9 kg ha^{-1} (5.8 kg N ha^{-1}) and 100.2 kg ha^{-1} (3.2 kg N ha^{-1}) respectively, Figure 3-2. At V8 corn stage, winter wheat and hairy vetch had 9.7 and 9.3 C:N ratio respectively, whereas BH winter wheat and hairy vetch had 14.9 and 12.8 C:N ratio respectively. Lastly, at BFKF, winter wheat and hairy vetch had 16.6 and 13.2 C:N ratio respectively. Moreover, C:N ratio analyzed by species from both years were less than 30, indicating that net mineralization was likely to occur (Allison, 1966), whereas C:N ratios above 25 have been related to N immobilization (Ranells and Wagger, 1996; Kaye and Hart, 1997; Kuo and Jellum, 2000). Depending on the components of a mix of legume and grass is most likely to have lower C:N ratio than monoculture, as described by Wagger (1989b) in a previous study, suggesting that a combination of grass and legume has lower potential for N immobilization than grass monoculture grown. The higher C/N ratios in the mixtures will result in slower N release (Thapa et al., 2018). Therefore, we can conclude from our findings that winter wheat and hairy vetch mix had potential for N immobilization which it showed low C:N ratio by BFKF sampling date, as C:N ratio is an important determinant of CC decomposition and N availability (Cabrera et al., 2005). Thus, N content in hairy vetch is most likely to be released faster than N content in winter wheat, varying according to CC termination time. Nitrogen released after CC decomposition might be available for the following cash crop which could potentially result in less N fertilizer to be applied. However, environmental and management factors can markedly influence decomposition dynamics, and it is difficult to accurately predict the amount of N that will become available, or when it will become available, to a subsequent cash crop (Crews and Peoples, 2005; McSwiney et al., 2010; Ruffo and Bollero, 2003).

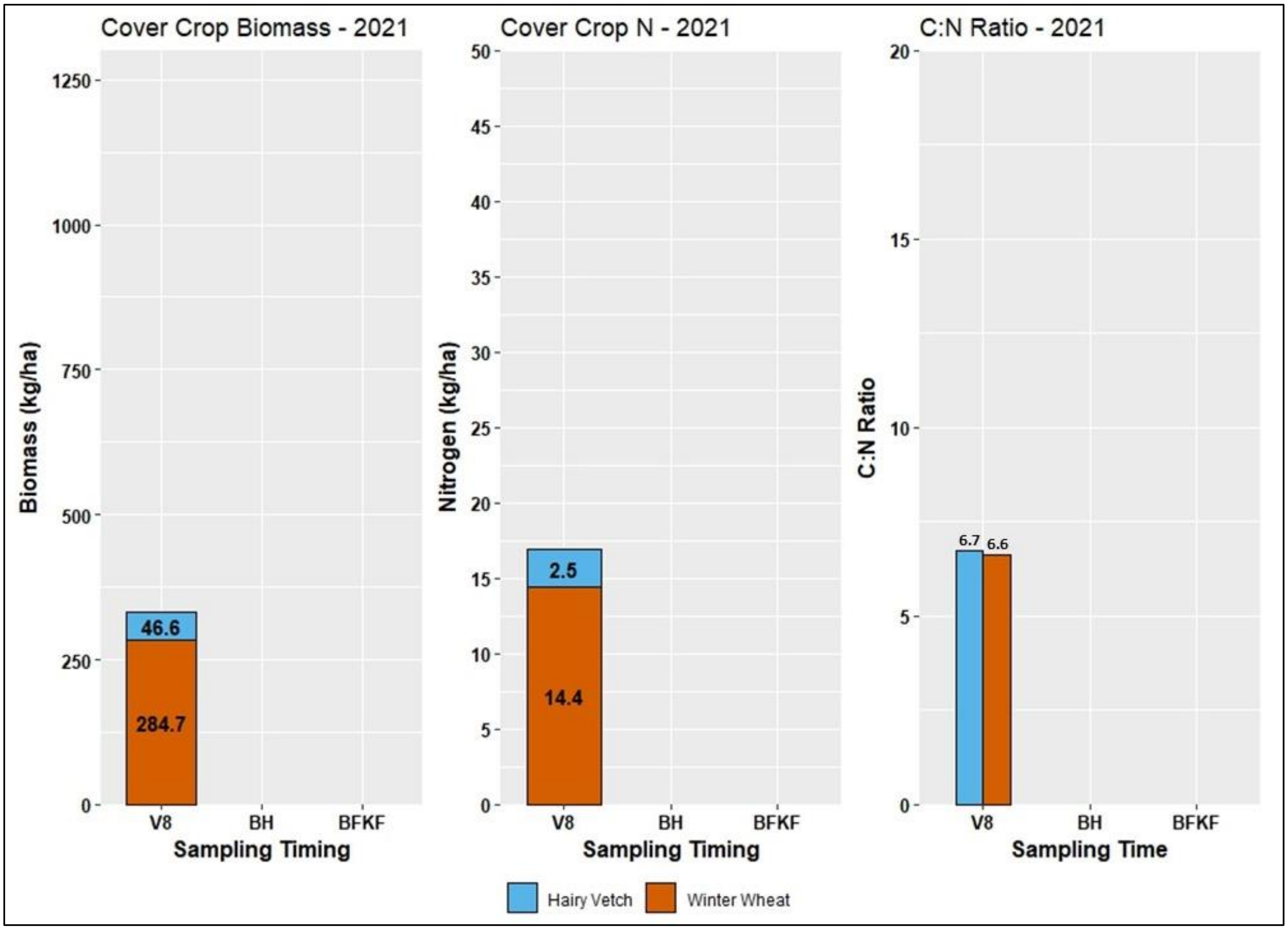

Figure 3-1. Cover crop (CC) total biomass, CC nitrogen (N) content and C:N ratio in 2021. Abbreviations: V8, V8 corn stage; BH, before harvest; BFKF, before first killing frost.

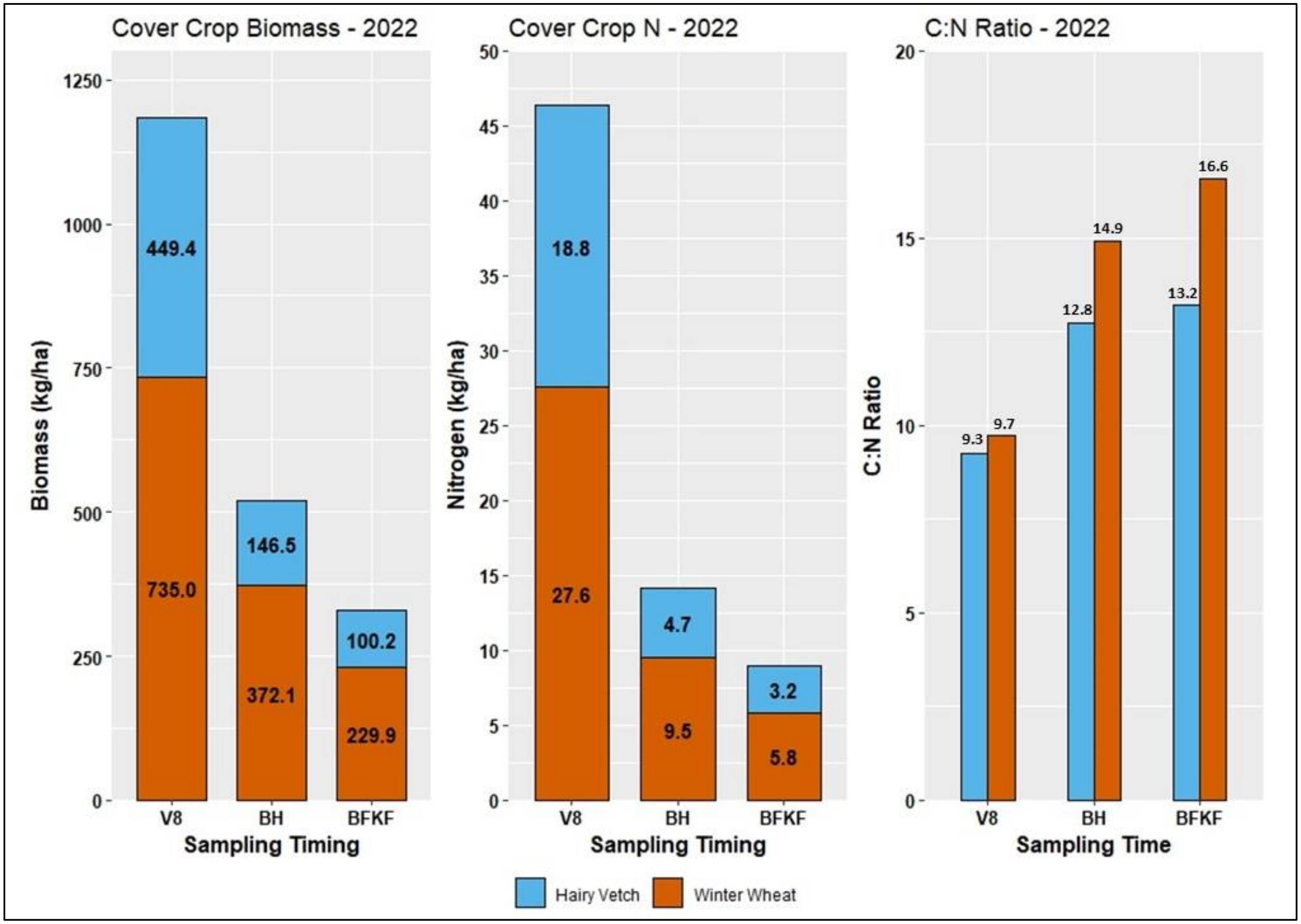

Figure 3-2. Cover crop (CC) total biomass, CC nitrogen (N) content and C:N ratio in 2022. Abbreviations: V8, V8 corn stage; BH, before harvest; BFKF, before first killing frost.

Corn Biomass

In 2021 there was no treatment effect on corn-N content (Figure 3-3) due low CC biomass production which impacted on low N capture throughout the crop season. Although CC established well and remained throughout the crop season in 2022, interseeded CC did not impact on corn-N content, except for total corn-N (p = 0.0492; Figure 3-3). Although CCs did not establish well in 2021 with less aboveground biomass, less total N in corn biomass under interseeded CC than NCC was noted in only in 2022, similarly to a previous study by Kramberger et al. (2014) that noted more N content in corn biomass under NCC than CC treatment that received mix of grass and legume, 75%:25% seeding ratio. According to our findings, we hypothesize that interseeded CC mix tie up N in its biomass, which could be released for the following cash crop.

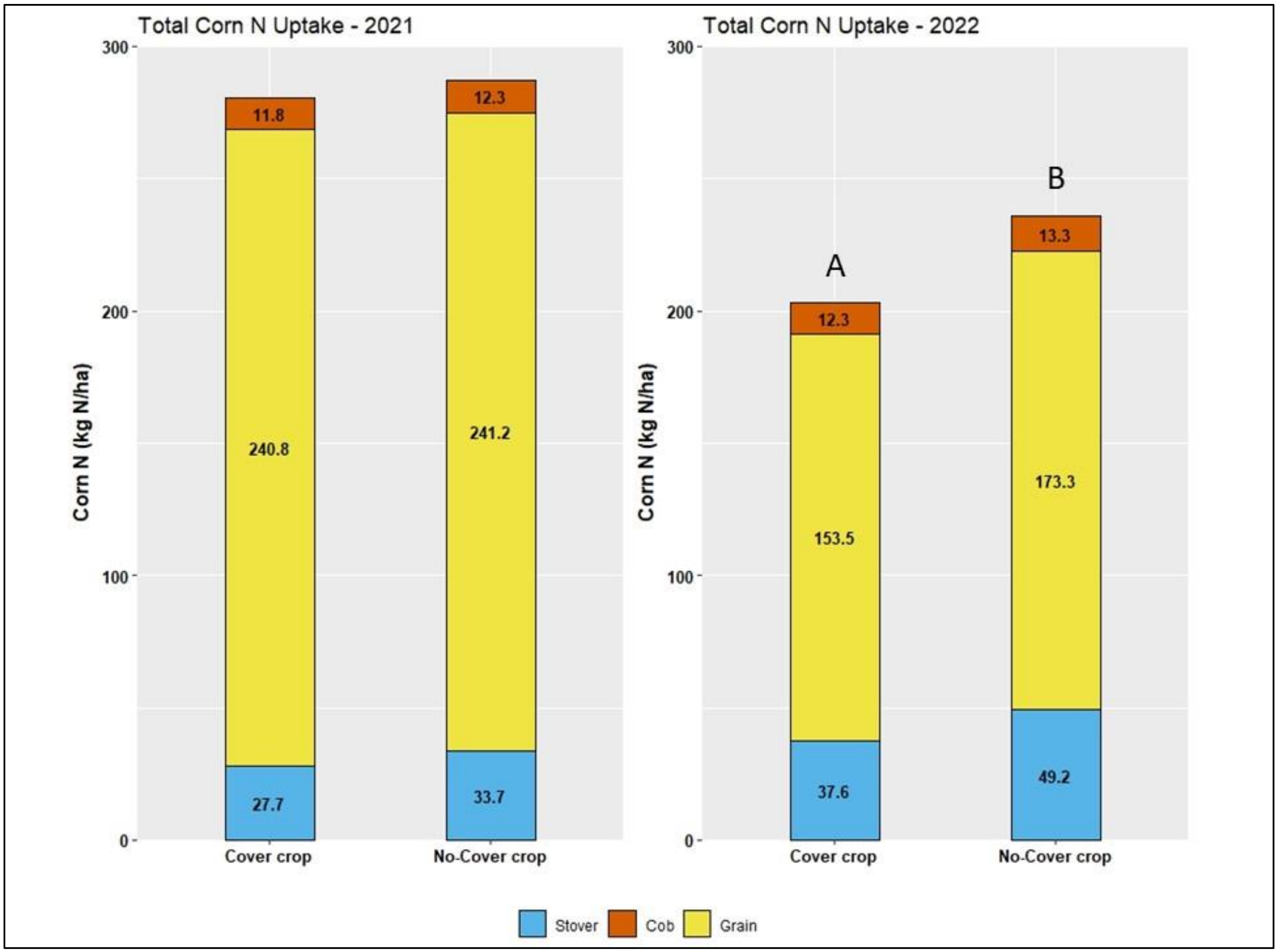

Figure 3-3. Total corn nitrogen (N) uptake per plant-part in 2021 and 2022. Different letters in 2022 represent significant differences with Tukey adjustment at the $p \leq 0.05$. Abbreviations: CC, cover crop; NCC, no-cover crop.

Corn Yield

In 2021, corn yield had no treatment effect. However, in 2022, CCs impacted negatively on corn yield ($p = 0.0202$, Table 3-2). Our findings from this study indicate the opposite of results from a previous study where CCs including hairy vetch and cereal rye were interseeded at 10 to 20 days after corn emergence and no corn yield penalty was identified (Abdin et al., 1998). Another study indicated a slight decrease on corn yield on treatments with CC in two out three site-experiments where CCs were evaluated (Black et al., 2023). Therefore, adding cover crops into cropping system might impact on corn grain yield, however to evaluate such difference, a long-term cover cropping system is needed to evaluate corn grain yield penalty by adding CCs.

Table 3-2. Corn grain yield for 2021 and 2022. Replications were included as random effects in the ANOVA model. Numbers followed by different letters represent significant differences with Tukey adjustment at the $p \leq 0.05$.

Site-year 2021	Yield (Mg ha^{-1})
Treatments	Mean (± SE)
CC	15.4 (1.5)
NCC	15.3 (1.0)
	p-value
CC x NCC	0.9600

Site-year 2022	Yield (Mg ha^{-1})
Treatments	Mean (± SE)
CC	14.9 (0.3) A
NCC	16.2 (0.9) B
	p-value
CC x NCC	**0.0202**

Abbreviations: CC, cover crop; NCC, non-cover crop; SE, standard error of the mean.

Soil-N Content

In 2021, there was no treatment effect by depth on each sampling time (Figure 3-4). Although CCs grew up to the V8 corn stage, corn canopy, PRE herbicide residual carry-over, interseeding time, and seeding rate may have contributed to total death of CCs. In April soil-N results in 2022 had no treatment effect. However, in November 2022 only soil-N content at 0-30 cm depth had a significant treatment effect ($p = 0.0092$, Figure 3-4), where NCC had 40% more N than CC treatment. In April 2023, soil-N content also showed treatment effect at depth of 0-30 cm ($p = 0.0045$), 60-91 cm ($p = 0.0463$), 91-122 cm ($p = 0.0185$) and 122-183 cm ($p = 0.0220$), shown on Figure 3-4. Moreover, corn-N content and yield for NCC treatment had treatment effect as discussed before in this chapter, then we can conclude the fact of NCC having more N available impacted on higher corn-N and yield results than CC treatment. It is also possible to identify possible downward N movement from 91-122 cm depth in April 2022 to 122-183 cm in November 2022, being potentially leached below the depth sampled.

Our soil results in 2021 were limited due to the evaluation of only one year of CCs establishment throughout the crop season, thus we observed no significant effect of interseeding CC on soil-N content. Similar results were described by Kramberger et al. (2014) suggested that mix of legume and grass (75%:25% respectively) led to lower N content in soil compared with the control. Interseeding CCs as monoculture or mix can lead to different rates of N uptake from soil at different depths, as a previous study conducted by Osterholz et al. (2021) described the impact of interseeded alfalfa in corn under different N rates applied, which reduced the overwinter and Spring nitrate leaching potential into groundwater. Legumes are more likely to increase N into the system due N fixation as described by Kramberger et al. (2014) by increasing legume seeding ratio in a mix with grass. However, legumes that are not winter hardy would be terminated by frost killing and decomposed before next crop season. In addition, the N dynamics under each CC species are important to consider for CC system design (Kaye et al., 2019) of how specific CC species could impact on capture N, decomposition, and mineralization. Moreover, Kaye et al. (2019) noted that it is possible that slightly earlier planting dates (e.g. September) or in locations with slightly warmer fall temperatures (or later frost dates) oat, and perhaps radish, canola, and pea, could be effective at reducing N leaching. Other studies have shown that CC species reduced N leaching (White et al. 2017) in long-term evaluation, where intensive soil sampling occurred and also with lysimeters installed, which allowed for better understanding of how N moves downward and how N pools are over crop season, considering corn system.

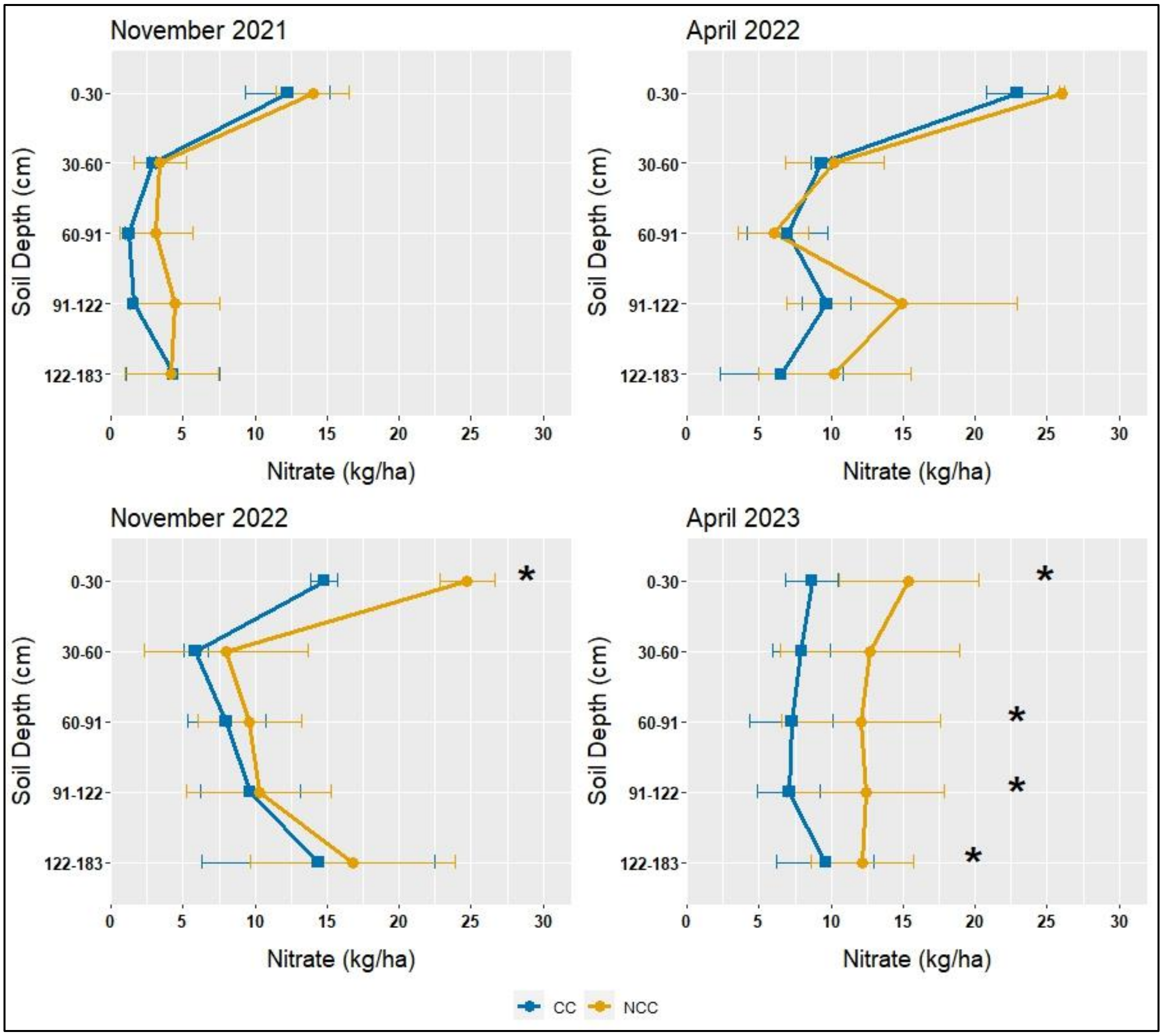

Figure 3-4. Soil nitrogen content per depth in November 2021, April 2022, November 2022 and April 2023. * represents significant differences at $p \leq 0.05$. Replication was considered random effect in the ANOVA model.

Abbreviations: CC, cover crop; NCC, no-cover crop

Partial Nitrogen Balance

For both CC and NCC treatments in 2021, net enrichment (N available < N unavailable) was observed on partial nitrogen balance, 3.5 kg N ha^{-1} and 3.3 kg N ha^{-1} (Table 3-3) respectively. Grain-N removal did not exceed the N fertilizer input and contributed to the soil-N enrichment result at lower depths of 122 – 183 cm. However, the partial N balance is incomplete due to not measurement of organic N that indicates N immobilization.

In 2022, partial N balance resulted in positive value only for NCC with 0.1 kg N ha^{-1}, where CC treatment had -11.6 kg N ha^{-1}, suggesting an enrichment of the system. However, the result for CC treatment does not suggest N loss due the N tied up by the growth of CC throughout the season, which was estimated at 9.0 kg N ha^{-1} at the end of season, which also suggests lower N content due the downward biomass accumulation throughout the season. Moreover, the unavailable N tied up on cover crops suggests that N will be potentially released after CC termination for the following crop season by mineralization process. N balance for NCC treatment suggests the opposite of observed on CC treatment, as due to no CC suggests N leaching potential. The increase of soil N from 2021 to 2022 followed a trend observed by Nyborg et al. (2018) that evaluated continuous annual cropping, where the measurements in the beginning of the study were lower than at end of study after a decade, suggesting that N was added on soil. Changes in soil N are influenced by crop management, but also by the initial soil-N (Ross et al., 2008). Moreover, less N was applied in 2022 to use the N surplus between 0-122 cm depth and also N ready to used in the lower depths of 122-183 cm was 14.4 and 16.8 kg N ha^{-1} for CC and NCC respectively (Table 3-3) suggesting that N moved downward from 2021 to 2022, contributing for higher N levels in that depth compared with same depth in 2021. The N balance for this study was estimated based on data collected throughout the season, which might increase uncertainty of N balance (Watson and Atkinson, 1999) as not all N pools were measured such as organic N. As noted by Van Faassen and Lebbink (1994), small errors in determining soil N and the difficulty to estimate parameters might result in uncertainties in absolute N, and consistency on maintaining same treatments and field management would provide results with more clarity and level for comparisons across years.

Table 3-3. Partial nitrogen balance for 2021 and 2022 considering N inputs, N outputs and soil N at beginning and at the end of the crop season. Soil-N was not included on N balance equation. Negative values on N balance mean net depletion and positive values mean soil N enrichment.

	2021		**2022**	
	CC	**NCC**	**CC**	**NCC**
	kg N ha^{-1}			
N Available				
N Fertilizer	263.2	263.2	200.0	200.0
Soil-N (0 - 122 cm)	17.9	24.9	38.3	52.6
N Unavailable				
Grain Removal	240.8	241.2	153.5	173.3
Corn-N	39.5	46.0	49.9	62.5
Soil-N (122 - 183 cm)	4.3	4.2	14.4	16.8
Cover Crop-N	-	-	9.0	-
N Balance	3.5	3.3	-11.6	0.1

Abbreviations: CC, cover crop; NCC, non-cover crop; N, nitrogen.

Conclusions

Our study faced challenges in the first year regarding cover cropping establishment and growth, most likely because of a low seeding rate, preemergence herbicide residual carry-over and late drill-interseeding timing. Taking into consideration the short window to grow CCs after corn harvest in Nebraska, interseeding CCs early in the season or early Fall is an option to extend the CC growing season. Interseeding earlier is expected to increase CC biomass production throughout the season, thus capturing surplus N early in the season. Therefore, future studies could also evaluate different early interseeding timings of CCs with different species in corn in Nebraska to achieve optimum establishment and biomass production throughout the crop season.

Our findings in 2021 did not show an impact of CCs on soil-N concentration across the depths. Soil-N results showed treatment effect only in November 2022 sampling at 0-30 cm, and differences on CC seeding rate, planting timing and residual herbicide impacted negatively on CC growth in 2021, resulting possibly in differences between 2021 and 2022. Moreover, more years of cover cropping would be necessary to estimate precisely the impact of CCs on soil-N, which also would provide more accurate N balance results with measurements of other soil parameters such as organic N. Although soil results estimated size of nitrate pool and its risks for leaching, measurement of water movement downward would provide better understanding of N dynamics in soil. Moreover, it is important to emphasize the benefits CCs can provide for the soil system regarding soil health improvement, soil aggregates stability, improvement of water infiltration and others, which can improve sustainable crop production. Due to only one year of CC growth throughout the season, we could not effectively estimate impact of CC on N pools in 2021 as estimated in 2022. Thus, from this project we were able to understand a cover cropping management regarding seeding rate and interseeding timing that would potentially improve CC growth across the crop season. Moreover, it is important for grower to choose PRE herbicide that would not impact on CC establishment. More research is needed to evaluate long-term effect of interseeding CCs in N dynamics across different soil depth. Measuring N losses in drainage water and denitrification might be beneficial for fine-tuning the N balance equation, combined with intensive early cover cropping in a long-term study. Moreover, research combining CC planting timing with decision for monoculture or mix of CCs species is also a need.

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