Spring 2022

Winter Cover Crop Impacts on Weed Dynamics in Eastern and Central Nebraska

Elizabeth Ann Oys
University of Nebraska - Lincoln

WINTER COVER CROP IMPACTS ON WEED DYNAMICS IN EASTERN AND CENTRAL NEBRASKA

by

Elizabeth Ann Oys

A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Agronomy

Under the Supervision of Professor Andrea D. Basche

Lincoln, Nebraska

May 2022

WINTER COVER CROP IMPACTS ON WEED DYNAMICS IN EASTERN AND CENTRAL NEBRASKA

Elizabeth Ann Oys, M.S.

University of Nebraska, 2022

Advisor: Andrea D. Basche

Reducing tillage in cropping systems causes weed management to be dependent on chemical and cultural methods for weed control. Over time, herbicide-resistant weeds have developed due to the continuous selection pressures from herbicides, particularly in the Midwest Corn Belt. Integrated weed management strategies, such as cover crops, can be used to mitigate some of these issues. Cover crops are primarily known for their soil health benefits, but there is evidence that cover crops can suppress weeds. However, less research has been done at the field-scale level to address cover crop impacts on the weed seedbank and aboveground weeds during the growing season. In response, two experiments were designed to investigate above and belowground weeds in eastern and central Nebraska. The soil seedbank was germinated from soil samples and weed density and biomass were measured at two points during the growing season. Our results show that cover crops did not influence the total seedbank density, but increased the density of *Amaranthus* spp. seeds in the seedbank. Aboveground, reductions in weed density and biomass reductions occurred at two sites. More importantly, larger pigweed seedbank densities in the cover crop treatments were not expressed aboveground, signifying cover crop suppression of the weed seedbank through reduced germination withdrawals. This research provides insight on above and belowground weed dynamics under cover crops

and shows that cover crops may be a viable integrated weed management tool for *Amaranthus* spp. management and mitigating risks of herbicide resistance over time by preventing seedbank withdrawals through germination.

ACKNOWLEDGEMENTS

There are many people who I would like to thank for their time, insight, and assistance these last two years, without whom this work would not be possible. I would first and foremost like to express my sincere thanks to my adviser Andrea Basche for her support and guidance, and for making me feel human during this challenging experience. I would also like to thank my committee member Katja Koehler-Cole for her insight and help on my thesis as well as providing opportunities for professional development, and I also would like to thank my other committee member Chris Proctor for his guidance on my thesis and field research. I have grown as a researcher thanks to this group of supportive people.

I would also like to thank the support I have received from my colleagues in the Basche lab for their time and support the past two years. I especially thank Fernanda Krupek for her patience as she has put up with my seemingly endless questions about statistics, on-farm research, and GIS. Thank you to the undergraduate assistants Aime Christian Tuyishime, Hannah Carden, Noella Bahatsi, Yvon Ukwishaka, and others for their efforts in the field and in the greenhouse. Thank you to the NRCS, SCAL, and ENREC staff for assistance at research sites as well as the NRCS Soil Health Initiative for funding the research. I would also like to thank Emily Robinson for her statistical assistance and time in helping with my research.

Finally, thank you to my family, friends (new and old), and partner who have been supportive and patient since day one of this journey, and were constant sources of motivation, kind words, and humor even during the toughest of days. This experience would have been a lot more difficult without you all.

GRANT INFORMATION

This material is based upon work supported by the Natural Resources Conservation Service (USDA-NRCS) Soil Health Initiative under agreement no. 68-6526-17-005. Any opinions, findings, conclusions, and recommendations given in this material are those of the author(s) and do not necessarily reflect the views of the USDA-NRCS Soil Health Initiative.

Table of Contents

List of Tables

List of Figures

Appendices

CHAPTER 1: LITERATURE REVIEW

Description of crop production and land use in Nebraska

The state of Nebraska is located in the Midwestern region of the United States and is well-known for its agricultural productivity, particularly in row crops and beef cattle production. The state's terrain and climate change along a precipitation gradient from east to west with five ecoregions: the Loess Hills, Loess and Glacial Drift, Central Loess Plains, Sandhills, Tablelands, and High Plains. Each ecoregion supports different agricultural activities, varying with precipitation levels. Corn and soybeans are grown throughout the east-half of the state, where rainfall averages about 35 inches per year (UNL HPRCC 2021), whereas winter wheat, dry edible beans, and sugar beets become more common further west as land becomes more arid (USDA NASS 2017). Currently in Nebraska, 9.3 million acres of the total 22.2 million acres in cropland production are irrigated, which makes crop production possible in more arid regions (USDA NASS 2017*)*. The state is also known for its prairie landscapes within these ecoregions, including tallgrass, mixed-grass, and shortgrass prairie. The presence of prairie systems has positively impacted Nebraska's agricultural potential due to rich natural soil fertility (Cunfer 2021).

Weed control concerns in no-till and reduced-till practices

In the most recent USDA Census, it was reported that 10.3 million acres of cropland in Nebraska were under no-tillage practices, the highest in the nation alongside Kansas in the total number of acres in no-tillage production (USDA NASS 2017). No-tillage and reduced-tillage are widespread practices in dryland acres due to concerns about soil erosion and maintaining soil moisture (Bekele, 2020). Additionally, this practice is becoming more widely used due to the soil health benefits it provides along with advantages in saving time, labor, machinery, and fuel input costs (Kawa, 2021).

When farmland shifts into no-tillage or reduced-tillage production, weed management methods also shift (Huggins & Reganold, 2008). Traditionally, tillage is used to prepare and level a seedbed for the cash crop, bury crop residue, and destroy and bury weeds or weed seed (Hobbs et al., 2008). The elimination or reduction of tillage causes weed control to be dependent predominantly on cultural methods such as crop rotation and the use and timing of herbicides (Huggins & Reganold, 2008). Furthermore, crop rotations in Nebraska are primarily simplified due to the demand for biofuels and livestock feed, which has led to most cropland acres being planted to corn and soybean (Hiller et al., 2009). Unlike simplified corn-soybean rotations, diversified crop rotations are more effective at reducing weed emergence because varying crop planting dates interrupt weed life cycles and induce different crop-weed competition, and these effects are more prominent in no-tillage systems (Weisberger et al., 2019). Diverse crop rotations combined with herbicide programs can be a more successful integrated weed management tool compared to simplified rotations (Doucet et al., 1999).

Dependence and cost of herbicide use in Nebraska

In 2020, an estimated 94% of all corn and 96% of all soybeans planted in Nebraska were genetically engineered (GE) hybrids or cultivars with herbicide resistance traits and insect tolerance traits (USDA ERS 2020), meaning humans have integrated modified genes into the crop to withstand or metabolize herbicides or insects that would otherwise kill or damage the crop, allowing for ease of herbicide applications and preservation of yields. These data show that producers prefer and utilize the herbicide trait platforms currently available on the market. This inevitably leads to the increased use of the herbicides these GE crops can tolerate.

Using the same herbicides year after year to cater to these trait platforms reduces herbicide diversity since the same or similar sites of action are constantly applied, increasing the risk for herbicide resistance developing in weeds (Jhala et al., 2014). In Nebraska, glyphosate is the most commonly used broad-spectrum herbicide followed by 2,4-D which is used to control broadleaf weeds (Sarangi & Jhala, 2018). Globally, glyphosate is the most popular herbicide, and its use has increased approximately 100-fold since its release to growers in 1974 (Myers et al., 2016).

According to a statewide survey of stakeholders in Nebraska in 2017, 74% of corn and 59% of soybean growers were using pre-emergence (PRE) herbicides before cash crop planting and more than 80% of growers utilized POST herbicides for in-season weed control after cash crop planting (Sarangi & Jhala, 2018). In general, herbicides are expensive and the average cost of weed management in GE corn and soybean were $90 and $81 ha^{-1} in Nebraska, respectively, according to the same study. The herbicide program cost from the survey does not account for the upfront premium cost of purchasing GE seed. In 2022, there is predicted to be an industry-wide herbicide shortage, which will significantly increase costs to growers (Johnson, Zimmer, & Young 2021). This shortage is estimated to increase the cost of some glyphosate products from about $5 per liter to about $21 per liter, putting further economic strain on growers.

Consequences of herbicide use

Currently, there is a wide range of concerns over herbicide use on the environment, human health, and weed ecology and management. The fate of herbicides in the field often contributes to surface and groundwater contamination, mainly through the leaching of water-soluble soil-applied herbicides (Ferreira Mendes et al., 2020). Runoff of these herbicides can lead to surface water contamination, eventually leading to the contamination of streams, lakes, and rivers. As the herbicides leach further into the soil profile, the risk for groundwater contamination increases. In a recent study spanning 46 states, 41% of the 1204 wells in aquifers were found to contain pesticide compounds (Bexfield et al., 2021). More specifically, the common herbicides atrazine, hexazinone, prometon, tebuthiuron, and metolachlor degradates were found in at least 5% of the wells. Of these contaminated samples, 1.6% were approaching concentrations of potential human-health concern. Exposure and bioaccumulation of these substances can have lethal or detrimental physiological consequences in aquatic and terrestrial organisms, including humans (Lushchak et al., 2018).

In context of row crops in the Midwest Corn Belt, one of the most prevalent concerns is the risk for herbicide resistance and herbicide-resistant weeds (HRW). Herbicide resistance (HR) is a plants ability to survive and continue to reproduction following the exposure to a dose of herbicide that would normally kill the wild-type weed, which can occur naturally through selection pressures from herbicides over time (Prather et al., 2000). HR involves single or multiple mutations or modifications in a plant biological pathway or enzymes so that the plant does not respond to the herbicide’s active ingredient and the target site is not affected. Plants with multiple mutations that cause resistance to different herbicide modes of action are known to have multiple resistance, and plants that have mutated to multiple active ingredients within the same mode of action are known to have cross-resistance.

Since the release of herbicides in 1944, HR has become a widely document issue globally and in the United States with an estimated 61 million acres infested with glyphosate-resistant (GR) weeds in 2012 (Fraser 2013, Peterson et al., 2018). Currently, the state of Nebraska has 16 individual herbicide-resistant weed (HRW) population reports, four of which had multiple resistance (International Survey of Herbicide Resistant Weeds 2021). Species of the *Amaranthaceae* weed family (*Amaranthus* spp.) made up nine of the reports and were the only species with multiple resistance. *Amaranthus tuberculatus* (common waterhemp) has had its HR evolution well-documented globally and is considered one of the most problematic weeds in the world (Tranel, 2021). This species is capable of both target-site and non-target-site resistance, including herbicide detoxification and metabolism. Sarangi and Jhala (2018) found that *Amaranthus* spp., particularly *Amaranthus palmeri* (Palmer amaranth) and *Amaranthus tuberculatus,* were ranked in the top five most problematic weeds across most surveyed districts in Nebraska. HRW impact growers economically by reducing profit (Orson, 1999), particularly glyphosate-resistant (GR) weeds. GR weeds force growers to utilize alternative herbicides for applications or tank-mix other herbicides with glyphosate to ensure efficacy (Sarangi & Jhala, 2018). With heavy weed pressure and HR weeds, growers may have to utilize tillage to eliminate weeds, increasing production and labor costs (Zhou et al., 2015) and defeating the goal of no-tillage in some cropland acres. HR weed populations are expected to increase in the future, which may only exacerbate these issues and related costs (Peterson et al., 2018).

Cover crops as an integrated weed management tool

Although herbicides are the most efficient ways to control weeds, alternative strategies are increasingly being explored to mitigate the risk of HR and problematic weed populations (Gage & Schwartz-Lazaro, 2019). With most of Nebraska's land under no-till production, there are environmental and HR issues resulting from the reliance of herbicides in simplified crop rotations. This has led to interest and the adoption of other integrated weed management strategies in Nebraska, such as cover crops. Cover crops are a cultural and biological tool that provide means of weed suppression through different mechanisms such as allelopathy, interplant competition, and changing the microenvironment in which weed seedlings germinate and emerge, thus reducing weed densities or biomass (Kruidhof et al., 2008; Liebman et al., 2021; Osipitan et al., 2018; Rueda-Ayala et al., 2015). These effects occur both while the cover crop is living and after termination.

While the cover crop is living, several competitive mechanisms can impact weeds. Allelopathy is assumed to occur when cover crops excrete toxins that act as a seed germination or seedling growth inhibitor (Kunz et al., 2016). Specifically, cereal rye (*Secale cereale*) is well known for its allelopathic abilities (Barnes & Putnam, 1986). Cover crops also act as a living mulch that competes for moisture, light, and nutrients with weeds (Kruidhof et al., 2008). Reduced light quantity and quality to the soil surface by the cover crop canopy can also impact weed seed germination and seedling growth (Teasdale, 1993; Teasdale & Daughtry, 1993). As light travels through the cover crop canopy, chlorophyll absorbs red light, which reduces the red to far-red light ratio. The ratio of red to far-red light has been shown to inactivate phytochrome, a photoreceptor that is crucial in phytochrome-mediated germination and growth (Devlin, 2016). Once the cover crop is terminated, the remaining residue can impact the microenvironmental conditions where weed seedlings germinate (Teasdale & Mohler, 2000). Soil moisture and temperature are influenced by cover crop residues due to the continued reduction in light transmittance through the residue, which suppresses weeds by either keeping weed seeds dormant or delaying germination, thus reducing weed biomass. The suppression abilities of cover crops are most often related to their biomass production, where at least 5 Mg ha^{-1} cover crop biomass is required for a 75% reduction in weed biomass (Nichols et al., 2020).

Perceptions of cover crops as a weed management tool

Despite the multiple mechanisms for weed suppression, growers do not always recognize cover crop potential as a weed suppressant, nor is it a driving factor in the adoption of cover crops (Drewnoski et al., 2015). In a 2018 survey conducted by the University of Nebraska, only 4% of respondents listed cover crops for weed management as a topic for researchers and extension to prioritize despite 747,000 acres of cover crops being planted in Nebraska (Sarangi & Jhala, 2018, USDA NASS 2017). A survey done in the Mid-southern region of the United States indicated that cover crops were the most frequent response for areas of research that should be explored for weed management in soybean but not a primary listed method for weed control in the region (Schwartz-Lazaro et al., 2018). These two surveys suggest that growers are interested in cover crops for weed control, but there is not enough information or research to implement them confidently. Another survey in the Mid-southern region of the United States from soybean, rice, and cotton crop consultants revealed that cover crops ranked second to least in importance, while herbicide systems ranked of greatest importance (Riar et al., 2013). This suggests that while consultants may have knowledge of cover crops and cover cropping systems, they do not recommend or perceive them as having weed management benefits. Instead, these surveys suggest cover crops are primarily perceived as a tool for maintaining and building soil health, and weed management in primarily focused on maximizing herbicide use.

Results from these surveys suggest that growers do not recognize the suppression abilities of cover crops even if they have an interest in cover crop and weed research. This represents a challenge to alter growers' perceptions of alternative weed control and shift away from reliance on herbicides and herbicide resistant seed trait technologies. Reducing, delaying, or preventing HR is time-sensitive for maintaining commercial cropping systems, crop yields, and herbicide efficacy as we know them today, and cover crops pose as a potential solution to these weed control issues.

Description of chapters

In my thesis, I seek to answer how cover crops influence weed dynamics in eastern and central Nebraska row cropping systems. To address this question, I conducted two separate experiments for weed seedbanks and in-season weeds to understand weed dynamics below and aboveground. Furthermore, there is precedent for understanding more about seedbanks under cover crops since this is a less studied area of cover crop research in the Corn Belt.

For the first experiment, described in Chapter 2, "Winter Cover Crop Impacts on Weed Seedbanks", I sample weed seedbanks at six sites across eastern and central Nebraska, both at research stations and field-scale on-farm sites. Using the germination method, I identified each weed seedling in the soil samples to species rank. We quantified the seedbank size, determined species composition, and evaluated community diversity metrics in all treatments and sites. Seedbanks are a lesser-studied topic under cover cropped systems, particularly in the Midwestern United States. Therefore, we were interested in gaining perspective of belowground weed dynamics in this system. The results show no impact of cover crops on the total weed seedbank density, however, increases in the proportion of pigweeds (*Amaranthus* spp.) occurred at half of the sites, indicating reductions in seedbank withdrawals through reduced weed seed germination. There were also no significant differences in diversity metrics (Shannon Hill diversity, richness, evenness). However, richness averaged greater in the cover crop at all sites and evenness greater at five of six sites, which may reveal important trends in the species diversity of seedbanks under cover crops. These data show no significant impact from cover cropping on seedbank size, but specific weed families like *Amaranthaceae* may be impacted by cover crop suppression. Additionally, indications that weed biodiversity improves under cover crops despite increases in pigweeds helps us make inferences about the sustainability of the weed management system.

In the second experiment described in Chapter 3, "Winter Cover Crop Impacts on Weed Growth During the Growing Season", I sampled in-season weeds by obtaining emerged weed density counts and sampling dried weed biomass at two different points during the growing season following winter cover crops and no cover crop treatments. Additionally, we collected soil moisture and temperature data to see if cover cropped soils have changes in these data that may affect weed abundance and size. Cover crops significantly reduced total weed density at two sites and reduced total weed biomass at two sites. However, all sites exhibited trends in reduced weed biomass during both sample periods. This may indicate that cover crops interfere with weed growth more than weed emergence. More importantly, we observed no differences in aboveground pigweeds in treatments at any site despite increases in pigweed seedbanks up to 355% in cover crop treatments, which may be indicative of cover crop suppression by reducing pigweed seed germination.

In the final chapter of my thesis, I summarize the outcomes of the two experiments and note differences in seedbank size and composition compared to in-season weeds. Together, the two experiments and the comparisons made give us insights on cover crop impacts on weed dynamics above and belowground. I also discuss the implications of these results on weed communities and other weed control issues relevant to the Midwest Corn Belt, such as herbicide resistance and finding strategies for integrated weed management, particularly in relation to *Amaranthus* spp.

References

Barnes, J. P., & Putnam, A. R. (1986). Evidence for Allelopathy by Residues and Aqueous Extracts of Rye (Secale cereale). *Weed Science, 34*(3), 384–390. https://doi.org/10.1017/s0043174500067035

Bekele, D. (2020). The Effect of Tillage on Soil Moisture Conservation: A Review. *International Journal of Research Studies in Agricultural Sciences*, *6*(10). https://doi.org/10.20431/2454-6224.0610004

Bexfield, L. M., Belitz, K., Lindsey, B. D., Toccalino, P. L., & Nowell, L. H. (2021). Pesticides and Pesticide Degradates in Groundwater Used for Public Supply across the United States: Occurrence and Human-Health Context. *Environmental Science and Technology*, *55*(1), 362–372. https://doi.org/10.1021/acs.est.0c05793

Cunfer, G. (2021). Soil fertility on an agricultural frontier: the US Great Plains. *Social Science History 45*(4):733-762. https://doi.org/10.1017/ssh.2021.25

Devlin, P. F. (2016). Plants wait for the lights to change to red. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(27), 7301–7303. https://doi.org/10.1073/pnas.1608237113

Doucet, C., Weaver, S. E., Hamill, A. S., & Zhang, J. (1999). Separating the effects of crop rotation from weed management on weed density and diversity. *Weed Science*, *47*(6), 729–735. https://doi.org/10.1017/s0043174500091402

Drewnoski, M., Muller, N., Saner, R., Jasa, P., Zoubek, G., Rees, J., Elmore, R., Redfearn, D., & Lesoing, G. (2015). Cover Crop Survey of Nebraska Farmers. *University of Nebraska Extension*, 2–4.

Fraser, K. (2013). Glyphosate Resistant Weeds – Intensifying. Stratus Agri-Marketing. Accessed on Jan. 17, 2022 from: http://www.stratusresearch.com/newsroom/glyphosate-resistant-weeds-intensifying

Ferreira Mendes, K., Paula Justiniano Régo, A., Takeshita, V., & Luiz Tornisielo, V. (2020). Water Resource Pollution by Herbicide Residues. *Biochemical Toxicology - Heavy Metals and Nanomaterials*, *April*. https://doi.org/10.5772/intechopen.85159

Gage, K. L., & Schwartz-Lazaro, L. M. (2019). Shifting the paradigm: An ecological systems approach to weed management. *Agriculture (Switzerland)*, *9*(8), 1–17. https://doi.org/10.3390/agriculture9080179

Hiller, T. L., Powell, L. A., McCoy, T. D., & Lusk, J. J. (2009). Long-term agricultural land-use trends in nebraska, 1866-2007. *Great Plains Research*, *19*(2), 225–237.

Hobbs, P. R., Sayre, K., & Gupta, R. (2008). The role of conservation agriculture in sustainable agriculture. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1491), 543–555. https://doi.org/10.1098/rstb.2007.2169

Huggins, B. D. R., & Reganold, J. P. (2008). No-Till: the Quite Revolution. *Scietific American*, *299*(1), 70–77. https://doi.org/10.1038/scientificamerican0708-70

International Herbicide-Resistant Weed Database (2021). Status of Herbicide Resistance in Nebraska. Accessed on Jan. 17, 2022 from: http://weedscience.org/Pages/USState.aspx?StateAbbr=NE

Jhala, A., Sandell, L., Knezevic, S., Kruger, G., & Wilson, R. (2014). Herbicide-Resistant Weeds in Nebraska. *University of Nebraska - Lincoln Extension*, *EC1278*.

http://extensionpublications.unl.edu/assets/pdf/ec1278.pdf

Johnson, B., Zimmer, M., Young, B. (2021). Herbicide Shortage – How to Plan for the 2022 Growing Season. Purdue University, Pest & Crop Newsletter. October 8, 2021. Accessed on Jan. 17, 2022 from: https://extension.entm.purdue.edu/newsletters/pestandcrop/article/herbicide-shortage-how- to-plan-for-the-2022-growing-season/

Kawa, N. C. (2021). A “Win-Win” for Soil Conservation? How Indiana Row-Crop Farmers Perceive the Benefits (and Trade-offs) of No-Till Agriculture. *Culture, Agriculture, Food and Environment*, *43*(1), 25–35. https://doi.org/10.1111/cuag.12264

Kruidhof, H. M., Bastiaans, L., & Kropff, M. J. (2008). Ecological weed management by cover cropping: Effects on weed growth in autumn and weed establishment in spring. *Weed Research*, *48*(6), 492–502. https://doi.org/10.1111/j.1365-3180.2008.00665.x

Kunz, C., Sturm, D. J., Varnholt, D., Walker, F., & Gerhards, R. (2016). Allelopathic effects and weed suppressive ability of cover crops. *Plant, Soil and Environment*, *62*(2), 60–66. https://doi.org/10.17221/612/2015-PSE

Liebman, M., Basche, A. D., Nguyen, H. T. X., & Weisberger, D. A. (2021). How can cover crops contribute to weed management? A modelling approach illustrated with rye (Secale cereale) and Amaranthus tuberculatus. *Weed Research*, *April*, 1–11. https://doi.org/10.1111/wre.12508

Lushchak, V. I., Matviishyn, T. M., Husak, V. V., Storey, J. M., & Storey, K. B. (2018).

Pesticide toxicity: A mechanistic approach. *EXCLI Journal*, *17*, 1101–1136. https://doi.org/10.17179/excli2018-1710

Myers, J. P., Antoniou, M. N., Blumberg, B., Carroll, L., Colborn, T., Everett, L. G., Hansen, M., Landrigan, P. J., Lanphear, B. P., Mesnage, R., Vandenberg, L. N., Vom Saal, F. S., Welshons, W. V., & Benbrook, C. M. (2016). Concerns over use of glyphosate-based herbicides and risks associated with exposures: A consensus statement. *Environmental Health: A Global Access Science Source*, *15*(1), 1–13. https://doi.org/10.1186/s12940-016-0117-0

Nichols, V., Martinez-Feria, R., Weisberger, D., Carlson, S., Basso, B., & Basche, A. (2020). Cover crops and weed suppression in the U.S. Midwest: A meta-analysis and modeling study. *Agricultural & Environmental Letters*, *June*, 1–7. https://doi.org/10.1002/ael2.20022

Orson, J. H. (1999). The Cost to the Farmer of Herbicide Resistance. *Weed Technology*, *13*(3), 607–611. https://doi.org/10.1017/S0890037X00046285

Osipitan, O. A., Dille, J. A., Assefa, Y., & Knezevic, S. Z. (2018). Cover crop for early season weed suppression in crops: Systematic review and meta-analysis. *Agronomy Journal*, *110*(6), 2211–2221. https://doi.org/10.2134/agronj2017.12.0752

Peterson, M. A., Collavo, A., Ovejero, R., Shivrain, V., & Walsh, M. J. (2018). The challenge of herbicide resistance around the world: a current summary. *Pest Management Science*, *74*(10), 2246–2259. https://doi.org/10.1002/ps.4821

Prather, T. S., Ditomaso, J. M., & Holt, J. S. (2000). Herbicide Resistance : Definition and Management Strategies. *University of California Division of Agriculture and*

Natural Resources, *Publication*.

Riar, D. S., Norsworthy, J. K., Steckel, L. E., Stephenson, D. O., Eubank, T. W., Bond, J., & Scott, R. C. (2013). Adoption of Best Management Practices for Herbicide-Resistant Weeds in Midsouthern United States Cotton, Rice, and Soybean. *Weed Technology*, *27*(4), 788–797. https://doi.org/10.1614/wt-d-13-00087.1

Rueda-Ayala, V., Jaeck, O., & Gerhards, R. (2015). Investigation of biochemical and competitive effects of cover crops on crops and weeds. *Crop Protection*, *71*, 79–87. https://doi.org/10.1016/j.cropro.2015.01.023

Sarangi, D., & Jhala, A. J. (2018). A Statewide Survey of Stakeholders to Assess the Problem Weeds and Weed Management Practices in Nebraska. *Weed Technology*, *32*(5), 642–655. https://doi.org/10.1017/wet.2018.35

Schwartz-Lazaro, L. M., Norsworthy, J. K., Steckel, L. E., Stephenson, D. O., Bish, M. D., Bradley, K. W., & Bond, J. A. (2018). A midsouthern consultant's survey on weed management practices in soybean. *Weed Technology*, *32*(2), 116–125. https://doi.org/10.1017/wet.2017.109

Teasdale, J. R. (1993). Interaction of Light, Soil Moisture, and Temperature with Weed Suppression by Hairy Vetch Residue. *Weed Science*, *41*, 46–51. https://doi.org/10.1017/S0043174500057568

Teasdale, J. R., & Daughtry, C. (1993). Weed Suppression by Live and Dessicated Hairy Vetch (Vicia Villosa). *Weed Science*, *41*, 207–212. https://doi.org/10.1017/S0043174500076074

Teasdale, John R., & Mohler, C. L. (2000). The quantitative relationship between weed emergence and the physical properties of mulches. *Weed Science*, *48*(3), 385–392. https://doi.org/10.1614/0043-1745(2000)048[0385:tqrbwe]2.0.co;2

Tranel, P. J. (2021). Herbicide resistance in Amaranthus tuberculatus†. *Pest Management Science*, *77*(1), 43–54. https://doi.org/10.1002/ps.6048

United States Department of Agriculture (USDA) – National Agriculture Statistics Service (NASS). (2017). USDA Census 2017. https://www.nass.usda.gov/AgCensus/

United States Department of Agriculture (USDA) - Economic Research Service (ERS). (2020). Adoption of Genetically Engineered Crops in the US. Accessed on Nov. 20, 2021 from: https://www.ers.usda.gov/data-products/adoption-of-genetically-engineered-crops-in-the-us.aspx#.U6zCYrEtqe0.

University of Nebraska (UNL) High Plains Regional Climate Center (HPRCC) (2021). ACIS Climate Maps. Accessed on Jan. 17, 2022 from: https://hprcc.unl.edu/maps.php?map=ACISClimateMaps

Weisberger, D., Nichols, V., & Liebman, M. (2019). Does diversifying crop rotations suppress weeds? A meta-analysis. *PLoS ONE*, *14*(7), 1–12. https://doi.org/10.1371/journal.pone.0219847

Zhou, X. V., Larson, J. A., Lambert, D. M., Roberts, R. K., English, B. C., Bryant, K. J., Mishra, A. K., Falconer, L. L., Hogan, R. J., Johnson, J. L., & Reeves, J. M. (2015). Farmer experience with weed resistance to herbicides in cotton production. *AgBioForum*, *18*(1), 114–125.

CHAPTER 2: WINTER COVER CROP IMPACTS ON WEED SEEDBANKS

Abstract

Cover crops are well-known for their soil benefits as well as the ability to provide aboveground weed suppression in corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.) dominated row crop systems in the Midwest Corn Belt. However, less is known about cover crop impacts on soil seedbank dynamics in these systems. We utilized four on-farm and two long-term research sites across eastern and central Nebraska that had winter cover crops established for four to seven years. Soil seedbanks were sampled in the early spring before cover crop termination and then germinated in the greenhouse for seven months where emerged seedlings were identified by species. Total seedbank density did not differ between cover crop treatments and the check, but three sites showed significant increases in the amount of pigweed seedlings in the cover crop seedbank. Palmer amaranth (*Amaranthus palmeri* S. Watson) was the most abundant species across all sites. Despite large amounts of pigweeds, species richness averaged greater in the cover crop at all sites. Therefore, our findings suggest that cover crops may not reduce seedbank density but rather influence its composition towards a more diverse seedbank that is pigweed-centric. More diverse weed communities may have positive weed and crop management implications with important ecological benefits. Observing increases in pigweed density may be indicative of cover crop pigweed suppression through reduced germination withdrawals in the seedbank, which may have herbicide resistance implications for pigweed management.

Introduction

Weed seedbanks are an important ecological and evolutionary aspect to weed population dynamics. While aboveground weed measurements are useful for comprehending impacts on crop yield, plant competition, and fecundity, understanding the size and composition of the soil seedbank can help us comprehend weed dynamics more completely and understand the belowground community from which weeds emerge. Additionally, weed seedbanks are sensitive to management practices (Buhler et al., 1997; Davis et al., 2005), including cover crops (Buchanan et al., 2016; Nichols et al., 2020). This chapter aims to understand how seedbanks respond to the use of cover crops in no-tillage and reduced tillage sites by assessing the size of the seedbank, species composition, and community diversity. Understanding how these aspects of the seedbank change can help us form cover crop-related weed management decisions and understand the prevalence and persistence of specific weed species under cover cropping.

The soil seedbank is dynamic and its persistence influences aboveground weed infestations, although it does not necessarily predict the number of emerged weeds (Cardina & Sparrow, 1996). Seedbanks are comprised of transient and persistent components (Thompson & Grime, 1979; Walck et al., 2005). The transient seedbank contains weed seeds that live within or on the soil for less than one year, whereas the persistent seedbank contains seeds that live longer than one year in the soil seedbank, which are mainly living dormant seeds. The longevity of the persistent seedbank, which will be referred to as seedbank persistence, can undermine the effects of aboveground weed management efforts over time as weed seeds germinate, essentially maintaining weed pressure.

Seedbank persistence is dependent on many factors, including physical seed characteristics such as seed size (Cideciyan & Malloch, 1982), density, and shape (Grundy et al., 2003). *Amaranthaceae* is a small-seeded weed family but these *Amaranthus* spp. are some of the most problematic species in the Midwest Corn Belt and globally. The persistence of this weed family in the seedbank ranges from twelve months (Omami et al., 1999), ten years (Burnside et al., 1981), or up to forty years (Kivilaan & Bandurski, 1981). Viability duration in the seedbank varies greatly for other species depending on physical characteristics (Conn et al., 2006; Toole & Brown, 1946).

Management practices like tillage can also influence seedbanks (Buhler et al., 1997; Davis et al., 2005; Gulden et al., 2011). Tillage buries weed seeds (Clements et al., 1996), and burial depth can induce or terminate weed seed dormancy (Omami et al., 1999). In no-tillage systems, weed seeds fall on the soil surface or into the soil profile within the first 5 cm (Clements et al., 1996). As a result, no-tillage soil can exhibit both increased seedbank density and emerged weed density (Cardina et al., 1991; Cardina et al., 2002; Webster et al., 1998). While seedbank density may increase, studies have shown that the total number of species (species richness) found in the seedbank may increase as well under no-tillage (Sosnoskie et al., 2006). Furthermore, reducing or eliminating tillage means increased dependence on the efficacy of chemical and cultural practices for weed control and management of the seedbank.

Cover crops are a cultural management practice that have known impacts on weeds (Büchi et al., 2020). These impacts can occur as a result of several processes, many of which are related to aboveground cover crop biomass. Rye (*Secale cereale*) and vetch

(*Vicia villosa*) cover crop biomass and their residue have shown to reduce soil temperatures and light transmittance to the soil, which can help delay weed emergence and keep weed seeds dormant longer (Teasdale 1993). Cover crops can also produce allelopathic chemicals that act as germination or growth inhibitors in the soil, which has been documented particularly in cereal rye residues (Kelton et al., 2012; Macías et al., 2019; Moonen & Bàrberi, 2006). Cover crops alone can have suppressive effects, but when coupled with herbicides, suppression efficacy increases by 70% compared to herbicide or cover crop use alone (Teasdale et al., 2005). In regions like the Corn Belt, where herbicides are widely used (Sarangi & Jhala, 2018), cover crops could complement herbicide programs and prove an effective weed suppression strategy.

In general, less is known about changes in seedbanks under cover crops compared to other cultural practices, such as crop rotation and tillage (Sosnoskie et al., 2006), especially in long-term studies. Primarily, research has demonstrated little change in seedbanks under cover cropping. No changes in seedbank density have been observed (Alonso-Ayuso et al., 2018; Buchanan et al., 2016), whereas other recent studies such as Nichols et al. (2020) found that cover crops significantly decreased seedbank density at two of five sites studied. In contrast, modelling studies have found that cover crops have the potential to reduce seedbanks in the long-term (Liebman et al., 2021a). Varying results across current literature indicate there is precedence in learning more about seedbank dynamics under cover crops.

Insights on specific weed species and their abundance important to assess weed community diversity, species richness, and population evenness. Inferences about the broader sustainability of the whole cropping system can be made based on these diversity metrics (Liebman et al., 2021b; Storkey & Neve, 2018). In general, ecosystems with increased biodiversity and species richness result in the improved ability to provide multiple ecosystem services (Lefcheck et al., 2015). Agricultural intensification and herbicide use are the primary drivers in weed biodiversity losses, which subsequently promotes the development of herbicide resistance (HR) and possible dominance of a few HR species that respond to the selection pressures (Fagúndez, 2014; Schütte et al., 2017). On the other hand, more diverse weed populations are less competitive with crops, positively associated with yield gain, less prone to being dominated by a few highly competitive weed species, and less likely to develop HR traits (Storkey & Neve, 2018). Furthermore, a more biodiverse weed community could allow growers to diversify herbicide modes of action (MOA) or site of action (SOA) with each herbicide application. In turn, this reduces selection pressures and slows the risk of weeds developing HR over time (Neve et al., 2014).

In this chapter, we wanted to assess how seedbanks and their composition change under cover cropping. The objectives of this study were to (1) quantify and compare the total seedbank densities in the cover crop treatments to the check (control), (2) determine differences or shifts in seedbank community composition in the treatments, and (3) assess how seedbank diversity changes under cover crops. We hypothesized that 1) seedbank densities in cover crops would be larger than the check due to reduced withdrawals from the seedbank over time, 2) specific weed communities would respond differently to cover cropping, and 3) weed community diversity metrics would improve under cover crops.

Materials and Methods

Site Descriptions

We studied weed seedbanks at six multi-year experiments across eastern and central Nebraska in 2021. The South-Central Agriculture Laboratory (SCAL) near Clay Center, NE and the Eastern Nebraska Research and Extension Center (ENREC) near Mead, NE are University of Nebraska – Lincoln research stations with long-term cover crop experiments (initiated in 2014). The remaining four locations were commercial on-farm sites in eastern and central Nebraska (initiated in 2016 or 2017) in Colfax, Greeley, Howard, and Merrick Counties. As a part of the Soil Health Initiative (SHI) launched in 2016, on-farm research locations partnered with the University of Nebraska (UNL) On-Farm Research Network and Nebraska Natural Resources Conservation Service (USDA-NRCS) to conduct evaluations of cover crops at field-scale. Summaries of the experimental design, management, and cropping systems for the six sites can be found in Appendix 1 through 3.

None of the six sites had previously assessed weeds because weed control was not an objective in these cover crop experiments. Therefore, there are no baseline measurements to use as a reference for our seedbank data or compare changes in seedbank trajectory over time.

Experimental design and plot management

Research stations

SCAL and ENREC experiments utilized a randomized complete block design with treatments of cereal rye (*Secale cereale*), hairy vetch (*Vicia villosa*), and no cover crop (check) at SCAL (n = 3 for each treatment) and cereal rye (*Secale cereale*) and no cover crop (check) at ENREC (n = 3 for each treatment). Cover crops were planted fall and terminated with herbicides in the spring within two weeks of the cash crop planting in the plots each year since 2014. Both locations utilized no-tillage management and a two-year corn-soybean crop rotation. In 2021, all plots sampled had a cash crop of corn. Experimental design, crop rotation history, and management information on these sites can be found in Appendix 2 through 4 and Appendix 6 through 7.

On-farm sites

All on-farm sites utilized a randomized complete block designs with treatments of multi-species cover crops (5-10 species) and no cover crop (check) (n = 4 for each treatment). Total field size at all four commercial farm locations was at least 20 hectares, and treatment strips were in randomized spatially balanced blocks and plot sizes varied. Cover crops were planted in the plots each year since 2016 or 2017. Cover crops were planted in the fall and terminated in the spring with PRE herbicides immediately before cash crop planting in each year. All on-farm sites followed a three-year corn-soybean-small grains rotation, where the previous crop was a small grain planted at Greeley, Colfax, and Howard Counties and field peas were planted at the Merrick County site in 2020. In 2021, three sites grew corn and one grew soybean. Experimental design, crop rotation history, and management information on these sites can be found in Appendix 2 through 4 and Appendix 6 through 7.

Sampling the soil weed seedbank

All of the sites in this experiment were under no-tillage, with the exception of the Merrick County on-farm experiment, which utilized strip tillage. For no-tillage systems in silt loam soil, an estimated 74% of the weed seedbank is concentrated in the upper 5 cm of the soil profile and 78% of the seedbank is within the upper 10 cm in ridge-tillage (Buhler et al., 1997; Clements et al., 1996). Five of the six research sites for this experiment have similar textures as the referenced studies (Appendix 1).

Larger seeded species can emerge from greater burial depths but typically emergence does not occur deeper than 8 cm, and smaller seeded species emerge from depths of about 1 cm (Grundy et al., 2003). Therefore, we assumed that the germinable seedbank would be no deeper than 10 cm at our sites with no-tillage and strip-tillage. Additional literature shows that 20 soil subsamples mixed into one composite sample will provide a sufficient estimation of the fields' seedbank density (Gross, 1990). Given these data from previous studies, we collected 20 soil subsamples to a depth of 10 cm to obtain a single composite sample for the soil seedbank in each plot.

Seedbank sampling at each site occurred before cover crop termination and planting of the cash crop and before pre-emergence herbicides were applied to the fields in April 2021. At SCAL and ENREC, a JMC® soil probe (PN031 JMC 36 inch sampler) with a diameter of 3.175 cm and tape marking 10 cm depth was used to extract 79.17 cm^3 of soil for each subsample, leading to a composite sample size of approximately 1,583 cm^3. At the four on-farm locations, a soil bulk density ring with a diameter of 7.25 cm and tape marking 10 cm depth was used to extract approximately 413 cm^3 of soil for each subsample, leading to a composite sample size of approximately 8,260 cm^3. Subsamples were obtained randomly due to the spatial distribution nature of weeds (Cardina et al., 1997) and the number of subsamples chosen (Colbach et al., 2000). Subsamples were taken at least 1 m from the plot boundary at SCAL and ENREC and at least 5 m from the plot boundary at the four on-farm sites, and were thoroughly hand-mixed together for one composite sample per plot. For ease of mixing the sampled soil, two composite samples of 10 subsamples each were used at the on-farm sites instead of one composite sample of 20 subsamples.

We justified increasing the volume of the soil subsample device at the on-farm sites due to the major differences in plot size compared to research stations. We determined that approximately 5.2 times more soil per plot at on-farm sites was sufficient to scale the experiment without exceeding practical time limitations before cover crop termination, human resources, and available space in the greenhouse. The composite samples were stored in air-tight plastic bags in coolers before being transported and stored in a refrigerator for 12-48 hours at 0°C prior to greenhouse processing.

Germination of the weed seedbank

This study utilizes the germinable soil seedbank method which allows for a more precise assessment of the seedbank composition by identifying seedlings by species (Gross, 1990)**,** although it may underestimate the total number of weeds and species present due to dormancy controls and not counting all physical seeds. In contrast, the physical extraction of seeds using elutriation or floatation methods may quantify the seedbank size more precisely, but not all seeds are viable and species or genus classification is not precise or sometimes feasible (Cardina & Sparrow, 1996). Additionally, the germination method is less time-intensive than physical extraction. Because this study intends to understand the shifts in the germinable seedbank due to the presence of cover crops, we determined the germination method to be the most useful and time-efficient method for the number of sites in this experiment.

After brief refrigeration, soil samples were weighed to ensure the composite samples were all relatively the same size before being brought to Greenhouse 3 on East Campus at the University of Nebraska – Lincoln. Each soil composite sample was then sieved into a five-gallon bucket through a wire screen with a sieve size (MTN Gearsmith ½” Classifier Sifting Pan) of 1.61 cm^2 to remove live plant matter, insects, and disaggregate large soil clods. After sieving, each sample was thoroughly mixed before being laid into growing trays (27.8 cm W x 54.5 cm L x 6.2 cm D). For SCAL and ENREC, one tray held one composite sample per plot. For on-farm sites, the two composite samples per plot were laid into separate trays due to the larger volume of soil per each sample. Soil held within the trays was approximately three cm depth.

Trays were watered twice daily and monitored to prevent over-saturated soil conditions that might induce seed decay over time. Greenhouse temperatures were controlled but varied depending on the month of the experiment. Generally, temperatures were maintained around 25°C, with maximums up to 40°C during midsummer daytime highs. Trays received 11-16 hours of natural sunlight daily and were supplemented with timed grow lights that turned on approximately one hour before sunset and one hour after sunrise. Germinated seedlings were identified by species, counted, and then discarded to obtain a running count of the germinable weed seedbank and to allow seedlings to emerge without shading of other weeds. Identification was done daily when germination rates were high and once per week when germination rates slowed. Approximately five days after seedlings ceased to emerge, all trays were dried for five days in the greenhouse. The soil was then resifted using the sieving process and laid out again to germinate more seedlings. The resifting occurred in July and September, for a total of three rounds of germination between April and October 2021. After the third round of germination, few seedlings germinated and soil was disposed of five days after the last weed emerged on November 1, 2021. Weed species identified are reported in the classification according to Gleason & Cronquist (1991), with codes representing the first three letters of the genus and the first two of the species.

Data analysis

Seedbank size and composition

Seedbank density data were converted to estimates of seeds m^{-2} based on the number of subsamples within the composite sample and subsample equipment size before being analyzed with a univariate approach with cover crop treatment as the main effect. Seedbank density variables were total seed density and subcategory densities of pigweed, grasses, and all other broadleaves. No outliers were removed prior to analysis in order to assess true total densities of seedbanks and their subcategories. Initial distribution of measured seedbank density data exhibited overdispersion and right skewness. Therefore we utilized a generalized linear mixed-effect model (Bolker et al., 2009) with a log-linked negative binomial distribution to account for overdispersion and address the underlying distribution of the data, which was fit using the *glmer.nb* function from the *lme4* package in R 4.1.2. (R Core Team, 2022; Bates et al., 2015). All other analyses packages mention here forward were conducted in R 4.1.2. Treatment, site, and their interaction were considered as fixed effects, and replicate nested with site was included as a random effect in order to account for variability between replicates within each site. Because site had a significant effect and there were confounding differences in field history, management, and herbicides, results are reported on an individual site basis. Pairwise comparisons were conducted by calculating the least-squares means, and contrasts were determined using the *emmeans* package (Lenth et al., 2021) at a level of $p < 0.1$. This level of significance corresponds to the University of Nebraska On-Farm Research Network significance levels (UNL OFR Network 2022), and accounts for the spatial variability at each on-farm site due to large sizes of plots.

Nonmetric multidimensional scaling

Seedbank community composition was compared between treatments within each site using nonmetric multidimensional scaling (NMDS) with the *vegan* package (Oksanen et al., 2019). Utilizing NMDS helps visually display similarities among treatment communities in ordination space. The distance between points represents dissimilarity, which was calculated using Bray-Curtis distances. Stress values were used to assess goodness-of-fit, and hulls were added into the plots to show treatment communities and overlap. This helps visualize if communities within each treatment were unique from one another. Rare species that made up less than 0.1% of the total seedbank were removed prior to NMDS (Poos & Jackson, 2012).

Seedbank community analyses

Metrics of species richness, evenness, and Shannon diversity were used as diversity and population metrics and were determined using the *vegan* package (Oksanen et al., 2019). Species richness refers to the total number of species present, whereas evenness refers to the distribution of individuals for each species present in the community, with a value of 0 to 1. Shannon diversity, interpreted as the effective number of species in a community, was determined using the exponential of Shannon diversity (Hill, 1973; Jost, 2006) with the following equation:

$$H' = \exp\left(-\sum_{i=1}^{S} p_i \ln(p_i)\right)$$

Where *S*: species richness; *i*: one unique species from the community; and p_i: relative abundance of the i^{th} species. Species evenness was determined as $\frac{H'}{\log(S)}$, also known as Pielou's evenness. Metrics were assessed with a linear mixed-effect model with the *lme4* package (Bates et al., 2015), and pairwise comparisons were determined with the *emmeans* package (Lenth et al., 2021).

Results

Seedbank size and composition

The seedbank germination experiment resulted in a total of 6561 seedlings emerged totaled across all sites. Total seedbank density ranged from 165 seeds m^{-2} at the Colfax County site to 4180 seeds m^{-2} at the Merrick County site (Figure 2-1). Cover crop treatments had no influence on total seedbank density at any site. Analysis of variance (Type II Wald Chi-Square tests) showed site had a significant effect in all generalized linear mixed effect models, and site and treatment had significant effects in the pigweed model. A total of 57 different species were identified, with great variability between sites. The most abundant weeds were summer annual weeds and relatively similar abundances of C_3 and C_4 plants were present. All sites had at least one *Amaranthus* spp. present in each treatment, with a single species making up to 67% of a treatment seedbank in some cases (Table 2-3). Furthermore, Palmer amaranth (*Amaranthus palmeri* S. Watson) made up 20.09% of the total seedlings counted across all sites, followed by green foxtail (*Setaria viridis* (L.) P. Beauv.) at 13.12% (Table 2-1). Greater numbers of pigweeds in the cover crop were found at the Merrick County ($p = 0.004$), Greeley County ($p = 0.05$), and SCAL (in the cereal rye ($p = 0.03$) and hairy vetch cover crop ($p = 0.09$) treatments) (Figure 2-2). This represented 355%, 243%, 180%, and 137% increases from the check, respectively. No grasses were found at ENREC or SCAL, and no differences in grass seedlings were detected at any of the four on-farm sites (Figure 2-3). The cover crop reduced broadleaf seedlings at the Merrick County site ($p = 0.008$), but no other sites (Figure 2-4).

Nonmetric multidimensional scaling (NMDS) of seedbank composition

Complete distinction (separation) of treatment communities was observed in the NMDS graphs at the Merrick and Greeley County sites, where cover crop communities were primarily based on *Amaranthus* spp. Across most sites, the NMDS reflected that the cover crop community composition leaned towards *Amaranthus* spp., as found in the models, but no other trends towards one specific species or weed family were observed consistently across all sites (Figure 2-5).

Seedbank community analyses

No statistical differences between cover crop and check treatments existed for species richness, evenness, and Shannon diversity except for a reduction in evenness in the cover crop at the Merrick County site (Table 2-2). While not significant, estimated mean species richness was numerically greater in the cover crop at all sites and evenness was greater at five of the six sites.

Discussion

Shifts in seedbank composition under cover crops

In our study, cover crops did not influence total seedbank density at any site. Similar findings in cover crop-seedbank studies have been reported (Alonso-Ayuso et al., 2018; Buchanan et al., 2016). However, other similar experiments have observed reductions in total seedbank density with the use of cover crops, especially when cover crops were used long-term (Moonen & Bàrberi, 2004; Nichols et al., 2020). We did, however, see increases in pigweeds with cover crop treatment at three sites (Figure 2-2). Biological reasons behind the large proportions of pigweeds in the cover crop seedbank could be attributed to a few possible mechanisms. Firstly, cover crops may suppress pigweed seed germination. This could result in fewer withdrawals from the seedbank in comparison to the check, since seedlings can freely germinate in non-cover cropped soils whereas cover crops induce conditions that lead to seed suppression. However, because we did not sample seedbanks prior to the first year of cover crop establishment, we cannot be certain of the seedbank trajectory and rates of withdrawals over time. In contrast of this idea that cover crops keeping large seedbanks in the soil, *Amaranthus tuberculatus* population modeling done by Liebman et al., 2021 indicates that with high herbicide efficacy, seed populations should decline under long-term rye cover cropping if the cover crop reduces and delays emergence. However, this study made conclusions based on a modeled ten-year period, whereas cover crops were present at our sites only four to seven years (Appendix 2 and 3).

The second reason relates to the fecundity of pigweed species. Cover crops often delay weed emergence (Moonen & Bàrberi, 2006), which can result in late emerging pigweeds with relatively earlier flowering and shorter vegetative stages. However, Wu & Owen (2014) have shown that despite later emergence dates, pigweeds can still produce the same or greater number of seeds as earlier emerging seedlings, therefore cover crops that delay weed emergence may not impact pigweed seed production like we might intuitively expect. Furthermore, pigweeds are known for their prolific seed production producing up to 2.3 million seeds plant^{-1} (Hartzler et al., 2004). This could contribute to the large number of pigweeds in the seedbank but would not explain why total seedbank sizes did not differ from the check. Because our study did not track the fecundity of pigweeds prior to collecting the soil seedbank, it is uncertain what biological mechanism caused this shift but based on the results of Chapter 3, it is likely due to reduced germination withdrawals and successful cover crop suppression of pigweed seeds.

Agroecological implications related to weed diversity

The large amounts of *Amaranthus* spp. present in the seedbank likely caused the Shannon diversity metric to be insensitive to lesser common species, therefore no statistical differences in Shannon diversity were observed at any sites. However, species richness was numerically greater in the cover crop treatments at all sites, which may reveal a trend of increased species diversity present in cover crops. Diverse weed communities are linked to improvements in crop yield and reduced risk to developing HR (Adeux et al., 2019). When evenness also exists in a diverse community, there is less interplant competition and populations are less likely to be overcome by a few aggressive or possibly herbicide-resistant species (Storkey & Neve, 2018). A simplified relationship between weed community diversity and relative abundance with relevant weed examples can be seen in Figure 2-6.

In general, agroecosystems are highly disturbed landscapes due to herbicides, tillage, and harvest for example. The intensity and frequency of disturbance and the resulting selection pressures can drive losses in ecological diversity and weed diversity (Storkey & Neve, 2018). Low weed diversity may limit options for growers to rotate herbicide MOA or SOA due to only a few highly adapted and dominant weed species in the field, thus herbicides induce greater selection pressures that may result in HR weeds. In natural ecosystems, diversity is usually restored over time after disturbances, however in agroecosystems selection pressures that are continually applied rarely allow for advancements past the early stages of ecological succession (Gliessman, 2014b). Furthermore, plant diversity in agroecosystems is usually perceived as a risk for crops by growers, mainly because of differences in philosophy between ecology and agronomy which results in differing management practices (Storkey & Neve, 2018). Plant diversity in agroecosystems can improve ecosystem stability and sustainability, resiliency to disturbance, increased potential for beneficial plant interactions, and increased resource efficiency (Gliessman, 2014a; Storkey & Neve, 2018). Weed diversity in specific has been shown to improve yields and reduce crop-weed competition (Adeux et al., 2019; Smith et al., 2010).

Several ways to improve weed diversity have been suggested, including cropping system diversification or the addition of cover crops which help diversify selection pressures to mitigate aggressive weed species (Liebman & Gallandt, 1997; Liebman et al., 2021; Palmer & Maurer, 1997), but other studies have found marginal evidence to support this (Adeux et al., 2022; Smith & Gross, 2007). Similarly, seedbank diversity does not tend to improve under cover crops (Alonso-Ayuso et al., 2018; Buchanan et al., 2016; Nichols et al., 2020). It is possible that highly disturbed agroecosystems do not allow for weed diversity to be improved due to continuous selection pressures that inhibit ecological succession (Gliessman, 2014a). In our study, we observed that cover crops tend to diversify the number of weed species present in the seedbank, which is possibly indicative of struggling ecological succession from diversifying the agroecosystem with cover crops, but it is difficult to know if crop rotation or other management practices had a stronger effect on species diversity due to the length of our study.

Seedbank management implications

Managing weed seedbanks can be complicated. Some agronomists believe that the depletion of the seedbank is what ultimately leads to successful weed control as well as best economic return (Oerke, 2006). While producers should always strive to minimize additions to the seedbank, the concept of seedbank elimination is complicated in cover cropped systems due to their ability to keep weed seeds dormant and suppress germination (Rueda-Ayala et al., 2015). Furthermore, weed scientists have found that climate change may increase the success of weeds with C_4 pathways, which include troublesome *Amaranthus* spp. (Ramesh et al., 2017). Likewise, rising CO_2 concentrations in the atmosphere may reduce glyphosate efficacy, which is the most popular herbicide used in Nebraska row crops (Sarangi & Jhala, 2018; Ziska et al., 1999). These issues threaten to increase deposits into the seedbank if weeds survive weed management strategies and begs the question of whether seedbank elimination is even feasible or economical (Schwartz-Lazaro & Copes, 2019). Therefore, effective management of weeds in a changing climate might look more like keeping weed seeds ungerminated or subject to natural decay and predation even if it means having a larger seedbank, instead of focusing efforts solely on aboveground weed control to target and eliminate seedbanks slowly.

Research limitations and future studies

Methods for quantifying the soil seedbank vary from physical extraction like flotation or elutriation to the germination method (Mahé et al., 2021). Because we wanted to assess the viable seedbank with minimal seed disturbance, we chose to use the greenhouse germination method with periods of soil stirring to stimulate additional germination flushes. The germination method may have underestimated the total number of species present, particularly without periods of cold stratification which may help break dormancy controls or cue germination in certain species (Gross, 1990). While this process only extracts viable seeds that have broken dormancy, it resulted in a total of 57 species and 6551 seedlings across the six sites. We concluded that we achieved sufficient enumeration of the seedbank, particularly in the detection of the most problematic and herbicide-resistant species like *Amaranthus palmeri.* Conclusions were based on comparisons made with similar seedbank studies in the Midwest, where total seedbank density and number of species exceeded (Nichols et al., 2020) or was similar to (Sosnoskie et al., 2006) other researchers results in germination experiments.

The total number of years cover crops were present at each site may have also impacted seedbanks. Cover crops can provide excellent habitat for invertebrate weed seed predators (Gallandt et al., 2005; Shearin et al., 2008; Ward et al., 2011). Longer-term cover crop usage (>10 years) coupled with predation and weed suppression could lead to seedbank density reductions (Liebman et al., 2021). Furthermore, the number of years that sites were in no-tillage production varied, and one site had strip-tillage. Reducing tillage is known to increase weed seedbank density but improve diversity, with no-tillage having the most notable effects (Sosnoskie et al., 2006). Lastly, crop rotation was not consistent across sites. On-farm sites were primarily corn-soybean rotations and diversified with a small grain or cool-season legume in 2020, whereas research station sites were consistently two-year corn-soybean rotations since the start of the initial experiment in 2014. Diversifying crop rotations or extending crop rotations can diversify seedbanks (Liebman et al., 2021b; Sosnoskie et al., 2006). Although rotations and tillage methods were similar overall, separation of these effects was not possible due to the length of this experiment (one year). Therefore, we could not address the trajectory of the seedbank prior to cover crops or establishment of a tillage method, and we did not sample weed seedbanks at each phase of the rotation.

Future cover crop seedbank studies should consider seedbank trajectory and weed fecundity over time. Furthermore, we suggest that crop rotation and tillage be separated from the effects of cover crops for further clarity on cover crop impacts. Insights on these aspects may better explain seedbank composition and diversity changes since we observed shifts in pigweed composition and richness under cover cropping, and provide growers with valuable information on seedbank management based on specific practices.

Conclusion

In this study, I was able to successfully quantify seedbank size and identify composition across six sites. Findings of my study show that cover crops do not have impacts on total seedbank size, but rather may have effects on specific species, particularly *Amaranthus* spp., where seedbank density increased up to 355% in the cover crop treatment. This is suggestive that cover crops suppress pigweed seeds through preventing germination, leading the seedbank to be larger in comparison to the check over time. It may be helpful to track pigweed fecundity under cover crops to confirm this in the future. Despite increases in pigweeds, mild trends in increasing species richness in the cover crops were observed. This may indicate that cover cropping promotes more diverse weed communities over time which can lead to weed management and crop productivity benefits. This experiment can be used to show how cover crops influence belowground weed communities and their composition without aboveground weed management interference, and has furthered knowledge in seedbank-cover crop dynamics.

References

Adeux, G., Vieren, E., Carlesi, S., Bàrberi, P., Munier-Jolain, N., & Cordeau, S. (2019). Mitigating crop yield losses through weed diversity. *Nature Sustainability*, *2*(11), 1018–1026. https://doi.org/10.1038/s41893-019-0415-y

Adeux, G., Yvoz, S., Biju-Duval, L., Cadet, E., Farcy, P., Fried, G., Guillemin, J. P., Meunier, D., Munier-Jolain, N., Petit, S., & Cordeau, S. (2022). Cropping system diversification does not always beget weed diversity. *European Journal of Agronomy*, *133*(November 2021). https://doi.org/10.1016/j.eja.2021.126438

Alonso-Ayuso, M., Gabriel, J. L., García-González, I., Del Monte, J. P., & Quemada, M. (2018). Weed density and diversity in a long-term cover crop experiment background. *Crop Protection*, *112*(May), 103–111. https://doi.org/10.1016/j.cropro.2018.04.012

Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1). https://doi.org/10.18637/jss.v067.i01

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J. S. S. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution*, *24*(3), 127–135. https://doi.org/10.1016/j.tree.2008.10.008

Buchanan, A. L., Kolb, L. N., & Hooks, C. R. R. (2016). Can winter cover crops influence weed density and diversity in a reduced tillage vegetable system? *Crop Protection*, *90*(December 2016), 9–16. https://doi.org/10.1016/j.cropro.2016.08.006

Büchi, L., Wendling, M., Amossé, C., Jeangros, B., & Charles, R. (2020). Cover crops to secure weed control strategies in a maize crop with reduced tillage. *Field Crops Research*, *247*(May 2019), 107583. https://doi.org/10.1016/j.fcr.2019.107583

Buhler, D. D., Hartzler, R. G., Forcella, F., Science, S. W., Jun, N. M., Buhler, D. D., & Forcella, F. (1997). Implications of Weed Seedbank Dynamics to Weed Management. *Weed Science*, *45*(3), 329–336. https://doi.org/10.1017/S0043174500092948

Burnside, O. C., Fenster, C. R., Evetts, L. L., & Mumm, R. F. (1981). Germination of Exhumed Weed Seed in Nebraska. *Weed Science*, *29*(5), 577–586. https://doi.org/10.1017/S0043174500063761

Cardina, J., Herms, C. P., & Doohan, D. J. (2002). Crop rotation and tillage system effects on weed seedbanks. *Weed Science*, *50*(4), 448–460. https://doi.org/10.1614/0043-1745(2002)050[0448:cratse]2.0.co;2

Cardina, J., Johnson, G. A., & Sparrow, D. H. (1997). The nature and consequence of weed spatial distribution. *Weed Science*, *45*(3), 364–373. https://doi.org/10.1017/s0043174500092997

Cardina, J., Regnier, E., & Harrison, K. (1991). Long-Term Tillage Effects on Seed Banks in Three Ohio Soils. *Weed Science*, *39*(2), 186–194. https://doi.org/10.1017/s0043174500071459

Cardina, J., & Sparrow, D. H. (1996). A Comparison of Methods to Predict Weed Seedling Populations from the Soil Seedbank. *Weed Science*, *44*(1), 46–51. https://doi.org/10.1017/S004317450009353X

Cideciyan, M. A., & Malloch, A. J. C. (1982). Effects of Seed Size on the Germination, Growth and Competitive Ability of Rumex Crispus and Rumex Obtusifolius. *The Journal of Ecology*, *70*(1), 227. https://doi.org/10.2307/2259875

Clements, David R., Benoit, Diane L., Murphy, Stephen D., Swanton, C. J. (1996). Tillage Effects on Weed Seed Return and Seedbank Composition. *Weed Science*, *44*(2), 314–322. https://doi.org/10.1017/S0043174500093942

Colbach, N., Dessaint, F., & Forcella, F. (2000). Evaluating field-scale sampling methods for the estimation of mean plant densities of weeds. *Weed Research*, *40*(5), 411–430. https://doi.org/10.1046/j.1365-3180.2000.00203.x

Conn, J. S., Beattie, K. L., & Blanchard, A. (2006). Seed viability and dormancy of 17 weed species after 19.7 years of burial in Alaska. *Weed Science*, *54*(3), 464–470. https://doi.org/10.1614/ws-05-161r.1

Davis, A. S., Renner, K. A., & Gross, K. L. (2005). Weed seedbank and community shifts in a long-term cropping systems experiment. *Weed Science*, *53*(3), 296–306. https://doi.org/10.1614/ws-04-182

Fagúndez, J. (2014). The paradox of aarable weeds: Diversity, conservation, and ecosystem services of the unwanted. *Agroecology, Ecosystems, and Sustainability*, 139–150. https://doi.org/10.1201/b17775

Gallandt, E. R., Molloy, T., Lynch, R. P., & Drummond, F. A. (2005). Effect of cover-cropping systems on invertebrate seed predation. *Weed Science*, *53*(1), 69–76. https://doi.org/10.1614/ws-04-095r

Gleason, H., & Cronquist, A. (1991). *Manual of Vascular Plants of Northeastern United States and Adjacent Canada* (2nd ed.). The New York Botanical Garden Press.

Gliessman, S. (2014a). Chapter 17: Agroecosystem Diversity. In *Agroecology: The Ecology of Sustainable Food Systems* (3rd ed., pp. 203–219). CRC Press.

Gliessman, S. (2014b). Chapter 18: Disturbance, Succession, and Agroecosystem Management. In *Agroecology: The Ecology of Sustainable Food Systems* (3rd ed., pp. 221–236). CRC Press.

Gross, K. L. (1990). A Comparison of Methods for Estimating Seed Numbers in the Soil. *Journal of Ecology*, *78*(4), 1079–1093. http://dx.doi.org/10.2307/2260953

Grundy, A. C., Mead, A., & Burston, S. (2003). Modelling the emergence response of weed seeds to burial depth: Interactions with seed density, weight and shape. *Journal of Applied Ecology*, *40*(4), 757–770. https://doi.org/10.1046/j.1365-2664.2003.00836.x

Gulden, R. H., Lewis, D. W., Froese, J. C., Van Acker, R. C., Martens, G. B., Entz, M. H., Derksen, D. A., & Bell, L. W. (2011). The Effect of Rotation and In-Crop Weed Management on the Germinable Weed Seedbank after 10 Years. *Weed Science*, *59*(4), 553–561. https://doi.org/10.1614/ws-d-11-00001.1

Hartzler, R. G., Battles, B. A., & Nordby, D. (2004). Effect of common waterhemp (Amaranthus rudis) emergence date on growth and fecundity in soybean . *Weed Science*, *52*(2), 242–245. https://doi.org/10.1614/ws-03-004r

Hill, M. O. (1973). Diversity and Evenness : A Unifying Notation and Its Consequences. *Ecology*, *54*(2), 427–432. https://doi.org/10.2307/1934352

Kelton, J.A., & Mosjidis, J. (2012). Allelopathic weed suppresion through the use of cover crops. In: Price AJ (Ed), Weed Control. INTECH Open Access Publisher, Rijeka. http://dx.doi.org/10.5772/34196

Kivilaan, A., & Bandurski, R. S. (1981). The One Hundred-Year Period for Dr . Beal's Seed Viability Experiment. *American Journal of Botany*, *68*(9), 1290–1292. https://doi.org/10.2307/2443054

Jost, L. (2006). Entropy and diversity. *OIKOS*, *2*(113), 363–375. https://doi.org/10.1111/j.2006.0030-1299.14714.x

Lefcheck, J. S., Byrnes, J. E. K., Isbell, F., Gamfeldt, L., Griffin, J. N., Eisenhauer, N., Hensel, M. J. S., Hector, A., Cardinale, B. J., & Duffy, J. E. (2015). Biodiversity enhances ecosystem multifunctionality across trophic levels and habitats. *Nature Communications*, *6*. https://doi.org/10.1038/ncomms7936

Lenth, R., Buerkner, P., Herve, M., Love, J., Miguez, F., Riebl, H., & Singmann, H. (2021). *Package "emmeans."* https://doi.org/10.1080/00031305.1980.10483031>.License

Liebman, M., Basche, A. D., Nguyen, H. T. X., & Weisberger, D. A. (2021). How can cover crops contribute to weed management? A modelling approach illustrated with rye (Secale cereale) and Amaranthus tuberculatus. *Weed Research*, *April*, 1–11. https://doi.org/10.1111/wre.12508

Liebman, M., & Gallandt, E. R. (1997). Many Little Hammers: Ecological Management of Crop-Weed Interactions. In L. E. Jackson (Ed.), *Ecology in Agriculture* (pp. 291–343). Academic Press. https://doi.org/10.1016/b978-0-12-378260-1.x5000-3

Liebman, M., Nguyen, H. T. X., Woods, M. M., Hunt, N. D., & Hill, J. D. (2021). Weed seedbank diversity and sustainability indicators for simple and more diverse cropping systems. *Weed Research*, *61*(3), 164–177. https://doi.org/10.1111/wre.12466

Macías, F. A., Mejías, F. J. R., & Molinillo, J. M. G. (2019). Recent advances in allelopathy for weed control: from knowledge to applications. *Pest Management Science*, *75*(9), 2413–2436. https://doi.org/10.1002/ps.5355

Mahé, I., Cordeau, S., Bohan, D. A., Derrouch, D., Dessaint, F., Millot, D., & Chauvel, B. (2021). Soil seedbank: Old methods for new challenges in agroecology? *Annals of Applied Biology*, *178*(1), 23–38. https://doi.org/10.1111/aab.12619

Moonen, A. C., & Bàrberi, P. (2004). Size and composition of the weed seedbank after 7 years of different cover-crop-maize management systems. *Weed Research*, *44*(3), 163–177. https://doi.org/10.1111/j.1365-3180.2004.00388.x

Neve, P., Busi, R., Renton, M., & Vila-Aiub, M. M. (2014). Expanding the eco-evolutionary context of herbicide resistance research. *Pest Management Science*, *70*(9), 1385–1393. https://doi.org/10.1002/ps.3757

Nichols, V., English, L., Carlson, S., Gailans, S., & Liebman, M. (2020). Effects of Long-Term Cover Cropping on Weed Seedbanks. *Frontiers in Agronomy*, *2*(December). https://doi.org/10.3389/fagro.2020.591091

Oerke, E. C. (2006). Crop losses to pests. *Journal of Agricultural Science*, *144*(1), 31–43. https://doi.org/10.1017/S0021859605005708

Oksanen, A. J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P. R., Hara, R. B. O., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Szoecs, E. (2019). Vegan. *Encyclopedia of Food and Agricultural Ethics*, 2395–

2396. https://doi.org/10.1007/978-94-024-1179-9_301576

Omami, E. N., Haigh, A. M., Medd, R. W., & Nicol, H. I. (1999). Changes in germinability, dormancy and viability of Amaranthus retroflexus as affected by depth and duration of burial. *Weed Research*, *39*(5), 345–354. https://doi.org/10.1046/j.1365-3180.1999.00149.x

Palmer, M. W., & Maurer, T. A. (1997). Does diversity beget diversity? A case study of crops and weeds. *Journal of Vegetation Science*, *8*(2), 235–240. https://doi.org/10.2307/3237352

Poos, M. S., & Jackson, D. A. (2012). Addressing the removal of rare species in multivariate bioassessments: The impact of methodological choices. *Ecological Indicators*, *18*, 82–90. https://doi.org/10.1016/j.ecolind.2011.10.008

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from: https://www.R-project.org/.

Ramesh, K., Matloob, A., Aslam, F., Florentine, S. K., & Chauhan, B. S. (2017). Weeds in a changing climate: Vulnerabilities, consequences, and implications for future weed management. *Frontiers in Plant Science*, *8*(February), 1–12. https://doi.org/10.3389/fpls.2017.00095

Rueda-Ayala, V., Jaeck, O., & Gerhards, R. (2015). Investigation of biochemical and competitive effects of cover crops on crops and weeds. *Crop Protection*, *71*, 79–87. https://doi.org/10.1016/j.cropro.2015.01.023

Sarangi, D., & Jhala, A. J. (2018). A Statewide Survey of Stakeholders to Assess the

Problem Weeds and Weed Management Practices in Nebraska. *Weed Technology*, *32*(5), 642–655. https://doi.org/10.1017/wet.2018.35

Schütte, G., Eckerstorfer, M., Rastelli, V., Reichenbecher, W., Restrepo-Vassalli, S., Ruohonen-Lehto, M., Saucy, A. G. W., & Mertens, M. (2017). Herbicide resistance and biodiversity: agronomic and environmental aspects of genetically modified herbicide-resistant plants. *Environmental Sciences Europe*, *29*(1). https://doi.org/10.1186/s12302-016-0100-y

Schwartz-Lazaro, L. M., & Copes, J. T. (2019). A review of the soil seedbank from a weed scientists perspective. *Agronomy*, *9*(7), 1–13. https://doi.org/10.3390/agronomy9070369

Shearin, A. F., Chris Reberg-Horton, S., & Gallandt, E. R. (2008). Cover Crop Effects on the Activity-Density of the Weed Seed Predator Harpalus rufipes (Coleoptera: Carabidae). *Weed Science*, *56*(3), 442–450. https://doi.org/10.1614/ws-07-137.1

Smith, R. G., Mortensen, D. A., & Ryan, M. R. (2010). A new hypothesis for the functional role of diversity in mediating resource pools and weed-crop competition in agroecosystems. *Weed Research*, *50*(1), 37–48. https://doi.org/10.1111/j.1365-3180.2009.00745.x

Smith, Richard G., & Gross, K. L. (2007). Assembly of weed communities along a crop diversity gradient. *Journal of Applied Ecology*, *44*(5), 1046–1056. https://doi.org/10.1111/j.1365-2664.2007.01335.x

Sosnoskie, L. M., Cardina, J., Herms, C. P., & Kleinhenz, M. (2006). Weed Seedbank Community Composition in a 35-Year-Old Tillage and Rotation Experiment. *Weed*

Science, *54*, 263–273. https://doi.org/10.21273/hortsci.39.4.845c

Storkey, J., & Neve, P. (2018). What good is weed diversity? *Weed Research, 58*(4), 239–243. https://doi.org/10.1111/wre.12310

Teasdale, John R., Pillai, P., & Collins, R. T. (2005). Synergism between cover crop residue and herbicide activity on emergence and early growth of weeds. *Weed Science*, *53*(4), 521–527. https://doi.org/10.1614/ws-04-212r

Teasdale, J. R. (1993). Interaction of Light, Soil Moisture, and Temperature with Weed Suppression by Hairy Vetch Residue. *Weed Science, 41*, 46–51. https://doi.org/10.1017/S0043174500057568

Thompson, K., & Grime, J. P. (1979). Seasonal Variation in the Seed Banks of Herbaceous Species in Ten Contrasting Habitats. *Journal of Ecology*, *67*(3), 893–921. http://www.jstor.com/stable/2259220

Toole, E. H., & Brown, E. (1946). Final Results of the Duvel Buried Seed Experiment. *Agricultural Research*, *72*(6). Accessed Dec. 15, 2021 from: https://naldc.nal.usda.gov/catalog/IND43970084

University of Nebraska – On-Farm Research Network (2022). University of Nebraska – Lincoln Institute of Agriculture and Natural Resources Extension. Available from: https://on-farm-research.unl.edu/

Walck, J. L., Baskin, J. M., Baskin, C. C., & Hidayati, S. N. (2005). Defining transient and persistent seed banks in species with pronounced seasonal dormancy and germination patterns. *Seed Science Research*, *15*(3), 189–196. https://doi.org/10.1079/ssr2005209

Ward, M. J., Ryan, M. R., Curran, W. S., Barbercheck, M. E., & Mortensen, D. A. (2011). Cover Crops and Disturbance Influence Activity-Density of Weed Seed Predators Amara aenea and Harpalus pensylvanicus (Coleoptera: Carabidae) . *Weed Science*, *59*(1), 76–81. https://doi.org/10.1614/ws-d-10-00065.1

Webster, T. M., Cardina, J., & Norquay, H. M. (1998). Tillage and seed depth effects on velvetleaf (Abutilon theophrasti) emergence. *Weed Science*, *46*(1), 76–82. https://doi.org/10.1017/s0043174500090202

Wu, C., & Owen, M. D. K. (2014). When Is the Best Time to Emerge: Reproductive Phenology and Success of Natural Common Waterhemp (Amaranthus rudis) Cohorts in the Midwest United States? . *Weed Science*, *62*(1), 107–117. https://doi.org/10.1614/ws-d-13-00079.1

Ziska, L. H., Teasdale, J. R., & Bunce, J. A. (1999). Future atmospheric carbon dioxide may increase tolerance to glyphosate. *Weed Science*, *47*(5), 608–615. https://doi.org/10.1017/s0043174500092341

Table 2-1: Species composition across all experimental sites. The 20 most frequent weed species found in this study. The remaining species represent 0.5% or less of the total species. Weeds classified by taxonomy of dicotyledonous (Dicot.) or monocotyledonous (Monocot.), life cycle type, and photosynthetic pathway.

Common Name	**Species Name**	**Species Code**	**Taxonomy Classification**	**Life Cycle**	**Photosynthetic Pathway**	**Percentage of Total (%)**
Palmer amaranth	*Amaranthus palmeri* S. Watson	AMAPA	Dicot.	Summer Annual	C_4	20.09
Green foxtail	*Setaria viridis* (L.) P. Beauv.	SETVI	Monocot.	Summer Annual	C_4	13.12
Scarlet pimpernel	*Anagallis arvensis* L.	ANAAR	Dicot.	Summer / Winter Annual	C_3	10.79
Lambsquarters	*Chenopodium album* L.	CHEAL	Dicot.	Summer Annual	C_3	7.514
Common yellow woodsorrel	*Oxalis stricta* L.	OXAST	Dicot.	Perennial	C_3	7.346
Redroot pigweed	*Amaranthus retroflexus* L.	AMARE	Dicot.	Summer Annual	C_4	7.103
Common waterhemp	*Amaranthus tuberculatus* (Moq.) Sauer	AMATU	Dicot.	Summer Annual	C_4	4.709
Marestail	*Erigeron canadensis* L.	ERICA	Dicot.	Winter / Summer Annual	C_3	4.207
Green carpetweed	*Mollugo verticillata* L.	MOLVE	Dicot.	Summer Annual	C_3/ C_4	3.963
Eastern black nightshade	*Solanum ptycanthum* Dunal	SOLPT	Dicot.	Summer Annual	C_3	3.826
Barnyard grass	*Echinochloa crus-galli* (L.) P. Beauv	ECHCR	Monocot.	Summer Annual	C_4	2.835
Yellow foxtail	*Setaria pumila* (Poir.) Roem. & Schult	SETPU	Monocot.	Summer Annual	C_4	2.713
Smooth crabgrass	*Digitaria ischaemum* (Schreb.) Screb. ex Muhl.	DIGIS	Monocot.	Summer Annual	C_4	1.966
Field pennycress	*Thlaspi arvense* L.	THLAR	Dicot.	Winter / Summer Annual	C_3	1.859
Large crabgrass	*Digitaria sanguinalis* (L.)	DIGSA	Monocot.	Summer Annual	C_4	1.463
Giant foxtail	*Setaria faberi* Herrm.	SETFA	Monocot.	Summer Annual	C_4	0.8078
Prostrate vervain	*Verbena bracteata* Cav. Ex Lag. & Rodr.	VERBR	Dicot.	Annual / Biennial	C_3	0.7468
False pimpernel	*Lindernia dubia* (L.) Pennell	LINDU	Dicot.	Summer Annual	C_3	0.6553
Tumble pigweed	*Amaranthus albus* L.	AMAL	Dicot.	Summer Annual	C_4	0.5944
Black medic	*Medicago lupulina* L.	MEDLU	Dicot.	Summer Annual	C_3	0.5030

Figure 2-1: Total seedbank density. Seedbank density reported in seeds m^{-2}, which was calculated from raw seedling counts. Mean estimates are least squares means and error bars represent standard error of the mean. Site had a significant effect in the model.

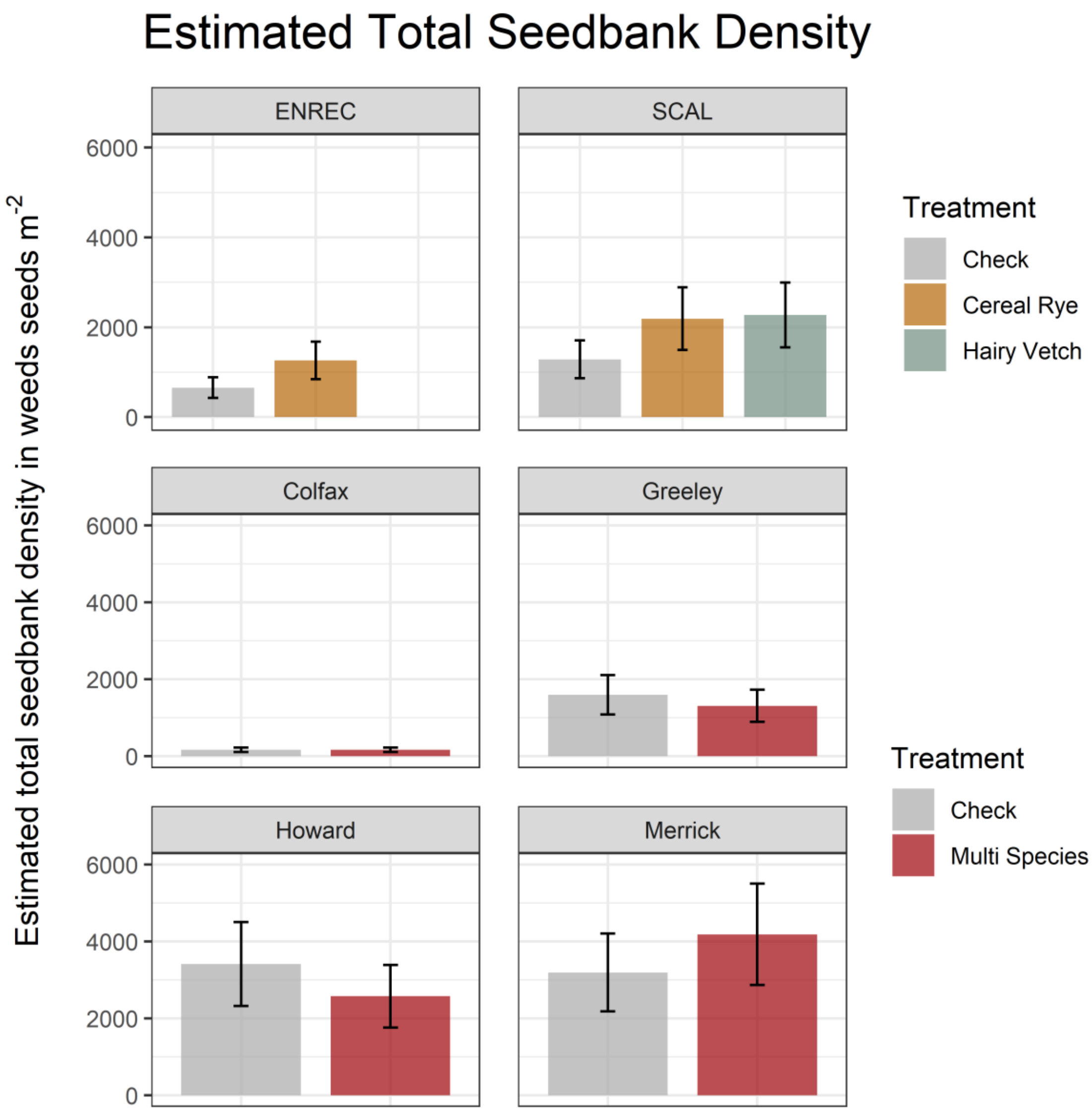


Figure 2-2: Pigweed seedbank density. Seedbank density reported in seeds m^{-2}, which was calculated from raw seedling counts. Asterisks represent level of significance with *, **, ***, **** representing levels of $p < 0.1$, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Mean estimates are least squares means and error bars represent standard error of the mean. Site had a significant effect in the model.

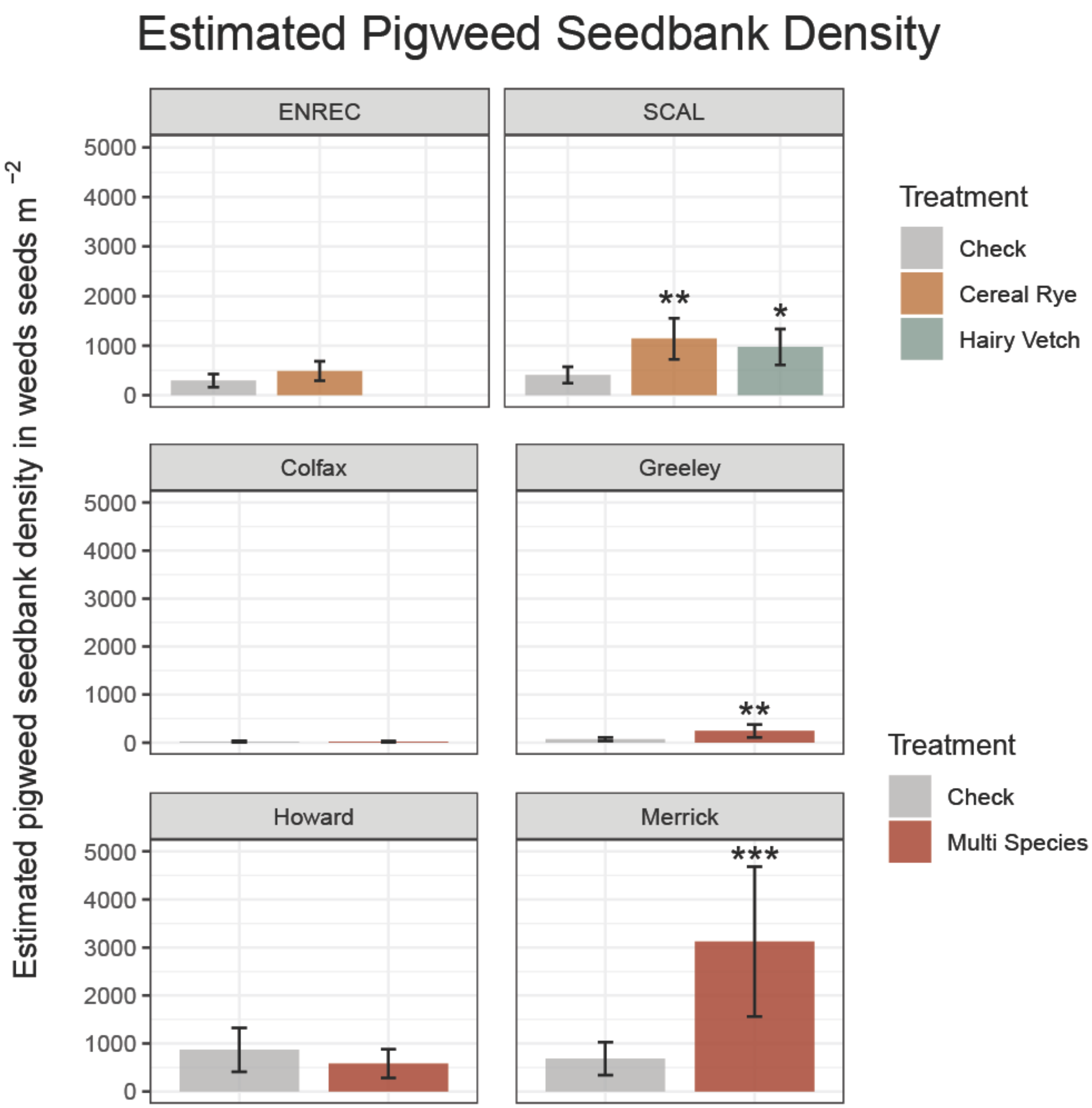


Figure 2-3: Grass seedbank density. Seedbank density reported in seeds m^{-2}, which was calculated from raw seedling counts. Mean estimates are least squares means and error bars represent standard error of the mean. Site had a significant effect in the model. (On-farm sites were the only ones to detect grasses in the seedbank, therefore graphs are not reported for research stations.)

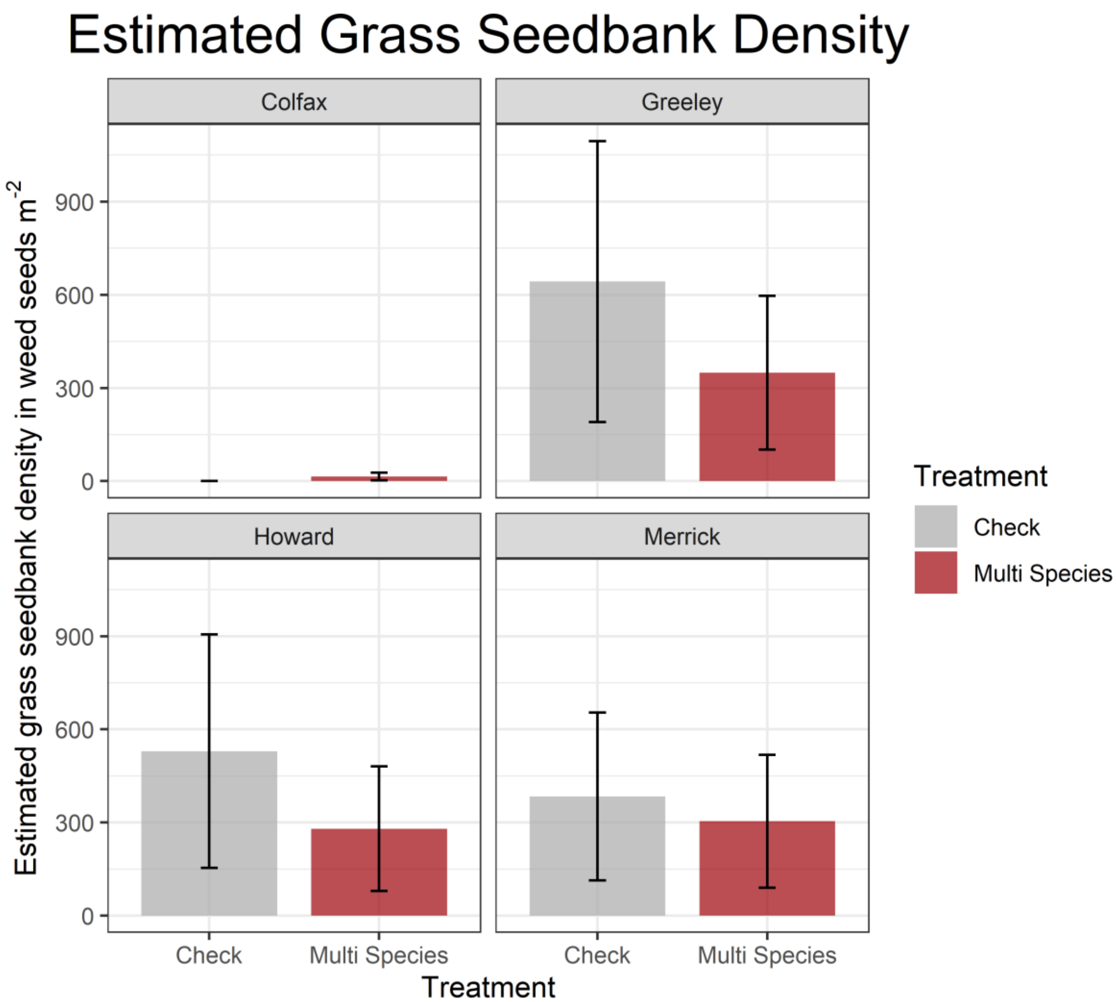


Figure 2-4: Broadleaf seedbank density. Seedbank density reported in seeds m^{-2}, which was calculated from raw seedling counts. Asterisks represent level of significance with *, **, ***, **** representing levels of $p < 0.1$, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Mean estimates are least squares means and error bars represent standard error of the mean. Site had a significant effect in the model for on-farm sites.

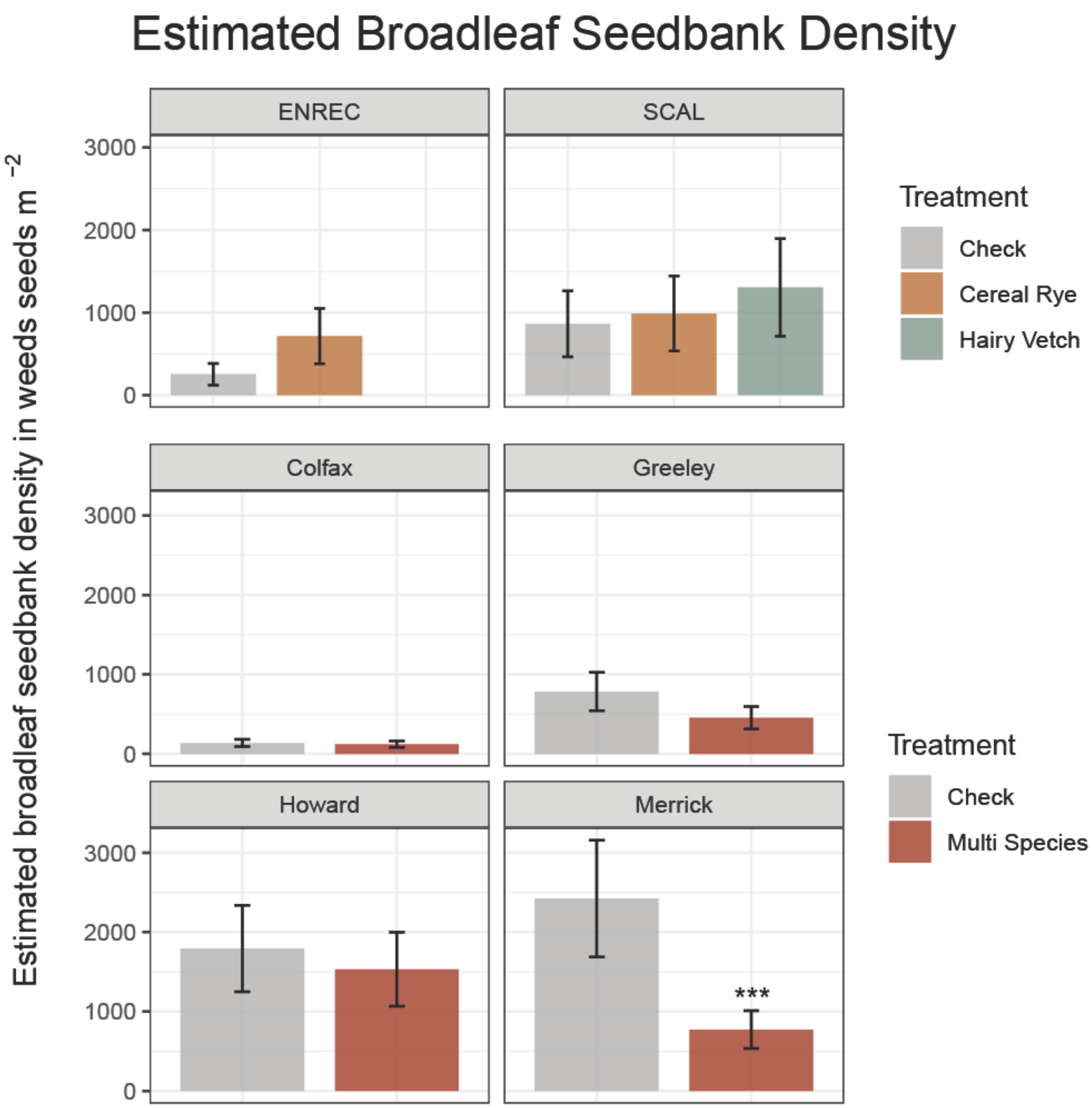


Figure 2-5: Nonmetric multidimensional scaling (NMDS) of seedbank species by site and treatment. Species representing at least 0.1% or more each sites total seedbank are presented for each respective site. Stress values represent goodness-of-fit (closer to zero is ideal). Bray-Curtis index was used for dissimilarity matrices. Species codes represent first three letters of genus and first two of species.

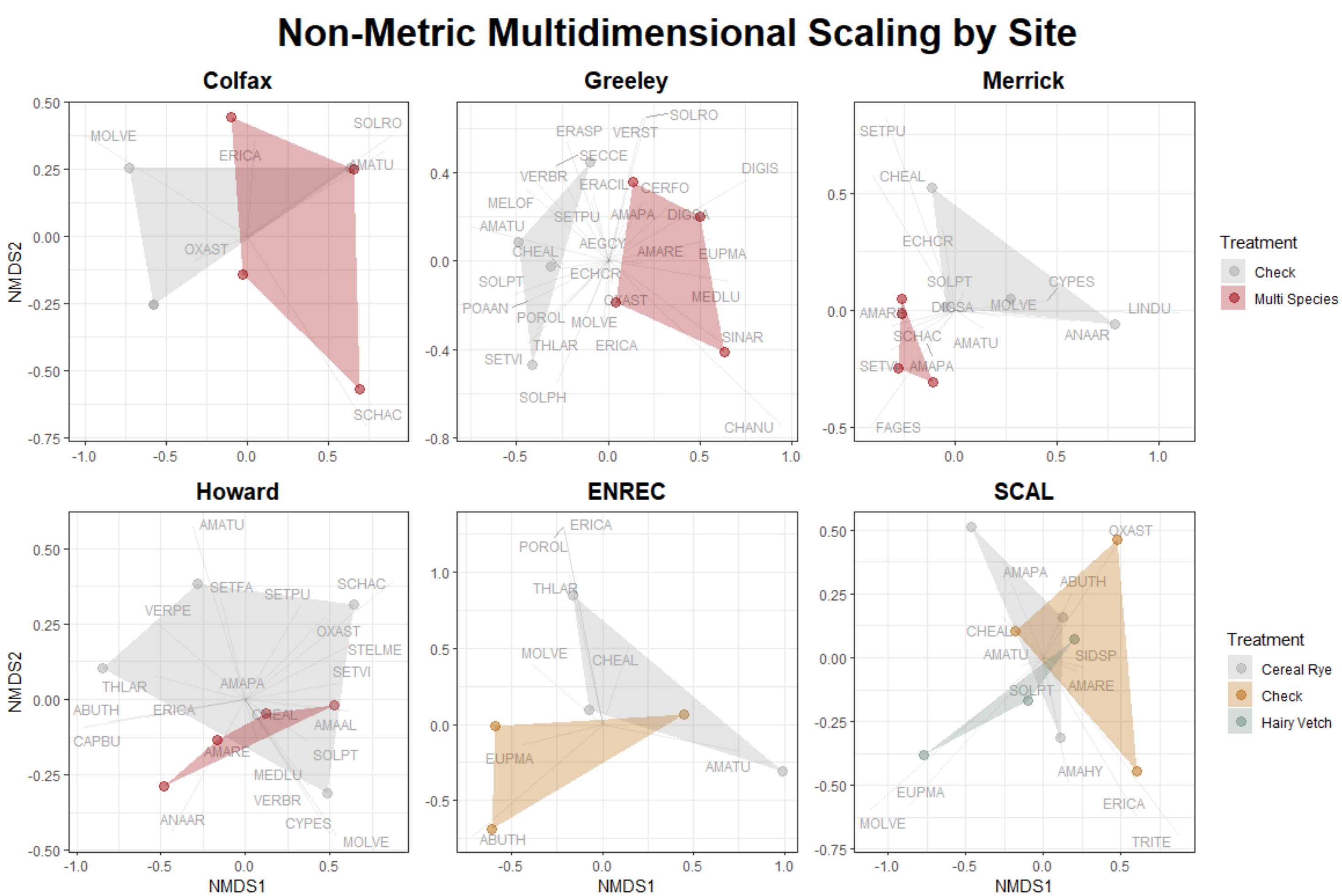


Table 2-2: Community analysis results for Shannon diversity, species richness, and species evenness by site. Estimated change represents difference between mean check score and mean cover crop score; negative estimated change represents greater score for cover crop treatment. SE represents standard error.

Seedbank Community Analyses

	Shannon Hill Diversity		Richness		Evenness	
	Estimated Change *(SE)*	*P-value*	Estimated Change *(SE)*	*P-value*	Estimated Change *(SE)*	*P-value*
Colfax	-0.69(1.53)	0.66	-1(1.65)	0.55	-0.19(0.12)	0.13
Greeley	0.11(1.53)	0.94	-0.25(1.65)	0.88	-0.05(0.1)	0.62
Howard	-1.15(1.53)	0.46	-2.75(1.65)	0.11	-0.03(0.1)	0.77
Merrick	2.1(1.53)	0.18	-1.25(1.65)	0.46	0.17(0.1)	0.09
ENREC	-1.34(1.31)	0.58	-1.67(1.68)	0.60	-0.06(0.17)	0.93
SCAL - Rye	-1.43(1.31)	0.54	-1.67(1.68)	0.60	-0.08(0.15)	0.84
SCAL - Hairy Vetch	-1.28(1.31)	0.61	-1(1.68)	0.82	-0.11(0.15)	0.75

Figure 2-6: Schematic showing a simplified relationship between weed abundance and species found in a community) and species evenness (the relative abundance of each species present). Communities with high richness and evenness are considered diverse, and theoretically diversity scores can theoretically be infinite, but niche saturation will eventually occur at different levels depending on the agroecosystem. Weed community diversity impacts are conceptualized based on agroecology principles of crop rotation diversity impacts on weeds and selection pressures in disturbed agroecosystems. Weed abundance on the x axis describes the number of emerged aboveground weeds. Shading on graph indicates ideal or problematic scenarios, with deepest shades of red indicating that there is greatest risk for potential yield loss in the cash crop. Ideal agroecosystems will have high weed diversity and low emerged weed density.

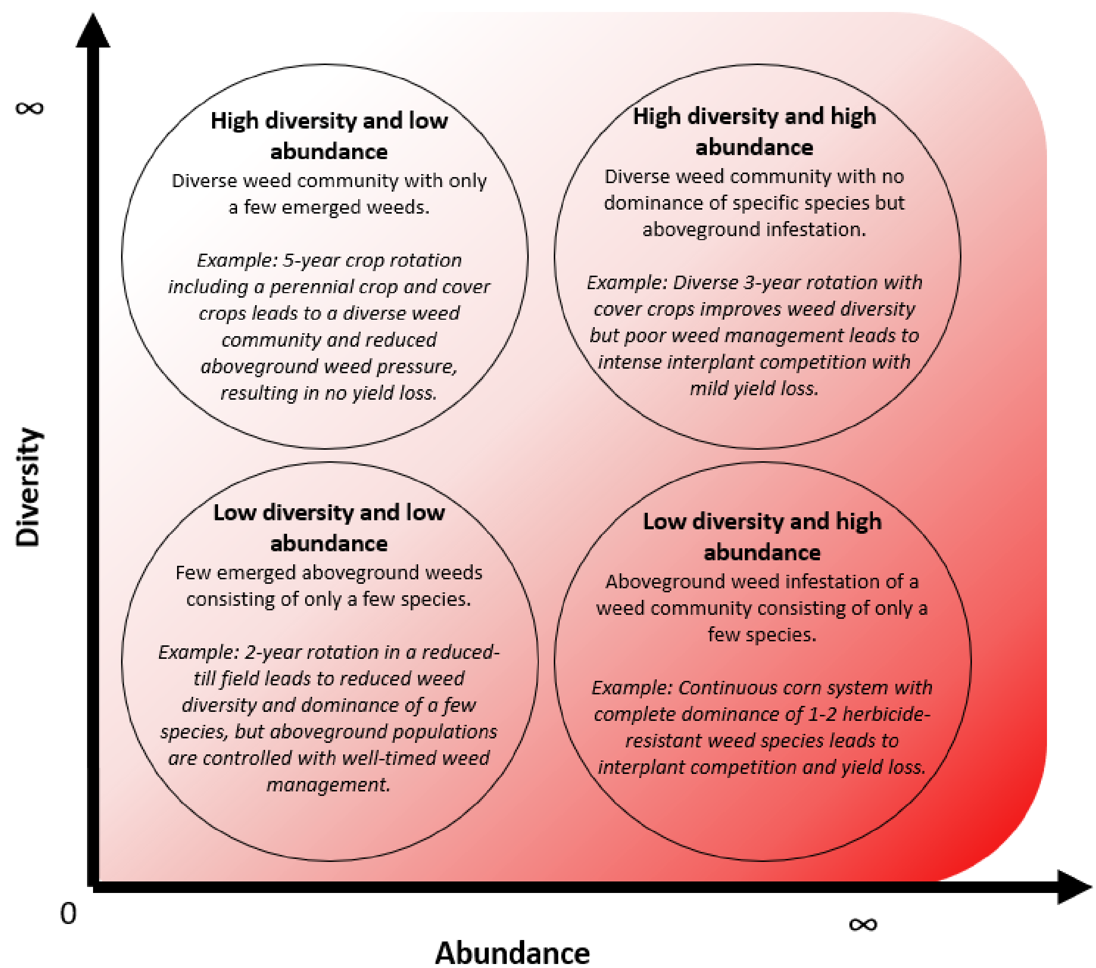


Table 2-3: *Amaranthus* species prevalence by site and treatment. Percentage of seedbanks represent percentage of each respective treatments seedbank at each site. Species are arranged in descending order of prevalence. AMAAL, AMAHY, AMAPA, AMARE, and AMATU represent species with common names of prostrate pigweed, tumble pigweed, Palmer amaranth, redroot pigweed, and common waterhemp, respectively.

Site	Treatment	Species	Species Code	Percent of Treatment Seedbank
Colfax	Check	*Amaranthus tuberculatus* (Moq.) Sauer	AMATU	29.7%
	Multi-Species Cover Crop	*Amaranthus tuberculatus* (Moq.) Sauer	AMATU	22.8%
Greeley	Check	*Amaranthus retroflexus* L.	AMARE	2.0%
		Amaranthus palmeri S. Watson	AMAPA	1.6%
		Amaranthus tuberculatus (Moq.) Sauer	AMATU	0.3%
	Multi-Species Cover Crop	*Amaranthus retroflexus* L.	AMARE	13.8%
		Amaranthus palmeri S. Watson	AMAPA	8.9%
Howard	Check	*Amaranthus tuberculatus* (Moq.) Sauer	AMATU	11.8%
		Amaranthus albus L.	AMAAL	2.1%
		Amaranthus palmeri S. Watson	AMAPA	1.7%
	Multi-Species Cover Crop	*Amaranthus retroflexus* L.	AMARE	14.2%
		Amaranthus palmeri S. Watson	AMAPA	5.0%
		Amaranthus albus L.	AMAAL	1.1%
		Amaranthus tuberculatus (Moq.) Sauer	AMATU	0.9%
Merrick	Check	*Amaranthus palmeri* S. Watson	AMAPA	15.9%
		Amaranthus retroflexus L.	AMARE	1.9%
		Amaranthus tuberculatus (Moq.) Sauer	AMATU	0.9%
		Amaranthus hybridus L.	AMAHY	0.1%
	Multi-Species Cover Crop	*Amaranthus palmeri* S. Watson	AMAPA	67.4%
		Amaranthus retroflexus L.	AMARE	6.2%
		Amaranthus tuberculatus (Moq.) Sauer	AMATU	0.9%
ENREC	Check	*Amaranthus tuberculatus* (Moq.) Sauer	AMATU	61.3%
	Cereal Rye	*Amaranthus tuberculatus* (Moq.) Sauer	AMATU	43.3%
SCAL	Check	*Amaranthus palmeri* S. Watson	AMAPA	19.7%
		Amaranthus retroflexus L.	AMARE	11.5%
		Amaranthus tuberculatus (Moq.) Sauer	AMATU	1.6%
	Cereal Rye	*Amaranthus palmeri* S. Watson	AMAPA	27.9%
		Amaranthus tuberculatus (Moq.) Sauer	AMATU	17.3%
		Amaranthus retroflexus L.	AMARE	8.7%
		Amaranthus hybridus L.	AMAHY	1.0%
	Hairy Vetch	*Amaranthus palmeri* S. Watson	AMAPA	21.3%
		Amaranthus tuberculatus (Moq.) Sauer	AMATU	13.9%
		Amaranthus retroflexus L.	AMARE	7.4%

CHAPTER 3: WINTER COVER CROP IMPACTS ON WEED GROWTH DURING THE GROWING SEASON

Abstract

Cool season cover crops have been increasingly explored as an integrated weed management strategy for supplemental weed suppression in Midwest Corn Belt cropping systems because they have the potential to directly compete with weeds for sunlight, water, and nutrients. They may also be a viable option for mitigating risks of herbicide resistance In order to assess impacts of cover crops on weeds during the growing season, we utilized four on-farm research sites and two long-term research stations to assess emerged weed density and biomass after cash crop planting during the critical weed control period and before crop canopy closure. Total weed biomass was reduced in the cover crop at all sites and sampling periods by 15-98%, but results were significant at only Greeley County for both sample periods and Howard County for the early sample period. Total weed density reductions were less consistent across sites, but significantly reduced in the cover crop at two on-farm sites. Across all six sites, trends of increased weed control were observed with increasing cover crop biomass. While we did not find strong or consistent weed control in our experiments, our results suggest that cover crops may provide more consistent weed suppression through weed growth interference instead of emergence, leading to weed biomass reductions. Furthermore, increases in pigweed seed density at three sites were not expressed aboveground in pigweed density, further pointing to the possibility of cover crop pigweed seed suppression discussed in Chapter 2 and we conclude the cover crops may be a viable tool for pigweed and herbicide resistance management.

Introduction

The need for effective integrated weed management strategies is crucial, particularly in no-tillage or reduced-tillage cropping systems in the Midwest Corn Belt where weed control primarily relies on the use and efficacy of herbicides. Herbicide efficacy is often reduced due to the frequent development of herbicide-resistance (HR) traits in weeds along with climate change factors such as rising CO_2 levels and shifting temperatures that can impact herbicide metabolism and detoxification in weeds (Peterson et al., 2018; Ramesh et al., 2017). Therefore, integrated weed management strategies are continuously being explored to mitigate this issue, such as cover crops. Cover crops are non-cash crop plants grown to provide continuous living plant cover on soil that would otherwise be fallow. While cover crops are most known for amending soil health, reducing soil erosion, and supplementing nutrients, they have also shown potential to reduce in-season weed density and biomass (Buchanan et al., 2016; Koehler-Cole et al., 2021; Nichols et al., 2020), which can lead to improvements in crop productivity (Adeux et al., 2021). However, few studies look at weed density and biomass under normal field management (i.e., herbicides applied) in established cover crop studies. This chapter seeks to understand how cover crops impact emerged weed density, weed biomass, and composition of pigweeds, grasses, and other broadleaves under herbicide-managed fields in eastern and central Nebraska in the Western Corn Belt.

The competitive effects of weeds for sunlight, water, and nutrient resources are most critical in the early growing season when the cash crop first emerges, which can lead to yield losses if weeds are not controlled (Ali et al., 2013; Knezevic & Datta, 2015). Cover crops can compete with weeds for these same resources before the cash crop is planted in the early growing season, which can reduce weed emergence and growth (Kruidhof et al., 2008; Rueda-Ayala et al., 2015). Early season weed control can be provided by cover crops and is comparable to chemical and mechanical weed control if high biomass is achieved and surface residue is persistent, regardless of cover crop species or type of mixture (Osipitan et al., 2018). Living cover crops are known to produce allelopathic chemicals, which act as germination or growth inhibitors for weed seedlings (Kunz et al., 2016; Moonen & Bàrberi, 2006). During the growing season, dead cover crop residue can act as a physical barrier for weed emergence and alter soil temperature through shading and reducing light penetration into the soil profile (Teasdale et al., 2007), leading to reduced weed germination and emergence (Buchanan et al., 2016). Additionally, cover crops may delay weed emergence and subject seeds to invertebrate predation over time (Liebman et al. 2021). Furthermore, evidence suggests that synergism occurs between cover crop residue and herbicides, which can reduce weed emergence more effectively than the use of cover crops or herbicides alone (Teasdale et al., 2005). Combined, these mechanisms can help secure weed control.

The suppressive effects of cover crops are attributed mainly to the amount of biomass accumulated before termination, which can be impacted by cover crop plant and termination dates (Wallace et al., 2019). Pre-harvest broadcast seeded cover crops typically produce more biomass than post-harvest drilled cover crops under ideal growing conditions, and *Secale cereale* (cereal rye) produces the most biomass regardless of planting date (Koehler-Cole et al., 2020). Furthermore, more cover crop biomass accumulates and better weed control occurs with delayed spring termination (Mirsky et al., 2011). A recent modelling analysis found that 5 Mg ha^{-1} cover crop biomass was needed to cause a significant reduction in weed biomass (Nichols et al., 2020). However, to achieve this amount of biomass in the Corn Belt required early fall planting and late spring termination. In Nebraska, termination would typically need to occur into June if planted at realistic fall windows (around October) in order to achieve 5 Mg ha^{-1} (Nichols et al., 2020). Consequently, there are risks of crop yield reductions with greater cover crop biomass due water use and/or high carbon to nitrogen (C:N) ratios that can temporarily immobilize nitrogen, and planting the cash crop later due to cover crop termination may also impact yields (Qin et al., 2021). These are important considerations and tradeoffs to growers if weed suppression is a goal of cover crop use.

The need for integrated weed management tools like cover crops is becoming more relevant and is being increasingly explored due to major challenges with HR (Gage & Schwartz-Lazaro, 2019; Kumar et al., 2020). HR is prevalent with herbicides like glyphosate, the herbicide of choice in Nebraska and globally (Jhala et al., 2014; Myers et al., 2016). Furthermore, weeds are predicted to tolerate and metabolize herbicides like glyphosate as climate change continues (Ziska et al., 1999). These are challenges for growers, researchers, industry, and policymakers to continue to explore and expand integrated weed management approaches that preserve yield, crop productivity, and efficacy of herbicides in the future.

Given the imperative need to find effective integrated weed management strategies to conserve current crop production systems, particularly in no-tillage or reduced-tillage, our study investigates cover crop impacts on weed density and biomass at different times during the growing season at both on-farm and long-term research experiments in the Western Corn Belt of Nebraska. Therefore, the objectives of this study were to (1) quantify total emerged weed density and density of pigweeds, grasses, and other broadleaves present and (2) quantify total weed biomass and biomass of pigweeds, grasses, and other broadleaves present at two different points in the growing season. We hypothesized that (1) weed density would be reduced under cover crops and (2) weed biomass would be reduced under cover crops.

Materials and Methods

Site Descriptions

We studied emerged weed densities and biomasses at six multi-year experiments across eastern and central Nebraska two different times during the 2021 growing season. The South-Central Agriculture Laboratory (SCAL) near Clay Center, NE and the Eastern Nebraska Research and Extension Center (ENREC) near Mead, NE are University of Nebraska – Lincoln research stations with long-term cover crop experiments (initiated in 2014). The remaining four locations were commercial on-farm sites in eastern and central Nebraska (initiated in 2016 or 2017) in Colfax, Greeley, Howard, and Merrick Counties. As a part of the Soil Health Initiative (SHI) launched in 2016, on-farm research locations partnered with the University of Nebraska (UNL) On-Farm Research Network and Nebraska Natural Resources Conservation Service (USDA-NRCS) to conduct evaluations of cover crops at field-scale. Summaries of the experimental design, management, and cropping systems for the six sites can be found in Appendix 1 through 3.

Experimental design and plot management

Research stations

SCAL and ENREC experiments utilized a randomized complete block design with treatments of cereal rye (*Secale cereale*), hairy vetch (*Vicia villosa*), and no cover crop (check) at SCAL (n = 3 for each treatment) and cereal rye (*Secale cereale*) and no cover crop (check) at ENREC (n = 3 for each treatment). Cover crops were planted in the plots each year since 2014. Cover crops were planted in the fall and terminated in the spring with glyphosate, within two weeks of cash crop planting each year. Both locations utilized no-tillage management and a two-year corn-soybean crop rotation. POST herbicides were utilized in all plots and sites (Table 3-1). In 2021, all plots sampled had a cash crop of corn. Experimental design, plot dimensions, crop rotation history, and management information on these sites can be found in Appendix 2 through 4 and Appendix 6 through 7.

On-farm sites

All on-farm sites utilized a randomized complete block designs with treatments of multi-species cover crops (5-10 species) and no cover crop (check) (n = 4 for each treatment). Total field size at all four commercial farm locations was at least 20 hectares, and treatment strips were in randomized spatially balanced blocks, and plot sizes varied. Cover crops were planted in the plots each year since 2016 or 2017. Cover crops were planted in the fall and terminated in the spring with PRE herbicides immediately before cash crop planting in each year. POST herbicides were utilized but differed at each site (Table 3-1).

All on-farm sites followed a three-year corn-soybean-small grains rotation, where the previous crop was a small grain planted at Greeley, Colfax, and Howard Counties and field peas were planted at the Merrick County site in 2020. In 2021, three sites grew corn and one grew soybean. Experimental design, plot dimensions, crop rotation history, and management information on these sites can be found in Appendix 2 through 4 and Appendix 6 through 7.

Cover crop biomass determination

At the research stations, cover crop biomass was obtained randomly two times per plot in a 1.5 m by 0.3 m quadrat. At on-farm sites, cover crop biomass was obtained two to three times per rep (an approximate rate of 1 sample per hectare) using 0.5 m x 1 m quadrats. All aboveground plant material matter was clipped at the soil surface, placed into paper bags, then dried at 50˚C until a constant weight was achieved. Cover crop biomass is estimated as Mg dry plant matter (DM) ha^{-1}.

Aboveground weed sampling

Aboveground weed observations were taken two times during the growing season: 1) assessment of early season weeds (after cash crop emergence but before a post-emergence (POST) herbicide application was made); 2) assessment of late season weeds (at least three weeks after POST and before canopy closure). Herbicides applied at each site can be found in Table 3-1. Weed density, weed biomass, soil moisture (% VWC), and soil temperature (degrees Celsius) samples were taken three times per replicate during both assessments.

Weed density was calculated by counting the number of emerged weeds in a quadrat (0.25 m^2 at research stations, 1 m^2 at on-farm sites). Soil moisture and temperature were taken in the same quadrat where weed density was determined. Once these data were obtained, a flag was placed and a GPS point was recorded to mark the exact location where weed density was counted in order to return to the same point in each plot for the later season weeds.

Weed biomass was taken at a point near each weed density sample at least one meter north (early season samples) or south (late season samples), so that weed density data would not be impacted by the removal of weed seedlings. Biomass was obtained by clipping seedlings at the soil surface, placing them in small envelopes classified by pigweeds, grasses, and all other broadleaves, then drying biomass at 50°C for five days until a constant weight was achieved. Weeds were measured without the envelope on a precision scale that measured to one ten-thousandth of a gram. Protocols described were followed for both early and late season weed observations (with the exception of late season weeds at the on-farm site in Merrick due to a storm lodging the cash crop which rendered the field unwalkable). Total weed measurements taken in the field were the sum of the subcategories of pigweeds, grasses, and other broadleaves in each respective plot.

Data analysis

Weed density data were converted to estimates of weeds m^{-2} and weed biomass data were converted to estimates of grams DM m^{-2} before analyses. Both datasets of weed density and weed biomass exhibited right skewedness due to inflation of counts of zero weeds or measurements of zero grams DM. In response, density data were $ln(x+1)$ transformed and biomass density data were $ln(x+0.1)$ before being analyzed using a linear mixed-effect model, which was fit using the *lmer* function from the *lme4* package in R 4.1.2 (Bates et al., 2015, R Core Team, 2022). All other analyses mentioned from here were conducted in R 4.1.2. Soil temperature and moisture data were analyzed with a linear mixed-effect model with the same package and function and Gaussian distributions were assumed. Main effects and all two and three-way interactions between treatment, site, and season (sampling period) were used as fixed effects. Replicate nested within site, and treatment nested within replicate and site were included as random effects to account for variability between replicates and within replicates respectively, with the residual variance accounting for the variation due to repeated measures over two different sampling periods. No correlation structure was included or necessary because there were only two sampling periods. Pairwise comparisons were conducted by calculating the least-squares means and contrasts were determined using the *emmeans* package (Lenth et al., 2021) at a level of $p < 0.1$.

Results

Cover crop biomass

The amount of cover crop biomass accumulated ranged from 0.11 up to 4.02 Mg ha^{-1} (Figure 3-1). The multi-species cover crop mixes at the on-farm locations accumulated more total biomass than monoculture mixes of rye and vetch at SCAL and ENREC due to an earlier plant date at the on-farm sites in late July and early August following a small grain harvest in 2020. Planting and termination dates varied across the sites and can be referenced in Appendix 8.

Weather

Between September 2020 and August 2021, average temperatures were similar or slightly cooler than the 30 year average, depending on the month. Deep freezes occurred in December, January, and February with temperatures reaching -36 ˚C at the coldest. Growing season (April-August) temperatures averaged similar to the 30 year average. Extreme reductions in precipitation occurred mainly between the months of September and December of 2020, which resulted in statewide moderate to severe drought. For example, at Howard and Merrick, October rainfall was 96% less than average. Heavy precipitation occurred in March across all sites, resulting in a 199-435% increase from 30 year average monthly precipitation data. In general, growing season precipitation was less than the 30 year average at most sites. Climate data for each site and the respective weather station can be viewed in Appendix 5.

Weed density

Results of the weed density measurements taken during the early and late growing season and results were dependent on site. At the Greeley County location, total weed densities (Figure 3-2) decreased in the cover crop treatment during early season ($p = 0.05$, -87%) and late season samples ($p = 0.05$, -89%). Late season total weed density reductions in the cover crop treatment also occurred at ENREC ($p = 0.09$, -73%). At Greeley County, total weed density reductions in the cover crop treatment were primarily driven by reductions in emerged grasses in the early season ($p = 0.05$, -90%) and late season ($p = 0.09$, -88%) sampling periods (Figure 3-4). Similarly, grasses were also reduced at Howard County during the early growing season ($p = 0.07$, -86%). A significant increase in broadleaves occurred at SCAL in the vetch cover crop treatment during the late season samples ($p = 0.06$) (Figure 3-5). Despite increases in pigweed seed densities in the cover crop treatments reported in Chapter 2: “Winter Cover Crop Impacts on Weed Seedbanks", no differences in emerged pigweed densities were observed at any of the six sites (Figure 3-3).

Weed biomass

Numerical weed biomass reductions in the cover crop treatments occurred at all sites and sampling times, and reductions ranged from 15% up to 98%. However, there were not often statistical differences in weed biomass between treatments due to variability in the replicates. Total weed biomass was significantly reduced in the cover crop treatment at the Greeley County location during both the early season (p = 0.06, -96.5%) and late season (p = 0.008, -99%) sample periods (Figure 3-6). Howard County also observed reductions in total weed biomass in the early season sample period (p = 0.1, -88%).

Similar to the trend in weed density, reductions in total biomass at Greeley County were driven by the reduction in grass biomass (Figure 3-8) during the early season (p = 0.02, -98%) and late season (p = 0.06, -96%) sample periods, as well as reduction in broadleaf biomass (Figure 3-9) during the late growing season (p = 0.005, 99%). Other significant reductions in weed biomass in the cover crop occurred at the ENREC site with reductions in late season pigweeds (Figure 3-7) (p = 0.09, -93%), the Howard County site with reductions in early season grasses (p = 0.09, -88%) which drove the significant reductions in total weed biomass, and the Merrick County site with reductions in early season broadleaves (p = 0.08, -99%).

Soil temperature and moisture

During the early growing season after crop emergence, soil temperature significantly increased in the cover crop at Greeley (p = 0.03) during the early growing season but no other differences were observed at any site, sampling period, or treatment (Table 3-2). No differences in soil moisture were determined at any site during either of the sampling periods (Table 3-3).

Discussion

Cover crop impacts on weed density and biomass

Numerical weed biomass reductions were observed at all sites and sampling periods ranging from 15-98%, but few results were statistically significant partly due to variability in the replicates. Variability could be attributed to the nature of the large, on-farm trials that introduced spatial variability as well as potential differences in the natural spatial distribution of emerged weeds in general (Cardina et al., 1997). Weed biomass reductions were more consistent across all sites than weed density reductions. This result aligns with findings from a meta-analysis by Nichols et al. (2020), which indicates that cover crops may be more effective in suppressing weed biomass than weed density in the Midwestern Corn Belt, because corn-soybean crop rotations impose time and weather constraints on the potential for winter cover crops to accumulate biomass. Our results potentially suggest that cover crops may have more impact on weed growth interference, thus reducing biomass, rather than disrupting germination and therefore weed density.

Low precipitation in September and October 2020 and statewide moderate to severe drought may have impacted fall cover crop germination and biomass accumulation (Figure 3-1) ahead of our cover crop biomass sampling in the 2021 growing season. The amount of biomass accumulation ranged from 0.11 to 4.02 Mg ha^{-1} depending on the site, and the amount of biomass may have contributed to limited significant differences in weed measurements. Additionally, we observed volunteer small grain emergence at three on-farm sites (Greeley, Howard, Colfax Counties) in both cover crop and check plots, which may have masked the true effects of the cover crop during our sampling year. However, sites like the Greeley County site saw consistent weed density and biomass even with 1.1 Mg ha^{-1} cover crop biomass, compared to the estimated 5 Mg ha^{-1} for significant reductions in weed biomass (Nichols et al., 2020). In contrast to Nichols et al. (2020), other studies have shown that lower cover crop biomass can still achieve reductions in weed density and biomass (Alonso-Ayuso et al., 2018; Wallace et al., 2019). It is also evident from our data that specific functional groups may be more sensitive to cover cropping, for example reductions in weed density and biomass at the Greeley County site were driven primarily by the reductions in grasses.

Stronger effects on weed density and biomass should be expected with greater cover crop biomass (Finney et al., 2016). However, to achieve more cover crop biomass there would need to be significant adjustments to cover crop planting and termination dates to maximize biomass potential in Nebraska, because cash crop harvest and planting dates often determine later cover crop plant dates in the fall, especially after corn, and earlier termination times in the spring (Nichols et al., 2020). Despite early cover crop planting dates at on-farm locations (late July and early August), total cover crop biomass did not exceed 5 Mg ha^{-1} but weed density and biomass reductions still occurred at two sites in the cover crop treatment.

Soil temperature and moisture under cover crops

There was no evidence in our data to show that soil temperature and moisture influenced weed biomass or weed density under cover crops. Increases in soil temperature in the cover crop were observed consistently throughout the sampling periods at Greeley County. Sandy soil texture at this site (Appendix 1) as well as hillslopes ranging from 3-30% may have impacted drainage at sampling points and therefore soil temperature at the time of sampling. Furthermore, there is evidence that topography combined with cover crops may influence weeds (Singh et al., 2020), which may have been a factor at on-farm sites where topography varied along replications, especially at Greeley County. Regardless, higher soil temperatures resulting in lower weed density and weed biomass are unusual at Greeley County given that cover crops typically result in cooler soil temperatures that reduce weed emergence and growth (Williams et al., 1998). Therefore, it is unknown if these weed reductions at Greeley County are truly attributed to cover crops or other factors such as the herbicide program.

Research limitations and call for future research

Our study considered the presence of cover crops as the main effect on weed biomass and density, but aboveground weeds are impacted by many other management practices, including crop rotations (Doucet et al., 1999; Weisberger et al., 2019). All sites in this study had a primary crop rotation of corn-soybean while the on-farm sites diversified the rotation with the inclusion of a small grain or cool season pulse crop in 2020 (Appendix 7). Diversified crop rotations, such as the on-farm sites, can provide better weed control by disrupting weed lifecycles through varied cash crop planting dates (Liebman & Nichols, 2020; Weisberger et al., 2019). However, crop rotation was not a controlled variable in our experiment, and weeds were sampled only during one phase of the rotation in 2021. To draw conclusions about crop rotation in this experiment, continuous weed assessments would need to take place over the whole rotation because emerged weed communities are indicative of specific management each year and each crop (Storkey & Neve, 2018). The inconsistency of cover crop impacts on weed biomass and density at the on-farm locations in our study could have been a result of weed measurements taken in summer annual crops (corn or soybean) following cool season crops, thus masking the potential impact of the cover crop treatments.

This study also looked at aboveground weeds in the context of typical Nebraska weed management systems (i.e., PRE and POST herbicides applied), therefore no herbicide-free controls existed and effects of herbicides were not separated from cover crops. Previous studies have noted that herbicides may override the effects of cover crops and discrepancies in cover crop termination timing may also impact results (Adeux et al., 2021). Additionally, while we recorded if herbicide residuals were present in PRE and POST applications (Table 3-1), the presence of residuals was not considered due to complexities in differing herbicide rates, brands, tank mixes, soil textures, and weather across the six sites. Residuals were used in herbicide programs at all on-farm sites for PRE applications and four sites for POST applications. Herbicide residuals may be an important factor for considering emerged weed populations in cover crops (Mccall, 2014) and effects should be considered in future cover crop-weed studies.

Our results must be taken in the context of the management practices in place, and future studies should attempt to separate these effects over longer-term studies. It may also be beneficial to track weed control prior to cover crop termination. We suspect that achieving ideal or larger amounts of cover crop biomass would result in greater weed suppression (Finney et al., 2016), therefore we still advise that growers and researchers maximize cover crop biomass potential if the primary goal is to maximize weed suppression.

Conclusion

In this study, I assessed weed density and biomass to understand in-season impacts of cover crops on aboveground weeds at two points during the growing season. I also attempted to understand impacts of soil temperature and moisture on these data. I found that weed density was reduced less consistently than weed biomass, potentially signifying that cover crops interfere with weed growth more than weed emergence. Total weed biomass was reduced at all site and sampling periods but results were often not statistically significant. Lack of significant results may also be indicative of total cover crop biomass achieved, which did not reach the amount cite in literature that is found to significantly reduce weed density and biomass. Despite this, we observed no differences in aboveground pigweed densities at sites where significant increases in pigweed seedbank densities occurred in the cover crop, showing that cover crops may suppress pigweed seed germination by preventing weed emergence even without optimal levels of cover crop biomass. Furthermore, other conditions such as experimental design (on-farm vs research station) and management practices (tillage, crop rotation) may have influenced results. Future studies should attempt to separate management practices effects from cover crops. Our study serves as an insight of cover crops impacts on aboveground weeds during the growing season and complements Chapter 2, which investigated belowground weed communities, and supports the idea that cover crops may be providing pigweed seed suppression through reduced germination withdrawals.

References

Adeux, G., Cordeau, S., Antichi, D., Carlesi, S., Mazzoncini, M., Munier-Jolain, N., & Bàrberi, P. (2021). Cover crops promote crop productivity but do not enhance weed management in tillage-based cropping systems. *European Journal of Agronomy*, *123*(November 2020). https://doi.org/10.1016/j.eja.2020.126221

Ali, A., Streibig, J., & Andreasen, C. (2013). Yield loss prediction models based on early estimation of weed pressure. *Crop Protection*, *53*, 125–131. https://doi.org/10.1016/j.cropro.2013.06.010

Alonso-Ayuso, M., Gabriel, J. L., García-González, I., Del Monte, J. P., & Quemada, M. (2018). Weed density and diversity in a long-term cover crop experiment background. *Crop Protection*, *112*(May), 103–111. https://doi.org/10.1016/j.cropro.2018.04.012

Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1). https://doi.org/10.18637/jss.v067.i01

Buchanan, A. L., Kolb, L. N., & Hooks, C. R. R. (2016). Can winter cover crops influence weed density and diversity in a reduced tillage vegetable system? *Crop Protection*, *90*(December 2016), 9–16. https://doi.org/10.1016/j.cropro.2016.08.006

Cardina, J., Johnson, G. A., & Sparrow, D. H. (1997). The nature and consequence of weed spatial distribution. *Weed Science*, *45*(3), 364–373. https://doi.org/10.1017/s0043174500092997

Doucet, C., Weaver, S. E., Hamill, A. S., & Zhang, J. (1999). Separating the effects of crop rotation from weed management on weed density and diversity. *Weed Science*, *47*(6), 729–735. https://doi.org/10.1017/s0043174500091402

Finney, D. M., White, C. M., & Kaye, J. P. (2016). Biomass production and carbon/nitrogen ratio influence ecosystem services from cover crop mixtures. *Agronomy Journal*, *108*(1), 39–52. https://doi.org/10.2134/agronj15.0182

Gage, K. L., & Schwartz-Lazaro, L. M. (2019). Shifting the paradigm: An ecological systems approach to weed management. *Agriculture (Switzerland)*, *9*(8), 1–17. https://doi.org/10.3390/agriculture9080179

Jhala, A., Sandell, L., Knezevic, S., Kruger, G., & Wilson, R. (2014). Herbicide-Resistant Weeds in Nebraska. *University of Nebraska - Lincoln Extension*, *EC1278*. http://extensionpublications.unl.edu/assets/pdf/ec1278.pdf

Knezevic, S. Z., & Datta, A. (2015). The Critical Period for Weed Control: Revisiting Data Analysis. *Weed Science, 63*(SP1), 188–202. https://doi.org/10.1614/ws-d-14-00035.1

Koehler-Cole, K., Elmore, R. W., Blanco-Canqui, H., Francis, C. A., Shapiro, C. A., Proctor, C. A., Ruis, S. J., Heeren, D. M., Irmak, S., & Ferguson, R. B. (2020). Cover crop productivity and subsequent soybean yield in the western Corn Belt. In *Agronomy Journal* (Vol. 112, Issue 4). https://doi.org/10.1002/agj2.20232

Koehler-Cole, K., Proctor, C. A., Elmore, R. W., & Wedin, D. A. (2021). Spring-planted cover crops for weed control in soybean. *Renewable Agriculture and Food Systems*. https://doi.org/10.1017/S1742170521000107

Kruidhof, H. M., Bastiaans, L., & Kropff, M. J. (2008). Ecological weed management by cover cropping: Effects on weed growth in autumn and weed establishment in spring. *Weed Research*, *48*(6), 492–502. https://doi.org/10.1111/j.1365-3180.2008.00665.x

Kumar, V., Obour, A., Jha, P., Liu, R., Manuchehri, M. R., Dille, J. A., Holman, J., & Stahlman, P. W. (2020). Integrating cover crops for weed management in the semiarid U.S. Great Plains: Opportunities and challenges. *Weed Science*, *68*(4), 311–323. https://doi.org/10.1017/wsc.2020.29

Kunz, C., Sturm, D. J., Varnholt, D., Walker, F., & Gerhards, R. (2016). Allelopathic effects and weed suppressive ability of cover crops. *Plant, Soil and Environment*, *62*(2), 60–66. https://doi.org/10.17221/612/2015-PSE

Lenth, R., Buerkner, P., Herve, M., Love, J., Miguez, F., Riebl, H., & Singmann, H. (2021). *Package "emmeans."* https://doi.org/10.1080/00031305.1980.10483031>.License

Liebman, M., Basche, A. D., Nguyen, H. T. X., & Weisberger, D. A. (2021). How can cover crops contribute to weed management? A modelling approach illustrated with rye (Secale cereale) and Amaranthus tuberculatus. *Weed Research*, *April*, 1–11. https://doi.org/10.1111/wre.12508

Liebman, M., & Nichols, V. A. (2020). Cropping system redesign for improved weed management: A Modeling Approach Illustrated with Giant Ragweed (Ambrosia trifida). *Agronomy*, *10*(2). https://doi.org/10.3390/agronomy10020262

Mccall, C. M. (2014). *Integrating cover crops and herbicides for horseweed and Palmer amaranth management in no-till soybean*. Kansas State University. Available from: https://krex.k-state.edu/dspace/handle/2097/38561

Mirsky, S. B., Curran, W. S., Mortenseny, D. M., Ryany, M. R., & Shumway, D. L. (2011). Timing of Cover-Crop Management Effects on Weed Suppression in No-Till Planted Soybean using a Roller-Crimper. *Weed Science, 59*(3), 380–389. https://doi.org/10.1614/ws-d-10-00101.1

Moonen, A. C., & Bàrberi, P. (2006). An ecological approach to study the physical and chemical effects of rye cover crop residues on Amaranthus retroflexus, Echinochloa crus-galli and maize. *Annals of Applied Biology*, *148*(1), 73–89. https://doi.org/10.1111/j.1744-7348.2006.00038.x

Myers, J. P., Antoniou, M. N., Blumberg, B., Carroll, L., Colborn, T., Everett, L. G., Hansen, M., Landrigan, P. J., Lanphear, B. P., Mesnage, R., Vandenberg, L. N., Vom Saal, F. S., Welshons, W. V., & Benbrook, C. M. (2016). Concerns over use of glyphosate-based herbicides and risks associated with exposures: A consensus statement. *Environmental Health: A Global Access Science Source*, *15*(1), 1–13. https://doi.org/10.1186/s12940-016-0117-0

Nichols, V., Martinez-Feria, R., Weisberger, D., Carlson, S., Basso, B., & Basche, A. (2020). Cover crops and weed suppression in the U.S. Midwest: A meta-analysis and modeling study. *Agricultural & Environmental Letters*, *June*, 1–7. https://doi.org/10.1002/ael2.20022

Osipitan, O. A., Dille, J. A., Assefa, Y., & Knezevic, S. Z. (2018). Cover crop for early season weed suppression in crops: Systematic review and meta-analysis. *Agronomy Journal*, *110*(6), 2211–2221. https://doi.org/10.2134/agronj2017.12.0752

Peterson, M. A., Collavo, A., Ovejero, R., Shivrain, V., & Walsh, M. J. (2018). The challenge of herbicide resistance around the world: a current summary. *Pest Management Science*, *74*(10), 2246–2259. https://doi.org/10.1002/ps.4821

Qin, Z., Guan, K., Zhou, W., Peng, B., Villamil, M. B., Jin, Z., Tang, J., Grant, R., Gentry, L., Margenot, A. J., Bollero, G., & Li, Z. (2021). Assessing the impacts of cover crops on maize and soybean yield in the U.S. Midwestern agroecosystems. *Field Crops Research*, *273*(March). https://doi.org/10.1016/j.fcr.2021.108264

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from: https://www.R-project.org/.

Ramesh, K., Matloob, A., Aslam, F., Florentine, S. K., & Chauhan, B. S. (2017). Weeds in a changing climate: Vulnerabilities, consequences, and implications for future weed management. *Frontiers in Plant Science*, *8*(February), 1–12. https://doi.org/10.3389/fpls.2017.00095

Rueda-Ayala, V., Jaeck, O., & Gerhards, R. (2015). Investigation of biochemical and competitive effects of cover crops on crops and weeds. *Crop Protection*, *71*, 79–87. https://doi.org/10.1016/j.cropro.2015.01.023

Singh, G., Kaur, G., Williard, K. W., Nelson, K. A., & Schoonover, J. E. (2020). Cover crops and topography differentially influence weeds at a watershed scale. *Weed Technology*, *34*(1), 73–81. https://doi.org/10.1017/wet.2019.83

Storkey, J., & Neve, P. (2018). What good is weed diversity? *Weed Research*, *58*(4), 239–243. https://doi.org/10.1111/wre.12310

Teasdale, J. R., Brandsæedter, L. O., Calegari, A., & Skora Neto, F. (2007). Cover crops and weed management. In U. and Blackshaw (Ed.), *Non-Chemical Weed Management: Principles, Concepts and Technology* (pp. 49–64). CAB International. https://doi.org/10.1079/9781845932909.0049

Teasdale, John R., Pillai, P., & Collins, R. T. (2005). Synergism between cover crop residue and herbicide activity on emergence and early growth of weeds. *Weed Science*, *53*(4), 521–527. https://doi.org/10.1614/ws-04-212r

Wallace, J. M., Curran, W. S., & Mortensen, D. A. (2019). Cover crop effects on horseweed (Erigeron canadensis) density and size inequality at the time of herbicide exposure. *Weed Science*, *67*(3), 327–338. https://doi.org/10.1017/wsc.2019.3

Weisberger, D., Nichols, V., & Liebman, M. (2019). Does diversifying crop rotations suppress weeds? A meta-analysis. *PLoS ONE*, *14*(7), 1–12. https://doi.org/10.1371/journal.pone.0219847

Williams, M. M., Mortensen, D. A., & Doran, J. W. (1998). Assessment of weed and crop fitness in cover crop residues for integrated weed management. *Weed Science, 46*(5), 595–603. https://doi.org/10.1017/s0043174500091153

Ziska, L. H., Teasdale, J. R., & Bunce, J. A. (1999). Future atmospheric carbon dioxide may increase tolerance to glyphosate. *Weed Science*, *47*(5), 608–615. https://doi.org/10.1017/s0043174500092341

Table 3-1: 2021 herbicide programs (PRE and POST). Reported by site with active ingredient rate in grams ai/ae ha^{-1}. Corresponding brand of herbicide with trade name and herbicide groups present included. Herbicides with residuals are reported with the letter "R" after active ingredient.

Location	**2021 Crop**	**PRE Herbicide(s) Active Ingredient Rate (g ai/ae ha^{-1}) (R = Residual Herbicide)**	**Trade Name(s)**	**Herbicide Groups**	**POST Herbicide(s) Active Ingredient Rate (g ai/ae ha^{-1}) (R = Residual Herbicide)**	**Trade Name(s)**	**Herbicide Groups**
Colfax	Corn	2123 ae glyphosate, 6.53 ae 2,4-dichloro-phenoxyacetic acid, 1157 ai metolachlor (R), 148 ai mesotrione (R), 1134 ai atrazine	Roundup PowerMAX 2,4-D Ravine	4, 5, 9, 15, 27	109 ai Mesotrione (R), 1315 ai S-metalachlor (R), 9 ai 3,6-dichloro-2-methoxybenzoic acid (R), 36 ai difluenzopyr, 844 ae glyphosate	Bellum Medal II Status Roundup	4, 9, 15, 19, 27
Greeley	Soybean	585 ae 2-ethylhexyl ester of 2,4-dichlorophenoxyacetic acid, 41 ai flumioxazin (R), 41 pyroxasulfone (R)	2,4-D LV6 Fierce	4, 14, 15	567 ae 3,6 dichloro-o-anisic acid (R)	XtendiMax	4
Howard	Corn	585 ai atrazine (R), 585 ai S-metolachlor (R), 77 ai mesotrione (R), 272 ae 3,6-dichloro-o-anisic acid (R), 1125 ae glyphosate	Lexar EZ Diflexx Durango DMA	4, 5, 9, 15, 27	363 ae 3,6 dichloro-o-anisic acid (R), 1125 ae glyphosate	Diflexx Durango DMA	4, 9
Merrick	Corn	50 ai saflufenacil (R), 431 ai dimethenamid-P (R), 1102 ae glyphosate	Verdict Buccaneer Plus	9, 15	658 ai glufosinate-ammonium	Liberty	10
ENREC	Corn	1846 ai glyphosate, 6378 ae 2-ethylhexyl ester of 2,4-dichloro-phenoxyacetic acid	Roundup WeatherMAX 2,4-D LV4 Ester	4, 9	744 ai glufosinate-ammonium	Liberty	10
SCAL	Corn	1923 ai glyphosate	Roundup PowerMAX	9	1547 ae glyphosate, 1733 ai S-metolachlor (R), 286 ai atrazine (R), 18 ai bicyclopyrone (R), 68 ai mesotrione, 612 ai S-metolachlor	Roundup PowerMAX Medal II EC Acuron	5, 9, 15, 27

Figure 3-1: Winter cover crop biomass by location and treatment. Biomass is reported in terms of megagrams of dry plant matter (DM) ha^{-1}.

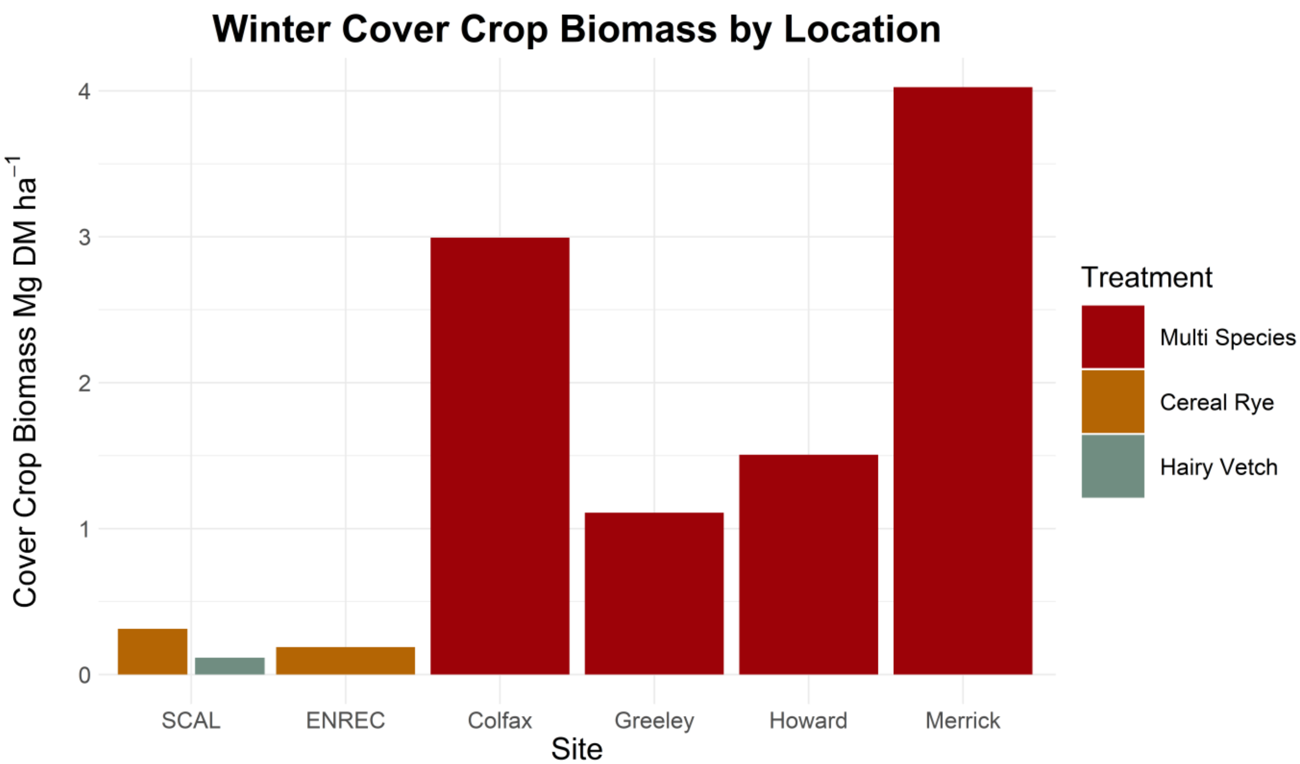


Figure 3-2: Total emerged weed density by site and sample period. Mean estimates are least squares means and error bars represent standard error of the mean. Asterisks represent level of significance with *, **, ***, **** representing levels of $p < 0.1$, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Estimates are reported in weeds m^{-2}. Colfax County data is not reported in graph due to no weeds present during the growing season. Merrick County did not have data in the late growing season due to a storm which lodged the corn field.

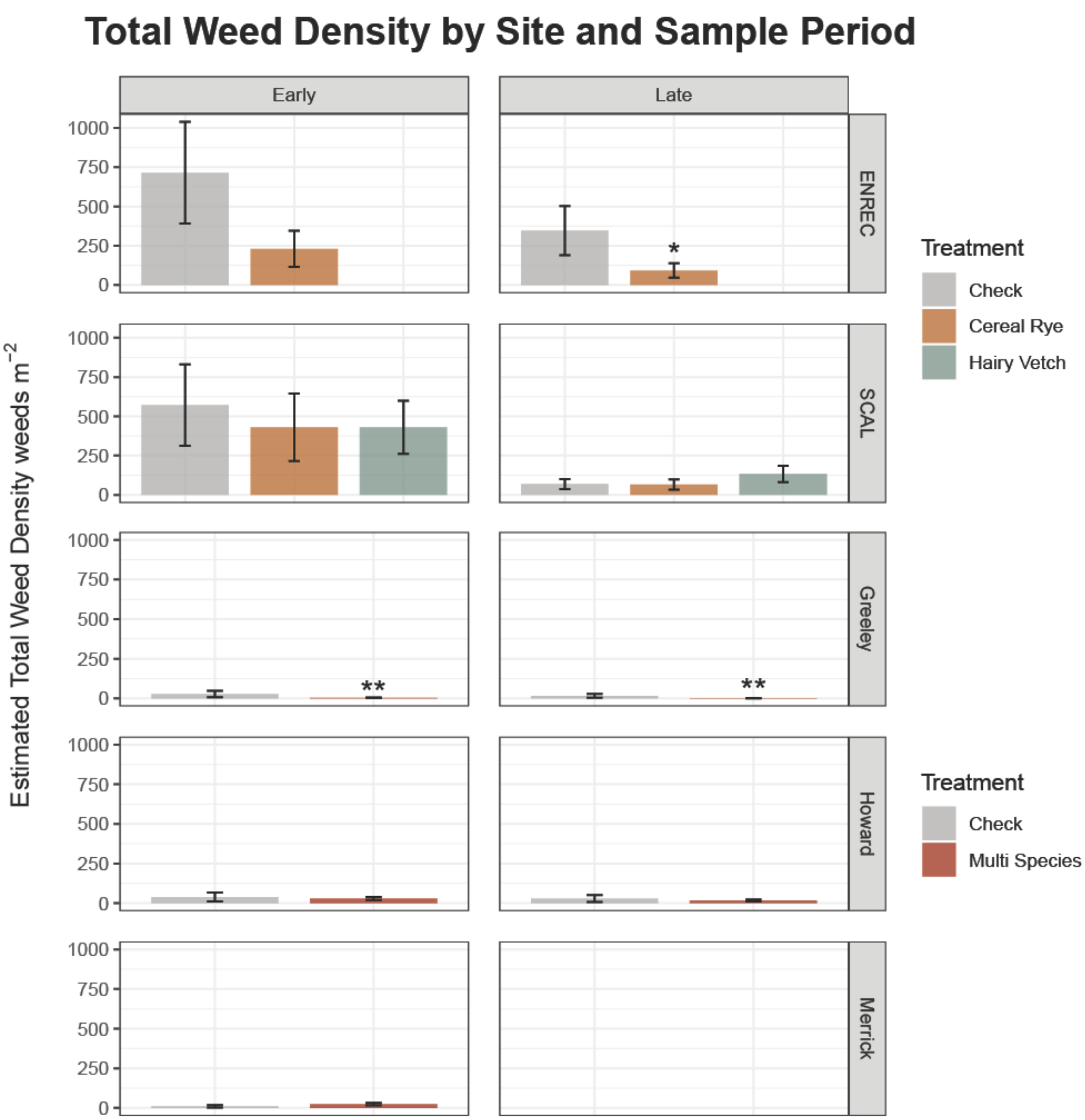


Figure 3-3: Emerged pigweed density by site and sample period. Mean estimates are least squares means and error bars represent standard error of the mean. Estimates are reported in weeds m^{-2}. Colfax County data is not reported in graph due to no weeds present during the growing season. Merrick County did not have data in the late growing season due to a storm which lodged the corn field. Greeley County did not find pigweeds in the late growing season.

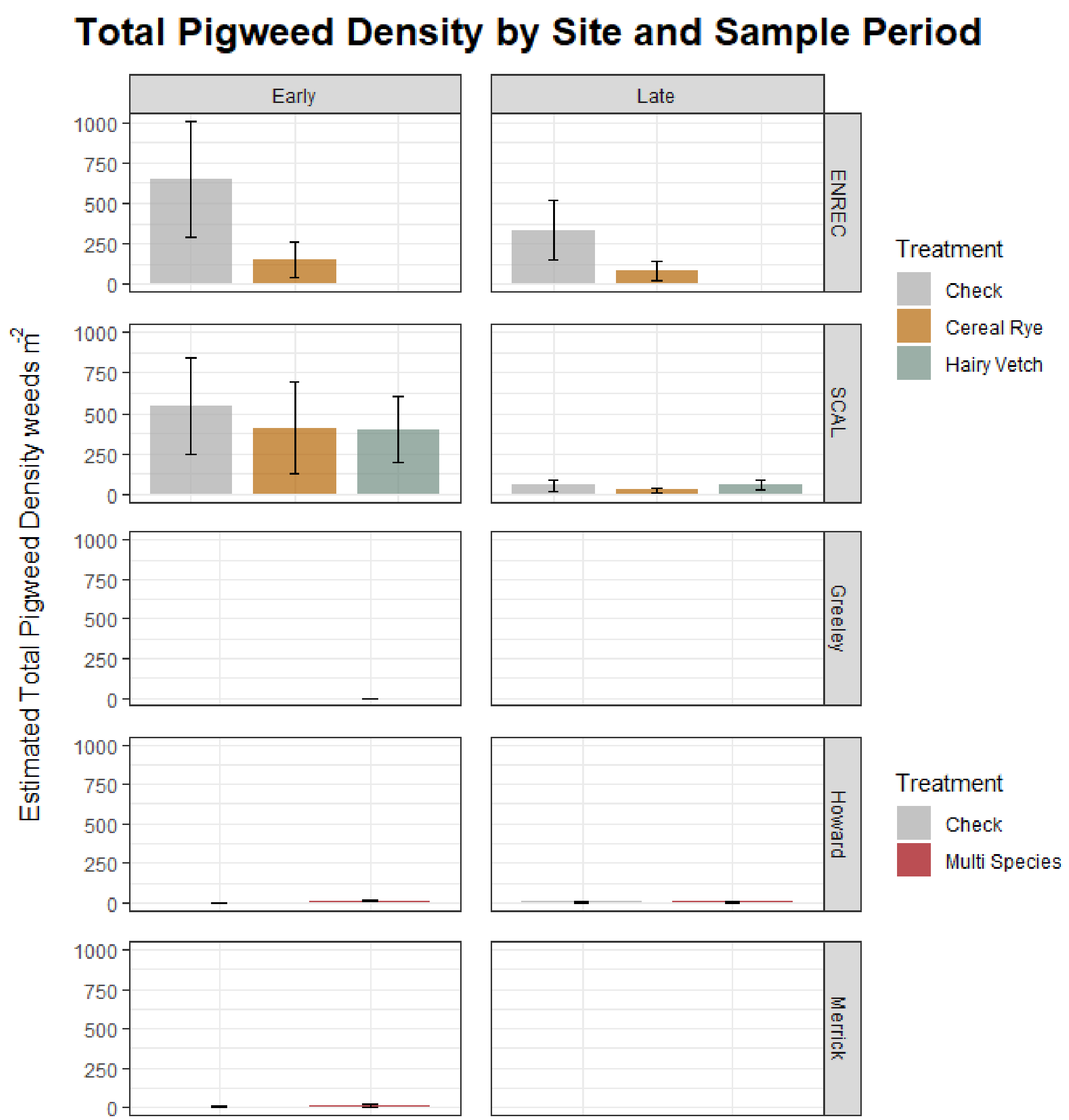


Figure 3-4: Emerged grass density by site and sample period. Mean estimates are least squares means and error bars represent standard error of the mean. Asterisks represent level of significance with *, **, ***, **** representing levels of $p < 0.1$, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Estimates are reported in weeds m^{-2}. Colfax County data is not reported in graph due to no weeds present during the growing season. Merrick County did not have data in the late growing season due to a storm which lodged the corn field.

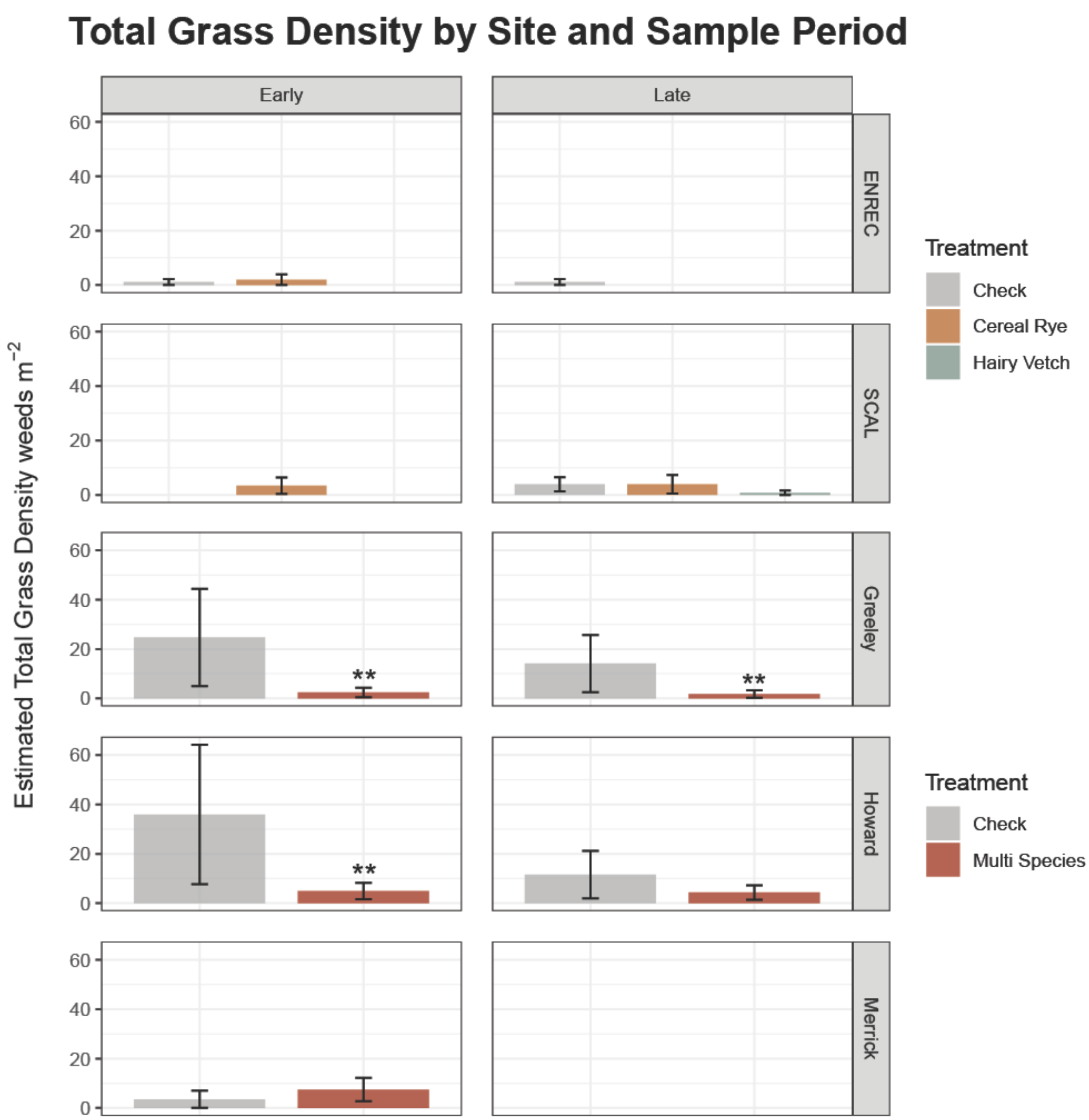


Figure 3-5: Emerged broadleaf density by site and sample period. Mean estimates are least squares means and error bars represent standard error of the mean. Asterisks represent level of significance with *, **, ***, **** representing levels of $p < 0.1$, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Estimates are reported in weeds m^{-2}. Colfax County data is not reported in graph due to no weeds present during the growing season. Merrick County did not have data in the late growing season due to a storm which lodged the corn field.

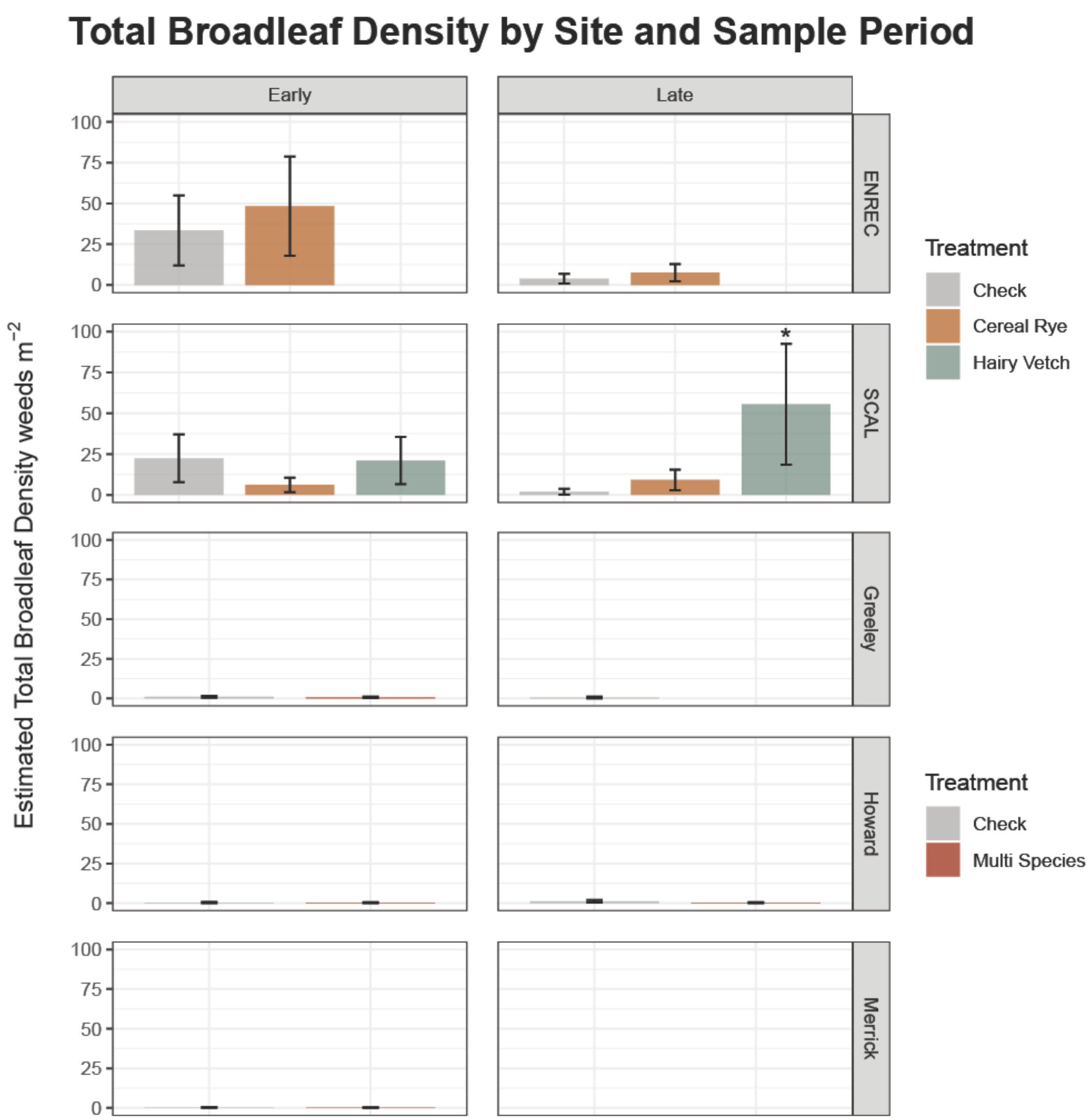


Figure 3-6: Total weed biomass by site and sample period. Mean estimates are least squares means and error bars represent standard error of the mean. Asterisks represent level of significance with *, **, ***, **** representing levels of $p < 0.1$, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Estimates are reported in grams dry matter (DM) m^{-2}. Colfax County data is not reported in graph due to no weeds present during the growing season. Merrick County did not have data in the late growing season due to a storm which lodged the corn field.

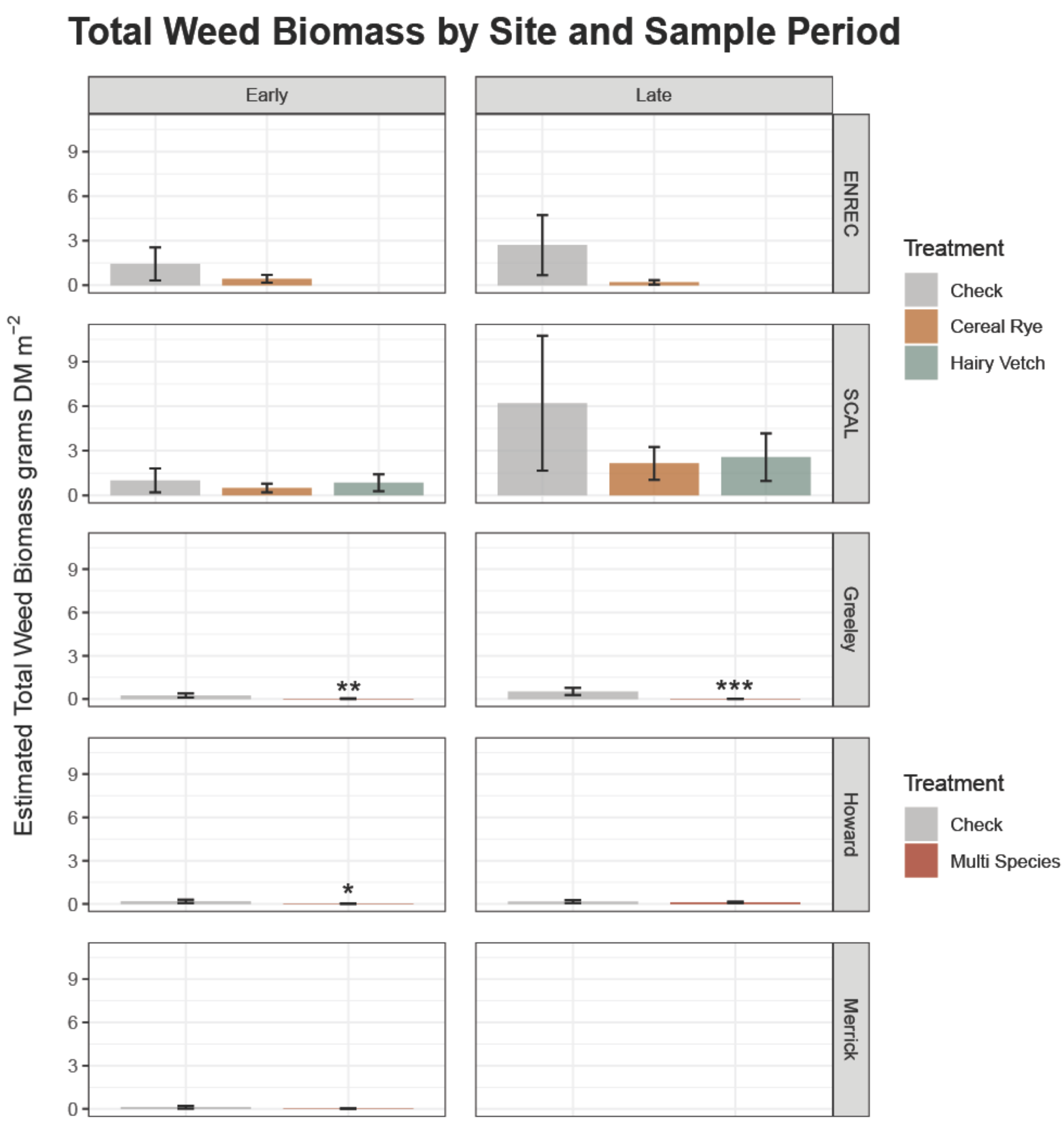


Figure 3-7: Pigweed biomass by site and sample period. Mean estimates are least squares means and error bars represent standard error of the mean. Asterisks represent level of significance with *, **, ***, **** representing levels of $p < 0.1$, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Estimates are reported in grams dry plant matter m^{-2}. Colfax County data is not reported in graph due to no weeds present during the growing season. Merrick County did not have data in the late growing season due to a storm which lodged the corn field. Greeley County did not find pigweeds in the late growing season.

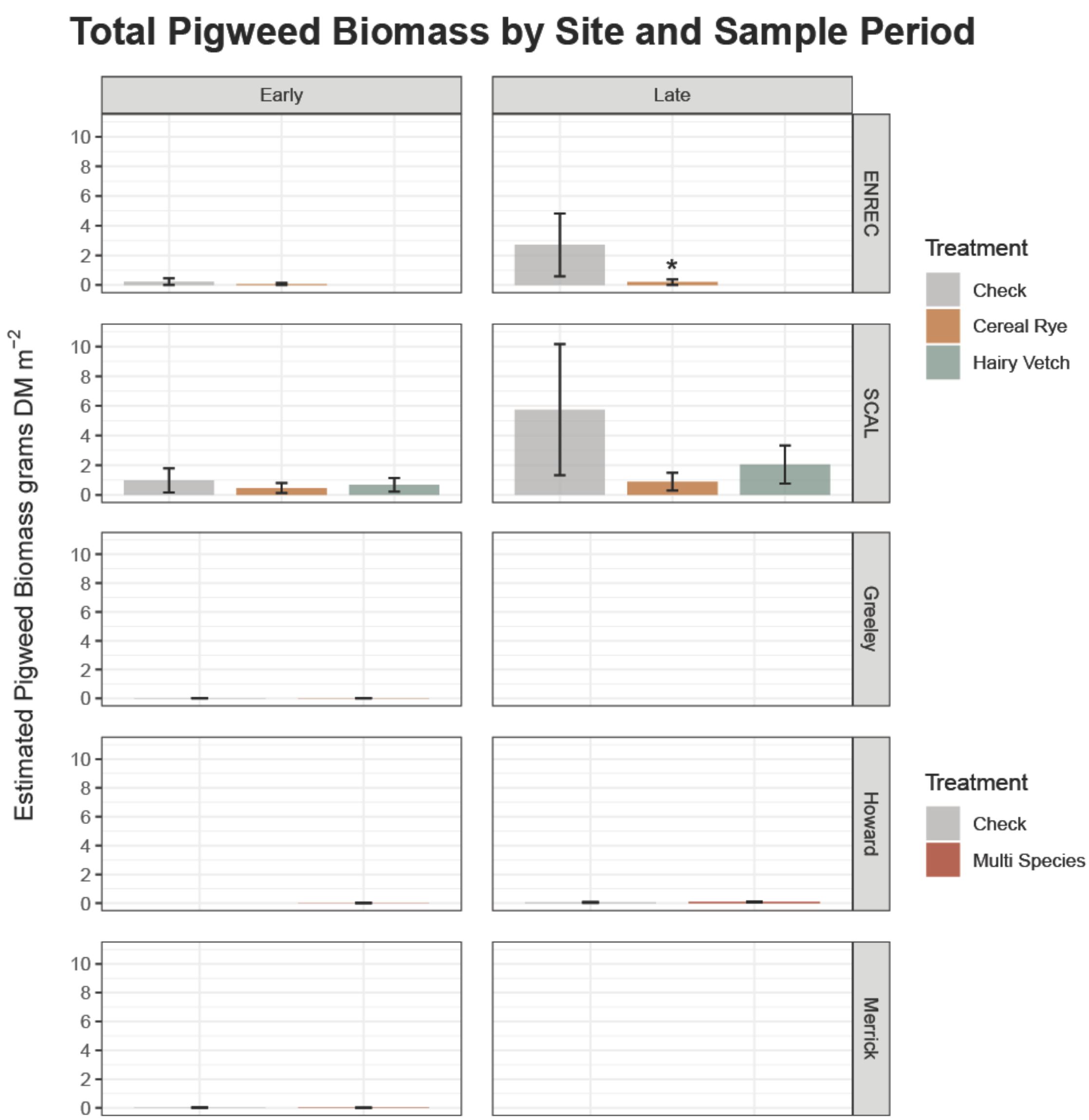


Figure 3-8: Grass biomass by site and sample period. Mean estimates are least squares means and error bars represent standard error of the mean. Asterisks represent level of significance with *, **, ***, **** representing levels of $p < 0.1$, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Estimates are reported in grams dry plant matter m^{-2}. Colfax County data is not reported in graph due to no weeds present during the growing season. Merrick County did not have data in the late growing season due to a storm which lodged the corn field.

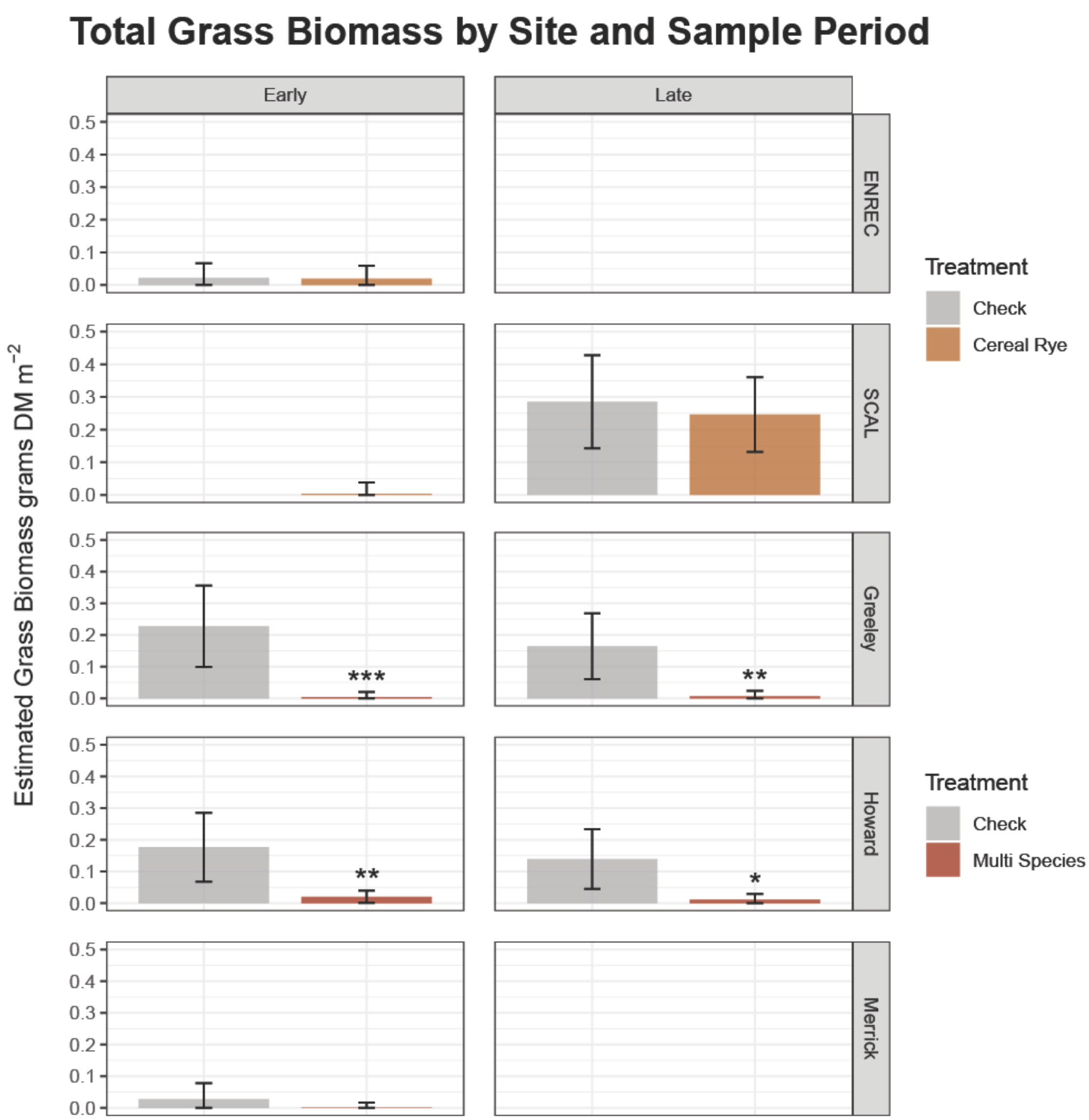


Figure 3-9: Broadleaf biomass by site and sample period. Mean estimates are least squares means and error bars represent standard error of the mean. Asterisks represent level of significance with *, **, ***, **** representing levels of $p < 0.1$, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Estimates are reported in grams dry plant matter m^{-2}. Colfax County data is not reported in graph due to no weeds present during the growing season. Merrick County did not have data in the late growing season due to a storm which lodged the corn field. ENREC observed no grasses in the late season.

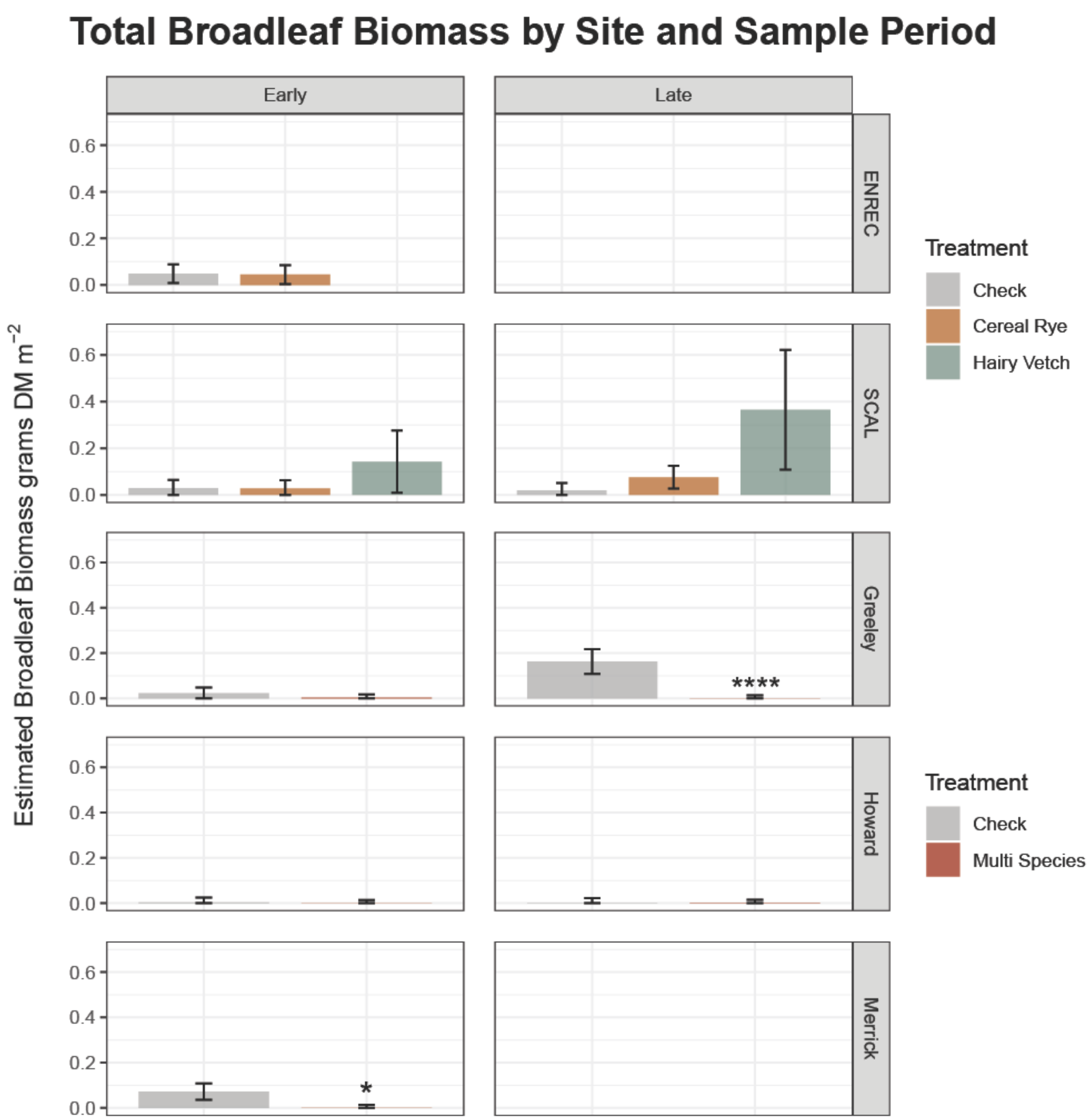


Table 3-2: Soil temperature by site and sample period. Mean estimates are least squares means with associated standard error. Letters denote significant difference at a level of $p < 0.1$. Estimates are reported in degrees Celsius. Merrick County did not have data in the late growing season due to a storm which lodged the corn field.

Site	Treatment	Early Soil Temperature (SE)	Late Soil Temperature (SE)
Colfax	Multi Species	21.8(1.98) A	26.5(1.98) A
	Check	21.7(2.11) A	26.5(2.11) A
Greeley	Multi Species	34.1(1.98) A	28.5(1.98) A
	Check	30.2(2.11) B	26.4(2.11) A
Howard	Multi Species	32.1(1.98) A	23.4(1.98) A
	Check	30.3(2.11) A	23.9(2.11) A
Merrick	Multi Species	30.3(1.98) A	NA
	Check	32.7(2.11) A	NA
ENREC	Cereal Rye	12.8(2.67) A	24.6(2.67) A
	Check	13.3(2.04) A	24.8(2.04) A
SCAL	Cereal Rye	30.1(2.67) A	27.1(2.67) A
	Hairy Vetch	32.3(1.39) A	26.6(1.39) A
	Check	30.6(2.04) A	26.9(2.04) A

Table 3-3: Soil moisture by site and sample period. Mean estimates are least squares means with associated standard error. Letters denote significant difference at a level of p < 0.1. Estimates are reported in percent volumetric water content (%VWC). Merrick County did not have data in the late growing season due to a storm which lodged the corn field.

Site	Treatment	Early Soil Moisture (SE)	Late Soil Moisture (SE)
Colfax	Multi Species	45.0(2.45) A	23.9(2.45) A
	Check	45.3(1.77) A	18.5(1.77) A
Greeley	Multi Species	29.2(2.45) A	24.7(2.45) A
	Check	33.1(1.77) A	23.9(1.77) A
Howard	Multi Species	45.2(2.45) A	40.8(2.45) A
	Check	45.7(1.77) A	43.8(1.77) A
Merrick	Multi Species	17.9(2.45) A	NA
	Check	19.2(1.77) A	NA
ENREC	Cereal Rye	41.4(2.63) A	25.0(2.63) A
	Check	41.8(2.07) A	22.6(2.07) A
SCAL	Cereal Rye	44.4(2.63) A	34.8(2.63) A
	Hairy Vetch	43.4(3.59) A	28.3(3.59) A
	Check	42.5(2.07) A	32.4(2.07) A

CHAPTER 4: CONCLUSION

The objective of my thesis sought to answer how cover crops influence above and belowground weed dynamics. I addressed this question through two separate experiments, one for cover crop impacts on aboveground weeds and a second to address belowground weeds in the weed seedbank.

In Chapter 2, we sampled the soil seedbank at six sites in eastern and central Nebraska and germinated out seeds from the soil samples for seven months in the greenhouse. We estimated the total seedbank density as well as the seedbank composition by species, which allowed us to utilize seedbank diversity metrics. Primarily, we focused on functional groups of weeds such as pigweeds, grasses, and other broadleaves. We found that while total seedbank size was not influenced by cover crops, the number of pigweed seeds in the cover crop seedbank was significantly greater at three sites and four cover crop treatments. Surprisingly, the increases in pigweeds did not drive increases in the total seedbank density, suggesting that pigweeds are sensitive to cover cropping and increases in pigweed seedbank density may be indicative of reduced germination withdrawals. Additionally, we utilized Shannon diversity, species richness, and species evenness to assess seedbank composition. This revealed that while increases in pigweeds occurred, species richness increased at all sites in the cover crop, although results were not statistically different. This trend may suggest that cover crops may positively influence the species diversity in seedbanks, which has positive weed and crop management benefits as well as positive ecological implications.

In Chapter 3, I assessed cover crop impacts on aboveground weeds through assessments of emerged weed density and biomass at two critical points in the cash crop growing season. We categorized total weed density and biomass into functional groups of pigweeds, grasses, and other broadleaves. Furthermore, we took samples of cover crop biomass, soil temperature, and soil moisture as supplemental data to provide possible explanations on aboveground weed dynamics. We found that weed density was not consistently impacted by cover crops across our experimental sites compared to weed biomass, which was reduced between 15 to 98% at all sites and sampling periods. Weed density and weed biomass reductions occurred at two sites. More importantly, there were no differences in aboveground pigweed densities despite significant increases in the cover crop at three sites, which may indicate that cover crops are potentially suppressing pigweed seed germination. This is an insightful finding that complements the data found in Chapter 2.

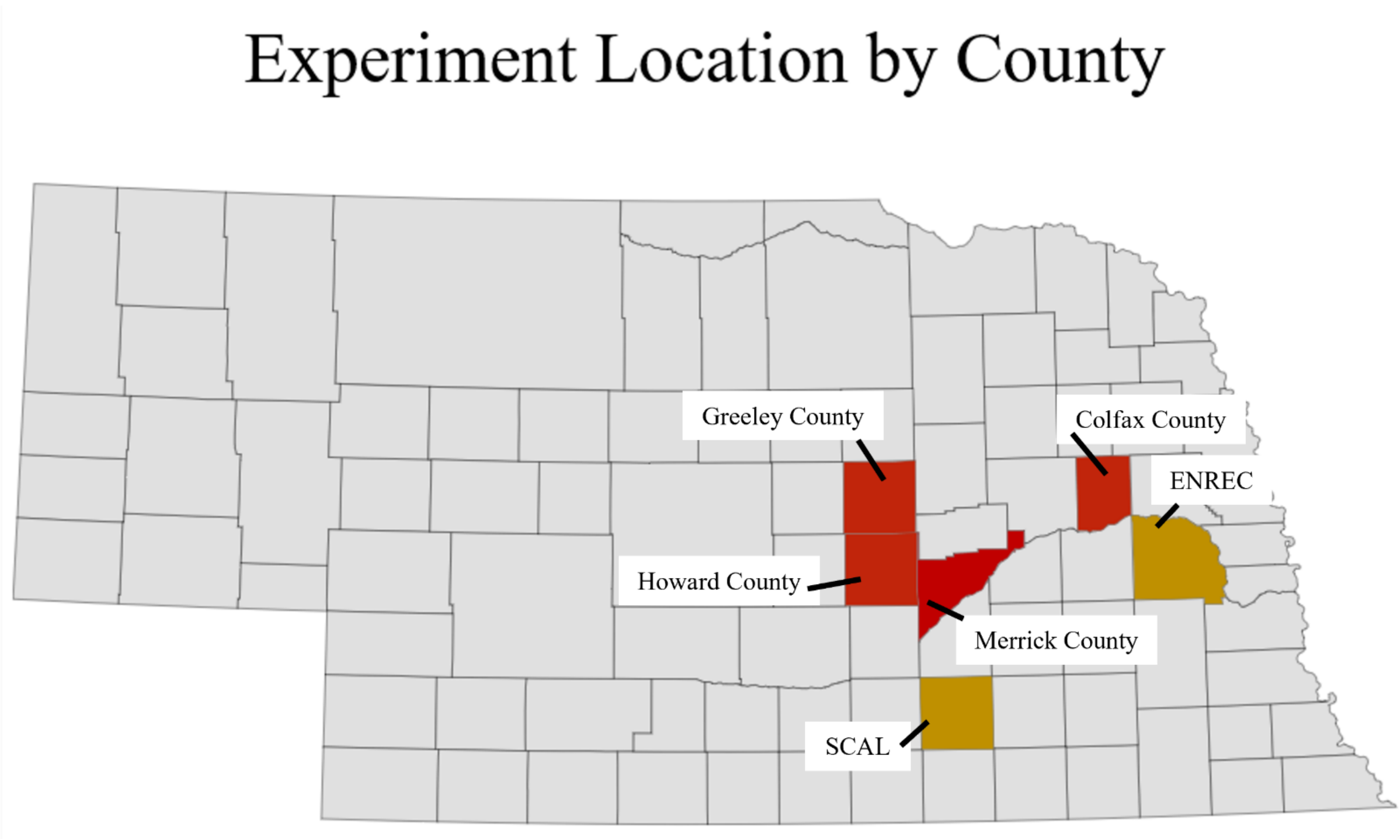
Together, Chapter 2 and Chapter 3 provide a more comprehensive understanding of cover crop impacts on weed dynamics. Sampling the soil weed seedbank allows us to understand the weed community from which aboveground weeds emerge, therefore helping us understand aboveground weed expressions. The results of this study regarding seedbank suppression can provide insights on cover crops influence on above and belowground weed communities in the context of herbicide-managed crop systems in the Corn Belt. Our results pertaining to the suppression of the pigweed seedbank show that cover crops may be a viable integrated weed management tool to suppress pigweed seedlings and mitigate herbicide resistance risks driven by these *Amaranthus* spp. Future studies regarding the weed suppression abilities of cover crops should include above and belowground experiments as it provides a more comprehensive approach to understanding weed and species dynamics.

Appendix

Appendix 1: Experiment site location, soil series, irrigation and tillage management, and rainfall overview.

Location	Coordinates	Primary Soil Series and Texture	Irrigation Type	Tillage Practice	30 Year Annual Average Precipitation (mm)
Colfax	41˚33’N -96˚57’W	Moody silty clay loam	Rainfed	No-till	725.9
Greeley	41˚36’N -98˚40’W	Gates silt loam, Hersh fine sandy loam	Pivot	No-till	650.0
Howard	41˚10’N -98˚34’W	Holdredge silty clay loam	Pivot	No-till	677.2
Merrick	41˚05’N -98˚19’W	Thurman loamy fine sand, Kenesaw silt loam	Pivot	Strip till	677.2
ENREC	41˚09’N -96˚24W	Sharpsburg silty clay loam	Rainfed	No-till	768.1
SCAL	40˚ 34’N -98˚ 08’W	Hastings silt loam	Pivot	No-till	769.9

Appendix 2: Map of experiment locations by county. Red represents on-farm trials and yellow represents research stations.



Appendix 3: Experimental design information, first year of cover crop establishment, species, and biomass, and previous and current crop rotation.

Location	Year Cover Crops Planted	Number of Replications (n)	Average Plot Size	Cover Crop Species	2021 Mean CC Biomass (Mg ha^{-1})	2021 Crop	2020 Crop
Colfax	2017	4	40 m x 623 m	Multi-species mix (cereal rye, radish, forage collards, winter peas, winter lentils, sunn hemp, buckwheat, spring oats, pearl millet, camelina)	2.99	Corn	Wheat
Greeley	2017	4	74 m x 412 m	Multi-species mix (oats, sorghum, pearl millet, radish, forage collards, rapeseed, buckwheat, mustard, sunn hemp, mung bean, winter pea, soybean)	1.10	Soybean	Rye
Howard	2016	4	52 m x 573 m	Multi-species mix (winter rye, radish, rapeseed, turnips, kale, lentils, Austrian winter peas, vetch)	1.51	Corn	Rye
Merrick	2017	4	24 m x 670 m	Multi-species mix (proso millet, grain sorghum, black oats, winter barley, flax, safflower, cowpeas, buckwheat, forage collards, canola, sunn hemp, sunflower)	4.02	Corn	Field Pea
ENREC	2014	3	4.5 m x 9 m	Rye	0.188	Corn	Soybean
SCAL	2014	3	6 m x 9 m	Rye or hairy vetch	0.314 (rye) 0.115 (hairy vetch)	Corn	Soybean

Appendix 4: Experiment sample dates by location for seedbank and aboveground weeds.

Location	**Seedbank Sampling Date**	**Early Season Weed Sampling Date**	**Late Season Weed Sampling Date**
Colfax	April 9, 2021	May 28, 2021	July 7, 2021
Greeley	April 16, 2021	June 3, 2021	July 6, 2021
Howard	April 15, 2021	June 2, 2021	July 8, 2021
Merrick	April 12, 2021	June 1, 2021	NA – Storm Damage
ENREC	March 31, 2021	May 27, 2021	July 3, 2021
SCAL	April 7, 2021	May 26, 2021	July 2, 2021

Appendix 5: Temperature and Rainfall Data by Site

A) Temperature and rainfall data for Colfax (Columbus, NE weather station).

Month (Sept. 2020-Aug. 2021)	Minimum Temperature (C)	Maximum Temperature (C)	Avg. Temperature (C)	30 Year Avg. Temperature (C)	Monthly Precipitation (mm)	30 Year Avg. Precipitation (mm)
September	2.2	36.7	17.3	18.9	32.3	61.2
October	-9.4	30.6	8.5	11.2	15.7	60.2
November	-11.7	28.3	5.5	3.3	37.8	30.2
December	-20.0	16.7	-2.3	-2.8	4.3	22.9
January	-15.0	14.4	-2.2	-4.8	17.0	16.5
February	-35.6	11.1	-10.0	-2.8	2.0	17.8
March	-5.0	25.0	7.1	4.0	171.5	40.1
April	-7.8	30.6	9.9	10.3	33.0	73.4
May	0.0	31.1	15.6	16.7	51.8	114.3
June	7.8	38.9	24.1	22.6	86.9	118.1
July	13.3	35.6	23.9	24.7	54.9	87.4
August	12.2	34.4	19.7	23.4	91.2	83.8
					Total: 598.4	*Total: 725.9*

B) Temperature and rainfall data for Greeley (Greeley, NE weather station)

Month (Sept. 2020-Aug. 2021)	Minimum Temperature (C)	Maximum Temperature (C)	Avg. Temperature (C)	30 Year Avg. Temperature (C)	Monthly Precipitation (mm)	30 Year Avg. Precipitation (mm)
September	-1.1	35.0	17.3	16.0	24.6	57.9
October	-11.7	31.7	8.5	7.8	25.4	51.1
November	-15.6	28.9	5.5	5.3	23.4	23.1
December	-18.9	18.9	-2.3	-1.3	21.8	16.3
January	-19.4	14.4	-2.2	-2.6	14.7	11.7
February	-36.7	13.3	-10.0	-10.8	10.9	14.5
March	-8.9	25.6	7.1	5.2	180.3	39.6
April	-10.0	28.9	9.9	7.8	39.4	64.8
May	-2.8	32.2	15.6	14.8	83.3	106.4
June	6.7	38.9	24.1	22.5	22.1	99.6
July	10.6	36.1	24.1	23.1	92.5	81.3
August	10.6	35.6	23.9	23.1	120.7	83.8
					Total: 659.1	*Total: 650.0*

C) Temperature and rainfall data for Howard and Merrick (Grand Island, NE weather station)

Month (Sept. 2020-Aug. 2021)	Minimum Temperature (C)	Maximum Temperature (C)	Avg. Temperature (C)	30 Year Avg. Temperature (C)	Monthly Precipitation (mm)	30 Year Avg. Precipitation (mm)
September	2.2	36.7	18.4	18.9	31.5	50.3
October	-7.8	32.8	10.2	11.4	2.0	50.5
November	-11.1	29.4	7.4	3.9	29.5	27.4
December	-15.6	20.6	0.2	-2.0	30.5	20.8
January	-16.7	17.8	-0.7	-3.7	33.5	16.0
February	-32.8	12.2	-9.2	-1.9	20.3	18.8
March	-5.0	26.7	7.8	4.6	219.7	41.1
April	-4.4	34.4	10.8	10.2	36.3	63.0
May	0.6	32.2	16.3	16.3	73.4	117.9
June	11.1	41.1	24.6	22.3	47.0	100.1
July	13.9	36.7	25.0	24.6	72.4	88.6
August	12.8	35.6	24.6	23.4	115.1	82.6
					Total: 711.2	*Total: 677.2*

D) Temperature and rainfall data for ENREC (Mead, NE weather station)

Month (Sept. 2020-Aug. 2021)	Minimum Temperature (C)	Maximum Temperature (C)	Avg. Temperature (C)	30 Year Avg. Temperature (C)	Monthly Precipitation (mm)	30 Year Avg. Precipitation (mm)
September	4.4	35.0	17.4	18.4	48.8	78.7
October	-8.9	30.6	8.5	11.3	17.3	57.7
November	-10.6	27.2	6.1	3.5	26.9	33.5
December	-18.9	16.1	-1.8	-2.7	24.6	27.7
January	-17.8	11.1	-2.9	-5.3	19.6	15.5
February	-33.3	10.6	-10.2	-3.2	13.2	19.6
March	-3.9	25.0	7.0	3.8	123.7	41.4
April	-8.3	32.8	9.5	9.9	50.5	73.2
May	0.5	30.0	15.5	16.1	110	118.6
June	9.4	37.8	23.2	22.0	101.6	124.5
July	13.3	35.6	23.6	24.0	71.9	79.2
August	10.6	34.4	23.3	22.8	184.9	98.6
					Total: 793	*Total: 768.1*

E) Temperature and rainfall data for SCAL (Clay Center, NE weather station)

Month (Sept. 2020-Aug. 2021)	Minimum Temperature (C)	Maximum Temperature (C)	Avg. Temperature (C)	30 Year Avg. Temperature (C)	Monthly Precipitation (mm)	30 Year Avg. Precipitation (mm)
September	2.2	35.0	17.6	18.6	30.7	68.6
October	-9.4	31.1	9.4	11.6	6.4	65.3
November	-10.0	27.8	7.3	4.2	41.7	30.7
December	-13.9	19.4	-0.1	-1.8	15.7	30.0
January	-18.3	16.1	-0.9	-3.8	33.5	10.9
February	-32.8	11.7	-9.6	-2.0	15.5	23.1
March	-3.3	25.0	7.3	4.3	162.6	39.4
April	-3.9	33.3	9.8	9.9	41.1	66.0
May	2.8	30.6	15.9	16.1	145.0	133.9
June	11.7	38.9	23.6	22.2	59.2	102.4
July	15.0	35.0	23.9	24.3	57.4	103.9
August	13.9	35.6	24.2	23.1	53.6	95.8
					Total: 662.4	*Total: 769.9*

Appendix 6: Previous crop (2020) and current crop (2021) fertilizer applications

Site	Previous Crop 2020 Fertilizer Applications	Current Crop 2021 Fertilizer Applications
Colfax	100 lb/a 11-52-0 30 gal/a 32% UAN	4000 gal hog manure (broadcasted across field) 7.5 gal/a 6-24-6 10 gal/a 32% UAN (aka 35.5 lb/a N) 40 gal/a 32% UAN Y-drop
Greeley	20 lb/a 32% UAN 10 lb/a thiosulfate (pivot applied)	75 lb/a MAP 11-25-0 50 lb/a Potash
Howard	117 lb/a 11-52-0 86 lb/a K-mag 27 lb/a Pel-lime 2 lb/a 36% Zinc	117 lb/ac 11-52-0 85 lb/ac K-Mag® 3 lb/ac of Zinc 26 lb/ac of Pel-lime 60 gal/ac UAN 2 gal/ac Thio-Sul®
Merrick	None (legume cash crop planted)	100 lb/a 0-0-60 20 gal/a 10-34-0-1 Zn 65 gal/a 28-0-0-5

Appendix 7: Table of past five years of crop rotation. Rotations are predominantly corn-soybean rotation. On-farm sites were diversified with a small grain or cool season legume in 2020.

Site	2017	2018	2019	2020	2021
Colfax	Soybean	Corn	Soybean	Wheat	Corn
Greeley	Soybean	Corn	Soybean	Rye	Soybean
Howard	Corn	Corn	Soybean	Rye	Corn
Merrick	Corn	Corn	Soybean	Field Pea	Corn
ENREC	Corn	Soybean	Corn	Soybean	Corn
SCAL	Corn	Soybean	Corn	Soybean	Corn

Appendix 8: Cash crop previous harvest and current plant dates, and cover crop planting and termination dates. Number of days fallow represents the number of days between cash crop harvest and winter cover crop planting in the summer or fall of 2020.

Site	Previous Crop Harvest Date	Cover Crop Planting Date	Number of Days Fallow	Cover Crop Termination Date	Current Crop Plant Date
Colfax	7/21/2020	8/6/2020	16	4/30/2021	4/30/2021
Greeley	7/25/2020	8/8/2020	14	4/28/2021	5/8/2021
Howard	7/23/2020	8/5/2020	13	5/4/2021	4/26/2021
Merrick	7/18/2020	7/25/2020	7	5/7/2021	5/22/2021
ENREC	10/8/2020	9/8/2020	0	4/3/2021	5/14/2021
SCAL	10/12/2020	11/6/2020	29	5/10/2021	5/6/2021