ESTABLISHMENT OF WILDFLOWER ISLANDS TO ENHANCE ROADSIDE HEALTH, ECOLOGICAL VALUE, AND AESTHETICS: PHASE II

FINAL REPORT

Jackson Ebbers[1], Heidi Hillhouse[1], John Guretzky[1], and Tom Weissling[2]

[1]Department of Agronomy and Horticulture

University of Nebraska-Lincoln

[2]Department of Entomology

University of Nebraska-Lincoln

Sponsored By

Nebraska Department of Transportation and U.S. Department of Transportation Federal Highway Administration

March 31, 2023

NEBRASKA
Good Life. Great Journey.
DEPARTMENT OF TRANSPORTATION

Technical Report Documentation Page

1. Report No. SPR-FY21(011)	2. Government Accession No.	3. Recipient's Catalog No.
4. Title and Subtitle Establishment of Wildflower Islands to Enhance Roadside Health, Ecological Value, and Aesthetics Phase II		**5. Report Date** March 31, 2023
		6. Performing Organization Code
7. Author(s) Jackson Ebbers, Heidi Hillhouse, John Guretzky, and Tom Weissling		**8. Performing Organization Report No.** If applicable, enter any/all unique numbers assigned to the performing organization.
9. Performing Organization Name and Address Department of Agronomy and Horticulture University of Nebraska-Lincoln 297 Plant Sciences Hall Lincoln, NE 68583-0915		**10. Work Unit No.**
		11. Contract # 26-6222-1007-001
12. Sponsoring Agency Name and Address Nebraska Department of Transportation Research Section 1400 Hwy 2 Lincoln, NE 68502		**13. Type of Report and Period Covered** Final Report July 1, 2020 – March 31, 2023.
		14. Sponsoring Agency Code

15. Supplementary Notes

If applicable, enter information not included elsewhere, such as translation of (or by), report supersedes, old edition number, alternate title (e.g. project name), or hypertext links to documents or related information.

16. Abstract

Roadsides play an ever-increasing role in sustaining biodiversity. In 2020, we launched a project on backslopes along Highways 2 and 77 evaluating seeding and mowing treatments to enhance beneficial forbs including milkweed (*Asclepias* spp.) species. Analyses showed that seeding increased forb diversity, total forb cover, and floristic quality. However, mowing effects were less clear. Forb cover was higher on plots that were mowed in October 2020 before seeding, but effects of pre-mowing varied with site. The combination of pre-mowing and seeding was most effective at increasing floristic quality of the Highway 2 site. However, mowing after seeding in early July did not improve forb establishment. Accumulation of dead plant matter on the soil surface (i.e., litter) appeared to have a strong impact on forb density in the first year of the study. This research provides information on the limitations and opportunities in planting native wildflower seed into roadsides with previously established vegetation. Insight from this report will help guide recommendations and future research on the best management practices for Nebraska roadsides.

17. Key Words diversity, grasses, forbs, insects, plants, milkweed, mowing, pollinators, seeding, sunflower, vegetation	**18. Distribution Statement** No restrictions. This document is available through the National Technical Information Service. 5285 Port Royal Road Springfield, VA 22161		
19. Security Classification (of this report) Unclassified	**20. Security Classification (of this page)** Unclassified	**21. No. of Pages** 46	**22. Price**

Form DOT F 1700.7 (8-72)   Reproduction of completed page authorized

DISCLAIMER

The contents of this report reflect the views of the authors, who are responsible for the facts and the accuracy of the information presented herein. The contents do not necessarily reflect the official views or policies neither of the Nebraska Department of Transportations nor the University of Nebraska-Lincoln. This report does not constitute a standard, specification, or regulation. Trade or manufacturers' names, which may appear in this report, are cited only because they are considered essential to the objectives of the report.

Table of Contents

List of Figures and Tables

Figure 1. The locations of the two study sites are displayed. The Highway 77 site (yellow) is located south of Cortland, Nebraska, and the Highway 2 site (blue) is located west of Nebraska City, Nebraska.

Figure 2. Eight treatment combinations implemented at Highway 77 and Highway 2 study sites. Treatments include various combinations of three treatment factors: pre-mowed and not pre-mowed, seeded and un-seeded, and year-after-mowed and not year-after-mowed.

Figure 3. Two mowing treatments were completed in October 2020 and early July 2021. (A) shows plots that were mowed in October 2020 while vegetation was dormant, and (B) shows the tractor and mower finishing a plot in the early July mowing.

Figure 4. The seeding treatment conducted in November 2020 is shown. (A) shows the equipment used to put seed in the ground in a previously mowed plot, and (B) shows an un-mowed plot where seed was drilled into the existing vegetation.

Figure 5. Photographs of vegetation sampling for cover using the 0.5 x 0.5-meter frame. Twelve frames per plot were used across 64 plots at two sites. (A) shows vegetation being observed in June 2021 following seeding in November 2020, and (B) shows a frame in a seeded plot in August 2022.

Figure 6. The plot setup is shown for vegetative litter sampling. Transects were randomized along the 18-foot plot edge. Fifteen measurements were taken on each of the three transects. A 2.5-foot margin was not sampled at each end of the plot.

Figure 7. Main effect of the seeding in November 2020 on total forb cover in summer 2021. The cover values are an average of plots within the two seeding treatments. Averages are obtained from the sum of cover for each forb species within a plot. Error bars represent the standard error of the sample. Both sites showed an increase in total forb cover on average in plots that were seeded. Only Highway 2 has a significant effect on forb cover from seeding (P = 0.01).

Figure 8. Main effect of pre-mowing in October 2021 on total forb cover in summer 2021. Both sites appear to show an increase in total forb cover in response to mowing before seeding, but only the Highway 77 site has a significant effect on total forb cover with pre-mowing (P = 0.03).

Figure 9. Main effect of seeding wildflowers on the total forb cover observed in summer 2022, the second season after planting and applying mowing treatments. The values are an average for both seeding treatments of the sum of forb species cover for each plot. Total forb cover remained higher in seeded plots one year after planting. The Highway 2 site had a significant effect of seeding wildflowers (P = 0.02).

Figure 10. Main effect of the pre-mowing treatment on the percentage of seeded forb cover for summer 2021. Only species included in the seed mix (Table 1) are included. The cover values are an average of the plots within the two pre-mowing treatments. The two highways are shown separately. Only Highway 77 had a significant effect of pre-mowing on seeded forb cover. The error bars represent standard error. Samples were collected from August to September of 2021.

5

Figure 11. Main effect of seeding wildflowers on the percent cover of forbs within the planting mix (Table 2.1) in summer 2022. Though both sites had an increase in the percentage of forb cover of seeded species, only Highway 2 had a significant effect of seeding (P = 0.01). Seeding occurred in November 2021. These data were obtained in August 2022.

Figure 12. Main effect of year-after mowing is shown for both sites in Summer 2022. This mowing treatment was implemented in early July 2021. Highway 77 does not appear to be affected by year-after mowing. The Highway 2 site shows some negative impacts to seeded species cover from mowing the previous summer, however, this is not significant (0.06).

Figure 13. Main effect of seeding on species richness for both sites in Summer 2021. Species richness is a count all plant species found within a plot. Plot species counts were averaged by seeding treatment for each site. Highway 2 had a significant increase in species richness in response to seeding wildflowers (P < 0.01). Seeding effects were weak for Highway 77 (P = 0.18).

Figure 14. Main effect of seeding on species richness for both sites in Summer 2022. Species richness is a count all plant species found within a plot. Plot species counts were averaged by seeding treatment for each site. Highway 2 had a significant increase in species richness in response to seeding wildflowers (P = 0.0002). Seeding effects were weak for Highway 77 (P = 0.092).

Figure 15. Main effect of seeding wildflowers on the floristic quality of both sites for summer 2021 and summer 2022. The floristic quality index for each plot was calculated by multiplying the average coefficient of conservatism value by the square root of the number of species present at that plot. The adjusted index floristic quality index was averaged by seeding treatment. Highway 2 after seeding appears to have a significant increase in average floristic quality in seeded plots (P < 0.01).

Figure 16. Interaction between the pre-mowing and seeding treatments are shown for the Highway 2 site in Summer 2021 for adjusted floristic quality. Average FQI for each treatment is calculated the same as Fig. 2.11. Pre-mowing and seeding wildflowers appear to lead to high floristic quality when compared to seeding without pre-mowing (P = 0.033).

Figure 17. Main effect of July 2021 mowing on grass cover in September 2021. Average percent cover is calculated by averaging the total percent cover of grass species for each plot within the two year-after mowing treatments. Highway 77 appears to have significantly less cover of grasses on plots mowed in July compared to plots not mowed in July of the same year (P < 0.01).

Figure 18. Main effect of pre-mowing on vegetative litter depth in millimeters for Spring 2021 with both sites combined. A plot average litter depth was derived from each measurement along three transects. Plots were then averaged by mowing treatment. The results for both sites were displayed together because both sites had a similar outcome. Data was collected in March of 2021 following the October 2020 pre-mowing treatment. Pre-mowed plots had a lower depth of vegetative litter at both sites compared to plots that were not mowed (P < 0.01).

Figure 19. Main effect of pre-mowing on vegetative litter depth (millimeters) in March 2022. Mowing occurred in October 2020. A plot average litter depth was derived from each

measurement along three transects. Plots were then averaged by mowing treatment. At both sites, pre-mowing appears to reduce the depth of litter present in plots (P < 0.05).

Figure 20. Main effect of year-after mowing on vegetative litter depth (millimeters) in March 2022. Mowing occurred in July 2021. Average litter depth was calculated using the same methods as Figure 2.15. Highway 2 mowed plots have significantly less litter than un-mowed plots (P < 0.01).

Figure 21. Vegetative litter depth and the total percent cover of forbs is compared for 2021. Each point represents a plot. Treatments are not included in the data. Vegetative litter depths on the y-axis were measured in March 2021, and the forb cover data were collected in September 2021. Forb cover appears to increase as vegetative litter depth decreases (r = -0.366, P = 0.003).

Figure 22. Vegetative litter depth and the total percent cover of forbs is compared for 2022. Each point represents a plot. Treatments are not included in the data. Vegetative litter depths on the y-axis were measured in March 2022, and the forb cover data were collected in September 2022. In 2022, there appears to be no correlation between litter depth and the amount of forb cover (r = 0.034, P = 0.79).

Figure 23. The frequency of common milkweed occurrences on seeded and unseeded plots across time is shown. Frequency was determined by the total number of frames containing common milkweed for each treatment at each sampling date. Common milkweed was the only milkweed species considered because it was the only species observed from the seed mix. Seeding occurred after the Summer 2020 sampling date. Milkweed frequency was considerably higher in seeded plots than un-seeded plots in Summer 2021 and Summer 2022.

Figure 24. Vegetative litter is shown in a plot following the early July mowing treatment. While much of the plot has reduced litter, strips of dense litter may be present between and outside the mower blade path.

Table 1. The 33 species of forbs in the wildflower mix are shown. Included is the species name, common name used in this study, functional group, life span, and bloom time.

Table 2. Percentage of each ground cover category observed in March 2021. There were significantly fewer litter observations and more bare ground observations on pre-mowed plots.

Table 3. Percentage of each ground cover category observed in March 2022. Bare ground observations remained higher for pre-mowed plots.

Table 4. Percentage of each ground cover category observed in March 2022. The year-after mowing treatment did not appear to significantly affect the occurrence of ground cover types.

Table 5. The frequency of common milkweed occurrences is shown in relation to the mowing treatment applied in summer 2021 and summer 2022. The pre-mowing treatment was applied in October 2020, and the year after mowing treatment was applied in July 2021. Frequency was calculated as the sum of frames containing common milkweed for each mowing treatment. There were no significant differences in the frequency of common milkweeds for any of the treatments in both 2021 and 2022. However, for both treatments at both years, un-mowed plots were observed to have a slightly higher frequency of milkweed compared to mowed plots.

Table 6. The frequency of observation for each species in the seed mix is shown for Summer 2021 and 2022 combined. Frequency was calculated by counting the number of frames that a species occurred in. Frequency for the 33 species in the wildflower mix is shown for both seeded and un-seeded plots.

INTRODUCTION

Roadside plantings of native grasses and forbs are frequently implemented on roadways across the United States with the purpose of increasing soil stabilization, wildlife habitat, and aesthetic value. The strong root systems of native warm season grasses are important for protecting infrastructure by preventing excessive soil erosion on roadsides. Native wildflowers are often incorporated into seed mixes to enhance pollinator habitat and aesthetic value. Habitat enhancement is important to pollinator conservation because many pollinator species are on the decline due to habitat loss and fragmentation of suitable habitats (Winfree et al. 2009). Other reasons for decline include infections from pathogens, lower genetic diversity due to small population sizes, climate change, and increasing use of pesticides in agriculture (Cameron et al. 2011; Goulson et al. 2015). Further research can improve our understanding of how pollinators interact with roadside forb plantings and how mowing can affect forbs seeded into a roadside setting.

Roadsides can provide important areas for pollinator habitat in landscapes, such as the tallgrass prairie ecoregion in Nebraska, that are largely privately owned. In many cases, roadside slopes lack the plant diversity to support a large variety of pollinating insects. Ideal pollinator habitat includes a diverse array of annual and perennial flowers, including legumes, shrubs, and trees that flower from early spring through the late fall (Gilbert, Vaughan, 2011). Using seed mixes that contain abundant season long blooms are more important to sustaining pollinators than diverse mixes that do not bloom throughout the entire growing season (Williams et al. 2015). In many revegetation projects, grasses are also included into seed mixes, however, this may negatively affect forb growth at sites where grasses are already established. Areas with high grass density typically have lower forb density due to competition for light and nutrients (Dickson, Busby, 2009).

The monarch butterfly (*Danaus plexippus*) is an iconic pollinator that has declined significantly in the last several decades. The eastern migratory population of monarchs has decreased by an estimated 84% in the last decade (Semmens et al. 2016). Monarch butterflies require milkweeds (*Asclepias spp.*) to provide food for their larvae. In 2014, the U.S. Fish and Wildlife service petitioned to list monarch butterflies under the Endangered Species Act (U.S. Fish and Wildlife Service, 2020), though it was decided in December 2020 that the monarch would not be listed, and no federal protection was warranted. In July 2022, the monarch was placed on the International Union for Conservation of Nature (IUCN) Red List as an endangered species (IUCN, 2022). This listing is important for the conservation of the species; however, it provides no federal protection or coverage under the Endangered Species Act.

Native prairie habitat often requires heterogeneous disturbance regimes to promote the highest possible diversity of plants. Historically, these disturbance regimes would have included a combination of grazing and periodic fire to create a mosaic of different habitat patches on the landscape. However, fire and grazing are not feasible in roadside settings due to safety concerns. Mowing is one form of disturbance that has been used to promote forb growth and limit the proliferation of grasses that compete with forbs for sunlight, water, and nutrients. In this study, our roadside sites were dominated with native warm season grasses, particularly big bluestem (*Andropogon gerardii*), Indiangrass (*Sorghastrum nutans*), and sideoats grama (*Bouteloua curtipendula*). Other research has shown that mowing can free desirable forbs from competition

with large grasses and restrain the growth of woody species (Jakobsson et al. 2018; Williams et al. 2007).

Current Nebraska Department of Transportation (NDOT) seed mixes for newly constructed roadsides are tailored to specific regions of Nebraska. Our study, for example, is in the Loess and Glacial Drift Region (Region B). Typical seed mixes here include 14 total species of plants, including eight native grasses, five native forbs, and a cover crop for early erosion control. According to the NDOT Roadside Vegetation Establishment and Management Handbook, about 10% of the total seed mix is forbs. On surfaced road shoulders, vegetation is mowed at a width of 15 feet from the edge of the pavement to a height of six inches.

Roadside rights-of-way are highly disturbed sites that frequently have less than ideal growth conditions for wildflowers. Un-approved mowing, soil compaction, herbicide drift from nearby fields, and invasive species are all potential challenges to establishing pollinator habitat along major highways. Our preliminary data before seeding these roadsides also shows that plant diversity is generally lacking along roadways. Bee abundance has been found to be significantly higher on restored roadsides compared to un-restored roadsides (Hopwood, 2008). Research on Nebraska roadsides will help managers determine the best practices to establish and promote the longevity of diverse wildflower resources. It is apparent that improving plant diversity on existing roadsides is necessary to support a diverse array of pollinating insects. In addition, native warm-season grasses are highly competitive with forbs for water, sunlight, and nutrients. Conflicting results have been found in past studies involving wildflower establishment and mowing. Enstminger et al. (2017) found that less frequent (no more than twice per year) and later season mowing after dormancy allows the forbs ample time to complete seed production for future reproduction. Alternatively, Williams et al. (2007) found that mowing forb seeded plots as often as every week led to greater root and shoot mass in forbs and double the abundance of forbs compared to un-mowed control plots.

Project Objectives and Summary of Findings

This project analyzed the efficacy of establishing a diverse wildflower seed mix on roadside backslopes at sites with pre-existing dominant cover of warm season grasses. We also determined if different mowing regimes can facilitate the germination and establishment of roadside wildflowers. Overall, this report determined if seeding backslopes and using mowing can benefit pollinator habitat and roadside aesthetics while still maintaining vegetative cover for the purpose of erosion control.

Our analyses showed that overall, our seeding treatment was effective at increasing the forb diversity and total forb cover for roadside backslopes. Floristic quality was also enhanced because of seeding wildflowers. These results were stronger for plantings established along a Highway 2 versus a Highway 77 site. Site orientation, rainfall, and soil compaction may have led to these differences in site responses. Highway 2 received a greater amount of rainfall throughout the study than Highway 77. Though both sites had compacted soils, Highway 77 plots had slightly more compacted soils on average.

Mowing effects were less clear throughout the study. Forb cover was higher on plots that were mowed in October 2020 before seeding, however, the benefits of pre-mowing varied based on the site. Pre-mowing and seeding together was the most effective treatment combination at

increasing the Floristic Quality of the Highway 2 site. Mowing after seeding in early July did not appear to have significant effects on the establishment of forbs. Accumulation of dead plant matter on the soil surface (i.e., litter) of the roadside was one factor that appeared to have a strong impact on forb density in the first year of forb establishment. Reducing litter depth was positively related to increasing forb cover.

This research provides information on the limitations and opportunities in planting native wildflower seed into roadsides with previously established vegetation. Insight from this report will help guide recommendations and future research on the best management practices for seeding and mowing Nebraska roadsides. Some limitations were observed during this study, such as below average rainfall throughout the duration of the research. This led to a low percentage of forbs flowering through this study. We assumed that perennial wildflower seedlings will flower in greater abundance and diversity under normal rainfall conditions. Although this report did not investigate the attractiveness of the treatments to pollinators, previous research has shown that increasing native floral resources will attract more pollinating insects (Blaauw & Isaacs, 2014; Blackmore & Goulsen, 2014; Hopwood, 2008; Schacht et al. 2017). In this report, we focus on the seeding and mowing treatments and how they may be used to increase wildflower density and diversity on Southeast Nebraska roadsides.

MATERIALS AND METHODS

Study Site Description

This study was conducted at two roadside sites in southeastern Nebraska (Figure 1). One site was located along Highway 77 starting about 5 miles southwest of Cortland, Nebraska in Gage County from mile marker 33 to mile marker 35. Highway 77 at this location is situated in a North-South orientation. The next site was located along Highway 2 starting about 3 miles west of Nebraska City, Nebraska in Otoe County from mile marker 502 to 494. Both sites were four lane highways with a vegetated median separating the lanes traveling in opposite directions and a paved shoulder.
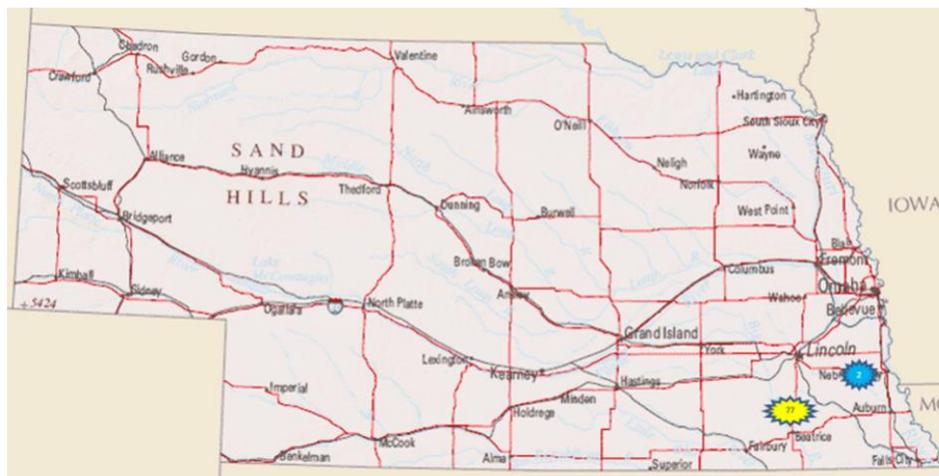

Figure 1. The locations of the two study sites are displayed. The Highway 77 site (yellow) is located south of Cortland, Nebraska, and the Highway 2 site (blue) is located west of Nebraska City, Nebraska.

This study was conducted from August 2020 to September 2022. The historical annual average temperature for Otoe County (Highway 2 site) is 10.6°C. Temperatures averaged 11.3°C in 2020, 11.7°C in 2021, and 10.7°C in 2022 in Otoe County. Both sites had their 16th warmest year since 1900 in 2021. The historical average yearly precipitation for Otoe County is 79 cm. Total precipitation for 2020 was 62.7 cm. In 2021, the total precipitation was above the historical average at 88.3 cm. In 2022, drier than average conditions were experienced with 59.1 cm of precipitation falling throughout the year (NOAA, 2023).

Gage County (Highway 77 site) has a historical annual average temperature of 10.7°C. In 2020, the average temperature for the year was 11.4°C, and in 2021 the average temperature was 12.1°C. The average yearly temperature in 2022 was 11.4°C. Gage County averages 76.2 cm of precipitation historically. In 2020, the county received 61.3 cm of precipitation, and in 2021, the county received 64 cm of precipitation. In 2022, conditions remained dry with 60.6 cm falling during the year. Unlike the highway 2 site, which had above average precipitation in 2021, the first year after planting seed, Highway 77 had below average precipitation for the duration of the study (NOAA, 2023).

Disturbances on roadside slopes are typically in the form of mowing. Vegetation on these roadsides is typically dominated by native warm-season grasses, including big bluestem (*Andropogon gerardii*), Indiangrass (*Sorghastrum nutans*), sideoats grama (*Bouteloua curtipendula*), switchgrasses (*Panicum virgatum*), and Eastern gamagrass (*Tripsacum dactyloides*). Native cool-season grasses are present with slender wheatgrass (*Elymus trachycaulus*) and Canada wildrye (*Elymus canadensis*) being the most common. Non-native cool-season grasses are also present on most slopes. The most common non-native cool-season grasses include Kentucky bluegrass (*Poa pratensis*), Smooth brome (*Bromus inermis*), and cheatgrass (*Bromus tectorum*). Many roadside slopes have multiple forbs present, with many of them being native wildflowers such as, Maximilian sunflower (*Helianthus maximiliani*), showy partridge pea (*Chamaecrista fasciculata*), Illinois bundleflower (*Desmodium illinoensis*), Canada goldenrod (*Solidago canadensis*), and heath aster (*Symphyotrichum ericoides*). Hairy vetch (*Vicia villosa*) and yellow sweet clover (*Melilotus officinalis*) are exotic legumes commonly found on roadsides that can become a nuisance in restoration projects.

Experimental Design

The design of this experiment was a randomized complete block design with four plots being allocated to each of eight different treatment combinations on both study sites (Figure 2). The Highway 2 and Highway 77 sites each had 32 plots for a total of 64 plots in the entire study. Treatments were organized in a 2x2x2 factorial arrangement with four replications of each treatment combination. There were three different treatment factors randomly assigned to each of the 32 plots at each location: pre-mowed or not pre-mowed, seeded or not seeded, and mowed the year after or not mowed the year after. Four plots at each site did not receive any treatments and were considered controls. Each plot was located on the backslope portion of the roadside to avoid traffic interference with the treatments and to provide the most safety possible during data collections.
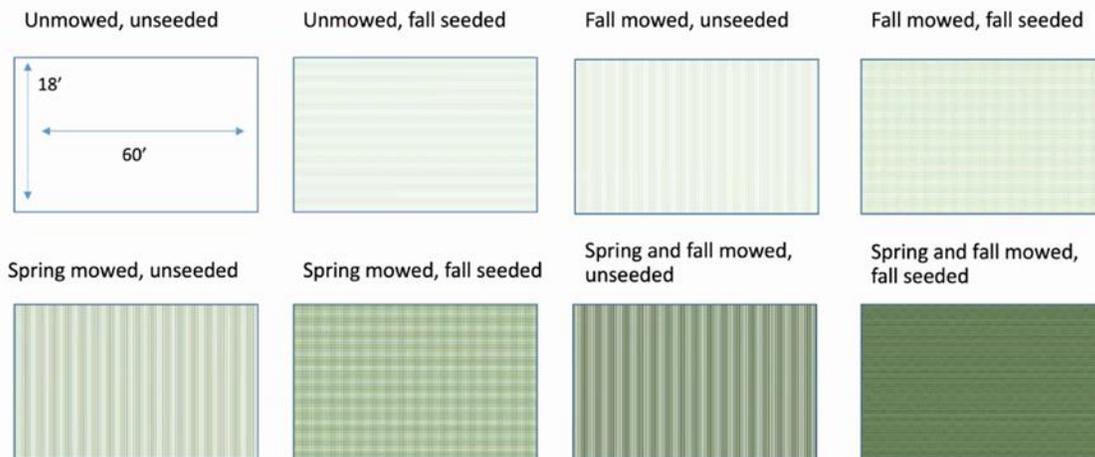
Figure 2. Eight treatment combinations implemented at Highway 77 and Highway 2 study sites. Treatments include various combinations of three treatment factors: pre-mowed and not pre-mowed, seeded and un-seeded, and year-after-mowed and not year-after-mowed.

The plot size for this study was 18 feet wide x 60 feet long running parallel to the highway (Figure 2). The plots had a 50-foot-wide gap between them where no data were collected. This area was considered a buffer to prevent treatment effects from mixing together. Each plot was marked with reflective fiberglass posts and flags. Plot locations were also stored in a handheld GPS device. Plots were arranged along multiple slope locations within each site, with each slope having between three and eight plots. Data collection was performed at least 1 foot inside the boundaries of the plots to account for the potential of treatments to miss the extreme edges of the plots.

Treatments

The first pre-mowing treatment was implemented in October 2020 (Figure 3A). Half of the 32 plots at each site were randomly selected to be mowed to a height of 6 to 8 inches, leaving 32 total plots mowed. A 15-foot-wide mower was pulled behind a tractor between the plot boundaries. The plots were 18 feet wide, so sampling was only performed within the mowed area of the plot. The second mowing treatment was conducted in early July 2021 and followed the same methods as the pre-seeding mowing treatment (Figure 3B). Half the plots were mowed, with some receiving both mowing treatments, some receiving just one of the mowing treatments, and some receiving no mowing at all. The seeding treatment, meanwhile, included drilling native wildflowers into half of the plots using a Great Plains Native Grass Series II drill (3P606NT; Figure 4).

Figure 3. Two mowing treatments were completed in October 2020 and early July 2021. (A) shows plots that were mowed in October 2020 while vegetation was dormant, and (B) shows the tractor and mower finishing a plot in the early July mowing.
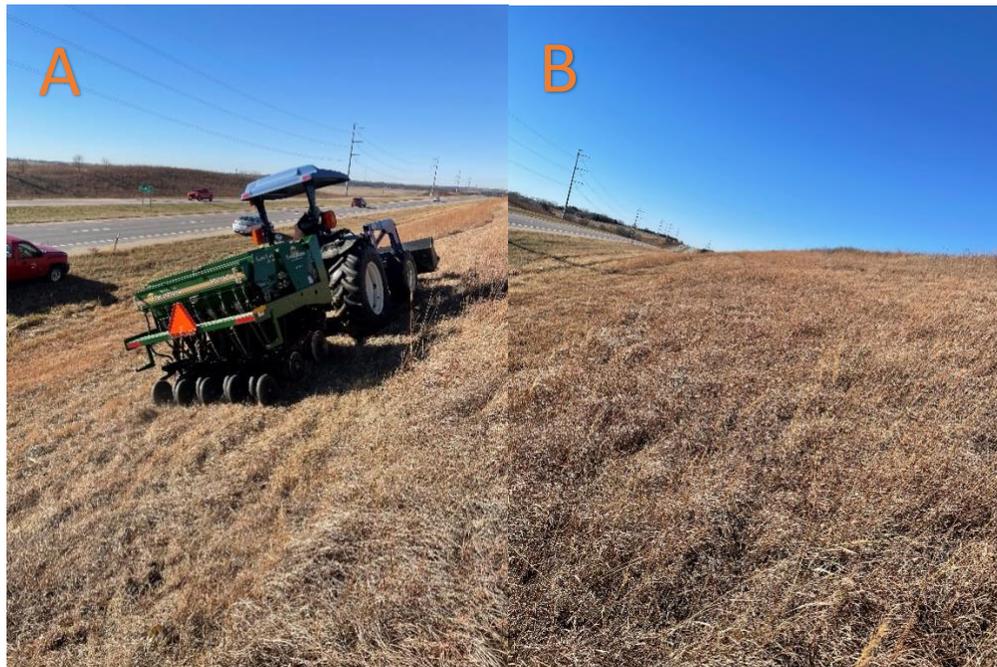

Figure 4. The seeding treatment conducted in November 2020 is shown. (A) shows the equipment used to put seed in the ground in a previously mowed plot, and (B) shows an un-mowed plot where seed was drilled into the existing vegetation.

The species mix included 33 species of native wildflowers with bloom times ranging from April to October. The seed mix was composed of an array of perennial and annual wildflowers obtained from Stock Seed Farms (Table 1).

Table 1. The 33 species of forbs in the wildflower mix are shown. Included is the species name, common name used in this study, functional group, life span, and bloom time.

Species	Common Name	Functional Group	Life Span	Bloom Time
Penstemon grandiflorus	Shell-leaf Penstemon	Forb	Perennial	Apr 15 – Jun 15
Zizia aurea	Golden Alexander	Forb	Perennial	Apr 15 – Jun 15
Achillea millefolium	Western Yarrow	Forb	Perennial	Apr 15 – Jun 15
Gaillardia pulchella	Indian Blanket Flower	Forb	Annual	Apr 15 – Jun 15
Rudbeckia hirta	Black-eyed Susan	Forb	Annual or Biennial	Apr 15 – Jun 15
Linum lewisii	Lewis Flax	Forb	Perennial	Jun 15 – Aug 15
Amorpha canescens	Leadplant	Woody	Perennial	Jun 15 – Aug 15
Asclepias syriaca	Common Milkweed	Forb	Perennial	Jun 15 – Aug 15
Asclepias tuberosa	Butterfly Milkweed	Forb	Perennial	Jun 15 – Aug 15
Solidago canadensis	Canada Goldenrod	Forb	Perennial	Jun 15 – Aug 15
Solidago missouriensis	Missouri Goldenrod	Forb	Perennial	Jun 15 – Aug 15
Astragalus canadensis	Canada Milkvetch	Legume	Perennial	Jun 15 – Aug 15
Coreopsis tinctoria	Plains Coreopsis	Forb	Annual	Jun 15 – Aug 15
Desmodium canadense	Showy Tick-trefoil	Legume	Perennial	Jun 15 – Aug 15
Heliopsis helianthoides	False Sunflower	Forb	Perennial	Jun 15 – Aug 15
Monarda fistulosa	Wild Bergamot	Forb	Perennial	Jun 15 – Aug 15
Echinacea angustifolia	Narrowleaf Purple Coneflower	Forb	Perennial	Jun 15 – Aug 15
Ratibida columnifera	Upright Prairie Coneflower	Forb	Perennial	Jun 15 – Aug 15
Ratibida pinnata	Grayhead Coneflower	Forb	Perennial	Jun 15 – Aug 15
Verbena hastata	Blue Vervain	Forb	Perennial	Jun 15 – Aug 15
Chamaecrista fasciculata	Showy Partridge-pea	Legume	Annual	Jun 15 – Aug 15
Dalea purpurea	Purple Prairie Clover	Legume	Perennial	Jun 15 – Aug 15
Desmanthus illinoensis	Illinois Bundleflower	Legume	Perennial	Jun 15 – Aug 15
Cleome serrulate	Rocky Mountain Bee Plant	Forb	Annual	Jun 15 – Aug 15
Helianthus maximiliani	Maximillian Sunflower	Forb	Perennial	Aug 15 – Oct 15
Helianthus pauciflorus	Stiff Sunflower	Forb	Perennial	Aug 15 – Oct 15
Oligoneuron rigidum	Stiff Goldenrod	Forb	Perennial	Aug 15 – Oct 15
Salvia azurea	Pitcher Sage	Forb	Perennial	Aug 15 – Oct 15
Symphyotrichum ericoides	Heath Aster	Forb	Perennial	Aug 15 – Oct 15
Symphyotrichum laeve	Smooth Blue Aster	Forb	Perennial	Aug 15 – Oct 15
Symphyotrichum novae-angliae	New England Aster	Forb	Perennial	Aug 15 – Oct 15
Silphium laciniatum	Compass Plant	Forb	Perennial	Aug 15 – Oct 15
Lespedeza capitata	Roundhead Bush Clover	Legume	Perennial	Aug 15 – Oct 15

Soil Bulk Density

In Spring 2022, the roadside soils were sampled to help gain an understanding of how germination and establishment of the seed mixture could be affected by soil compaction and other to be determined properties. Highway 2 plots had an average bulk density of 1.50 g/cm^3, and Highway 77 plots had an average bulk density of 1.57 g/cm^3. Both sites were on average more compacted than typical prairie soils, which generally fall between 1.0 and 1.4 g/cm^3.

Vegetation Sampling

Vegetation was sampled by identifying the frequency and cover of each plant species located within a 0.5 m x 0.5 m quadrat (Figure 5). An observer started at one end of the plot and randomly tossed the quadrat into the plot, being sure not to bias the toss. To be counted, each quadrat had to lie entirely inside the plot boundaries, otherwise the quadrat had to be re-tossed. Treatments were not administered outside the plot boundaries, so this ensured that the data accurately represented the treatments being applied to a particular plot. Only species rooted within the boundaries of the quadrat were counted in the frequency and cover assessments.
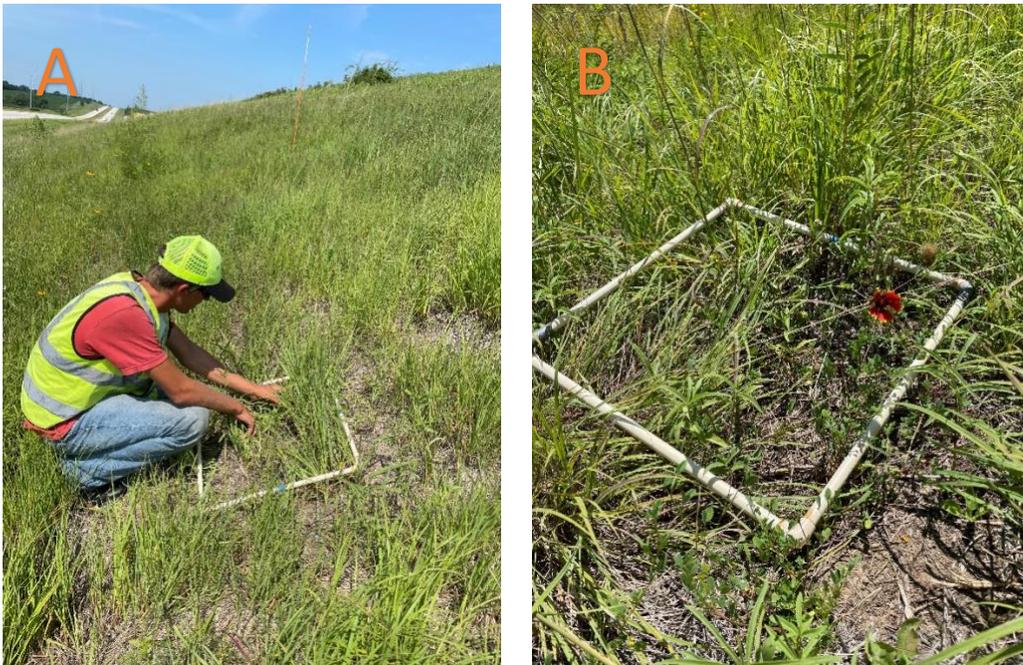
Figure 5. Photographs of vegetation sampling for cover using the 0.5 x 0.5-meter frame. Twelve frames per plot were used across 64 plots at two sites. (A) shows vegetation being observed in June 2021 following seeding in November 2020, and (B) shows a frame in a seeded plot in August 2022.

Daubenmire Cover Classes (1 = 0-5%, 2 = 6-25%, 3 = 25-50%, 4 = 50-75%, 5 = 75-95%, 6 = 95-100%) were used to assess the percent cover of each species present. The total cover of each species could total more than 100% because the vegetation at these sites was often layered, with some dominant species creating a canopy above the less robust species. Twelve quadrats were sampled per plot with six tosses being across the top half of the plot and six being made across the bottom half in a stratified random manner.

Litter Cover and Depth Sampling

Dead plant material on the soil surface, i.e., litter, sampling was conducted by randomly selecting three transects within the 18 x 60-foot plots along the 18-foot edge (Figure 6). One-foot margins along the top and bottom of the plots were not included in the transects to account for treatments not being perfectly aligned with the edge of the plots. The transects were determined to be at 2, 11, and 14 feet. There was also a 2.5-foot margin at each end of the plot, which left each transect running a total of 55 feet parallel to the long edge of the plot. Litter sampling was conducted in March 2021 and March 2022 before plants began to grow significantly. Figure 6 shows the layout of the transects for the vegetative litter sampling.

A step point tool was placed 2.5 feet inside the plot boundary, and the point was allowed to extend forward and rest on the ground. The observer then recorded the cover type at the point of the tool. Litter, plant base (basal cover), and bare ground were recorded in this study. Litter in this case was defined as the previous year's plant material lying flat on the ground. When litter cover was observed, a ruler was used to measure the depth of the material in millimeters at the location of the point. To determine the location of the next observation, the observer took two steps from the previous point along the transect. The researcher was expected to make about 15 observations per transect or 45 observations per plot. No points were taken outside of the 2.5-foot margin at the edge of the plots.
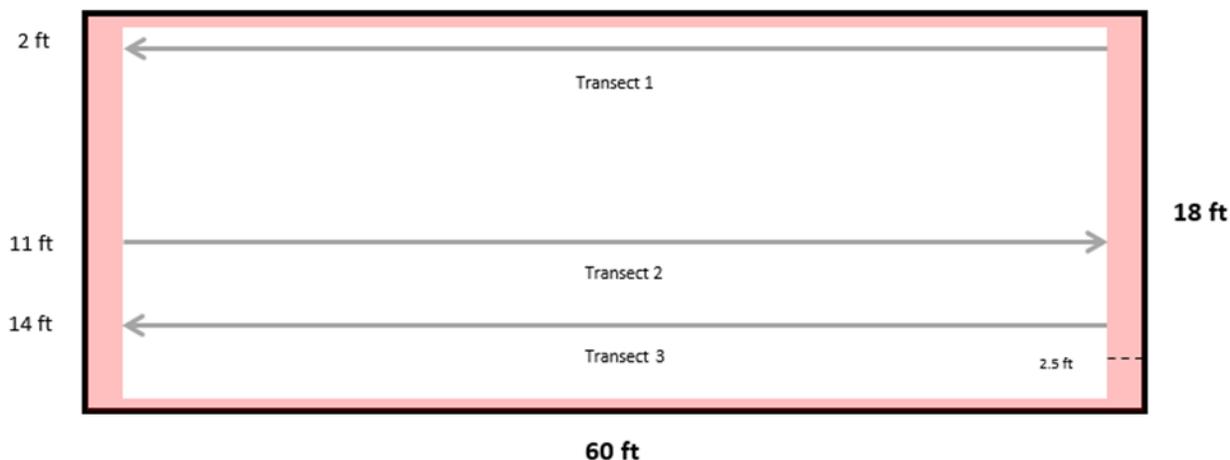

Figure 6. The plot setup is shown for vegetative litter sampling. Transects were randomized along the 18-foot plot edge. Fifteen measurements were taken on each of the three transects. A 2.5-foot margin was not sampled at each end of the plot.

Data Analysis

Data analyses were performed using RStudio version 2022.07.0+548 ©2009-2022. The Tidyverse package was used to manipulate data into usable forms. To determine the treatment

effects on vegetation and plant litter, the doBy package was used to perform an ANOVA, where all treatment interactions were shown. Significance was determined at alpha = 0.05. Correlation between various types of data were show by performing Pearson's Product Moment Correlation tests. The two study sites were sampled independently to account for variation in growing conditions between the two sites. The samples taken from each plot were averaged to obtain a per plot average of cover, frequency, species richness, and floristic quality. Plot data was then averaged by the treatment being analyzed. Floristic Quality was calculated using the methods from Taft et al. 1997. The coefficients of conservatism (C) were obtained from the Nebraska Natural Heritage Program plant species list (Gerry Steinauer personal communication). All figures of results were created using ggplot in the RStudio Tidyverse package. Tables were created in Microsoft Word.

RESULTS

Total Forb Cover

The total percent cover of forbs was assessed to determine the effects of seeding wildflowers and different mowing regimes on the density of forbs present at a site. In 2021, there were no significant interactions between seeding and mowing treatments on forb cover. The treatment effects were only observed for individual treatments separately. Seeding alone increased the total forb cover for both sites (Figure 7). Highway 2 had 19.5% cover in seeded plots and 12.3% in un-seeded plots (P = 0.01). Highway 77 seeded plots had 17.9% forb cover, and un-seeded plots had 13.1% seeded cover on average (P = 0.06). Only the Highway 2 site showed a significant increase in forb cover from seeding; however, the Highway 77 site was trending in a similar direction.
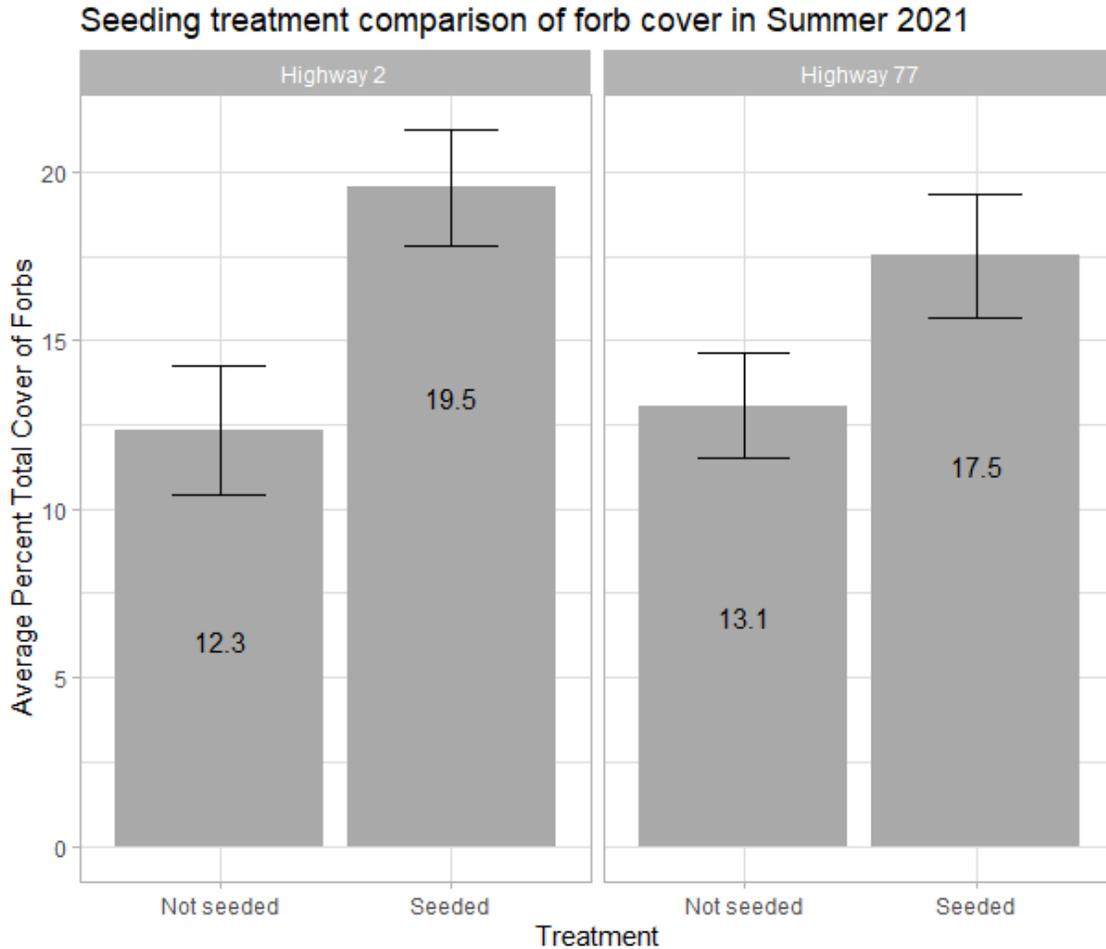
Seeding treatment comparison of forb cover in Summer 2021

Figure 7. Main effect of the seeding in November 2020 on total forb cover in summer 2021. The cover values are an average of plots within the two seeding treatments. Averages are obtained from the sum of cover for each forb species within a plot. Error bars represent the standard error of the sample. Both sites showed an increase in total forb cover on average in plots that were seeded. Only Highway 2 has a significant effect on forb cover from seeding ($P = 0.01$).

Pre-mowing appeared to have a positive effect on forb growth at the Highway 77 site (Figure 8), where pre-mowed plots had an average cover of 17.9%, while plots that were not mowed only averaged 12.7% ($P = 0.03$). This positive effect of pre-mowing on forb cover was not observed at the Highway 2 site. There appears to be a difference in conditions at each site leading to inconsistent effects from mowing and seeding treatments.
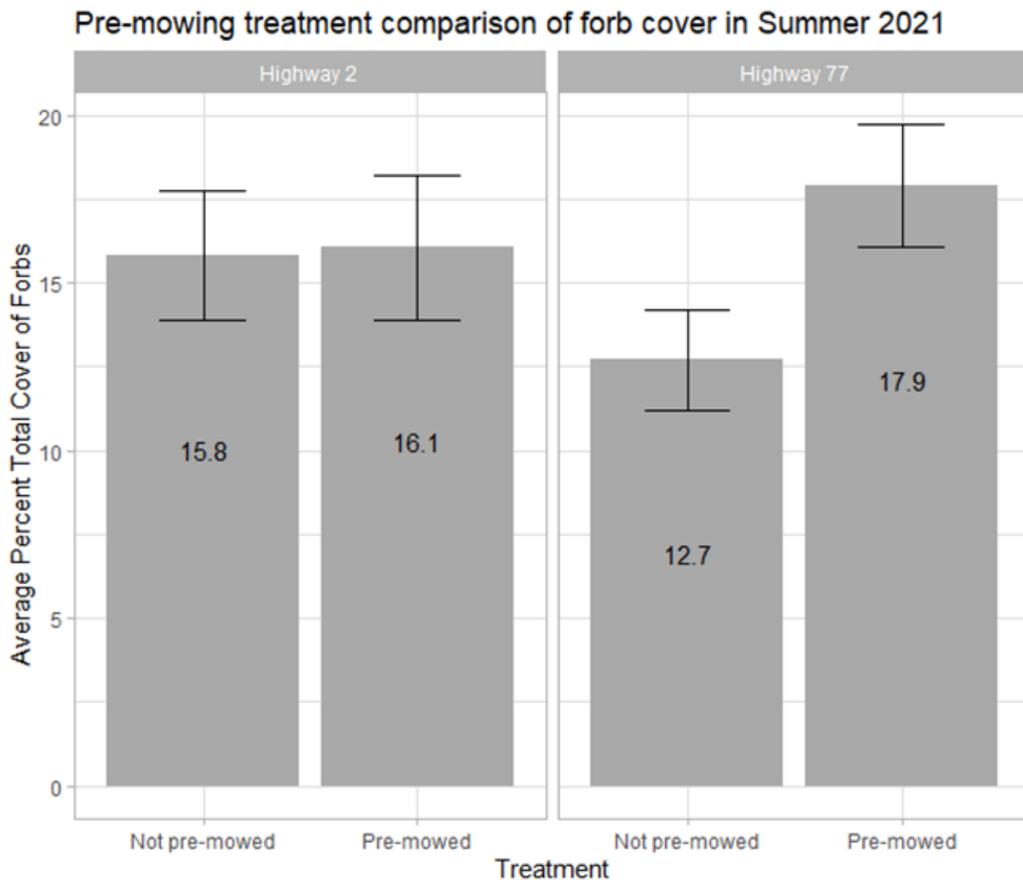
Figure 8. Main effect of pre-mowing in October 2021 on total forb cover in summer 2021. Both sites appear to show an increase in total forb cover in response to mowing before seeding, but only the Highway 77 site has a significant effect on total forb cover with pre-mowing (P = 0.03).

Vegetation was again assessed for total forb cover in summer 2022, the season after planting and implementing the final mowing treatment. Forb cover appeared to have recovered to similar levels in response to both mowing treatments at both sites in the season after mowing. The effects of mowing appeared to be temporary with forb cover being unaffected by pre- or post-seeding mowing in 2022. Like Summer 2021, mowing treatments and seeding treatments did not appear to interact. The positive effects of seeding remained evident in 2022, as Highway 2 seeded plots had 29.4% forb cover and un-seeded plots had 18.9% total forb cover (P = 0.02). Highway 77 seeded plots had 22.7% forb cover on seeded plots compared to 16.8% cover on un-seeded plots in 2021, but this was not a significant effect (P = 0.16). Overall, forb cover showed increases from 2021 to 2022 for all plots, but seeded plots on average remained higher in forb coverage (Figure. 9).
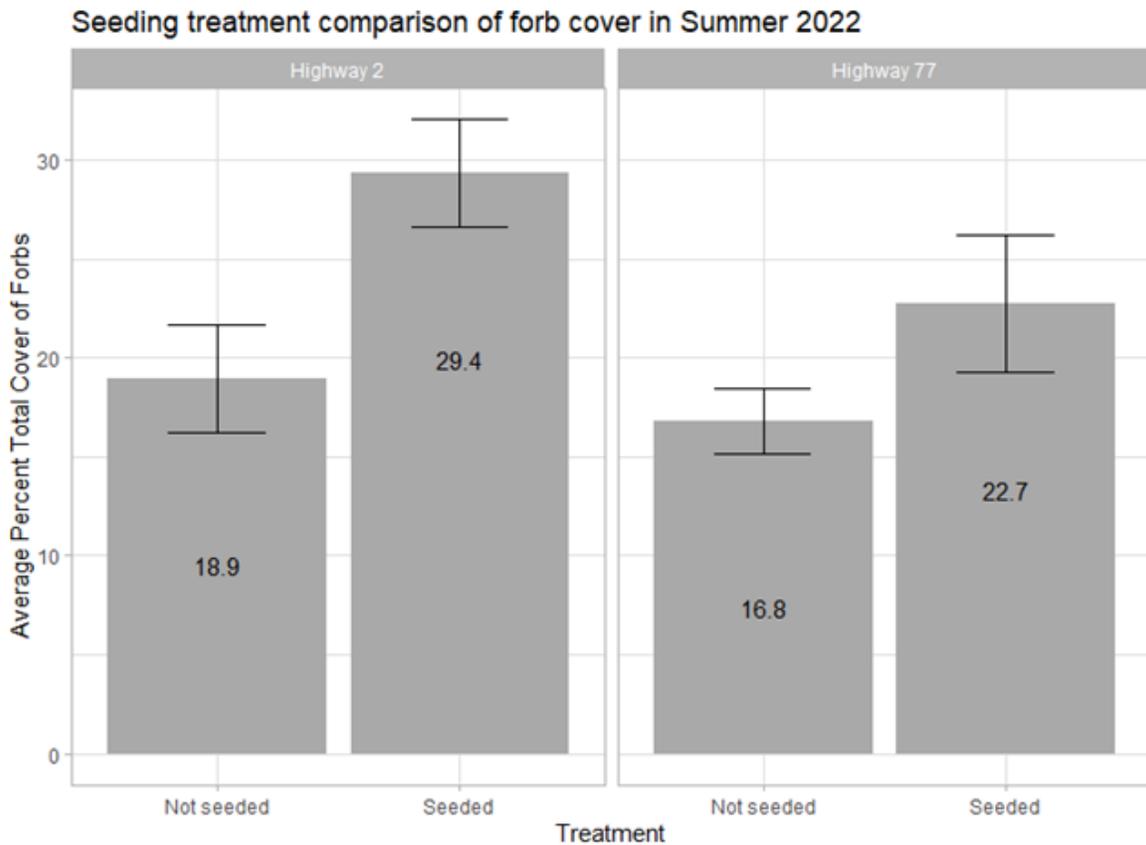
Figure 9. Main effect of seeding wildflowers on the total forb cover observed in summer 2022, the second season after planting and applying mowing treatments. The values are an average for both seeding treatments of the sum of forb species cover for each plot. Total forb cover remained higher in seeded plots one year after planting. The Highway 2 site had a significant effect of seeding wildflowers (P = 0.02).

Cover of Seeded Forbs Only

To assess the establishment of the seed mix and the effects of mowing on seeded forbs, the percent cover of seeded species was analyzed using only those species within the wildflower seed mix (Table 1). In the first season after planting (summer 2021), the Highway 2 site showed a small increase in seeded species forb cover from 9.71% on un-seeded plots to 12.8% cover on seeded plots (P = 0.18). There were also no significant effects from October pre-mowing or July year-after mowing on seeded species forb cover. The early July mowing did lead to about 3% less seeded species cover on average for both sites compared to plots that were not mowed. Highway 77 had a small increase in forb cover from 10.1% on un-seeded plots to 13.8% on plots seeded with wildflowers (P = 0.07). The Highway 77 site, however, did have a higher percentage of coverage from these seeded species on plots that were mowed before seeding (Figure 10). Highway 77 plots that were not pre-mowed had an average of 9.47% cover, while plots that were mowed before seeding averaged 14.5% cover of species within the seed mix (P = 0.02). There were no interactions between treatment factors for either site in 2021.
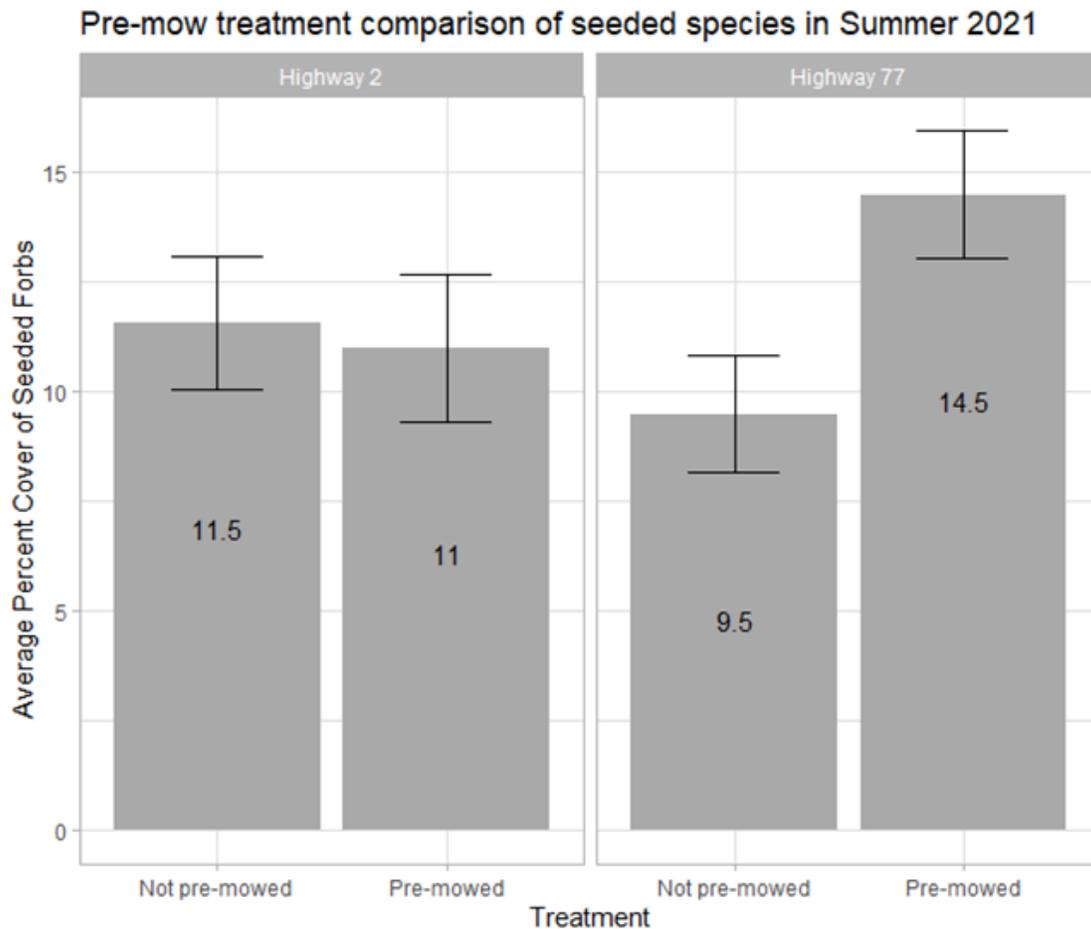
Figure 10. Main effect of the pre-mowing treatment on the percentage of seeded forb cover for summer 2021. Only species included in the seed mix (Table 1) are included. The cover values are an average of the plots within the two pre-mowing treatments. The two highways are shown separately. Only Highway 77 had a significant effect of pre-mowing on seeded forb cover. The error bars represent standard error. Samples were collected from August to September of 2021.

Percent cover of seeded forbs was assessed again in summer 2022. No significant interactions between multiple treatment factors were observed in 2022. For wildflowers in the seed mix, seeding appears to have a positive effect on the percent cover. Highway 2 seeded plots had an average percent cover of 17.4%, while plots that were not seeded had 11.2% cover of these species in the mix (Figure 11), which was found to be a significant difference (P = 0.01). Highway 77 also had a higher percent cover of these species with 16.3% cover in seeded plots and 13.8% cover in un-seeded plots, however, this difference was not significant for the site.
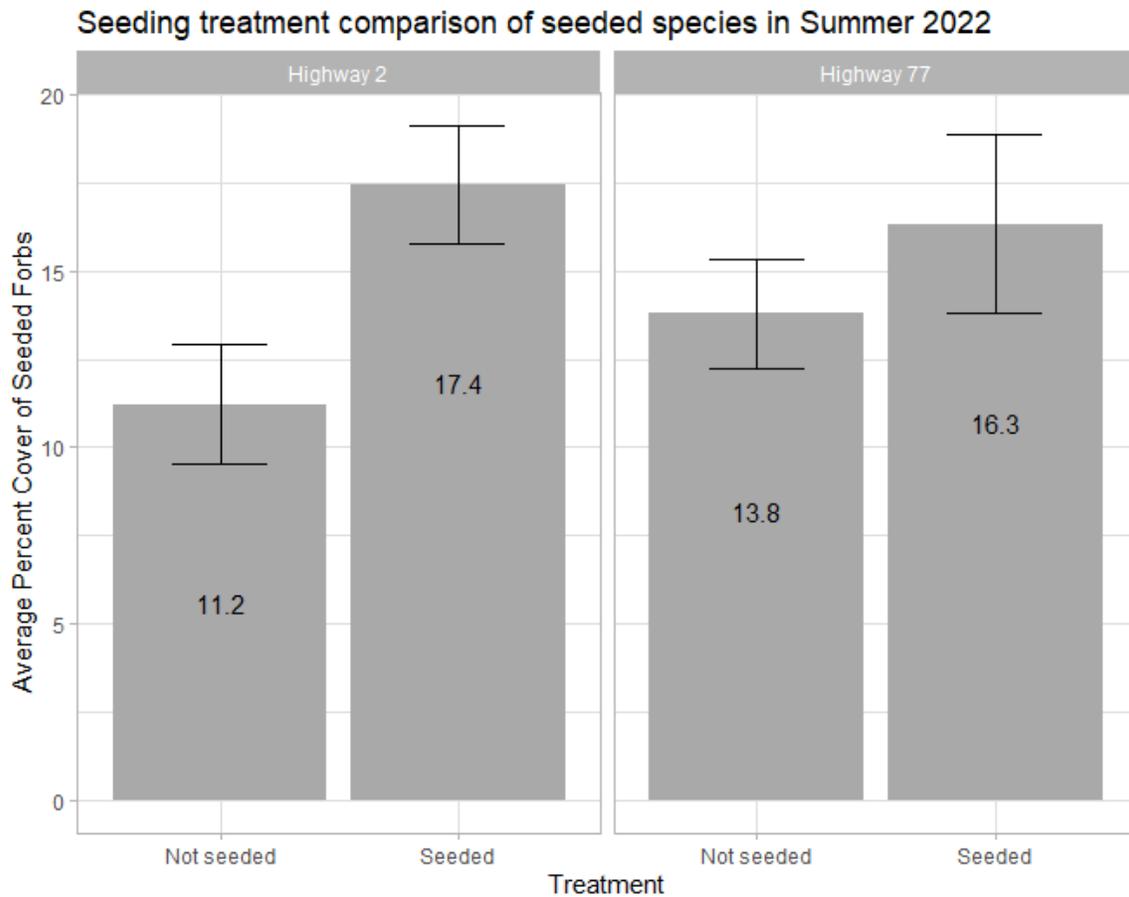
Figure 11. Main effect of seeding wildflowers on the percent cover of forbs within the planting mix (Table 2.1) in summer 2022. Though both sites had an increase in the percentage of forb cover of seeded species, only Highway 2 had a significant effect of seeding (P = 0.01). Seeding occurred in November 2021. These data were obtained in August 2022.

Pre-mowing had no significant effects on seeded species cover for either site. The year-after mowing treatment also did not show a significant effect at Highway 77 (Figure 12). The year-after mowing treatment, though, showed negative effects on cover of seeded species at Highway 2 with 16.6% cover of seeded forbs on plots that were not mowed compared to 12% cover on plots that were mowed the second year (P = 0.06).
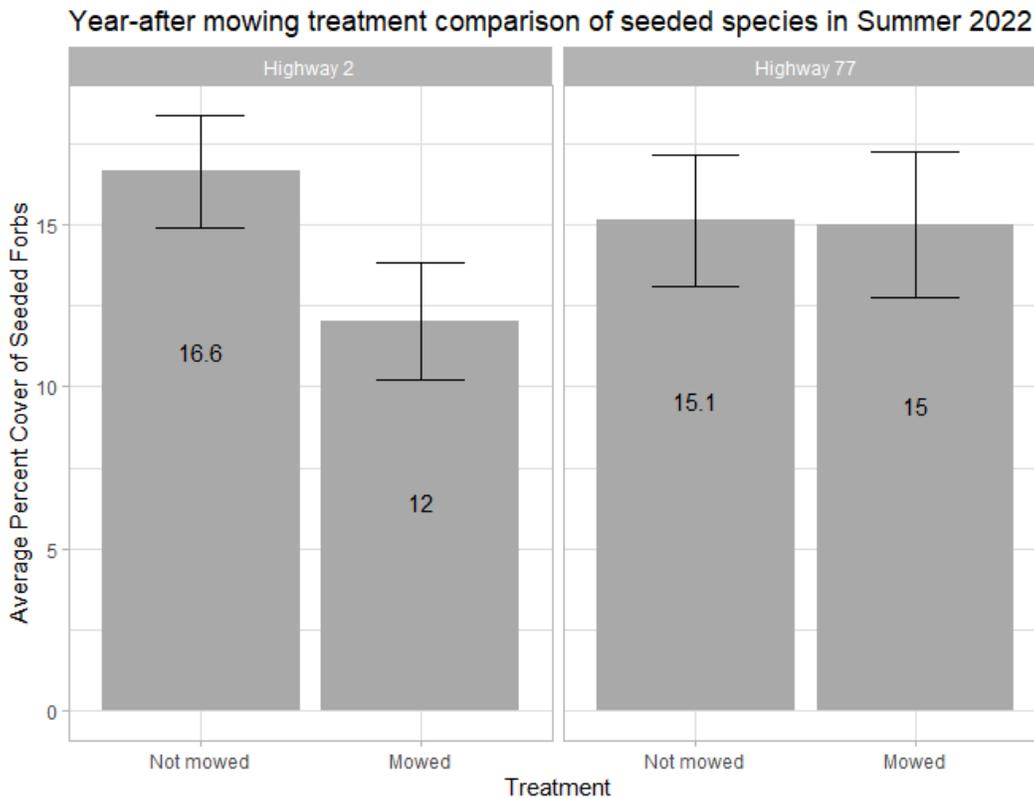
Figure 12. Main effect of year-after mowing is shown for both sites in Summer 2022. This mowing treatment was implemented in early July 2021. Highway 77 does not appear to be affected by year-after mowing. The Highway 2 site shows some negative impacts to seeded species cover from mowing the previous summer, however, this is not significant (0.06).

Species Richness

To determine the effects of our treatments on plant diversity, species richness was assessed by counting the total number of species found within each plot. In summer 2020, before any treatments were applied, the average species richness for the Highway 2 site was 14.9 species, and the average for the Highway 77 site was 20.9 plant species. In Summer 2021, after all treatments had been applied, pre- and post-seeding mowing on the Highway 2 site had no effects on species richness. No interactions between seeding and mowing treatments were observed. The only significant treatment effect was seeding alone ($P < 0.01$) with 21.2 species in seeded plots and 15 species in un-seeded plots (Figure 13). Highway 77 also did not have any effects of mowing or treatment interactions on species richness. Seeding did not significantly affect species richness, with only a slight increase in number of species observed from 19.1 species on un-seeded plots to 21.6 species on seeded plots ($P = 0.18$).
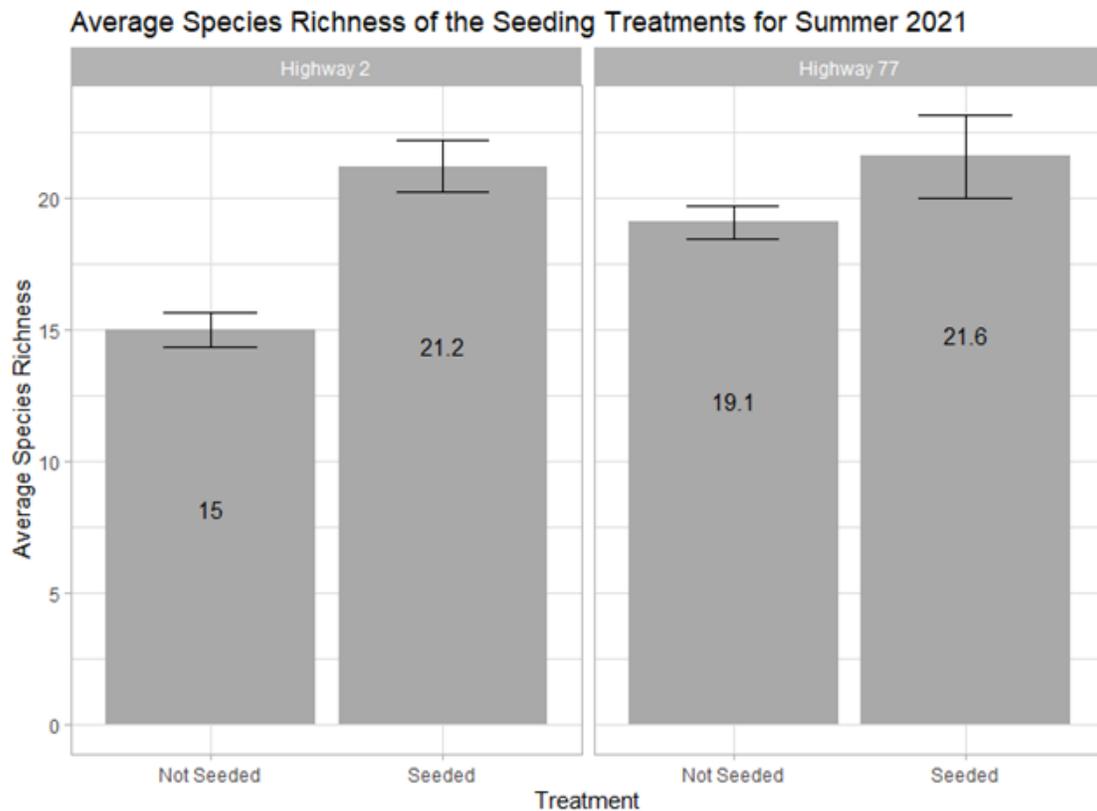
Average Species Richness of the Seeding Treatments for Summer 2021

Figure 13. Main effect of seeding on species richness for both sites in Summer 2021. Species richness is a count all plant species found within a plot. Plot species counts were averaged by seeding treatment for each site. Highway 2 had a significant increase in species richness in response to seeding wildflowers (P < 0.01). Seeding effects were weak for Highway 77 (P = 0.18).

In summer 2022, species richness remained higher on seeded plots compared to plots that were not seeded with wildflowers (Figure 14). Highway 2 seeded plots averaged 23.6 species, and un-seeded plots averaged 16.4 species (P < 0.01). Highway 77 seeded plots had 22.8 species, while un-seeded plots had 20.4 species on average (P = 0.09). Like 2021, no significant effects from mowing were observed at either site. No significant interactions between seeding and mowing treatments were observed in 2022.
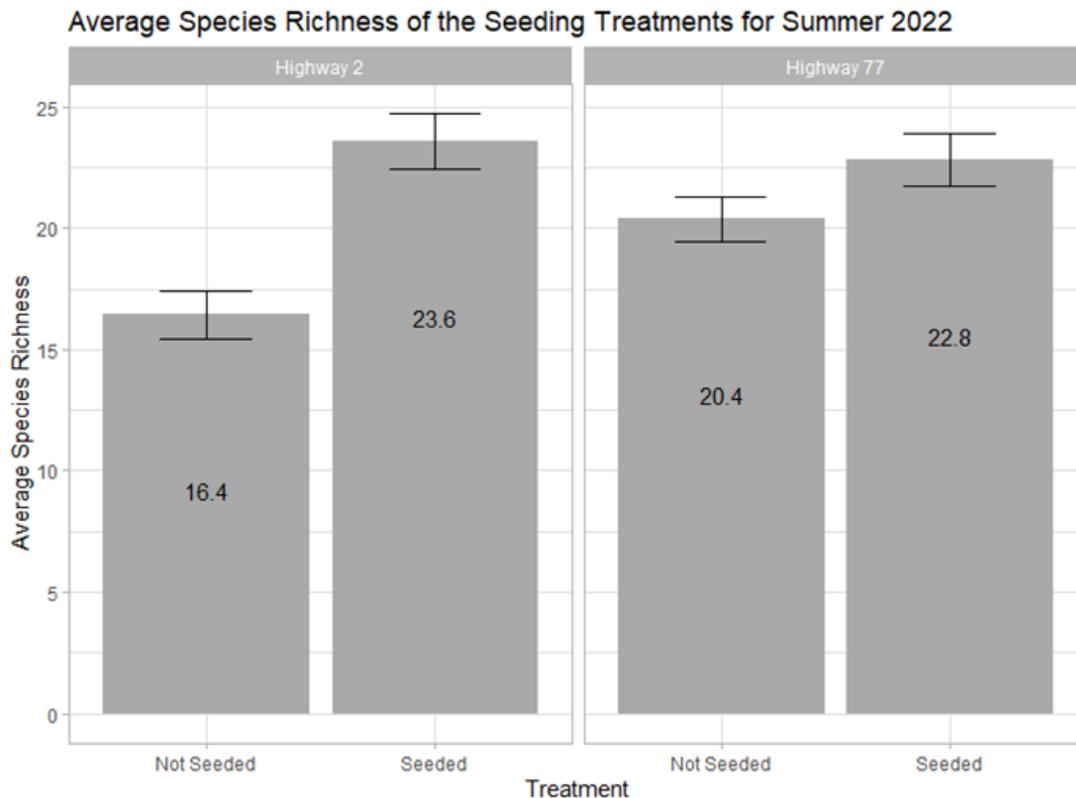
Figure 14. Main effect of seeding on species richness for both sites in Summer 2022. Species richness is a count all plant species found within a plot. Plot species counts were averaged by seeding treatment for each site. Highway 2 had a significant increase in species richness in response to seeding wildflowers (P = 0.0002). Seeding effects were weak for Highway 77 (P = 0.092).

Floristic Quality Assessment

A floristic quality assessment was performed for each plot to determine the quality of vegetation at each site. In Summer 2021, wildflower seeded plots had an average FQI of 15, and un-seeded plots averaged 11.6 (P < 0.01). Highway 77 had a small increase in average FQI on seeded plots with an average FQI of 12 on un-seeded plots and 13.5 on seeded plots, however this was not a significant increase (P = 0.12). Figure 15 shows the seeding treatment effects on floristic quality.
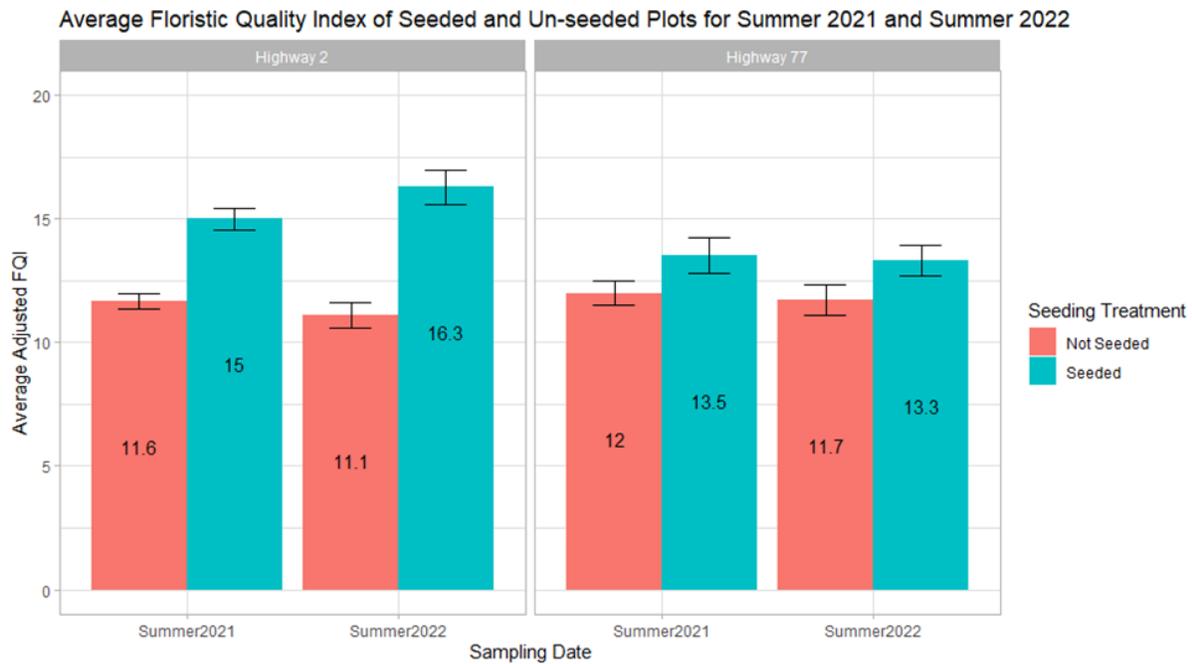
Figure 15. Main effect of seeding wildflowers on the floristic quality of both sites for summer 2021 and summer 2022. The floristic quality index for each plot was calculated by multiplying the average coefficient of conservatism value by the square root of the number of species present at that plot. The adjusted index floristic quality index was averaged by seeding treatment. Highway 2 after seeding appears to have a significant increase in average floristic quality in seeded plots ($P < 0.01$).

There was an observed interaction between the pre-mowing treatment and the seeding treatment on Highway 2 in 2021. Pre-mowing appears to lead to a significantly higher FQI on seeded plots compared to seeded plots that were not mowed before seeding. Pre-mowed seeded plots had an average FQI of 16, while seeded plots that were not pre-mowed averaged 14 ($P = 0.03$). Pre-mowing did not affect the FQI of un-seeded plots in 2021. Figure 16 illustrates the interaction between pre-mowing and seeding for Highway 2 in 2021.

In Summer 2022, the effects of seeding on floristic quality remained significant on Highway 2 with an average FQI of 16.3 on seeded plots and an average FQI of 11.1 ($P < 0.01$) on un-seeded plots (Fig. 15). Seeding alone was the only significant treatment effect on this site in 2022, and the pre-mowing interaction with seeding from 2021 was no longer observed. Highway 2 did show some evidence of increased floristic quality from the pre-mowing treatment alone with an average FQI of 14.6 on pre-mowed plots and 12.8 on un-mowed plots, however, this was not significant ($P = 0.06$). On Highway 77, seeding effects were still observed with an average FQI of 13.3 on seeded plots and 11.7 ($P = 0.08$) on un-seeded plots (Fig. 15). No significant interactions or mowing effects were observed for Highway 77 in 2022.
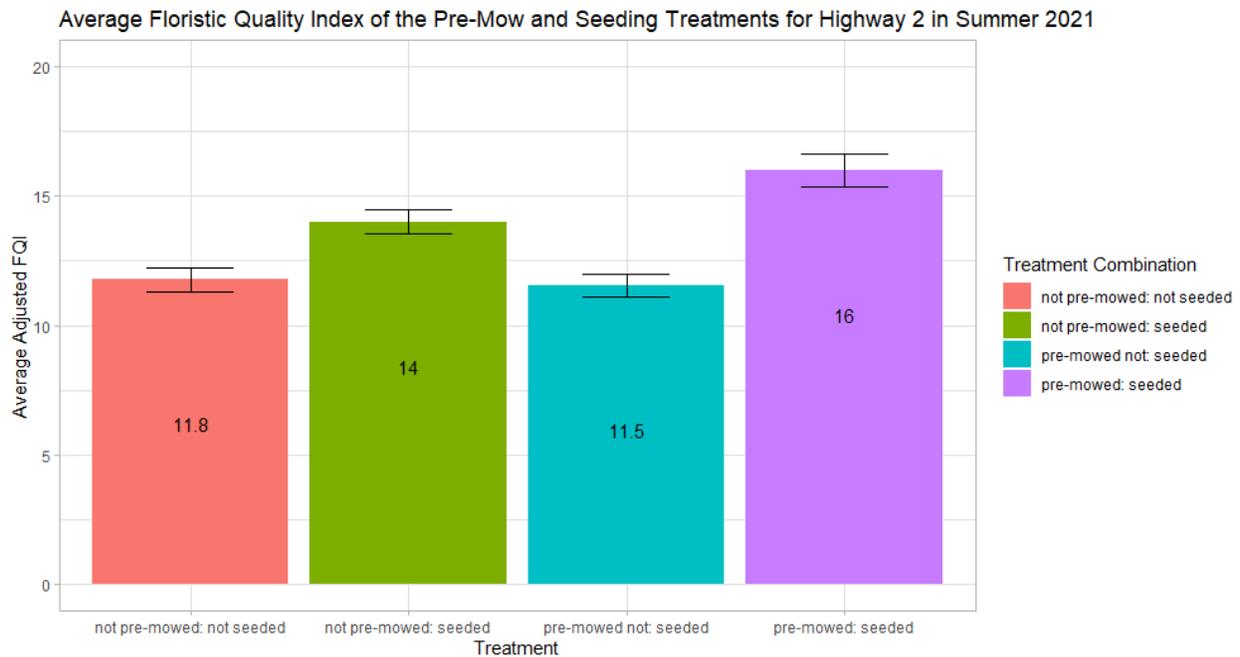
Figure 16. Interaction between the pre-mowing and seeding treatments are shown for the Highway 2 site in Summer 2021 for adjusted floristic quality. Average FQI for each treatment is calculated the same as Fig. 2.11. Pre-mowing and seeding wildflowers appear to lead to high floristic quality when compared to seeding without pre-mowing ($P = 0.033$).

Mowing Effects on Grass Cover

Total cover of grass species in plots was analyzed to determine mowing effects on grass cover and the potential for removing grass cover to aid in establishment of forbs. Grass cover data collected in June 2021 was assessed to determine the impacts fall mowing may have on reducing grass cover. Both sites showed no significant effects of pre-mowing in October 2020 on the June 2021 grass cover. The average grass cover for both sites of pre-mowed plots was 48.2% and 51.7% in non-pre-mowed plots ($P = 0.19$). It appears that mowing dead vegetation in October has little effect on the density of grass regrowth in June.

The second mowing treatment was implemented in July 2021. In August 2021, Highway 2 had no significant effects from pre-mowing or year-after mowing treatments on grass cover, and there were no interactions between the mowing treatments (Fig. 17). Highway 77 showed less grass cover on plots that were mowed in July 2021 compared to plots that were not mowed (Fig. 17). Year-after mowed plots at this site had an average of 43.5% grass cover, while plots that were not mowed averaged 51.1% grass cover ($P < 0.01$).
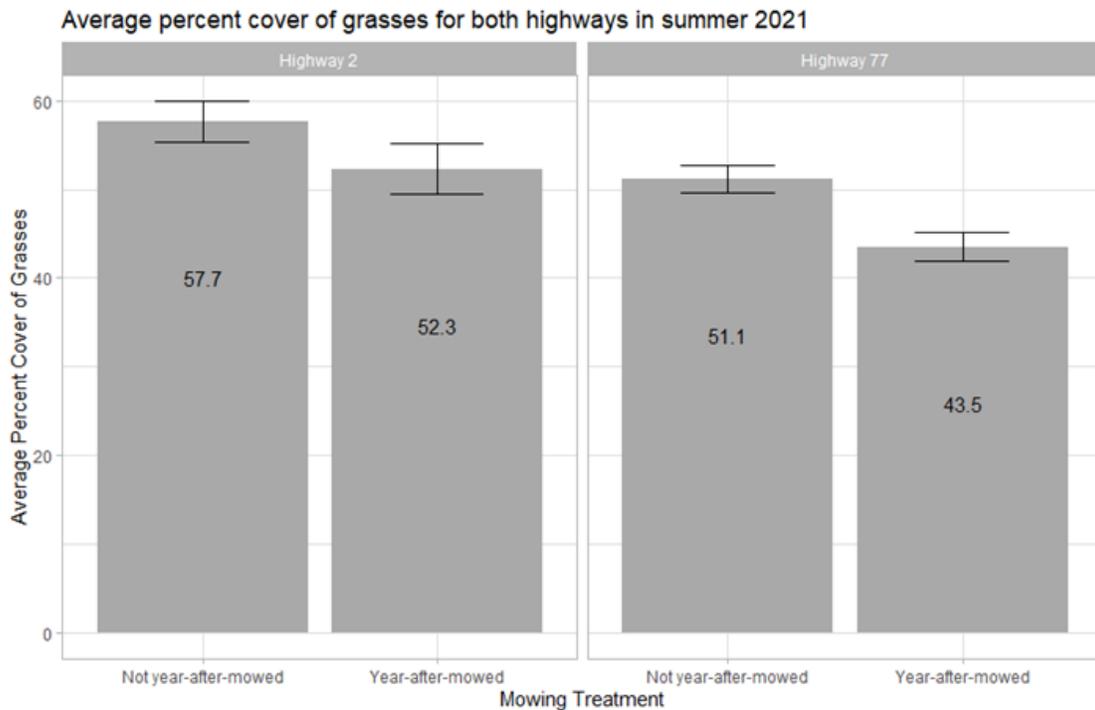
Figure 17. Main effect of July 2021 mowing on grass cover in September 2021. Average percent cover is calculated by averaging the total percent cover of grass species for each plot within the two year-after mowing treatments. Highway 77 appears to have significantly less cover of grasses on plots mowed in July compared to plots not mowed in July of the same year ($P < 0.01$).

In Summer 2022, no significant effects of pre-mowing or year-after mowing treatments alone were observed for either site. Highway 77 had no interaction between the two mowing treatments. Highway 2 plots mowed twice did have slightly lower grass cover on average than other mowing treatment combinations, however, this was not significant. Plots at Highway 2 that were mowed twice had 41.1% grass cover on average, compared to the other combinations of mowing treatments, which averaged 46.6% on average ($P = 0.06$).

Mowing and Vegetative Litter

In March 2021, plots were sampled to determine how vegetative litter compares on mowed and un-mowed plots. For both sites, vegetative litter depth was found to be significantly lower on plots that received pre-mowing than plots that were not pre-mowed ($P < 0.01$). The average depth on pre-mowed plots was 28.5 mm, while the average on non-pre-mowed plots was 49.9 mm (Figure 18). Mowing appears to remove some of the litter from the plot boundaries.
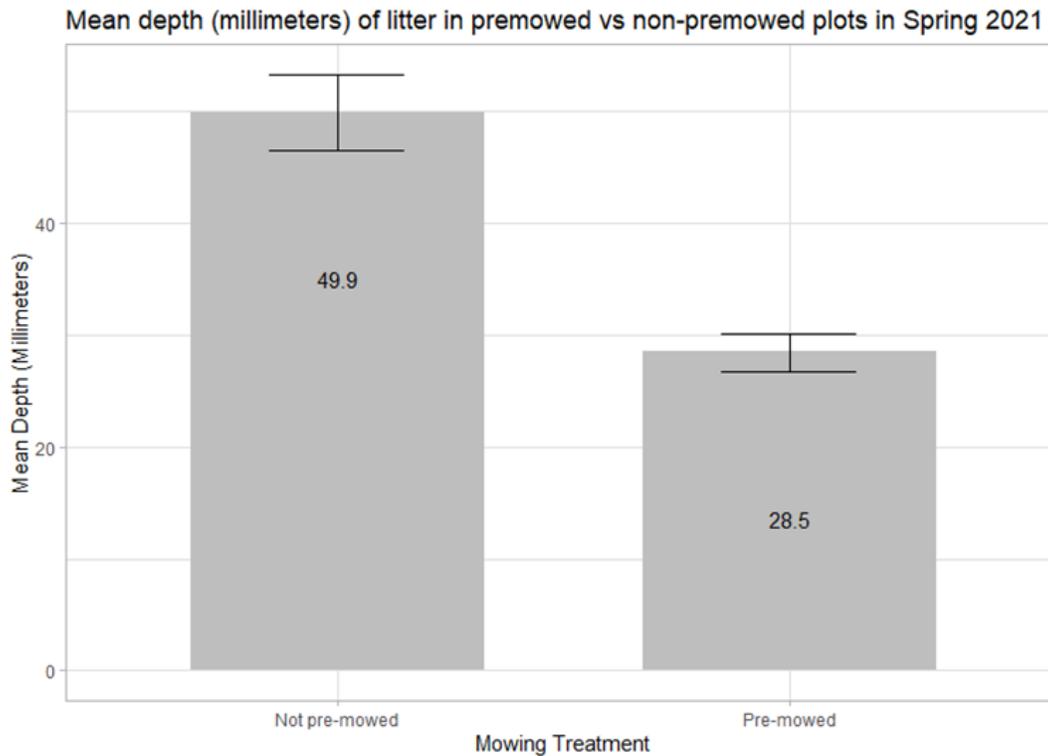
Figure 18. Main effect of pre-mowing on vegetative litter depth in millimeters for Spring 2021 with both sites combined. A plot average litter depth was derived from each measurement along three transects. Plots were then averaged by mowing treatment. The results for both sites were displayed together because both sites had a similar outcome. Data was collected in March of 2021 following the October 2020 pre-mowing treatment. Pre-mowed plots had a lower depth of vegetative litter at both sites compared to plots that were not mowed (P < 0.01).

Litter was sampled again in March 2022 to determine the effects both mowing treatments have on litter cover. On both highways, the pre-mowing treatment effects on litter depth were still observed (Fig. 19). On Highway 2, non-pre-mowed plots had a mean litter depth of 49.2 mm, and pre-mowed plots had 38.4 mm of litter (P = 0.013). On Highway 77, non-pre-mowed plots averaged 33.7 mm of litter while pre-mowed plots had 23.1 mm of litter (P = 0.01). The second mowing in July 2021 also appeared to reduce litter depth in 2022 (Fig. 20). On Highway 2, year-after mowing in July 2021 led to a significant reduction in litter depth from 53.8 mm on un-mowed plots to 33.8 mm on mowed plots (P < 0.01). Year-after mowing effects were not as evident at Highway 77, where mowed plots had 25.7 mm of litter compared to 31.1 mm in un-mowed plots (P = 0.16). There were no observed interactions between mowing treatments for either site.

Figure 19. Main effect of pre-mowing on vegetative litter depth (millimeters) in March 2022. Mowing occurred in October 2020. A plot average litter depth was derived from each measurement along three transects. Plots were then averaged by mowing treatment. At both sites, pre-mowing appears to reduce the depth of litter present in plots ($P < 0.05$).
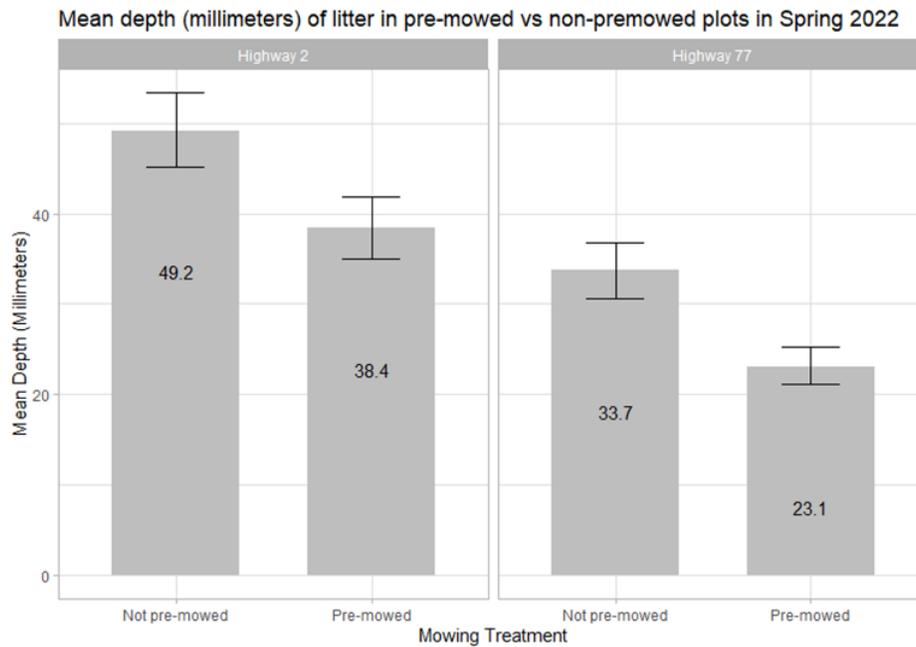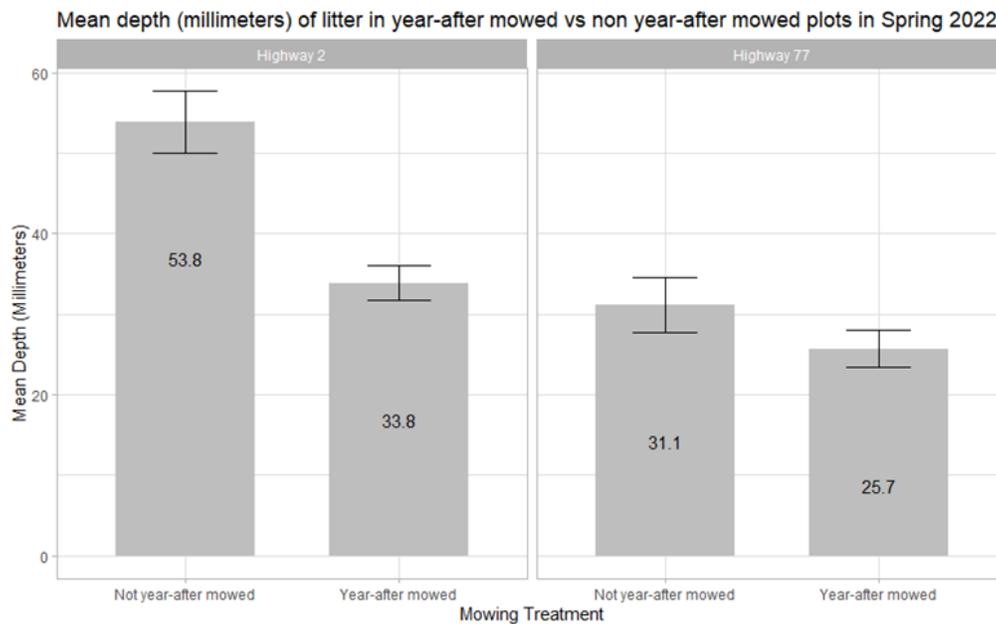

Figure 20. Main effect of year-after mowing on vegetative litter depth (millimeters) in March 2022. Mowing occurred in July 2021. Average litter depth was calculated using the same methods as Figure 2.15. Highway 2 mowed plots have significantly less litter than un-mowed plots ($P < 0.01$).

Counts of ground cover types were recorded for three categories: litter cover, basal cover, and bare ground. Data from both sites was combined because of similar results. In Spring 2021, following the October 2020 mowing treatment, vegetative litter occurred in 96% of observations in un-mowed plots compared to 92.1% of observations in mowed plots ($P < 0.01$). Bare ground was observed in 1.1% of points taken in un-mowed plots and 4.5% in mowed plots ($P < 0.01$). Basal cover was not affected by mowing in October. Table 2 shows the percentage of occurrence for each of the three cover types at both pre-mowing treatments.

Table 2. Percentage of each ground cover category observed in March 2021. There were significantly fewer litter observations and more bare ground observations on pre-mowed plots.

Mowing Treatment	Percent Litter Cover	Percent Basal Cover	Percent Bare Ground
Not pre-mowed	96%	2.9%	1.1%
Pre-mowed	92.1%	3.4%	4.5%
P-value	0.002	0.502	0.004

Ground cover was assessed again in spring 2022 after the final mowing treatment was implemented in July 2021. Results from both sites were combined again. Significant effects of pre-mowing in October 2020 were only observed for bare ground (Table 3). Plots that were not pre-mowed had 1.81% occurrence of bare ground compared to 3.83% on mowed plots ($P = 0.01$). The second mowing treatment in July 2021 had no significant effects on the counts of the three cover categories (Table 4).

Table 3. Percentage of each ground cover category observed in March 2022. Bare ground observations remained higher for pre-mowed plots.

Mowing Treatment	Percent Litter Cover	Percent Basal Cover	Percent Bare Ground
Not pre-mowed	89.7%	8.48%	1.81%
Pre-mowed	87.6%	8.61%	3.83%
P-value	0.07	0.89	0.01

Table 4. Percentage of each ground cover category observed in March 2022. The year-after mowing treatment did not appear to significantly affect the occurrence of ground cover types.

Mowing Treatment	Percent Litter Cover	Percent Basal Cover	Percent Bare Ground
Not year after mowed	89.7%	7.62%	2.71%
Year-after mowed	87.6%	9.46%	2.93%
P-value	0.09	0.06	0.79

Reducing vegetative litter cover appears to positively affect the percentage of forb cover present (Fig. 21) in summer 2021. A correlation coefficient of -0.366 was observed when comparing litter depth and forb cover (P < 0.01). Results show that vegetative litter over 50 mm generally leads to about 5-10% forb cover. Reducing litter depth to 25 mm increases forb cover to 20-30%. There appeared to be no positive correlation, though, between reduction in litter cover and forb cover in 2022 (Fig. 22). Forb cover does not seem to be affected by mowing treatments and associated litter cover one year after mowing is implemented. Reducing litter may be most beneficial for newly germinating seedlings than already established forbs.

Figure 21. Vegetative litter depth and the total percent cover of forbs is compared for 2021. Each point represents a plot. Treatments are not included in the data. Vegetative litter depths on the y-axis were measured in March 2021, and the forb cover data were collected in September 2021. Forb cover appears to increase as vegetative litter depth decreases (r = -0.366, P = 0.003).
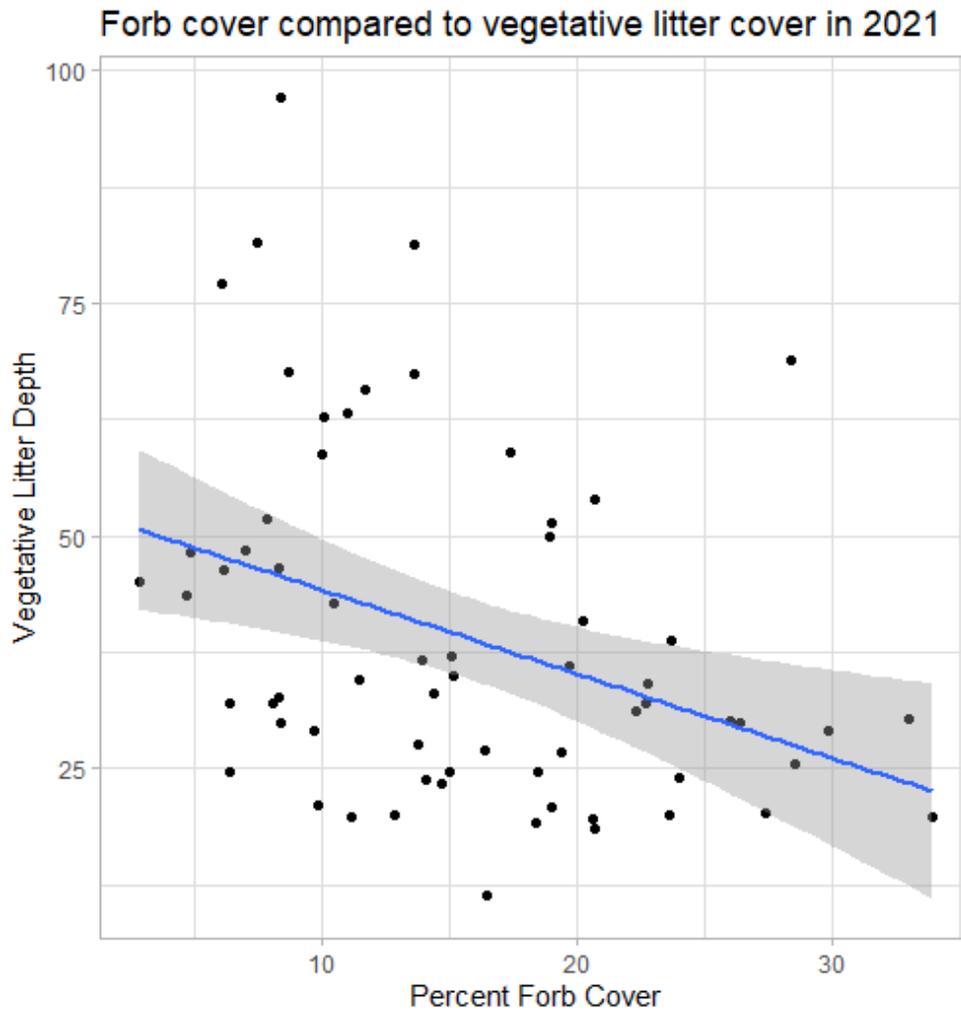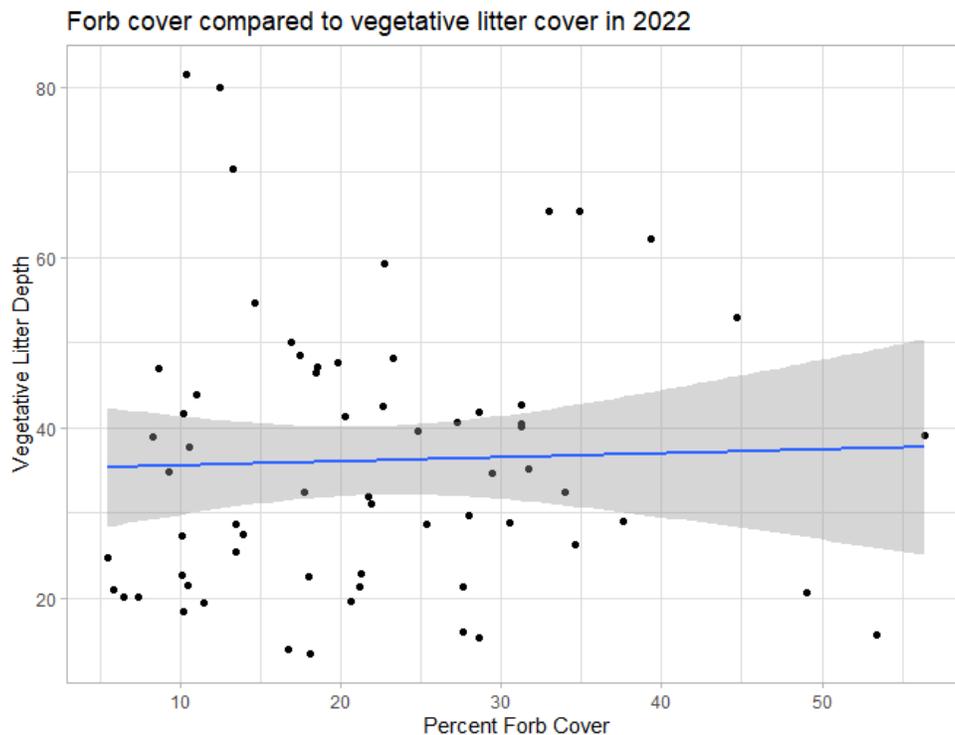
Figure 22. Vegetative litter depth and the total percent cover of forbs is compared for 2022. Each point represents a plot. Treatments are not included in the data. Vegetative litter depths on the y-axis were measured in March 2022, and the forb cover data were collected in September 2022. In 2022, there appears to be no correlation between litter depth and the amount of forb cover ($r = 0.034$, $P = 0.79$).

Milkweed Frequency

Throughout the duration of the study, three species of milkweed were observed within the roadside plots: common milkweed (*Asclepias syriaca*), whorled milkweed (*Asclepias verticillata*), and spider milkweed (*Asclepias viridis*). The two species of milkweed included in the seed mix (Table 1) were common milkweed and butterfly milkweed (*Asclepias tuberosa*), however, no butterfly milkweed plants were observed during the study. In summer 2020 before any treatments were applied, only six total frames contained common milkweed across all 64 plots. In summer 2021 after treatments had been applied to the plots, seeded plots had 26 total occurrences of common milkweed compared to just one occurrence in all un-seeded plots at both sites combined ($P < 0.01$). Mowing treatments did not have any significant impact on the frequency of common milkweed in 2021 or 2022. For pre-mowing and year-after mowing treatments, plots that were not mowed had slightly higher occurrences of milkweeds in both 2021 and 2022 (Table 5). In 2022, 94 total frames contained common milkweed across all treatments compared to only 27 frames in 2021. Conditions seem to have been more favorable for common milkweed in 2022 regardless of the seeding treatment applied. Common milkweed had a frequency of 68 on seeded plots and 26 on un-seeded plots in Summer 2022 ($P < 0.01$).

Frequency of common milkweed increased across the duration of the study with the strongest increases occurring in plots that received the seeding treatment (Fig. 23).
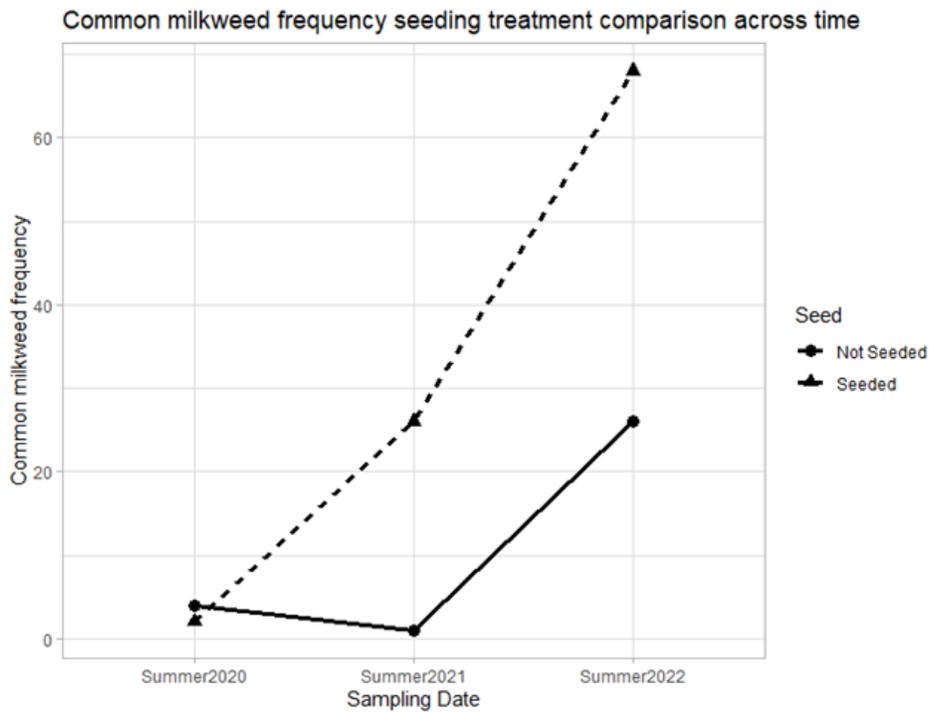

Figure 23. The frequency of common milkweed occurrences on seeded and unseeded plots across time is shown. Frequency was determined by the total number of frames containing common milkweed for each treatment at each sampling date. Common milkweed was the only milkweed species considered because it was the only species observed from the seed mix. Seeding occurred after the Summer 2020 sampling date. Milkweed frequency was considerably higher in seeded plots than un-seeded plots in Summer 2021 and Summer 2022.

Table 5. The frequency of common milkweed occurrences is shown in relation to the mowing treatment applied in summer 2021 and summer 2022. The pre-mowing treatment was applied in October 2020, and the year after mowing treatment was applied in July 2021. Frequency was calculated as the sum of frames containing common milkweed for each mowing treatment. There were no significant differences in the frequency of common milkweeds for any of the treatments in both 2021 and 2022. However, for both treatments at both years, un-mowed plots were observed to have a slightly higher frequency of milkweed compared to mowed plots.

Mowing Treatment	2021 Frequency	2022 Frequency
Not pre-mowed	16	51
Pre-mowed	11	43
Not year after mowed	18	51
Year after mowed	9	43

Seeded Forb Encounters

In summer 2020 before seeding, Maximilian sunflower was the most commonly encountered forb at both sites. A total of 427 frames contained the species in 2020. By the end of the study in summer 2022, a total of 394 frames contained Maximilian sunflower. Un-seeded plots had 215 frames and seeded plots had 179 frames containing the species. The year-after mowing treatment performed in July 2021 was the only treatment to have a significant effect on Maximilian sunflower in summer 2022. Mowed plots had 166 frames while un-mowed plots had 228 frames containing the species ($P = 0.0459$).

Showy partridge pea was found in 338 total frames at both sites in summer 2020 before seeding. Partridge pea became the most frequently encountered species by the end of the study in summer 2022 with 513 total frames containing the species. Seeding did not appear to significantly increase showy partridge pea as un-seeded plots had 244 frames and seeded plots had 269 frames containing the species. None of the treatments or combinations of treatments significantly influenced partridge pea encounters.

Plant species already present at the sites were chosen to ensure that some flowers would be adapted to roadside conditions and to provide easily established options for pollinators. Nineteen of the 33 species included in the seed mix were present at the site before seeding took place. Only one encounter of leadplant was observed during the study, and it was before seeding occurred. Following seeding, 30 of the 33 species were observed in 2021 and 2022, with only two species being completely excluded from our observations. This shows that our seed mix was in fact the cause of the increases in plant diversity at seeded plots. The two species that were not observed were Rocky Mountain bee plant and butterfly milkweed.

Table 6 shows the seeded species ranked in order of most occurrences to least occurrences for 2021 and 2022 combined. Partridge pea and Maximilian sunflower were the most encountered forbs regardless of whether the plot was seeded or not. Nineteen of the species on the list were observed in 2021 and 2022 in un-seeded plots. Thirty of the species were observed in seeded plots.

Table 6. The frequency of observation for each species in the seed mix is shown for Summer 2021 and 2022 combined. Frequency was calculated by counting the number of frames that a species occurred in. Frequency for the 33 species in the wildflower mix is shown for both seeded and un-seeded plots.

Top 10 most abundant forbs from the seed mix by seeding treatment in Summer 2021 and 2022		
Rank	Not Seeded	Seeded
1	Partridge pea --- 444	Partridge pea --- 501
2	Maximilian sunflower --- 419	Maximilian sunflower --- 365
3	Canada goldenrod --- 114	IL bundleflower --- 188
4	IL bundleflower --- 110	Canada goldenrod --- 164
5	Heath aster --- 94	Grayhead coneflower --- 121
6	Blackeyed Susan --- 79	Black Samson --- 111
7	Grayhead coneflower --- 48	Blackeyed Susan --- 106
8	False sunflower --- 35	Common milkweed --- 94
9	Smooth blue aster --- 35	Golden Alexander --- 88
10	Common milkweed --- 27	Heath aster --- 88
11	Pitcher sage --- 26	False sunflower --- 69
12	Tick trefoil --- 25	Blue flax --- 67
13	Rigid goldenrod --- 20	Smooth blue aster --- 62
14	Stiff sunflower --- 8	Stiff sunflower --- 58
15	Black Samson --- 4	Indian blanketflower --- 56
16	Missouri goldenrod --- 4	Pitcher sage --- 41
17	Purple prairie clover --- 1	Upright prairie coneflower --- 26
18	Roundhead bush clover --- 1	New England aster --- 22
19	Yarrow --- 1	Rigid goldenrod --- 21
20	Blue flax --- 0	Shell leaf penstemon --- 14
21	Blue vervain --- 0	Purple prairie clover --- 13
22	Canada milkvetch --- 0	Compass plant --- 12
23	Compass plant --- 0	Wild bergamot --- 8
24	Golden Alexander --- 0	Canada milkvetch --- 7
25	Indian blanketflower --- 0	Yarrow --- 6
26	Leadplant --- 0	Roundhead bushclover --- 4
27	New England aster --- 0	Blue vervain --- 3
28	Plains coreopsis --- 0	Tick trefoil --- 3
29	Shell leaf penstemon --- 0	Missouri goldenrod --- 2
30	Upright prairie coneflower --- 0	Plains coreopsis --- 1
31	Butterfly milkweed --- 0	Leadplant --- 0
32	Rocky Mountain bee plant --- 0	Rocky Mountain bee plant --- 0
33	Wild bergamot --- 0	Butterfly milkweed --- 0

DISCUSSION

Planting wildflowers on roadside slopes was effective at increasing total forb cover, however, the effects of seeding were strongest at the Highway 2 site for 2021 and 2022. Forb cover increased from 2021 to 2022 at both sites for both seeded and un-seeded plots. This increase was especially pronounced on seeded plots with Highway 2 having a 9.9% increase in total forb cover on seeded plots from 2021 to 2022 and Highway 77 having an increase of 4.8%. This observation was expected because many of the observed plants from the seed mix were very small in 2021 after planting. By 2022, many of the perennial plants had visually grown.

Many of the species in the seed mix were already present on the study sites before planting. These species were selected because they were already known to be successful at becoming established on highly disturbed roadside sites in southeastern Nebraska. Our seed mix included some dominant forb species already present on these roadside sites, however, Dickson and Busby (2009) suggest limiting the use of some dominant species, such as Maximilian sunflower and partridge pea because they can inhibit the growth of other desirable species. Reducing the use of dominant species could help promote greater overall forb diversity and cut back costs associated with using more seed. Prior to seeding, Highway 2 had an average of 7.7% cover of Maximilian sunflower, and Highway 77 had 2.4% cover of the species. Seed purchased for restoring a single site can be adjusted by determining which species are already present in large quantities. Planting additional Maximilian sunflower at a site like Highway 2 may not be necessary due to its high prevalence before seeding.

Most of the wildflower seedlings from the seed mix did not bloom in the two-year period of sampling. More time may be needed to see the wildflower species reach full maturity after planting. Studies have shown that native perennial wildflower cover and species richness will peak in the third growing season after planting and begin to decline in cover in years following (Schmidt et al. 2020; Korpela et al. 2013). Another study found that perennial wildflower abundance increased only in the second and third years after planting, while annual species only increased in the first year after planting (Carvell et al. 2022). It appears that at least three years are needed to see the full establishment of perennial wildflowers species.

As expected, seeding a 33 species native wildflower mix led to increases in plant species richness on both sites. These increases in species richness were the most pronounced at the Highway 2 site. Before seeding, Highway 2 plots averaged just 14.9 total plant species. Species richness at this site peaked in summer 2022 with 23.6 species per plot on average, compared to just 16.4 species on un-seeded plots. The effects of seeding were not as evident on Highway 77, which received less rainfall than Highway 2 during the study. These plots receiving seed treatments will have the greatest potential to provide pollinators with season-long floral resources. Ebeling et al. (2008) shows that high diversity flowering plant communities in grasslands will support high diversity and stability in pollinator communities. Providing a wide array of flowers will benefit pollinator species that specialize in using specific species or groups of plants (Hanberry et al. 2020). Many Lepidopteran caterpillars are specialist species utilizing only one genus of plant, which highlights the need for diverse seed mixes (Gilgert & Vaughn, 2011).

The most dominant forb before implementing treatments was Maximilian sunflower, Maximillian sunflower was found more frequently in un-seeded plots than plots that were seeded

despite the species being included in the planting mix. The occurrences of Maximilian sunflower do not appear to change much throughout the study for either seeding treatment. This suggests low germination success of Maximillian sunflower seeds in the planting mix. It is unknown why conditions did not favor germination of Maximillian sunflower seeds in this study. Given the high frequency of Maximilian sunflower already existing at the sites before seeding, it is assumed that seeds are sitting dormant in the soil until germination conditions are more favorable for the germination. According to Dietz et al. (1992), Maximilian sunflower can develop to maturity in one growing season under normal growing conditions. Site conditions appear to be favorable for existing plants, but not for newly planted seed. It is still possible that seeds will germinate in growing seasons after the conclusions of this study. In a southern Great Plains study, it was observed that Maximilian sunflower was one of the first forb species to wilt and dry out during drought conditions, and the greatest emergence of seedlings occurred in the fourth growing season after planting (Berg, 1990). Dry conditions may have led to poor emergence of newly planted seed, while existing plants are able to persist due to more developed root systems. The year after mowing treatment (July 2021) was the only treatment found to significantly affect the frequency of Maximilian sunflower occurrence in summer 2022. Mowed plots had 37.4% fewer occurrences of the species than plots that were not mowed. July mowing appears to limit regrowth of the species one year later. Though mowing could impair the floral resources from Maximilian sunflower, reducing this species could allow greater overall wildflower species diversity of a site. Dietz et al. suggests a conservative seeding rate of 0.3 to 0.6 kg/ha to limit competition with other species in the seed mix.

Showy partridge pea was the highest occurring forb species in summer 2021 and summer 2022. Frequency of partridge pea increased by 51.8% from the beginning of the study before seeding to the end of the study in summer 2022. No treatments were found to significantly affect the frequency of occurrence of the species. However, partridge pea was found slightly more frequently on seeded plots compared to un-seeded plots in summer 2022. Partridge pea appears to have increased in frequency without the help of seeding or mowing treatments. Partridge pea is an annual, so plants would have reproduced from seed. Partridge pea has been found to be highly adaptable to disturbed sites with a wide range of pH and low nutrient concentrations (Marcy & Martin, 1991). They also noted that the ability of the species to colonize nutrient deficient sites allows it to compete with other plant species, especially grasses, that are not as tolerant of low nutrient sites.

Milkweeds were studied in this project to determine the potential for increasing habitat for monarch butterflies, whose larvae use milkweeds as a source of food. Loss of milkweed plants in their breeding grounds across the eastern half of the United States likely has a larger impact on monarch butterfly populations than climate change and deforestation of wintering habitat in Mexico (Flockhart et al. 2015). Declines in milkweed plants are largely attributed to increasing use of glyphosate tolerant crops and associated use of glyphosate herbicides (Hartzler, 2010; Pleasants and Oberhauser, 2013). Roadside right-of-way areas provide potential stretches of land to increase milkweed stems in the monarch's migratory range. In 2010, 20% of total milkweeds along the migration path of eastern monarchs were estimated to be on roadsides (Pleasants & Oberhauser, 2013). Two species of milkweed were planted from our seed mix (Table 1), common milkweed and butterfly milkweed. Butterfly milkweed was not observed during this study. Spider milkweed and whorled milkweed were found within plots growing as

volunteer species. There was also one small patch of Sullivant's milkweed (*Asclepias sullivantii*) growing in the ditch outside of the research plots. Though these volunteer species were not included in the seed mix, they could be added to future seed mixes looking to add additional milkweed diversity in southeastern Nebraska. In an Iowa study comparing monarch egg density on nine different species of milkweeds, egg counts were found to be highest on common milkweed (Pocius et al. 2018). They found that Sullivant's milkweed had similar egg counts to common milkweed, while butterfly milkweed had much lower egg counts. Given that no occurrences of butterfly milkweed were observed from the seed mix and the low use by monarchs, selection of a different species may make more economical sense.

Common milkweed was observed only 6 times in summer 2020 before any treatments were applied. This highlights the need for increasing milkweed density on these roadsides. In summer 2021, milkweed frequency was significantly higher in seeded plots than un-seeded plots. Many of these milkweeds were small seedlings. Seeded plots still had a relatively low frequency of occurrence. Of the 384 frames observed in seeded plots, only 26 contained a milkweed plant. Only one frame contained a milkweed in un-seeded plots. In summer 2022, the total frequency of common milkweeds increased for both seeded and un-seeded plots. Sixty-eight frames in seeded plots contained common milkweed compared to 26 frames in un-seeded plots. It is unknown why there was an increase in milkweeds in un-seeded plots. Many of the milkweed stems were still quite small in 2022. Even though milkweed germination from seed was relatively low in this study, much of the seed from the mix could still germinate in the years following our vegetation monitoring. Given the dry conditions of the sites, milkweed frequency was still on the increase from year one to year two after planting. Up to 90% of common milkweed seeds remain viable in the soil five years after planting (Bagi, 2008). Future studies should consider monitoring newly planted sites for at least five years after planting to capture germination of seeds that may remain dormant until conditions favor those species. Once established, common milkweed can reproduce vegetative clones by sending out rhizomes that expand as much as 3 meters in the first year (Bagi, 2008). It may take several years to significantly increase the density of milkweeds after a restoration.

Mowing did not significantly impact common milkweed occurrence in 2021 and 2022, however, un-mowed plots always had a slightly higher occurrence of milkweeds compared to mowed plots. Given that the pre-mowing treatment occurred in late October before seeding occurred and most plants were dormant, it seems unlikely that the pre-mowing treatment led to a reduction in milkweed stems. Evidence from other mowing studies suggests that monarch butterfly egg densities are higher on common milkweed plants mowed in July than controls that were not mowed (Fischer, 2015; Knight et al. 2019). Our results showed an overall slight decrease in milkweed counts resulting from the early July mowing. However, milkweed establishment in our plots was low. More research would be needed on mature, more dense stands of milkweeds to determine how growing season mowing affects milkweeds on Nebraska roadsides.

Pre-mowing plots in October 2020 before seeding wildflowers was successful at reducing the depth of vegetative litter in plots that were sampled for basal cover in March 2021. For both sites combined, litter depth was 21.4 mm lower in pre-mowed plots compared to plots that were not mowed. It appears that some of the plant litter is removed from the plots when a single 15-foot-wide strip is mown. Mowing in October after warm season grasses entered dormancy

reduced the vegetative litter layer that accumulates in un-mowed plots where vegetation is left standing. This reduction in dead biomass had no effects on live standing biomass of grasses the summer following the initial mowing treatment. In addition to reducing the depth of the litter layer in plots, fall mowing increased the occurrence of bare ground by 3.4%. Increasing bare ground and reducing litter cover has the potential to increase light penetration to newly germinating seedlings. The effects of pre-mowing on litter cover and bare ground appeared to be sustained when measured again in March 2022. Litter accumulation in response to pre-mowing in October 2020 remained significantly lower on mowed plots more than one year later. These results suggest that mowing dead vegetation can lead to a reduction in total dead biomass for at least two growing seasons. The July 2021 mowing did not lead to any differences in bare ground. Litter depth, however, was lower in July mowed plots compared to plots not mowed in July. Mowing live vegetation in July prevented grasses from growing to maturity.

In summer 2021, there was a positive correlation of forb growth compared with vegetative litter depth. As litter depth decreases, the percentage of forb cover appears to increase in the first season after planting. Removing litter can free newly emerging seedlings from sunlight restrictions and increase the value of a site as pollinator habitat. Our results are supported by Pei et al. (2023), where reducing litter depth positively impacts forb growth. Reducing litter accumulation has the potential to increase the habitat value of a site by increasing both the forb cover and species diversity. This could allow for the greatest potential to have a variety of floral resources that benefit multiple pollinator species throughout the length of the growing season.

Litter depth in March 2022 after both mowing treatments had been implemented showed no interaction with forb cover. Perhaps litter cover has less effect on already established forbs than newly emerging forbs from seed. Drier conditions in 2022 could have also affected the growth of forbs in Summer 2022. Removal of litter during drought conditions could possibly lead to increased evaporation of soil moisture, negatively impacting the growth of forbs. Prairie sites with litter removed lost about 3% more total soil moisture in July and August compared to sites where vegetative litter was not removed (Deutsch & Willms, 2010).

Mowing in our study was effective at removing plant biomass from the research plots, however, it should be noted that we only made one pass with the mower through each plot. If wider areas are being sown with wildflowers, multiple passes from the mower could lead to some of the biomass landing in the planted area (Figure 24). Consideration should be taken to avoid large clumps or strips of dead biomass that could poorly impact seedling germination. One option to remove cut biomass is haying. A study comparing grassland simulated mowing and haying treatments on grassland sod units in a controlled greenhouse setting found that sod units where biomass was cut and removed averaged 10 species (Jutila & Grace, 2002). By contrast, they found that cutting vegetation leaving biomass on the soil surface led to only 3.1 species per sod unit. Haying may come with some drawbacks, however. First, haying will require more equipment to travel over the seeded area, potentially leading to more soil compaction. The safety of using bailing equipment on steep roadside slopes would also need to be taken into consideration. One final consideration with haying is the cost of additional labor and equipment associated with baling and removing bales from the sites.

Figure 24. Vegetative litter is shown in a plot following the early July mowing treatment. While much of the plot has reduced litter, strips of dense litter may be present between and outside the mower blade path.

CONCLUSION

Data from two growing seasons after implementing seeding and mowing treatments suggests that seeding and pre-mowing can be effective at increasing the forb density and diversity on southeast Nebraska roadsides. These results, however, can be variable under differing site conditions. We conclude:

- Seeding wildflowers is effective at increasing wildflower density, diversity, and floristic quality.
- Mowing in October before seeding increases forb cover and floristic quality.
- Mowing in early July after seeding is effective at limiting the growth of grasses, however, this reduction in live grasses does not appear increase forb cover.
- Pre-mowing and year-after mowing are effective at reducing the depth of litter across most of a plot boundary. This litter may still be present in concentrated strips. Areas where multiple mower passes will be made will likely have strips of dense litter throughout the mowed area.
- A reduction in vegetative litter is positively correlated with greater forb cover in the first season after seeding. Reducing litter likely allows new seed to germinate free from competition for sunlight.
- Reducing vegetative litter cover does not appear to impact forb cover the second growing season after planting.

- Seeding common milkweed greatly increases the occurrence of common milkweeds present on roadsides. Butterfly milkweed had no establishment in this project.
- Mowing in October and early July did not significantly impact the frequency common milkweeds.

RECOMMENDATIONS

This research confirmed that seeding wildflowers on Nebraska's roadsides is effective at increasing forb diversity, forb cover, and floristic quality of roadside sites. However, the success of establishment can be highly variable dependent on the site conditions. Rainfall is likely one reason for the limited success of establishment on Highway 77, however, there may be other unknown characteristics that impact the germination of wildflowers. In cases of limited funding for roadside restoration, soil conditions may be assessed to select only the sites with the best likelihood of forb establishment. Floral resources were observed to be low for seeded forbs. As other research has suggested, it may take several growing seasons to see a wider array of blooming flowers especially in dry conditions. Thirty-one of the 33 species in the seed mix were observed at least one time during the study. Further refinement of species with poor establishment could further cut costs. One of the species from the mix that was not observed, butterfly milkweed, has been found to have lower use by monarch butterflies compared to other milkweed species. To increase the diversity of milkweed resources, species already present on roadsides may be selected, such as Sullivant's or whorled milkweed. These species could be added to seed mixes to supplement the existing plants already present on roadsides. Fall mowing before seeding seems to have some positive effects on forb cover in the first year after seeding. Pre-seeding mowing can be used as a site preparation tool to redistribute litter and open bare ground for seed to germinate. It should be noted that the litter is not completely removed from the site with mowing. Mowers that move litter out of the seeded area may create patchy growth elsewhere from excess strips of litter. Haying is another option to remove dead plant litter, but it will likely involve more costs and soil compaction. Early July mowing did not aid in growth of seeded forbs, and likely led to a slight reduction in forbs already present at the site. Additional monitoring of newly restored sites would provide more insight to the long-term sustainability of roadside plantings.

REFERENCES

Bagi, I. (2008). Common milkweed (L.). In *The Most Important Invasive Plants in Hungary, Institute of Ecology and Botany, Hungarian Academy of Sciences, Vácrátót* (pp. 151-159).

Berg, W. A. (1990). Native forb establishment and persistence in a grass-forb seeding in the southern plains. In *Proceedings of the 12th North American Prairie Conference: Recapturing a Vanishing Heritage. University of Northern Iowa, Cedar Falls, IA* (pp. 179-181).

Blaauw, B. R., & Isaacs, R. (2014). Flower plantings increase wild bee abundance and the pollination services provided to a pollination-dependent crop. *Journal of Applied Ecology*, *51*(4), 890-898.

Blackmore, L. M., & Goulson, D. (2014). Evaluating the effectiveness of wildflower seed mixes for boosting floral diversity and bumblebee and hoverfly abundance in urban areas. *Insect Conservation and Diversity*, *7*(5), 480-484.

Cameron, S. A., Lozier, J. D., Strange, J. P., Koch, J. B., Cordes, N., Solter, L. F., & Griswold, T. L. (2011). Patterns of widespread decline in North American bumble bees. *Proceedings of the National Academy of Sciences*, *108*(2), 662-667.

Carvell, C., Mitschunas, N., McDonald, R., Hulmes, S., Hulmes, L., O'Connor, R. S., ... & Redhead, J. W. (2022). Establishment and management of wildflower areas for insect pollinators in commercial orchards. *Basic and applied ecology*, *58*, 2-14.

Deutsch, E. S., Bork, E. W., & Willms, W. D. (2010). Soil moisture and plant growth responses to litter and defoliation impacts in Parkland grasslands. *Agriculture, ecosystems & environment*, *135*(1-2), 1-9.

Dickson, T. L., & Busby, W. H. (2009). Forb species establishment increases with decreased grass seeding density and with increased forb seeding density in a Northeast Kansas, USA, experimental prairie restoration. *Restoration Ecology*, *17*(5), 597-605.

Dietz, D. R., Wasser, C. H., Dittberner, P. L., & Martin, C. O. (1992). Maximilian sunflower (Helianthus maximiliani): section 7.4. 3, *US Army Corps of Engineers wildlife resources management manual*.

Ebeling, A., Klein, A. M., Schumacher, J., Weisser, W. W., & Tscharntke, T. (2008). How does plant richness affect pollinator richness and temporal stability of flower visits?. *Oikos*, *117*(12), 1808-1815.

Entsminger, E. D., Jones, J. C., Guyton, J. W., Strickland, B. K., & Leopold, B. D. (2017). Evaluation of mowing frequency on right-of-way plant communities in Mississippi. *Journal of Fish and Wildlife Management*, *8*(1), 125-139.

Fischer, S. J. (2015). Enhancing monarch butterfly reproduction by mowing fields of common milkweed. *The American Midland Naturalist*, *173*(2), 229-240.

Flockhart, D. T., Pichancourt, J. B., Norris, D. R., & Martin, T. G. (2015). Unravelling the annual cycle in a migratory animal: breeding-season habitat loss drives population declines of monarch butterflies. *Journal of Animal Ecology*, *84*(1), 155-165.

Gilgert, W., & Vaughan, M. (2011). The value of pollinators and pollinator habitat to rangelands: connections among pollinators, insects, plant communities, fish, and wildlife. *Rangelands*, 33(3), 14-19.

Goulson, D., Nicholls, E., Botías, C., & Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science*, *347*(6229).

Hanberry, B. B., Debano, S. J., Kaye, T. N., Rowland, M. M., Hartway, C. R., & Shorrock, D. (2020). Pollinators of the Great Plains: Disturbances, stressors, management, and research needs. *Rangeland Ecology & Management*. doi:10.1016/j.rama.2020.08.006

Hartzler, R. G. (2010). Reduction in common milkweed (*Asclepias syriaca*) occurrence in Iowa cropland from 1999 to 2009. *Crop Protection*, *29*(12), 1542-1544.

Hopwood, J. L. (2008). The contribution of roadside grassland restorations to native bee conservation. *Biological conservation*, *141*(10), 2632-2640.

IUCN. 2022. *The IUCN Red List of Threatened Species*. Version 2022-1. Accessed on 9/27/2022 at: https://www.iucnredlist.org. Accessed on 9/27/2022

Jakobsson, S., Bernes, C., Bullock, J. M., Verheyen, K., & Lindborg, R. (2018). How does roadside vegetation management affect the diversity of vascular plants and invertebrates? A systematic review. *Environmental Evidence*, *7*(1), 17.

Jutila, H. M., & Grace, J. B. (2002). Effects of disturbance on germination and seedling establishment in a coastal prairie grassland: a test of the competitive release hypothesis. *Journal of ecology*, *90*(2), 291-302.

Knight, S. M., Norris, D. R., Derbyshire, R., & Flockhart, D. T. (2019). Strategic mowing of roadside milkweeds increases monarch butterfly oviposition. *Global Ecology and Conservation*, *19*, e00678.

Korpela, E. L., Hyvönen, T., Lindgren, S., & Kuussaari, M. (2013). Can pollination services, species diversity and conservation be simultaneously promoted by sown wildflower strips on farmland? *Agriculture, Ecosystems & Environment*, *179*, 18-24.

Marcy, L. E., & Martin, C. O. (1991). *Partridge Pea (Cassia Fasciculata)*. US Army Engineer Waterways Experiment Station.

Nebraska Department of Transportation. (2022). *NDOT Roadside Vegetation Establishment and Management*. Retrieved July 27, 2022, from https://dot.nebraska.gov/media/4016/veg-manual.pdf

NOAA National Centers for Environmental information, Climate at a Glance: County Time Series, published March 2022, retrieved on February 24, 2023 from https://www.ncdc.noaa.gov/cag/

Pei, C. K., Hovick, T. J., Limb, R. F., Harmon, J. P., & Geaumont, B. A. (2023). Invasive grass and litter accumulation constrain bee and plant diversity in altered grasslands. *Global Ecology and Conservation*, *41*, e02352.

Pleasants, J. M., & Oberhauser, K. S. (2013). Milkweed loss in agricultural fields because of herbicide use: effect on the monarch butterfly population. *Insect Conservation and Diversity*, *6*(2), 135-144.

Pocius, V. M., Pleasants, J. M., Debinski, D. M., Bidne, K. G., Hellmich, R. L., Bradbury, S. P., & Blodgett, S. L. (2018). Monarch butterflies show differential utilization of nine Midwestern milkweed species. *Frontiers in Ecology and Evolution*, *6*, 169.

Schacht, W., Soper, J. M., & Wienhold, C. (2017). Improving Wildflower Longevity in Roadside Seeding Areas.

Semmens, B. X., Semmens, D. J., Thogmartin, W. E., Wiederholt, R., López-Hoffman, L., Diffendorfer, J. E., ... & Taylor, O. R. (2016). Quasi-extinction risk and population

targets for the Eastern, migratory population of monarch butterflies (*Danaus plexippus*). *Scientific Reports*, *6*(1), 1-7.

Taft, J. B., Wilhelm, G. S., Ladd, D. M., & Masters, L. A. (1997). *Floristic quality assessment for vegetation in Illinois, a method for assessing vegetation integrity* (p. 29). Westville, Illinois: Illinois Native Plant Society.

U.S. Fish and Wildlife Service. (2020). Assessing the status of the monarch butterfly. Retrieved November 23, 2020, from https://www.fws.gov/savethemonarch/ssa.html

Williams, D. W., Jackson, L. L., & Smith, D. D. (2007). Effects of frequent mowing on survival and persistence of forbs seeded into a species-poor grassland. *Restoration Ecology*, *15*(1), 24-33.

Williams, N. M., Ward, K. L., Pope, N., Isaacs, R., Wilson, J., May, E. A., ... & Peters, J. (2015). Native wildflower plantings support wild bee abundance and diversity in agricultural landscapes across the United States. *Ecological Applications*, *25*(8), 2119-2131.

Winfree, R., Aguilar, R., Vázquez, D. P., LeBuhn, G., & Aizen, M. A. (2009). A meta-analysis of bees' responses to anthropogenic disturbance. *Ecology*, *90*(8), 2068-2076.