

THE GROWTH AND BENEFITS OF INTERSEEDED COVER CROPS IN ORGANIC  
CORN SYSTEMS

By

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Approved:

A handwritten signature in blue ink, appearing to read "Matthew D. Ruark". The signature is fluid and cursive, with the first name "Matthew" and last name "Ruark" clearly distinguishable.

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Matthew D. Ruark; Department of Soil Science

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## **Abstract**

Cover cropping is a commonly used practice in organic systems for its agroecosystem benefits and effectiveness in reducing soil erosion, maintaining soil organic matter without inorganic additives, improving the biological community within the soil, and managing nutrients and soil fertility. In the Upper Midwest winter rye (*Secale cereal L.*) is a commonly used cover crop following corn (*Zea mays L.*) but organic farmers are interested in using diverse cover crop species for varying agroecosystem benefits. This study aims to determine the effects of other cover crop species from all three functional groups (grasses, legumes, and brassicas) on weed suppression, soil nitrogen, and corn yield when interseeded into corn at V3. Annual ryegrass (*Lolium multiflorum Lam.*), red clover (*Trifolium pratense L.*), and oilseed radish (*Raphanus sativus L.*) were selected to represent the three functional groups of cover crops in both single and mixed species applications. All cover crop treatments effectively suppressed weeds, reducing total weed biomass by 50% - 90%, in comparison to a no cultivation no cover weedy check while standard in-season cultivation reduced total weed biomass by 60% - 80%. In addition to achieving effective weed control, there was no effect on in-season soil nitrate and no reduction in corn silage yields. Cover crop growth was variable between site years with mixtures containing oilseed radish having the most biomass in field 1, but no difference between mixtures and single species application in field 2. Interseeding cover crops allows farmers to adopt various cover crop species and is a viable method of weed management in organic corn systems without negatively affective soil nitrate level or corn silage yields.

## **Introduction**

As of 2021, there are roughly 4,000,000 total acres of corn planted in Wisconsin and 40,000 of those are certified organic acres, a 10% increase from 2016 (USDA NRCS, 2022). This growth emphasizes farmers' interest in transitioning to organic production systems. Addressing the main barriers to transitioning to organic, including fertility, weed management, and pest concerns while maintaining soil health requires research on innovative management strategies. In 2014 the Midwest was identified as ideal for expanding organic grain operations and barriers to optimizing organic production were identified including high costs of organic fertilizer, weed competition, and low supply of livestock manure for fertilizer (Reaves and Rosenblum, 2014). Organic farmers rely heavily on tillage for weed management (Pimental et al., 1993) and these tillage practices degrade the soil, reducing its water holding capacity, ability to cycle nutrients efficiently, and increasing the risk of nutrient runoff and soil loss via erosion. Soil erosion from unsustainable management and nutrient leaching impacts human and environmental health by contaminating drinking water and contributing to degrading ecosystem health (Kladivko et al., 2014; Krueger et al., 2013). Agroecological management practices address some of these environmental and human health concerns that are a result of conventional practices.

Cover crops are a key tool in organic management. They have the potential to reduce the need for imported fertility sources, enhance weed suppression, decrease reliance on cultivation, and improve soil health. By reducing the exposure of soil, cover crops can reduce the need for nutrient imports by sequestering nutrients that may otherwise be lost to runoff, erosion, or leaching (Thapa et al., 2018). The varying benefits of cover crops are associated with three functional groups of species: grasses, legumes, and brassicas. Each functional group and species within them have their advantages and disadvantages to agroecosystems.

Legume cover crops have the potential to reduce reliance on N inputs by supplying N to the cash crop and retaining less N than non-legume species (Tonitto et al., 2006). Grass cover crops immobilize N, making it unavailable to subsequent cash crops, but surpass other species in terms of ease of establishment and weed suppression. Additionally, they accumulate more biomass throughout the growing season than other species, benefiting the overall soil health (Finney et al., 2016, Wagger, 1989). Brassicas mitigate soil compaction and erosion in addition to increasing N retention and weed suppression (Blesh et al., 2019). When grown in mixes, it is possible that the N credit provided by legume cover crops may offset the immobilization of N by grass species, reducing the need for increased fertilizer inputs. Organic growers are interested in adopting cover crop mixtures, with species from multiple functional groups, rather than single species to enhance agroecosystem services (Silva et al., 2021). Cover crop mixtures can increase the functional diversity of crop rotations, suppress weeds, and offset the nutrient and yield tradeoffs associated with species such as winter rye (Schipanski & Drinkwater, 2011).

Cover crop adoption has increased steadily since 2015 across the United States (CTIC & SARE, 2020), however organic growers in Wisconsin face barriers unique to this region due to climate and environmental pressures (Reaves and Rosenblum, 2016). These barriers, especially in corn systems, include short growing seasons, weed management, nutrient management, and understanding of best management practices with which to maximize cover crop biomass and associated benefits.

In the Upper Midwest cover crop establishment is heavily constrained by the weather and crop rotations. Standard cover crop adoption requires cover crops to be planted after cash crop harvest in the fall - this leaves a limited and varying window of time to get cover crops in the ground with enough time to establish before winter. Grasses are usually favored over legumes



and brassicas for their rapid establishment and ability to take up excess nitrogen from the ecosystem (Lavergne et al., 2021). In the Upper Midwest winter rye (*Secale cereale L.*) has proven to put on substantial and reliable biomass regardless of the lower soil temperatures and limited growing degree days following corn harvest (Cates et al 2018; Baker and Griffis, 2009). In dairy production systems it is common practice to apply manure and plant winter rye in the fall after corn harvest. While a fall planting of winter rye has proven effective for reducing erosion and nutrient leaching (Kasper & Singer, 2011), especially in dairy production systems, this single species fall planting does not allow for farmers to harness the full potential of all cover crop functional groups to provide numerous agroecosystem benefits throughout the year. Organic farmers, in comparison to conventional farmers, are interested in utilizing multiple cover crop functional groups at once for multiple agroecosystem services (Wayman et al., 2016).

Interseeding cover crops is a viable option to mitigate the barriers to cover crop implementation, particularly by eliminating the issue of having a short growing season in the fall. With the exception of winter hardy grasses, fall planting dates run the risk of exposure to unpredictable harsh conditions and therefore poor cover crop establishment (Brooker et al., 2020; Noland et al., 2018). By planting early in the growing season of corn, interseeded cover crops are given time to establish throughout the summer and flourish again in the fall after harvest. Interseeding allows farmers to harness the benefits of ecosystem services that are provided by all cover crop functional groups, including biological weed suppression (den Hollander et al., 2007; Donovan et al., 2001; Sarrantonio & Gallandt, 2003).

Many farmers cite weed management as a barrier to both adopting cover crops into their management practices, and to transitioning to organic agriculture (Silva et al., 2021). Agroecological weed management takes a multi-tactic approach with four main goals: to

organically reduce weed seeds in soil, weed seedling establishment, weed seed production by established plants, and prevent or reduce weed seed spread (Cordeau, 2022; Silva et al., 2021; Gaba et al., 2013). Interseeding various functional groups of cover crops expands the potential to suppress summer annual weeds through outcompeting for resources (Silva, 2014; Scholberg et al., 2009; Uchino et al. 2012). Being able to rely on cover crops for weed suppression would allow farmers to reduce their reliance on tillage and mechanical cultivation. Mechanical cultivation has long been a favored management method for weeds in the organic community (Bond & Grundy, 2001; Merfield, 2023). Research conducted at the University of Wisconsin - Madison has shown that heavy reliance on mechanical weed management leads to increased soil loss and higher weed seed bank counts within the soil (Drewitz & Stoltenberg, 2018). Weed competition has the potential to decrease soil nitrate levels and corn yields, therefore effective management of these weeds are a high priority and concern for organic farmers when making management decisions.

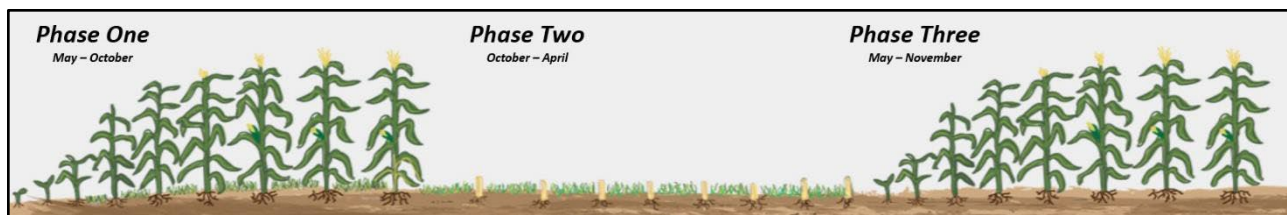
Decreasing soil nitrate levels is a concern in corn production systems because corn is a high nitrogen demand crop. While interseeded cover crops may reduce weed competition with cash crops and therefore the nutrient demands of variable weed species, it is unclear what the nutrient demands of the cover crops are and how they contribute to the nutrient cycle of the agroecosystem. There is a lack of research and data to accurately make nutrient management recommendations to farmers in Wisconsin who have implemented cover crops into their management practices. Diversification of standard corn-soybean rotations by using cover crops has the potential to reduce reliance on mineral fertilizers while maintaining current crop yields (Davis et al., 2012). Due to poor soil management, conventionally managed corn-soybean rotations can require up to 90% more fertilizer than diversified systems that incorporate manure

and better soil health practices (Liebman and Schulte, 2015). As more farmers transition to organic production systems and look to ways to meet nutrient demands, diversification of corn rotations with interseeded cover crops has the potential to reduce the need for synthetic fertilizer inputs depending on species selection, establishment, and management. In order to make accurate nutrient management recommendations however, research on cover crop nutrient demands in tandem with the reduction in weed nutrient competition is vital to improving overall agroecosystem nutrient efficiency without reducing corn yield potential.

To maximize individual ecosystem services and accurately measure the growth and benefits of each individual species, this experiment is limited to three cover crop species. Higher cover crop plant density decreases individual cover crop plant productivity and different functional groups can disproportionately dominate mixtures, impeding overall production (He et al., 2005). Three cover crop species were chosen as representatives of the three cover crop functional groups (grasses, legumes, and brassicas) as each offer varying ecosystem services. Their growth, establishment, and ecosystem services were assessed individually and in mixtures when interseeded in corn (*Zea mays L.*). Annual ryegrass (*Lolium multiflorum Lam.*) was selected for its rapid establishment, weed suppression, and winter hardiness (Caswell et al., 2019, Brooker et al., 2020). Red clover (*Trifolium pratense L.*) was chosen for its ability to over winter and grow well under low light conditions (Caswell et al., 2019; J. Stute & Shelley, 2009). Additionally, as a legume, red clover has the potential to reduce the need for nitrogen fertilizer inputs due to its ability to biologically fix nitrogen from the atmosphere (Sarrantonio & Gallandt, 2003; Peoples et al., 2009; J. Stute & Shelley, 2009). Finally, oilseed radish (*Raphanus sativus L.*) was selected for its rapid establishment, ability to scavenge nitrogen, and reduce soil compaction (Ruark et al., 2018; USDA- NRCS 2012). The agroecosystem benefits of these

experimental species and mixes were compared to winter rye planting following corn harvest, which is a standard cover cropping practice in Wisconsin.

The overall goal of this study is to assess the agroecosystem benefits of cover crop functional groups when interseeded at V3 into organic corn systems in the Upper Midwest. Specific objectives of this study are to: 1) quantify interseeded cover crop growth and nitrogen uptake, 2) determine the level of weed suppression by cover crops, 3) determine the effect of cover crops on available soil nitrogen, and 4) determine the effects of cover crops on current and subsequent crop yields. The study is executed over three phases, however due to seasonal constraints, this thesis will cover the first two. The first phase starts when corn is planted and ends when it is harvested for silage. The second phase starts at corn silage harvest and goes through cover crop termination the subsequent spring. The final phase of the experiment examines the effects of interseeded cover crops on subsequent crop yields (Figure 1), but is not covered in this thesis.

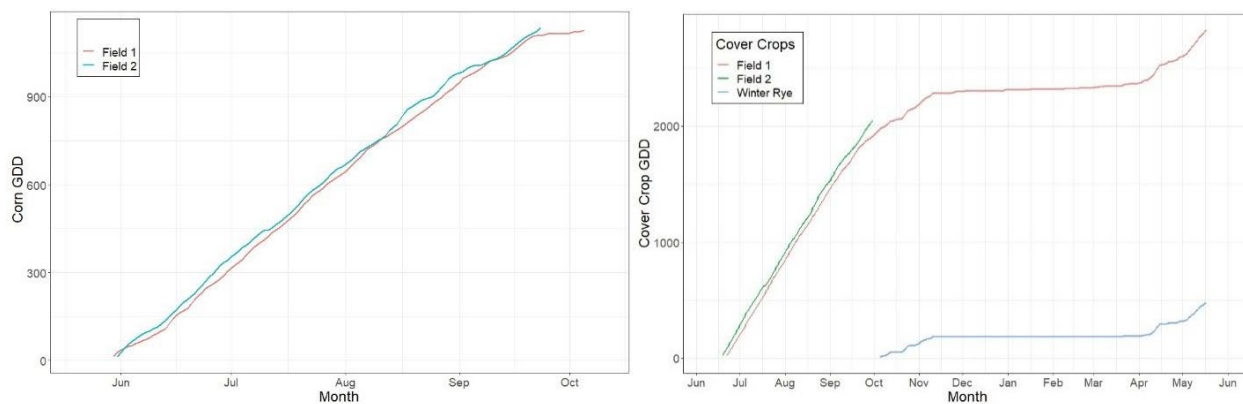


**Figure 1. Timeline of experimental phases one, two, and three.** Phase one begins at corn planting in late May and ends at corn silage harvest in late September/early October. Phase two begins at corn harvest and continues until cover crop termination in late April/early May. Phase three examines subsequent crop yields beginning at corn planting and ending at corn grain harvest.

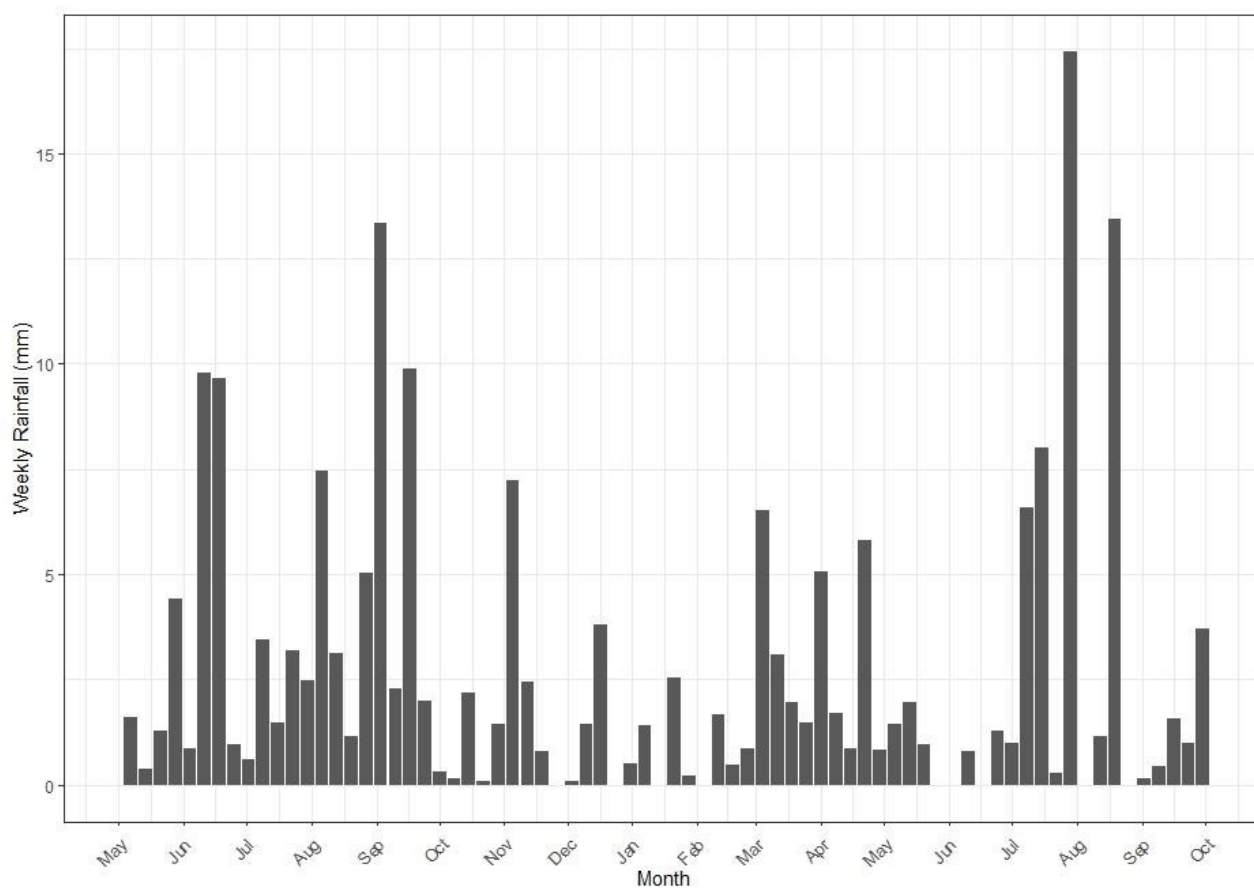
## **Materials and Methods**

### *Field Description and Design*

A two-year field study was conducted at the University of Wisconsin Arlington Agricultural Research Station (43°18'9.47"N, 89°20'43.32"W) from 2022-2023 on two certified organic fields located less than 2 km apart. Both fields were following three years of alfalfa (*Medicago sativa*) which was terminated prior to the start of this study. The first field (field 1) was used from 2022-2023 across two growing seasons and the second field (field 2) was used in 2023 to replicate the first phase of the experiment. Due to the timing of this experiment, there is only one year of data from field 1 for phase two of the experiment. The soil at each field is a Plano silt loam (fine-silty, mixed, superactive, Mesic Typic Argiudoll). The study site has a mean annual temperature of 7.77°C and a mean annual precipitation of 90.2 cm (National Climate Data Center). The growing degree days for both years were similar throughout the first phase for all cover crops and corn (Figure 1). There was an early season drought in the second year of the study during corn and cover crop planting beginning in late May and continuing through June (Figure 2). Common weeds in Wisconsin grain and forage systems include: common lambsquarters (*Chenopodium album L.*), redroot pigweed (*Amaranthus retroflexus L.*), dandelion (*Taraxacum officinale*), yellow foxtail (*Setaria pumila*), and velvetleaf (*Abutilon theophrasti*) (Drewitz & Stoltenberg, 2018). Routine soil analyses were done on both fields at the start of the experiment. Field 1 had a soil pH (1:1 water) of 6.2, soil P was 51.1 ppm, soil K was 116.8 ppm, and OM% was 3.6%. Field 2 had a soil pH (1:1 water) was 7.0, soil P was 101 ppm, soil K was 166 ppm, and OM% was 3%.



**Figure 2** Accumulated grown degree days (GDDs) for both site years from corn planting to harvest for corn silage and cover crop planting to cover crop termination. GDDs were calculated with temperature data from MSU Enviroweather and Wisconet.  $GDD = (T_{max} + T_{min})/2 - T_{base}$  where  $T_{base} = 10\text{ }^{\circ}\text{C}$  for corn,  $0\text{ }^{\circ}\text{C}$  for annual ryegrass, red clover, and oilseed radish (Baraibar et al., 2018), and  $4\text{ }^{\circ}\text{C}$  for winter rye.



**Figure 3** Weekly precipitation (mm) for Arlington, WI for field 1 and field 2 from the corn planting in year 1 to corn harvest in year 2 (May 2022- October 2023). Rainfall data was gathered through MSU Enviroweather and Wisconet.

The experimental design was a randomized complete block replicated four times. There were 10 whole plot treatments, each 4.6 x 45.7 m. Cover crop treatments consisted of four single species applications, four mixed species treatments (all two-way and three-way combinations), one no cover but with cultivation (NCWC) treatment, and one no cover and no cultivation treatment (NCNC).

To quantify the effects of cover crops across the seasons, field management and data collection was divided into three phases to track the seasonality of cover crop agroecological effects. Phase 1 covers the first growing season up to corn silage harvest. Phase 2 begins when the corn is harvested, and winter rye planted and continues up until cover crop termination. Phase 3 tracks the subsequent season following cover crop implementation and begins at cover crop termination, ending when corn is harvested for grain. This thesis will cover the experiment and results of the first two phases.

### **Phase One (corn planting – silage harvest)**

Phase one was executed across two years and two different fields in 2022 and 2023. Prior to corn planting in 2022 liquid dairy manure (<1% dry matter) was injected into the soil at a rate of 59,650 L ha<sup>-1</sup>. This applied 51 kg ha<sup>-1</sup> of phosphorus, 81 kg ha<sup>-1</sup> of potassium, 0 kg ha<sup>-1</sup> of sulfur and 134 kg ha<sup>-1</sup> of nitrogen, with an estimated nitrogen credit of 67 kg ha<sup>-1</sup> within the first year of injection. The manure analysis for 2023 indicated 2.51% dry matter with total nutrient application of 31 kg ha<sup>-1</sup> of phosphorus, 80 kg ha<sup>-1</sup> of potassium, 14 kg ha<sup>-1</sup> of sulfur, and 52 kg ha<sup>-1</sup> of nitrogen with an estimated nitrogen credit of 26 kg ha<sup>-1</sup> within the first year of injection.

Shallow tillage (10 cm) was conducted with a tandem disk prior to corn planting to kill the alfalfa after manure application to prep the field for corn planting.

Corn (Blue River 30K84) was planted in field 1 on 31 May 2022 and in field 2 on 30 May 2023, both at 79,012 seeds ha<sup>-1</sup> at a depth of 5 cm in 0.9 m wide rows. Between V1 and V3 all plots except the NCNC treatment received mechanical weed management with tine weeding or a rotary hoe four times in 2022, and two times in 2023 (Table 1).

**Table 1** Phase one field activity timeline for field 1 (2022 – 2023) and field 2 (2023)

Field Activity	Equipment	Field 1	Field 2
		2022	2023
Manure application	Jamesway tanker	May 6	April 27
Tillage	Tandem Disk	May 13	April 10
Tillage	Field cultivator	-	April 26
Tillage	Field cultivator	May 17	May 17
Tillage	Field cultivator	May 31	May 30
Planting	JD 1750 Organic Planter	May 31	May 30
Weeding	Tine weeder	June 3	June 8
Weeding	Tine weeder	June 10	-
Weeding	Tine weeder	June 15	-
Weeding	Rotary hoe	June 20	June 15
CC Planting	Dawn Interseeder	June 22	June 19
Weeding	Field cultivator	June 27	June 23



Weeding	Field cultivator	July 7	June 30
30 DAI CC and Weed Sampling		July 22	July 19
Harvest CC and Weed Sampling		October 3	September 19
Silage Harvest	Klaus Chopper	October 5	September 21
Harvest Soil Sample		October 7	October 3

Cover crops were drill seeded with a Dawn Interseeder (Dawn Equipment, Sycamore, IL) at the V3 stage on June 22, 2022 in field 1 and June 19, 2023 in field 2. Two rows of cover crops (19 cm spacing) were planted between each set of corn rows. The fall winter rye and NCWC treatments received in row cultivation two times after cover crop planting to control weeds.

Seeding rates for individual and mixtures are provided in Table 2.

**Table 2** Cover crop treatment numbers, species, and seeding rate.

Treatment	Species	Seeding Rate (kg ha <sup>-1</sup> )
1	Annual Ryegrass (AR)	22
2	Red Clover (RC)	11
3	Oilseed Radish (OR)	11
4	AR + RC	11 + 11
5	RC + OR	11 + 2
6	AR + OR	17 + 2
7	AR + RC + OR	11 + 9 + 2
8	Cultivation	---
9	Fall Winter Rye	67
10	No Cultivation No Cover (NCNC)	---

### Phase Two (corn silage harvest – spring cover crop termination)

Following corn silage harvest, winter rye was drill seeded following corn harvest in 19 cm rows. The winter rye was mowed back to a height of < 15 cm once (May 11, 2023) and again prior to manure treatment application to control the growth (May 17, 2023). Composted pelletized poultry manure was spread at rates of 0, 27, 54, 81, 108, and 136 kg-N ha<sup>-1</sup> randomly in 7.6m strips across all cover crop treatments using a manure spreader.

Cover crops were terminated as late as possible to allow for maximum agroecosystem benefits. Cover crops were terminated and poultry manure was incorporated on May 17, 2023 in field 1 with a disk cultivator. Winter rye typically would be terminated earlier in its growth stage, however we kept the termination date the same for all treatments. The field was then prepped for corn planting using a tandem disk.

**Table 3** Phase Two field activity for field 1.

Field Activity	Equipment	Field 1
		2022
Rye Planting	JD 1590 NT Drill	October 5
		2023
Winter Rye Mowing		May 11
Weeding	Tandem Disk	May 11
Winter Rye Mowing		May 17
Cover Crop Termination	Tandem Disk	May 17

## Soil and Biomass Analysis

### **Phase One**

Soil was sampled by collecting cores from a random distribution using a 2.5 cm diameter probe. Soil was sampled four times in phase one; at corn planting, at cover crop planting, 30 days after interseeding (30DAI), and at corn harvest. Soil samples were collected as composites of 4-8 sub samples per block prior to cover crop planting and 6 sub samples per plot after cover crop planting. At corn planting, cover crop planting, and corn harvest soil was sampled (0-15cm) for routine analysis (organic matter, phosphorus, potassium, and pH). At 30 DAI, samples were collected in single species treatments, NCWC, and NCNC plots at 30cm in field 1 and analyzed for nitrate and ammonium. In field 2 NCWC and NCNC plots were also sampled at a depth of 30-60cm and analyzed for nitrate and ammonium. At corn planting and corn harvest soil samples were collected (0-60 cm) and analyzed for nitrate and ammonium - N with a potassium chloride extraction at the Soil and Forage Analysis Laboratory in Madison, Wisconsin. All samples were dried for at least 7 days and ground to pass through a 1mm sieve.

In field 1 in 2022 there was a clear emergence pattern between wheel track and non-wheel track rows (from the interseeder) for the cover crops. This created an unintentional split plot uniformly across all treatments. For cover crop and weed biomass samples we collected samples separately within each row to quantify the impact of management on cover crop growth and weed suppression. Cover crop and weed biomass was collected as composites of 6 random 0.44 x 0.44 m (0.36 m<sup>2</sup>) quadrats subsamples per cover crop treatment in both wheel track and non-wheel track rows in field 1. Annual ryegrass, red clover, grass weeds, and broadleaf weeds were clipped at ground level and placed into individual bags separated by species. For the oilseed

radish, both above and below ground biomass was collected and placed into a bag. All bags were dried at 65°C for at least one week. The cover crops and weeds were then weighed and dry weight biomass was scaled to a kg ha<sup>-1</sup> using a 0.44 x 0.44 m (0.36 m<sup>2</sup>) sample area to account for the space between corn rows without cover crops.

Corn was hand harvested for silage once it had reached 65% moisture content (October 5, 2022 in field 1 and September 21, 2023 in field 2). Stalks were cut with a machete 20 cm above the ground from the middle two rows of each plot from 1.5 m at 65°C for at least one week. Dry weight biomass was collected and yield was standardized to 65% moisture content. All biomass samples were ground, milled, and rolled in tin capsules to be analyzed for total nitrogen and carbon content. Between 4-5 mg of milled biomass was rolled into 6 x 3 mm tin capsules and analyzed using a Flash EA 1112 CN Automatic Elemental Analyzer.

## **Phase Two**

Soil was sampled prior to cover crop termination. Six sub samples were taken and homogenized into a composite sample for each plot at depths 0-15cm, 0-30 cm, and 30-60 cm. Samples were dried for a minimum of seven days before being ground through a 1mm sieve and analyzed. 0-15 cm samples received a routine analysis while samples from 0-30 cm and 30-60 cm depths were analyzed for nitrate and ammonium.

Canopy cover for each cover crop treatment was assessed using Canopeo prior to termination. Photos were taken 1 meter above the ground of 3 random representative sections of both tire and non-tire track rows for each plot. Photos were uploaded into Canopeo's website (canopeoapp.com)

Prior to termination, cover crop and weed biomass was collected as composites of six random 0.44m x 0.44m (0.36 m<sup>2</sup>) quadrats subsamples in both wheel track and non-wheel track rows and separated by species in all treatments except the winter rye cover crop treatment. Cover crop and weed biomass from Winter Rye plots were collected as a composite of 3 random 0.44 m x 0.44 m (0.36 m<sup>2</sup>) quadrats sub samples in both wheel track and non-wheel track rows. Winter rye samples were taken two times, once on May 11, 2023 prior to mowing, and again at cover crop termination on May 17, 2023. Between mowing times, the rye had regrown to a height of 1 meter on average. In order to keep all cover crop termination timing the same, winter rye was mowed in order to manage the height of the crop and prevent it from reaching its reproductive stages. In field 1 cover crops were terminated on May 17, 2023, two weeks prior to corn planting.

All cover crop and weed biomass samples were dried at 65°C for at least one week. The cover crops and weeds were then weighed and dry weight biomass was scaled to a kg ha<sup>-1</sup> using a 0.44 x 0.44 m sample area (0.36 m<sup>2</sup>) to account for the space between corn rows without cover crops.

### Statistical Analysis

All statistical analysis was conducted with RStudio version 2023.06.1 using R statistical software version 4.3.1. Assumptions for normality and equal variance were tested using QQ-plots and plotting residuals from untransformed and transformed data. If assumptions were not met, square root transformations were conducted when necessary and values were back transformed before being reported. For field 1, square root transformations were made to cover crop biomass and nitrogen uptake, weed biomass and nitrogen uptake, and canopy cover. In field

2, cover crop and weed nitrogen uptake, and soil nitrate received square root transformations before analysis. For field 1, analysis of variance (ANOVA) was conducted to determine the effect of wheel track row, cover crop treatment, and their interaction on cover crop biomass, cover crop canopy cover, and weed biomass using linear mixed effects models (lme4 & lmerTest). For this analysis block, treatment, row, and species were all random effects with plot number as a fixed effect. Non-wheel track row data was analyzed using ANOVA to assess cover crop treatments on cover crop growth, weed suppression, soil nitrate, nitrogen content, and corn yield using generalized linear mixed effects models (lme4). Tukey HSD was used as a means separation test (tukeyhsd()).

## **Results and Discussion**

### **Phase One**

#### *Wheel Track Row*

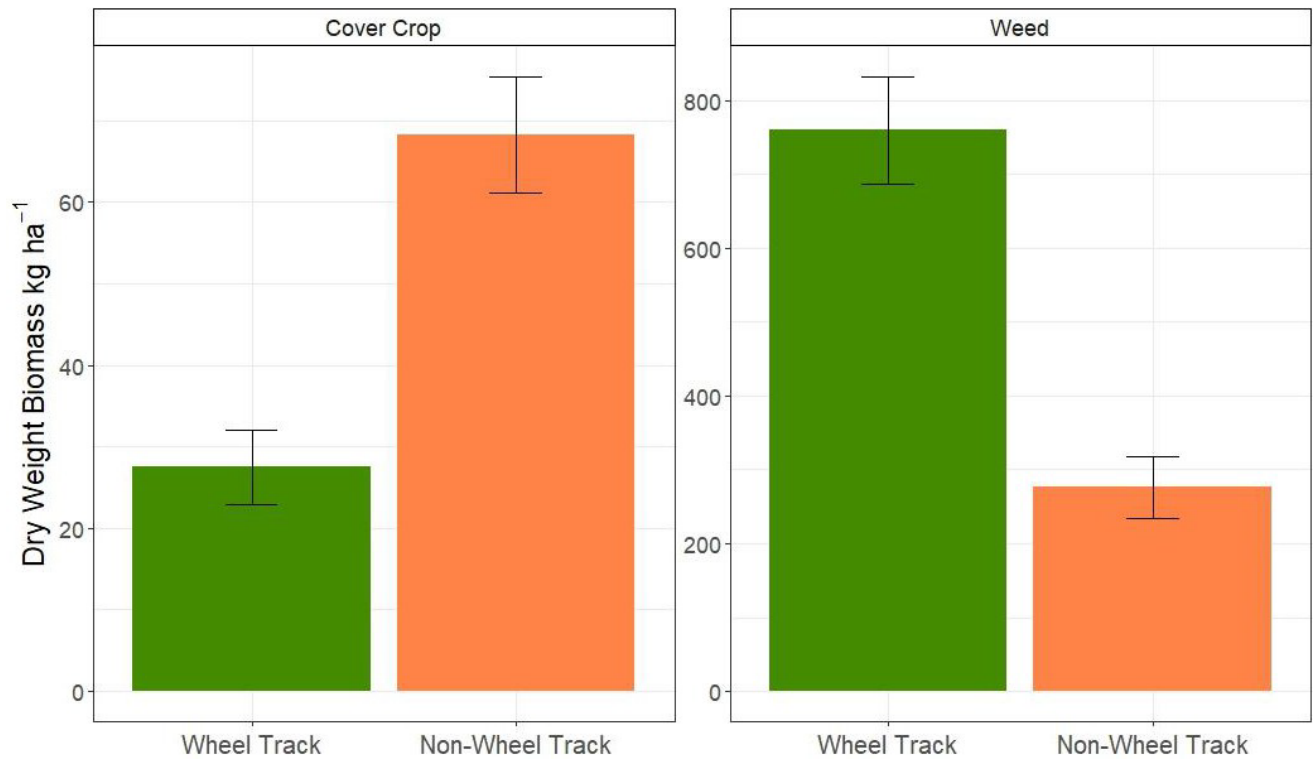
In field 1 we observed a lack of growth in two inner rows across all cover crop treatments. This was likely a combination of environmental and management factors that resulted in the wheel track effect because it did not occur in field 2. From early emergence cover crops were growing better in rows where tractors had not driven (Figure 4). We measured cover crop and weed growth separately between wheel track and non-wheel track rows.



**Figure 4. Oilseed Radish single species treatment in field 1, 10 days after interseeding.** Inner rows one, two, and three were planted with oilseed radish (a). Emergence in wheel track rows (b) was decreased in comparison to non-wheel track rows (c).

The average total cover crop biomass at corn harvest across all treatments in wheel track rows ( $27 \text{ kg ha}^{-1}$ ) was less than in non-wheel track rows ( $68 \text{ kg ha}^{-1}$ ). Conversely, there was more total weed biomass in wheel track rows ( $759 \text{ kg ha}^{-1}$ ) than in non-wheel track rows ( $276 \text{ kg ha}^{-1}$ ).

ha<sup>-1</sup>) across all treatments (Figure 5). Due to poor cover crop establishment in wheel track rows, there was higher weed density as a result of a lack of competition from cover crops.



**Figure 5. Average cover crop (left) and weed (right) biomass across all cover crop treatments in wheel track rows compared to non-wheel track rows in field 1.** Above ground biomass of all cover crops and weeds were harvested in addition to below ground biomass from oilseed radish from six quadrats per row within each treatment. Biomass was collected the day before corn harvest (10/4/2022). Error bars represent the standard error from the mean.

The wheel track effect allowed us to better compare weed management strategies. As cover crops did not establish well it functions as another control treatment comparing reduced weed management to standard cultivation and interseeded cover crops. Standard cultivation treatments were cultivated two more times after cover crop planting in field 1. The wheel track rows did not receive this extra cultivation and all cover crop treatments had higher weed biomass



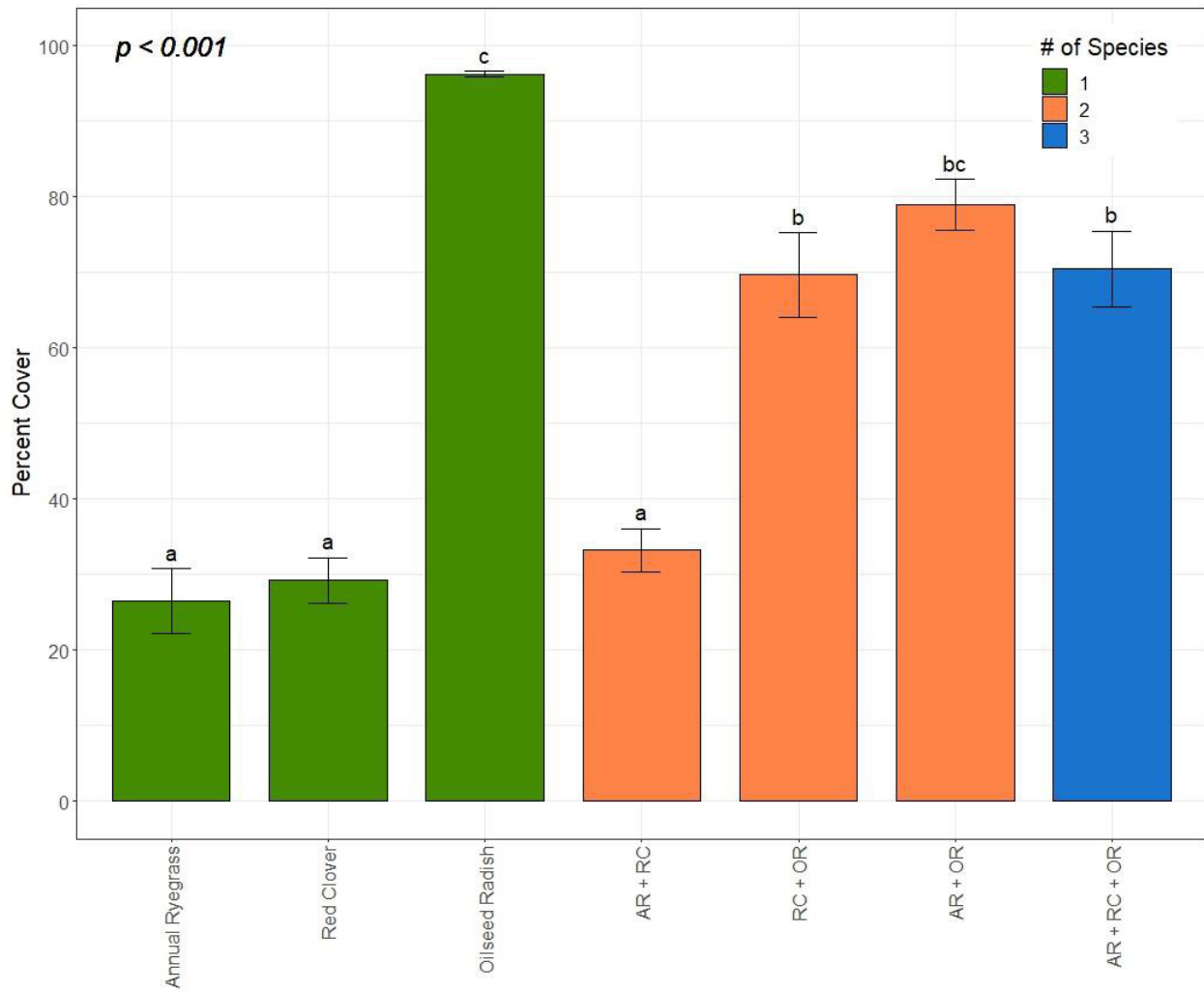
in wheel track rows than in non-wheel track rows. The wheel track row shows that extra cultivation passes are required for weed management without cover crops.

The wheel track effect is likely the result of a combination of environmental and management factors including tire pressure, rainfall, and soil compaction. We did not collect any soil compaction measurements but there were cover crop seeds sitting on the soil surface in wheel track rows. It is possible that the planter was not able to plant the cover crop seeds deep enough to germinate. To avoid this effect farmers should ensure they have adequate soil conditions and check that the planter is getting the seed deep enough during planting. Data from the wheel track row in field one was not analyzed in this project in field 1 due to the difference in growth. In field 2 there was no significant difference between wheel track row and non-wheel track row on cover crop growth so both rows were analyzed equally.

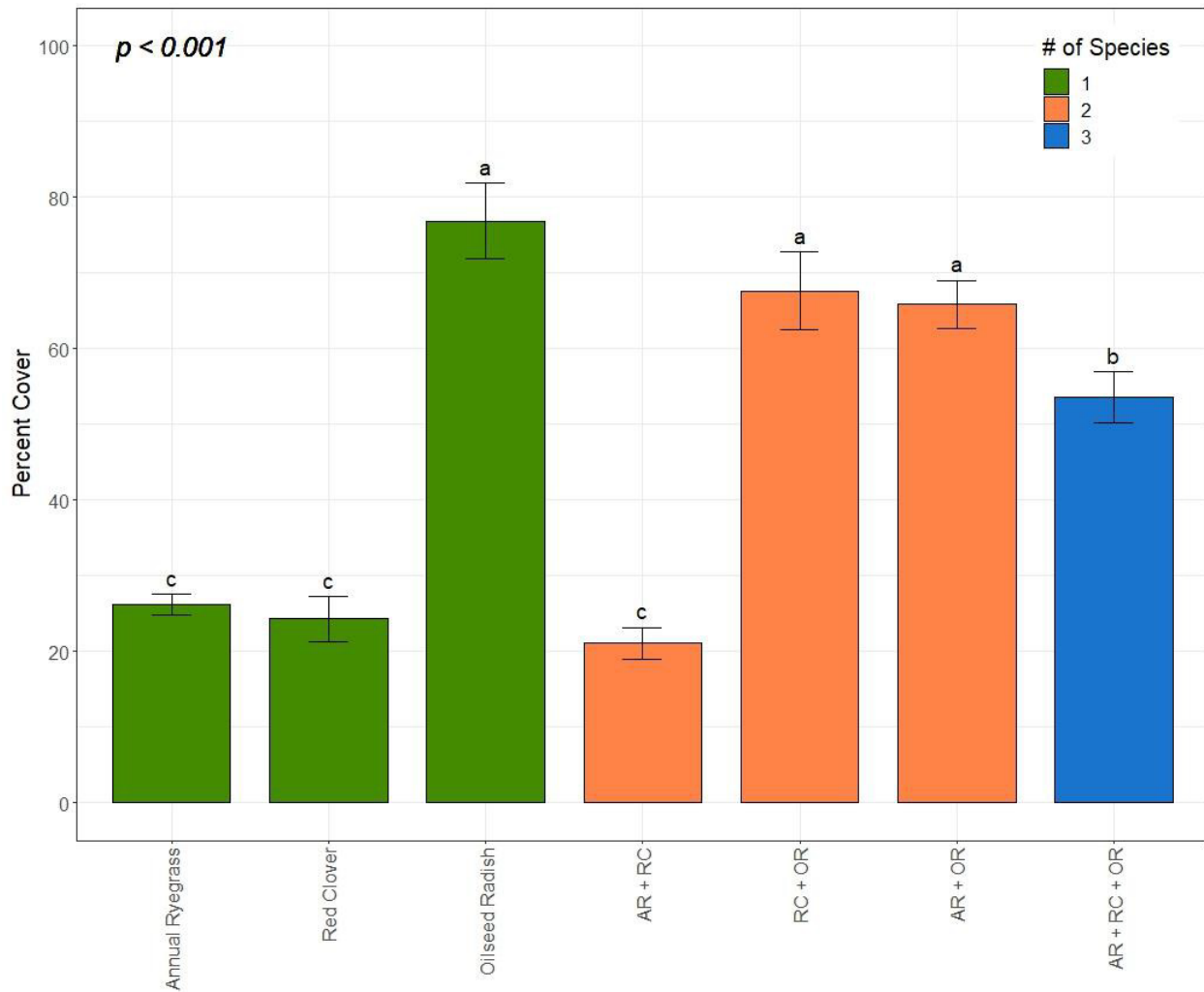
### Canopy cover

Cover crop treatments containing oilseed radish had higher canopy cover percentages 30 days after interseeding (30 DAI) than treatments without in both years (Figures 6 and 7). Oilseed radish established well in both years when interseeded at V3 into standing corn and established more rapidly than annual ryegrass and red clover. Annual ryegrass and red clover are both smaller seeds than oilseed radish and previous research has shown that seedlings emerge at a faster rate in large seeded species (Benvenuti et al., 2001). Previous studies have found annual ryegrass to have a higher plant density than oilseed radish or clover 30 DAI (Brooker et al., 2020), but in both years the canopy cover was lowest in the single species application of annual ryegrass as well as in the two species mixture with red clover.

In field 2 there was a drought around the time of planting which likely effected the early season growth of both annual ryegrass and red clover. Research on annual ryegrass establishment and growth in Lexington, KY found that annual ryegrass produced less biomass in drier conditions (Stanton & Haramoto, 2019). While annual ryegrass was expected to have higher canopy cover due to ease of establishment, previous research has shown that legumes are more difficult to establish than other cover crop functional groups. Drill interseeding red clover has shown higher rates of establishment than broadcasting (Caswell et al., 2019), however red clover it still did not establish well in either year. When oilseed radish was grown in mixtures, the total percentage of canopy cover decreased as more species were introduced into the mixture despite all mixtures having the same seeding rate of oilseed radish. This trend was observed both years but was more evident in field two where the three species mixture had 23% lower canopy cover than the single species oilseed radish treatment, and 12% - 15% less than the other mixtures containing oilseed radish (Figure 7).



**Figure 6. Canopy cover percentage (%) 30 days after interseeding (30 DAI) in year one (2022).** Canopy cover was taken using Canopeo. Error bars represent the standard error from the mean. Across all treatments columns with the same letter are not significantly different ( $\alpha = 0.05$ ).



**Figure 7. Canopy cover percentage (%) 30 days after interseeding (30 DAI) in year two (2023).** Canopy cover was taken using Canopeo. Error bars represent the standard error from the mean. Across all treatments and both rows, columns with the same letter are not significantly different ( $\alpha = 0.05$ ).

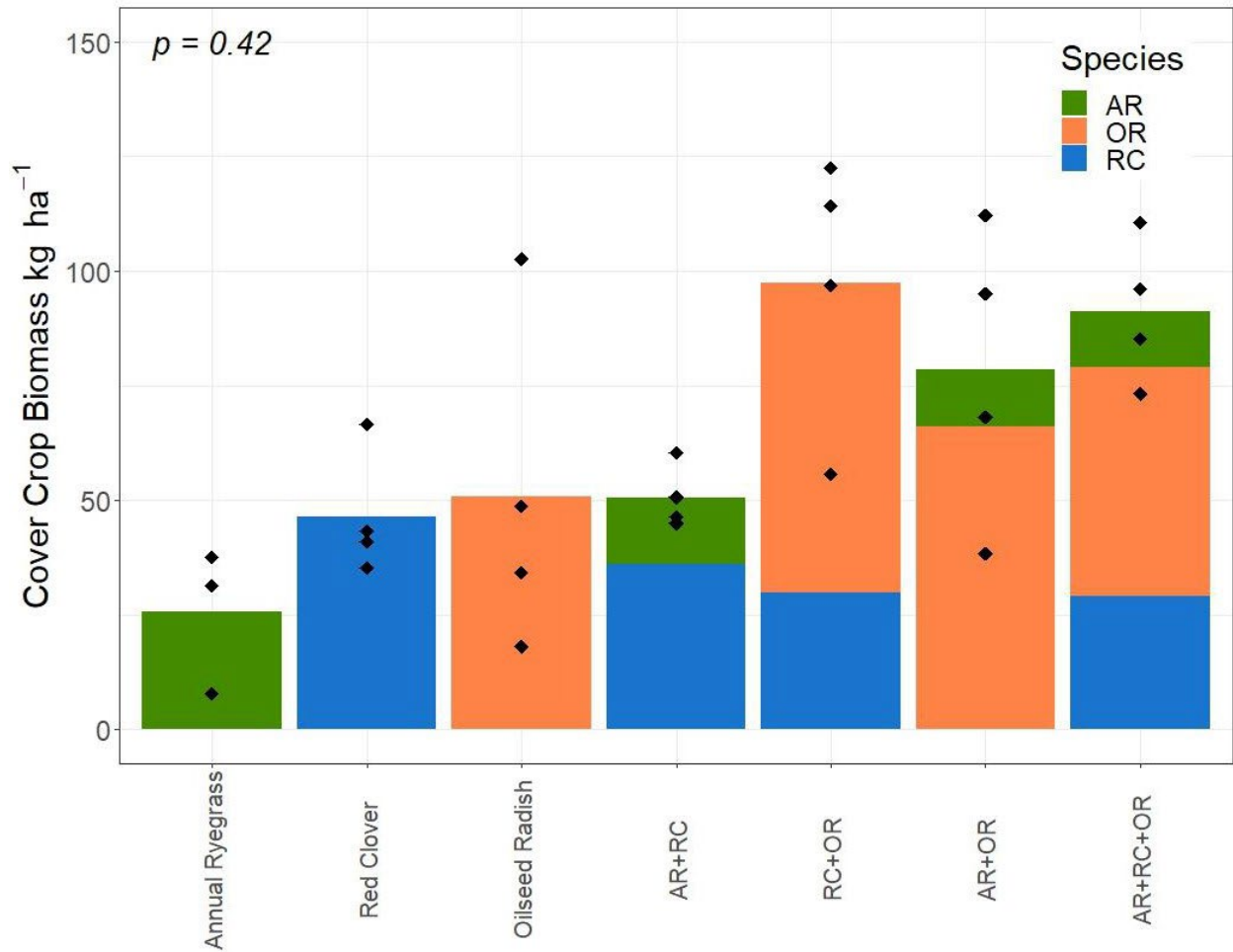
### Cover crop and weed biomass

Total cover crop biomass production was low and highly variable across all three species and both site years compared to other studies. This is a result of both management and environmental factors. While drill interseeding can benefit growth and emergence because of higher seed to soil contact (Wallace et al., 2020), it limited the potential total biomass production by increasing plant density. Decreasing plant density by distributing the same number of seeds

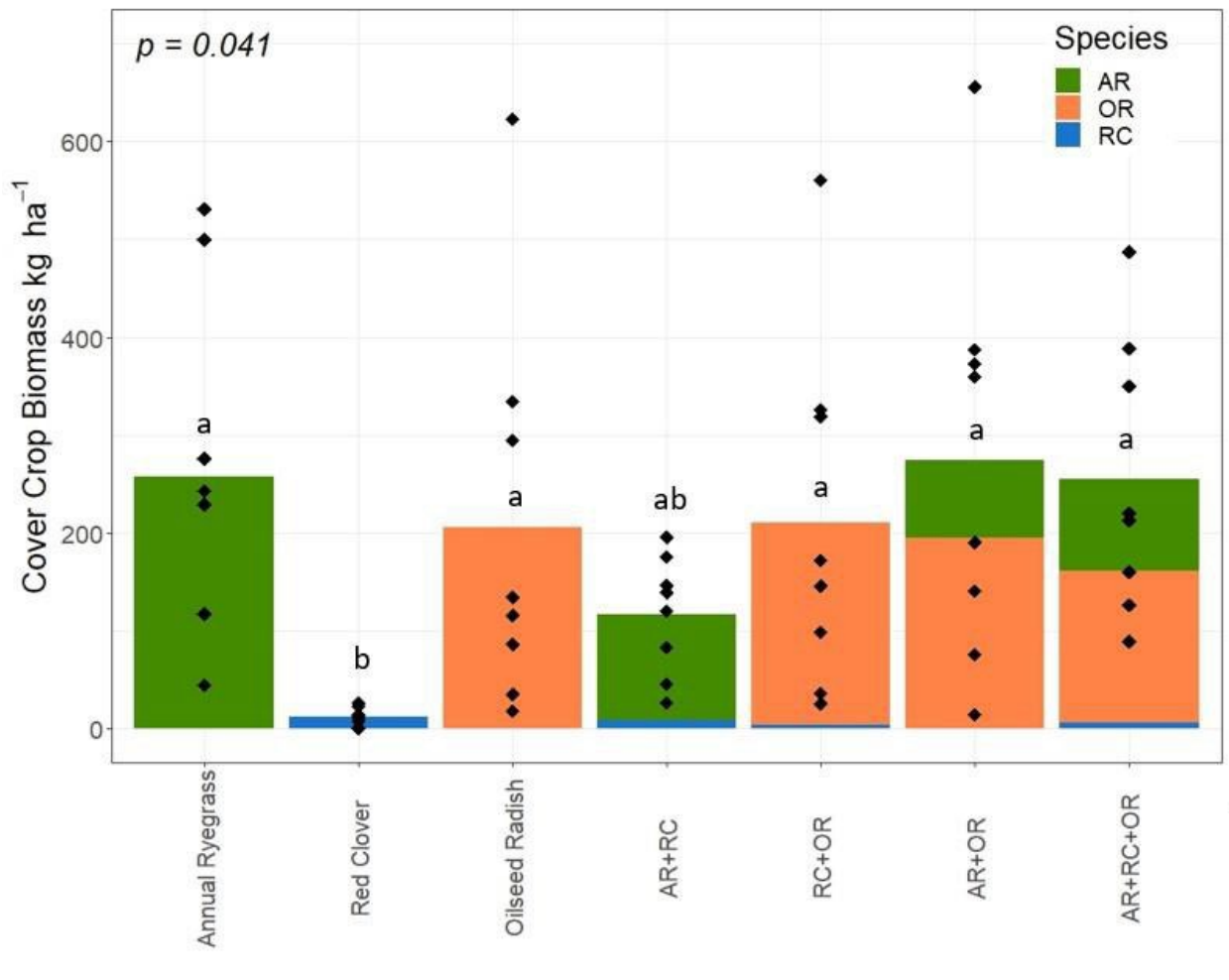
across a greater area by broadcast seeding or planting more than two rows of cover crops, allows for individual plants to accumulate more biomass. Other interseeding studies range from planting two rows of cover crops (Stanton & Haramoto, 2019; Wallace et al., 2020) to four rows (Caswell et al., 2019) while broadcast seeding trials have higher seeding rates.

In this study cover crop growth and establishment was variable across site years; in field 1 there was more total biomass in mixtures compared to monocultures (Figure 8) however in field 2 there was no difference between cover crop mixtures containing oilseed radish and the annual ryegrass and oilseed radish single species treatments (Figure 9). The species composition of each mixture indicates the reduction in total biomass in field 2 in mixtures is due to the lack of establishment of red clover. There was a drought around the time of planting in field 2 which affected red clover growth and total biomass production in mixtures containing red clover.

In field 1 there was no statistical difference across cover crop treatments, regardless of the number of species in the treatment, for total biomass production ( $p = 0.42$ ) due to variability in the data, however, cover crop mixtures produced more total biomass than single species applications (Figure 8). Previous studies have found that cover crops grown in mixtures have more biomass relative to single species applications (Dean & Weil, 2009; Lavergne et al., 2021, Kahn & McVay, 2019) regardless of species composition. In field 2 all cover crop treatments produced similar amounts of biomass with the exception of the red clover single species treatment (Figure 9).



**Figure 8. Average cover crop biomass (kg ha<sup>-1</sup>) from year one (2022).** Above ground biomass of all cover crops was harvested in addition to below ground biomass from oilseed radish from six quadrats per row within each treatment. Each dot represents total treatment biomass per plot. Cover crop biomass was collected the day before corn harvest (10/4/2022). There were no differences between the means ( $p = 0.42$ ) due to variability.



**Figure 9. Average cover crop biomass (kg ha<sup>-1</sup>) from year two (2023).** Above ground biomass of all cover crops was harvested in addition to below ground biomass from oilseed radish from six quadrats per row within each treatment. Each dot represents total treatment biomass per plot. Cover crop biomass was collected two days before corn harvest (9/19/2023). Columns with the same letter are not significantly different ( $\alpha = 0.05$ )

Oilseed radish established rapidly in both years when interseeded at V3 into standing corn. Cover crops mixtures typically produce more biomass than single species applications (Dean & Weil, 2009; Lavergne et al., 2021, Kahn & McVay, 2019), although Murrell et al found that brassicas do not produce as much biomass in mixtures compared to single species application (Murrell et al., 2017). In this study however, single species applications of oilseed radish produced similar amounts of biomass in comparison to mixed species applications. The

seeding rate for oilseed radish by itself was 11 kg ha<sup>-1</sup> while it was only 2 kg ha<sup>-1</sup> in all mixtures. While it had the most biomass compared to other species, oilseed radish produced less than 300 kg ha<sup>-1</sup> in both years and previous research has found it capable of producing above 1,000 kg ha<sup>-1</sup> at the same seeding rate (Brooker et al., 2020; Belfry & Van Eerd, 2016). Oilseed radish has not shown any difference in biomass production when planted at rates between 10 and 22 kg ha<sup>-1</sup> (Ngouajio & Mutch, 2004), but brassicas have proven to produce more biomass relative to seeding rate (Murrell et al., 2017). Despite the changes in seeding rate, oilseed radish produced similar quantities of biomass in mixtures and single species applications unlike annual ryegrass and red clover. On farm research comparing cover crop species interseeded in monoculture applications have reported that oilseed radish produces similar, if not more, biomass than annual ryegrass when interseeded into corn (Brooker et al., 2020). Despite this study having a higher seeding rate for annual ryegrass (16 kg ha<sup>-1</sup> vs. 22 kg ha<sup>-1</sup>), oilseed radish still produced equal if not more biomass in single species applications than annual ryegrass in both fields. There was no difference in nitrogen content between oilseed radish and the other cover crops. The nitrogen uptake was higher, however, due to the higher total biomass of oilseed radish. Previous studies have also found that the oilseed radish takes up more nitrogen than other cover crops because total biomass, not nitrogen content (Dean & Weil, 2009).

Annual ryegrass growth was inconsistent across the two site years. Annual ryegrass produced more biomass in single species applications than in mixtures in field 2. In field 1, however, it had the lowest overall biomass at corn harvest. Previous studies have found annual ryegrass can produce anywhere from 0 to 612 kg ha<sup>-1</sup> at similar planting rates (Brooker et al., 2020; Caswell et al., 2019; Stanton & Haramoto, 2019; Wallace et al., 2020). Research has found that grasses tend to dominate mixtures (Murrell et al., 2017), however annual ryegrass had the



lower biomass than oilseed radish despite having a higher seeding rate in mixtures. In year two there was better establishment and growth of annual ryegrass in mixtures, but it still was less than 50% of the overall biomass despite being planted at a higher rate than other species. Despite previous research finding that annual ryegrass produces less biomass in drier conditions (Stanton & Haramoto, 2019), in this study it performed better in drought conditions in the second year. While that study was examining the effects of herbicides for weed control on annual ryegrass growth, they concluded that environmental factors had a greater influence than herbicide treatments.

Red clover biomass production in the fall was the most variable out of all species across both years. Red clover is known to be difficult to establish in interseeding, however drill interseeding has shown to have better rates of establishment than broadcast (Caswell et al., 2019). Despite drill interseeding however, it still did not establish well in either year. In field 2 there was a lack of rain prior to and immediately following cover crop planting, resulting in the poor establishment of red clover in comparison to field 1. Previous research has found that the success and establishment of small legumes such as red clover is heavily influenced by rainfall events and soil moisture (Brooker et al., 2020; Keeling et al., 1996). However, another study reported red clover yields of 688 - 1184 kg ha<sup>-1</sup> in dry conditions (Queen et al., 2009). Red clover was broadcast interseeded into winter wheat, and biomass increased after wheat was harvested indicating that competition for light is an important factor in total biomass accumulation with red clover. The combination of low precipitation early in the season and less light penetration through the corn canopy later in the season would explain lack of establishment and growth in field 2. In field 1 red clover produced more biomass than annual ryegrass in single species applications. In field 1 mixtures containing red clover produced less biomass when planted with

oilseed radish than with annual ryegrass, likely because of competition for light, but overall biomass trends followed seeding rate trends unlike annual ryegrass and oilseed radish. Previous studies have also found that mixtures containing legumes have less biomass than mixtures without and that cool season legumes have poor establishment compared to grasses and brassicas (Kahn & McVay, 2019; Florence et al., 2019). Other legumes have been found to have higher nitrogen content than grass cover crops (Ranells & Wagger, 1997), but there was no difference in nitrogen content in red clover compared to annual ryegrass and oilseed radish (Table 4).

**Table 4. 2022 Nitrogen and carbon content, C:N ratio, and nitrogen yield of cover crops.** ANOVA results from total nitrogen yield of whole cover crop treatments are represented by letters in the last column. Letters indicate significant differences in values between treatments ( $\alpha = 0.05$ ). Cover crop species did not differ in nitrogen uptake.

Treatment	Species	<u>N Content</u>	<u>C Content</u>	<u>C:N</u>	<u>N Yield</u>	
		-----%-----			<i>Species</i>	<i>Total</i>
					-----kg ha <sup>-1</sup> -----	
Annual Ryegrass	AR	3.39	28.78	8.5	0.84	0.84 b
Red Clover	RC	3.37	28.71	8.5	1.5	1.5 b
Oilseed Radish	OR	3.78	27.5	7.3	1.0	1.0 b
AR + RC	AR	2.83	29.28	10.3	0.5	0.9 b
	RC	2.26	25.53	11.3	0.4	
RC + OR	RC	2.59	28.37	11.0	1.2	1.1 b
	OR	3.28	29.36	9.0	0.7	
AR + OR	AR	2.38	26.56	11.1	0.3	0.7 b
	OR	4.03	29.92	7.4	0.4	
AR + RC + OR	AR	3.74	31.48	8.4	1.8	4.0 a
	RC	2.99	31.77	10.6	1.7	
	OR	3.78	28.05	7.4	0.9	
p-value					0.947	0.005

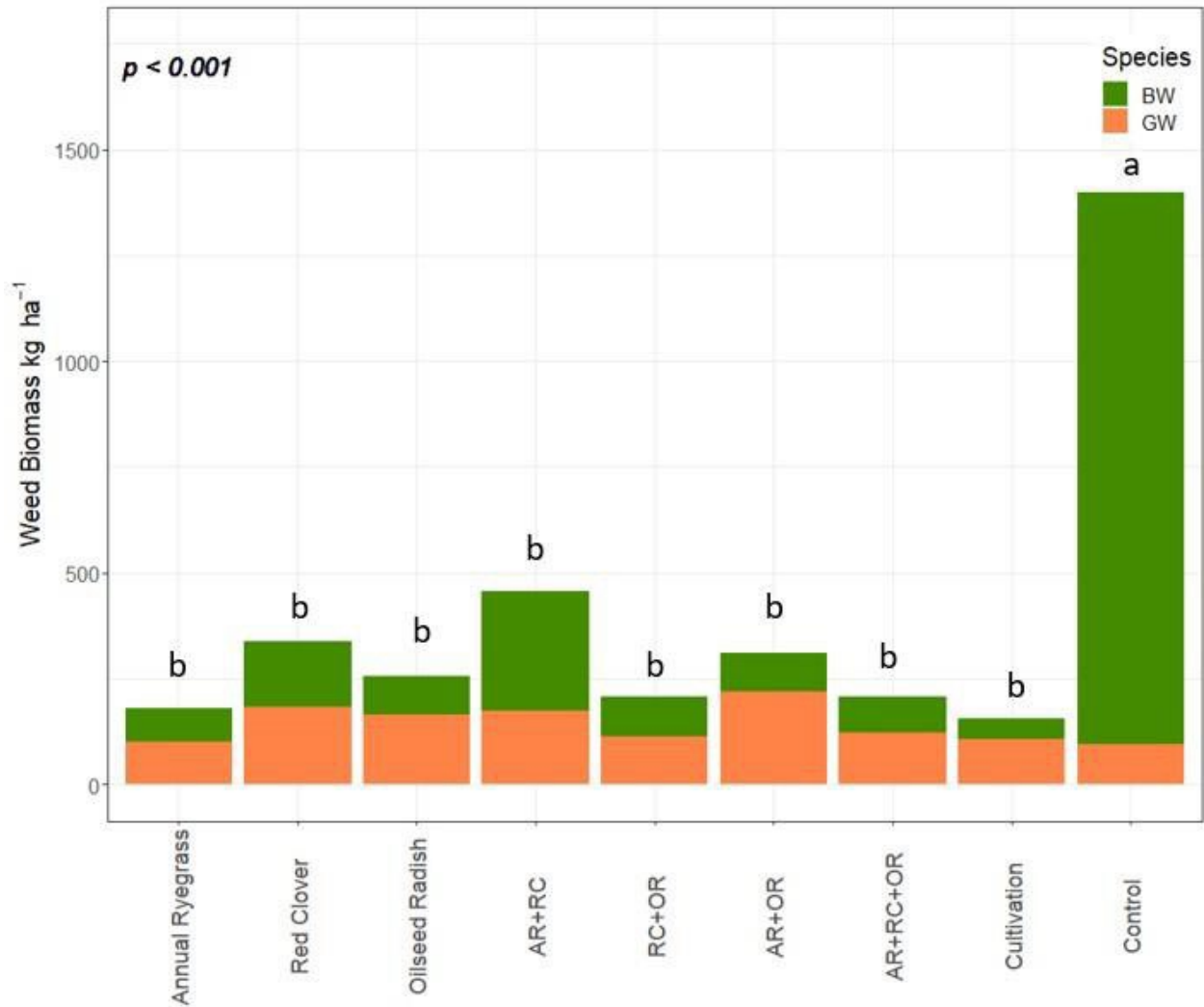
Across both fields, total weed biomass was greater than cover crop biomass across all treatments. However, cover crops treatments had similar weed biomass to the cultivation treatment in both years. In field 1 there were more grass weeds in cover crop treatments (Figure 10) in comparison to field 2 which had more broadleaf weeds (Figure 11). Additionally, cover

crop treatments had a higher ratio of grass weed to broadleaf weeds in comparison to the NCNC treatment which had fewer grass weeds (Figure 10). Broadleaf weeds typically have larger seeds than grass weeds and emerge sooner (Benvenuti et al., 2017) and were managed by cultivation prior to cover crop planting leaving space for grass weeds to emerge. Weed biomass from field 1 was analyzed for carbon and nitrogen content (Table 5). There was no statistical difference in nitrogen content between broadleaf weeds and grass weeds ( $p = 0.08$ ) so treatments with greater overall weed biomass had higher total nitrogen uptake (Table 5).

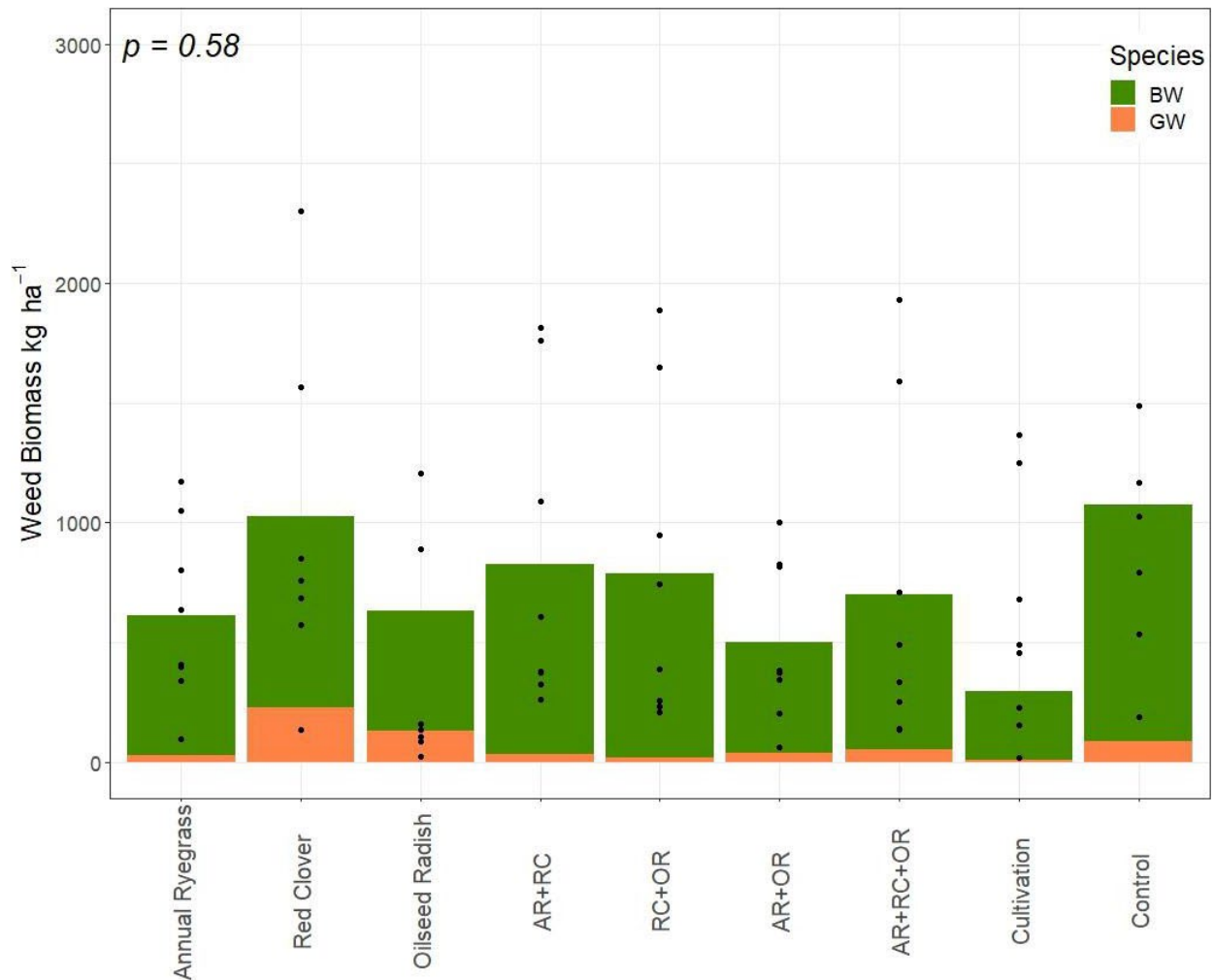
**Table 5. Weed nitrogen content, uptake, and C:N ratio.** Each treatment is separated into weed species. ANOVA results from total nitrogen yield of whole cover crop treatments are represented by letters in the last column. Letters indicate significant differences in values between treatments ( $\alpha = 0.05$ ). Comparing nitrogen content of grassweeds and broadleaf weeds indicates that broadleaf weeds have a higher N content (2.76%) compared to grass weeds (2.53%) ( $p = 0.04$ ).

Treatment	Species	N Content	C Content	C:N	Nitrogen Uptake		
		-----%-----			Species	Total	
					-----kg ha <sup>-1</sup> -----		
Annual Ryegrass	BW	2.91	32.39	11.14	5.2	7.0 b	
	GW	2.57	31.04	12.09	3.3		
Red Clover	BW	2.77	35.48	12.82	9.5	14.6 b	
	GW	2.48	36.16	14.57	5.9		
Oilseed Radish	BW	2.45	31.32	12.78	3.0	8.8 b	
	GW	2.23	32.51	14.59	5.9		
AR + RC	BW	3.09	32.72	10.59	14.1	16.9 b	
	GW	2.86	31.05	10.86	5.2		
RC + OR	BW	3.00	35.70	11.88	8.1	13.5 b	
	GW	2.41	34.11	14.16	5.3		
AR + OR	BW	2.84	34.29	12.08	8.9	22.6 a	
	GW	2.69	35.09	13.03	13.7		
AR + RC + OR	BW	2.59	37.32	14.41	6.1	11.8 b	
	GW	2.52	33.03	13.10	5.7		
Cultivation	BW	2.81	35.72	12.69	1.9	5.8 b	
	GW	2.70	34.19	12.67	3.7		
NCNC	BW	2.71	36.01	13.31	31.1	33.0 a	
	GW	2.54	34.26	13.47	1.9		
p-value						<0.001	

In field 2 there was higher weed biomass overall, regardless of type, and more variability across the field and the single species red clover treatment had the highest total weed biomass out of all cover crop treatments ( $952 \text{ kg ha}^{-1}$ ). There was no statistical difference between cover crop treatments, cultivation, and NCNC in total weed biomass. The NCNC treatment had the highest total weed biomass with an average of  $1,733 \text{ kg ha}^{-1}$  while the cultivation treatment had the lowest total biomass ( $580 \text{ kg ha}^{-1}$ ). The annual ryegrass ( $612 \text{ kg ha}^{-1}$ ) and oilseed radish ( $631 \text{ kg ha}^{-1}$ ) single species treatments had the lowest average weed biomass out of all the cover crop treatments. Due to field size limitations, there were no borders between cover crop treatments and the edge of the field, so weed encroachment was likely a cause of this variability and increase in weed density/biomass/competition. The decrease in grass weeds in field 2 may be a factor of the number of times we cultivated prior to cover crop planting or a difference in weed seed bank diversity. Fall weed nitrogen content was greater in broadleaf weeds than in grass weeds, but overall nitrogen uptake was determined by total biomass production. The NCNC weedy control treatment had the most above ground weed biomass and therefore the highest nitrogen uptake.



**Figure 10. Average weed biomass (kg ha<sup>-1</sup>) from year one (2022).** Above ground biomass of weeds from six quadrats per row within each treatment and separated out by broadleaf weed or grassweed. Weed biomass was collected the day before corn harvest (October 5, 2022). Across all treatments and both rows, columns with the same letter are not significantly different ( $\alpha = 0.05$ )



**Figure 11. Weed biomass (kg ha<sup>-1</sup>) from year two (2023).** Above ground biomass of weeds from six quadrats per row within each treatment and separated out by broadleaf weed or grassweed. Weed biomass was collected the day before corn harvest (September 19, 2022). There was no statistical significance between the means ( $p = 0.63$ ).

It has been found that an increase in the number of cover crop species is directly correlated to agroecosystem benefits, however not always with an increase in weed suppression (Finney & Kaye, 2016; Smith et al., 2020; He et al., 2005). Previous studies have also indicated, however, that above ground biomass is a better determinant of weed suppression than number of species (Florence et al., 2019). As cover crops tend to produce more biomass in mixtures than in

monocultures it is likely that the type of cover crop species is more important than the number. In this study there was no difference between single species and mixtures on weed suppression despite mixtures producing more cover crop biomass. Cover crop establishment was more of a factor on weed suppression than diversity or type. When red clover established well in field 1 it suppressed weeds as well as other species, however its poor establishment in field 2 led to higher and more variable weed production.

### Soil nitrogen response

Annual ryegrass, red clover, and oilseed radish had no effect on in-season soil or spring nitrate levels. Single species and control treatments were sampled at a depth of 0-30cm 30 DAI in both fields. At corn harvest all treatments were sampled at depths of 0-30cm and 30-60cm. The cover crops had no effect on soil nitrate levels at any depth (Table 6). In field 1 there was low overall biomass from cover crop treatments which would result in lower overall nitrogen demand. Previous studies have found that legumes seeded in the fall as cover crop have increased soil nitrogen levels (Lavergne et al., 2021), however there was no increase in soil nitrogen levels in field 1 when red clover established well. Other studies did not find any effect on fall soil nitrate levels in the top 20 – 30 cm of the soil profile (Belfry & Van Eerd, 2016; Wallace et al., 2020). Research conducted in Maryland on brassicas used for cover crops found both a decrease in fall soil nitrate as well as an increase in spring available nitrogen (Dean & Weil, 2009). Despite having the most biomass in field 1, there was no evidence that oilseed radish scavenged nitrogen. In field 1 the NCNC reduced fall soil nitrate at 30-60 cm due to the higher total biomass in these plots, and higher percentage of broadleaf weeds. In field 2 the lack of cover crop effect on soil nitrate levels despite producing more biomass indicates there may be little competition for nitrogen in the system.

**Table 6. In-season soil nitrate (NO<sub>3</sub>-N) by treatment.** 2022 was the only year with soil nitrate reported at harvest. 30 days after interseeding (30 DAI) single species, cultivation, and no cultivation no cover (NCNC) treatments were sampled at a depth of 0-30 cm. At corn harvest, all treatments were sampled at depth 0-30 cm and 30-60 cm. Treatments with the same letter are not significantly different ( $\alpha = 0.05$ ).

Treatment	Soil nitrate (NO <sub>3</sub> -N)			
	30 DAI		Harvest	
	<u>2022</u>	<u>2023</u>	<u>2022</u>	
	-----cm-----			
	0-30	0-30	0-30	30-60
	----- mg kg <sup>-1</sup> -----			
Annual Ryegrass	72.5	55.0	19.9	15.3 a
Red Clover	56.1	73.8	26.8	15.9 a
Oilseed Radish	56.1	71.9	23.5	12.6 a
AR + RC	-	-	28.1	16.5 a
RC + OR	-	-	20.0	13.2 a
AR + OR	-	-	18.3	12.6 a
AR + RC + OR	-	-	16.1	15.3 a
Cultivation	66.2	67.4	20.9	12.7 a
NCNC	43.4	48.8	15.8	4.0 b
	<b>p-value</b>			
Treatment	0.3445	0.2804	0.06897	0.003172

### Corn yield effects

Cover crop treatments had no effect on corn silage yield in either field 1 or field 2 (Table 7). Corn silage was standardized to 65% moisture both years. In field 1 corn silage was analyzed for nitrogen uptake and there was no effect of cover crop treatments on nitrogen content or uptake.



**Table 7. Corn yield effects by treatment.** Corn silage yield was standardized to 65% moisture. Corn silage was measured for nitrogen content and uptake in year 1. There was no significant difference ( $\alpha = 0.05$ ) between corn yields in either year across all treatments.

Treatment	Yield		N Content	N Uptake
	2022	2023	2022	
	----- Mg ha <sup>-1</sup> ----		%	Mg ha <sup>-1</sup>
Annual Ryegrass	52.1	51.6	1.50	0.782
Red Clover	55.9	45.1	1.72	0.966
Oilseed Radish	52.5	51.2	1.65	0.870
AR + RC	50.8	43.9	1.65	0.837
RC + OR	48.3	62.8	1.53	0.747
AR + OR	56.2	54.2	1.57	0.881
AR + RC + OR	54.3	60.8	1.63	0.877
Cultivation	53.0	59.6	1.59	0.842
NCNC	43.9	51.6	1.51	0.662
	<b>p – value</b>			
Treatment	0.351	0.165	0.370	0.223

In both fields cover crop treatments did not decrease corn silage yields. Previous studies have found that cover crops can be interseeded as early as V2 without resulting in a reduction in yield (Brooker et al., 2020; Gieske et al., 2017; Wallace et al., 2020). Organic farmers are interested in interseeding legumes in order to utilize the biological nitrogen fixation provided by the cover crop to benefit yields. Unfortunately, red clover interseeded both in single and mixed species applications does not provide enough nitrogen to the agroecosystem in-season or in the spring to be considered as a beneficial source of nitrogen. In field 1 all cover crop treatments and cultivation had higher yields than the NCNC treatment. In field 2 however, the red clover and red clover plus annual ryegrass treatments had the lowest overall yield. While not statistically significant, the difference in yields is agronomically significant to a farmer and this study would benefit from a longer-term study.

## Phase Two

### Cover crop and weed biomass

Annual ryegrass and red clover survived the winter and grew into the spring. Winter rye also survived the winter and was harvested two times (WR1 and WR2) during the spring with a mowing in between each harvest. The two species mixture containing annual ryegrass and red clover had the most biomass out of the seven experimental treatments but winter rye put on the most biomass overall (Table 8). Previous studies have found that annual ryegrass will over winter and can double in biomass in the spring compared to the fall (Caswell et al., 2019; Wallace et al., 2020). In field 1 spring annual ryegrass produced up to ten times the amount of biomass collected in the fall.

**Table 8. Spring cover crop biomass separated by species and row.** Cover crops were harvested from six quadrats per row and separated into species. Winter rye was harvested twice, once on 4/28/2023 (WR1) and again on 5/16/2023 (WR2). The winter rye treatment was mowed immediately after the first sampling date. All cover crops were samples on 5/16/2023 prior to termination. Letters indicate significant differences in values between treatments ( $\alpha = 0.05$ ).

Treatment	Species	Spring Cover Crop Biomass	
		<i>Species</i>	<i>Total</i>
		-----kg ha <sup>-1</sup> -----	
Annual Ryegrass	AR	291.4	291.4 bc
Red Clover	RC	118.6	118.6 bc
Oilseed Radish	OR	0	0
AR + RC	AR	175.0	378.2 b
	RC	203.1	
RC + OR	RC	39.7	39.7 c
	OR	0	
AR + OR	AR	155.1	155.1 bc
	OR	0	
AR + RC + OR	AR	109.2	219.1 bc
	RC	109.9	
	OR	0	
Winter Rye	WR 1	2344	6433 a
	WR 2	4089	
p-value			< 0.001

Red clover successfully overwintered but did not produce as much biomass as annual ryegrass in the single species application. However, in mixtures with annual ryegrass red clover produced more biomass than the single species treatment. While previous studies and fall biomass would suggest that biomass production is reduced with the introduction of legumes, results from this study there is an advantage to increased biodiversity in cover crop treatments for winter hardiness and spring biomass production.

All cover crop treatments suppressed spring weeds better than cultivation (Table 9). The cultivation treatment had the highest spring weed biomass (131 kg ha<sup>-1</sup>) while the winter rye treatment had the lowest (5.5 kg ha<sup>-1</sup>). Winter rye suppressed weeds better than other cover crop treatments, but most cover crop treatments suppressed spring weeds better than in season cultivation with the exception of annual ryegrass and two species mixtures containing annual ryegrass.

**Table 9. Spring weed biomass separated by species.** Weeds were harvested from six quadrats per row and separated into type (broadleaf or grassweed). The winter rye treatment is the total weeds between both sampling dates. All treatments were sampled on 5/16/2023 prior to being terminated. Letters indicate significant differences in values between treatments ( $\alpha = 0.05$ ).

Treatment	Species	Spring Weed Biomass	
		Species	Total
		-----kg ha <sup>-1</sup> -----	
Annual Ryegrass	BW	56.6	60.1 ab
	GW	3.6	
Red Clover	BW	31.3	33.3 bc
	GW	2.0	
Oilseed Radish	BW	21.3	25.8 bc
	GW	4.5	
AR + RC	BW	44.8	48.8 abc
	GW	4.0	
RC + OR	BW	26.5	32.8 bc
	GW	6.3	
AR + OR	BW	40.8	56.9 ab
	GW	16.1	

AR + RC + OR	BW	20.8	22.3 bc
	GW	1.6	
Cultivation	BW	130.5	131.2 a
	GW	0.7	
Winter Rye	BW	5.9	5.5 c
	GW	0.0	
NCNC	BW	29.4	32.0 bc
	GW	2.6	
p-value			< 0.001

There was not a lot of weed biomass in the spring compared to the fall. However, all cover crop treatments, included the oilseed radish that winter killed, suppressed weeds despite the amount of above ground cover crop biomass produced. Despite having higher spring cover crop biomass, annual ryegrass and two species mixtures containing annual ryegrass had higher weed biomass than other cover crop treatments. The in-season cultivation treatment had the highest weed biomass produced in the spring across all treatments because of the increased soil disturbance that likely brought more weed seeds to the surface. Studies comparing organic no-till to organic operations that use tillage as a method of weed control found that no-till fields have lower weed seed banks than fields that regularly till or cultivate (Buhler et al., 1994; Mulugeta & Stoltenberg, 1997). Fall plantings of brassicas have been found to have better weed suppression in the spring compared to fallow treatments despite not being winter hardy because fall weed competition is the dominant determinant of spring weed emergence (Lawley et al., 2012). In this experiment oilseed radish treatments suppressed spring weeds just as well as annual ryegrass and red clover despite not being winter hardy suggesting that interseeding oilseed radish can provide year-round weed suppression. This is likely not an effect of a reduction in summer weed seed production because the cover crops suppressed summer annuals rather than spring/winter weeds. However, this could result in a reduction in winter/spring annual weed seed production.

Soil nitrogen response

Soil nitrogen decreased in all treatments over the winter, however treatment effects were only seen at the 0-30 cm depth (Table 10). Soil was sampled prior to cover crop termination. Winter rye reduced spring soil nitrate levels more than any other treatment at both 0-30 cm and 30-60 cm. While the interseeded cover crop treatments had no effect on soil nitrate levels, the production of above ground biomass in treatments containing annual ryegrass and red clover would imply that some of the decrease in soil nitrate from the fall could be attributed to cover crop growth due to the positive association of cover crop biomass and nitrogen use (Khan & McVay, 2019). Treatments with no cover crop growth (oilseed radish and NCNC treatments) likely lost soil nitrate to leaching. Despite the relatively low C:N ratio in the oilseed radish, there is no evidence that it increased soil NO<sub>3</sub>N levels in the spring.

**Table 10. Soil nitrate levels at cover crop termination.** Treatments were sampled at depths 0-30 cm and 30-60 cm with ANOVA results as affected by treatment. Mean values followed by different letters were determined using Fisher's LSD.

Treatment	<u>Spring Soil Nitrate (NO<sub>3</sub>-N)</u>	
	----- cm -----	
	<u>0 - 30</u>	<u>30 - 60</u>
	-----mg kg <sup>-1</sup> -----	
Annual Ryegrass	10.2 a	11.0 a
Red Clover	11.1 a	15.4 a
Oilseed Radish	13.0 a	10.1 a
AR + RC	14.6 a	9.9 ab
RC + OR	14.1 a	14.4 a
AR + OR	11.6 a	10.7 a
AR + RC + OR	13.0 a	12.5 a
Cultivation	12.5 a	12.0 a
Winter Rye	3.2 b	2.3 b
NCNC	11.8 a	9.6 ab
	p-value	
Treatment	0.035	0.109

## **Conclusions**

Cover crop establishment is a primary concern for farmers when considering cover crop adoption, therefore it is important to note that successful establishment for the purpose of weed suppression may be a result of both management and abiotic factors. When interseeded at V3 oilseed radish was the most consistent species in terms of establishment and growth while annual ryegrass and red clover were more variable. Interseeded cover crops suppress weeds without negatively affecting in-season soil nitrate or corn yields in the organic corn production systems in the Upper Midwest. When planted at V3 and with favorable conditions, annual ryegrass, red clover, and oilseed radish are able to establish under the corn canopy and suppress weeds just as well as in-season cultivation, reducing the need for tillage to manage weed both in season and in the spring. This reduction in summer and fall tillage combined with successful fall weed suppression also leads to a reduction in spring weeds. The extent of this effect is variable year to year depending on environmental conditions and field management. In this study red clover did not provide any measurable benefit to soil nitrogen or corn yield in season nor in the spring. Future studies examining seeding rate and density of cover crops would provide more data to give agronomic recommendations for farmers. Planting more than two rows of cover crops in between corn rows may lead to more consistent establishment and weed suppression.

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