Establishment of Wildflower Islands to Enhance Roadside Health and Aesthetics

Project MO-58

Final Report

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By

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Wildflowers are crucial in the ecological function of the low-input roadside plant communities in terms of water and nutrient cycling, nutrient inputs such as nitrogen, total plant canopy cover, stand longevity, and provision of habitat for numerous small animals. Further, wildflowers provide critical foraging and nesting resources for birds, insects, and other wildlife. Unfortunately, habitat loss from agricultural and urban development has led to rapid population declines in wild bees and other pollinators across the US, thereby jeopardizing not only food production but also the sustainability of our natural landscapes (Kearns & Inouye, 1997). One way to mitigate wild bee decline is to establish more habitat corridors on public rights-of-way, such as roadsides. Planting pollinator-friendly native wildflowers on roadsides provides nutrient-rich forage and nesting resources for bees and is aesthetically pleasing. With 97,256 miles of public roadways in Nebraska (~4 million miles of roadways in the United States), roadsides play ever increasing roles in sustaining biodiversity within our state and beyond.
Federal guidelines state that wildflowers are to be used in roadside seeding mixtures, and NDOT includes a diversity of wildflower species in its seeding mixtures. However, these complex seeding mixtures are often expensive because of the diversity of species and high seed price of many of these native species, particularly the wildflowers which compose roughly 10% of the total seeds but represent 30% of the total cost of seed mixtures. Further, wildflowers on roadsides are typically seeded with competitive grasses and are costly to establish and manage long term. This research explored ways to improve wildflower establishment by separating wildflower seeds from the conventional seed mixture with includes both wildflower and grass seeds. Additionally, wildflower plots were seeded at different patch or island sizes to assess cost-effective ways of reducing competition by nonnative weeds and enhancing the longevity of roadside habitat. Optimal patch sizes and treatment groups included 100% wildflower mix seeded to the entire 3 m x 18.3 m plot (treatment 100), only 50% of the plot seeded in one continuous patch (treatment 50) or in two small patches (treatment 25x2) compared to current practices of seeding wildflower-grass mixtures (treatment conventional). Ecological benefits of roadside habitat, wild bee abundance, diversity, and nesting activity was assessed and compared across seeding practices and patch size treatments. Floral diversity and abundance were also analyzed to compare plant-pollinator interactions across treatments.
Conventional roadside seeding methods yielded plots with lower abundance and richness of forbs and bees compared to plots seeded with wildflowers only (treatments 100, 50, 25x2) but only in the first year of establishment. Bee richness was highest in the late season, while forb abundance and richness were highest in the mid-season. No differences were observed across differently sized wildflower-only patches likely because of the recent establishment of plots. In fact, only ~50% of seeded forbs had established and roughly 14 plants out of the 40 species in the seed mixture did not establish in either survey years and may therefore be replaced in future seed mixtures. Our results indicate that wildflower segregation in strips or islands may be a cost-effective method of improving wildflower establishment and persistence in diverse roadside mixtures. As plots mature and become vulnerable to weed encroachment, the effect of patch size may become more distinguished across treatment groups, therefore, further monitoring and research may be necessary to further address issues with low establishment and high competitive pressure from volunteer species. This data contributes to NDOT's ongoing pursuit to more effectively establish wildflowers on roadsides and to better understand the role floral enhancements have on supporting and sustaining vulnerable wildlife, such as our pollinator communities.</td></tr>
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Introduction

In the Midwest, agricultural and urban expansion has converted grassland ecosystems into a mosaic of crop fields, impervious surfaces, and fragmented natural lands. Bees provide critical pollination services to many crops and natural flora that support diverse native wildlife, however, pollinator decline has been on the rise, particularly in the Midwest, and is a major concern to Nebraskans. Pollinating bees have co-adapted with complex plant communities that make up tall-, short-, and mixed-grass prairie ecoregions. However, agricultural and urban encroachment can fragment remnant prairies and natural landscapes and disrupt plant-pollinator networks (Tscharntke et al., 2005). Thus, underutilized lands such as crop field margins, right of ways, and roadsides play ever increasing roles in sustaining biodiversity in these areas. Marginal lands, such as roadsides, have the potential to connect fragmented landscapes and act as habitat corridors that connect isolated plant and pollinator communities particularly those surrounded by large crop fields (Krewenka et al., 2011). Over 4 million miles (6.5 million kilometers) of roadway in the United States (97,256 miles of public roadways in Nebraska) can provide and an estimated 9.6 million acres (3.9 million hectares) of potential pollinator habitat (Wojcik & Buchmann, 2012) or serve as critical corridor habitat or refugia (partial habitat) for bee species that establish near agricultural field margins, prairie woodlands, or urban settings (Hopwood et al., 2015).

Suitability of roadside habitat for pollinators depends broadly on vegetation composition, abundance and establishment, physical soil structure, and adjacent landscapes (Hopwood et al., 2015). Roadside habitats with abundant and diverse flowering plants throughout the season provide critical foraging resources (nectar, pollen), nest sites, materials for nest construction, and protection from chemicals (Tarpy, 2003; Oldroyd & Fewell, 2007; Whitehorn et al., 2011). Bees use a variety of plant materials including flower petals, resins, fibers, and wood to construct their brood chambers within their nests (Michener, 2007). Understanding these diverse nesting and foraging requirements of bees is a critical component of establishing pollinator habitat on roadsides.

According to federal guidance, wildflowers are to be used in roadside seeding mixtures. Studies show roadsides restored to native vegetation can promote and support wild bee communities better than those which are left weedy and dominated by nonnative plants (Hopwood, 2008). Therefore, sufficient wildflower establishment is necessary to maintain the longevity and function of pollinator habitat on roadsides. Further, wildflowers compose roughly 10% of the total seeds in the seeding mixtures used by NDOT. Despite the low percentage of wildflowers in the seeding mixtures, wildflower seeds represent 30% of the total cost of seed. The expense of wildflower seeds represents challenges for widespread adoption of seeding wildflowers throughout all our roadways. Additionally, a previous study completed by the University of Nebraska-Lincoln (UNL) in collaboration with NDOT (Project M329) has shown that wildflowers compose less than 10% of the botanical composition of roadsides 10 years following seeding, suggesting either poor establishment of wildflowers and or high pressure from competitive grasses and "weedy" plants (Soper et al., 2018). One way to mitigate competition and promote better floral establishment is to plant isolated wildflower patches that are bordered by native grasses. Grasses will likely encroach into flower patches naturally, but wildflower mixtures without the incorporation of grass seeds will have a better chance at establishment and persistence because of the reduction in competition from grasses and weeds.

Project objectives

This project examined whether the segregation of wildflower seeds from grasses would improve wildflower establishment. Further, we planted differently-sized wildflower patches to assess encroachment by competitive grasses and weeds and examine how density of wildflower seeding may impact the longevity of wildflower patches when compared to conventional seeding practices. Wildflower patch treatments included: 1) the entire blackslope seeded to the NDOT mixture which consists of a mix of wildflowers and grasses (conventional seeding); 2) 50% of blackslope seeded as described in treatment 1 and 50% of area seeded in two strips (or small patches (25%x2)) to a pollinator mixture of wildflowers; 3) 50% of backslope seeded as described in treatment 1 and 50% of area seeded in a single strip (or medium-sized patch) to a pollinator mixture of wildflowers; and 4) the entire backslope seeded to a pollinator mixture of wildflowers or 100% of the area (large patch) (Figures 1 and 2). Lastly, to assess functional diversity, we compared the diversity and abundance of blooming flowers or forbs and the foraging bees utilizing them as well as nesting activity by bees among the differently-sized wildflower patches.

Our results indicate segregating wildflowers from grasses in the seeding of roadside habitats does improve wildflower establishment and promote abundance and richness of forbs in all wildflower treatments (100, 50, 25x2) compared to the conventional seeding practice. This could be partially explained by the higher number of wildflower species in the wildflower only mix compared to the number of wildflower species in the conventional mix, however, roughly ~50% of seeded forbs in the wildflower only mix had established during the first two years. Bee richness was highest in the late season, while forb abundance and richness were highest in the mid-season, however, no differences in forb and bee measures were observed across differently sized wildflower-only patches (100, 50, and 25X2) likely because these plots and the newly seeded plants have not fully established and matured. As plots mature and become vulnerable to weed encroachment, the effect of patch size may become more distinguished across treatment groups.

Best management practices for establishing pollinator habitat on roadsides are still being discussed and adapted as we learn more about how wild pollinator communities react to different management techniques and planted seed mixtures (Hopwood et al., 2015). This research provided further insight into the role floral enhancements and patch size play in attracting bees on Nebraska roadsides and will help develop recommendations on how to better manage roadsides to support and sustain healthy wild bee communities.

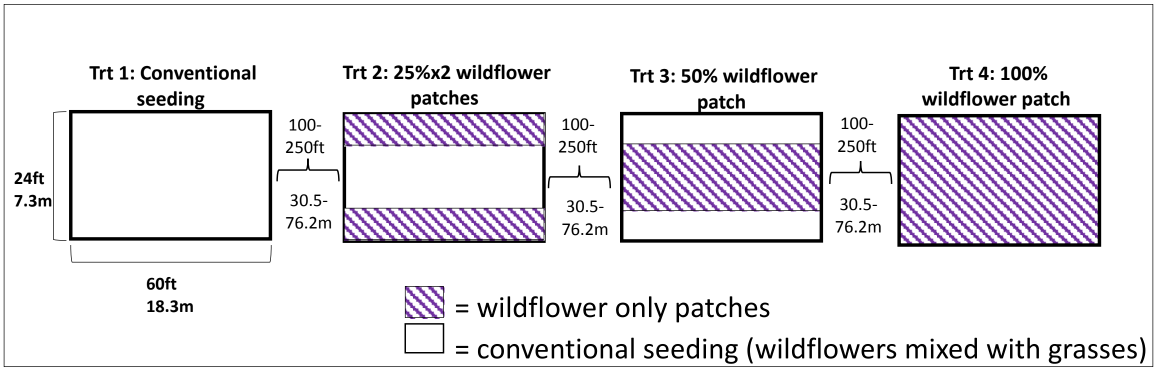
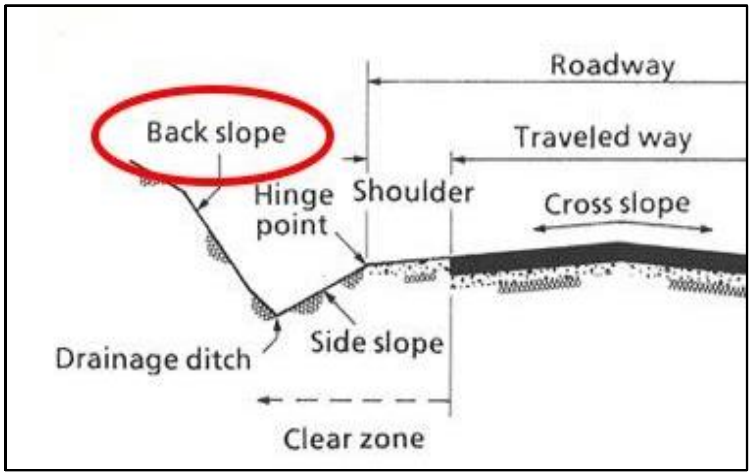


Figure 1. Experimental plot design in each replicated block of wildflower seeding treatments. Each plot represents a different treatment type: 1) NDOT conventional seeding mix of wildflowers mixed with grasses (conventional), 2) two small wildflower only patches that each make up 25% of the plot (25x2), 3) one medium wildflower only patch that covers 50% of the plot continuously (50), and 4) one large wildflower only patch covering entire plot (100).

Figure 2. Photos of experimental plots. Plots showing vegetation growth just after seeding (A); one month after seeding (B); and established plots (100% wildflower treatment) in mid-season of 2017 (C) and 2018 (D).

Figure 3. Diagram of a roadside (cross section).
Red circle depicts the location of a back slope were plots were seeded with wildflower treatments (US DOT, 2019).



Material and Methods

Study site description

In April 2017, the investigators collaborated with NDOT staff (Carol Wienhold) to identify a suitable study site. The study site selected is an 11 km stretch of Nebraska Highway 75 within Nebraska Department of Transportation (NDOT) Landscape Region B (Figure 4) between the village of Union, NE and Nebraska City, NE. The site occurs in southern Cass and northern Otoe counties. Nebraska Department of Transportation splits the state into six landscape regions to make appropriate seeding and landscaping decisions for each region. Landscape region B is comprised of flat to rolling plains with mostly silt loam soil with clay subsoil (NDOT, 2019) and is within USDA Plant Hardiness Zone 5 (USDA, 2012, NDOT, 2019). Native vegetation in this region is dominated by Tallgrass prairie species including grasses such as big bluestem (*Andropogon gerardii*), Indiangrass (*Sorghastrum nutans*), switchgrass (*Panicum virgatum*), and Canada wildrye (*Elymus canadensis*), and wildflowers such as maxmillian sunflower (*Helianthus maximiliani*), blackeyed susan (*Rudbeckia hirta)*, and upright prairie coneflower (*Ratibida columnifera*). Fragments of remnant prairies exist in this region along with woodlands which include a variety of trees, such as oaks, hickories, cottonwoods, and willows (dot.nebraska.gov, *no date*). The annual average temperature is 11.7 C with rainfall averaging 85.6 cm inches of rain and 68.58 cm of snow per year (www.usclimatedata.com). The hottest month is July at 30.5 C as the average high and 18.3 C as the average low. The coldest month is typically January with a 1.1 C average high and -10 C average low (www.usclimatedata.com).

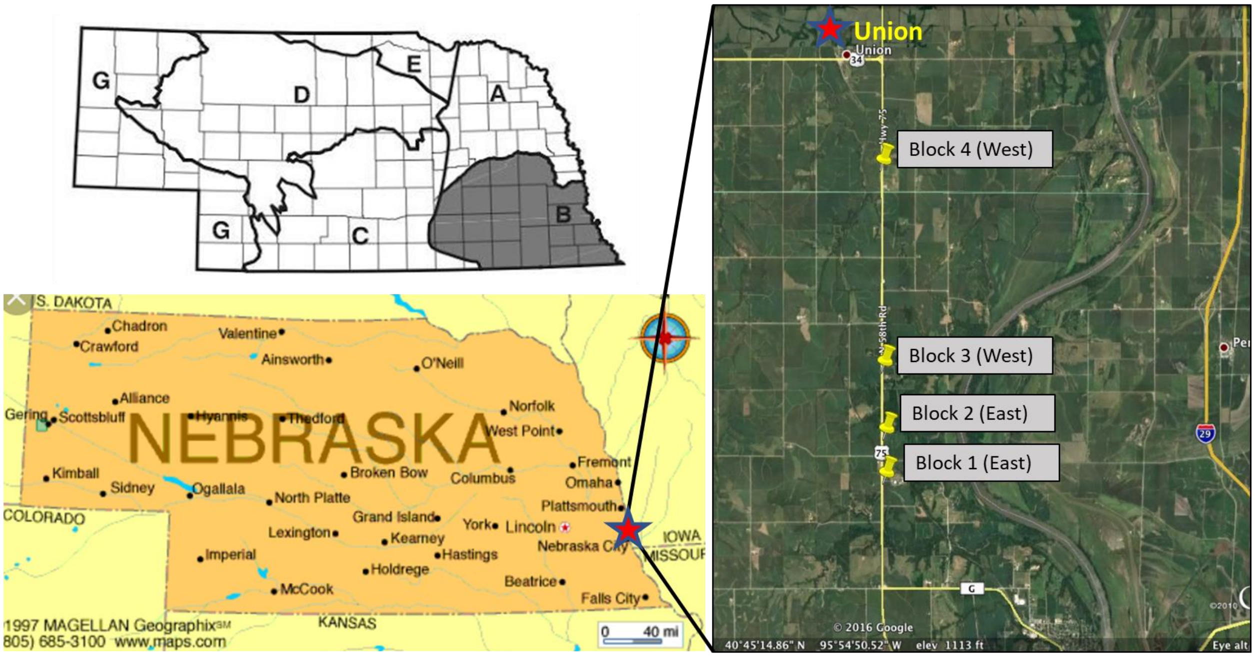


Figure 4. Map of Nebraska depicting NDOT landscape regions.
Project study sites were in region "B" (highlighted grey) (NDOT, 2019). Maps indicate location of replicated blocks on Highway 75 near Union and whether plots were on the West or East side of the road.

Experimental design

The experiment was designed as a randomized complete block with four plots in each replication. Plots were located on the backslope and approximately 24 x 60 feet (7.3 x 18.3 m) and separated from each other by 100-250 feet (30.5-76.2 m). Four replicated blocks were established for a total of 16 plots. Each plot within a block was randomly assigned a treatment as follows: 1) NDOT conventional seeding mix of wildflowers mixed with grasses (conventional), 2) two small wildflower only patches that each made up 25% of the plot (25x2), 3) one medium wildflower only patch that covered 50% of the plot continuously (50), and 4) one large wildflower only patch which covered the entire plot (100) (Figures 1-3).

Seeding of plots

A wheat cover crop was planted in fall of 2016 to prepare field sites. Plots with treatments 2-4 ("25x2", "50", "100") were planted on April 26, 2017 and biotic earth (Biotic Earth BlackTM) was applied to enrich the soil and encourage germination. The Nebraska Department of Transportation (NDOT) conventional seed mix of grasses and wildflowers was planted at the same time on the rest of the slopes, around all plots, and for the entirety of treatment 1 "conventional" plots. The NDOT "conventional" seeding mixture used for this project consisted of 11 grass species that range from 0.3- 4 lbs of seed/acre and 11 forb species that range from 0.05-1 lbs of seed/acre (Table 1). The wildflower seed mix used in treatments 2-4 ("25x2", "50", and "100") was selected by Jonathan Soper in collaboration with Nebraska Department of Transportation (NDOT) to include early through late season blooming forb species that range from 11.5 lbs of seed/acre (Table 2). Oats were planted in the spring and wheat in the fall at 14 lbs of seed/acre as cover crops providing soil stability.

Table 1. List of plants in the seed mixture used in "conventional" treatments.
Seed mixtures were provided by NDOT-approved seed distributors. Table includes plant type (grass/flower), time of season for blooming flowers, minimum purity rate as reported by seed companies, application rate of seeds using a mechanical drill, and the project years in which the plant was observed during vegetation surveys.

"Conventional" seed mixture	Plant type (Bloom time- *wildflowers only*)	Minimum Purity	Approved Mechanical Drill Application Rate in lb. of Pure Live Seed/Acre	Year(s) observed in transect surveys
Canada wildrye (*Elymus canadensis*)	grass	85	4	2018
Slender wheatgrass *(Elymus trachycaulus)*	grass	85	3	2017, 2018
Western wheatgrass *(Pascopyrum smithii)*	grass	85	3	2017, 2018
Switchgrass *(Panicum virgatum*)	grass	90	0.75	2017, 2018
Indiangrass *(Sorghastrum nutans)*	grass	75	2	2017, 2018
Little bluestem *(Schizachyrium scoparium)*	grass	60	2.5	2017, 2018
Big bluestem (*Andropogon gerardii*)	grass	60	2.5	2017, 2018
Sideoats grama (*Bouteloua curtipendula*)	grass	75	3	2017, 2018
Sand dropseed (*Sporobolus cryptandrus*)	grass	85	0.3	-
Prairie cordgrass (*Spartina pectinata*)	grass	85	0.5	-
Partridge pea (*Chamaecrista fasciculata)*	Flower/Mid-season	90	0.05	2017, 2018
Purple prairie clover (*Dalea purpurea*)	Flower/Mid-season	90	0.2	2017, 2018
Grayhead prairie coneflower (*Ratibida pinnata*)	Flower/Mid-season	90	0.25	2017, 2018
Butterfly milkweed (*Asclepias tuberosa*)	Flower/Mid-season	75	0.3	2017, 2018
Common milkweed (*Asclepias syriaca*)	Flower/Mid-season	75	0.2	2017, 2018

Mexican red hat (*Ratibida columnifera*)	Flower/Mid-season	90	0.25	2017, 2018
Pale purple coneflower (*Echinacea pallida*)	Flower/Mid-season	85	0.3	2017, 2018
Blue flax (*Linum lewisii*)	Flower/Mid-season	90	1	2017, 2018
Maximilian sunflower (*Helianthus maximiliani*)	Flower/Mid-season	85	0.25	2017, 2018
Spiked gayfeather/blazing star (*Liatris spicata*)	Flower/Mid-season	90	0.2	-
Plains coreopsis (*Coreopsis tinctoria*)	Flower/Mid-season	85	0.2	2017, 2018
Oats (Avena sativa)/ Wheat (Triticum spp.)	Cover crop	90	14	-

Table 2. List of plants in the seed mixture used in "wildflower" treatments.
Seed mixtures were provided by NDOT-approved seed distributors. Table includes the time of season for blooming flowers, minimum purity rate as reported by seed companies, application rate of seeds using a mechanical drill, and the project years in which the plant was observed during vegetation surveys.

Wildflower seed mixture	Bloom time (early, mid or late season)	Minimum Purity	Approved Mechanical Drill Application Rate in lb. of Pure Live Seed/Acre	Year(s) observed in transect surveys
Blackeyed Susan (*Rudbeckia hirta*)	Early	85	0.3	2017, 2018
Black samson (*Echinacea angustifolia*)	Mid	85	0.25	2018
Blanket flower (*Gailardia spp*.)	Early	85	1	2017, 2018
Blue flax (*Linum lewisii*)	Mid	85	1	2017, 2018
Blue vervain (*Verbena hastata*)	Mid	75	0.1	2017, 2018
Blue wild indigo (*Baptisia australis*)	Early	60	0.25	-
Butterfly milkweed (*Asclepias tuberosa*)	Mid	75	0.2	2017, 2018
Canada goldenrod (*Solidago canadensis*)	Mid	85	0.1	2017, 2018
Canada milkvetch (*Astragalus canadensis*)	Mid	75	0.1	2018
Canada tick clover (*Desmodium canadense*)	Mid	90	0.3	2017, 2018
Common milkweed (*Asclepias syriaca*)	Mid	75	0.3	2017, 2018
Compass plant (*Silphium laciniatum*)	Late	75	0.4	-
False sunflower (*Heliopsis helianthoides*)	Mid	75	0.1	2018
Golden alexander (*Zizia aurea*)	Early	75	0.2	2017

Grayhead coneflower (*Ratibida pinnata*)	Mid	85	0.1	2017, 2018
Heath aster (*Aster ericoides*)	Late	75	0.02	-
Illinois bundleflower (*Desmanthus illinoensis*)	Mid	90	0.3	2017, 2018
Leadplant (*Amorpha canescens*)	Mid	85	0.1	-
Maximilian sunflower (*Helianthus maximiliani*)	Late	85	0.25	2017, 2018
Mexican red hat (*Ratibida columnifera*, red)	Mid	85	0.75	2017, 2018
Missouri goldenrod (*Solidago missouriensis*)	Mid	75	0.1	-
New England aster (*Symphyotrichum novae-angliae*)	Late	85	0.2	2017
New Jersey tea (*Ceanothus americanus*)	Late	75	0.15	-
Pale purple coneflower (*Echinacea pallida*)	Mid	75	0.25	2017, 2018
Pitcher sage (*Salvia azurea*)	Late	75	0.3	-
Plains coreopsis (*Coreopsis tinctoria*)	Mid	85	0.1	2017, 2018
Prairie cinquefoil (*Drymocallis arguta*)	Mid	60	0.03	2018
Purple prairie clover (*Dalea purpurea*)	Mid	85	0.5	2017, 2018
Rattlesnake master (*Eryngium yuccifolium*)	Late	75	0.1	-
Rocky mountain bee plant (*Cleome serrulata*)	Mid	85	0.4	2017, 2018
Rough gayfeather (*Liatris aspera*)	Late	75	0.1	-
Roundhead lespedeza (*Lespedeza capitata*)	Late	75	0.1	2017, 2018
Shell-leaf penstemon (*Penstemon grandiflorus*)	Early	85	0.15	2018
Showy partridge pea (*Chamaecrista fasciculata*)	Mid	90	0.2	2017, 2018
Smooth blue aster (*Symphyotrichum laeve*)	Late	85	0.02	2018
Spiderwort (*Tradescantia bracteata*)	Late	75	0.25	-
Stiff goldenrod (*Solidago rigida*)	Late	75	0.1	-
Stiff sunflower (*Helianthus pauciflorus*)	Late	75	0.1	-
Thickspike blazing star (*Liatris pycnostachya*)	Late	85	0.15	-
Upright prairie coneflower (*Ratibida columnifera*)	Mid	85	0.5	2017, 2018
Western ironweed (*Vernonia baldwinii*)	Early	85	0.2	2017
Western yarrow (*Achillea millefolium*)	Early	75	0.2	2017, 2018
White false indigo (*Baptisia bracteata*)	Early	75	0.2	-
White prairie clover (*Dala candida*)	Mid	85	0.5	2017
Wild bergamot (*Monarda fistulosa*)	Mid	75	0.1	2018
Wild rose (*Rosa arkansana*)	Early	65	0.4	-
Oats *(Avena sativa)*/ Wheat (*Triticum spp.*)	Cover crop	90	10	2016

Site management

Guidelines from “NDOT Roadside Vegetation Establishment and Management” document includes a regime for roadside managers to completely mow the backslopes every 4 or 5 years (dot.nebraska.gov, n.d.). The document highlights the importance of mowing time on wildflower seed dispersal and supporting pollinating organisms and states that mowing of foreslopes, ditches, and backslopes should not occur from May 1st- October 1st of any given year. All research plots were managed by our research team. Selective cutting of weedy forbs that were 5 ft tall or taller on all plots and still flowering occurred in late August 2017 to help prevent the weedy species from producing mature seed. Additionally, in April 2018, each plot was mowed with the help of Jon Soper (NDOT) with a small mower to help open the canopy and aid in wildflower establishment.

Vegetation frequency of occurrence sampling

Frequency of occurrence surveys on all vegetation, including non-blooming forbs and grasses, were carried out two times through the growing season in 2017 (in June and September) and twice in 2018 (July and September) growing season. The frequency of occurrence of seeded species and volunteer species (not seeded) was estimated using a frequency rod. The rod, consisting of 22 five-centimeter segments, was randomly placed and sampled 15 times in each of the wildflower-only seeded and conventionally-seeded areas (Figure 5). At each sampling point, the number of segments containing forbs and grasses were counted and species identified. These surveys were carried out to determine how the establishment of wildflower islands impacted establishment of wildflowers and associated floral resources, and plant species composition and diversity of roadside grasslands. A 5% frequency of occurrence of an individual species was a minimum of one plant per linear meter (Jonathan Soper, personal communication). Volunteer forbs and grasses, not incorporated in seed mixtures, were not individually identified but were categorized as "weedy" forbs or grasses. In addition to the general vegetation occurrence assessment, forb surveys were conducted parallel to bee surveys to assess the abundance and richness of flowering plants in each plot.

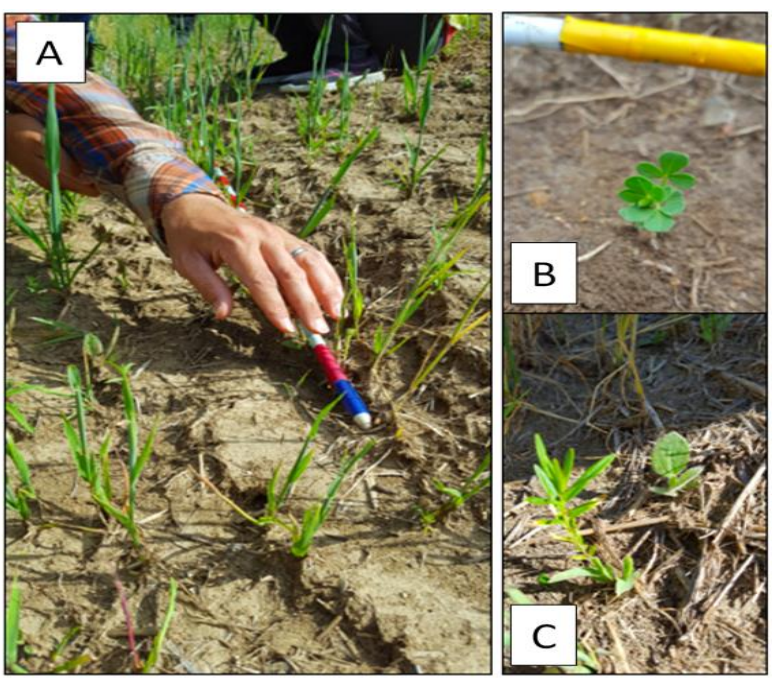


Figure 5. Photographs of vegetation sampling and example seedlings observed in plots. A sampling rod was placed on seeded rows and used to count vegetation frequency of occurrence (A); clover seedling (B); butterfly milkweed and black-eyed Susan seedlings (C).

Forb and bee surveys

To survey blooming forbs and the bees visiting them, transects were conducted at each plot every two weeks from May through October in 2017 and 2018. Four mini-transects (5 ft length x 3 ft width) randomly distributed vertically and across the length of the plot were used to survey foraging bees and identify their associated flowering plants (Figure 6). All blooming flowers along transects were quantified by counting the number of inflorescences, or cluster of flowers on one or many stems, to determine forb abundance. Forbs were also identified to their lowest taxonomic rank (genera or species) to compare plant richness across treatments. Species of the flowers on which bees were foraging were identified and recorded. We also attempted to collect all bees along transects during a 3-minute sampling period using a sweep net and collection vials. When a bee was caught, it was assigned a unique label that indicated which flower and plot it was associated with. If collection of a bee was not possible, then a visual observation was made complete with floral association when possible. When bees could not be identified to genus in the field, they were counted for abundance, while bees that were identified to genus were counted for bee richness. Bee abundance was measured by summing the total bees caught and visual counts of foraging bees per plot per collection. Bee richness was determined only using bees physically caught and identified to the species or genus level. Collected specimens were curated and identified to genus or species using several taxonomic keys: Bees of the Tall Grass Prairie (Arduser 2018 edition) and Discover Life (discoverlife.org). Identifications were verified by bee taxonomist Michael Arduser (Missouri Department of Conservation) and voucher specimens representing each genus will be retained at the University of Nebraska State Museum Entomology Collection for reference.

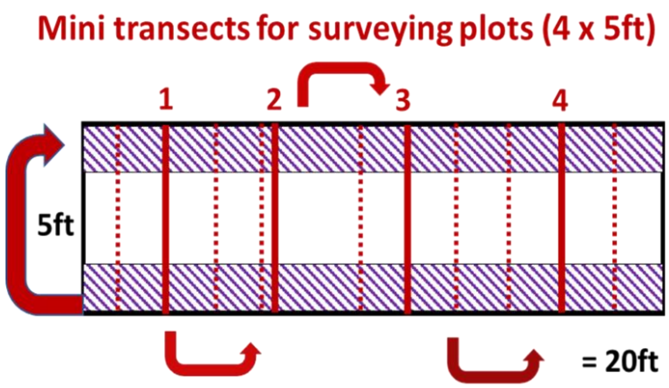


Figure 6. Diagram illustrating mini transects and a photograph of the main surveyor.
Kayla Mollet who led forb and bee surveys and data collection on this project received her Master's of Science degree from the University of Nebraska-Lincoln in August 2019.

Trap nests for bees

Bee nest trapping is one way of assessing habitat suitability for some wild bees. Nest traps attract bees that nest above ground and are made with empty tubes or pithy stems or by drilling holes of varying sizes (diameter: 2.4-12.7mm, depth: 2.7cm) into blocks of wood. Bundles of nesting materials (~ 15 hollow stems, 15 paper tubes, and 1 wood block with 60 holes) were provided in each plot to assess nesting capacity and establishment preference across treatments (Figure 6). Trap nests were installed in early spring and collected before onset of winter. The total number of utilized holes in blocks, tubes, and stems were counted and the nesting material was categorized as: 1) mud-sand composite, 2) cut leaf or flower petals, 3) plant resins, and 4) shredded straw, or 5) unknown substrate. Often bees can be identified through the type of nesting substrate used to secure brood chambers. For example, leaf-cutting bees (*Megachile* sp.) may use cut leaves and petals while other bees, such as mason bees (*Osmia sp.)* and some wasps utilize a mud-sand composite (Cane et al, 2007). Stems packed with shredded straw or grass indicates wasp nesting (Latter, 2012) and were counted as such. In Fall, occupied nests were placed in emergence cages separated by plot in an unheated storage unit in Lincoln, NE to over-winter. Insects emerging from the stems would be attracted to the light secured to one side of the emergence cage. Once insects move toward the light the cage prevents them from accessing the stems and allows for easy collection. Cages were monitored for emerging insects monthly through the winter and then weekly after March.

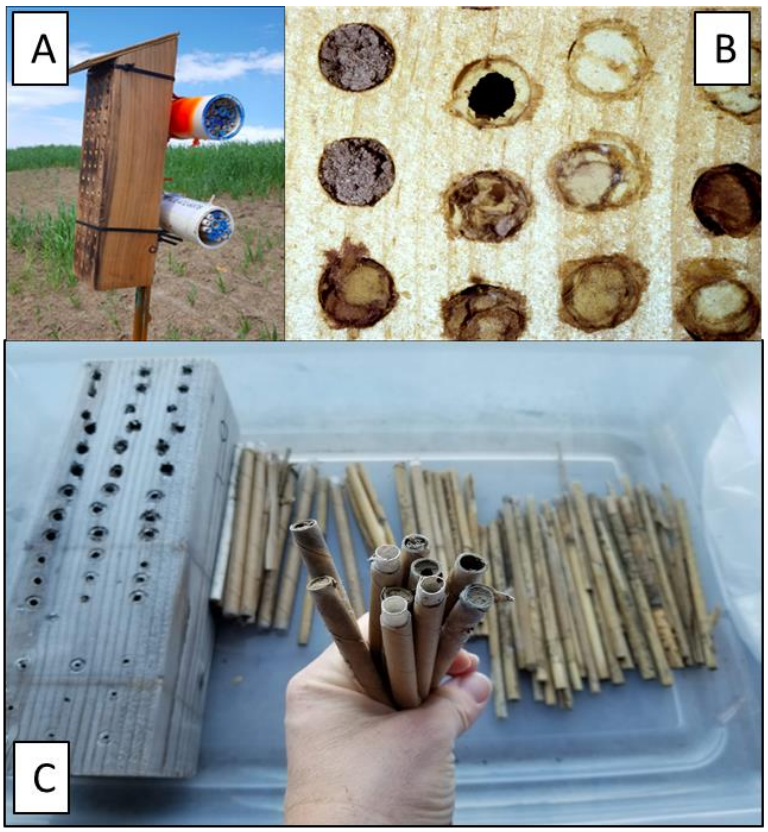


Figure 7. Photographs of trap nests. Trap nests consisted of stems or tubes and blocks containing ~60 holes of varying diameters. Trap nests were fastened to a post in the center of each plot, so it was elevated approximately 5ft (1.5 meters) from the ground nests (A). Hole entrances in tubes and blocks were examined for bee nesting activity. Image (B) shows holes covered by mud and cut leaves indicating the presence of mason bees (*Osmia sp.)* and leaf-cutting bees (*Megachile* sp.). Trap nests were collected in the Fall and placed in emergence cages (C).

Statistical Analysis

Vegetation frequency of occurrence data

Plant communities were measured for all flowering and non-flowering vegetation in each plot. Frequency of occurrence of forbs and grasses were assessed and compared across treatment groups, sampling period, and block using three measures: 1) total seeded forbs, 2) total volunteer forbs, and 3) total grasses. Additionally, forbs for 2017 and 2018 (pooled) were ranked to determine the top 10 most frequently detected forb species. An analysis of variance (ANOVA) was used to determine if planting treatment groups, sampling period, and block significantly influenced the establishment of seeded and unseeded volunteer forbs and grasses. Post hoc means separation tests were used when statistical significance was determined at alpha=0.05. Statistical analyses were performed using SPSS (version 25.0, SPSS Incorporated, Chicago, IL).

Abundance and richness of forbs and bees

All plots were seeded in 2016, and data were collected from early June to mid-October in 2017 and mid-May to mid-October in 2018. Year 1 and 2 results of the project were significantly different given the time needed for seed germination and establishment of some biennial plants, therefore, 2017 and 2018 data were separately analyzed. Forb and bee data collected from mini-transects were pooled together by plot on each collection date. Abundance and richness data for plants and bees were compared among treatments (conventional, 25x2, 50 and 100), seasons [early (May and June), middle (July and August), and late (September and October) each with 3-4 collection dates], replicated blocks, and their interaction effects. Data not normally distributed were log or square-root transformed and statistically analyzed using Analysis of Variance (ANOVA) statistical models followed by post-hoc Tukey's Honest Significant Difference (HSD) means separation tests. Significance was determined at alpha = 0.05. Data analysis was completed using R statistical computing program (Version 1.1.463 – © 2009-2018 RStudio, Inc.).

Trap nest occupancy and emergence

To assess suitability of plots as bee habitat, trap nests were quantified for nest occupancy and compared among treatments and years. Emerged bees and wasps, or those individuals that overwintered and emerged within the emergence cages the following summer, were quantified and compared among treatments for 2017 only. Data were square root transformed for normality. Three trap occupancy response variables were used to determine statistical differences in trap nest occupancy across treatments and year using Analysis of Variance (ANOVA) statistical models followed by post-hoc Tukey's Honest Significant Difference (HSD) means separation tests. These response variables included total block occupancy (referring to holes that were utilized from wooden block nests), tube occupancy (referring to holes utilized from stems or tube nests including bamboo, paper, or phragmites), and total occupancy (sum of block and tube occupancy). Data analysis was completed using R statistical computing program (Version 1.1.463 – © 2009-2018 RStudio, Inc.).

Results

Vegetation frequency of occurrence

Establishment of vegetation in plots was measured by quantifying the frequency of occurrence scores for the surveyed plants over the collection periods and across treatments. Surveyed plants were placed in three categories. Plant frequency of all three plant categories (seeded forbs, volunteer forbs, and seeded or volunteer grasses) significantly increased over time from sampling collections 1 (June 2017), 2 (September 2017), and 3 (July 2018) indicating new growth and establishment over the two year period. Frequency of occurrence for seeded forbs differed among collection periods ($F_{2,213}$ =24.92, $p< 0.05$) but not treatment or replicated block. Frequency of volunteer forbs was affected by both collection period and block ($F_{6,332}$ =2.432, p= 0.03) likely due to unintended herbicide drift from adjacent crop fields into block 1 plots (Supplementary figure S2). As a result, the mean (±SE) occurrence of the volunteer forbs was significantly lower in collection period 1 and 2 and highest in period 3 ($F_{2,166}$=32.734, p<0.05) and pairwise comparisons indicate differences by collection period*block were only between blocks 1 vs 2 and 1 vs 4 in collection periods 2 and 3. The majority of the volunteer forbs (>70%, as listed in Supplementary Table S1), consisted of plants known to be pollinator friendly, 30% of which were plants that bees were caught on in this study. Forb occurrence data were ranked and the top 10 seeded forb species were then compared across treatments. Data indicate significant differences by collection date ($F_{1,139}$ =48.254 , p<0.05), but no statistical differences were observed across treatment ($F_{3,\ 139}$ =1.399, ns) indicating that the most frequently detected seeded forbs were distributed relatively evenly across treatments (Table 3).

Table 3. Lists of the most frequently observed blooming plants from the seeded wildflower mixture and the most visited flowers by bees in 2017 and 2018. Photographs are of the listed top 10 most abundant seeded wildflower surveyed in the plots.

Top 10 most abundant seeded wildflower	
1	Maximilian sunflower (*Helianthus maximiliani*)
2	Blanket flower (*Gailardia spp* .)
3	Partridge pea (*Chamaecrista fasciculata*)
4	Upright prairie coneflower (*Ratibida columnifera*)
5	Blackeyed Susan (*Rudbeckia hirta*)
6	Plains coreopsis (Coreopsis tinctoria)
7	Butterfly milkweed (*Asclepias tuberosa*)
8	Blue flax (*Linum lewisii*)
9	Purple prairie clover (*Dalea purpurea*)
10	Common milkweed (*Asclepias syriaca*)

Top 5 Bee-visited plants in 2017		
	Plant name	# Bees
1	Blanket flower (*Gailardia spp.*)	47
2	Partridge pea (*Chamaecrista fasciculata*)	19
3	Plains coreopsis (*Coreopsis tinctoria*)	13
4	Mexican Hat Coneflower (*Ratibida columnifera*)	12
5	Black-eyed Susan (*Rudbeckia hirta*)	7

Top 5 Bee-visited plants in 2018		
	Plant name	# Bees
1	Maxmillian sunflower (*Helianthus maximiliani*)	93
2	Annual sunflower (*Helianthus annuus*)	55
3	Blanket flower (*Gailardia spp.*)	53
4	Partridge pea (*Chamaecrista fasciculata*)	50
5	Birds-foot trefoil (*Lotus corniculatus*)	21

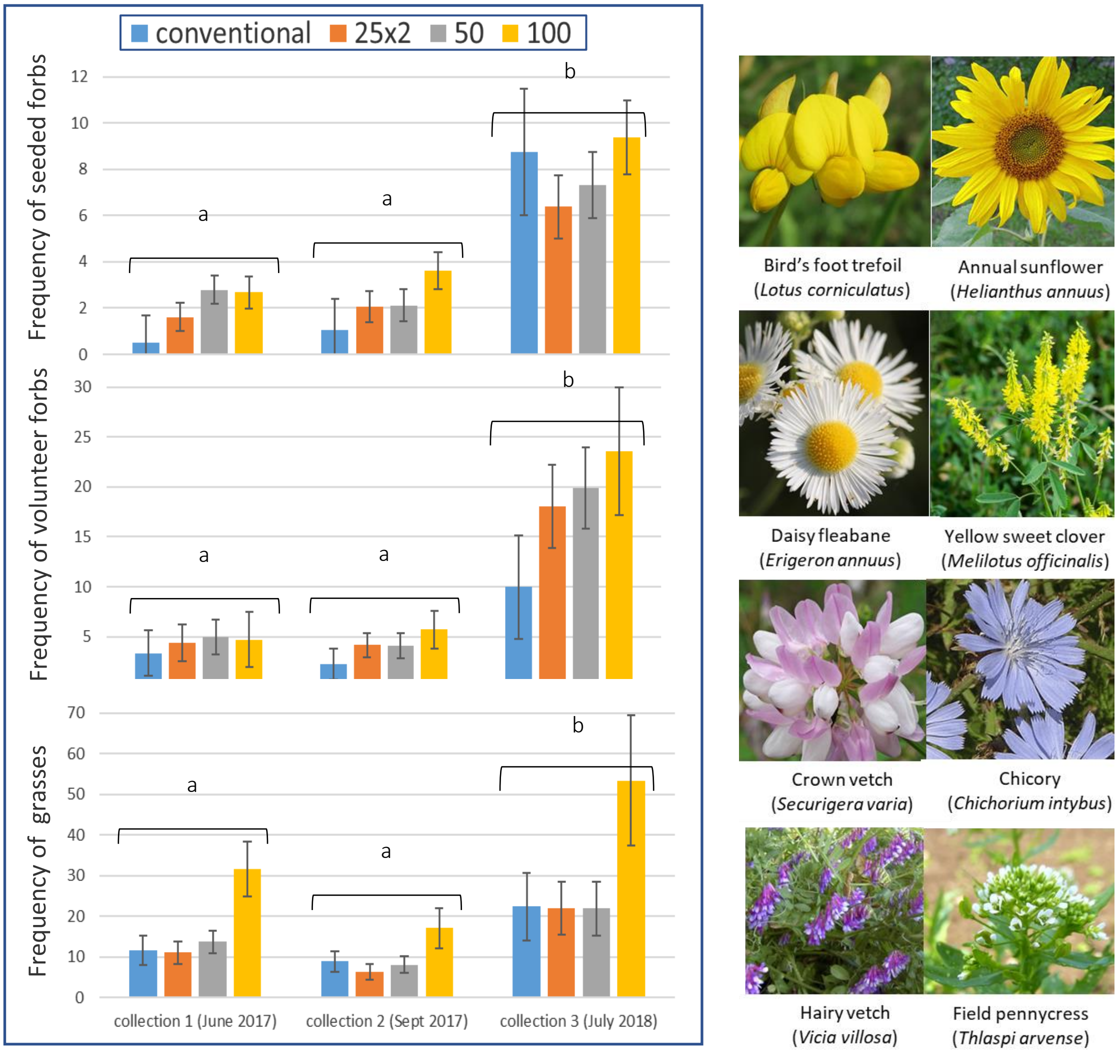


Figure 8. The frequency of occurrence of seeded forbs, volunteer forbs, and grasses. Figure shows data collected over 3 periods and grouped by treatment (conventional, 25x2, 50, 100). Different letters denote significant differences at alpha=0.05. Results indicates all vegetation became more established in study plots over time. Data indicate high pressure from competitive grasses and volunteer forbs. Photographs (right) show several volunteer forbs commonly found in 2017 and 2018.

Blooming forb abundance

A total of 60 blooming forbs were identified during bee and forb surveys over the two years. Thirty-three of these forbs (55%) were volunteer species, while the other 27 species (45%) originated from the wildflower seed mix. Of the 45 plant species that were in the wildflower only seed mix, 14 species (31%) were observed blooming in 2017 while 26 species (58%) were blooming by 2018 (Table 2, S1). Forb abundance was significantly higher in mid-season compared to other times of the season. Additionally, forb abundance in plots seeded with wildflowers in medium (50) and large (100) patches were significantly higher than in conventionally-seeded plots for 2017 data. However, there were no statistical differences observed in forb abundance across treatments in 2018 (Figure 9).

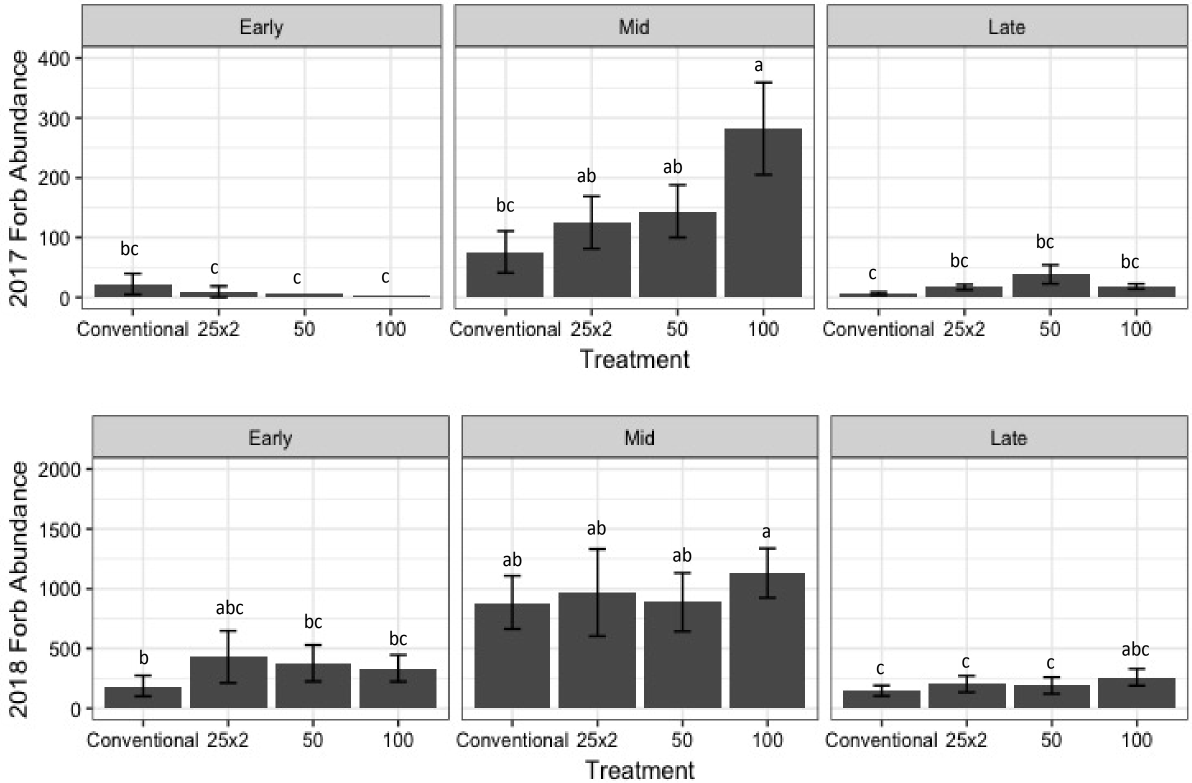


Figure 9. The average number of blooming forbs across treatment (conventional, 25x2, 50, 100) and by season (Early, Mid, Late) for each year (2017, 2018). Different letters denote significant differences at alpha<0.05. Results indicate significant differences in forb abundance between treatments ($F_{3,110}$=3.992, p=0.00967) and lower forb abundance was found in the conventional treatment when compared to the 50% (p=0.066) and 100% (p=0.0066) seeded treatment plots. Additionally, forb abundance was significantly higher in mid-season ($F_{2,110}$=18.58, p=1.12e-7) compared to early (p=2.48e-5) and late (p=6.7e-6) seasons.

Blooming forb richness

Forb richness was calculated by averaging the number of distinct species per collection per plot. In all plots, blooming forb richness was significantly higher in mid-season collections compared to early and late seasons in 2017 and 2018 (Figure 10). These results are likely due to a number of volunteer forbs establishing in the plots, many of which bloom mid-season. Despite high weed pressure in all plots, conventionally seeded plots yielded significantly lower forb diversity than compared to all other treatments in 2017, but not in 2018 as more seeded and volunteer forbs began to establish.

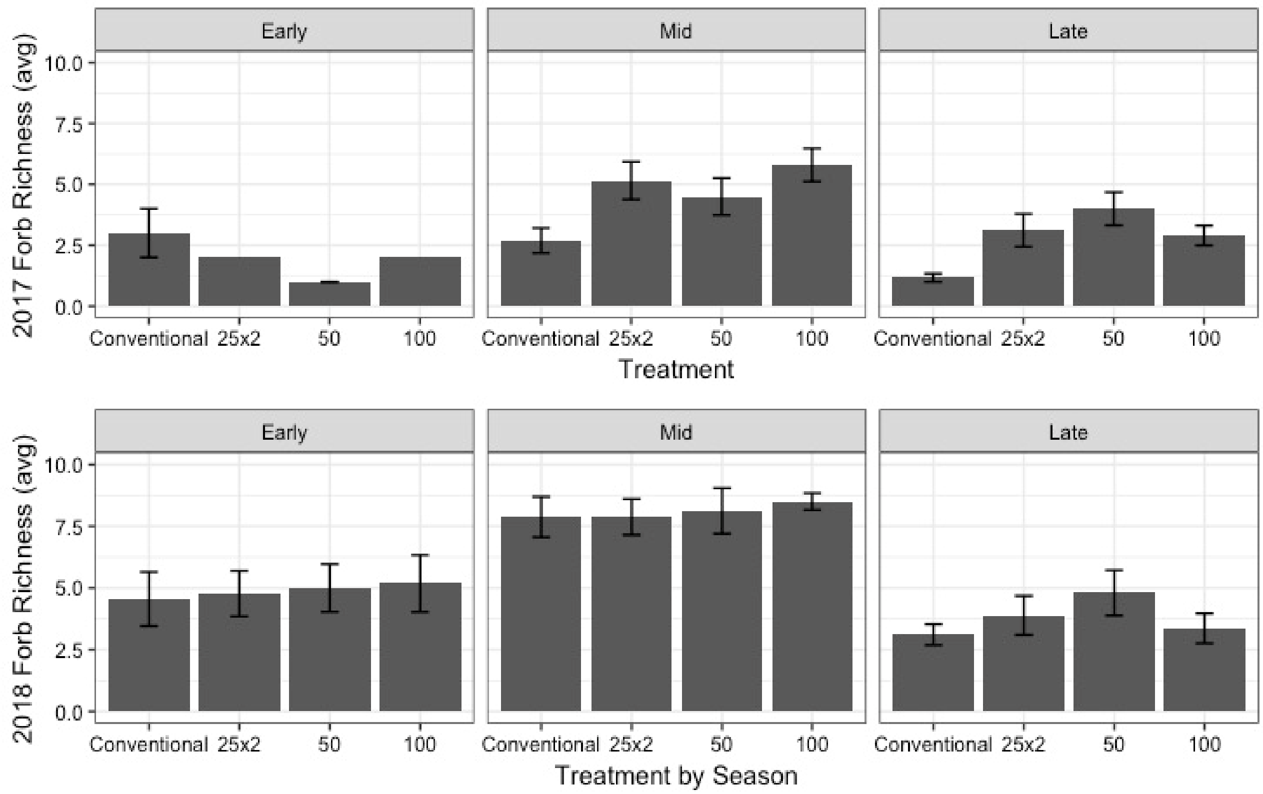
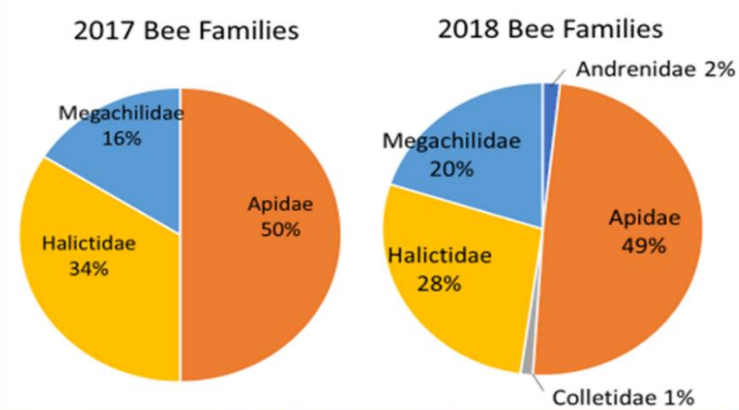


Figure 10. The average number of blooming forbs across treatment (conventional, 25x2, 50, 100) and by season (Early, Mid, Late) for each year (2017, 2018). Significantly more forb species were observed in mid-season collections compared to early and late seasons in 2017 and 2018 ($F_{2,89}$=5.884, p=0.000693 and $F_{2,139}$=41.595, p=6.95e-15, respectively). Statistically fewer forb species were found in conventional treatments compared to all other treatments ($F_{3,89}$=5.884, p=0.001) in 2017, but not in 2018 ($F_{3,139}$=0.439 ns).

Bee abundance and richness

Over two years (2017, 2018) a total of 510 bees across all research plots were identified to genus. In 2017, 106 bees were collected during bee surveys and in the following year there were roughly 4 times the number of bees collected from the previous year (a total of 404 bees in 2018). This data supports previous studies that show positive correlations between wildflower establishment in restored habitat and increased pollinator abundance and diversity. Visual observations made up 248 bees, while 265 bees were vial collected and curated. The bees surveyed through this project represent 25 different genera from 5 different bee families (Andrenidae, Apidae, Colletidae, Halictidae, and Megachilidae) (Figure 11, Table 3). Nine unique genera were found in 2017 and 28 bee genera were found in 2018. Foraging bees were observed utilizing 27 different species of flowering plants and the most visited were seeded wildflowers and not volunteer weeds, further highlighting the positive impact of establishing wildflowers on roadsides on local pollinator communities (Figure 11).

Abundance of bees in plots followed similar patterns as the forb abundance data. In 2017, bee abundance was lower in conventionally seeded plots compared to all other plots seeded with wildflowers, however, statistical differences were only observed within block 2. In 2018, there were significant differences between blocks 2 and 3 in late season collections compared to other seasons but there were no differences among treatments. Further, bee abundance was highest in mid- and late-seasons compared to early season collections in 2017 and 2018 (Figure 12). Richness of bees, as measured by the number of unique genera, showed no differences across treatments or blocks, however, bee richness was significantly greater in early and late season compared to mid-season collections, indicating the importance of providing floral resources during those times (Figure 12,13).



2017 & 2018 (combined)		
Family	# of Genera	# of Bees
Apidae	10	249
Halictidae	7	144
Andrenidae	3	10
Megachilidae	3	102
Colletidae	1	5

Figure 11. Profile of the bee community utilizing research plots. Diagrams illustrate the percent of bees (n=510) separated by family surveyed across all treatments for each year (2017, 2018), the number of genera represented within each bee family, the number of bees surveyed, and photographs of common bees within each family.

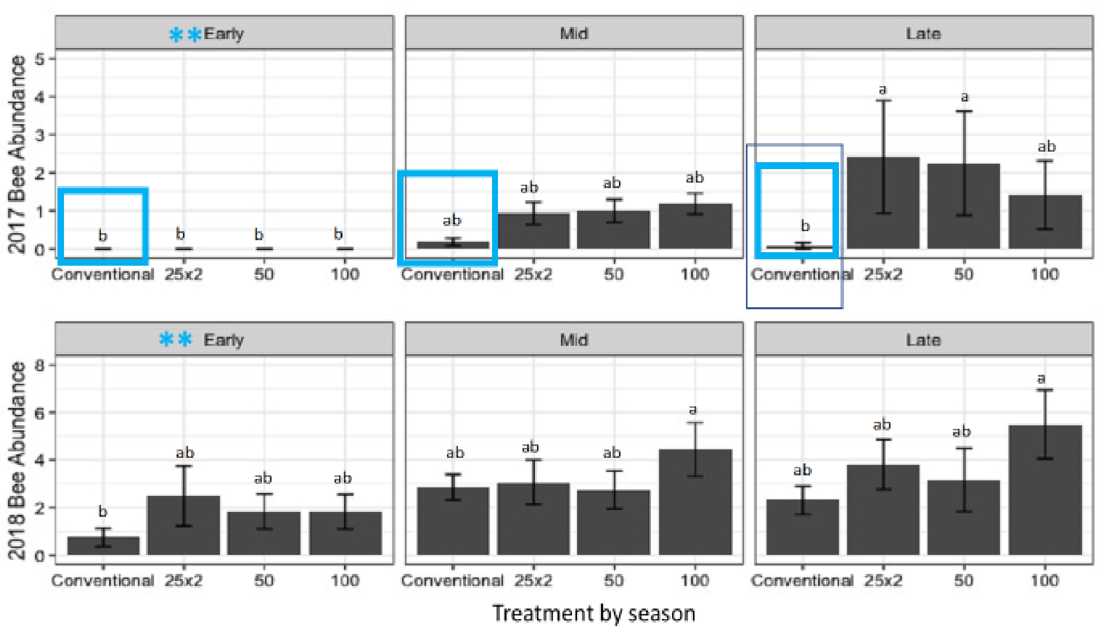


Figure 12. Abundance of bees surveyed in research plots. Graph illustrates the average number of bees across treatment (conventional, 25x2, 50, 100), by season (Early, Mid, Late), and for each year (2017 & 2018). Bee surveys from 2017 show lower abundance of bees in conventionally seeded plots ($F_{3,101}$=6.846, p=3.03e-4) than compared to all other wildflower treatments (25x2 ((1.4±3.3) p=0.00229); 50 ((1.4 ±3.1) p=0.00123); 100 ((1.2 ±2.1) p=0.00279) (denoted with blue boxes). There were no bees observed in the early season in 2017 and significantly fewer bees observed in early 2018 than compared to mid (p=0.0099) and late (p=0.0045) season collections (denoted with blue **). And while, in 2018, 100% wildflower seeded plots had generally higher bee abundance in mid- and late seasons there were no statistical differences observed across treatments ($F_{3,123}$=0.974, ns).

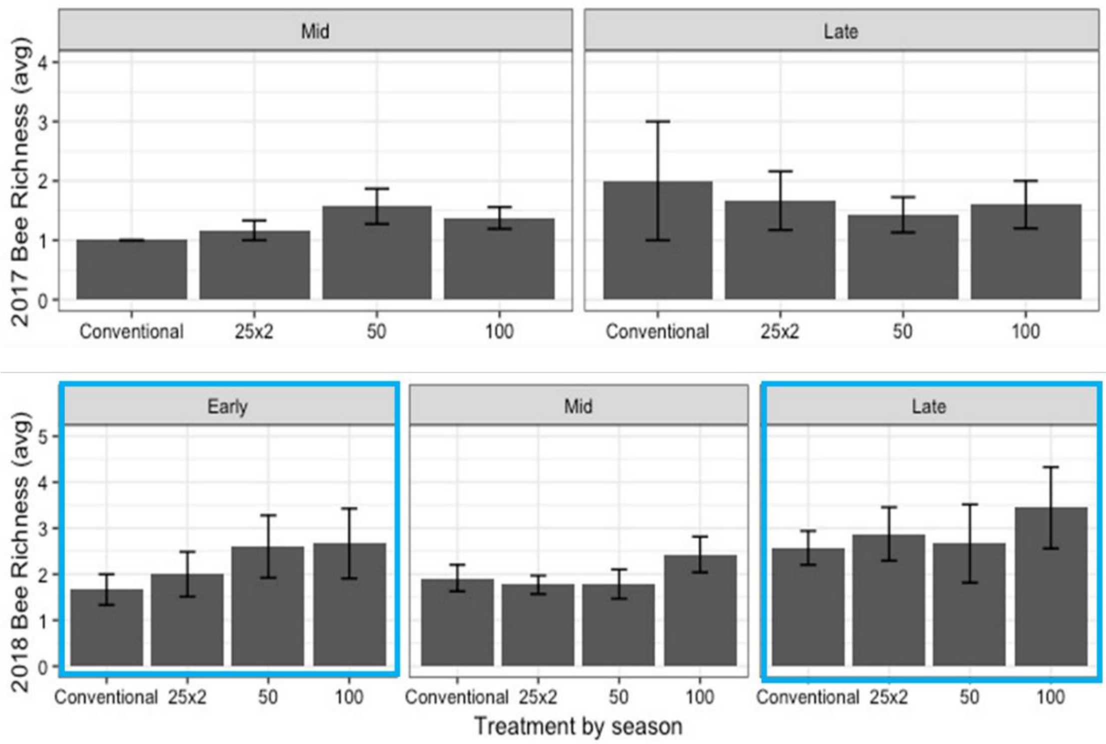


Figure 13. Richness of the bee community surveyed in research plots. Graph illustrates the average number of unique bee genera across treatment (conventional, 25x2, 50, 100), by season (Early, Mid, Late), and for each year (2017 & 2018). No bees were collected in early 2017 and no differences were observed in the number of bee genera across treatment or seasons. In 2018, no treatment ($F_{3,98}$=1.056, ns) effect was observed but there were significantly fewer bee genera observed in transects mid-season ($F_{2,98}$=3.147, p=0.0474) than compared to early and late season collections (denoted with blue boxes).

Trap nest occupancy and emergence

Fall collection of trap nests indicated no differences in the number of occupied nests across treatments or years. However, the use of varying substrates by insects to cover hole entrances of occupied tube or block nests was different indicating the types of insects nesting in traps was different across treatments. For example, conventional and 25x2 treatments had higher occupancy of nests with straw (19%) indicating activity by wasps; whereas, 100% wildflower treatment trap nests exhibited fewer wasps (3% straw) and more utilization by bees as indicated by the nest entrances covered with cut leaves (39%), mud (46%), and resin (11%) (Figure 14). Trap nest emergence data were recorded for one year only (2017). Three distinct genera (*Coelioxys, Heriades, Megachile)* of bees emerged in late June 2018 that had overwintered from 2017 field season. All bee genera belonged to the family Megachilidae which includes leaf-cutting, mason, and resin bees. Additionally, there was greater nesting activity by bees across treatments with 73, 86, 80, and 96% of occupied nests filled with either mud, cut leaf or resin in conventional, 25x2, 50, and 100 treatments, respectively. Nests from conventionally seeded plots had the most wasps emerge followed by nests collected from 50 and 100 wildflower treatments. Nests from 25x2 wildflower plots had the lowest emergence of bees compared to all other treatments while nests from 100 wildflower plots had the highest number of "field emerged" or preoccupied cells where bees had already developed and emerged in the field during the 2017 growing season indicating higher nesting utilization and bee establishment in these plots (Figure 14).

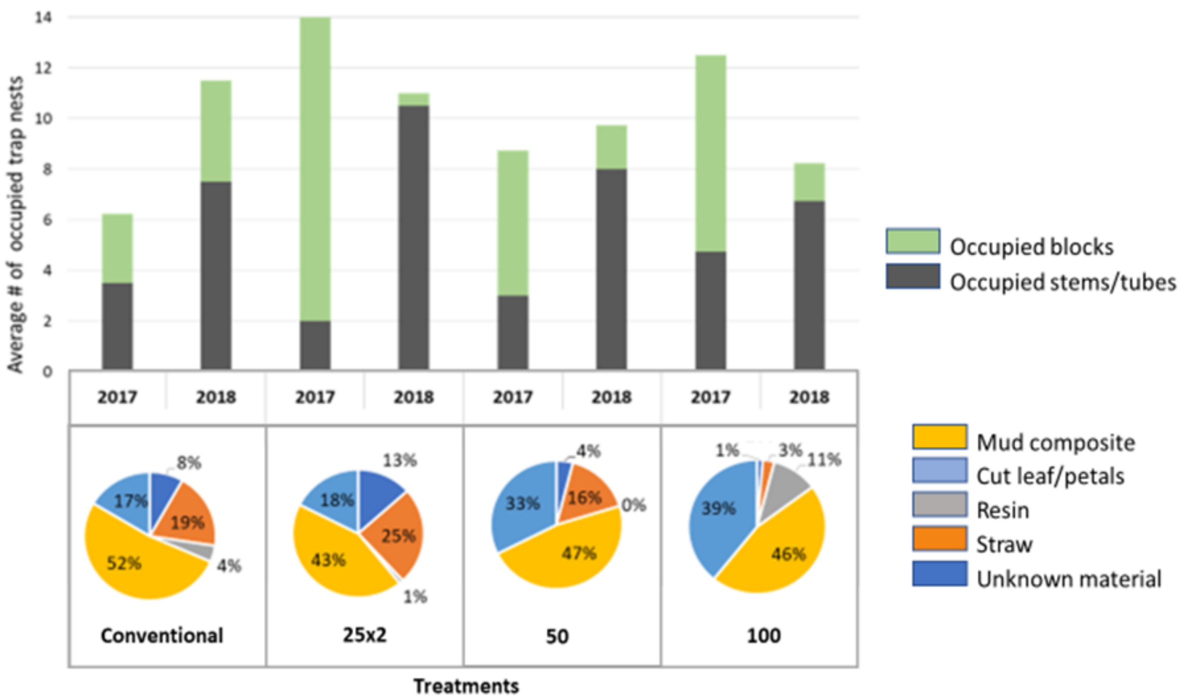


Figure 14. Bee nesting activity in research plots. Graph illustrates trap nest occupancy, or the average number of occupied blocks or tubes across treatment (conventional, 25x2, 50, 100) and year (2017, 2018). Occupancy is determined by whether nest entrances are covered which signifies the presence of brood. The substrates used to cover nest entrances, including mud, leaf, resin, and straw or grass, are often species-specific and can be used to identify nest occupants. Conventional and 25x2 treatments had higher more nests with straw (19%) indicating wasp activity; whereas, 100% wildflower treatment nests exhibited more nests with cut sections leaf or flower petals (39%) and resin (11%) indicating activity by leaf-cutting and resin or cellophane bees.

Summary and interpretation of findings

From this two-year project, our data suggests that the conventional seeding mixtures (wildflowers and grasses seeded together) are not as effective in promoting wildflower establishment than segregating wildflower seeds from grasses and seeding patches of dense wildflower stands. Other findings include:

- Vegetation occurrence and forb surveys indicate that plant communities were significantly more established and exhibited higher forb abundance and richness in

2018 compared to 2017. This was as expected because plots were seeded in the spring of 2017 and establishment of seeded native plants, especially perennials, is generally greater in the second year (The Xerces Society, 2019).

- Of the 46 species in the wildflower only seed mix, 25 (54%) were recorded in the vegetation occurrence surveys in 2017, four species (9%) were recorded in 2018 but not 2017, and 17 species (37%) were not found at all in surveys.
- Forb abundance and richness in all treatments were highest during mid-season for both years, but during mid-season average blooming forbs were 30% less abundant in 2017 than in 2018.
- There was a two-fold increase in floral richness from 32 unique flowering plants in 2017 to 56 flowering plants in 2018. And although there were few significant treatment effects, there were generally more species of forbs in 100% seeded plots and little differences between the 25x2 and 50% seeded plots, indicating that small patches (25x2 and 50) are comparable to 100% seeded plots despite containing 50% less wildflower seed.
- It is possible however, that smaller patches may lose forbs more quickly than 100% seeded plots as grasses encroach over time. Due to the short-term nature of this project, we were unable to assess longevity of the varying wildflower patch sizes. Further vegetation occurrence and forb surveys in these plots would be necessary to fully examine this.
- Strong seasonal effects due to low forb presence in early and late season indicate improvements could be made on wildflower seed selection. Specifically, the addition of more spring and fall blooming plants to the seed mixture would boost and evenly distribute floral resources throughout the season to better support pollinators.

Management implications

From this project, we were able to show that increases in forb abundance and richness from 2017 to 2018 (increased 3-fold) directly promoted bee abundance and nesting in wildflower seeded plots. Additionally, roughly 40% of seeded forbs had not yet established during those two years, thus subsequent forb surveys may help refine seed mixtures to remove species that do not germinate or establish well. Different species of bees are active throughout early, late, and mid-season, so adding more early season forbs into the seed mix can help attract and sustain early season bees as well as sustaining a more diverse bee community overall. Despite increases in forb and bee measures among wildflower plots, the volunteer weeds and grasses were highly competitive and presented major challenges in all plots. Therefore, further studies should continue to examine ways to reduce competitive weedy species and encourage better establishment of seeded plants. Removing poor-performing species from the wildflower seed mixture and increasing the seeding rate of species that established well on roadsides may allow for more dense wildflower growth rendering encroachment by volunteer species more difficult. Another refinement to the seed mixtures that may reduce competition by volunteer weeds is the introduction of quick growing annuals that may temporary secure space for slow growing or biennial plants to later establish. Other management methods to reduce competition that could be tested include mowing regimes, changes to topsoil practices, and burning or herbicide applications. While our results are in alignment with previous studies and provide further support that roadside habitat enhancements are effective at attracting bees (Hopwood, 2008; Wojcik & Buchmann, 2012), future studies could more closely examine persistence of individual seeded wildflowers to determine the duration of floral patch establishment and their ability to sustain diverse bee communities over longer periods of time.

Supplementary Tables & Figures

Supplementary Table S1. Inventory of plants found vegetation frequency of occurrence surveys. Table includes data from all plots categorized by plant type (forb or grass), bloom phenology, whether the plant was in the conventional or wildflower seed mix or a volunteer species, and which year(s) it was present (2017, 2018) (**denotes plants used as cover crop options, typically seeded in the spring (oats) or fall (wheat). For this project, wheat was seeded in the Fall in 2016.)*

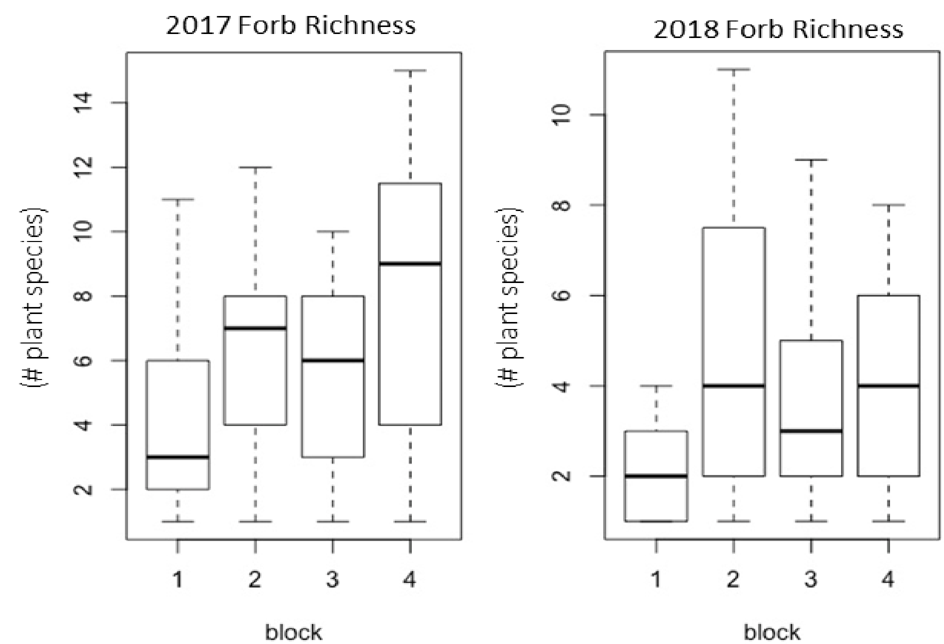
Plant species	Common name	Plant Type	Bloom time**	Conventional mix	Wildflower mix	Volunteer species	2017	2018
Andropogon gerardii	Big bluestem	grass		X			X	X
Elymus canadensis	Canada wildrye	grass		X				X
Sorghastrum nutans	Indiangrass	grass		X			X	X
Schizachyrium scoparium	Little bluestem	grass		X			X	X
Avena sativa	Oats*	grass		X			X	X
Spartina pectinata	Prairie cordgrass	grass		X				
Sporobolus cryptandrus	Sand dropseed	grass		X				
Bouteloua curtipendula	Sideoats grama	grass		X			X	X
Elymus trachycaulus	Slender wheatgrass	grass		X			X	X
Panicum virgatum	Switchgrass	grass		X			X	X
Pascopyrum smithii	Western wheatgrass	grass		X			X	X
Triticum	Wheat*	grass		X				
Melilotus officinalis	Yellow sweet clover	forb	Early, Mid, Late			X	X	X
Helianthus annuus	Annual sunflower	forb	Mid, Late			X	X	X
Rudbeckia hirta	Blackeyed susan	forb	Early		X		X	X
Linum lewisii	Blue flax	forb	Mid	X	X		X	X
Verbena hastata	Blue vervain	forb	Mid		X		X	X

Baptisia australis	Blue wild indigo	forb	Early		X			
Asclepias tuberosa	Butterfly milkweed	forb	Mid	X	X		X	X
Solidago canadensis	Canada goldenrod	forb	Mid		X		X	X
Astragalus canadensis	Canada milkvetch	forb	Mid		X			X
Desmodium canadense	Canada tickclover	forb	Mid		X		X	X
Asclepias syriaca	Common milkweed	forb	Mid		X		X	X
Silphium laciniatum	Compass plant	forb	Late		X			
Heliopsis helianthoides	False sunflower	forb	Mid		X			X
Zizia sp.	Golden alexander	forb	Early		X		X	
Ratibida pinnata	Grayhead coneflower	forb	Mid	X	X		X	X
Symphyotrichum ericoides	Heath aster	forb	Late		X			
Desmanthus illinoensis	Illinois bundleflower	forb	Mid		X		X	X
Gaillardia sp.	Indian blanketflower	forb	Early		X		X	X
Amorpha canescens	Leadplant	forb	Mid		X			
Helianthus maximiliani	Maximillian sunflower	forb	Late	X	X		X	X
Ratibida columnifera, red	Mexican redhat	forb	Mid	X	X		X	X
Solidago missouriensis	Missouri goldenrod	forb	Mid		X			
Symphyotrichum novae-angliae	New England aster	forb	Late		X		X	
Ceanothus americanus	New jersey tea	forb	Late		X			
Echinacea pallida	Pale purple coneflower	forb	Mid	X	X		X	X
Salvia azurea	Pitcher sage	forb	Late		X			
Coreopsis tinctoria	Plains coreopsis	forb	Mid		X		X	X
Drymocallis arguta	Prairie cinquefoil	forb	Mid		X			X
Echinacea purpurea	Purple coneflower	forb	Mid		X			X
Dalea purpurea	Purple prairie clover	forb	Mid		X		X	X
Eryngium yuccifolium	Rattle-snake Master	forb	Late		X			

Cleome serrulata	Rocky mountian bee plant	forb	Mid		X		X	X
Silphium integrefolium	Rosinweed	forb	Mid, Late		X			X
Lespedeza capitata	Roundhead lespedeza	forb	Late		X		X	X
Penstemon grandiflorus	Shell-leaf Penstemon	forb	Early		X			X
Chamaecrista fasciculata	Showy partridge-pea	forb	Mid	X	X		X	X
Symphyotrichum laeve	Smooth blue aster	forb	Late		X			X
Tradescantia sp.	Spiderwort	forb	Late		X			
Oligoneuron rigidum	Stiff goldenrod	forb	Late		X			
Helianthus pauciflorus	Stiff sunflower	forb	Late		X			
Liatris spicata	Thickspike blazing star	forb	Late	X	X			
Ratibida columnifera	Upright prairie coneflower	forb	Mid		X		X	X
Vernonia baldwinii	Western ironweed	forb	Mid		X		X	
Achillea millefolium	Western yarrow	forb	Early		X		X	X
Baptisia australis	Blue false indigo	forb	Early		X			
Dalea candida	White prairieclover	forb	Mid		X		X	
Monarda fistulosa	Wild bergamont	forb	Mid		X			X
Rosa arkansana	Wild rose	forb	Early		X			
Medicago sativa	Alfalfa	forb	Mid			X		X
Convolvulus arvensis	Bindweed	forb	Early, Mid, Late			X	X	X
Lotus corniculatus	Bird's foot trefoil	forb	Early, Mid, Late			X	X	X
Medicago lupulina	Black medic	forb	Early, Mid, Late			X		X
Solanum rostratum	Buffalo burr	forb	Early, Mid, Late			X		X
Nepeta cataria	Catnip	forb	Early, Mid, Late			X		X
Chichorium intybus	Chicory	forb	Early, Mid, Late			X	X	X
Glechoma hederacea	Creeping charlie	forb	Early			X		X

Securigera varia	Crown vetch	forb	Early, Mid, Late			X	X	X
Erigeron annuus	Daisy fleabane	forb	Early, Mid, Late			X	X	X
Hesperis matronalis	Dames rocket	forb	Early			X	X	X
Taraxacum officionales	Dandelion	forb	Early, Late			X		X
Thlaspi arvense	Field pennycress	forb	Early			X	X	X
Gaura sp.	Gaura	forb	Early, Mid, Late			X	X	X
Vicia villosa	Hairy vetch	forb	Early, Mid, Late			X	X	X
Verbascum sp.	Mullein	forb	Mid			X		X
Trifolium pratense	Red clover	forb	Early, Mid, Late			X	X	X
Festuca arundinacea	Tall fescue	grass	Early, Mid			X	X	
Croton texensis	Texas croton	forb	Early, Mid, Late			X		X
Prunus americana	Wild plum	shrub	Early			X	X	
Oxalis sp.	Wood sorrel	forb	Early, Mid, Late			X		X

Supplementary Figure S2. Box and whisker plots of forb richness among plots organized by replicated block and separated by year. Forb richness, or the median number of unique flowering plant species, is depicted by the black line, while the lower and upper 25% quartiles make up the box and the whiskers extending from the box depict maximum and minimum number of species. Block 1 had significantly lower forb diversity compared to all other blocks in both years ($F_{3,89}$=4.602, p=.004845 and $F_{3,139}$=12.727, p=2.13e-7, respectively) likely because block 1 plots were exposed to the off-target herbicide drift from the adjacent crop field in the Spring of 2017.



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