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Establishment of Wildflower Islands to Enhance Roadside Health, Ecological Value, and Aesthetics

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

by

Jackson Ebbers

A THESIS

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

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University of Nebraska, 2024

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Roadsides provide an abundant opportunity to increase connectivity of fragmented landscapes with diminishing floral resources for pollinating insects. The ecological value of these sites is often overlooked as quality habitat for pollinators, particularly monarch butterflies, which have been experiencing severe declines due to loss of habitat and loss of milkweeds that provide food for their larvae. Land managers across the nation are realizing the potential of roadsides to provide high quality floral resources for the benefit of insects and other wildlife. Current wildflower seed mixes used by state transportation departments are often low diversity and may only be implemented following road construction with no follow-up management. Mowing is often used on roadsides to manage vegetation for safety and aesthetic reasons. The timing and frequency of mowing can impact the density and diversity of wildflowers present on a site. This research seeks to determine the efficacy of seeding a diverse, native wildflower seed mix into backslopes with well-established perennial grasses. We monitored two sites in southeast Nebraska in 2021 and 2022. Two mowing regimes: October pre-seeding mowing and July post-seeding mowing were studied to determine the effects that mowing may have on existing perennial grasses. Our results indicate that seeding wildflowers is effective at increasing wildflower density, diversity, and floristic

quality of a site. Milkweed abundance also increased because of seeding. Mowing dormant vegetation in October before seeding appears to increase forb cover and floristic quality of one site, particularly in the first season after planting. This benefit of pre-seeding mowing appears to stem from a reduction in vegetative litter in the plots. Post-seeding mowing provided no benefit to wildflower density or diversity. Neither mowing treatment led to a reduction of perennial grass cover by the second season of the study, confirming that grasses can recover quickly following disturbance. The temporary reduction of litter following October mowing, however, may enable forbs to germinate in the spring following seeding.

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GRANT INFORMATION

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Figure 2.13. 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 < 0.01). Seeding effects were weak for Highway 77 (P = 0.092).

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Figure 2.18. Main effect of pre-mowing on vegetative litter depth (millimeters) in March 2022. Mowing occurred in October 2020. Each site is shown separately. 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 (Highway 2, P = 0.013; Highway 77, P = 0.009).

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Figure 2.20. 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. The vegetative litter depth on the y-axis was collected in March 2021, and the forb cover data was collected in September 2021. Litter depth for each plot was calculated by averaging litter depth in millimeters at each point taken along three transects. For each frame of forb cover data, the sum of forb cover was calculated. These frames were then averaged by plot. Forb cover appears to increase as vegetative litter depth decreases in the first season after seeding wildflowers (r = -0.366, P = 0.003).

CHAPTER 1

LITERATURE REVIEW

Roadside Habitat Enhancement

Roadside plantings of native grasses and forbs are used for soil stabilization on roadside slopes, habitat for wildlife, and aesthetic value. Vegetation with strong root systems prevents the loss of soil due to erosion, which protects infrastructure associated with roads. Plantings of native forbs and grasses are also useful for enhancing habitat quality for pollinators and improving the aesthetics of our roadways. 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). Infections from pathogens, lower genetic diversity due to small population sizes, climate change, and increasing use of pesticides in agriculture also contribute to pollinator decline (Cameron et al. 2011; Goulson et al. 2015).

Perhaps the biggest challenge of pollinator habitat enhancement in Nebraska is availability of public lands. Nebraska is 97% privately owned land, making large-scale habitat enhancement difficult. The Conservation Reserve Program (CRP) is a voluntary land retirement program administered by the United States Department of Agriculture (USDA) that provides compensation to landowners to convert cropland to grasslands (Stubbs, 2014). Many of these contracts involve seeding mixtures of native grasses and forbs to benefit wildlife, including pollinators, and to prevent soil erosion. CRP is an important source of pollinator habitat in areas where row crops dominate the landscape, such as southeast Nebraska. From its peak enrollment in 2007, CRP acres on former cropland have dropped in subsequent signups largely due to rising commodity prices and stagnant CRP rental rates (Stubbs, 2014). One study has found that the most common barriers that prevent landowners from enrolling in government conservation programs includes: lack of knowledge on programs or implementation of conservation practices, program eligibility requirements, and lack of economic benefit from retiring production lands (Reimer & Prokopy, 2014). Lack of voluntary conservation on agricultural lands highlights the importance of roadsides as potential pollinator habitat. The use of roadways for habitat enhancement provides an opportunity for conservation of pollinators and plant diversity in a landscape dominated by row crops.

Another challenge of managing diverse plant communities for pollinator health is the fact that suitable habitat with diverse stands of season-long blooming wildflowers is extremely fragmented in many agricultural landscapes. Fragmented habitat can be difficult to define depending on the type of wildlife in question. For pollinators, especially migratory species like the monarch butterfly (*Danaus plexxipus* L.), floral resources are needed in a continuous stretch across their entire migration route. A landscape could be considered fragmented when agricultural lands cut grasslands into isolated patches. A lack of habitat corridors connecting small patches of habitat leads to fragmentation, which in Nebraska is most often caused by extensive row cropping. It has been found that even small-scale fragmentation of suitable habitat can have significant effects on the movements of native bees. For example, bumblebees (*Bombus sp.*) will avoid 1.5 m^2 wildflower patches that are separated from continuous floral resources by a

5 m mowed strip (Goverde et al. 2002). Fragmented habitat can also lead to changes in the composition of flowering plants, which may lead specialist pollinators, or insects that collect pollen and nectar from a specific group of plants, to consume a more generalist diet, in which the insects collect pollen and nectar from a larger array of plant species (Xiao et al. 2016). Small scale patches of wildflowers (1.5 m^2) will have lower visitation rates by pollinators, leading to reduced pollen dispersal among the flowers, which in turn leads to an increase in inbreeding and lower genetic diversity in plants (Goverde et al. 2002). Ison and Wagenius (2014) suggested that spatial isolation of Black samson (*Echinacea angustifolia* DC.) can lead to about 25% lower seed production in plants that are located greater than 50 meters from neighboring plants. This drop in seed production could be due to a lack of pollen movement by insects to isolated flowers. The abundance of wild bee visits has also been linked to the distance a patch of plants is located from a tract of grassland. Bee abundance decreases with increasing distance of up to 1000 meters from a semi-natural grassland (Steffan-Dewenter & Tscharntke, 1999). Utilizing roadways for enhancement of pollinator habitat can also provide travel corridors for wildlife, including small mammals, reptiles, and grassland birds in areas where habitat is fragmented (Säumel et al. 2016). These travel corridors provided by roadside habitat can help decrease the negative impacts experienced by wildlife and plant diversity because of fragmentation.

Land use change from native prairie to cropland and urban development may be the largest contributor to the loss of biodiversity in species across the Great Plains region, including major losses of pollinating insects. An estimated 1.3 million acres of grassland was lost in the Western Corn Belt between 2006 and 2011 (Wright & Wimberley, 2013). They found that conversion of grasslands, including native prairie, pasture, hay lands and CRP lands, to cropland was the main land use change at 1.0 – 5.4% of grass converted to corn and soybeans annually during this time. Pollinating insects become increasingly stressed with increasing conversion of grasslands to cropland and urbanization due to losses of nectar sources (Winfree et al. 2009).

Many pollinating insects rely on the nectar or pollen of wildflowers as a source of food. While gathering nectar, many pollinators inadvertently transfer pollen from one flower to another (Hopwood, 2013). Grains of pollen from the male anther of one flower can be transferred to the female stigma of a flower from the same species, resulting in the production of seeds through cross-pollination (Barrett & Harder, 1996). Barrett and Harder (1996) stated that successful pollination results in fertilization of the ovules and the ability to grow seeds or fruit. They noted that plants can also reproduce by self-pollination where pollen from the anther is deposited on the stigma of a flower on the same plant, but cross-pollination leads to more genetic variation of plants.

In addition to the benefits for pollinating insects, roadside habitat management can be an important tool in carbon sequestration. Carbon sequestration occurs when soil and vegetation remove carbon dioxide (CO_2) from the atmosphere and convert it to a solid form (Da Silva et al. 2010). The process of photosynthesis takes in carbon and stores it in the leaves, stems, and roots of plants. Organic carbon is then stored in soil as plant material decays. The Federal Highway Administration (FHWA, 2010) estimated that the more than 2 million hectares of right-of-way land in the United States stores 90 million metric tonnes of carbon at 3.6 million metric tonnes stored per year. The FHWA also estimated that the amount of storage could be up to seven times higher by converting open areas along highways to native perennial grasses and increasing tree cover in forested areas. In some cases, the cost of roadside vegetation management can be offset by the value of carbon sequestration because carbon credits can be sold by state transportation departments for income (Da Silva et al. 2010).

Erosion control is another benefit to roadside vegetation management because it prevents runoff from damaging structures such as culverts, bridges, signs, and the roads themselves. Roots from vegetation help hold the soil together and prevent soil from washing the backslopes into the ditches. Non-native grasses, such as smooth bromegrass (*Bromus inermis* Levss) and Kentucky bluegrass (*Poa pratensis* L.), can be desirable for erosion control due to their rapid establishment and robust root systems; however, the competitive nature of non-native grasses can displace desirable native forbs, shrubs, and grasses (Beyers, 2004). Vegetation along roadways can also be important for water quality in lakes and streams. Roadside vegetation can reduce nutrient and sediment loads from nearby agricultural fields by sequestering excess nutrients and trapping sediment before it reaches the ditch (Streeter & Schilling, 2020).

Habitat enhancement that benefits pollinators can also benefit other wildlife populations, including gallinaceous game birds, songbirds, herbivorous mammals, and even fish that consume insects along streams (Gilgert, Vaughan, 2011). Gilgert and Vaughan (2011) explained that many birds and mammals consume the seeds or other plant materials associated with forbs that are pollinated by insects. By connecting fragmented habitats, small wildlife populations will be able to disperse more evenly, allowing individuals to find more potential mates and reduce the effects of genetic drift (Banks et al. 2007).

Roadsides in Nebraska

The United States has over 6.5 million km of public roadways, with over 156,000 km of public roads in Nebraska (Wojcik & Buchmann, 2012). Given that pollinator habitat is fragmented across the state, roadsides may provide an opportunity to connect habitat patches. The greatest species richness of roadside vegetation in Nebraska is typically found in the western portions of the state, particularly in the sandhills, while the lowest species richness is typically found in areas dominated by row crops (Soper et al. 2019). Roadsides in row crop dominated landscapes should be a focus of restoration to provide pollinator habitat in locations where diversity is limited. Previous research on wildflower establishment in Nebraska has focused on re-vegetating sites after construction has occurred, meaning there is little to no vegetation established at the time of seeding. A previous study conducted in southeast Nebraska on newly constructed roadside slopes found that segregating rows of wildflowers from the native grasses led to greater forb establishment than planting a mixture of grasses and wildflowers evenly across the site (Wu-Smart & Schacht, 2019). They also found that mid-season blooming forbs were 30% more abundant in the second year of the study than the first year after planting. Results from a study in central Nebraska indicated that mowing in July and September only benefited the frequency of occurrence for black-eyed Susan (*Rudbeckia hirta* L.) and upright prairie coneflower coneflower [*Ratibida columnifera* (Nutt.)

Wooton & Standl.] of the 10 species seeded (Schacht et al. 2017). They also found that inter-seeding forbs into established perennial grasses was only marginally successful compared to segregated rows of wildflowers.

Nebraska Department of Transportation Management Practices

The Nebraska Department of Transportation (NDOT) typically designs seed mixes to match the climatic and soil conditions found within a specific region of the state. The NDOT has identified six different landscape regions across the state, each having its own seed mix for use on roadsides. The six regions are: Loess Hills, Loess Hills and Glacial Drift, Central Loess Plains & Rainwater Basins, Sandhills, Shale-Plains Tablelands, and High Plans (Figure 1.1). Nebraska Department of Transportation biologists have identified unique combinations of forbs and grasses that will have the highest likelihood of establishing in each region. Native species are preferred due to the ecological, economic, aesthetic and safety benefits from native plantings. Native plants are also desired because they benefit biodiversity and enhance resilience of roadsides to invasive species (Isaacs et al. 2009). Economic benefits from native species include prevention of soil erosion around roadside structures and the prevention of invasive plant establishment. Native plants, especially wildflowers, also make our roadsides more visually appealing to visitors. In addition, native plants provide safety benefits, such as enhanced visibility and traction for vehicles that have left the roadway (Steinfeld et al. 2007).

The NDOT typically seeds roadsides with 14 total species of plants in the Loess and Glacial Drift region (Region B), which is where this study was performed (Table

1.1). Eight are native grasses, five are native forbs, and the final species planted is common oat (*Avena sativa L.*) or wheat (*Triticum aestivum L.*). Common oat or wheat is used after construction due to their ability to establish quickly for erosion control and are generally only used for temporary cover. In most NDOT plantings, about 10% of the total seed includes forbs.

On surfaced road shoulders, NDOT requires a minimum mowing width of 1.52 m and a maximum of 4.57 m from the pavement. The NDOT vegetation management protocols allow managers to alter the 1.52 m minimum mowing width to avoid destroying flowers in bloom. Vegetation is mowed to a height of no shorter than 15 cm above the ground. This helps prevent forb seedlings from being mowed before they can produce seed. In some areas, mowing is halted from May 1 to October 1 to avoid destroying nectar-producing flowers that pollinators use for food. Private landowners are prohibited from mowing roadsides without permission in most cases; however, this is difficult to enforce, and it provides a challenge to establishing wildflower islands on public roadways.

Pollinators and Roadsides

Pollinating insects provide many ecosystem services that benefit both humans and wildlife. Conservation of wild bee populations is important in sustaining pollinator-dependent crops, many of which provide food for humans and livestock (Williams et al. 2015). One third of all agricultural output in the United States depends on pollination (McGregor, 1976). Pollination is also beneficial to many native wildland plants and can increase the ecological services provided by them (Potts et al. 2010). A healthy plant community is in turn beneficial to other wildlife and even pollinators by providing nectar and seed sources for food.

Habitat heterogeneity is important for maintaining healthy populations 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). Most species of bees in North America are ground-nesting, so the presence of bare ground patches near rich and abundant floral resources is ideal for survival (Hopwood, 2008). Some species of bumble bees use abandoned mammal dens or layers of plant litter to make their nests (Gilbert & Vaughan, 2011).

Ground nesting bees generally prefer nesting sites on south to west-facing slopes with at least 10% bare ground present (Hopwood, 2008). According to Hopwood (2013), patches of bare ground suitable for bees are often found near the base of native bunch grasses, while areas with dense stands of nonnative sod-forming grasses, such as smooth bromegrass tend to have fewer ground-nesting bees. Bee abundance tends to increase as soil temperatures increase and soil moisture drops (Buckles & Harmon-Threatt, 2019). Hopwood (2008) suggested that roadside slopes as narrow as 18 meters with patches of bare ground and diverse floral resources could provide beneficial pollinator habitat. This suggests that even the narrowest right-of-way zones can provide some habitat for insects.

Blackmore and Goulsen (2014) have shown that bumblebees are 50 times more abundant on seeded plots of existing grassland vegetation mown each autumn versus unseeded grassland plots mowed multiple times per year at variable intervals. These benefits, however, are not always immediate. They noted that bumblebee abundance nearly doubled in seeded plots from the first year to the second year after sowing wildflowers. In a pollination dependent crop setting, native bees and syrphid flies (Syrphidae family) increased in abundance annually across a four-year period in fields with wildflower sown margins, while fields with grassy margins not sown with wildflowers had no significant increase in native pollinator abundance (Blaauw & Isaacs, 2014). By contrast, non-native honeybee (*Apis melifera* L.) abundance was not impacted by sowing wildflowers into field margins. These results indicate that native pollinators need multiple years to respond positively to wildflower plantings.

Leonhardt and Blüthgen (2012), found that bumblebees tend to collect pollen with substantially more protein and essential amino acids when compared with honeybees, suggesting that bumblebees search for higher quality food sources rather than quantity. They also observed bumblebees visiting twice as many plant species as honeybees despite the fact they prefer higher quality food sources, and they frequently changed the species they were visiting when compared to honeybees, which visited a relatively constant array of flower species. It is important that native bumblebees have a healthy supply of late blooming wildflowers before winter arrives. Goldenrods (*Solidago sp.*) and asters (*Symphyotrichum sp.*) provide bumblebee queens a source of nectar after the first frosts in the fall, which allows them to store fat for hibernation (Gilgert & Vaughan, 2011).

Monarch Butterfly Conservation

Research on roadside habitat is important as many pollinator species are on the decline. Perhaps one of the most well-known pollinators found on roadsides is the monarch butterfly. The Eastern migratory population of monarchs has decreased by an estimated 84% in the last decade (Semmens et al. 2016). In 2014, the U.S. Fish and Wildlife Service (USFWS) petitioned to list monarch butterflies under the Endangered Species Act (U.S. Fish and Wildlife Service, 2020). However, the USFWS decided in December 2020 that the monarch would not be listed due to being a lower priority than some other species being considered for listing. Precluding the monarch butterfly from being listed on the Endangered Species Act means that no federal protections are in place to conserve the species. In July 2022, the monarch was placed on the International Union for Conservation of Nature (IUCN) Red List as an endangered species (IUCN, 2022). Being listed as endangered under the IUCN Red List does not provide any federal protections for the species, however, it does provide some insight into the overall status of the species and helps bring awareness to declining populations. In September of 2023, the IUCN made another decision to alter the listing of the monarch butterfly from Endangered to Vulnerable (IUCN, 2023). This decision was based on a report by Thogmartin et al. (2020) which stated that declines in monarch populations appeared to slow or stabilize after the year 2014. This reduction in the rate of decline is presumed to be from an overall stabilization in use of herbicides and loss of monarch habitat from land use change (Thogmartin et al. 2017). Regardless of the current listing of the monarch butterfly, it is apparent that conservation efforts are needed to protect the species from further declines. Further conservation efforts will be needed for improving the quantity and quality of floral resources available to monarchs within their migration routes.

Monarchs rely on milkweeds for laying their eggs and as a source of nectar for adults and foliage for larvae. It has been estimated that 850 million milkweed plants have been lost from row crop fields, and an additional 11 million have been lost from grasslands since 1999 (Pleasants, 2017). Pleasants (2017) estimates that 40% of the total milkweeds have been lost from 1999 to 2014 with the increased use of glyphosate-tolerant crops and the associated drift of herbicides to plants adjacent to crop fields. Monarch use of milkweed plants has also been found to vary between different species of milkweeds. Baker and Potter (2018) found that tall, broadleaf milkweed species, such as common milkweed (*Ascelpias syriaca* L.*)* and showy milkweed (*Ascelpias speciosa* Torr.) were used more by monarchs. They also found that taller milkweed species (greater than 1.5 m), such as common milkweed and swamp milkweed (*Asclepias incarnata* L.) in general attracted more monarchs than shorter milkweed species (less than 0.6 m), such as whorled milkweed (*Asclepias venticillata* L.) and spider milkweed (*Asclepias viridis* Walter.), suggesting that monarchs may have a greater ability to find taller host plants.

One issue in managing roadsides for monarch habitat is the potential for the nectar plants to accumulate heavy metals from pollution and de-icing salts in their biomass. According to Mitchell et al. (2020), roadside milkweed plants along rural highways had higher levels of sodium and zinc with increasing traffic volume. They found that the levels of these potentially toxic elements decreased with increasing distance from the roadside. Mitchell et al. (2020) found that roadside soils and milkweeds are influenced by traffic and road management. However, they concluded that the concentrations of toxic elements in milkweeds are generally not high enough to negatively impact monarch butterflies. No specific threshold was given for the concentrations of toxic elements that could impact monarchs.

Roadside Mowing

Ideal pollinator habitat contains a wide diversity of native forbs that provide multiple nectar sources throughout the entire growing season. Different blooming forb species can have great variation in the pollinator species they attract, highlighting the need for a wide diversity of wildflower species (Campbell et al. 2019). One example of the need for plant diversity comes from a study by Nichols et al. (2019). They found that bumblebees and solitary bees both utilized flower species in the family Asteraceae, however, bumblebees also heavily utilized species of Geraniaceae, while solitary bees preferred to use Apiaceae species. Blaauw and Isaacs (2014) found that increasing flower species richness from one flower species per m^2 to four flower species per m^2 led to double the wild bee species richness. Management practices should aim to promote the greatest heterogeneity across the entire growing season.

One of the challenges of roadside forb establishment is implementing effective and practical disturbance regimes to limit the growth of tall native grass species, such as big bluestem (*Andropogon gerardii* Vitman), Indiangrass [*Sorghastrum nutans* L. (Nash)], switchgrass (*Panicum virgatum* L.), and sideoats grama [*Bouteloua curtipendula*

(Michx.) Torr.], while promoting the growth of a variety of native forbs. Previous research has suggested that tall native warm-season grasses limit the diversity and cover of native forbs due to competition for sunlight, rather than competition for water and nutrients (Hautier et al. 2009; McCain et al. 2010). McCain et al. (2009) found that a 50% reduction in aboveground biomass of big bluestem led to a 45% increase in light penetration to the soil, while removing 50% of switchgrass biomass led to a 35% increase in light penetration. Historically fire and grazing by bison (*Bison bison* L.) would have been the primary disturbances of the tallgrass prairie. Bison tend to favor grasses over forbs when grazing, leading to greater potential for an abundant and diverse forb community on the landscape (Knapp et al. 1999). Buckles and Harmon-Threatt (2019) compared bee abundance on burned, mowed, and hayed sites, and found that burning alone achieved the greatest abundance of bees. However, burning is not always a practical option for roadside habitat management. A management practice that may be used to reduce competition from existing vegetation and enhance pollinator and beneficial insect habitat on roadsides is mowing. Mowing can help reduce the competition for light from large statured grasses, aiding forb establishment (Williams et al. 2007). In addition to limiting growth of large grasses, mowing can restrain woody plant abundance (Jakobsson et al. 2018). Gilgert and Vaughan (2011) suggest using mowing on one third or less of overall habitat each year to provide refugia for recolonizing of forbs.

The timing and frequency of mowing regimes can impact the survival of forb species. Mowing once in the fall after most plants have become dormant allows the forbs ample time to complete seed production (Entsminger et al. 2017). In contrast, 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 non-mowed control plots by the fourth season after planting. Williams et al. (2007) increased mower height each time plots were mowed to account for forb growth. The timing of mowing should also be considered to avoid mortality of pollinator eggs, especially for monarch butterflies. Reproduction of monarchs can be maximized by mowing common milkweed plants once before peak egg laying occurs in late spring to early summer (Knight et al. 2019). Monarchs have been observed to lay more eggs on freshly resprouted milkweeds than larger and older milkweeds, and mowing appears to extend the breeding season of monarchs (Fischer, 2015). Fischer (2015) also noted that mowing milkweeds in upstate New York on July 1 led to greater regrowth of milkweed plants and provided more suitable habitat for monarchs to lay their eggs when compared to mowing August 17.

Mowing can have varying effects on the establishment and retention of forbs depending on the species, frequency, timing, and mowing height. For example, species that reproduce asexually through rhizomes, such as western yarrow (*Achillea millefolium* L.), tend to not be impacted by mowing treatments (Dewey et al. 2006). However, species spreading through seed are only negatively impacted if they are not given a chance to flower and produce seed. Specifically, Dewey et al. (2006) found that Pacific aster (*Symphyotrichum chilense* Nees) tends to be more negatively impacted by mowing treatments than western yarrow because Pacific asters reproduce more by seed than asexually. Another study focusing on tallgrass prairie forb response to mowing found that overwinter seedling mortality of native wildflowers was 3% in plots mowed every two weeks during the growing season compared to 29% in non-mowed plots (Williams et al. 2007). Forbs were excluded from being destroyed by the mower by gradually increasing the height of the mower blades from 13.5 cm in May to 27 cm by July. One study found that mowing common ragweed (*Ambrosia artemisiifolia* L.) at a height of 10 cm from the ground reduced the amount of pollen produced by about 56%, however, little research has been done to determine the effects of mowing on the pollen production of other forbs (Simard & Benoit, 2011). Neigebauer et al. (2000) determined that mowing significantly reduced root biomass of black-eyed Susan, and non-mowed plants had a greater root dry weight in the upper 2.5 cm of soil compared to plants that were mowed. Root depth was also found to increase linearly with increasing mowing height. The increase in root depth and density with increasing mowing height suggests that soil stability may be better maintained in areas where forbs are not mowed close to the ground.

Mowing has been used as an effective tool for limiting the growth of non-native cool-season grasses. A study on the front-range of Colorado found that mixed-grass prairie vegetation containing exotic annual grasses, exotic perennial grasses, warm-season perennial grasses, and native forbs mowed in late May and early June had significantly less cheatgrass (*Bromus tectorum* L.) coverage than the same vegetation with no mowing used at any time of the year (Prevéy et al. 2014). The vegetation was mowed before the inflorescences had ripened, preventing successful reproduction of cheatgrass and promoting the growth of native grasses, such as western wheatgrass (*Pascopyrum smithii* Rydb.) and blue grama [*Bouteloua gracilis*

(Willd. ex Kunth) Lag. ex Griffiths]. Though mowing was effective at removing non-native grasses, Prevéy et al. (2014) found that mowed plots had greater coverage of non-native forbs, such as redstem filaree [*Erodium cicutarium* (L.) L'Hér. ex Aiton] and field bindweed (*Convolvulus arvensis* L.).

Smooth bromegrass is one of the most common introduced cool-season grasses seen on Nebraska's roadsides, which can create a challenge for promoting forb diversity and abundance on sites where smooth brome is dominant. A study conducted in South Dakota found that smooth bromegrass rhizome biomass, bud formation, and tiller recruitment were significantly reduced by mowing three times during the growing season for two consecutive years (Xu et al. 2016). In Minnesota, brome stands mowed in May had a 16% reduction in tiller density, however, this was not considered a significant reduction from pre-treatment tiller densities (Willson & Stubbendieck, 1996). There is little evidence that single standalone mowing treatments can reduce smooth brome coverage in the long-term.

Haying is another management option often used on roadsides to manage vegetation. A greenhouse study simulating haying and mowing found that greater species richness was obtained when grassland vegetation was cut and removed from the soil surface than when grasses were cut and left on the soil surface (Jutila & Grace, 2002). By contrast, study in Kansas found that annual haying of re-seeded tallgrass praire in December led to a plant community dominated by perennial grasses with few native forbs (Foster & Lovett, 2003). The NDOT currently allows haying on roadside rights-of-ways to individuals that obtain a permit. Harvest of roadside hay must be completed between July 15 and September 15 while many plants are flowering (NDOT, 2022).

Forb Seeding

One aspect of forb plantings that is important to consider is the use of diverse seed mixes. A species rich seed mix can increase habitat heterogeneity and will be more attractive to a diverse range of pollinator species that may specialize in obtaining pollen from specific wildflower species (Hanberry et al. 2020). It is also important to have flowers in bloom throughout the entire growing season to ensure pollinators have a food source for as long as possible. Using seed mixes that contain abundant season-long blooms can be more important than only having high flower diversity that does not bloom through the entire growing season (Williams et al. 2015). For example, many caterpillars in the order Lepidoptera are considered specialists where some only occupy a specific genus or even species of plant, which further highlights the importance of high plant diversity (Gilgert & Vaughan, 2011). Dickson and Busby (2009) suggest limiting the density of some dominant forb species, such as Maximillian sunflower (*Helianthus maximilliani* Schrad.), showy partridge pea [*Chamaechrista fasciculata* (Michx.) Greene], and upright prairie coneflower because they can decrease the abundance of other desirable forbs.

Though weeds are often managed with the use of herbicides, they can also be suppressed by increasing the seeding rate of wildflowers from 11 to 17 kg ha-1 (Corley et al. 1993). Corley et al. (1993) also suggests using higher seeding rates of aggressive species, such as yarrow; showy partridge pea; Indian blanketflower (*Gaillardia pulchella*

Foug.); black-eyed Susan; and sunflowers (*Helianthus* sp.); to help keep costs down in high seeding rate projects.

In some revegetation projects, non-native species of forbs are used due to their long flowering periods. It has been found that many native insects prefer native flower species instead of non-native (Memmott & Waser, 2002). Native plants also have an advantage because they do not require fertilizers to become established, and they often require less water to survive than non-native plants due to their deep root systems (Skousen & Venable, 2008). Roadsides that contain native wildflowers support a higher diversity and abundance of bees than roadsides planted to non-native grasses and flowers (Hopwood, 2008). The species richness of Lepidopteran flower visitors was also found to be higher on native plants versus non-natives (Burghardt et al. 2010).

Non-native grasses can have negative effects on native plant density and species richness, which can limit the success of habitat restoration (Flory & Clay, 2010). A study conducted by Drobney et al. (2020) on restored prairie in Iowa used seed mixes containing as high as 68% perennial forbs with warm-season grass seed density as low as 14% in a high species richness treatment containing 58 total species and a low species richness planting containing only 10 species. They compared the invasion by non-native forbs and grasses into these areas between the high species richness mix and the low richness mix and found that the higher richness mix led to an increase in the invasion by non-native cool season grasses, such as smooth bromegrass and Kentucky bluegrass when compared to sites that were seeded with lower forb densities over the course of 10 years. In contrast, the study found that non-native forbs decreased throughout the 10-year study.

There is evidence that certain forb species, such as leadplant (*Amorpha canescens* Pursh), purple prairie clover (*Dalea purpurea* Vent.), grayhead coneflower [*Ratibida pinnata* (Vent.) Barnhart], and golden Alexander [*Zizia aurea* (L.) W.D.J. Koch] may be more attractive to bees than other native forbs (Harmon-Threatt, Hendrix, 2015). These forbs may serve as keystone species for pollinating insects. Golden Alexander is an early blooming forb while purple prairie clover, grayhead coneflower, and leadplant are mid- to late-season blooming species. One study in the United Kingdom determined that flowers in the Asteraceae family contained the largest amounts of pollen per individual flower (Hicks et al. 2016). In addition to having a diverse array of wildflower species present, it is equally important to have flowering plants in bloom throughout the entire growing season to promote pollinator diversity (Williams et al. 2015). Heterogeneity can be achieved in multiple forms, including species composition, bloom timing, and plant structure. According to Williams et al. (2015), more research is needed to determine if habitat enhancement increases pollinator populations or simply attracts them from other areas to the plots. Though it is important to have both perennial and annual forbs in a high-quality pollinator habitat restoration project, it has been found that perennial meadows can produce up to 20 times more nectar and 6 times more pollen than meadows containing only annual flowers (Hicks et al. 2016).

Forb establishment can be largely impacted by the amount of pre-existing vegetation present. Areas with high grass density typically have lower forb density due to competition for light and nutrients (Dickson & Busby, 2009). Roadside backslopes often lack the disturbance regimes necessary to limit the growth of tall warm-season grasses. In mesic grasslands, the removal of big bluestem and switchgrass increased the total forb productivity and species richness but had no effect on inorganic soil nitrogen or soil moisture, suggesting that light availability is the main factor limiting forb growth with large grasses present (McCain et al. 2010). In seeding mixes dominated by grass seed, forb species richness and abundance will rapidly decrease with time (Dickson & Busby, 2009). When seeding forbs into roadside settings, it can be beneficial to seed wildflowers into segregated rows to prevent excessive competition with grasses rather than uniformly mixing them (Schacht et al. 2017). According to Dickson and Busby (2009), forb seeds sown in locations away from dominant grasses could mimic the natural patchiness that comes with competition between robust grasses and forbs.

Timing of seeding may also be important to consider in wildflower seeding projects. When comparing early-summer seeding with late-summer seeding on Iowa roadsides, Williams and Smith (2007) determined that seeding in early-summer led to higher establishment, biomass, and species richness of prairie plants with 2.2 times more prairie plants in early-summer seeded roadsides. Their results also showed that plants in early-summer seeded roadsides had a 12% mortality rate from the first growing season to the second compared to 26% plant mortality in late-summer seeded roadsides. When comparing floral displays in a grassland setting, Blackmore and Goulson (2014) found there were 1.4 times more flowers the second year after sowing compared to the first growing season after sowing.

Challenges of Roadside Pollinator Management

The consensus of most previous roadside research suggests that the benefits of roadside habitat management outweigh the costs; however, there are still several potential pitfalls to management of roadsides (Phillips et al. 2020). Perhaps the most obvious problem is pollinator-vehicle collisions. At major bottleneck areas along monarch migration routes, between 25% and 42% of individuals crossed a major highway below a 6-meter critical threshold for vehicle collisions (Alvarez et al. 2019). A study in Canada used road mortality data on Lepidopterans, Hymenopterans, and Dipterans from a 2 kilometer stretch of highway to estimate that mortality could be in the hundreds of billions across North America (Baxter-Gilbert et al. 2015). Turbulence from high-speed traffic can also make it difficult for pollinators to forage close to the edge of the road, and lower traffic roads tend to have a higher density of pollinators present (Phillips et al. 2019). Alvarez et al (2019) also determined that monarch butterflies could be killed by the wind vortices of speeding trucks.

Another potential barrier to enhancing wildflower abundance and diversity on roadsides is the high cost of forb seed. In mixes containing 10% wildflower seed, 30% of the total cost comes from the flowers alone (Schacht et al. 2017). Seeding rate of forbs should be considered because a higher seeding rate may not lead to higher floral resources (Wilkerson et al. 2014). Using more seed than the slope can support just adds unnecessary costs and wastes forb seed that could be used in another area. Competition from un-desirable weeds and grasses can suppress wildflowers within a few years of planting (Norcini & Aldrich, 2004), so re-seeding is often necessary to maintain high levels of floral diversity and density. Forb seed can also be difficult to evenly distribute when planting with a drill due to the varying sizes of wildflower seeds in diverse mixes. This can affect the amount of seed used because small species such as heath aster [*Symphyotrichum ericoides* (L.) G.L. Nesom] will exit the drill more quickly than larger species such as compass plant (*Silphium laciniatum* L.).

Roadsides receive relatively high amounts of pollution from passing traffic, which can negatively impact the survival of pollinator species. Bees have very poor vision, so they usually rely on olfactory senses to locate floral resources (Chittka & Raine, 2006). Air pollution can interfere with their ability to use olfaction for finding nectar sources along roadsides. Increases in air pollution can lead to reductions in the floral hydrocarbons that bees use to locate flowers for pollination, and the distance that these signals can be detected is greatly reduced from multiple kilometers to less than 200 meters (McFrederick et al. 2008). Pollution from vehicles is also stored in roadside soil. Concentrations of lead, zinc, copper, and cadmium have been observed to be the greatest in the upper 5 cm of soil and within 2 m of the roadway (Dierkes & Geiger, 1999). Dierkes and Geiger (1999) determined that older embankments with higher traffic density had the highest concentrations of pollutants. According to Wingeyer et al. (2018), concrete grinding residues (CGR) on roadside slopes contains high concentrations of calcium, sodium, magnesium, and potassium. The National Pollutant Discharge Elimination System (NPDES) permit allows an application rate of 11 Mg CGR ha^{-1}, but Wingeyer et al. (2018) found that application of up to 90 Mg CGR ha^{-1} on loam and silt loam soils did not negatively impact roadside vegetation or soil quality.

Invasion of non-native species, such as sweet clover (*Melilotus sp.*), hairy vetch (*Vicia villosa,* Roth), and cool-season grasses may be a threat to wildflower plantings due to competition for nutrients and sunlight. Yellow sweet-clover [*Melilotus officinalis* (L.) Lam.] and white sweet clover (*Melilotus alba,* Medikus, orth. Var.) are common roadside legumes native to Eurasia (Lesica & DeLuca, 2000). Both species are biennial nitrogen fixing legumes that may be abundant at one site in some years and not in others (Stubbendieck et al. 2003). Sweet-clover seeds can remain viable in the soil for decades, making it difficult to predict invasions (Kline, 1984). Sweet clover has been found to be an attractive nectar source for pollinating insects (Dibble et al. 2020; Simanonok et al. 2022). Though sweet-clover is often planted in the Great Plains as a forage crop, it is considered invasive across much of its introduced range due to its ability to tolerate a wide range of conditions and outcompete native forbs. Sweet-clover can be controlled using prescribed fire, herbicides, and mowing. Burning in May has been found to effectively reduce sweet-clover cover, and mowing in July, though less effective than burning, has also been found to reduce sweet clover cover when burning is not an option (Kline, 1984). In contrast, Kline (1984) found that dormant season burns stimulated germination and second year survival of sweet-clover plants.

A potential downside to using roadsides for habitat management is the possibility of other humans disturbing research plots or managed slopes. Roadside wildflower plantings are easily accessible to the public, which can lead to destruction of flowers or plot boundaries. Coordination between roadside managers and research personnel is important in preventing accidental mowing or removal of plot boundary indicators. In many states, private landowners are prohibited from mowing public roadways; however, many landowners still mow these public resources along fields and in ditches. It is also possible that accidental chemical drift from agricultural fields can kill planted wildflowers and grasses.

Common Roadside Forbs of Southeast Nebraska

One common forb found in southeast Nebraska is Maximilian sunflower, which is a perennial wildflower in the Asteraceae family. Maximilian sunflower grows well in medium sandy to clayey loams in areas that receive at least 35.5 cm of precipitation annually (Farrar, 2012). It is a highly competitive forb that spreads through seed and rhizomes, and it normally reaches maturity in one growing season. Flowering typically occurs in September and October in the central United States. Maximilian sunflower has a high tolerance for fire and drought but has low tolerance to grazing and saline soils (Dietz et al. 1992). The optimum soil temperature for germination is between 20°C and 30°C (Owens & Call, 1985).

Another common species found on roadside slopes of the central United States is showy partridge pea, an annual legume of the Fabaceae family native to the tallgrass prairie (Foote & Jackobs, 1966). According to the USDA-NRCS (2002), showy partridge pea flowers bloom from July to September and reach a height of 30 to 90 cm. They also state that it can grow on a variety of soils from slightly acidic to moderately alkaline with high levels of disturbance. Showy partridge pea is a valuable food species for pollinating insects and birds, especially the northern bobwhite quail (*Colinus virginianus* L.) (Marcy & Martin, 1991).

Canada goldenrod (*Solidago canadensis L.*) is a common perennial forb of the Asteraceae family. It is most common on disturbed sites, such as roadsides or abandoned farmland, with moist soil (Gould et al. 2013). Canada goldenrod flowers from June through August, making it a vital nectar source for pollinating insects in late summer (USDA-NRCS, 2012). Many native bees rely on this late season source of nectar to build up fat stores for winter hibernation. It spreads aggressively through seed and rhizomes, and it can tolerate high concentrations of heavy metals and moderate salt concentrations, which can lead to its invasive nature in some areas (Gould et al. 2013).

One of the more common early blooming species in southeast Nebraska is black-eyed Susan, which flowers from mid-April to mid-June. Black-eyed Susan is unique because it can function as an annual, a biennial, or a short-lived perennial. It is adapted to clay, loam, and sandy soils that are acidic with mild disturbance (Brackie, 2019). Black-eyed Susan is well adapted to dry or moist conditions and prefers full sunlight (Farrar, 2012). A central taproot makes up its root system, and it can only reproduce by seed (Illinois Wildflowers, 2019).

Heath aster is a common late-blooming species of wildflower found throughout much of the United States. It is a native perennial in the Asteraceae family that spreads through seed or rhizomes, and it thrives under drought conditions due to its extensive rhizomes (Farrar, 2012). Heath aster prefers clayey loam soil and can tolerate alkaline soils and drought conditions (USDA-NRCS, 2004). USDA-NRCS (2004) also mentioned that it can become weedy in some situations due to its positive response to fire and its resistance to grazing by livestock.

Common milkweed is a perennial forb of the Asclepiadaceae Family that spreads through seeds and rhizomes (Stubendieck et al. 2003). Common milkweed grows in a variety of moderately dry to moist soils in disturbed sites (Farrar, 2012). Milkweed species contain a milky latex substance that is mildly toxic upon consumption to most animals including livestock and some insects. According to Stubendieck et al. (2003) common milkweed is an important species for monarch conservation because monarchs lay their eggs on milkweeds and the larvae feed on the plants.

Literature Cited

Alvarez, B. X. M., Carrera-Treviño, R., & Hobson, K. A. (2019). Mortality of monarch butterflies (Danaus plexippus) at two highway crossing “Hotspots” during autumn migration in Northeast Mexico. *Frontiers in Ecology and Evolution*, *7*.

Baker, A. M., & Potter, D. A. (2018). Colonization and usage of eight milkweed (Asclepias) species by monarch butterflies and bees in urban garden settings. *Journal of Insect Conservation*, *22*(3), 405-418.

Banks, S. C., Piggott, M. P., Stow, A. J., & Taylor, A. C. (2007). Sex and sociality in a disconnected world: a review of the impacts of habitat fragmentation on animal social interactions. *Canadian Journal of Zoology*, *85*(10), 1065-1079.

Barrett, S. C., & Harder, L. D. (1996). Ecology and evolution of plant mating. *Trends in Ecology & Evolution*, *11*(2), 73-79.

Baxter-Gilbert, J. H., Riley, J. L., Neufeld, C. J., Litzgus, J. D., & Lesbarrères, D. (2015). Road mortality potentially responsible for billions of pollinating insect deaths annually. *Journal of Insect Conservation*, *19*(5), 1029-1035.

Beyers, J. L. (2004). Postfire seeding for erosion control: effectiveness and impacts on native plant communities. *Conservation Biology*, 18(4), 947-956.

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.

Brakie, M. (2019). Plant Guide for black-eyed Susan (Rudbeckia hirta). USDA-Natural Resources Conservation Service, *East Texas Plant Materials Center. Nacogdoches, TX*.

Buckles, B. J., & Harmon-Threatt, A. N. (2019). Bee diversity in tallgrass prairies affected by management and its effects on above-and below-ground resources. *Journal of Applied Ecology*, *56*(11), 2443-2453.

Burghardt, K. T., Tallamy, D. W., Philips, C., & Shropshire, K. J. (2010). Non-native plants reduce abundance, richness, and host specialization in lepidopteran communities. *Ecosphere*, *1*(5), 1-22.

Campbell, J. W., Kimmel, C. B., Grodsky, S. M., Smithers, C., Daniels, J. C., & Ellis, J. D. (2019). Wildflower plantings harbor increased arthropod richness and abundance within agricultural areas in Florida (USA). *Ecosphere*, *10*(10), e02890.

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.

Chittka, L., & Raine, N. E. (2006). Recognition of flowers by pollinators. *Current opinion in plant biology*, 9(4), 428-435.

Corley, W. L., Murphy, T. R., & Reynolds, K. R. (1993). Weed management options for wildflower meadows and beauty spots. *SNA Res. Conf. Proc. 38*, 315-316.

Da Silva, A. M., Braga Alves, C., & Alves, S. H. (2010). Roadside vegetation: estimation and potential for carbon sequestration. *iForest-Biogeosciences and Forestry*, 3(5), 124.

Dewey, D. W., Johnson, P. G., & Kjelgren, R. K. (2006). Effects of irrigation and mowing on species diversity of grass and wildflower mixtures for the Intermountain West. *Native Plants Journal*, *7*(3), 267-278.

Dibble, A. C., Drummond, F. A., & Stack, L. B. (2020). Bee visitation on flowers in Maine, United States, reveals the relative attractiveness of plants through space and time: part I. *Environmental Entomology*, *49*(3), 726-737.

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.*

Dierkes, C., & Geiger, W. F. (1999). Pollution retention capabilities of roadside soils. *Water Science and Technology*, *39*(2), 201-208.

Drobney, P., Larson, D. L., Larson, J. L., & Viste-Sparkman, K. (2020). Toward improving pollinator habitat: reconstructing prairies with high forb diversity. *Natural Areas Journal*, *40*(3), 252-261.

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.

Farrar, J. (2012). *Field Guide to Wildflowers of Nebraska and the Great Plains*. Iowa City: Published for the Nebraska Game and Parks Commission by the University of Iowa Press.

Federal Highway Administration. (2010a). Carbon Sequestration Pilot Program: Estimated Land Available for Carbon Sequestration in the National Highway System. Retrieved 10/12/2020, from Office of Planning, Environment, and Realty: http://www.fhwa.dot.gov/environment/climate_change/mitigation/resources_and_publications/carbon_sequestration/index.cfm

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

Flory, S. L., & Clay, K. (2010). Non-native grass invasion alters native plant composition in experimental communities. *Biological Invasions*, 12(5), 1285-1294.

Foote, L. E., & Jackobs, J. A. (1966). Soil factors and the occurrence of partridge pea (Cassia fasciculata Michx.) in Illinois. *Ecology*, 47(6), 968-975.

Foster, J., & Lovett, J. (2003). Haying effects on a restored prairie plant community in northeastern Kansas. *Transactions of the Kansas Academy of Science (1903)*, 198-206.

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.

Gould, K., Smreciu, A., & Wood, S. (2013). Solidago canadensis: Canada goldenrod, common goldenrod.

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).

Goverde, M., Schweizer, K., Baur, B., & Erhardt, A. (2002). Small-scale habitat fragmentation effects on pollinator behaviour: experimental evidence from the bumblebee Bombus veteranus on calcareous grasslands. *Biological Conservation*, *104*(3), 293-299.

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,* 78, 220-234

Harmon-Threatt, A. N., & Hendrix, S. D. (2015). Prairie restorations and bees: The potential ability of seed mixes to foster native bee communities. *Basic and Applied Ecology*, *16*(1), 64-72.

Hautier, Y., Niklaus, P. A., & Hector, A. (2009). Competition for light causes plant biodiversity loss after eutrophication. *Science, 324*(5927), 636-638.

Hicks, D. M., Ouvrard, P., Baldock, K. C., Baude, M., Goddard, M. A., Kunin, W. E., Mitschunas, N., Memmott, J., Morse, H., Nikolitsi, M., Osgathorpe, L. M., Potts, S. G., Robertson, K. M., Scott, A. V., Sinclair, F., Westbury, D. B., & Stone, G. N. (2016). Food for Pollinators: Quantifying the Nectar and Pollen Resources of Urban Flower Meadows. *PloS one*, *11*(6), e0158117.

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

Hopwood, J. L. (2013, June). Roadsides as habitat for pollinators: management to support bees and butterflies. In *Proceedings of the 7th International Conference on Ecology and Transportation (IOECT): Canyons, Crossroads, Connections. Scottsdale, Arizona, USA*, 1-18.

Illinois Wildflowers. (2019). Black-eyed Susan-Rudbeckia hirta. Accessed on 12/10/2020 at: https://www.illinoiswildflowers.info/prairie/plantx/be_susanx.htm

Isaacs, R., Tuell, J., Fiedler, A., Gardiner, M., & Landis, D. (2009). Maximizing arthropod-mediated ecosystem services in agricultural landscapes: the role of native plants. *Frontiers in Ecology and the Environment*, *7*(4), 196-203.

Ison, J. L., & Wagenius, S. (2014). Both flowering time and distance to conspecific plants affect reproduction in Echinacea angustifolia, a common prairie perennial. Journal of Ecology, 102(4), 920-929.

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

IUCN. 2023. *The IUCN Red List of Threatened Species*. Accessed on 11/28/2023 at: https://www.iucnredlist.org.

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.

Kline, V. M. (1984). Response of sweet clover (Melilotus alba Desr.) and associated prairie vegetation to seven experimental burning and mowing treatments. *Proceedings of the Ninth North American Prairie Conference 1984*, 149-152.

Knapp, A. K., Blair, J. M., Briggs, J. M., Collins, S. L., Hartnett, D. C., Johnson, L. C., & Towne, E. G. (1999). The keystone role of bison in North American tallgrass prairie: Bison increase habitat heterogeneity and alter a broad array of plant, community, and ecosystem processes. *BioScience*, *49*(1), 39-50.

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.

Leonhardt, S. D., & Blüthgen, N. (2012). The same, but different: pollen foraging in honeybee and bumblebee colonies. *Apidologie*, *43*(4), 449-464.

Lesica, P. L., & DeLuca, T. H. (2000). Sweetclover: a potential problem for the Northern Great Plains. *Journal of Soil and Water Conservation*, *55*(3), 259-261.

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

McCain, K. N., Baer, S. G., Blair, J. M., & Wilson, G. W. (2010). Dominant grasses suppress local diversity in restored tallgrass prairie. *Restoration Ecology*, *18*, 40-49.

McFrederick, Q. S., Kathilankal, J. C., & Fuentes, J. D. (2008). Air pollution modifies floral scent trails. *Atmospheric Environment*, 42(10), 2336-2348.

McGregor, S. E. (1976). Insect pollination of cultivated crop plants (No. 496). *Agricultural Research Service, US Department of Agriculture*.

Memmott, J., & Waser, N. M. (2002). Integration of alien plants into a native flower–pollinator visitation web. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *269*(1508), 2395-2399.

Neigebauer, A. L., Horst, G. L., Steinegger, D. H., & Davis, G. L. (2000). Shoot and root characterization of Rudbeckia hirta L. mowed at different heights. *HortScience*, *35*(7), 1247-1248.

Nichols, R. N., Goulson, D., & Holland, J. M. (2019). The best wildflowers for wild bees. *Journal of Insect Conservation*, *23*, 819-830.

Norcini, J. G., & Aldrich, J. H. (2004). Establishment of native wildflower plantings by seed. University of Florida Cooperative Extension Service, *Institute of Food and Agricultural Sciences, EDIS.*

Owens, D. W., & Call, C. A. (1985). Germination characteristics of Helianthus maximilianai Schrad. and Simsia calva (Engelm. and Gray) Gray. *Rangeland Ecology & Management/Journal of Range Management Archives*, 38(4), 336-339.

Prevéy, J. S., Knochel, D. G., & Seastedt, T. R. (2014). Mowing reduces exotic annual grasses but increases exotic forbs in a semiarid grassland. *Restoration Ecology*, *22*(6), 774-781.

Phillips, B. B., Gaston, K. J., Bullock, J. M., & Osborne, J. L. (2019). Road verges support pollinators in agricultural landscapes, but are diminished by heavy traffic and summer cutting. *Journal of Applied Ecology*, *56*(10), 2316-2327.

Pleasants, J. (2017). Milkweed restoration in the Midwest for monarch butterfly recovery: estimates of milkweeds lost, milkweeds remaining and milkweeds that must be added to increase the monarch population. *Insect Conservation and Diversity*, 10(1), 42-53.

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in ecology & evolution*, *25*(6), 345-353.

Reimer, A. P., & Prokopy, L. S. (2014). Farmer participation in US Farm Bill conservation programs. *Environmental management*, *53*, 318-332.

Säumel, I., Weber, F., & Kowarik, I. (2016). Toward livable and healthy urban streets: Roadside vegetation provides ecosystem services where people live and move. *Environmental Science & Policy*, *62*, 24-33.

Schacht, W., Soper, J., & Wienhold, C. (2017). *Improving Wildflower Longevity in Roadside Seeding Areas* (No. M329). Nebraska Department of Transportation.

Wu-Smart, J., & Schacht, W. (2019). *Establishment of Wildflower Islands to Enhance Roadside Health and Aesthetics* (No. MO58). Nebraska Department of Transportation.

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.

Simanonok, S. C., Otto, C. R., & Iovanna, R. (2022). Forbs included in conservation seed mixes exhibit variable blooming detection rates and cost-effectiveness: implications for pollinator habitat design. *Restoration Ecology*, *30*(8), e13657.

Simard, M. J., & Benoit, D. L. (2011). Effect of repetitive mowing on common ragweed (Ambrosia Artemissifolia L.) pollen and seed production. *Annals of agricultural and environmental medicine*, *18*(1).

Skousen, J. G., & Venable, C. L. (2008). Establishing native plants on newly-constructed and older-reclaimed sites along West Virginia highways. *Land Degradation & Development*, *19*(4), 388-396.

Soper, J. M., Raynor, E. J., Wienhold, C., & Schacht, W. H. (2019). Evaluating composition and conservation value of roadside plant communities in a grassland biome. *Environmental management*, *63*, 789-803.

Steinfeld, D. E., Riley, S. A., Wilkinson, K. M., Landis, T. D., & Riley, L. E. (2007). *A manager's guide to roadside revegetation using native plants* (No. FHWA-WFL-TD-07-006). United States. Federal Highway Administration. Western Federal Lands Highway Division.

Streeter, M. T., & Schilling, K. E. (2020). Assessing and mitigating the effects of agricultural soil erosion on roadside ditches. *Journal of Soils and Sediments*, *20*(1), 524-534.

Stubbendieck, J. L., Coffin, M. J., & Landholt, L. M. (2003). *Weeds of the Great Plains*. Lincoln, Neb.: Nebraska Dept. of Agriculture, Bureau of Plant Industry in cooperation with the University of Nebraska-Lincoln.

Stubbs, M. (2014, August). Conservation Reserve Program (CRP): status and issues. Washington, DC, USA: Library of Congress, Congressional Research Service.

Thogmartin, W. E., Szymanski, J. A., & Weiser, E. L. (2020). Evidence for a growing population of eastern migratory monarch butterflies is currently insufficient. *Frontiers in Ecology and Evolution*, *8*, 43.

Thogmartin, W. E., Wiederholt, R., Oberhauser, K., Drum, R. G., Diffendorfer, J. E., Altizer, S., ... & Lopez-Hoffman, L. (2017). Monarch butterfly population decline in North America: identifying the threatening processes. *Royal Society open science*, *4*(9), 170760.

United States Department of Agriculture. (2002) *Showy Partridge Pea Chamaecrista fasiculata (Michx.) Greene* [Fact sheet]. Natural Resources Conservation Services. https://plants.usda.gov/factsheet/pdf/fs_chfa2.pdf

United States Department of Agriculture. (2012) *Canada Goldenrod Solidago canadensis L.* [Fact sheet]. Natural Resources Conservation Services. https://plants.usda.gov/factsheet/pdf/fs_soca6.pdf

United States Department of Agriculture. (2004) *White Heath Aster Symphyotrichum ericoides (L.)* [Fact sheet]. Natural Resources Conservation Services. https://plants.usda.gov/plantguide/pdf/pg_syere.pdf

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

Wilkerson, M. L., Ward, K. L., Williams, N. M., Ullmann, K. S., & Young, T. P. (2014). Diminishing returns from higher density restoration seedings suggest trade-offs in pollinator seed mixes. *Restoration Ecology*, *22*(6), 782-789.

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, D. W., & Smith, D. D. (2007). The effects of seeding time on native plant establishment in a prairie reconstruction. *Tallgrass Prairie Center.*

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.

Willson, G. D., & Stubbendieck, J. L. (1996). Suppression of smooth brome by atrazine, mowing, and fire. *Agronomy--Faculty Publications*, 369.

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.

Wingeyer, A., Mamo, M., Schacht, W., McCallister, D., & Sutton, P. (2018). Vegetation and soil responses to concrete grinding residue application on highway roadsides of eastern Nebraska. *Journal of environmental quality*, *47*(3), 554-561.

Wojcik, V. A., and S. Buchmann. 2012. Pollinator conservation and management on electrical transmission and roadside rights-of-way: a review. *Journal of Pollination Ecology*. 7, 16–26.

Wright, C. K., & Wimberly, M. C. (2013). Recent land use change in the Western Corn Belt threatens grasslands and wetlands. Proceedings of the National Academy of Sciences, 110(10), 4134-4139.

Xiao, Y., Li, X., Cao, Y., & Dong, M. (2016). The diverse effects of habitat fragmentation on plant–pollinator interactions. *Plant Ecology*, *217*(7), 857-868.

Xu, L., Young, J., Boe, A., Hendrickson, J. R., & Troelstrup Jr, N. H. (2016). Impacts of Mowing Treatments on Smooth Bromegrass (Bromus inermis) Belowground Bud Bank. *International Rangeland Congress*.

CHAPTER 2

FORB SEEDING INTO ROADSIDE SLOPES FOR POLLINATOR HABITAT

Introduction

Pollinator habitat enhancement is difficult to implement in Nebraska because 97% of land is privately owned. Much of the existing habitat for pollinators is fragmented, which reduces the pollen dispersal of flowers and leads to more inbreeding of plants and lower plant genetic diversity (Goverde et al. 2002). Roadsides provide a large percentage of the state's public land that will not be further developed or put into row crops, which makes them prime areas to study habitat enhancement. Many states currently use wildflowers in restorative seedings on roadsides thanks to the passing of the Highway Beautification Act of 1965, which was signed by President Lyndon B. Johnson (Lucey & Barton, 2010). The First Lady, Ladybird Johnson, stressed the importance of using native wildflowers to enhance the aesthetics and ecological value of roadsides. The Texas Department of Transportation reports that by seeding native plants on roadsides, maintenance costs from mowing are reduced by 25% (Markwardt, 2005).

Previous research has found that roadsides restored with native wildflower plantings had significantly higher bee abundance with an average of 80 individual bees and 15 species compared to non-restored sites that only averaged 34 total bees in 12 species (Hopwood, 2008). Hopwood (2008) also found that the amount of traffic or width of the road did not significantly affect the abundance of bees, suggesting that high traffic roadside strips as narrow as 18 meters can be suitable sites for restoration. There are some challenges to using roadsides as restoration sites for pollinators, such as invasive species, disturbances from traffic or agricultural activities, poor soil conditions, and cost of wildflower seed. A survey also found that the greatest limitations state agencies encounter when assessing and monitoring monarch butterfly (*Danaus plexippus* L.) habitat on roadsides are time, funding, and plant identification expertise (Cariveau et al. 2019).

The planting of milkweeds (*Ascelpias sp.*) on roadsides may have significant positive benefits to the protection of the monarch butterfly, which has suffered estimated declines of 84% for the eastern migratory population in the last decade (Semmens et al. 2016). Milkweed plants are important to monarchs because they serve as the host for caterpillars. One study found that milkweeds, especially common milkweeds (*Asclepias syriaca* L.), were present at many sites regardless of whether they were planted or not, with swamp milkweed (*Asclepias incarnata* L.) and butterfly milkweed (*Asclepias tuberosa* L.) showing up at higher densities in areas that were seeded (Lukens et al. 2020). This may suggest that milkweeds in general have a high ability to colonize sites even when they are not planted. Milkweeds have unfortunately been in great decline along with the monarch butterflies that use them. Between 1999 and 2010, it has been estimated that 81% of milkweeds on agricultural lands have been lost due to increasing use of glyphosate herbicides (Pleasants & Oberhauser, 2013). Incorporating milkweeds in roadside restorations may be an important way to provide monarch larvae food sources free from herbicide destruction. Monarch habitat is also generally a low priority for roadside managers. In Iowa, a survey of local roads managers indicated that their vegetation management decisions were most often influenced by safety and maintenance costs, while pollinator habitat and wildlife were most frequently stated as having no impact on their management decisions (Nemec et al. 2021).

It has been found that diverse wildflower mixes are important for providing season long nectar sources for pollinators and that large displays of flowers blooming throughout the growing season are important for attracting bees (Williams et al. 2015). Williams et al. (2015) also noted that annuals are important in wildflower mixes because they are more likely to provide larger floral displays in the first season after planting than perennial wildflowers, which may take several seasons to bloom. There are certain wildflower species that may become dominant over other less vigorous species. Dickson and Busby (2009) found that certain species of forbs, such as Maximillian sunflower (*Helianthus maximiliani* Schrad.), showy partridge pea [*Chamaecrista fasciculata* (Michx.) Greene], and upright prairie coneflower [*Ratibida columnifera* (Nutt.) Wooton & Standl.] may decrease the abundance of other desirable forbs, leading to decreased diversity of wildflower species that may become established. This is due to the competitive nature of these species. It may be beneficial to limit the amount of seed used in restorations of these dominant species to promote the establishment of a more diverse stand of wildflowers. These species do still provide benefits to pollinators while blooming. Angelella et al. (2019) found that showy partridge pea was particularly attractive to bumblebees while Maximillian sunflower was attractive to an array of non-bumblebee species. Simanonok & Otto (2021) also found that Maximilian sunflower is preferentially selected by bees in Midwest grasslands.

Native plant species are typically recommended for restorations because native plants are well adapted to the local climate and soil conditions and do not require fertilizers or pesticides to establish (Skousen and Venable, 2008). Another study found that non-native plants were visited by fewer insects, including pollinating and non-pollinating insects, when compared to native plants; however, pollinating insects specifically did not seem to show a preference for native versus non-native plants (Memmott & Waser, 2002). According to Memmott and Waser (2002), native plants had a flowering period of about 60 days compared to 125 days for non-native species; however, insects in general still showed preference for native plants, and pollinators still showed about even preference for native and non-native plants. From the standpoint of providing nectar for pollinating insects, non-native plants may be able to meet the needs of most pollinators, but some non-native flower species may be difficult to establish due to in-compatibility with the local climate, while other species may become invasive due to a lack of natural pests or other limiting factors. Using diverse mixes of wildflowers that bloom throughout the growing season can compensate for the shorter flowering period of most native wildflowers.

The Floristic Quality Assessment (FQA) is a metric commonly used to determine the overall quality of a plant community at a site, where each native plant is assigned a value, known as the coefficient of conservatism (C), from 0-10 (Spyreas, 2019). Each non-native plant is assigned a 0. Species with higher C values generally indicate higher quality sites. A weighted value, called the Floristic Quality Index (FQI) is obtained to represent the overall quality of plant species at a site. Heads et al. (2022) found that a positive relationship exists between bumble bee (*Bombus sp.*) richness and floristic quality, where sites with higher FQI values generally have a greater bee richness. A similar relationship was found with butterflies, where grassland sites with high FQI values contained a greater abundance and diversity of butterflies than sites with lower FQI values (Farhat et al. 2014).

Mowing is an important tool that can be used in roadside vegetation management to suppress un-desirable plant species; however, most roadside mowing aims to increase driver visibility and to provide room for vehicles to pull off the road during emergencies (Hopwood, 2013). The Nebraska Department of Transportation (NDOT) uses roadside mowing to manage vegetation in several ways: removing weeds before they produce seed, preventing volunteer trees from becoming hazards to vehicles, removal of vegetation from guardrails and signs, and providing a manicured look to urban areas for aesthetic enhancement (NDOT, 2022). Though mowing can have some benefits to roadside vegetation, timing, and frequency of mowing needs to be assessed to determine the optimal time to mow without causing harm to native vegetation, insects, and other wildlife.

Reducing the frequency of mowing can increase the use of roadsides by pollinators and promotes the growth and blooming of native wildflowers. Frequent mowing during the growing season is often unnecessary and costly, and it may reduce the number of blooming wildflowers and suppress growth of native plants (Hopwood et al. 2015). Entsminger et al. (2017) suggests that mowing only once per year can retain adequate plant growth for erosion control and allows native plants to flower and produce seed. They also suggest that reducing mowing to one time per year can reduce competition from nonnative grasses, which can compete with native plants for sunlight, moisture, nutrients, and space.

The timing of mowing can also be altered to protect other wildlife. For example, in Minnesota, mowing more than 2.5 m from the shoulder is prohibited from July 31 to August 31 to protect nesting birds (Hopwood, 2013). One study in tallgrass prairie found that mowing in late June following fire events helped to maintain plant species diversity and suppress woody species better than plots that were not subject to any mowing (Collins et al. 1998). Mowing in late fall can allow forbs to flower and produce seed before they are destroyed by mowing (Entsminger et al. 2017), leading to greater reproduction potential of wildflowers. Mowing during elongation or reproductive growth phases of wildflowers likely negatively impacts their ability to flower and produce new stems.

Mowing differs from other forms of disturbances, such as grazing, burning, and haying. With mowing, cut plant material falls into the remaining stubble before either blowing away or making its way to the soil surface as vegetative litter. One study found that thatch from dead ripgut brome (*Bromus diandrus*, Roth) had a greater impact on the growth of native forbs than competition from the living ripgut brome due to the sunlight being blocked by the thatch, suggesting that vegetative litter cover may be a bigger factor than competition for resources between forbs and exotic annual grasses (Molinari & D’Antonio, 2020). Litter production resulting from mowing has been found to have similar effects on native grasslands. Mowing often has only short-term benefits on vegetation structure and has been observed to contribute to higher litter accumulation over time in Conservation Reserve Program fields planted to native grasses (McCoy et al. 2001). Accumulated plant litter may reduce bare ground and light availability, suggesting that removal of litter would increase germination of forb seedlings and overall species diversity of grassland sites (Ruprecht et al. 2010). Haying is one management approach that would remove the available plant material that forms litter on the soil surface. Jutila and Grace (2002) found that simulated having events in a greenhouse setting led to greater species diversity than simulated mowing events where litter is left on the soil surface. The NDOT issues haying permits to individuals annually, but hay must be removed between July 15 and September 15 while many wildflowers are in bloom (NDOT, 2022). Additional passes of heavy equipment on roadside slopes could also lead to greater soil compaction and reduced health of vegetation.

Previous research on roadside pollinator habitat has focused on seeding wildflowers into newly constructed roadside sites. Little research has been conducted to assess how seeding or mowing can enhance older roadside sites. This study focusses on enhancing already established roadside vegetation to make the sites more attractive to pollinating insects. The goal is to determine if seeding wildflowers directly into grass dominated roadside backslopes can increase forb diversity and cover for pollinators. We will also monitor forb and grass cover to determine if different mowing treatments can promote forb growth. We will inter-seed native wildflowers into the existing vegetation, which is primarily tall native warm-season grasses. We will also assess forb response to two mowing treatments. One mowing treatment will occur in October before seeding, and the other will occur in early July following forb seeding. We hypothesize that seeding wildflowers into existing vegetation combined with mowing in October before seeding and mowing again in July after seeding will provide the greatest overall forb cover, diversity, and floristic quality among the eight treatment combinations. We also predict that a combination of pre-mowing and year-after mowing treatments will lead to the greatest reduction in vegetative litter at a site. We anticipate that a negative relationship will exist between litter cover and forb cover, where increasing litter cover will correlate to decreasing forb cover.

Materials and Methods

Study Site Description

This study was conducted from August 2020 to September 2022 at two roadside sites located in southeastern Nebraska (Figure 2.1). The first site was located along Highway 77 about 8 km southwest of Cortland, Nebraska in Gage County from mile marker 33 to mile marker 35. Highway 77 is situated in a north-south orientation at the location of the plots. The second site was located along Highway 2 starting about 4.8 km west of Nebraska City, Nebraska in Otoe County from mile marker 502 to 494. Highway 2 is situated in an east-west orientation where the plots are located. Both highways were four lanes with a grassed median separating the lanes traveling in opposite directions. 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. Total roadside width for sites used at Highway 2 varied from 29 to 49 meters from the edge of the pavement to the top of the backslope. Highway 77 roadsides varied from 16.5 to 32 meters from the pavement to the top of the backslope.

Gage County (i.e., the location of the Highway 77 site) has an annual average temperature of 10.7°C from 1895 to 2022. In 2020, the average temperature for the year was 10.8°C, and in 2021 the average temperature was 11.9°C. The average 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 they received 64 cm of precipitation. During the growing season of 2021 (April-September) Gage County had 41.6 cm of precipitation, which is 15.1 cm lower than the average between 1895 and 2022 for those six months. In 2022, the annual precipitation was 60.6 cm, which is 15.6 cm below the yearly average (NOAA 2022).

The historical annual average temperature for Otoe County (i.e., the location of the Highway 2 site) is 10.6°C from 1895 to 2022. Temperatures in 2020 averaged 11.3°C, and in 2021 they averaged 11.8°C for 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, however, during the growing season (April-September) this county received 50.9 cm of precipitation, which is 6.8 cm below the historical average for those six months. The annual precipitation for 2022 was 59.1 cm. Temperature and precipitation data for both sites are shown in Tables 2.1 and 2.2 (NOAA, 2022).

Disturbances on roadside slopes are typically created in the form of mowing. Vegetation on these roadsides is dominated by native warm-season grasses, including big bluestem (*Andropogon gerardii* Vitman), Indiangrass [*Sorghastrum nutans* (L.) Nash], sideoats grama [*Bouteloua curtipendula* (Michx.) Torr], switchgrass (*Panicum virgatum* L.), and Eastern gamagrass (*Tripsacum dactyloides* L.). Native cool-season grasses are present with slender wheatgrass [*Elymus trachycaulus* (Link) Gould ex Shinners] and Canada wildrye (*Elymus canadensis* L.) being common. Non-native cool-season grasses are also present on most slopes, and they can become the most dominant vegetation form in some cases. The most common non-native cool-season grasses include Kentucky bluegrass (*Poa pratensis* L.), smooth bromegrass (*Bromus inermis* Leyss.), and cheatgrass (*Bromus tectorum* L.). Many roadside slopes have several forbs present, with many of them being native wildflowers such as, Maximilian sunflower, showy partridge pea, Illinois bundleflower [*Desmanthus illinoensis* (Michx.) MacMill. Ex B. L. Rob & Fernald], Canada goldenrod (*Solidago canadensis* L.), and heath aster [*Symphyotrichum ericoides* (L.) G.I. Nelson]. Hairy vetch (*Vicia villosa* Roth) and yellow sweet-clover [*Melilotus officinalis* (L.) Pall.] are exotic legumes found at these sites.

Experimental Design

The design of the experiment was a randomized complete block design with four plots being allocated to each of eight different treatment combinations on both study sites. 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 2 x 2 x 2 factorial arrangement. Three different treatment factors that were 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.

The plot size for this study was 5.5 meters wide x 18.3 meters long running parallel to the highway. The plots had a 15.2-meter-wide gap between them where data was not collected. Each plot was marked with reflective fiberglass posts and flags. Plot locations were also stored in a handheld global positioning system unit to help locate plots upon return for repeated sampling. Plots were arranged along multiple slope locations within each site, with each slope having between three and eight plots. Traffic travels east-west on Highway 2. At Highway 2, twenty-eight plots were located on the north side of the highway, and only four were located on the south side. Highway 77 traffic travels north-south. At Highway 77, nine plots were located on the west side of the highway, and 23 were located on the east side. Data collections were performed at least 0.5 meter inside the boundaries of the plots to account for the potential of treatments to miss the extreme edges of the plots.

Treatments

For this study, mowing will be conducted in October after many plants have become dormant (pre-mowing) and early July when many newly planted seedlings will be below the height of the mower blades (year-after mowing). The first pre-mowing treatment was implemented in October of 2020. Half of the 32 plots at each site were randomly selected to be mowed to a height of 15 to 20 cm, leaving 32 total plots mowed across two sites. A 4.6-meter-wide mower was pulled behind a tractor between the plot boundaries. Since the plots were 5.5 meters wide, sampling was only performed within the mowed area of the plot. The second mowing treatment was conducted in July of 2021 and followed the same methods as the pre-seeding mowing treatment. 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. Figure 2.2 illustrates the eight different treatment combinations for the study.

The next treatment included seeding native wildflowers into half of the plots. The wildflowers were no-till drilled using a Great Plains Native Grass Series II drill (3P606NT). The species mix included 31 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 (Murdock, Nebraska). Table 2.3 lists the species, bloom times, and seeding rates for the wildflower mix.

Soil Conditions

In Spring of 2022, soil was sampled to help gain an understanding of how germination and flowering of the seed mixture could be affected by roadside soil conditions. Obtaining soil samples occurred in two parts. First, four 2.5 cm diameter cores were taken to a depth of 15 cm in each plot. Samples were taken in a zig-zag pattern to represent the entire plot (Figure 2.3). The four cores from a plot were combined into one composite sample and stored in a paper bag. The material in each bag was then mixed by hand and oven dried. Each sample was sent to the Ward Laboratories (Kearney, Nebraska) for an analysis of soil properties. The second portion of sampling was performed by taking two 5 cm diameter cores to a depth of 15 cm from each plot to assess bulk density (Fig. 2.3). The core tool for bulk density had a sliding hammer attachment to help press the tool into the ground. The two samples from each plot were combined into one composite sample. Each combined sample was oven dried and weighed. The following formula was used to compute soil bulk density:

$$bulk\ density = mass \div volume$$

Soil textures at Highway 2 included silty clay, silty clay loam, and loams soils. Highway 77 had clay loam, silty clay loam, silt loam, and loam soils. Soil properties were combined and averaged for all plots at both sites. Table 2.4 shows the values for the observed soil properties.

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. The observer started at one end of the plot and randomly tossed the quadrat into the plot about 2 meters, 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.

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 totals of cover for 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

Litter sampling was conducted by using a step-point tool along three randomly selected transects within the 5.5 m x 18.3 m plots. One-quarter meter margins along the top and bottom of the plots were not included in the transects to account for treatments not reaching the exact edge of the plots. The transects were determined to be at 0.3, 3.4, and 4.3 meters from the bottom of the 5.5-meter edge of the plots. There was also a 0.8-meter margin at each end of the plot, which left each transect running a total of 16.8 meters parallel to the long edge of the plot. Litter sampling was conducted in March 2021 and 2022 while many plants were still dormant. Figure 2.4 shows the layout of the transects for the vegetative litter sampling.

The step point tool was placed 0.8 meters 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 dead plant material from the previous year lying flat on the ground. When litter cover was observed, a ruler was used to measure the depth of the material 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 15 observations per transect or 45 observations per plot. No points were taken outside of the 0.8-meter margin at the edge of the plots.

Data Analysis

Data analyses were performed using RStudio version 2022.07.0+548 (R Core Team, 2020). The Tidyverse package (Wickham et al. 2019) was used to manipulate data into usable forms. The two study sites were analyzed independently to account for variation in growing conditions between the two sites. Each year of data was also analyzed separately due to variations in the growing conditions encountered in the three years of this study. The 'aov' function within Tidyverse was used to create analysis of variance (ANOVA) tables. Treatment main effects and interactive effects were analyzed simultaneously. All effects were considered significant at a $P \leq 0.05$, and tests with P-values between 0.05 and 0.10 were considered trending towards significant. The 'summaryBy' function within the doBy package (Højsgaard & Halekoh, 2022) was used to calculate treatment means when the ANOVA indicated significant or trending towards significant effects. The differences in means were further analyzed using Tukey's Honest Significance Test.

To calculate vegetative cover of forbs and grasses, filters were used to isolate specific groups of plants, sampling dates, and the site being tested. Cover was presented as a percentage, though these percentages do not necessarily total 100% due to the presence of bare ground, litter, and multiple layers of vegetation. Cover data of individual plant species falling into the plant group being tested were summed for each of the 12 frames collected in a plot. For example, the total cover of forbs is the sum of each cover value for each forb species found in a frame. The cover value of each frame in a plot was then averaged to obtain a plot cover value. Each plot was then assigned to the appropriate treatment, and an average cover was obtained for each treatment combination. The difference in treatment means for forb cover was tested using a three-way analysis of variance (ANOVA). Seeding, pre-mowing, and year-after mowing were analyzed as main effects. In addition to the main effects, the interaction between these treatments was analyzed simultaneously. A two-way ANOVA was used to compare grass cover across the two mowing treatments because seeding was not considered in the analysis of grass cover.

To determine the effects of treatments on plant diversity, species richness was assessed to determine the total number of species found within each plot. Species richness was calculated simply by counting the total number of species encountered in each plot. The plot species richness was then averaged by treatment. The difference in treatment means was tested using a three-way ANOVA. Seeding, pre-mowing, and year-after mowing were analyzed individually as main effects. In addition to the main effects, the interaction between these treatments was analyzed. The ANOVA tables were created using the ‘aov’ function within Tidyverse.

The amount of vegetative litter present at a site was also analyzed and compared across mowing treatments. Litter depth in millimeters was used to determine the amount of litter present on a site and the potential effects that litter had on the plant community. The litter depth points taken for each plot were averaged to obtain the mean litter depth at each plot. Each plot was then assigned to the appropriate mowing treatment combination, and the average litter depth of each mowing treatment was obtained for both sites. A one-way ANOVA was used to test the effects of pre-mowing on vegetative litter in the spring of 2021, before the year-after mowing treatment was implemented. In 2022, litter was analyzed again to determine the effects that pre- and year-after mowing treatments have on vegetative litter. This second year of litter cover data was tested using a two-way ANOVA where the two mowing treatment main effects and interaction between the mowing treatments were tested. A percentage of bare ground, basal cover, and litter cover was also obtained for each plot by determining the number of points taken at each of the three categories. The percentages of each category were then averaged by treatment. Correlation between forb cover and vegetative litter cover was also tested using the Pearson’s Product Moment Correlation tests. The forb cover values from each plot were compared to the litter depth values for each plot.

Floristic Quality Indices were also calculated for each plot to determine the overall resistance to disturbance and quality of these roadside sites. A Floristic Quality Index (FQI) was calculated for each plot using the methods from Freyman et al. (2016). To obtain FQI, the Mean Coefficient of Conservatism (Mean C) value was first calculated using the following equation:

$$Mean\ C = \sum C \,/\, n$$

where *C* is the Coefficient of Conservatism value for one plant species and *n* represents the number of species present at a site. These *C* values are averaged for each plot. Coefficient of Conservatism (C) values for Nebraska flora were obtained from the

Nebraska Natural Heritage Program plant species list (Gerry Steinauer, pers. comm.). These values represent the quality of the plant community at a site. Each plant in the state is assigned a *C* value from 0-10, where species with greater *C* values would be indicative of a higher quality site. All non-native species are given a value of 0. The FQI for one plot was then calculated using the equation below:

$$FQI = Mean\ C \times \sqrt{n}$$

Floristic quality considers the overall quality of the plant community and the species richness of the vegetation present, but it does not account for the proportion of a species that is present at a site. Sites with FQI values from 1-19 are generally considered low quality sites, while 20-35 would be considered high quality, and values above 35 would be considered exceptional sites (Lotze, 2019). Statistical tests were conducted using a three-way ANOVA, and the main effects and interactions were tested between the treatment combinations.

Results

Warm-season grasses were by far the most dominant vegetation type recorded at both sites throughout the study. Figure 2.5 depicts vegetation structure of Highway 2 and Highway 77 in Summer 2020 before treatments were applied to the sites. These percentages do not total 100% due to the presence of bare ground and dead vegetative litter. Warm-season grasses had an average cover of 52.9% at Highway 2 and 45.6% at Highway 77. Native wildflowers comprised 11.8% cover at Highway 2, while Highway 77 had 14.4% wildflower cover before applying treatments. Cool-season grasses comprised 8.3% of the cover at Highway 2 compared to 10.4% cover at Highway 77. Other forbs and woody plants also represented a small portion of the vegetative cover at both sites.

Seeding and Mowing Effects on Total Forb Cover

Total percent cover of forbs was assessed to determine the effects of seeding wildflowers and different mowing regimes on the density of forbs. No significant interactions between seeding and mowing treatments were observed on total forb cover in 2021 for either site (Table 2.1A). Seeding alone increased total forb cover on seeded plots for both sites with Highway 2 having 19.5% cover in seeded plots and 12.3% cover in non-seeded plots ($P = 0.014$), while Highway 77 had 17.5% cover in seeded plots and 13.1% forb cover in non-seeded plots, which was trending towards significance ($P = 0.06$). Only the Highway 2 site showed a significant increase in forb cover from seeding; however, the Highway 77 site trended in a similar direction (Fig. 2.6). Pre-mowing had a positive effect on forb growth in the Highway 77 site (Fig. 2.7), where pre-mowed plots had an average cover of 17.9%, while plots that were not mowed averaged 12.7% ($P = 0.03$). This positive effect of pre-mowing on forb cover was not observed at the Highway 2 site.

Vegetation was again assessed for total forb cover in Summer 2022, the season after planting and implementing the final mowing treatment. Forb cover no longer showed significant differences in response to pre-mowing and year-after mowing treatments. Like Summer 2021, there were no significant interactive effects between seeding and mowing treatments for either site (Table 2.2A). The positive effects of seeding remained evident in 2022, as Highway 2 seeded plots had 29.4% forb cover, and non-seeded plots had 18.9% total forb cover (P = 0.02). Highway 77 seeded plots had 22.7% forb cover compared to 16.8% cover on non-seeded plots in 2022, but this was not a significant effect (P = 0.158). Figure 2.8 shows the comparison of forb cover on seeded and non-seeded plots for both sites in 2022.

Seeding and Mowing Treatment Impacts to Cover of Seeded Wildflowers

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 mix (Table 2.3). In the first season after seeding (Summer 2021), the Highway 2 site showed no significant increase in seeded species forb cover from 9.7% on non-seeded plots to 12.8% on seeded plots (P = 0.182). There were also no significant main effects from pre-mowing or mowing after planting on seeded forb species cover. Highway 77 had a small increase in forb cover from 10.1% on non-seeded plots to 13.8% on plots seeded with wildflowers (P = 0.067), which is trending towards significance. The Highway 77 site, however, did have a higher percentage of cover from these seeded species on plots that were mowed before seeding (Fig. 2.9). Highway 77 plots that were not pre-mowed had an average of 9.5% cover, while plots that were mowed averaged 14.5% cover of species within the seed mix (P = 0.015). There were no interactions between treatment factors for either site in 2021 (Table 2.3A).

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 (Fig. 2.10), which was found to be a significant difference (P = 0.013). Highway 77 cover of seeded species was 16.3% in seeded plots and 13.8% in non-seeded plots (Fig. 2.10); however, this difference was not significant for the site. 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 for either site. The Highway 2 site showed a slight decrease in forb cover in response to the year-after mowing treatment 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 = 0.057; Fig. 2.11), which is trending towards significance. Table 2.4A shows the main and interactive treatment effects for seeded forb cover in 2022.

Species Richness

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, and no interactions between seeding and mowing treatments were observed (Table 2.5A). The only significant treatment effect was seeding alone (P < 0.001) with 21.2 species in seeded plots and 15 species in non-seeded plots at Highway 2 (Fig. 2.12). Highway 77 also did not have any effects of mowing or treatment interactions on species richness. Seeding did not significantly affect species richness either, with only a slight increase in number of species observed from 19.1 species on non-seeded plots to 21.6 species on seeded plots (P = 0.176).

In Summer 2022, species richness remained higher on seeded plots compared to plots that were not seeded with wildflowers (Fig. 2.13). Highway 2 seeded plots averaged 23.6 species, and non-seeded plots averaged 16.4 species (P < 0.001). Highway 77 seeded plots had 22.8 species, while non-seeded plots had 20.4 species on average (P = 0.092). Like 2021, no significant effects from mowing were observed at either site, and no significant interactions between seeding and mowing treatments were observed in 2022 (Table 2.6A).

Abundance of Seeded Wildflowers

Prior to seeding in November 2020, the Summer 2020 data shows that 17 of the 31 species from the seeding mix (Table 2.3) were already present on these roadside slopes. Maximilian sunflower was the most frequently encountered forb in 2020, occurring in 89% of plots. Showy partridge pea was the second most common forb in 2020, occurring in 84% of plots. After seeding in November 2020, two species from the seed mix were not observed in any of the sampling periods, butterfly milkweed and Rocky Mountain bee plant [*Cleome serrulata* (Pursh) Roalson & J.C. Hall]. Twenty-nine species from the mix were observed in each sampling period following seeding. After the final sampling in Summer 2022, Maximilian sunflower occurred in 88% of plots, while showy partridge pea occurred in 100% of the 64 total plots. Common milkweed occurred in 61% of plots in 2022, compared to just 9% in 2020 before seeding.

In summer 2021, non-seeded plots contained a total of 16 species from the seed mix with a combined 657 occurrences of these species. Twenty-nine of the species were recorded in seeded plots with a total of 993 occurrences of these species. Significantly more occurrences of seeded species were recorded in seeded plots than non-seeded plots ($P = 0.001$). No other treatment effects or interactions were observed (Table 2.7A). Table 2.9 shows the number of frames that contained each species of the seed mix in seeded and non-seeded plots. As expected, seeding wildflowers does increase the frequency of desirable wildflowers at a site.

In 2022, non-seeded plots had a total of 718 occurrences of seeded wildflowers with 18 of the 31 species present. Total seeded species forb occurrence increased in seeded plots to 1199 total frames containing these species. Significantly more occurrences of seeded species were recorded in seeded plots than non-seeded plots ($P < 0.001$). Five of the 31 species in the seed mix were not detected in these plots, however, the overall frequency of seeded wildflowers increased (Table 2.10). No other treatment effects or interactions were observed (Table 2.7A).

Maximilian sunflower frequency was analyzed by itself because this species was present in the greatest amount compared to other native forbs before any treatments were applied. This allows us to determine if any significant increases in Maximilian sunflower were a result of the treatments. Dickson and Busby (2009) found that including aggressive species, such as Maximilian sunflower, may not be necessary where plants are already well established due to the potential for this species to outcompete other native forbs. In Summer 2020 before seeding, Maximilian sunflower was the most frequently 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. Non-seeded plots had a frequency of 215 frames and seeded plots had a frequency of 179 frames containing the species. No treatment effects or interactions were observed for Maximilian sunflower frequency in 2021 (Table 2.8A). The year-after mowing treatment performed in July 2021 was the only treatment to have a significant effect on the frequency of Maximilian sunflower in Summer 2022 (Table 2.8A). Mowed plots had a frequency of 166 frames while non-mowed plots had 228 frames containing the species ($P = 0.046$).

I also analyzed showy partridge pea individually because it became the most frequently encountered forb across all treatments after seeding and mowing treatments were implemented. This allows us to determine if the significant increase in frequency of showy partridge pea was a result of the treatments. 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 does not appear to be the cause of the increase in showy partridge pea frequency as non-seeded plots had a frequency of 244 frames and seeded plots had a frequency of 269 frames containing the species. None of the treatment combinations or lack of treatments significantly influenced the frequency of partridge pea (Table 2.9A).

Milkweed Abundance

Throughout the duration of the study, three species of milkweed were observed within the roadside plots: common milkweed, whorled milkweed (*Asclepias verticillata* L.), and spider milkweed (*Asclepias viridis* Walter). The two species of milkweed included in the seed mix (Table 2.3) were common milkweed and butterfly milkweed; 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 non-seeded plots at both sites combined ($P < 0.001$). 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 2.8 shows the counts of milkweed occurrences for mowing treatments in 2021 and 2022. 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 non-seeded plots in Summer 2022 ($P = 0.004$). Frequency of common milkweed increased across the duration of the study with the strongest increases occurring in plots that received the seeding treatment (Fig. 2.22). No other treatment effects or interactions were observed in 2021 or 2022 (Table 2.10A).

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 non-seeded plots averaged 11.6 ($P < 0.001$) at Highway 2. Highway 77 did not have any significant differences in average FQI from 12 on non-seeded plots to 13.5 on seeded plots ($P = 0.108$). Figure 2.14 shows the seeding treatment effects on floristic quality. 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 ($P = 0.033$). The interactive effects between pre-mowing and seeding were less apparent at Highway 77 with an average FQI of 14.1 on pre-mowed and seeded plots and 13 on plots that were seeded but not pre-mowed ($P = 0.743$). It is unknown why Highway 2 responded better to seeding and mowing practices than Highway 77 regarding floristic quality. It does appear that mowing before seeding wildflowers may improve the overall floristic quality of a site. Pre-mowing did not affect the FQI of non-seeded plots in 2021. Fig. 2.15. illustrates the interaction between pre-mowing and seeding for Highway 2 in 2021. The early July year-after mowing treatment had no significant impacts on FQI regardless of the seeding treatment. Table 2.11A shows the significance tests for the treatment main effects and interactions. Though seeding and pre-mowing successfully increased floristic quality at Highway 2, all observed FQI values were considered low according to Lotze (2019).

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 11.1 on non-seeded plots (Fig. 2.14; $P < 0.001$). Seeding was the only significant treatment effect on this site in 2022, and the pre-mowing interaction with seeding from 2021 was only observed at the trending significant level (Table 2.12A). Highway 2 did show some evidence of an increase in floristic quality from the pre-mowing treatment alone with an average FQI of 14.6 on pre-mowed plots and 12.8 on non-mowed plots ($P = 0.057$). On Highway 77, seeding effects were still observed with an average FQI of 13.3 on seeded plots and 11.7 ($P = 0.079$) on non-seeded plots (Fig. 2.14). At Highway 77, evidence of interaction was observed between the seeding and year-after mowing treatment. Seeded plots that were year-after mowed had an average FQI of 12.6, while seeded plots that were not mowed had an average FQI of 14 (Table 2.12A).

Mowing Effects on Grass Cover

Total cover of grass species 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 (Table 2.13A). The average grass cover for Highway 2 was 48.2% on pre-mowed plots and 51.3% on non-mowed plots ($P = 0.449$). The average grass cover for Highway 77 was 48.3% on pre-mowed plots and 52.1% on non-mowed plots ($P = 0.278$). Mowing dead vegetation in October has little effect on the density of grass regrowth by June.

The second mowing treatment was implemented in early July 2021. In August 2021, Highway 2 had no significant effects from the year-after mowing treatment on grass cover, and there were no interactions between the mowing treatments (Fig. 2.16; Table 2.14A). By September of 2021, there was evidence that pre-mowing may have reduced grass cover at Highway 2 because plots that were not pre-mowed had 58.3% cover, while pre-mowed plots had 51.7% cover, which is trending towards significance ($P = 0.072$). Highway 77 showed less grass cover on plots that were mowed in July 2021 compared to plots that were not mowed (Fig. 2.16). 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 ($P = 0.002$). No interactions between pre-mowing and year-after mowing were observed at Highway 77 (Table 2.14A).

In Summer 2022, no significant effects of pre-mowing or year-after mowing treatments alone were observed for either site (Table 2.15A). Highway 77 had no interaction between the two mowing treatments, while Highway 2 showed some evidence of interaction between pre- and post-seeding mowing (Table 2.15A). Plots at Highway 2 that were mowed twice had 41.1% grass cover, compared to the other combinations of mowing treatments, which averaged 46.6% ($P = 0.057$).

Mowing and Vegetative Litter

In March 2021, vegetative litter was compared between pre-mowed and not pre-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 ($P < 0.001$). The average depth on pre-mowed plots was 28.5 mm, while the average on non-pre-mowed plots was 49.9 mm (Fig. 2.17). The mower blades moved a portion of the vegetative litter outside the plot boundaries where it was not detected by sampling. The remaining litter fell within the plot boundaries, where it was incorporated into the litter pool. Much of the litter within the plots was consolidated into a single strip in the center of the plot between the mower blades, leaving open spaces for seedling germination throughout most of the plot.

Litter was sampled again in March 2022 to determine the effects both mowing treatments had on litter cover. On both sites, the pre-mowing treatment effects on litter depth were still observed (Fig. 2.18). 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 ($P = 0.009$). The second mowing in July 2021 reduced litter depth in 2022 (Fig. 2.19). On Highway 2, year-after mowing in July 2021 led to a significant reduction in litter depth from 53.8 mm on non-mowed plots to 33.8 mm on mowed plots ($P < 0.001$). 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 non-mowed plots ($P = 0.158$). There were no observed interactions between pre- and year-after seeding mowing treatments for either site (Table 2.17A).

Counts of ground cover types were recorded for three categories: litter cover, basal cover, and bare ground. Data from both sites were combined because of similar results. In Spring 2021, following the October 2020 mowing treatment, vegetative litter occurred in 96% of observations in non-mowed plots compared to 92.1% of observations in mowed plots (P = 0.002). Bare ground was observed in 1.1% of points taken in non-mowed plots and 4.5% in mowed plots (P = 0.004). Basal cover was not affected by mowing in October. Table 2.5 shows the percentage of occurrence for each of the three cover types at both pre-mowing treatments.

Ground cover counts were 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 2.6). Plots that were not pre-mowed had 1.8% occurrence of bare ground compared to 3.8% on mowed plots (P = 0.012). The second mowing treatment in July 2021 had no significant effects on the counts of the three cover categories (Table 2.7).

Reducing vegetative litter cover appeared to positively affect the percentage of forb cover present (Fig. 2.20) in Summer 2021. A correlation coefficient of -0.366 was observed when comparing litter depth and forb cover (P = 0.003). Results show that vegetative litter over 50 mm generally led to about 5-10% forb cover. Cutting litter depth to 25 mm generally increased forb cover 20-30%. There appeared to be no positive correlation between a reduction in vegetative litter cover and forb cover in 2022 (Fig. 2.21). Forb cover was not affected by mowing treatments and associated litter cover one year after mowing was implemented (Table 2.18A).

Soil Bulk Density

Soil bulk density was determined for each plot in spring 2022 and compared to the amount of forb cover present at both sites. Soil bulk density did not have any significant effects on forb cover for either site in 2021 or 2022 (Table 2.18A). Bulk density was slightly higher than typical prairie soils, which generally fall between 1.0 and 1.4 g/cm^3. Highway 2 had an average bulk density of 1.50 g/cm^3, while Highway 77 averaged 1.57 g/cm^3. The correlation coefficient between soil bulk density and percent forb cover was -0.052 in 2021 for both sites combined (P = 0.683). In 2022, the correlation coefficient was -0.046 at both sites (P = 0.72). Figure 2.23 shows that soil bulk density had little effect on forb cover for both sites.

Discussion

Roadside habitat enhancement in southeast Nebraska has the potential to connect fragmented pollinator habitat and provide floral resources for an array of pollinating insects, including migratory populations of the monarch butterfly. Our data supports the hypothesis that seeding wildflowers into existing vegetation on roadside slopes can increase forb cover, species richness, and floristic quality of a site. The hypothesis that seeding combined with both pre- and year-after mowing treatments will achieve the greatest forb abundance and diversity was not supported by the data. Seeding alone increased forb cover, richness, and floristic quality. Pre-mowing in October also showed evidence of benefitting forb cover and floristic quality when combined with seeding. It appears that pre-mowing can increase forb cover and floristic quality in the first season after planting wildflowers. Fall mowing before seeding effectively reduced cover from competitive grasses and vegetative litter covering the ground. The hypothesis that vegetative litter and forb cover would have a negative relationship is supported by the data. Reducing litter in the first year after planting wildflowers can lead to increased forb cover. A variety of benefits can be drawn from restoring native vegetation on roadsides. This can include pollinator habitat, erosion control, and aesthetic value. Similar research has also shown that roadsides can provide key habitat for struggling pollinator communities in heavily fragmented landscapes (Cariveau et al. 2019; Hopwood, 2008; Hopwood, 2013).

Dry conditions undoubtedly had a significant impact on the germination and establishment of wildflower seedlings throughout this study. An increase in annual species is often expected because of drought pressure (Stampfli et al. 2018); however, aside from showy partridge pea, annual species made up a small very small proportion of the species composition at our sites. Control plots did not show any significant changes in plant composition throughout the study. Perhaps sufficient moisture was present for existing, deep-rooted perennial vegetation to persist, while newly planted wildflower seeds did not have the necessary moisture for full establishment at both sites. The importance of moisture in the first year after planting is highlighted by the difference in establishment of wildflowers at Highway 2 versus Highway 77. The Highway 2 site received above average rainfall in 2021, while Highway 77 received below average precipitation in 2021. Total rainfall for both sites from 2020 to 2022 can be seen in Table 2.1. For Highway 77, precipitation was well below the 76.2 cm average for all three years of the study. In 2021, the first season after planting, Highway 77 received 64 cm of precipitation compared to 88.3 cm at Highway 2. Seed was not planted until November 2020, however, a dry 2020 likely led to low soil moisture for newly germinating seedlings in spring 2021. Highway 2 was also drier than the 79.0 cm average for this site in 2020 and 2022. The 88.3 cm received in 2021 at Highway 2 was above the average for the site. This precipitation was important as it came during the first season after planting seed, allowing seedlings to germinate. The difference in precipitation in 2021 is a likely explanation for differences in forb establishment at the two sites. Though precipitation for Highway 2 was slightly less than Highway 77 in 2022, the higher total amount of moisture at Highway 2 in 2021 likely allowed seed to germinate more effectively. Throughout the study, Highway 2 outperformed Highway 77 in terms of forb cover and floristic quality.

Seeding and Mowing Impacts on Wildflower and Grass Cover

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. At Highway 2, a 9.9% increase in total forb cover on seeded plots was observed from 2021 to 2022, and Highway 77 had an increase of 4.8% forb cover in seeded plots. 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 wildflowers had visually grown. The number of seedlings observed had also increased by the second season, highlighting that not all seed germinates in the first year after planting.

Many of the species in the seed mix were already present on the study sites before planting. These species were selected because they are already known to be successful at growing on highly disturbed roadside sites in southeastern Nebraska. In 2021, the cover of forbs contained in the seed mix was only about 3% higher at both sites in seeded plots compared to non-seeded plots. In 2022, seeded species forb cover on Highway 2 was

6.2% higher in seeded plots compared to non-seeded plots. Highway 77 experienced less growth with seeded plots only having 2.5% more cover from these species in 2022.

The seed mix included some dominant forb species already present on these roadside sites. However, Dickson and Busby (2009) suggested limiting the use of some dominant species, such as Maximilian sunflower and showy partridge pea because they can limit 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. Given the already high presence of Maximilian sunflower at these sites, adding additional seed from this species may negatively impact the overall diversity of the site. However, this high existing cover of Maximilian sunflower may not be the case at other roadside sites. 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 the Highway 2 site may not be necessary due to its high prevalence before seeding.

Many seedlings in seeded plots had visibly grown from 2021 to 2022. 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.

The response of forb cover to the mowing treatments was difficult to determine due to inconsistencies between the sites. In Summer 2021, pre-mowing significantly increased total forb cover at Highway 77. While Highway 2 did not have any significant benefits from October mowing in terms of forb cover, the mowing did not lead to a reduction in forb growth. Evidence from Highway 77 shows that pre-mowing may be beneficial to forb growth. More evidence shown below suggests that dormant season mowing can increase floristic quality and reduce vegetative litter from roadside sites during the first growing season after implementation of treatments. These potential benefits of dormant season mowing are short lived as the positive effects were not observed in 2022.

Mowing in early July after seeding did not benefit to forb growth at either site. At Highway 2, year-after mowing reduced forb cover. Mowing in early July also appears to limit the growth of grasses, however, this reduction in growth of live grasses does not appear to improve the growth of forbs. Throughout the study, we included the cover of existing forbs that also contribute to plant cover and nectar sources for pollinators. These larger plants were likely destroyed by the mower blades during July mowing. Growing season mowing has been found to interrupt the reproduction and seed spread of wildflowers (Entsminger et al. 2017). The most common forb from our seed mix prior to applying treatments, Maximilian sunflower, decreased in frequency because of mowing. These existing floral resources are still an important part of the pollinator habitat available on roadsides. By removing existing forbs, floral resources may be set back until seedlings in the wildflower mix reach maturity. These existing forbs provide nectar sources for pollinators while newly seeded wildflowers become established.

Pre-mowing led to slight decreases in grass cover in Summer 2021, however this was not significant. The reduction in vegetative litter from grasses because of mowing during the fall is more likely to benefit forbs than reducing living grass biomass. Year-after mowing during early July 2021 when many warm-season grasses are nearing maturity had relatively little impact on grass regrowth. By the next sampling period in September 2021, grass cover was within 5% on year-after mowed and not year-after mowed plots at Highway 2. Highway 77 mowed plots had about 8% less cover than plots that were not mowed that summer. It appears that grasses at Highway 77 had less recovery after defoliation than Highway 2, possibly due to greater drought stress. Native warm-season grasses appear to recover quickly after disturbance even during drier than average conditions. By Summer 2022, no impacts of mowing were observed at either site for either mowing treatment. This is not surprising as these grasses store most of their carbohydrates underground in roots and rhizomes. Big bluestem plants defoliated only once per year have similar tiller densities and rhizome weights of plants that are not cut (Owensby et al. 1974). Single defoliation events during the growing season may not limit native warm-season grasses enough to significantly benefit forb growth.

Species Richness

As expected, seeding the 31 species native wildflower mix led to increases in plant species richness on both sites. These increases in species richness were 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 non-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). Some examples of at-risk lepidopteran insects that use specific species or groups of plants in southeast Nebraska include: monarch butterflies and milkweeds, the Whitney underwing (*Catocala whitneyi* Dodge) and leadplant (*Amorpha canescens* Pursh), the mottled duskywing (*Erynnis martialis* Scudder) and New Jersey tea (*Ceanothus americanus* L.), and the regal fritillary (*Speyeria idalia* Drury) and violets (*Viola sp.*) (Schneider et al. 2018). Each of these lepidopteran insects listed are considered Tier-1 at-risk species in Nebraska. Though violets and New Jersey tea were not used in this study, roadside vegetation managers should consider adding specific plant species to seed mixes to benefit at-risk specialist pollinators that rely on these species for reproduction. Roadside vegetation managers should reference their state wildlife action plans to determine if plant species can be added to seed mixes that will benefit at-risk pollinators with specific host plant requirements. Sites with high plant diversity may also be able to better resist invasions from un-desirable plants (Yurkonis, 2013). The duration of our study did not capture flowering of most of the species that were observed in the plots. However, their presence indicates that floral diversity can be enhanced by seeding these roadside sites.

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. Seventeen of the 31 species included in the seed mix were present at the sites before seeding took place. Following seeding, 28 of the 31 species were observed in 2021 and 2022, with only two species being completely excluded from our observations. This shows that our seed mix was 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.

Abundance of Seeded Wildflowers

The most dominant forb before implementing treatments was Maximilian sunflower, which was found more frequently in non-seeded plots than plots that were seeded despite being included in the planting mix. The occurrences of Maximilian sunflower did not change significantly throughout the study for either seeding treatment. This suggests either low germination success of Maximillian sunflower seeds in the planting mix or a high enough natural presence that sampling could not detect changes in the cover of the species. 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 will be sitting dormant in the soil until germination conditions are favorable. 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 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 reduce the floral resources from Maximilian sunflower, reducing this species could allow greater wildflower species diversity of a site. Maximilian sunflower is generally very competitive with other native forbs because of its ability to aggressively spread by rhizomes and natural seeding in disturbed sites (Dietz et al. 1992). Under different conditions, Maximilian sunflower may have very high germination after seeding. The use of aggressive forb species, including Maximillian sunflower, should be limited in most seed mixes to minimize competition with other forbs and promote greater diversity of wildflowers at restored sites (Dickson & Busby, 2009). 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. Maximilian sunflower may be left out of the seed mix entirely in sites where establishment is already high, such as the sites used in this study. This could potentially reduce seed costs and prevent excess competition from species that are already dominant at the site.

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 non-seeded plots in Summer 2022. Partridge pea increased in frequency without the help of seeding or mowing treatments. Partridge pea is an annual species, 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 values (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.

Occurrence of Milkweeds in Response to Seeding and Mowing

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 monarchs were found to be on roadsides, and this figure is likely higher now as milkweed habitat becomes increasingly more constrained by cropland conversion (Pleasants & Oberhauser, 2013).

Two species of milkweed were planted from our seed mix (Table 3), 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* Englam. Ex A.Gray) 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 to increase milkweed diversity of restoration projects in southeastern Nebraska. In an Iowa study comparing monarch egg density on nine different species of milkweeds, egg counts were 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 more adaptable species found in Nebraska's roadsides may make more economical sense.

Common milkweed was observed only six 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 non-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 non-seeded plots. Some of the seed began to germinate in the first growing season. Pre- and post-seeding mowing were not found to affect the frequency of common milkweeds in 2021. In Summer 2022, the total frequency of common milkweeds increased for both seeded and non-seeded plots. Sixty-eight frames in seeded plots contained common milkweed compared to 26 frames in non-seeded plots. It is unknown why there was an increase in milkweeds in non-seeded plots. Many of the milkweed stems were still observed to be quite small. Drought conditions are likely the reason for poor growth of milkweeds in the second growing season. Mowing still had no effect on milkweed frequency 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 can 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 three 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, non-mowed plots always had a slightly higher occurrence of common milkweed compared to mowed plots (Table 2.8). 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. There may be some evidence that monarch butterflies prefer milkweeds that have recently regenerated from mowing. 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 insignificant decrease in milkweed counts resulting from the early July mowing. More research is needed on mature, more dense stands of milkweeds to determine how growing season mowing affects milkweeds on Nebraska roadsides.

Vegetative Litter and Forb Growth

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 (Fig. 2.17). It appears that some of the plant litter is removed from the plots when a single 4.6-meter-wide strip is mown. Mowing in October after many warm season plants had entered dormancy reduced the vegetative litter layer that accumulates in non-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 a single mowing event during dormancy can lead to a reduction in total dead biomass for at least two growing seasons. The percentage of bare ground observations also remained higher in March 2022 in response to the pre-mowing treatment. 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 temporarily prevented some grass from growing to maturity. Similarly, defoliation from grazing in eastern Nebraska during the elongation phase starting in mid-June leads to reduced biomass accumulation of big bluestem (Mousel et al. 2003). Mowing in July had a similar effect of grazing as the growing points of new tillers were removed. Removal of live growing points effectively limited growth and biomass accumulation of competitive grasses. This reduction in growth from tall warm-sesaon grasses, however, did not appear to significantly increase the density of forbs. Molinari & D’Antonio (2020) have found that thatch accumulation from exotic annual grasses has more impact on forb growth than competition for resources.

Additional research has suggested that tall native warm-season grasses limit the diversity and cover of native forbs due to competition for sunlight, rather than competition for water and nutrients (Hautier et al. 2009; McCain et al. 2010). Our research shows that litter accumulation from native warm-season grasses seems to have a similar impact on forb growth as exotic annual grasses. Removal of dead litter seems to be more beneficial to wildflowers than reducing competition from living grasses.

In summer 2021, there was a positive correlation of forb growth compared with vegetative litter depth. As litter depth decreased, the percentage of forb cover appeared 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. Litter depth and forb species richness are directly associated, where more litter leads to less forb richness (Pei et al. 2023). 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. Reduced litter cover did not appear to benefit forb growth one year after planting wildflowers. 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 can lose about 3% more total soil moisture in July and August compared to sites where vegetative litter is not removed (Deutsch & Willms, 2010). There seems to be a balance between having enough litter to hold in soil moisture and allowing enough sunlight to penetrate to germinating forbs that needs to be reached to maximize wildflower growth.

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 accumulating in the planted area. Consideration should be taken to avoid large clumps or strips of dead biomass that could lead to poor 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 per sod unit (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. Flatter slopes may need to be used to avoid tipping equipment on slopes. One final consideration with haying is the cost of additional labor and equipment associated with baling and removing bales from the sites.

Floristic Quality

Our data shows that seeding alone is effective at increasing the overall floristic quality of the roadside sites, however, the effects of seeding are stronger on Highway 2 than Highway 77. Seeding wildflowers increased the overall species richness of native plants in the plots, which contributes to increasing the floristic quality. Our plot locations were almost entirely surrounded by cropland. This leaves little chance that increases in floristic quality were due to spread from nearby wildflower seed sources and were likely due to sowing seed at these sites. Soper et al. (2019) found that floristic quality on Nebraska roadsides is generally much higher on sites adjacent to rangelands compared to roadsides surrounded by cropland. This further highlights the need for restoration in the tallgrass prairie region where few natural wildflower seed sources exist to provide pollinator habitat. Perhaps funds for pollinator habitat enhancement can be focused on landscapes that have little to no natural pollinator habitat available.

The FQI of Highway 2 seeded plots increased from 15 in 2021 to 16.3 in 2022. Though only a small increase, McIndoe et al. (2008) supports that floristic quality values tend to rise with increasing time after a restoration as the site shifts from native and exotic weeds to more desirable native wildflowers. Highway 77 seeded plots experienced a slight decrease (0.2) in FQI from 2021 to 2022 but overall, the site performed more poorly than Highway 2 in terms of wildflower establishment. As was mentioned above, the poor performance of Highway 77 may be attributed to less precipitation than Highway 2. Presumably some of the seed sown at both sites may be able to sit dormant during drought conditions until more moisture is received.

Year-after mowing in July did not have any significant impacts on floristic quality. At Highway 2, combining seeding and pre-mowing did lead to a significant increase in FQI in 2021, the first season following seeding. In 2022, there was evidence that pre-mowing alone may have increased FQI at this site. It appeared that under the higher moisture conditions at Highway 2, pre-mowing had a positive effect on floristic quality. Given that the second mowing treatment in early July had no effects on floristic quality at either site, it appeared that the removal of dormant plant litter had a more positive effect on site quality than cutting live vegetation in July.

Even though seeding at both sites and seeding and pre-mowing combined at Highway 2 improved the overall floristic quality, the FQI values averaged below 20 for all treatments. Values less than 20 are considered low quality sites (Lotze, 2019). This is partially due to the presence of numerous introduced species, which have a C value of 0. The more non-native plants present at a site, the lower the mean C value will be, indicating a lower quality site. Given that no site prep was conducted except mowing half the plots, we expect the abundance of introduced plants to remain constant before and after implementing treatments. The average C value for species in the seed mix was only 4.2. Species with a lower C value are expected to easily colonize low quality sites. Many of the species selected are already present on these disturbed roadsides, and though they are not necessarily indicators of high-quality sites, they are beneficial to pollinators and function well at persisting through disturbance and difficult growing conditions. Given the cost of seed and generally difficult growing conditions present on roadsides, it is logical to use native species that will provide the most long-term benefits to pollinators.

Management Implications

Our research shows that seeding native wildflowers on southeastern Nebraska roadsides can effectively increase forb diversity, forb cover, and floristic quality. The success of establishment, however, can vary widely depending on the conditions present at a site. Soil conditions appear to be acceptable for forb establishment at both sites. Rainfall differences, especially in the first year of establishment, are the likely reason for Highway 2 having better forb establishment than Highway 77. However, there may be other unknown characteristics that impact the germination of wildflowers. Floral resources from these newly seeded forbs were observed to be low. As other research has suggested, it may take several growing seasons for some perennial wildflowers to bloom. Twenty-nine of the 31 species in the wildflower seed mix were observed at least one time during the study. Further refining the seed mix can help cut costs by eliminating species that have a low likelihood of establishment. One of the species from the seed mix that was not observed, butterfly milkweed, has been found to have lower use by monarch butterflies than other species. To increase the diversity of milkweed resources, species already present on these roadsides, such as Sullivant's or whorled milkweed, can be substituted, if available, into the seed mix to increase the benefits for monarchs.

Fall mowing before sowing wildflowers seems to have some positive effects on the initial forb establishment in the first growing season after seeding. Fall mowing before seeding functions as a site preparation tool that redistributes vegetative litter and opens bare ground for seed to germinate. Mowing, however, may not completely remove the cut material and thereby litter from a site. Mowing in a manner that reduces the buildup of clumps of dead plant matter on the soil surface will prevent patchy forb growth when strips of vegetative litter are left between the mower blades. These strips of litter can be dense, making it difficult for wildflowers to germinate. Haying is another potential option to remove dead plant litter, but it will involve more costs and soil compaction. Mowing in early July after planting seed in November did not increase the cover of seeded forbs, and even led to a slight reduction in cover of forbs already present at the site. It seems that removing the dead vegetative litter in fall is more beneficial to forb cover than mowing tall warm-season grasses during the growing season, which are well-adapted to periodic disturbance and have time to recover before dormancy.

Perhaps future research on roadside pollinator habitat restorations can assess the site conditions that lead to successful growth of wildflower species. A better understanding of the conditions that make some sites more successful than others would allow roadside vegetation managers to target these higher quality sites for seeding. Using only quality sites with high chances of success would improve efficiency in use of restoration funds considering generally expensive wildflower seed.

Conclusions

Data from two growing seasons after implementing seeding and mowing treatments suggested that seeding and pre-mowing while plants are dormant can be effective at increasing the forb cover and diversity on roadsides in southeast Nebraska. Seeding alone increased wildflower cover, diversity, and floristic quality. Pre-seeding mowing in October also appeared to increase forb cover at Highway 77 and floristic quality at Highway 2 in the first season after planting wildflowers. Though the benefits from pre-mowing were inconsistent at each site, there was evidence to show that it can be an effective site preparation tool to help with germination of forbs. Soil conditions, including soil compaction, were similar at both sites, so it seems likely that precipitation was the main cause of differences in wildflower growth at the two sites. Post-seeding mowing in early July while vegetation was actively growing did not appear to benefit forb growth and may even reduce the density of existing wildflowers at a site. Mowing in July does temporarily limit the cover of tall perennial grasses; however, this single disturbance had no impact on grass cover by Summer 2022. It appeared that mowing dormant vegetation to reduce litter has a greater positive impact on forb growth than mowing live vegetation in early July. This reduction in litter opens the soil surface for newly planted seed to germinate while still retaining some cover to hold soil moisture. Seeding common milkweed led to a significant increase in milkweed frequency across both sites. Mowing does not appear to benefit the frequency of milkweed occurrence. Seeding wildflowers into established vegetation on roadside slopes appears to be a viable option to add ecological and aesthetic value to roadsides provided enough moisture falls to allow growth. Single mowing events during the growing season do not benefit forb growth but mowing in October before seeding may be able to aid initial germination of forbs without damaging existing floral resources.

Literature Cited

Angelella, G. M., Stange, L., Scoggins, H. L., & O'Rourke, M. E. (2019). Pollinator refuge establishment and conservation value: impacts of seedbed preparations, seed mixtures, and herbicides. *HortScience*, *54*(3), 445-451.

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*, 179-181.

Cariveau, A. B., Anderson, E., Baum, K. A., Hopwood, J., Lonsdorf, E., Nootenboom, C., ... & Snell-Rood, E. (2019). Rapid assessment of roadsides as potential habitat for monarchs and other pollinators. *Frontiers in Ecology and Evolution*, *7*, 386.

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.

Collins, S. L., Knapp, A. K., Briggs, J. M., Blair, J. M., & Steinauer, E. M. (1998). Modulation of diversity by grazing and mowing in native tallgrass prairie. *Science*, *280*(5364), 745-747.

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.

Farhat, Y. A., Janousek, W. M., McCarty, J. P., Rider, N., & Wolfenbarger, L. L. (2014). Comparison of butterfly communities and abundances between marginal grasslands and conservation lands in the eastern Great Plains. *Journal of Insect Conservation*, *18*, 245-256.

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.

Freyman, W. A., Masters, L. A., & Packard, S. (2016). The Universal Floristic Quality Assessment (FQA) Calculator: an online tool for ecological assessment and monitoring. *Methods in Ecology and Evolution*, *7*(3), 380-383.

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.

Goverde, M., Schweizer, K., Baur, B., & Erhardt, A. (2002). Small-scale habitat fragmentation effects on pollinator behaviour: experimental evidence from the bumblebee Bombus veteranus on calcareous grasslands. *Biological Conservation*, *104*(3), 293-299.

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*.

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

Hautier, Y., Niklaus, P. A., & Hector, A. (2009). Competition for light causes plant biodiversity loss after eutrophication. *Science*, *324*(5927), 636-638.

Heads, S. W., Harmon-Threatt, A., Dana, C., & Jared, M. (2022). Monitoring and evaluating the status of bumble bees in Illinois. *Prairie Research Institute, University of Illinois.*

Højsgaard, S. & Halekoh, U. (2022). doBy: Groupwise Statistics, LSmeans, Linear Estimates, Utilities. R package version 4.6.13. https://CRAN.R-project.org/package=doBy.

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

Hopwood, J. L. (2013). Roadsides as habitat for pollinators: management to support bees and butterflies. In *Proceedings of the 7th International Conference on Ecology and Transportation (IOECT): Canyons, Crossroads, Connections. Scottsdale, Arizona, USA*, 1-18.

Hopwood, J., Black, S., & Fleury, S. (2015). *Roadside best management practices that benefit pollinators: Handbook for supporting pollinators through roadside maintenance and landscape design* (No. FHWA-HEP-16-059). United States. Federal Highway Administration.

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.

Lotze, N. (2019). Floristic Quality Assessment. *WeConservePA.* Retrieved on April 21, 2024 from https://library.weconservepa.org/library_items/1390-Floristic-Quality-Assessment.

Lucey, A., & Barton, S. (2010). Public perception and sustainable roadside vegetation management strategies. *Newark, Delaware, University of Delaware University Transportation Center*, 1-15.

Lukens, L., Kasten, K., Stenoien, C., Cariveau, A., Caldwell, W., & Oberhauser, K. (2020). Monarch habitat in conservation grasslands. *Frontiers in Ecology and Evolution*, *8*, 13.

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

Markwardt, D. (2005). Texas roadside wildflowers. *Native plants journal*, *6*(1), 69-71.

McCain, K. N., Baer, S. G., Blair, J. M., & Wilson, G. W. (2010). Dominant grasses suppress local diversity in restored tallgrass prairie. *Restoration Ecology*, *18*, 40-49.

McCoy, T. D., Kurzejeski, E. W., Burger Jr, L. W., & Ryan, M. R. (2001). Effects of conservation practice, mowing, and temporal changes on vegetation structure on CRP fields in northern Missouri. *Wildlife Society Bulletin*, 979-987.

McIndoe, J. M., Rothrock, P. E., Reber, R. T., & Ruch, D. G. (2008, January). Monitoring tallgrass prairie restoration performance using floristic quality assessment. In *Proceedings of the Indiana Academy of Science 117*(1), 16-28.

Memmott, J., & Waser, N. M. (2002). Integration of alien plants into a native flower–pollinator visitation web. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *269*(1508), 2395-2399.

Molinari, N. A., & D'Antonio, C. M. (2020). Where have all the wildflowers gone? The role of exotic grass thatch. *Biological Invasions*, *22*(3), 957-968.

Mousel, E. M., Schacht, W. H., & Moser, L. E. (2003). Summer grazing strategies following early-season grazing of big bluestem. *Agronomy Journal*, *95*(5), 1240-1245.

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

Nemec, K., Stephenson, A., Gonzalez, E. A., & Losch, M. (2021). Local decision-makers' perspectives on roadside revegetation and management in Iowa, USA. *Environmental Management*, *67*(6), 1060-1074.

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/

Owensby, C. E., Rains, J. R., & McKendrick, J. D. (1974). Effects of one year of intensive clipping on big bluestem. *Rangeland Ecology & Management/Journal of Range Management Archives*, *27*(5), 341-343

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.

R Core Team (2020). R: A language and environment for statistical computing. R *Foundation for Statistical Computing, Vienna, Austria*. from https://www.R-project.org/.

Ruprecht, E., Enyedi, M. Z., Eckstein, R. L., & Donath, T. W. (2010). Restorative removal of plant litter and vegetation 40 years after abandonment enhances reemergence of steppe grassland vegetation. *Biological Conservation*, *143*(2), 449-456.

Schmidt, A., Kirmer, A., Kiehl, K., & Tischew, S. (2020). Seed mixture strongly affects species-richness and quality of perennial flower strips on fertile soil. *Basic and applied ecology*, *42*, 62-72.

Schneider, R., M. Fritz, J. Jorgensen, S. Schainost, R. Simpson, G. Steinauer, and C. Rothe- Groleau. (2018). Revision of the Tier 1 and 2 Lists of Species of Greatest Conservation Need: A Supplement to the Nebraska Natural Legacy Project State Wildlife Action Plan. The Nebraska Game and Parks Commission, Lincoln, NE

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.

Simanonok, S. C., Otto, C. R., & Buhl, D. A. (2021). Floral resource selection by wild bees and honey bees in the Midwest United States: Implications for designing pollinator habitat. *Restoration Ecology*, *29*(8), e13456.

Skousen, J. G., & Venable, C. L. (2008). Establishing native plants on newly-constructed and older-reclaimed sites along West Virginia highways. *Land Degradation & Development*, *19*(4), 388-396.

Soper, J. M., Raynor, E. J., Wienhold, C., & Schacht, W. H. (2019). Evaluating composition and conservation value of roadside plant communities in a grassland biome. *Environmental management*, *63*, 789-803.

Spyreas, G. (2019). Floristic Quality Assessment: a critique, a defense, and a primer. *Ecosphere*, *10*(8).

Stampfli, A., Bloor, J. M., Fischer, M., & Zeiter, M. (2018). High land-use intensity exacerbates shifts in grassland vegetation composition after severe experimental drought. *Global Change Biology*, *24*(5), 2021-2034.

Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H (2019). Welcome to the tidyverse. *Journal of Open Source Software*, 4(43), 1686.

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.

Yurkonis, K. A. (2013). Can we reconstruct grasslands to better resist invasion?. *Ecological Restoration*, *31*(2), 120-123.

Tables

Table 1.1. The seed mix used by the NDOT is shown for newly constructed highways in Region B, Loess and Glacial Drift (NDOT, 2022).

Species	Common Name	Functional Group	Life Span	Lbs. of PLS/acre
Elymus canadensis	Canada Wildrye	Grass	Perennial	4
Elymus trachycaulus	Slender Wheatgrass	Grass	Perennial	3
Pascopyrum smithii	Western Wheatgrass	Grass	Perennial	4
Sorghastrum nutans	Indiangrass	Grass	Perennial	2
Panicum virgatum	Switchgrass	Grass	Perennial	1.5
Angropogon gerardii	Big Bluestem	Grass	Perennial	3
Schizachyrium scoparium	Little Bluestem	Grass	Perennial	2.5
Bouteloua curtipendula	Sideoats Grama	Grass	Perennial	4
Desmanthus illinoensis	Illinois Bundleflower	Legume	Perennial	0.2
Chamaecrista fasciculata	Partridge Pea	Legume	Annual	0.2
Rudbeckia hirta	Black-eyed Susan	Forb	Biennial	0.4
Linum lewisii	Lewis's Flax	Forb	Perennial	1
Cleome serrulate	Rocky Mountain Bee Plant	Legume	Annual	0.3
Ratibida pinnata	Grayhead Prairie Coneflower	Forb	Perennial	0.25
Avena sativa/Triticum aestivum	Oat/Wheat	Grass	Annual	10

Table 2.1. Total precipitation (centimeters) is shown for 2020, 2021, and 2022 at both sites. Below average precipitation was experienced at both sites except for 2021 at Highway 2. Precipitation averages are based on data from 1895 to 2022.

Total Precipitation (centimeters)				
Site	**2020**	**2021**	**2022**	**Average (1895-2022)**
Highway 2	62.7	88.3	59.1	79.0
Highway 77	61.3	64	60.6	76.2

Table 2.2. Average annual temperature (Celsius) is shown for Highway 2 and 77. Both sites had above average temperatures for the duration of the study. Average temperatures are based on data from 1895 to 2022.

Average Annual Temperature (Celsius)				
Site	**2020**	**2021**	**2022**	**Average (1895-2022)**
Highway 2	11.3	11.7	10.7	10.6
Highway 77	11.4	12.1	11.4	10.7

Table 2.3. The 31 species of forbs in the wildflower mix are shown. Included is the species name, common name used in this study, functional group, life span, bloom time, and Pounds of Pure Live Seed per acre.

Species	Common Name	Functional Group	Life Span	Bloom Time	Lbs. of PLS/acre
Penstemon grandiflorus	Shell-leaf Penstemon	Forb	Perennial	Apr 15 – Jun 15	0.25
Zizia aurea	Golden Alexander	Forb	Perennial	Apr 15 – Jun 15	0.4
Achillea millefolium	Western Yarrow	Forb	Perennial	Apr 15 – Jun 15	0.1
Gaillardia pulchella	Indian Blanket Flower	Forb	Annual	Apr 15 – Jun 15	0.6
Rudbeckia hirta	Black-eyed Susan	Forb	Annual or Biennial	Apr 15 – Jun 15	0.1
Linum lewisii	Lewis Flax	Forb	Perennial	Jun 15 – Aug 15	0.6
Amorpha canescens	Leadplant	Woody	Perennial	Jun 15 – Aug 15	0.4
Asclepias syriaca	Common Milkweed	Forb	Perennial	Jun 15 – Aug 15	0.5
Asclepias tuberosa	Butterfly Milkweed	Forb	Perennial	Jun 15 – Aug 15	0.5
Solidago canadensis	Canada Goldenrod	Forb	Perennial	Jun 15 – Aug 15	0.06
Solidago missouriensis	Missouri Goldenrod	Forb	Perennial	Jun 15 – Aug 15	0.25
Astragalus canadensis	Canada Milkvetch	Legume	Perennial	Jun 15 – Aug 15	0.3
Coreopsis tinctoria	Plains Coreopsis	Forb	Annual	Jun 15 – Aug 15	0.07
Desmodium canadense	Showy Tick-trefoil	Legume	Perennial	Jun 15 – Aug 15	0.6
Heliopsis helianthoides	False Sunflower	Forb	Perennial	Jun 15 – Aug 15	0.3
Monarda fistulosa	Wild Bergamot	Forb	Perennial	Jun 15 – Aug 15	0.15
Echinacea angustifolia	Narrowleaf Purple Coneflower	Forb	Perennial	Jun 15 – Aug 15	0.45

Ratibida columnifera	Upright Prairie Coneflower	Forb	Perennial	Jun 15 – Aug 15	0.3
Ratibida pinnata	Grayhead Coneflower	Forb	Perennial	Jun 15 – Aug 15	0.15
Verbena hastata	Blue Vervain	Forb	Perennial	Jun 15 – Aug 15	0.15
Chamaecrista fasciculata	Showy Partridge-pea	Legume	Annual	Jun 15 – Aug 15	0.5
Dalea purpurea	Purple Prairie Clover	Legume	Perennial	Jun 15 – Aug 15	0.25
Desmanthus illinoensis	Illinois Bundleflower	Legume	Perennial	Jun 15 – Aug 15	0.3
Cleome serrulata	Rocky Mountain Bee Plant	Forb	Annual	Jun 15 – Aug 15	0.75
Helianthus maximiliani	Maximillian Sunflower	Forb	Perennial	Aug 15 – Oct 15	0.25
Helianthus pauciflorus	Stiff Sunflower	Forb	Perennial	Aug 15 – Oct 15	0.4
Oligoneuron rigidum	Stiff Goldenrod	Forb	Perennial	Aug 15 – Oct 15	0.2
Symphyotrichum laeve	Smooth Blue Aster	Forb	Perennial	Aug 15 – Oct 15	0.25
Symphyotrichum novae-angliae	New England Aster	Forb	Perennial	Aug 15 – Oct 15	0.15
Silphium laciniatum	Compass Plant	Forb	Perennial	Aug 15 – Oct 15	0.75
Lespedeza capitata	Roundhead Bush Clover	Legume	Perennial	Aug 15 – Oct 15	0.25
					Total Lbs. PLS/acre: 10.28

Table 2.4. Soil properties are shown for both sites combined. Values denoted with an asterisk are averages. All other values are displayed as a range.

Soil Property	Value
pH	7.0 (neutral) *
Organic Matter	2.2% *
Nitrates	0.56 ppm *
Phosphorus	21 ppm *
Cation Exchange	24 meq/100 grams *
Sodium	1% *
Potassium	347-354 ppm
Sulfate	6.1-6.4 ppm
Magnesium	621 – 661 ppm
Calcium	3502-3534 ppm

Table 2.5. Effects of the pre-mowing treatment on the counts of different cover types in Spring 2021. The three categories of cover were vegetative litter, basal cover, and bare ground. For both treatments, percentages were derived for each plot on the number of points observed for each cover category. The cover percentages for each plot were averaged across the two treatment options. Data were collected in March 2021 following the October 2020 mowing. The percentage of litter cover decreases with mowing, while bare ground seems to increase with mowing.

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

Table 2.6. Effects of the pre-mowing treatment on the counts of different cover types in Spring 2022. The three categories of cover were vegetative litter, basal cover, and bare ground. Cover percentages were obtained using the same methods as Table 2.5. Data was collected in March 2022. Plots mowed in October 2020 continue to have a higher percentage of bare ground compared to plots that were not mowed.

Mowing Treatment	Percent Litter Cover	Percent Basal Cover	Percent Bare Ground
Not pre-mowed	89.7%	8.5%	1.8%
Pre-mowed	87.6%	8.6%	3.8%
P-value	0.074	0.891	0.012

Table 2.7. Effects of the year-after mowing treatment on the counts of different cover types in Spring 2022. The three categories of cover were vegetative litter, basal cover, and bare ground. Cover percentages were obtained using the same methods as Table 2.5. Data was collected in March 2022. The mowing occurred in July 2021. The amount of bare ground does not appear to be influenced by mowing in July. The occurrence of basal cover is slightly higher on mowed plots than non-mowed plots.

Mowing Treatment	Percent Litter Cover	Percent Basal Cover	Percent Bare Ground
Not year after mowed	89.7%	7.6%	2.7%
Year-after mowed	87.6%	9.5%	2.9%
P-value	0.088	0.057	0.785

Table 2.8. 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 milkweed 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, non-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

Table 2.9. The frequency of occurrence in 2021 is shown for each species in the wildflower seed mix comparing seeded and non-seeded plots. Frequency is a count of the number of frames that a plant species occurs in each treatment. Twelve frames are recorded for each of the 64 plots.

Abundance of forb species from the seed mix in Summer 2021		
Rank	**Not Seeded**	**Seeded**
1	Maximilian sunflower – 204	Partridge pea – 232
2	Partridge pea – 200	Maximilian sunflower – 186
3	Canada goldenrod – 55	IL bundleflower – 83
4	Blackeyed Susan – 51	Canada goldenrod – 78
5	IL bundleflower – 49	Black samson – 57
6	Grayhead coneflower – 30	Blackeyed Susan – 55
7	False sunflower – 21	Grayhead coneflower – 50
8	Smooth blue aster – 18	Blue flax – 36
9	Tick trefoil – 16	False sunflower – 33
10	Rigid goldenrod – 7	Smooth blue aster – 32
11	Stiff sunflower – 3	Golden alexander – 27
12	Common milkweed – 1	Indian blanketflower – 27
13	Missouri goldenrod – 1	Common milkweed – 26
14	Purple prairie clover – 1	Stiff sunflower – 23
15	Black samson – 0	Rigid goldenrod – 9
16	Blue flax – 0	Canada milkvetch – 6
17	Blue vervain – 0	Compass plant – 6
18	Canada milkvetch – 0	Purple prairie clover – 6
19	Compass plant – 0	New England aster – 4
20	Golden Alexander – 0	Shell leaf penstemon – 4
21	Indian blanketflower – 0	Yarrow – 4

22	Leadplant – 0	Blue vervain – 2
23	New England aster – 0	Missouri goldenrod – 2
24	Plains coreopsis – 0	Tick trefoil – 2
25	Roundhead bushclover – 0	Plains coreopsis – 1
26	Shell leaf penstemon – 0	Roundhead bushclover – 1
27	Upright prairie coneflower – 0	Wild bergamot – 1
28	Wild bergamot – 0	Leadplant – 0
29	Yarrow – 0	Upright prairie coneflower – 0
30	Butterfly milkweed – 0	Butterfly milkweed – 0
31	Rocky Mountain bee plant – 0	Rocky Mountain bee plant – 0
Total	**657**	**993**

Table 2.10. The frequency of occurrence in 2022 is shown for each species in the wildflower seed mix comparing seeded and non-seeded plots. Frequency is a count of the number of frames that a plant species occurs in each treatment. Twelve frames are recorded for each of the 64 plots.

Abundance of forb species from the seed mix in Summer 2022		
Rank	**Not Seeded**	**Seeded**
1	Partridge pea – 244	Partridge pea – 269
2	Maximilian sunflower – 215	Maximilian sunflower – 179
3	IL bundleflower – 61	IL bundleflower – 105
4	Canada goldenrod – 59	Canada goldenrod – 86
5	Blackeyed Susan – 28	Grayhead coneflower – 71
6	Common milkweed – 26	Common milkweed – 68
7	Grayhead coneflower – 18	Golden Alexander – 61
8	Smooth blue aster – 17	Black samson – 54
9	False sunflower – 14	Blackeyed Susan – 51
10	Rigid goldenrod – 13	False sunflower – 36
11	Tick trefoil – 9	Stiff sunflower – 35
12	Stiff sunflower – 5	Blue flax – 31
13	Black samson – 4	Smooth blue aster – 30
14	Missouri goldenrod – 3	Indian blanketflower – 29
15	Roundhead bushclover – 1	Upright prairie coneflower – 26
16	Yarrow – 1	New England aster – 18
17	Blue vervain – 0	Rigid goldenrod – 12
18	Canada milkvetch – 0	Shell leaf penstemon – 10
19	Compass plant – 0	Purple prairie clover – 7
20	Golden Alexander – 0	Wild bergamot – 7
21	Indian blanketflower – 0	Compass plant – 6

22	Leadplant – 0	Roundhead bushclover – 3
23	New England aster – 0	Yarrow – 2
24	Plains coreopsis – 0	Tick trefoil – 1
25	Roundhead bushclover – 0	Blue vervain – 1
26	Purple prairie clover – 0	Canada milkvetch – 1
27	Upright prairie coneflower – 0	Missouri goldenrod – 0
28	Wild bergamot – 0	Leadplant – 0
29	Shell leaf penstemon – 0	Plains coreopsis – 0
30	Butterfly milkweed – 0	Butterfly milkweed – 0
31	Rocky Mountain bee plant – 0	Rocky Mountain bee plant – 0
Total	**718**	**1199**

Figures

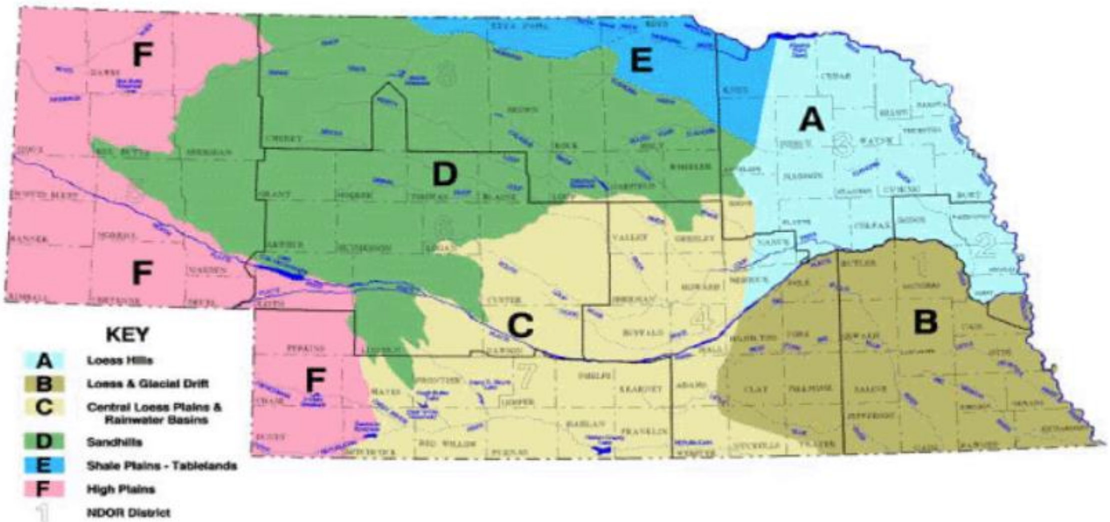

Figure 1.1. Map of the Landscape Regions used by the Nebraska Department of Transportation. This study is located within Region B: Loess and Glacial Drift (NDOT, 2022).

Figure 2.1. The locations of the two study sites are displayed. The Highway 77 site is located south of Cortland, Nebraska, and the Highway 2 site is located west of Nebraska City, Nebraska.

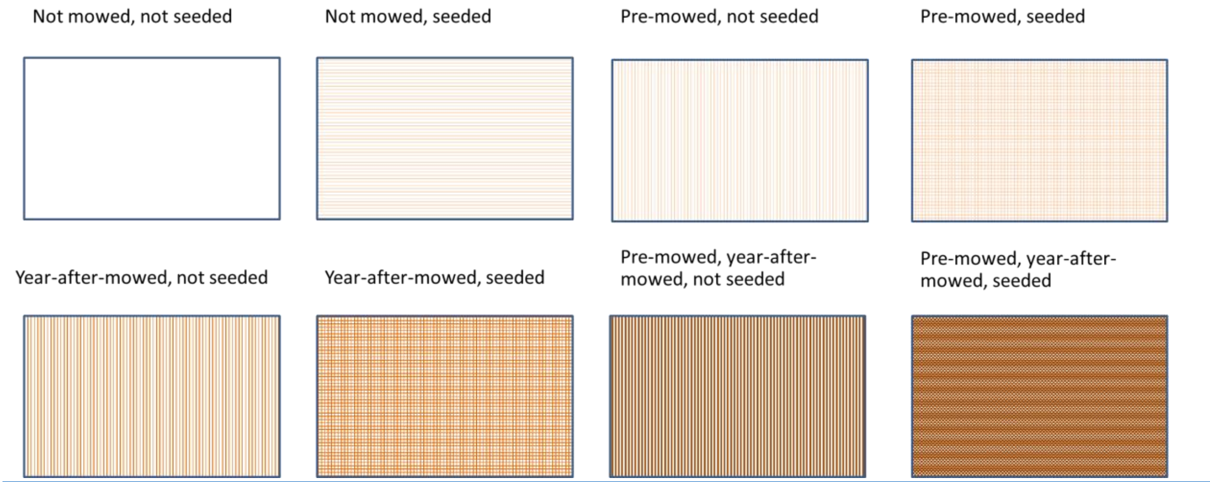

Figure 2.2. The eight treatment combinations are illustrated. Treatments include various combinations of the three treatment factors: pre-mowed and not pre-mowed, seeded and non-seeded, and year-after-mowed and not year-after-mowed. These treatment factors were combined for a total of eight treatments. Plots receiving no seeding or mowing treatments were considered controls.

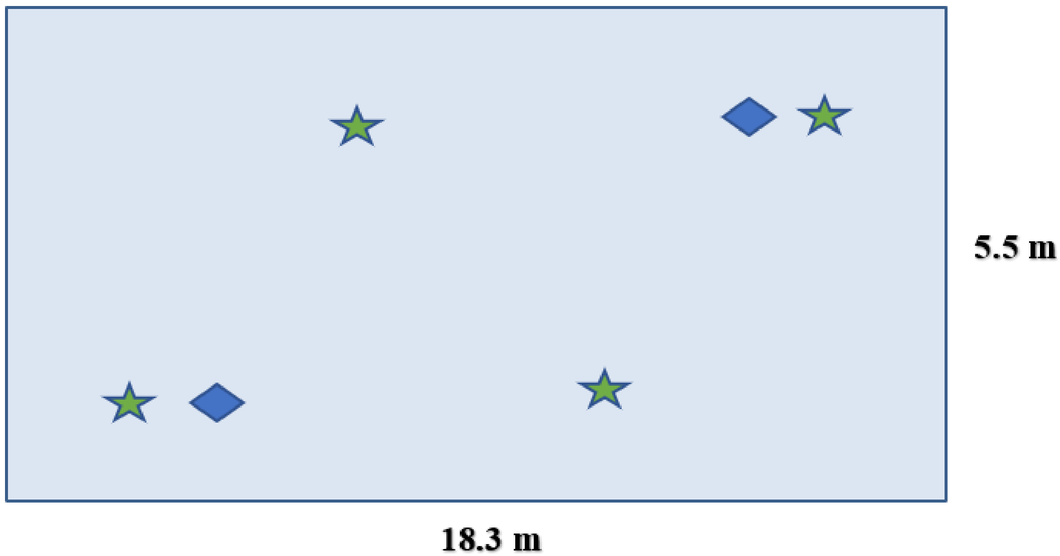

Figure 2.3. The locations of soil samples within the plots are shown. Four 2.5 cm diameter soil cores were taken in a zig-zag pattern to represent the entire plot. Two bulk density cores with a diameter of 5 cm were taken from each plot. Stars represent the small cores, and diamonds represent the larger bulk density cores.

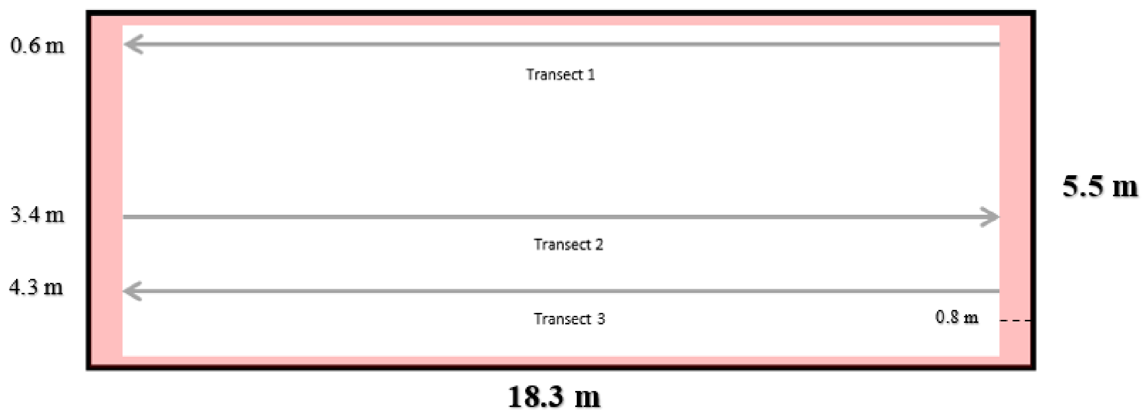

Figure 2.4. The plot setup is shown for vegetative litter sampling. Transects were randomized along the 5.5-meter plot edge. Fifteen measurements were taken on each of the three transects. A 0.8-meter margin was not sampled at each end of the plot.

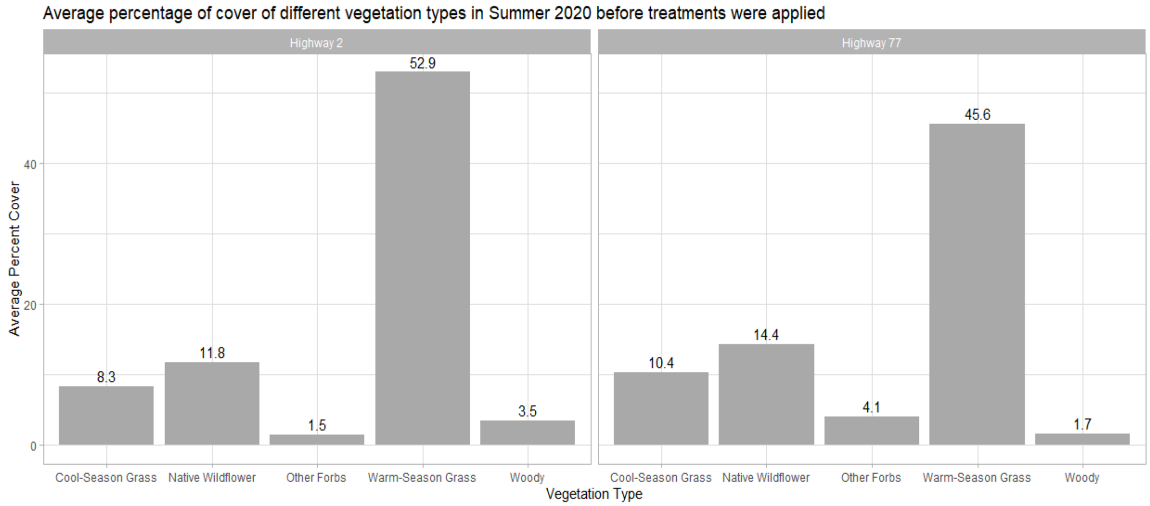

Figure 2.5. The average percent cover of the vegetation types are shown for Highway 2 and Highway 77 in Summer 2020. This information is before any treatments were applied to give a representation of the vegetative structure on roadsides in southeastern Nebraska. Warm-season grasses were by far the most dominant vegetation on these sites.

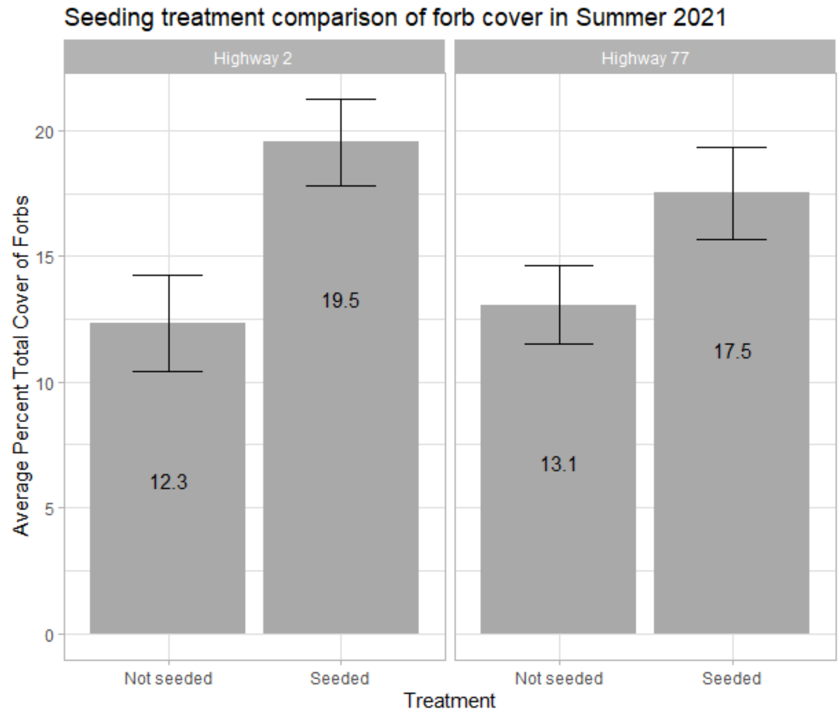

Figure 2.6. Main effect of the seeding in November 2020 on total forb cover for Summer 2021. The cover values are an average of plots within the two seeding treatments. Averages are obtained from the sum of cover for each forb species within a plot. Error bars represent the standard error of the sample. Both sites are represented in the figure, with both sites showing an increase in total forb cover on average in plots that were seeded. Highway 2 had a significant effect on forb cover from seeding (P = 0.014), while Highway 77 was trending towards significance (P = 0.06).

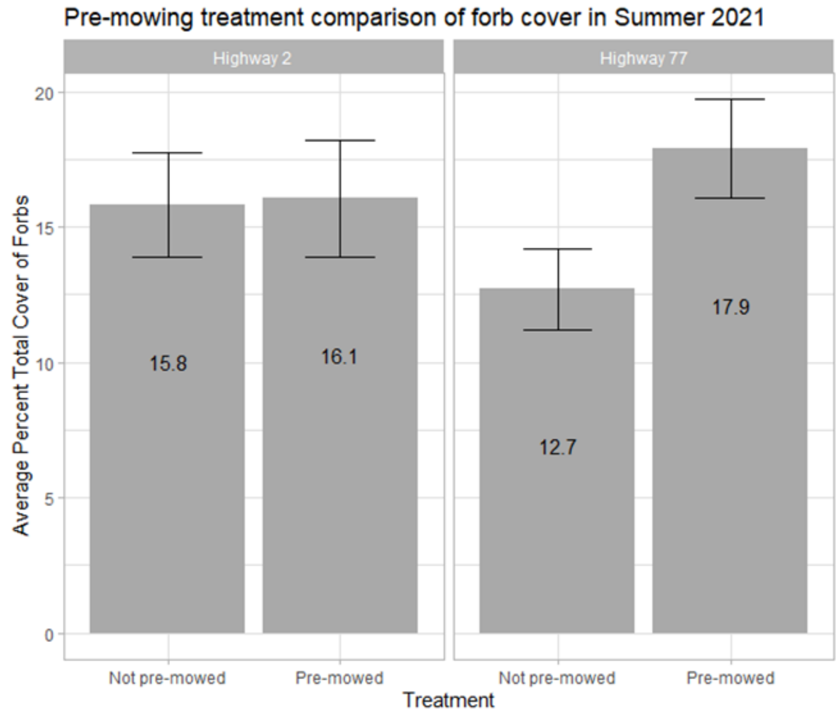

Figure 2.7. Main effect of Pre-mowing in October 2021 on total forb cover in Summer 2021. Treatment cover averages were obtained using the same methods as Fig. 2.4. Both sites appear to show an increase in total forb cover in response to mowing before seeding, but only the Highway 77 site has a significant effect on total forb cover with pre-mowing ($P = 0.03$).

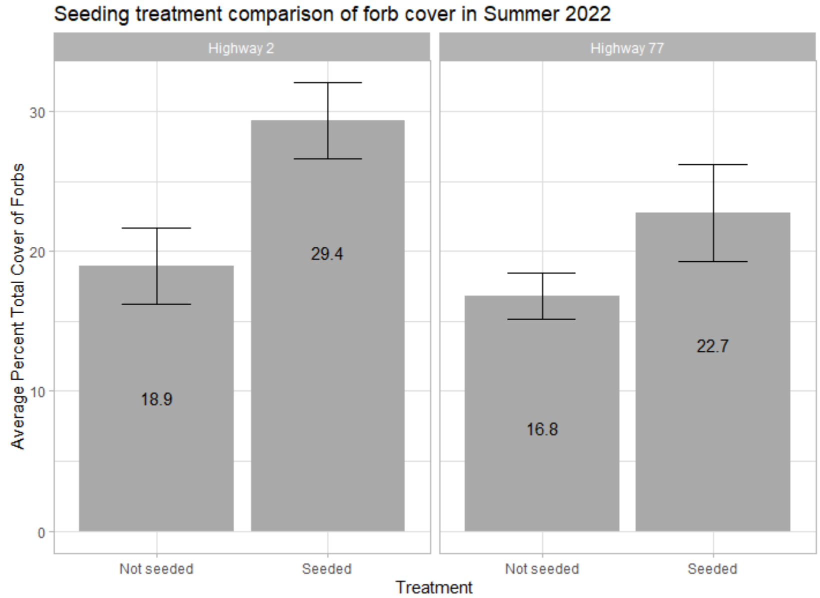

Figure 2.8. Main effect of seeding wildflowers on the total forb cover observed in Summer 2022, the second season after planting and applying mowing treatments. The values are an average for both seeding treatments of the sum of forb species cover for each plot. Planting wildflowers on roadsides increases the total forb cover. The Highway 2 site had a significant effect of seeding wildflowers (P = 0.02).

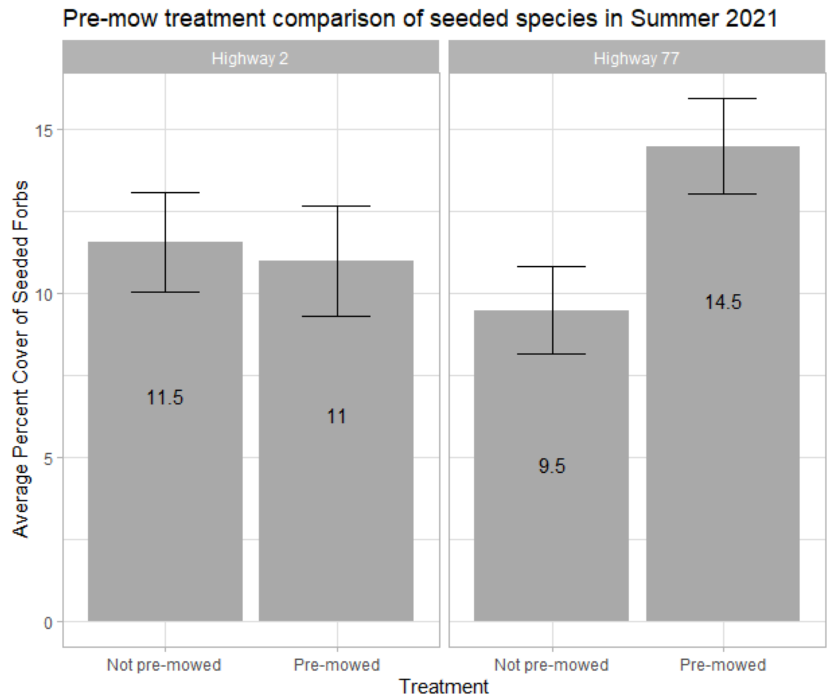

Figure 2.9. Main effect of the pre-mowing treatment on the percentage of seeded forb cover for Summer 2021. Only species included in the seed mix (Table 2.3) are included. The cover values are an average of the plots within the two pre-mowing treatments. The two highways are shown separately. Only Highway 77 had a significant effect of pre-mowing on seeded forb cover (P = 0.015). Samples were collected in August and September of 2021.

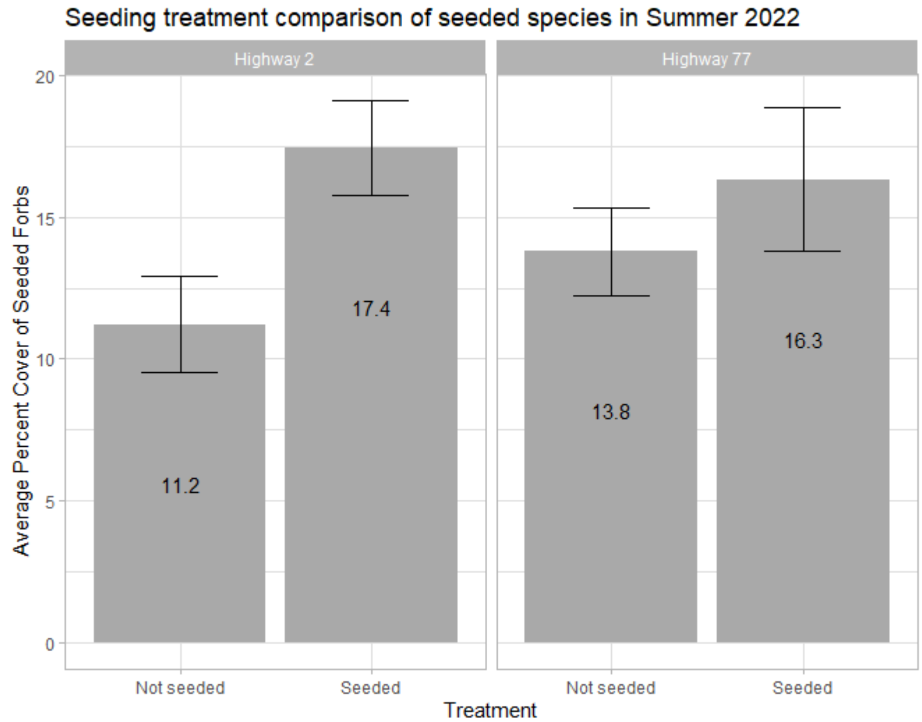

Figure 2.10. Main effect of seeding wildflowers on the percent cover of forbs within the planting mix (Table 2.3) in Summer 2022. The cover values are an average of plots within the two seeding treatments. Highway 2 had a significant effect of seeding ($P = 0.013$). Seeding occurred in November 2021. This data was obtained in August 2022.

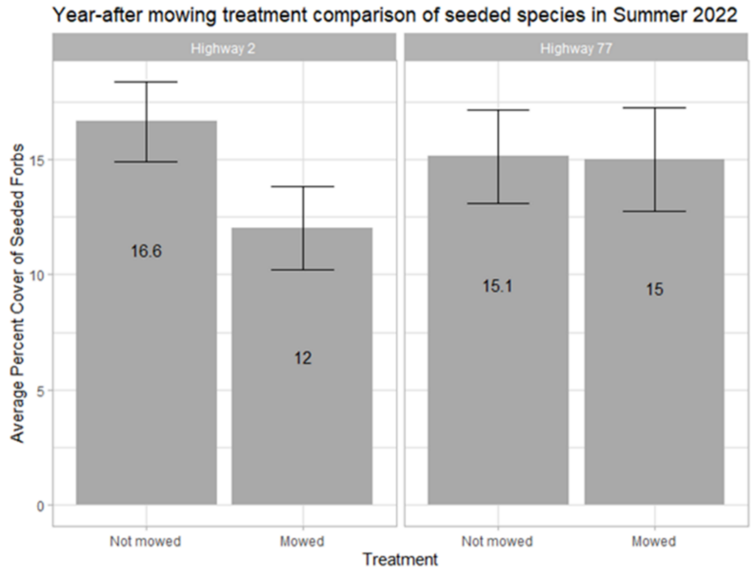

Figure 2.11. Main effect of year-after mowing treatment on seeded species cover in Summer 2022. Year after-mowing led to reduced cover of seeded species at Highway 2 in the second growing season after planting (P = 0.057). The year-after mowing treatment occurred in July 2021.

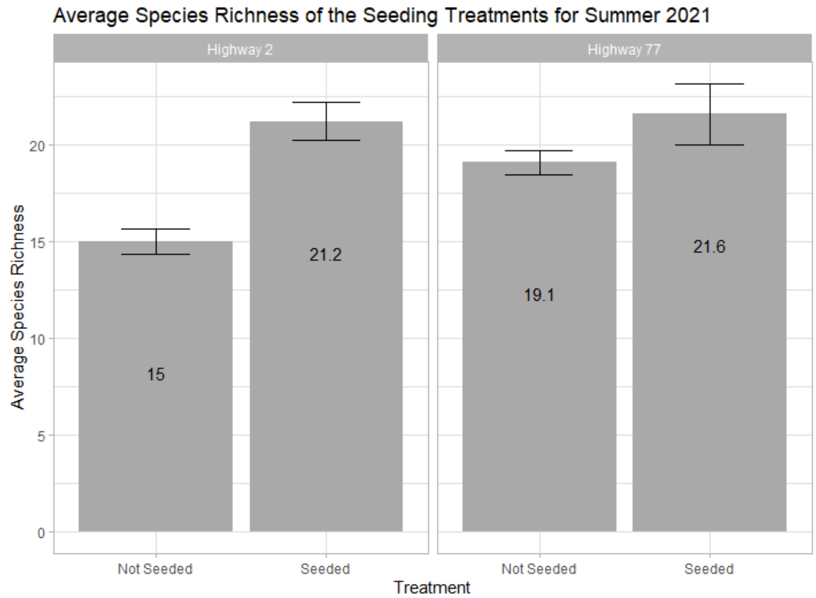

Figure 2.12. Main effect of seeding on species richness for both sites in Summer 2021. Species richness is a count all plant species found within a plot. Plot species counts were averaged by seeding treatment for each site. Highway 2 had a significant increase in species richness in response to seeding wildflowers (P < 0.001).

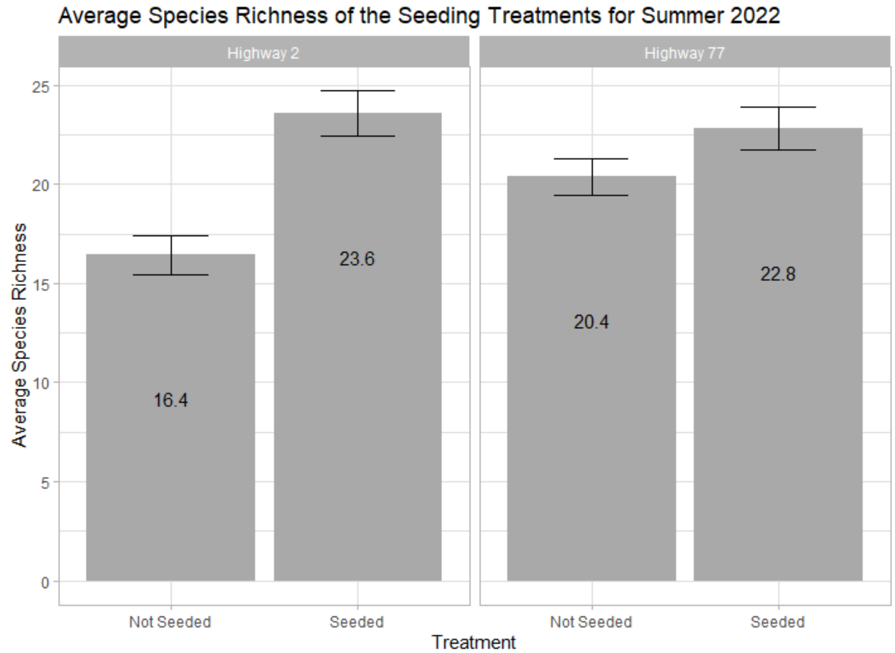

Figure 2.13. Main effect of seeding on species richness for both sites in Summer 2022. Species richness is a count all plant species found within a plot. Plot species counts were averaged by seeding treatment for each site. Highway 2 had a significant increase in species richness in response to seeding wildflowers ($P < 0.001$). Seeding effects were trending towards significance for Highway 77 ($P = 0.092$).

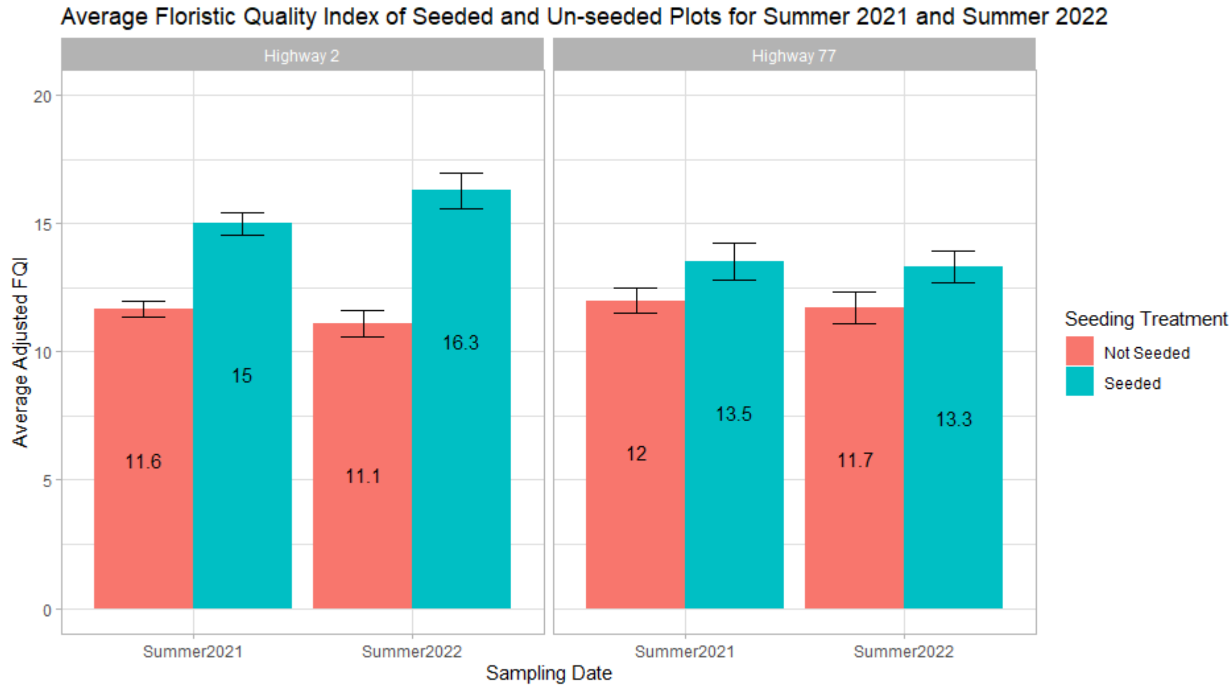

Figure 2.14. Main effect of seeding wildflowers on the floristic quality of both sites for Summer 2021 and Summer 2022. The floristic quality index for each plot was calculated by multiplying the average coefficient of conservatism value by the square root of the number of species present at that plot. 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 ($P < 0.001$).

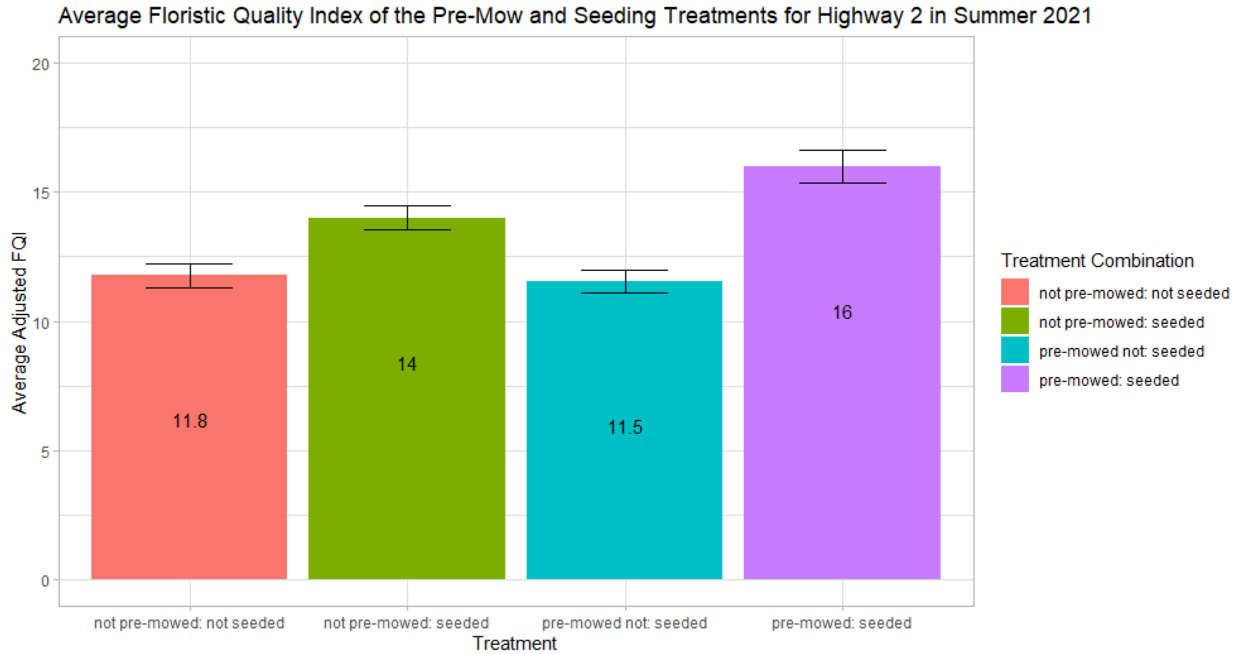

Figure 2.15. Interaction between the pre-mowing and seeding treatments are shown for the Highway 2 site in Summer 2021 for adjusted floristic quality. Average FQI for each treatment is calculated the same as Fig. 2.11. Pre-mowing and seeding wildflowers leads to higher floristic quality when compared to seeding without pre-mowing (P = 0.033).

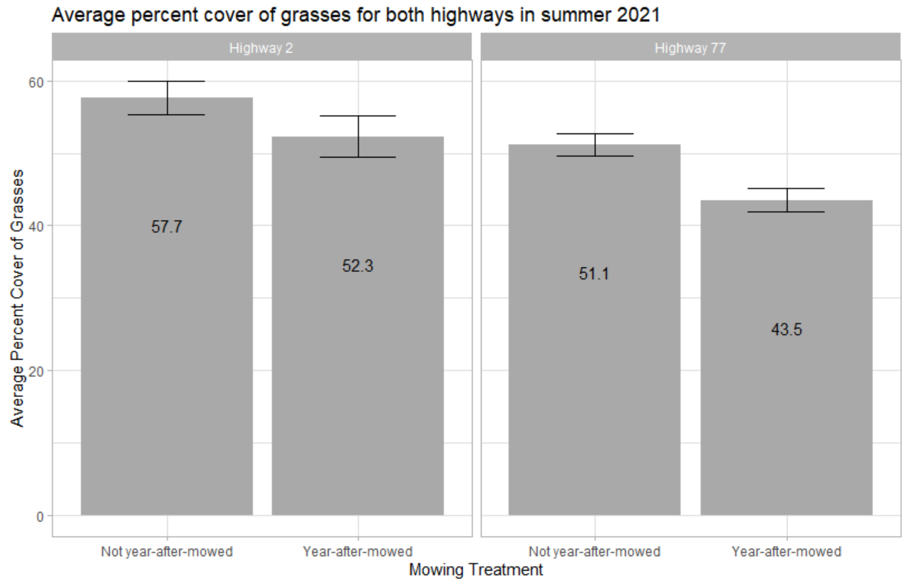

Figure 2.16. Main effect of July 2021 mowing on grass cover in September 2021. Average percent cover is calculated by averaging the total percent cover of grass species for each plot within the two year-after mowing treatments. Highway 77 had significantly less cover of grasses on plots mowed in July compared to plots not mowed in July of the same year (P = 0.002).

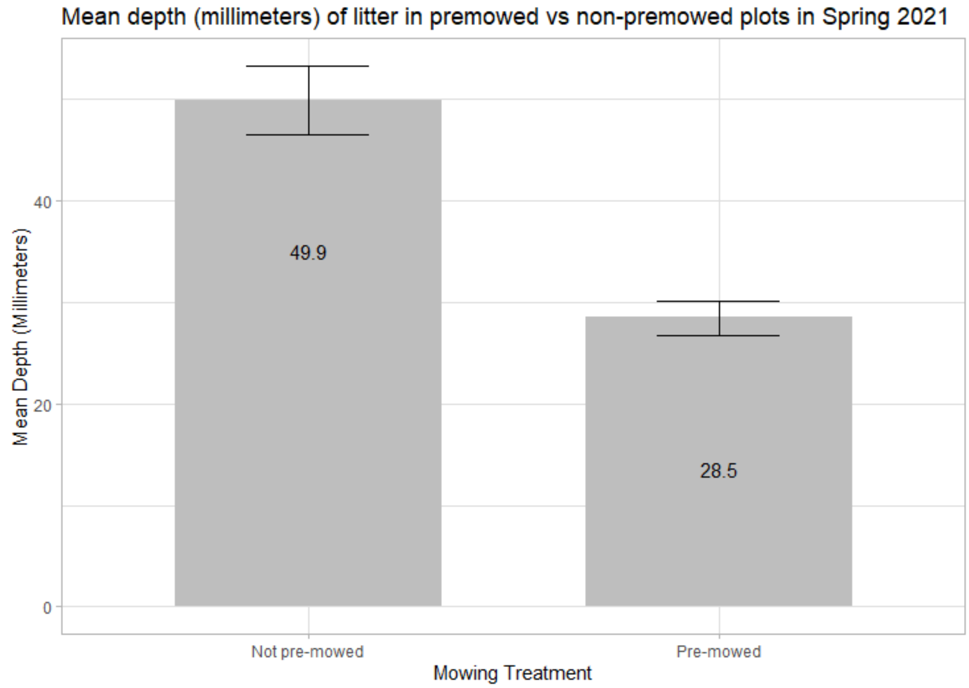

Figure 2.17. Main effect of pre-mowing on vegetative litter depth in millimeters for Spring 2021 with both sites combined. A plot average litter depth was derived from each measurement along three transects. Plots were then averaged by mowing treatment. The results for both sites were displayed together 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 ($P < 0.001$).

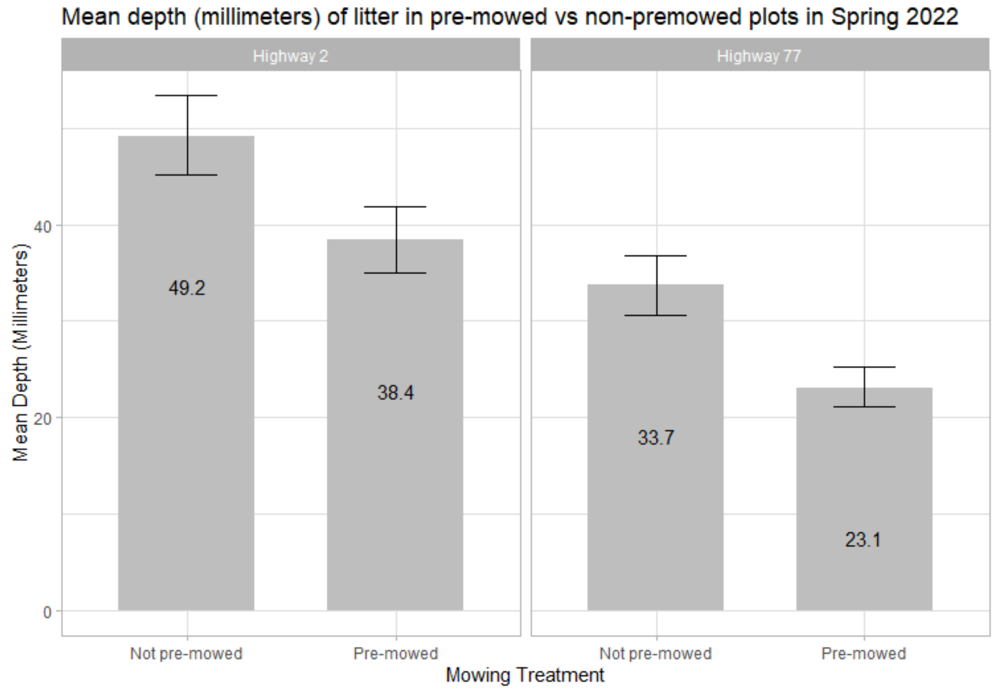

Figure 2.18. Main effect of pre-mowing on vegetative litter depth (millimeters) in March 2022. Mowing occurred in October 2020. Each site is shown separately. A plot average litter depth was derived from each measurement along three transects. Plots were then averaged by mowing treatment. At both sites, pre-mowing reduces the depth of litter present in plots (Highway 2, P = 0.013; Highway 77, P = 0.009).

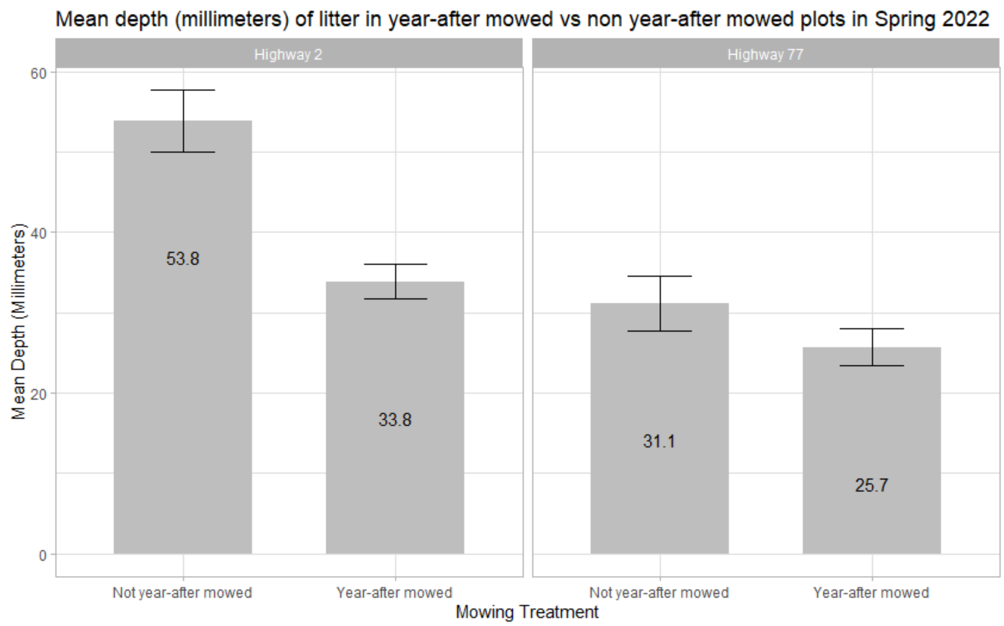

Figure 2.19. Main effect of year-after mowing on vegetative litter depth (millimeters) in March 2022. Mowing occurred in July 2021. Average litter depth was calculated using the same methods as Figure 2.15. Highway 2 mowed plots have significantly less litter than non-mowed plots ($P < 0.001$).

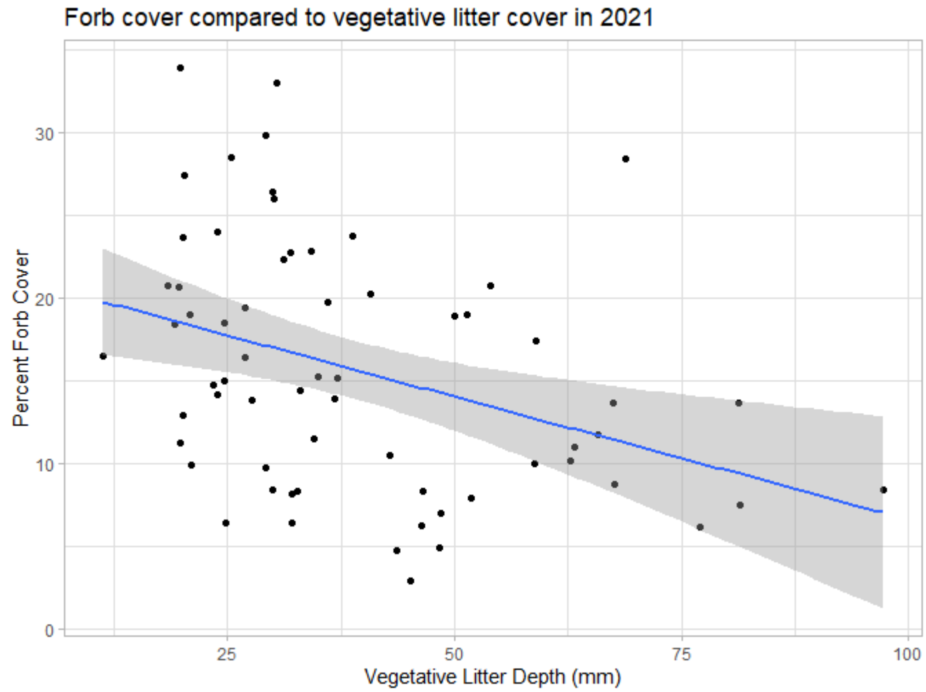

Figure 2.20. Vegetative litter depth and the total percent cover of forbs is compared for 2021. Each point represents a plot. Treatments are not included in the data. The vegetative litter depth on the x-axis was collected in March 2021, and the forb cover data on the y-axis was collected in September 2021. Litter depth for each plot was calculated by averaging litter depth in millimeters at each point taken along three transects. For each frame of forb cover data, the sum of forb cover was calculated. These frames were then averaged by plot. Forb cover increases as vegetative litter depth decreases in the first season after seeding wildflowers ($r = -0.366$, $P = 0.003$).

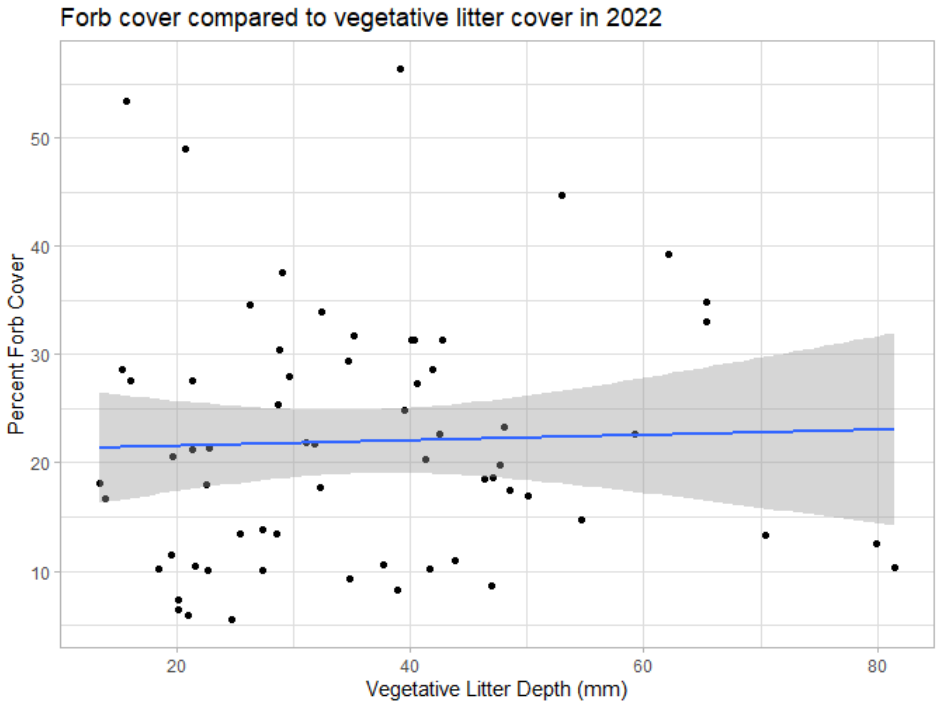

Figure 2.21. Vegetative litter depth and the total percent cover of forbs is compared for 2022. Each point represents a plot. Treatments are not included in the data. The vegetative litter depth on the x-axis was collected in March 2022, and the forb cover data on the y-axis was collected in September 2022. Average litter depth and percent forb cover were calculated using the same methods as Figure 2.20. In 2022, there is no correlation between litter depth and the amount of forb cover ($r = 0.034$, $P = 0.79$).

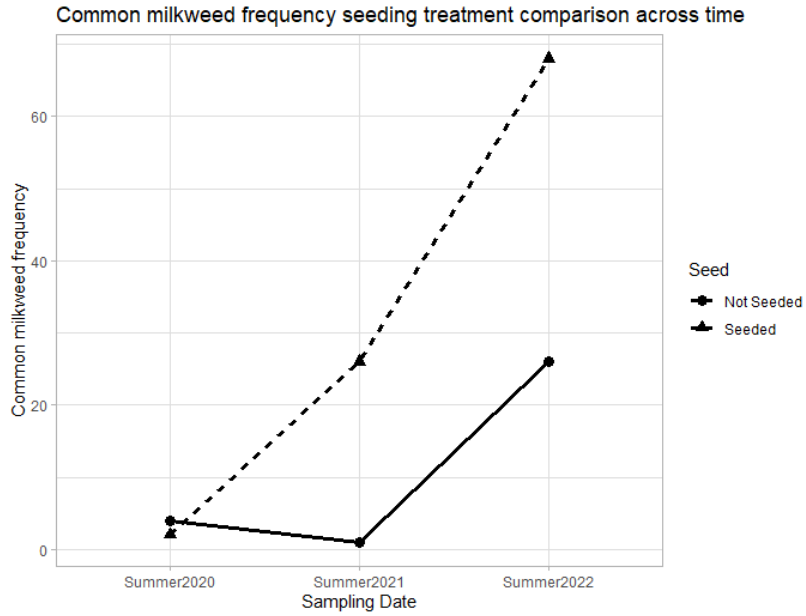

Figure 2.22. The frequency of common milkweed occurrences on seeded and unseeded plots across time is shown. Frequency was determined by the total number of frames containing common milkweed for each treatment at each sampling date. Common milkweed was the only milkweed species considered because it 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 non-seeded plots in Summer 2021 and Summer 2022, and frequency increased further with time.

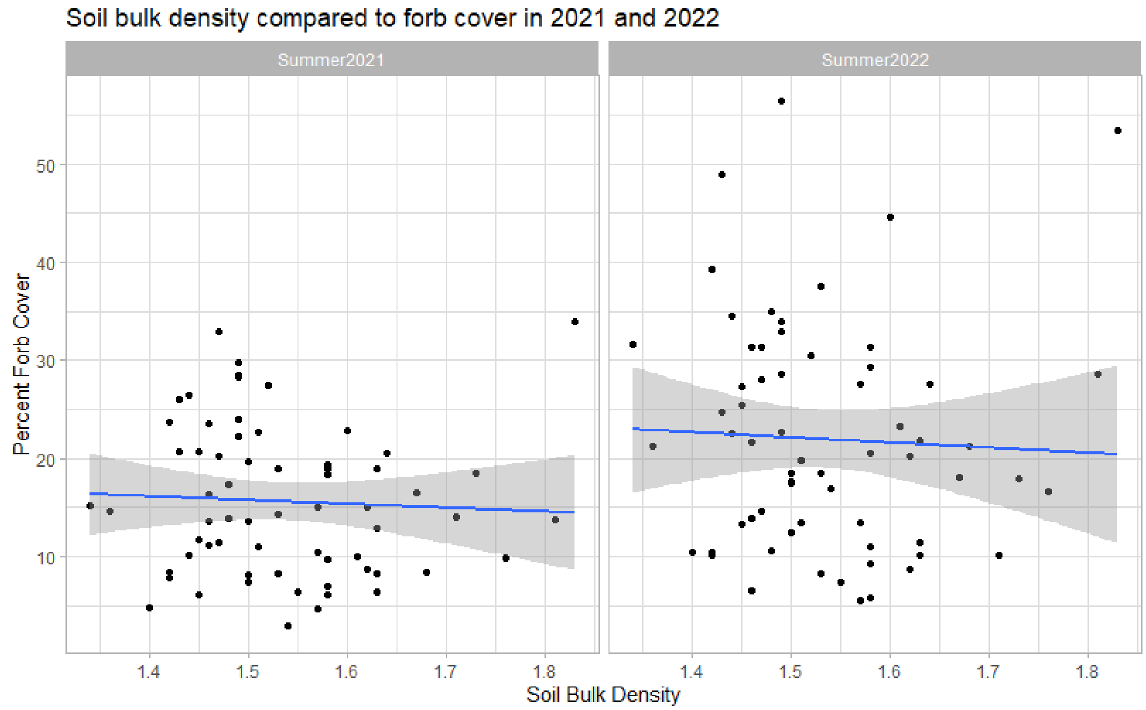

Figure 2.23. The correlation between forb cover and soil bulk density is shown for both sites combined in 2021 and 2022. Soil bulk density does not have any effect on forb cover at these sites. The correlation coefficient for 2021 was -0.052 (P = 0.68). The correlation coefficient for 2022 was -0.046 (P = 0.72).

APPENDIX

Table 2.1A. Results from a three-way ANOVA comparing forb cover under different seeding and mowing treatment combinations and main effects in 2021. The seeding main effect was significant at Highway 2 and trending towards significance at Highway 77. The pre-mow main effect was significant at Highway 77.

Treatment Effects on Total Forb Cover - 2021

	Highway 2			Highway 77		
Treatment	**DF**	**F-value**	**P-value**	**DF**	**F-Value**	**P-Value**
Pre-mow	1	0.007	0.935	1	5.293	0.030**
Seeding	1	7.022	0.014**	1	3.895	0.060*
Year-after Mow	1	1.731	0.201	1	1.317	0.263
Pre-mow:Seeding	1	0.146	0.706	1	1.905	0.180
Premow:Year-after Mow	1	0.043	0.838	1	0.197	0.661
Seeding:Year-after Mow	1	0.633	0.434	1	1.668	0.209
Pre-mow:Seeding:Year-after Mow	1	0.476	0.497	1	0.001	0.974

Statistically significant effects are shown with **
Effects that are trending towards significance are shown with *

Table 2.2A. Results from a three-way ANOVA comparing forb cover under different seeding and mowing treatment combinations and main effects in 2022. The seeding main effect at Highway 2 was the only significant treatment effect in 2022.

Treatment Effects on Total Forb Cover – 2022

	Highway 2			Highway 77		
Treatment	**DF**	**F-value**	**P-value**	**DF**	**F-Value**	**P-Value**
Pre-mow	1	0.016	0.899	1	1.469	0.237
Seeding	1	6.189	0.020**	1	2.123	0.158
Year-after Mow	1	0.514	0.481	1	0.207	0.653
Pre-mow:Seeding	1	0.353	0.558	1	0.440	0.513
Premow:Year-after Mow	1	0.003	0.958	1	0.002	0.967
Seeding:Year-after Mow	1	0.001	0.976	1	0.183	0.673
Pre-mow:Seeding:Year-after Mow	1	0.309	0.584	1	0.378	0.545

Statistically significant effects are shown with **
Effects that are trending towards significance are shown with *

Table 2.3A. Results from a three-way ANOVA comparing cover of seeded forb species under different seeding and mowing treatment combinations and main effects in 2021. The seeding main effect at Highway 77 was the only significant treatment effect in 2021. The pre-mowing main effect at Highway 77 was trending towards significance.

Treatment Effects on Seeded Species Forb Cover – 2021

	Highway 2			Highway 77		
Treatment	**DF**	**F-value**	**P-value**	**DF**	**F-Value**	**P-Value**
Pre-mow	1	0.065	0.801	1	6.881	0.015**
Seeding	1	1.886	0.182	1	3.692	0.067*
Year-after Mow	1	2.650	0.117	1	2.457	0.130
Pre-mow:Seeding	1	0.325	0.574	1	0.843	0.368
Premow:Year-after Mow	1	0.449	0.509	1	0.289	0.596
Seeding:Year-after Mow	1	0.756	0.393	1	0.929	0.345
Pre-mow:Seeding:Year-after Mow	1	0.110	0.743	1	0.091	0.766

Statistically significant effects are shown with **
Effects that are trending towards significance are shown with *

Table 2.4A. Results from a three-way ANOVA comparing cover of seeded forb species under different seeding and mowing treatment combinations and main effects in 2022. The seeding main effect at Highway 2 was the only significant treatment effect in 2022. The pre-mowing main effect at Highway 77 was trending towards significance.

Treatment Effects on Seeded Species Forb Cover – 2022

	Highway 2			Highway 77		
Treatment	**DF**	**F-value**	**P-value**	**DF**	**F-Value**	**P-Value**
Pre-mow	1	2.033	0.167	1	1.464	0.238
Seeding	1	7.192	0.013**	1	0.627	0.436
Year-after Mow	1	3.989	0.057*	1	0.001	0.974
Pre-mow:Seeding	1	0.875	0.359	1	0.041	0.841
Premow:Year-after Mow	1	0.487	0.492	1	0.255	0.618
Seeding:Year-after Mow	1	0.495	0.489	1	0.003	0.956
Pre-mow:Seeding:Year-after Mow	1	0.035	0.854	1	0.026	0.874

Statistically significant effects are shown with **
Effects that are trending towards significance are shown with *

Table 2.5A. Results from a three-way ANOVA comparing species richness under different seeding and mowing treatment combinations and main effects in 2021. The seeding main effect at Highway 2 was the only significant treatment effect in 2021.

Treatment Effects on Species Rishness – 2021

	Highway 2			Highway 77		
Treatment	**DF**	**F-value**	**P-value**	**DF**	**F-Value**	**P-Value**
Pre-mow	1	0.578	0.454	1	0.005	0.945
Seeding	1	25.195	0.000**	1	1.940	0.176
Year-after Mow	1	0.928	0.345	1	0.393	0.537
Pre-mow:Seeding	1	0.126	0.726	1	0.310	0.583
Premow:Year-after Mow	1	1.874	0.184	1	0.238	0.630
Seeding:Year-after Mow	1	0.023	0.880	1	1.242	0.276
Pre-mow:Seeding:Year-after Mow	1	0.311	0.582	1	0.078	0.783

Statistically significant effects are shown with **
Effects that are trending towards significance are shown with *

Table 2.6A. Results from a three-way ANOVA comparing species richness under different seeding and mowing treatment combinations and main effects in 2022. The seeding main effect at Highway 2 was the only significant treatment effect in 2022.

Treatment Effects on Species Richness – 2022

	Highway 2			Highway 77		
Treatment	**DF**	**F-value**	**P-value**	**DF**	**F-Value**	**P-Value**
Pre-mow	1	2.142	0.156	1	0.731	0.401
Seeding	1	19.282	0.000**	1	3.081	0.092*
Year-after Mow	1	0.095	0.761	1	0.245	0.625
Pre-mow:Seeding	1	0.095	0.761	1	0.585	0.452
Premow:Year-after Mow	1	0.053	0.819	1	0.051	0.824
Seeding:Year-after Mow	1	0.006	0.939	1	3.081	0.092*
Pre-mow:Seeding:Year-after Mow	1	0.000	1.000	1	3.081	0.092*

Statistically significant effects are shown with **
Effects that are trending towards significance are shown with *

Table 2.7A. Results from a three-way ANOVA comparing wildflower frequency of seeded species under different seeding and mowing treatment combinations and main effects at both sites combined. The seeding main effect was the only significant treatment effect in 2021 and 2022.

Treatment Effects on Seeded Species Frequency at Both Sites

	2021			2022		
Treatment	**DF**	**F-value**	**P-value**	**DF**	**F-Value**	**P-Value**
Pre-mow	1	1.976	0.160	1	1.490	0.222
Seeding	1	11.882	0.001**	1	21.373	0.000**
Year-after Mow	1	1.540	0.251	1	1.222	0.269
Pre-mow:Seeding	1	1.686	0.194	1	0.089	0.766
Premow:Year-after Mow	1	0.248	0.618	1	1.180	0.278
Seeding:Year-after Mow	1	1.918	0.166	1	1.398	0.237
Pre-mow:Seeding:Year-after Mow	1	0.096	0.756	1	0.126	0.722

Statistically significant effects are shown with **
Effects that are trending towards significance are shown with *

Table 2.8A. Results from a three-way ANOVA comparing frequency of Maximilian sunflower under different seeding and mowing treatment combinations and main effects at both sites combined. Year-after mowing significantly reduced the number of Maximilian sunflower occurrences in 2022.

Treatment Effects on Maximilian Sunflower Frequency at Both Sites

	2021			2022		
Treatment	**DF**	**F-value**	**P-value**	**DF**	**F-Value**	**P-Value**
Pre-mow	1	0.310	0.580	1	0.108	0.743
Seeding	1	0.310	0.580	1	1.405	0.241
Year-after Mow	1	0.978	0.327	1	4.168	0.046**
Pre-mow:Seeding	1	0.310	0.580	1	0.625	0.433
Premow:Year-after Mow	1	0.000	1.000	1	0.004	0.948
Seeding:Year-after Mow	1	0.749	0.390	1	0.850	0.361
Pre-mow:Seeding:Year-after Mow	1	1.850	0.179	1	2.099	0.153

Statistically significant effects are shown with **
Effects that are trending towards significance are shown with *

Table 2.9A. Results from a three-way ANOVA comparing frequency of showy partridge pea under different seeding and mowing treatment combinations and main effects at both sites combined. No effects were observed from treatments.

Treatment Effects on Showy Partridge Pea Frequency at Both Sites

	2021			2022		
Treatment	**DF**	**F-value**	**P-value**	**DF**	**F-Value**	**P-Value**
Pre-mow	1	0.151	0.699	1	0.226	0.636
Seeding	1	1.073	0.305	1	0.835	0.365
Year-after Mow	1	1.073	0.305	1	0.162	0.689
Pre-mow:Seeding	1	0.419	0.520	1	2.471	0.122
Premow:Year-after Mow	1	0.419	0.520	1	0.012	0.913
Seeding:Year-after Mow	1	1.073	0.305	1	0.835	0.365
Pre-mow:Seeding:Year-after Mow	1	0.017	0.897	1	0.108	0.743

Statistically significant effects are shown with **
Effects that are trending towards significance are shown with *

Table 2.10A. Results from a three-way ANOVA comparing frequency of common milkweed under different seeding and mowing treatment combinations and main effects at both sites combined. Common milkweed abundance was significantly higher on seeded plots than non-seeded plots in 2021 and 2022.

Treatment Effects on Common Milkweed Frequency at Both Sites

	2021			2022		
Treatment	**DF**	**F-value**	**P-value**	**DF**	**F-Value**	**P-Value**
Pre-mow	1	0.764	0.386	1	0.322	0.573
Seeding	1	19.105	0.000**	1	8.871	0.004**
Year-after Mow	1	2.476	0.121	1	0.322	0.573
Pre-mow:Seeding	1	1.498	0.226	1	0.322	0.573
Premow:Year-after Mow	1	2.476	0.121	1	1.629	0.207
Seeding:Year-after Mow	1	1.498	0.226	1	0.724	0.398
Pre-mow:Seeding:Year-after Mow	1	1.498	0.226	1	0.020	0.888

Statistically significant effects are shown with **
Effects that are trending towards significance are shown with *

Table 2.11A. Results from a three-way ANOVA comparing floristic quality index under different seeding and mowing treatment combinations and main effects in 2021. The seeding main effect at Highway 2 showed that seeded plots had significantly higher floristic quality than non-seeded plots. The pre-mowing main effect was trending towards significance. There was also significant interaction between seeding and pre-mowing at Highway 2.

Treatment Effects on Floristic Quality – 2021

	Highway 2			Highway 77		
Treatment	**DF**	**F-value**	**P-value**	**DF**	**F-Value**	**P-Value**
Pre-mow	1	3.243	0.084*	1	0.644	0.430
Seeding	1	46.509	0.000**	1	2.780	0.108
Year-after Mow	1	1.829	0.189	1	0.092	0.765
Pre-mow:Seeding	1	5.113	0.033**	1	0.110	0.743
Premow:Year-after Mow	1	0.242	0.627	1	0.027	0.871
Seeding:Year-after Mow	1	2.405	0.134	1	1.371	0.253
Pre-mow:Seeding:Year-after Mow	1	0.736	0.399	1	0.083	0.775

Statistically significant effects are shown with **
Effects that are trending towards significance are shown with *

Table 2.12A. Results from a three-way ANOVA comparing floristic quality index under different seeding and mowing treatment combinations and main effects in 2022. The seeding main effect at Highway 2 was significant, and the Pre-mowing main effect was approaching significance. At Highway 77, the seeding main effect and the interactive effect between seeding and year-after mowing were approaching significance.

Treatment Effects on Floristic Quality – 2022

	Highway 2			Highway 77		
Treatment	**DF**	**F-value**	**P-value**	**DF**	**F-Value**	**P-Value**
Pre-mow	1	4.003	0.057*	1	0.731	0.874
Seeding	1	35.587	0.000**	1	3.081	0.079*
Year-after Mow	1	0.198	0.661	1	0.245	0.834
Pre-mow:Seeding	1	0.000	0.995	1	0.585	0.741
Premow:Year-after Mow	1	0.413	0.527	1	0.051	0.807
Seeding:Year-after Mow	1	0.074	0.788	1	3.081	0.082*
Pre-mow:Seeding:Year-after Mow	1	0.237	0.631	1	3.081	0.215

Statistically significant effects are shown with **
Effects that are trending towards significance are shown with *

Table 2.13A. Results from a one-way ANOVA comparing the effects of pre-mowing on grass cover in June of 2021. No significant effects were observed.

Pre-mowing Effects on Grass Cover – June 2021

	Highway 2			Highway 77		
Treatment	**DF**	**F-value**	**P-value**	**DF**	**F-Value**	**P-Value**
Pre-mow	1	0.589	0.449	1	1.223	0.278

Statistically significant effects are shown with **
Effects that are trending towards significance are shown with *

Table 2.14A. Results from a two-way ANOVA comparing the treatment effects of pre-mowing and year-after mowing on grass cover in 2021. The pre-mow main effect was approaching significance at Highway 2, while the year-after mowing treatment was significant at Highway 77.

Mowing Treatment Effects on Grass Cover – 2021

	Highway 2			Highway 77		
Treatment	**DF**	**F-value**	**P-value**	**DF**	**F-Value**	**P-Value**
Pre-mow	1	3.499	0.072*	1	2.811	0.105
Year-after Mow	1	2.330	0.138	1	12.270	0.002**
Premow:Year-after Mow	1	0.900	0.351	1	0.192	0.665

Statistically significant effects are shown with **
Effects that are trending towards significance are shown with *

Table 2.15A. Results from a two-way ANOVA comparing the treatment effects of pre-mowing and year-after mowing on grass cover in 2022. At Highway 2, there was interaction between pre-mowing and year-after mowing that was trending towards significance.

Mowing Treatment Effects on Grass Cover – 2022

	Highway 2			Highway 77		
Treatment	**DF**	**F-value**	**P-value**	**DF**	**F-Value**	**P-Value**
Pre-mow	1	0.141	0.710	1	0.097	0.757
Year-after Mow	1	0.387	0.539	1	0.745	0.395
Premow:Year-after Mow	1	3.958	0.057*	1	0.928	0.344

Statistically significant effects are shown with **
Effects that are trending towards significance are shown with *

Table 2.16A. Results from a one-way ANOVA comparing the effects of pre-mowing on vegetative litter cover in 2021 for both sites combined. The pre-mow treatment significantly reduced litter cover in plots at both sites.

Pre-mowing Effects on Vegetative Litter Cover – 2021

Treatment	**DF**	**F-value**	**P-value**
Pre-mow	1	31.400	0.000**

Statistically significant effects are shown with **
Effects that are trending towards significance are shown with *

Table 2.17A. Results from a two-way ANOVA comparing the treatment effects of pre-mowing and year-after mowing on vegetative litter cover in 2022. The pre-mow main effect significantly reduced litter depth at both sites. The year-after main effect significantly reduced litter at Highway 2.

Mowing Treatment Effects on Vegetative Litter Cover – 2022

	Highway 2			Highway 77		
Treatment	**DF**	**F-value**	**P-value**	**DF**	**F-Value**	**P-Value**
Pre-mow	1	7.112	0.013**	1	7.910	0.009**
Year-after Mow	1	24.200	0.000**	1	2.101	0.158
Premow:Year-after Mow	1	0.431	0.517	1	0.068	0.797

Statistically significant effects are shown with **
Effects that are trending towards significance are shown with *

Table 2.18A. Results of the Pearson's Moment Correlation Tests. Litter depth and forb cover were compared in 2021 and 2022. Soil bulk density was also compared to forb cover for 2021 and 2022. The correlation coefficient is represented by '**r**' in the table. There was a significant negative correlation between litter depth and forb cover in 2021.

Pearson's Moment Correlation Tests for 2021 and 2022

	2021			2022		
Sampling Year	**DF**	**r**	**P-value**	**DF**	**r**	**P-Value**
Litter Depth: Forb Cover	62	-0.366	0.003**	62	0.034	0.790
Soil Bulk Density: Forb Cover	62	-0.052	0.683	62	-0.046	0.720

Statistically significant effects are shown with **
Effects that are trending towards significance are shown with *