DEPARTMENT OF TRANSPORTATION

Cost-effective roadside revegetation methods to support insect pollinators

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Roadsides contain promising habitat for insect pollinators, yet roadside restorations can be expensive and are rarely evaluated for effectiveness. Where do we establish pollinator-friendly revegetation to maximize benefits? How effective are current revegetation practices at providing habitat for pollinators? We address these questions with two studies. Chapter 2 examines the impact of roadside-adjacent habitat that has been identified as pollinator-friendly for bumble bees. We use pollinator habitat maps to examine associations between the amount of nearby pollinator-friendly habitat and bumble bees (abundance and richness). We also regroup land covers to more specifically align with bumble bee habitat needs and compare the ability of both land cover categorizations to predict bumble bee metrics. This study can help refine predictors in mapping efforts to prioritize locations for pollinator habitat enhancements. Chapters 3 and 4 combine detailed insect and floral surveys of sites with known revegetation history to test efficacy of current revegetation methods for providing habitat for insect pollinators. We show which plants establish after seeding and how communities change as they age. We find that native flowering plants are more likely to establish in roadsides when they are planted, but native and non-native seeded sites converge in the plant community through time. Bumble bee and butterfly abundance and diversity is tied to flowering plant abundance and diversity, regardless of their status as native plants. This work identifies where pollinator-friendly restorations should be implemented and how current seeding practices could be modified to improve benefits to pollinators while reducing costs.

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FINAL REPORT

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EXECUTIVE SUMMARY

Roadside rights-of-way contain millions of acres of promising habitat for insect pollinators (Phillips et al., 2020) and have been shown to support diverse pollinator communities and species of conservation concern (Ries et al., 2001; Saarinen et al., 2005). Minnesota roadsides provide habitat for several species of butterflies of conservation concern (including monarchs) and the rusty patched bumble bee, which was listed as federally endangered in 2017 (Cariveau et al., 2019; Evans et al., 2019). Transportation departments and roadside management organizations have limited resources to allocate to roadside revegetation and management. Transportation projects often destroy existing plant communities in the course of construction and accordingly have revegetation plans budgeted into projects. Building back better plant communities that support healthy pollinator communities has the potential to be an important conservation tool. However, many questions related to how we can best manage roadside vegetation to support pollinators remain unanswered. Our project addressed two research questions to help improve our ability to apply limited resources to aid pollinator conservation in roadsides. 1) Where on the landscape should we prioritize implementing pollinator-friendly habitats? 2) And what plant communities are created by current revegetation practices, and how do pollinators use them?

1) Where on the landscape should we prioritize implementing pollinator-friendly habitats?

To examine the question of where on the landscape to prioritize pollinator-friendly habitat implementation, we quantified how habitat designated as pollinator friendly affected the abundance and richness of bumble bees in roadside habitat. We chose to focus this study on bumble bees due to the known conservation needs of many bumble bee species and the ability to rapidly survey bumble bees using non-lethal methods. Our surveys focused on Washington County, in the Twin Cities metropolitan region of Minnesota, which has mapped habitat that is likely to be beneficial to pollinators (Washington County Pollinator Habitats, 2018). We used this map to verify whether pollinator-friendly land cover was positively associated with bumble bees. We also compared the existing pollinator-friendly mapping, which aims to provide habitat for a taxonomically broad range of pollinating insects, including all bees as well as butterflies and others, to a version adapted to more specific habitat preferences for bumble bees. We demonstrated that:

- Generalized pollinator-friendly habitat mapping helps prioritize areas for pollinator restoration.
- Map refinement may be needed to more effectively target particular groups of conservation concern.
- Floral area has a positive association with bumble bee metrics, which indicates the importance of managing roadsides for high floral abundance.

2) What plant communities result from current revegetation practices and how do pollinators use them?

Our second study takes a detailed look at the floral communities that establish over the years that follow post-construction seeding and the bumble bees and butterflies that are using them. Using construction plans that were implemented between 2 and 20 years ago, we selected sites that were seeded with native seed mixes and compared them with sites that were seeded with non-native seed mixes. Over the course of 2.5 months, we surveyed these sites six times for vegetation (focusing on actively flowering forbs, the available food for pollinators), and for bumble bees, butterflies, and other insects.

With respect to plants, we demonstrated that:

- Seeding native forbs is necessary to get native forbs. Natural colonization is insufficient.
- Many introduced species that were never planted are present at all types of sites. Native sites are not resistant to colonization by introduced species.
- The effect of seeding on the plant community is short lived. The community is similar between native and non-native sites a few years after seeding, as seeded plants become less common and invasive plants become more common. Sites become more grass dominated with time.
- We highlight which plant species present in seed mixes are successfully establishing and which may fail to establish.

With respect to pollinators, we demonstrate that:

- Roadsides host diverse bumble bee and butterfly communities (as well as other insects) and provide important floral resources.
- More blooming flowers and greater floral diversity supports greater bumble bee and butterfly diversity in roadsides.
- Native flowers (and native seeded sites) do not attract a greater diversity of generalist bumble bees and butterflies than non-native flowers (and non-native sites). However, native flowers may be important for specialist pollinator species not examined in this study.

There are substantial opportunities for road management agencies to improve how roadsides are managed if pollinator conservation is a goal. First, while many other studies show no effect of the broader landscape on pollinator diversity, our study found a moderate effect. Using mapping efforts to target areas for habitat implementation could increase the likelihood of there being a base pollinator population in the area to receive the benefit of a restoration or could help target particular pollinator groups of conservation concern. Regardless, pollinators are certainly using sites with high floral abundance and diversity. Second, our results suggest that roadsides could be managed with a “more flowers everywhere” strategy without raising costs, and such a change would benefit pollinators. Seed mixes tend to fall into two categories: very diverse (and expensive) native mixes, or not very diverse (and not so expensive) non-native mixes. Seed mixes could be reworked to bolster the floral diversity in the commonly used non-native mixes by adding a few species of pollinator preferred forb species that respond well to seeding (e.g., wild bergamot). Native seed mixes could be streamlined by reducing the number of species in the mix based on which species are actually establishing in these roadsides; there is no advantage and a significant cost associated with sowing expensive seeds that never establish. Overall, this could potentially lead to a greater proportion of Minnesota roadsides that have native pollinator-friendly vegetation. Finally, we discuss how future research should consider effective methods of managing invasive species in roadside restorations.

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

Many insects, including pollinators such as bumble bees and butterflies, are declining (Cameron et al., 2011; Colla et al., 2012; Hallmann et al., 2017; Schultz et al., 2017). Many factors contribute to pollinator decline including disease, agrochemical application, and, perhaps most importantly, habitat loss (Potts et al., 2010; Sánchez-Bayo and Wyckhuys, 2019). Roadside rights-of-way contain millions of acres of promising habitat for insect pollinators (Phillips et al., 2020) and can support diverse pollinator communities and species of conservation concern (Cariveau et al., 2019; Ries et al., 2001; Saarinen et al., 2005). Transportation departments, which manage roadsides, have limited resources for roadside revegetation and management. Transportation projects often necessarily destroy existing plant communities in the course of construction, and accordingly have revegetation plans budgeted into projects. Building back plant communities that support healthy pollinator communities has the potential to be an effective conservation tool. Our project aims to address two important research questions to improve our ability to apply limited resources to aid pollinator conservation in roadsides. 1) Where on the landscape should we prioritize implementing pollinator-friendly habitats? 2) And what plant communities are created by current revegetation practices, and how do pollinators use them?

Question 1) Where on the landscape should we prioritize implementing pollinator-friendly habitats?

The study described in Chapter 2 assesses bumble bee communities at roadside sites that varied in the proportion of surrounding pollinator/bumble bee-friendly land cover. We explore associations between the bumble bee abundance and species richness, the floral area of a given site, and the surrounding landscape. This chapter verifies the utility of general pollinator and more specific bumble bee habitat designations in predicting impact on bumble bees, increasing confidence in the use of these types of land cover data to optimize placement of pollinator-friendly habitats on the landscape.

Question 2) What floral communities are created by current revegetation practices, and how do pollinators use them?

The study described in Chapter 3 took a detailed look at the floral communities that establish over the years that follow post-construction seeding. We compared sites that were seeded with native seed mixes to sites that were seeded with non-native seed mixes and also surveyed additional sites of unknown seeding history to represent “typical” roadsides in the area. Over the course of one season, we repeatedly surveyed 19 projects that each had these three site types (native seeded, non-native seeded, and typical sites) for a total of 57 sites. The native and non-native seeded sites varied in age since construction between 2 and 20 years. In this chapter, we described how the floral communities differed among sites of different seeding histories and site age. We also analyzed which specific species included in seed mixes required seeding to establish, and which ones failed to establish.

The study described in Chapter 4 took a detailed look at the insect community supported at the roadside sites described in Chapter 3 and explored relationships between the floral community and the insect community. Specifically, we targeted bumble bee and butterfly surveys as well as sweep netting surveys concurrently with the vegetation surveys described in Chapter 3. We explored foraging patterns of bumble bees and butterflies, highlighting plant species that were important food sources in roadsides. We explored relationships between floral abundance/diversity and pollinator diversity. In addition, we assessed the value of native flower species for attracting pollinators. Together, Chapters 3 and 4 provided a detailed look at how current revegetation practices affected the floral community and provided insights into how current practices could be modified to save money while enhancing pollinator habitat.

The final chapter synthesizes the findings of the prior three chapters to provide management suggestions and future research directions that come from this work.

CHAPTER 2: IMPACTS OF LANDSCAPE FACTORS ON BUMBLE BEES

2.1 INTRODUCTION

Declines in bumble bees have been observed worldwide and are attributed to agricultural intensification, habitat loss, insecticides, fungicides, and disease (Cameron et al., 2011; Colla and Packer, 2008; Grixti et al., 2009; Morales et al., 2016; Williams and Osborne, 2009). Habitat conservation and restoration is one of the actions identified as necessary for recovery of declining bumble bee species such as the federally endangered rusty patched bumble bee (U.S. Fish and Wildlife Service 2021) . Surrounding land use is known to have an impact on bumble bee communities (Carvell et al., 2008; Rundlöf et al., 2008; Samuelson et al., 2018). When habitat enhancements are being planned, accounting for the surrounding landscape could increase the impact of the enhancements. For example, enhancements in areas surrounded by high quality bumble bee habitat may be most successful as there are larger populations and more species of bumble bees than in poor quality habitats such as agriculture.

Currently, high-quality bumble bee habitat is not well delineated as there are few efforts to map habitat extent and location. Washington County, in the Twin Cities Metropolitan region of Minnesota, has mapped habitat that is likely to be beneficial to pollinators. This mapping effort was led by the Board of Water and Soil Resources, Washington County Natural Resources, and Metro Blooms. Potential pollinator-friendly areas were mapped using Minnesota Land Cover Classification System groupings ("Minnesota Land Cover Classification System," 2004) with land covers such as grasslands, wooded swamps, woodlands, and vegetated wetlands designated as pollinator friendly based on expert input (Washington County Pollinator Habitats 2018). This map was used to identify areas best suited for pollinator habitat restoration efforts (sweet spots) based on their proximity to larger areas with pollinator-friendly habitat. Washington County is approximately 1095 km^2 and has a population of approximately 260,000 people (Metropolitan Council, 2021). Despite its proximity to the major metropolitan areas of Saint Paul and Minneapolis, 39% of land in Washington County is public, including parks, preserves, and undeveloped land (Metropolitan Council, 2017). These public lands could ease logistical barriers to pollinator habitat restoration.

While there are commonly shared habitat needs among insect pollinators, such as the presence of floral resources, there are also differences in habitat needs within this taxonomically-diverse grouping. For example, among the butterflies and moths, there are different requirements for suitable host plants for larvae, while among bees, there are different nesting habitat requirements and floral needs (Holland et al., 2015). Bumble bees require floral resources from spring through fall as well as nesting and overwintering habitat. A broad approach to pollinator habitat creation and restoration may be best suited to support pollinators overall, but more specific habitat planning could help land managers target species of conservation priority, such as the rusty patched bumble bee (Liczner and Colla, 2020)

Across North America, roadsides have the potential to provide habitat for pollinators and may play an important role in pollinator conservation (Hopwood, 2008). In the US, the potential habitat area along roads is estimated to be almost 4,000,000 ha (Wojcik and Buchmann, 2012). The value of this habitat to pollinators will be affected by the surrounding habitat as well as the resources present, with the presence of host plants being particularly important (Wojcik and Buchmann 2012). By examining bumble bee floral use within roadside habitats, we can provide recommendations for vegetation management to support bumble bees.

We quantified how habitat designated as pollinator friendly surrounding roadsides affected the abundance and richness of bumble bees in roadside habitat within Washington County, Minnesota. We also compared the existing pollinator-friendly mapping, which aims to provide habitat for a taxonomically broad range of pollinating insects including all bees as well as butterflies and others, with a version adapted to possible habitat preferences for bumble bees. We examined the impact of these two habitat designations on bumble bee abundance and species richness. We also collected data on plant use by bumble bees in roadsides to inform management for roadsides to support pollinators.

2.2 METHODS

2.2.1 Site selection

We selected survey sites along county, state, or federal roads in Washington County using randomly generated points. We designated sample locations using ArcGIS (ESRI 2011) by placing random points along major roads and highways with the Functional Class Roads data layer produced by the Metropolitan Council (Metropolitan Council and NCompass Technologies 2018). Initially, we generated 100 random points that were at least 2 km from each other so that unsuitable sites could be removed without reducing the number of usable sites to less than 25. We chose the distance of 2 km to minimize the chance of observing the same *Bombus* individual at more than one site as this is beyond the typical foraging range of most bumble bees (Redhead et al., 2015). We selectively removed unsuitable habitats, defined as sites with no suitable foraging habitat along the roadway within 500 m of the random point by examining aerial imagery from ArcGIS and visiting sites before surveys began. We selected 35 sites at the start of our surveys (Figure 1). Surveys took place within 400 m of a random point along the roadway, starting approximately 4.5 m in from the road edge to reduce the chance of surveying along frequently mown areas. We completed six rounds of surveys at 27 sites. We dropped five sites after the first two rounds due to an absence of bumble bees, and road construction prevented several rounds of sampling at three sites.

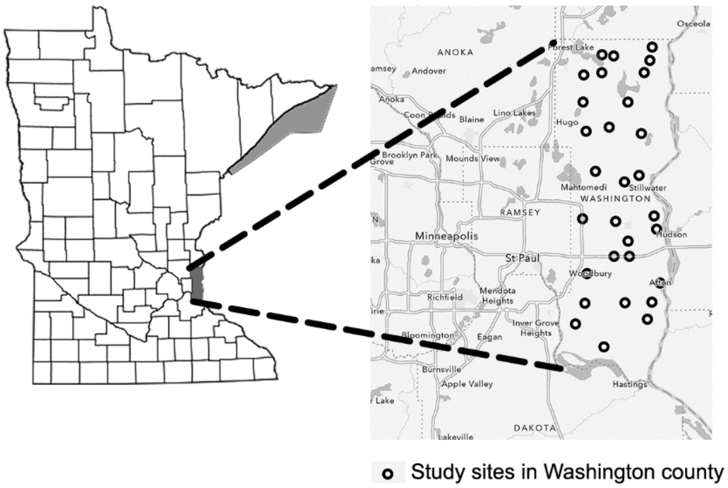

Figure 2.1. Study site locations in Washington County, Minnesota.

2.2.2 Mapping

Pollinator mapping for Washington County used the Minnesota Land Cover Classification System combined with ground truthing to identify pollinator-friendly land cover with expert input ("Minnesota Land Cover Classification System," 2004)("Minnesota Land Cover Classification System," 2004). Land cover categories identified as pollinator friendly included land with less than 26% impervious surface, wooded areas, native prairie, grasses and meadows, and woodlands (Appendix A). We use the term "pollinator friendly" to denote this land cover designation throughout the report. To refine the pollinator mapping, we created an alternative map based on previous research indicating that wooded areas, developed areas with vegetation, and wetlands can be positively associated with bumble bee communities (Evans et al., 2019b; Sepp et al., 2004). The bumble bee version designated habitats as bumble bee friendly with up to 75% impervious surface when there were trees, grasses, or forbs present, and included a wider range of wetland habitat types than the original pollinator-friendly mapping (Appendix A). We use the term "bumble bee friendly" to denote this land cover designation throughout the report. We summarized land cover data within 1 km of survey sites (Figure 2). A 1 km buffer distance was chosen to encompass the foraging range of bumble bees, which has been documented varying from 700 m to 2500 m with most bees foraging within 1 km of their nest (Dramstad, 1996; Hagen et al., 2011; Walther-Hellwig and Frankl, 2000).

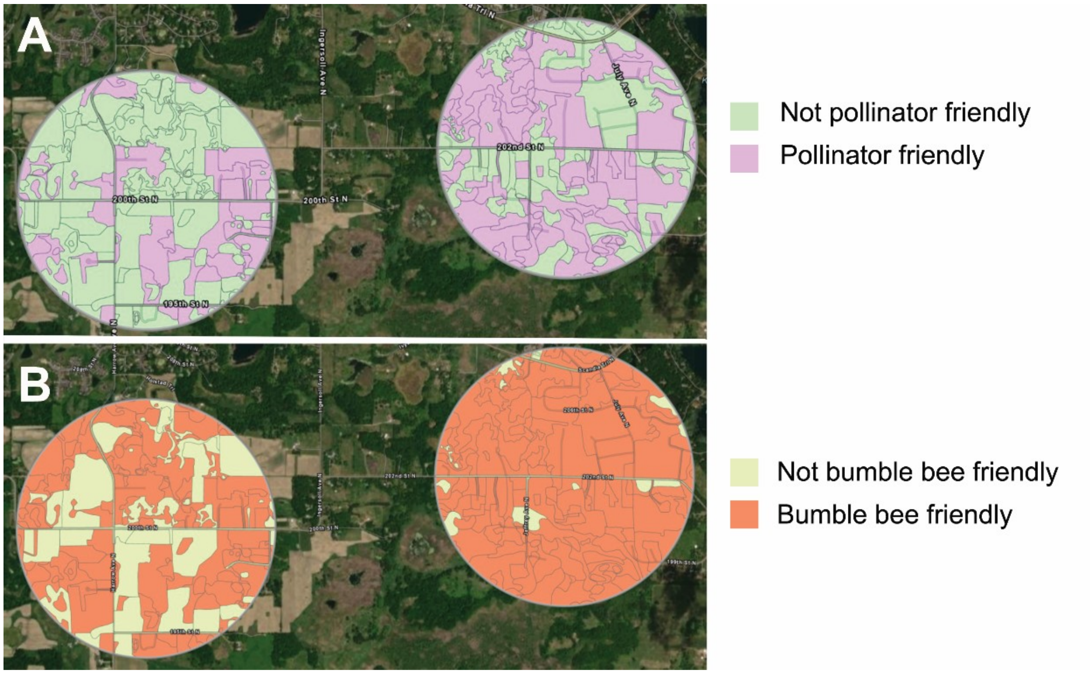

Figure 2.2. Pollinator-friendly and bumble bee-friendly land cover categories within 1 km of two sites. A. Original pollinator-friendly land cover designations for two study sites, with 31% and 62% pollinator-friendly land cover. B. Bumble bee-friendly land cover designations for the same two sites, with 63% and 95% and bumble bee-friendly land cover.

2.2.3 Bumble bee community assessment

At each site, we surveyed bumble bees along a meandering 250 m transect running parallel to the road. Meandering transects allowed us to survey flowering plants that were not located directly on straight transect lines (Droege et al., 2016), increasing the chance of detecting *Bombus* at our sites. Surveyors trained to walk at a consistent pace along the transect to cover 250 m in 15 minutes. Transect locations varied during each survey round so that observers could focus on areas with blooming flowers in the survey area. Observers rechecked their pacing every two weeks to ensure that they consistently covered 250 m in 15 minutes of walking. Observers trained extensively to visually identify *Bombus* spp. to species or species group level by practicing using photographs, specimens, and trial surveys. Most species could be reliably identified, but *B. auricomus* and *B. pensylvanicus* were identified to the level of species group. During surveys, we recorded the species (or species group) and sex of all bumble bee individuals observed within 1 m of the transect. For each survey, we caught one individual of each species that we detected and took a photograph for species verification (by E. Evans), noting the original field identification. For uncommon species, which included *B. affinis, B. terricola, B. fervidus, B. borealis*, *B. ternarius*, and *B. perplexus*, we attempted to net and photograph all individuals detected for species verification. In addition, photographs were taken of any unknown species. We used these photographs to quantify overall identification error rate. All surveys were non-lethal as we were surveying in an area known to be occupied by the federally endangered *B. affinis*. We obtained the appropriate permit from the USFWS to allow handling of bumble bees. Minimal handling of bumble bees allowed for rapid assessment, enabling more repetitions of surveys over the season at more sites than methods requiring handling of each individual for identification.

We surveyed from June 14-August 25, 2021 between 9:50-15:00 h when there was no rain, temperatures were 15 C or above, and wind was below 25 mph. We surveyed five days per week, weather permitting. We collected data for a total of six survey rounds, with a survey round being defined as a complete set of all sites being visited at least once. For survey rounds three through six, we dropped all sites at which no *Bombus* spp. were observed in the first two rounds, leaving 31 sites. Four other sites were not visited for all six rounds due to road construction, leaving 27 sites for standardized survey methods.

2.2.4 Vegetation assessment

In conjunction with bee surveys, we surveyed vegetation along the same transects once during each round. We walked the same 250 m meandering transect as the bee observer, stopping every 25 m to survey a 1 m^2 quadrat, alternating which side of the transect to drop the quadrat every time. We surveyed 10 quadrats at each site. For each quadrat, we counted the number of floral units of each blooming plant species observed. For each plant species, we predefined the definition of a single "floral unit". For example, for a common dandelion, *Taraxacum officinale*, a floral unit was one blooming flower head consisting of multiple individual florets. For a species such as Canada goldenrod, *Solidago canadensis*, a floral unit was a panicle consisting of multiple branches with flower heads.

We estimated the total area of blooming flowers at each site on each survey date using our counts of blooming floral units in each quadrat. To get a mean area of each floral unit for every plant species, we either measured ten floral units in the field and calculated the mean, used measures from previous studies at the UMN Bee Lab, or we searched online at MN Wildflowers, an online field guide to the flora of MN, for an average floral diameter (https://www.minnesotawildflowers.info/). To estimate floral area at each site, we multiplied the area of each floral unit by the number of floral units of each species recorded within the quadrat and added together the total area of blooming flowers in the 10 quadrats from each site. For each site, we then extrapolated the data from the ten quadrats to estimate total area of blooming flowers for the entire 250 m^2 transect by multiplying the floral area from the 10 m^2 of quadrat surveys by 25. We used this to estimate total area of blooming flowers.

2.2.5 Analysis

We measured the impact of habitat designated as pollinator or bumble bee friendly on bumble bees with two levels of measures. The first level was measures on the whole bumble bee assemblage. We examined overall abundance across all survey rounds and species richness, the total number of species or species groups over survey rounds at each site. We used generalized linear regression models to examine the impact of land cover on these overall bumble bee measures using R with the package MASS (Table 1) (Venables and Ripley, 2002). We chose gaussian, poisson, or negative binomial error distributions based on lowest AIC values. We used negative binomial models for overall abundance and a gaussian distribution for richness models. To determine which land use cover designation was a better predictor of these bumble bee measures, we compared the models using AIC.

We also examined measures of the bumble bee assemblage at the species level. We estimated maximum abundance for each species at each site per survey round as well as overall occupancy and detection per species with a Bayesian multiple species N-mixture model using JAGS (Plummer, 2003)(Plummer, 2003). Estimates are based on four chains of 20,000 iterations. We used vague priors for mean occupancy and detection and uniform priors for mean abundance. Due to limited suitability of these models for species with low abundances, we limited the data set to include bumble bee species with overall abundances greater than 10 individuals: *auricomus/pensylvanicus* group*, bimaculatus*, *fervidus*, *griseocollis*, *impatiens*, *rufocinctus,* and *vagans*. We then conducted a generalized linear mixed model with maximum estimated abundance as the response variable and floral area and either the amount of surrounding pollinator-friendly or bumble bee-friendly land cover as predictor variables (Table 1). The model with bumble bee-friendly land cover had the lowest AIC value and we hereafter report these results for the species abundance models. We scaled the predictor variables as they had dramatically different magnitudes. As there were many zeros in the sample rounds for each species, we used a zero-inflated negative binomial model (Brooks et al., 2017).

Table 2.1. Models examining impact of land cover on bumble bee communities.

Bumble bee metric	Model
Overall bumble bees per site	glm.nb(abundance ~ pollinator friendly land cover + floral area)
	glm.nb(abundance ~ bumble bee friendly land cover + floral area)
	lm(richness ~ pollinator friendly land cover + floral area)
	lm(richness ~ bumble bee friendly land cover + floral area)
Individual bumble bee species per survey round	glm.nb zero-inflated(estimated abundance ~ bumble bee friendly land cover + (1|site))

2.3 RESULTS

2.3.1 Bumble bees and vegetation

We observed 2,586 bumble bees representing 11 species across all 35 sites (Table 2). We captured and photographed 536 bumble bees to verify field identifications of foraging bumble bees by observers, and 16 bumble bees to identify unknown bee species. Of the 536 field identified bees, 93% were correctly identified. All photographs of bees that were identified in the field as *B. auricomus/pensylvanicu*s species group were verified as *B. auricomus.* Species varied in their frequency of identification error

(Table 2). We observed four rusty patched bumble bees, *Bombus affinis* (Table 3). We included information on all observations due to their status as a federally endangered species. Two of these sightings were outside of standardized survey efforts and were not included in our analyses.

Table 2.2. Observed abundances of bumble bee species across all sites and validation of identification. We compared field identifications made during observations of bees foraging on flowers with verifications from capturing and photographing one of each species observed during each survey. We note what species mistaken field identifications were corrected to, and note if the species was mistaken for another in its field identification

Common name	Scientific name	Abundance	Field ID matched verified ID	Corrected to	Corrected from
Rusty patched	*affinis*	2	2/2		
black and gold	*auricomus*	32	20/20		*auricomus* group
two-spotted	*bimaculatus*	232	77/80		2 *rufocinctus,* 1 unknown
boreal	*borealis*	9	8/8		
lemon cuckoo	*citrinus*	10	7/8	1 *impatiens*	1 unknown
golden northern	*fervidus*	13	13/13		
brown belted	*griseocollis*	118	46/47	1 *rufocinctus*	
common eastern	*impatiens*	1,051	131/132	1 *vagans*	1 citrinus, 2 unknown
red belted	*rufocinctus*	649	124/129	2 *bimaculatus*, 3 *vagans*	1 griseocollis, 7 *vagans*, 10 unknown
tri-colored	*ternarius*	4	4/4		
half black	*vagans*	453	79/87	7 *rufocinctus*	1 *impatiens*, 3 *rufocinctus*
unknown	sp.	12	12	1 *bimculatus,* 1 *citrinus*, 2 *impatiens*, 8 *rufocinctus*	

Table 2.3. Locations and plant associations for 2021 observations of the rusty patched bumble bee, *Bombus affinis*. Two of these observations were outside of standardized survey efforts.

Date	Observed foraging on		Location	
August 18	*field thistle*	*Cirsium discolor*	44.84046, -92.9739	US-10
June 15	*crown vetch*	*Securigera varia*	44.84046, -92.9739	US-10
July 21	*creeping thistle*	*Cirsium arvense*	45.06787, -92.863	State Hwy 15
August 5	*false sunflower*	*Heliopsis helianthoides*	44.92113, -92.9478	County Rd 16

We estimated the occupancy and detection for species with more than ten individuals over all surveys (Table 4). Bumble bee species varied in their overall probability of occupying a site from 41% +/- 10% for *B. auricomus* to 92% +/- 5% for *B. impatiens*. Detection probability also varied among species, with the lowest detection probability for *B. fervidus*, at 7% +/- 5% and while most other species had directions between 20% and 32% +/- 6%.

Table 2.4. Estimated occupancy and detection probabilities for bumble bee species with more than ten observed individuals over all surveys. The probability of detection reported is the maximum over six survey rounds, with the survey round noted. ψ = occupancy. p= detection probability. SE= standard error. CI= confidence interval.

Species	ψ ± SE	95% CI (ψ)	p ± SE	95% CI (p)	Survey round
auricomus grp.	0.406 ± 0.101	0.221 - 0.614	0.197 ± 0.107	0.043 - 0.448	6
bimaculatus	0.870 ± 0.060	0.734 - 0.963	0.276 ± 0.065	0.157 - 0.409	3
fervidus	0.567 ± 0.195	0.240 - 0.952	0.068 ± 0.050	0.010 - 0.196	1
griseocollis	0.613 ± 0.089	0.433 - 0.780	0.209 ± 0.068	0.095 - 0.355	1
impatiens	0.924 ± 0.047	0.812 - 0.988	0.155 ± 0.023	0.111 - 0.200	4
rufocinctus	0.866 ± 0.060	0.727 - 0.960	0.321 ± 0.053	0.217 - 0.426	3
vagans	0.740 ± 0.078	0.572 - 0.877	0.322 ± 0.045	0.237 - 0.412	5

We collected bumble bees from 56 flowering plant species, with 15 flowers accounting for 94% of observations (Table 5). Of the most frequently visited flowers, four were of native origin. Floral area at survey sites varied from 0.13 m^2 per 250 m^2 transect (<0.001 percent cover) to 39 m^2 per 250 m^2 transect (16% cover). The mean floral cover of sites with seven to eight bumble bee species present was 8%. The mean floral cover of sites with two to six bumble bee species present was 3%.

Table 2,5. Fifteen most frequently visited flowers. [a] = native to Minnesota

Common name	Scientific name	Bumble bee abundance
spotted knapweed	*Centaurea stoebe*	580
bird's foot trefoil	*Lotus corniculatus*	362
field thistle[a]	*Cirsium discolor*	326
crown vetch	*Securigera varia*	284
white sweet clover	*Melilotus alba*	232
creeping thistle	*Cirsium arvense*	202
wild bergamot[a]	*Monarda fistulosa*	83
spear thistle	*Cirsium vulgare*	81
field sow thistle	*Sonchus arvensis*	74

purple loosestrife	*Lythrum salicaria*	50
Canada goldenrod complex[a]	*Solidago canadensis* complex	33
common tansy	*Tanacetum vulgare*	29
St. John's wort	*Hypericum perforatum*	28
red clover	*Trifolium pratense*	24
common milkweed[a]	*Asclepias syriaca*	17

2.3.2 Relation of bumble bees to land cover and floral abundance

Total bumble bee abundance was positively associated with the amount of land cover designated as pollinator friendly (z=1.96, p=0.05) and bumble bee friendly (z=2.29, p=0.02) within a 1 km radius (Figure 3). The two models with the different land covers were similar in their ability to predict overall bumble bee abundance (pollinator friendly AIC: 285.71, bumble bee friendly AIC: 285.12). Bumble bee richness was positively related to bumble bee friendly land cover (t=2.32, p=0.03). There was a trend towards a positive relationship with total abundance and pollinator-friendly land cover, but it was not significant (t=1.64, p=0.12). The model using bumble bee-friendly land cover was a slightly better predictor of bumble bee richness than pollinator-friendly land cover (pollinator friendly AIC: 93.50, bumble bee friendly AIC: 90.89). We included the average floral area at the survey site across six surveys as a covariate in these models. The floral area at survey sites had a positive association with bumble bee richness for both land covers (pollinator friendly t=3.54, p=0.002, bumble bee friendly: t=3.23, p=0.004) and a positive association for bumble bee abundance in the model with pollinator-friendly land cover (pollinator friendly z=2.28, p=0.02, bumble bee friendly: z=1.77, p=0.07).

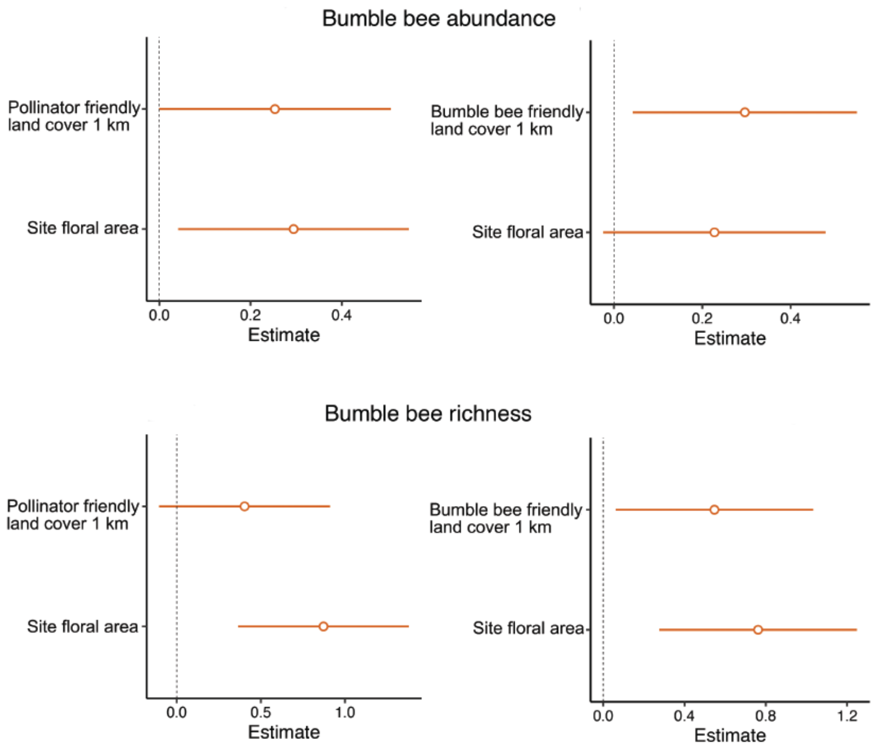

Figure 2.3. Overall bumble bee abundance and species richness related to land cover and floral cover. Estimates of fixed effect variables with 95% confidence intervals (CI) from models with proportion of land cover at 1 km from survey sites. Effects of land cover and floral cover variables are significant when the 95% CI does not cross zero.

Examination of the relationship of the maximum estimated abundances for individual species per survey round to land cover found no relationship to the pollinator-friendly or bumble bee-friendly land covers (Figure 4). There was a trend towards a positive relationship for both land cover designations that was slightly stronger for bumble bee-friendly land cover.

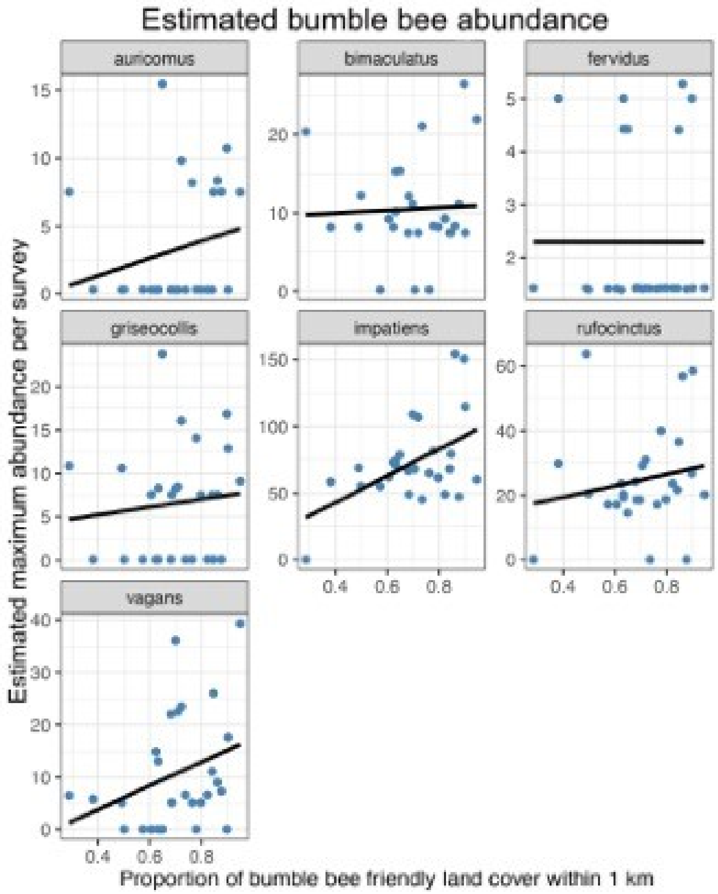

Figure 2.4. Estimated maximum abundance per survey round in relation to the amount of pollinator-friendly or bumble bee-friendly land cover. Abundance was estimated using a multiple species N-mixture model that included bumble bee species with more than ten individuals across all surveys.

2.4 MANAGEMENT IMPLICATIONS AND SUGGESTIONS

Restoring and maintaining habitat is important for pollinator conservation. Mapping efforts to identify areas to target for restoration can provide important information to land managers considering where restoration may be most effective, but questions remain as to whether identified pollinator-friendly lands have a positive impact on pollinator communities, and if assumptions about suitability for a general pollinator population apply to specific groups of interest, such as bumble bees. We found that habitat designated as pollinator friendly was positively associated with one of the overall bumble bee measures we examined, bumble bee abundance, but not with bumble bee richness or estimated abundance per species per survey round. However, the refinement of the land cover designations to include habitats previously identified as being associated with bumble bee community measures resulted in an additional positive association of land cover with bumble bee richness. Bumble bee richness is an important measure to examine as abundance alone does not provide information on species that may be of conservation concern. The lack of relationship of either land cover designation with estimated abundances for individual species at each survey round may indicate that additional refinement of land cover groupings is needed to predict land cover associations for individual bumble bee species. In particular, the nesting habitat needs of different bumble bee species differ (Pugesek and Crone, 2021). While the bumble bee-friendly land cover map provided a better match with overall bumble bee measures, it could be a less suitable match for other pollinators of conservation concern, such as floral specialists. We still recommend generalized pollinator-friendly habitat mapping to prioritize areas for pollinator restoration but recognize that refinement of these maps may be needed to more effectively target particular groups of conservation concern. The association of floral area with bumble bee metrics indicates the importance of managing roadside for high floral abundance.

The abundance and diversity of flowers at a site is well documented to have a positive impact on many types of pollinators. We found that sites with greater than 8% floral cover supported a higher diversity of bumble bee species. Although bumble bees often use a wide variety of non-native as well as native flowers, native plants that perform well in roadsides could be important for creating pollinator habitat in roadsides (Hanley et al., 2014; Lanterman et al., 2019). The frequent use of field thistle (*Cirsium discolor*), wild bergamot (*Monarda fistulosa*), Canada goldenrod complex (*Solidago canadensis* complex), and common milkweed (*Asclepias syriaca*) in roadside habitat in this study indicate their potential utility in roadside pollinator planting plans if establishment rates indicate they are suitable candidates.

CHAPTER 3: THE INFLUENCE OF NATIVE AND NON-NATIVE SEED MIXES ON ROADSIDE PLANT COMMUNITIES

3.1 INTRODUCTION

3.1.1 Highlights

- We compared the plant community (flowering forbs and common grass species) between sites that were seeded with native vs sites seeded with non-native seeds of varying age since planting.
- Seeding native forbs is necessary to get native forbs. Natural colonization is insufficient.
- Many introduced species that were never planted are present at all types of sites. Native sites are not resistant to colonization by introduced species.
- The effect of seeding is on the plant community is short lived. The community is similar between native and non-native sites a few years after seeding. This is because we lose seeded plants through time and invasive plants become more common. Sites become more grass dominated with time.
- We highlight which plant species present in seed mixes are successfully establishing and which are not.

3.1.2 Abstract

The plant community in roadside right-of-ways is an important component of transportation infrastructure. The right plant community can reduce erosion, filter runoff, provide wildlife habitat, and improve aesthetics. Roadside revegetation after construction is often viewed as an opportunity to improve the plant community by planting native grasses and forbs. However, the cost of native seed mixes is substantially more expensive than those used in conventional roadside plantings. In this study, we sought to measure the payoff of this investment by repeatedly surveying the flowering plant community (and several common grass species) across a single growing season in sites seeded with native seed mixes and non-native seed mixes along roadsides in Minnesota. We also surveyed nearby “typical” roadsides with unknown construction histories for comparative purposes. Sites varied in age (years since establishment) from 2-20 years old. Our study shows that native seeded sites have substantially more native flowering forbs than non-native seeded sites. Non-native sites have few flowering native forbs, indicating natural colonization is insufficient for native forb establishment. Both native and non-native sites had high levels of introduced forbs; native sites are not resistant to the invasion of introduced species. In terms of site succession, older sites have fewer floral resources than

younger sites. While native seed mixes do boost the occurrence of native flowers for several years, the native and non-native sites converge in their quantity of floral resources (and overall plant community) through time. Though many desired species establish with seeding, they do not necessarily persist over decades. To make recommendations with respect to certain native plants, we quantified the strength of the seeding effect for all species present in seed mixes in our study. There are a number of native forb species that are substantially more likely to occur where they were seeded than where they were not, suggesting their inclusion in seed mixes is worth the investment. However, we also identify species that were seeded but never detected or were detected at similar rates in places where they were not seeded, suggesting their inclusion in seed mixes may be less likely to pay off. This information can be used to improve seed mix design. Overall, our results demonstrate that seeding native forbs into roadside revegetation projects is an important prerequisite to having native forbs establish—these plants are not establishing in roadside sites on their own. If the goal is persistence of native forbs along roadsides across decades, roadside managers may need to consider additional interventions.

3.1.3 Introduction

With ~6.6 million kilometers of roads, paved roads and their adjacent right-of-ways (ROWs) cover ~1% of the total land area of the United States (Federal Highway Administration, 2007). Most of these roads were initially constructed prior to the 1970s without much thought towards their environmental impacts. ROWs were often planted with non-native vegetation and were readily colonized by invasive species. This history, coupled with frequent disturbance and environmental stressors, means that many ROWs are characterized by weedy non-native vegetation. Most modern road construction projects involve improving existing infrastructure rather than the creation of new roads and often destroy the existing roadside plant communities (Federal Highway Administration, 2007). The revegetation component of such projects presents an opportunity to improve the vegetation communities along roadsides.

There has been a major push by federal, state, and local agencies towards revegetating roadsides with native plant communities. There are a number of widely-touted benefits for establishing native plant communities along roadsides including low maintenance weed and erosion control, improved infiltration, reduced snow drift, aesthetics, enhancing plant diversity and wildlife habitat, and filtering nutrients, pesticides, and sediment (Federal Highway Administration, 2007; IOWADOT, 2022; Phillips et al., 2019a). In particular, the potential conservation value of roadsides for pollinators has been of considerable interest (Cariveau et al., 2019; Hopwood, 2008; Ries et al., 2001) Pollinators have been in worldwide decline due to a variety of causes, with habitat loss as the primary factor (Simon G. Potts et al., 2010). Roadsides may be detrimental to wildlife due to collisions, pollution, habitat fragmentation, and the facilitation of human movement and resource extraction (Trombulak and Frissell, 2000)But roads are not going away, so there has been a push to consider possible benefits of roadside habitat, for instance in pollinator conservation. Roadsides can be particularly beneficial in landscapes that are otherwise mostly unsuitable for pollinators, such as intensively farmed landscapes (Phillips et al., 2019b). Roadsides can support important floral resources and because they are not tilled, are important habitat for ground nesting bees.

Regardless of the proposed benefits of establishing native vegetation along roadsides, one of the major knowledge gaps regarding roadside revegetation projects is how effective current practices are at producing the desired plant community (see Auestad et al., 2016; Auffret and Lindgren, 2020; Bugg et al., 1997; Haan et al., 2012; Karim and Mallik, 2008; Mallik and Karim, 2008; Nordbakken et al., 2010). Roadsides are fundamentally different than many other potential restoration sites. Roadsides often have imported and compacted soils (Berli et al., 2003; Forman et al., 2003), are chemically altered by exhaust and de-icing salts (Mitchell et al., 2020), are primarily "edge" habitats, and facilitate plant invasion(von der Lippe and Kowarik, 2007) The most common funding model of road construction projects in the United States results in sufficient funding for up-front costs but no continued financial support for long term maintenance of projects. This is also generally true of many restoration projects as well (Barak et al., 2021; Bash and Ryan, 2002; Galatowitsch and Bohnen, 2020). Accordingly, many roadside revegetation projects are planted at the end of the construction but are never surveyed afterwards to determine whether the seeded species establish and persist, and what non-seeded plants colonize sites. Furthermore, the benefits of native plant communities can never be realized if the communities never actually establish. Are expensive native seeds being sown only to never establish in disturbed roadside areas? Can desirable species able to colonize sites naturally? Are sites seeded with native plants more resistant to the establishment of invasive species? Having answers to these questions will improve our ability to make cost-effective seeding decisions for roadside projects.

The primary goal of our study is to assess the efficacy of current revegetation practices at producing native floral communities after road construction disturbances. To do so, we repeatedly surveyed roadside plant communities that were seeded with native and non-native seed mixes after road construction projects that were 2-20 years old in Minnesota. Additionally, we surveyed "typical" roadsides nearby these sites that were not involved in the construction projects, that provides a useful comparison for our construction sites. Our surveys focused on flowering forbs and eight common grass species. In this chapter, we assess how the plant communities of sites seeded with native plants differ from sites seeded with non-native plants and assessed how these communities change with age. Furthermore, we assess which seeded plant species establish and persist in restorations and which do not.

3.2 METHODS

3.2.1 Site Selection

We obtained construction plans for projects in Dodge, Brown, Scott, Wright, Carver, Ramsey, and Washington County, MN that ranged from 2-20 years old (Figure 1; Table B1). Plans were obtained from State and County transportation employees. Construction plans are the road construction "blueprints" with detailed maps of the project and includes information on what seed mixes were used in all the areas affected by the construction (we know exactly which species were seeded in each site). Our criteria for inclusion of a project in our study required that a single project contain at least one site seeded with a native seed mix and one site seeded with a non-native seed mix. Within these projects, we preferred selecting upland sites (as opposed to wetlands) for consistency in habitat comparisons. We selected one continuous area seeded with a native seed mix and one continuous area seeded with a non-native seed mix within each project. We georeferenced sections of these plans in ArcGIS and delineated the boundaries of each study site. All projects were related to improving existing infrastructure. Our sites included right-of-ways, land adjacent to exit ramps, bridges, roundabouts, and upland areas near containment ponds. Sites were a variety of shapes and areas (min = 285.2 meters^2, max = 5443.8 m^2, mean = 1380.7m^2). For each project, we also selected one "typical" roadside site at least 1 km from our other two sites, but along the same road as the other sites. We selected the first site outside of 1km that was seemingly maintained similarly to our other sites (e.g. we rejected sites that appeared to be weekly mowed turfgrass). For example, we would not select a regularly mowed turfgrass lawn, but instead a right-of-way that was likely occasionally mowed. We do not know the construction or revegetation history of these typical sites. Accordingly, within each project, we have three sites: native, non-native, and typical. Our final dataset included 19 projects consisting of 57 sites.

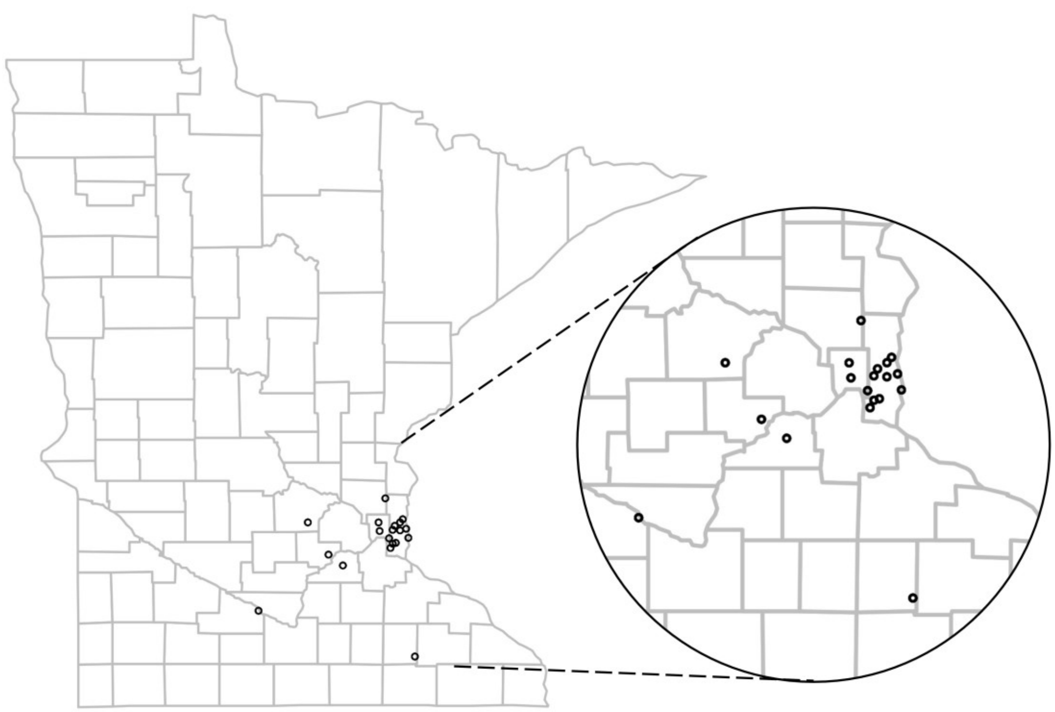

Figure 3.1. Study site locations in Dodge, Brown, Scott, Wright, Carver, Ramsey, and Washington County, MN.

3.2.2 Plant Surveys

We conducted six plant surveys per site, between 28 May 2021 and 24 August 2021. We visited sites in the same general order to ensure approximately even time between visits. However, we shuffled the order of sites within each day to make sure our insect surveys were random with respect to time of day. We visited each site once every 10-14 days over the course of the study. We conducted plant surveys by using 1x1m quadrats systematically placed throughout the site. We started on one edge of the site and surveyed plants in a quadrat placed every 5m as we zig-zagged through the site at a ~35° angle. Given the irregular shapes of our sites, this method ensured we sampled across the full width of sites. Thus, if we had a long, rectangular site that had an elevation gradient (as many ditches have) our quadrats were randomly dispersed with respect to that gradient. We identified any plants which were flowering in each quadrat. We identified goldenrods at the genus level (*Solidago* spp.). We identified the presence of several native and introduced grass species if they had seed heads present. We surveyed for the native grasses big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), indian grass (*Sorghastrum nutans*), Canada wild rye (*Elymus canadensis*), and the introduced grasses reed canary grass (*Phalaris arundinacea*), smooth brome (*Bromus inermis*), quack grass (*Elymus repens*), and foxtail grasses (*Setaria* spp.). When we refer to the plant community, we are referring to those flowering forbs plus the eight grass species mentioned above, however it should be noted that due to the methods described here we did not survey the entire plant community (only flowering forbs and a subset of grass species, only noted when their seed heads were present). On our first visit of the season, we estimated the percent cover of forbs, grass, and bare ground in each quadrat into arcsin square root cover categories (0, 1, 5, 25, 50, 75, 95, 99, 100). At the end of the field season, we took three soil compaction measurements at 10cm of soil depth with an Agtronix penetrometer and collected triplicate soil samples of the top 10cm of soil which we performed particle size analysis. Minnesota had a drought during our field season (https://www.dnr.state.mn.us/climate/journal/drought-2021.html#:~:text=Overview,drought%20in%2010%2D30%20years.)

3.2.3 Statistical Methods

We classified each species of plant that we observed as native or introduced utilizing the Minnesota Department of Natural Resources Vascular Plant Checklist (https://www.dnr.state.mn.us/eco/mcbs/plant_lists.html). Soil penetration data were averaged across 3 samples in each site to create a mean soil penetration value for each site (min = 917, max = 2413 kPa). Quadrat level plant data were aggregated to create a date-specific, site level metric of abundance for each species' flowers (or seed heads) as the number of quadrats in which a species was observed divided by the total number of quadrats assessed in the survey in which that species was found. The resulting values represent the prevalence of each species' flowers at a 1 meter resolution—or proportional occurrence of a species' flowers at the 1m resolution (continuous 0-1). Flower and seed head abundances were then used to calculate species richness and Shannon diversity index for each site-date of sampling. Shannon Diversity is calculated by the following formula:

$$H = -\sum_{i=1}^{s} p_i \ln p_i$$

Where H is the diversity index, s is the number of species, and p_i is the proportion of individuals of each species belonging to the ith species of the total number of individuals. We used Shannon diversity to represent diversity in this study because it is equally sensitive to rare and abundant species (Morris et al. 2014). In addition, all community metrics were calculated for the whole floral community, and for both the native and introduced species floral communities.

We performed a model-based analysis of all plant community data using a multivariate generalized linear model (mvGLM) fitted to the abundances of all plants with binomially distributed errors (mvGLM; Wang et al., 2012). Models were fitted and evaluated using the mvabund package. Explanatory variables in our model included seeding treatment, site age, treatment by age interaction, sample date (as linear and quadratic components), soil compaction, and site area. Statistical significance of changes is evaluated with permutation-based tests, resulting significance estimates that are robust to the paired nature of our sample design (sites paired within projects)(Fieberg et al., 2020; Wang et al., 2012). We evaluate significance of community change across site types (native seeded, non-native seeded) and age as fixed effects through a log-likelihood ratio test and permutation-based p-values. The strengths of this type of analysis (mvGLM) is that it allows for an evaluation of the entire community of plants (as opposed to a single value derived from community data—like richness or diversity) and offers a more robust statistical testing framework than ordination or distance based methods (Hui and Taskinen, 2014; Jupke and Schäfer, 2020).

To visualize species contributions to the age and phenology components of the multivariate response, we also modeled the multivariate community along those two axes using principal response curves (PRC) (van den Brink and ter Braak, 1998). PRC is a specialized version of a multivariate analysis of distance between treatment groups (difference in community composition), that enables a graphical output more easily interpreted than visualizations of other multivariate approaches. The method produces a distance metric (canonical coefficient C_{dt}) for the treatment group from the control which is plotted against the axes of interest to visualize community differences along that axis. Individual species contributions are represented as species weights, with species of large magnitudes showing large contributions to the observed community distance, and the sign of each species weight indicating the species being overrepresented in the treatment group (weight with same sign as canonical coefficient) or overrepresented in the control group (weight with opposite sign as canonical coefficient). PRC calculation and visualization was performed in R using the package vegan.

To assess the establishment of seeded species in these communities, we modeled the presence of each seeded species in our dataset as a function of its inclusion in the seed mix used at the site. This was accomplished using a GLM with binomially distributed errors. Because these data contained many zeros in the response data (cases where a seeded species was not found in any of our sampling), we fitted these data in a Bayesian framework using the R package rstanarm (Goodrich et al., 2020). Fit checks and model diagnostics were checked using the R package shinystan (Muth et al., 2018)Credible intervals (95%) for the posterior distributions represent estimates of the odds of finding a seeded plants flower in a site where that species was seeded (e.g., an odds-ratio of 10 for species x indicates species x was 10 times more likely to be found in a site where it was seeded than one where it was not).

3.3 RESULTS

Across all sites, we sampled 8315 quadrats, made 5427 flowering forb observations within those quadrats, and identified 120 forb species. For native seeded sites, on average 61% of quadrats sampled had at least one flower present, 25% had a least one native flower present, and 41% had at least one introduced flower present. In non-native seeded sites, on average 47% of sites had at least one flower present, 5% had at least one native flower present, and 45% had at least one introduced flower present. The mean percent occurrence of the 20 most common plants observed in native, non-native, and typical sites are in Table 1.

Table 3.1: The most common flowering plants and grasses detected in vegetation surveys, broken down by site type. Percent occurrence is the percent of quadrats in which that species was detected across all six sampling visits. Forbs were only identified by their flowers and grasses by their seed heads. “No flowering veg” does not necessarily indicate bare soil, but that no forbs were flowering and none of the surveyed grasses species had seed heads. *Asterisks denote introduced plant species. (G) indicates grasses. (C) indicates “colonizing” plants that are not included in any of the seed mixes used in our study.

	Native Sites		Non-native Sites		Typical Sites	
Rank	Common Name	% occurrence	Common Name	% occurrence	Common Name	% occurrence
1	no flowering veg	27.6	no flowering veg	34.3	no flowering veg	36.1
2	birds foot trefoil*(C)	17.2	reed canary grass* (G, C)	15.7	smooth brome* (G)	26.1
3	reed canary grass* (G, C)	14.4	smooth brome* (G)	14.3	reed canary grass* (G, C)	13.3
4	black-eyed susan	8.4	alfalfa*	11.5	birds foot trefoil*(C)	10.4
5	crown vetch*(C)	6.2	birds foot trefoil*(C)	11.3	quackgrass*(C)	6.0
6	smooth brome* (G)	5.7	Canada thistle*(C)	5.8	spotted knapweed*(C)	4.6
7	Canada wild rye (G)	5.5	crown vetch*(C)	5.2	Switchgrass (G)	4.5
8	Switchgrass (G)	5.1	white sweet clover*(C)	5.1	big bluestem (G)	3.4
9	annual fleabane (C)	5.0	red clover*	3.3	ox-eye daisy*(C)	3.2
10	Canada thistle*(C)	4.7	white clover*	3.2	canada thistle*(C)	2.7
11	goldenrods	4.2	spotted knapweed*(C)	2.7	hoary alyssum*(C)	2.3
12	yellow sweet clover*(C)	3.9	yellow sweet clover*(C)	2.2	Canada wild rye (G)	1.9
13	alfalfa*	3.4	Switchgrass (G)	2.2	leafy spurge*(C)	1.9
14	white sweet clover*(C)	3.3	alsike clover*	1.9	perennial sow thistle*(C)	1.3
15	rough cinquefoil (C)	3.1	hoary alyssum*(C)	1.8	white sweet clover*(C)	1.2
16	quackgrass* (G, C)	2.5	big bluestem (G)	1.8	little bluestem	1.1
17	red clover*	2.3	Queen Anne’s lace*(C)	1.5	goldenrods	1.0
18	big bluestem (G)	1.8	annual fleabane(C)	1.2	foxtails* (G, C)	0.9
19	white clover*	1.8	ox-eye daisy*(C)	1.1	annual fleabane (C)	0.9
20	common st. johnswort*(C)	1.6	quackgrass* (G, C)	1.1	bull thistle*(C)	0.8

The plant communities along roads seeded with native plants were significantly different than the communities seeded with non-native plants (Deviance: 56.51; P = 0.01). Time-since-restoration (Dev:111.33; P = 0.01) and date (Dev:202.41; P = 0.01) both significantly affected the plant community. Soil compaction (Dev:52.13; P = 0.634), site area (Dev:29.15; P = 0.743) and the interaction of seeding treatment and site age (Dev:15.95, P = 0.485) did not significantly influence the plant community. The significant statistical outcomes identified here are explored with data visualizations, described below.

The significant effects of seed mix and date on the percentage of quadrats with flowering forbs is explored in Figure 2. Native seeded sites have more native flowers than non-native seeded sites. However, native and non-native sites have similar levels of introduced flowers. Typical sites had fewer native and introduced flowers. Floral resources were lowest at the beginning of our study but increased through the season. There is a midseason peak in flowering in all sites, and then a second peak that is largely driven by native flowers (e.g. goldenrod) apparent late in the season. Similar overall patterns are apparent when visualizing the effect of seeding treatment and date on richness (Figure B1) and Shannon Diversity Index (Figure B2).

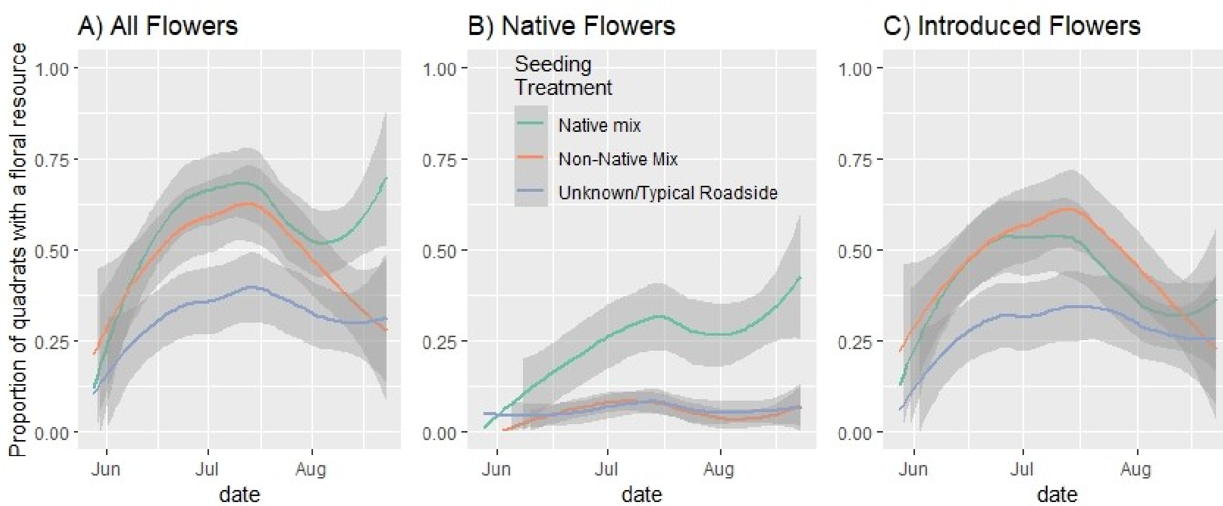

Figure 3.2. Seasonal change in the percentage of quadrats with A) at least one flower present, B) at least one native flower present, and C) at least one introduced flower present, broken down by seeding treatment. Lines represent an average computed by a Loess smoother and shaded area is the 95% confidence interval.

The significant effects of seed mix and site age on the percentage of quadrats with flowering forbs is explored in Figure 3. More recently seeded sites have more floral coverage than older sites, and non-native seeded sites lose floral resources more quickly than native sites. Similar overall patterns are apparent when visualizing the effect of seeding treatment and age on richness (Figure B3) and Shannon Diversity Index (Figure B4). Older sites have more grass than younger sites (Figure B5).

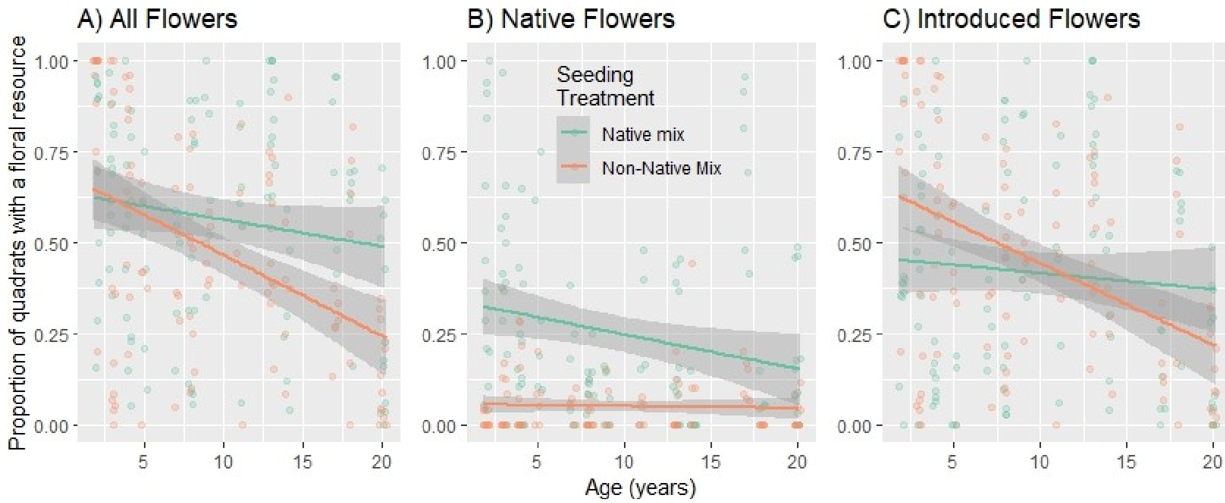

Figure 3.3. Change in floral coverage of sites displayed as time since seeding (e.g site age, in years). We show the percentage of quadrats with A) at least one flower present, B) at least one native flower present, and C) at least one introduced flower present, broken down by seeding treatment. The line is a simple linear trendline and the shaded area is a 95% confidence interval. Because typical sites have unknown history (and accordingly, no known "age") they are not included in this plot.

Principal response curves of the plant community showed that the native planted sites were most different from non-native planted sites at young ages (Figure 3a). The species weights illustrate the individual species contributions to treatment differences across age of sites. For example, black eyed susan is the species that is most represented by the black line, indicating it is most represented at young, native sites. Alfalfa is the species most opposite of the black line, indicating it is most represented at young, non-native sites. There was a strong signal of difference in the plant communities across the growing season, with difference from control increasing into later sampling periods (Figure 3b). Here the species weights illustrate the individual species contributions to treatment differences across the season.

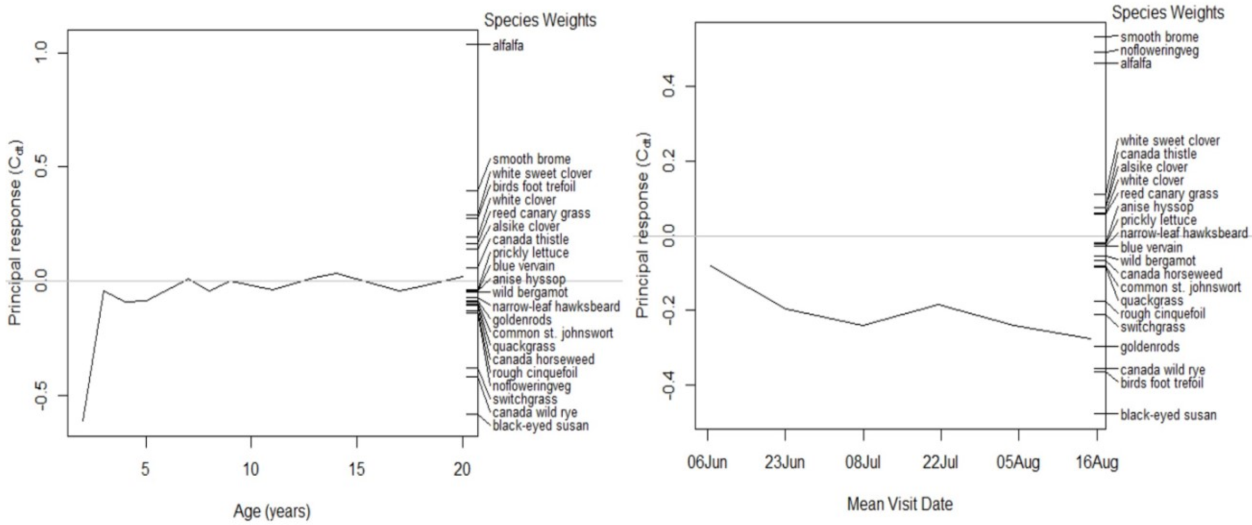

Figure 3.4. Principal response curves for multivariate responses of age and phenology. Principal response shows the multivariate distance of the plant community of native seeded sites (black line) from the sites seeded with nonnative mixes (gray line at y = 0). Species weights show individual species contributions to the overall pattern observed, with negative values indicating that a species is more prevalent in native seeded plots along the axis being evaluated. To declutter visualizations, species weights display only species with weights greater than 0.04.

We assessed how frequently each species occurred in quadrats in sites where it was seeded and in sites where it was not seeded, summarized by the strength of the "seeding effect" (odds ratio) for species which were seeded and detected in our study (Table 2). For example, white prairie clover was 26.64 times more likely to be detected in sites where it was seeded versus sites where it was not seeded, occurred in 0.32% of quadrats where it was seeded, and was not detected in any site where it was not seeded.

Table 3.2: List of seeded species ranked by strength of seeding effect (odds ratio). The odds ratio is interpreted as how many more times likely the species is to be encountered in sites where it was seeded versus sites where it was not. The percent occurrence is the percentage of quadrats in which the species was encountered in sites where it was seeded and was not seeded. Standard deviation is abbreviated as SD. *Asterisks denotes introduced species, (G) denotes grass species. The forbs that were included in seed mixes but never detected in our survey are as follows: *Amorpha canescens, Asclepias incarnata, Bidens frondosa, Bouteloua curtipendula, Chamaecrista fasciculata, Coreopsis palmata, Doellingeria umbellate, Euthamia graminifolia, Eutrochium maculatum, Helianthus pauciflorus, Liatris aspera, Liatris pycnostachya, Mimulus ringens, Oligoneuron rigidum, Penstemon grandifloras, Physostegia virginiana, Rudbeckia laciniate, Symphyotrichum ericoides, Symphyotrichum leave, Symphyotrichum lancelolatum, Symphyotrichum novae-angliae.* Of these species, *Physostegia virginiana,* and the *Symphyotrichum* spp. may have primarily bloomed after our surveys ended in late August.

				unseeded		seeded	
Species	Common Name	odds ratio	SD	% occurrence	SD	% occurrence	SD
Dalea candida	white prairie clover	26.64	2.88	0.00	0.00	0.32	1.57
Rudbeckia hirta	black eyed susan	19.25	1.19	0.67	2.35	10.64	20.58
Dalea purpurea	purple prairie clover	13.92	2.30	0.01	0.18	0.56	3.26
Desmodium canadense	showy tick trefoil	9.12	3.21	0.00	0.00	0.14	0.73
Asclepias tuberosa	butterfly milkweed	9.11	3.26	0.00	0.00	0.11	0.61
Agastache foeniculum	anise hyssop	8.81	2.01	0.03	0.46	0.47	2.51
*Medicago sativa**	Alfalfa*	6.82	1.11	2.61	10.84	12.95	27.63
Monarda fistulosa	Wild bergamot	6.63	1.28	0.35	2.07	1.96	6.07
Elymus canadensis	Canada wild rye (G)	6.31	1.15	0.98	6.50	6.97	18.19
*Trifolium pratense**	red clover*	5.02	1.21	1.78	7.14	5.55	15.91
Helenium autumnale	sneezeweed	3.68	3.45	0.03	0.45	0.21	1.02
Solidago spp.	goldenrod spp.	2.73	1.17	1.39	6.90	3.31	12.45
Verbena stricta	hoary vervain	2.46	1.34	0.40	2.19	1.00	3.19
Asclepias syriaca	common milkweed	2.28	1.96	0.09	0.73	0.40	1.36
Sorghastrum nutans	indian grass (G)	1.83	1.43	0.26	2.25	0.31	3.01
*Trifolium repens**	white clover*	1.49	1.20	1.55	9.15	2.33	11.70

Verbena hastata	blue vervain	1.48	1.42	0.45	3.08	0.75	2.00
*Bromus inermis**	smooth brome (G)*	1.38	1.07	14.84	23.15	16.22	21.91
Schizachyrium scoparium	little bluestem (G)	1.15	1.29	0.86	5.12	0.95	4.81
Astragalus canadensis	Canada milkvetch	1.14	2.29	0.12	1.38	0.10	0.61
Heliopsis helianthoides	false sunflower	1.09	1.91	0.20	1.07	0.25	1.16
Andropogon gerardii	big bluestem (G)	0.94	1.15	2.54	9.36	1.79	7.44
Zizia aurea	golden alexander	0.93	2.23	0.07	0.97	0.13	0.76
Panicum virgatum	switchgrass (G)	0.92	1.12	3.95	11.74	3.89	13.64
Anemone canadensis	Canada anemone	0.07	52.68	0.01	0.11	0.00	0.00
Thalictrum dasycarpum	tall meadow rue	0.00	19176.68	0.01	0.16	0.00	0.00
*Trifolium hybridum**	alsike clover*	0.00	19369.75	0.85	4.83	0.00	0.00

3.4 DISCUSSION

Seeding native forbs is necessary to get native forbs. Natural colonization is insufficient.

There are several lines of evidence from our results section that, together, show that seeding is an important prerequisite for establishment of native forbs. First of all, our statistical analysis demonstrates that the plant communities are significantly different between native and non-native seeded sites. The strongest evidence that seeding is necessary to get native forbs comes from our “seeding table” (Table 2), which shows the effect of seeding as odds ratios (likelihood a species is to be detected where it was seeded versus where it was not; an odds-ratio of 6 means a species is 6 times more likely to be found where seeded, odds-ratio of 1 means equal likelihood of presence where seeded and not). There are six species of native forbs that were at least 8 times as likely to be detected where they were seeded, and 11 species of native forbs that are at least twice as likely to be found where they were seeded versus where they were not (Table 2). Further evidence comes from Figures 1b, B1b, and B2b, which focuses on the native forb community in our sites. These figures clearly show that native seeded sites have more native floral coverage (Figure 2), more native forb species (Figure B1), and more native forb diversity (Figure B2) than the non-native and typical sites. The low level of native forbs in non-native and typical sites indicates native forbs are generally not colonizing roadside sites on their own. Possible reasons for this include that these species are not present in the local species pool, or that the species are poor colonizers (need a long time to establish), or that they are poor competitors (they are colonizing and being outcompeted). Previous experimental work on prairie plants suggests that non-native species tend to outcompete native species in nitrogen-rich soils (Gaya Shivega and Aldrich-Wolfe, 2017; Seabloom et al., 2003), and roadsides are known to accumulate nitrogen from exhaust (Bettez et al., 2013). Future work should strive to untangle the drivers for this lack of establishment and explore potentially counterproductive role fertilizing has on native species establishment. Table 1 shows the most common plants encountered at each of the three sites, and besides the annual fleabane (which is native), native forbs are not abundant in non-native and typical sites.

Many introduced species that were never planted are present at all types of sites. Native sites are not resistant to colonization by introduced species.

While seeding native forbs is a necessary prerequisite to establishment of native forbs (see above), native sites are not resistant to invasion. Though native forbs are much more common where natives are seeded, the floral community at all types of sites is predominantly introduced species (Table 1), and not necessarily introduced species that were intentionally planted. Many of the common forbs in our sites were never intentionally seeded there (e.g. birds foot trefoil, crown vetch, sweet clover species, thistle species, spotted knapweed). Reed canary grass was also not intentionally seeded in any sites yet was common in all three types of sites. Thus, even though there is a much higher chance to find a native forb in the native seeded sites, most of the flowering community in native seeded sites is still comprised of introduced species. Figure 2c, B1c, and B2c focuses on the introduced flowering forb community at all three site types. Native and non-native sites also have similar floral coverage of introduced species, which is slightly more than the typical sites (Figure 2). In addition, all sites showed similar richness (Figure B1) and diversity of introduced species (Figure B2).

The effect of seeding on the plant community is short lived. Many seeded plants establish but do not persist, and introduced species move into both native and non-native seeded sites.

There are several lines of evidence from our results that provide insights into how seeded communities change through time. It is important to note that this study did not follow projects across years, but instead relied on a sampling of sites spanning a range of years since planting. We use this variation in site age to assess age effects and find strong evidence for loss of native flowering forbs through time. Overall, our statistical tests indicate that site age significantly effects the plant community. Figure 3b, B3b, and B4b broadly demonstrate that younger native seeded sites have more native flowers than older native sites, indicating that native flowers are lost through time at native sites. Non-native sites of all ages have low native forbs. Similar patterns, showing a loss of floral coverage, richness, and diversity are apparent whether focusing on all types of flowers (panel A) or just introduced flowers (panel C) in Figure 3, B3 and B4. The PRC age plot (Figure 4a) shows that native seeded and non-native seeded plant communities are quite different at young sites (as the black line is far from the gray line early on). But overall, the plant communities converged at older sites (as the black line is close to the gray line after a few years). This pattern is likely because sites are losing some of the planted species that made them different (e.g. alfalfa in non-native plantings, black eyed Susan in native plantings) and gaining common invasive species (e.g. reed canary grass, birds foot trefoil) through time. The pattern of losing flowering species is not necessarily surprising as some of the plants in the seed mixes are early successional species or not intended to persist for years (e.g. alfalfa). Overall, sites tend to become more grass dominated with time, and these grass species may be outcompeting forbs (Figure B5). This is similar to general a pattern of succession in North American grasslands, where grasses come to dominate over time (Inouye et al., 1987). It suggests that future studies may need to consider other management techniques, such as controlled burning (Howe, 1995) or interseeding (Rossiter et al., 2016) if roadside managers wish to maintain higher densities of flowering plants for pollinators.

We highlight which plant species present in seed mixes are successfully establishing and which are not.

Our "seeding table" (Table 2) shows a ranked list of species that are most dependent on seeding for their establishment. The caption for Table 2 includes a list of species that were seeded, could have been detected (i.e. species that flower or grasses we surveyed for), but were never detected in our study. Importantly, we do not have equal representation of different seed mixes in our study (Table B1). Native plants such as wild bergamot and goldenrods responded well to seeding and were well used by pollinators (Chapter 2, 4). Non-native plants such as alfalfa and white and red clover responded fairly well to seeding and were well used by pollinators (Chapter 2, 4).

Seed mix cost

Seed mixes used in native sites in our study cost 3.2 times as much as those used in non-native sites. Using price estimates from 2019, the average price per acre for non-native seed mixes used in our study was $232 (range $139-480). The average price per acre for native seed mixes used in our study was $736 (range $558-1094). The specific seed mixes used at sites in our study and their associated costs are listed in Table B1.

Limitations to our study

As with any scientific investigation, our study had logistical limitations. Here we highlight some limitations in an effort to minimize misunderstandings related to the present study. Our study is not a randomized experiment, and thus native and non-native sites likely had differences beyond just the seed mixes used. In our results, these other drivers are confounded with apparent effects of seed mix choice. Specific guidelines outlining what criteria should be used (and may have been used in our sites) when deciding on seed mix design are outlined in MacDonagh and Hallyn, 2010.

Additionally, we are unaware of the precision of the seeding treatments that were used in these sites, and it is possible the original seeding was imprecise or seed movement occurred after seeding (e.g. a mower carry seeds from one site to another). We do not have detailed management histories (e.g. mowing regimes, burns) for all of our sites, and as such, that remains unaccounted for. It should be noted that the Washington County sites were mowed annually in August. We did not have the sufficient replication to address questions related to fertilizer treatments, erosion blankets, and other implementation techniques utilized at the time of construction. However, other research suggest nitrogen enriched soils may favor non-native species (Gaya Shivega and Aldrich-Wolfe, 2017; Seabloom et al., 2003). Finally, our study did not track individual sites over the course of years. A longitudinal study at specific sites could provide much more detail as to the successional changes in the plant communities over the course of years.

Vegetation surveys focused on actively flowering forbs. Accordingly, forbs that have longer flowering phenology (i.e. that flower over the course of months) are more likely to be detected, and plants that had short flowering phenology are less detectable. Some of our results (e.g. percent occurrence in Table 1) averages across space and time. For example, birds foot trefoil flowered over the course of months (and was therefore in a greater percentage of our quadrats), whereas goldenrod species only started flowering by our final round of surveys. Had our surveys continued into the fall or started earlier, this would have increased the percent occurrence and/or detection of certain species (early: *Penstemon grandifloras, Zizia aurea;* late: *Symphyotrichum* spp. *Helenium autumnale, Physostegia virginiana)*. Our focus on forbs and pollinators meant that we also had limited data on grasses. Grasses were only identified when they had seed heads present, and we only surveyed for eight common species of grasses. The goldenrods (*Soldiago* spp.) were identified at the genus level, and accordingly our analysis of goldenrods could include several species, some of which were seeded and some of which were not (e.g. *Solidago canadensis*). Finally, our study occurred during a drought year which may have disproportionately affected some plant species. For example, there were several sites visited in June where it was clear non-native clovers (*Trifolium* spp.) were present but had recently died and were not flowering. These species likely would have been more common in our surveys had it occurred in a wetter year.

Management recommendations

- Seeding native forbs is necessary for native forbs to establish
- Refining seed mixes to contain pollinator-friendly vegetation that also successfully establishes can reduce the cost of seed mixes. Consider bolstering non-native mixes with a few native forbs that have good seeding success. Seeded non-native forbs (e.g. some clovers, alfalfa) establish well, and are used by pollinators. They could be included in other non-native seed mixes.
- Continued intervention (e.g. invasive species control) and monitoring may be necessary to maintain high floral diversity in native sites.

CHAPTER 4: RELATIONSHIP BETWEEN THE FLORAL COMMUNITY AND BUMBLE BEES AND BUTTERFLIES

4.1 INTRODUCTION

4.1.1 Highlights

- Roadsides host diverse bumble bee and butterfly communities and provide important floral resources.

- More blooming flowers and greater floral diversity supports greater bumble bee and butterfly diversity in roadsides.

- Native flowers (and native seeded sites) do not attract a greater diversity of generalist bumble bees and butterflies than non-native flowers (and non-native sites). However, native flowers may be important for specialist pollinator species not examined in this study.

4.1.2 Abstract

Insects are declining, and loss of habitat and foraging resources is an important factor underlying their decline. Roadsides are publicly managed lands that can provide quality habitat for bumble bees and butterflies. Understanding how the floral community influences the bumble bee and butterfly community can provide insights into how to manage roadside vegetation to improve pollinator conservation. In this study, we leverage detailed plant surveys from roadsides (detailed in Chapter 3) to understand how the floral community influences the bumble bee and butterfly community. Roadside habitats in our study had diverse bumble bee and butterfly communities, including occasional sightings of the endangered rusty patched bumble bee. Our surveys revealed that bumble bee and butterfly diversity is positively correlated with floral resource abundance, and floral diversity. Sites with a greater proportion of the floral resources coming from native plants did not have higher bumble bee or butterfly diversity. However, several species of plants that were commonly used by feeding bees and butterflies were native. Our data suggests increasing the diversity of the floral community and abundance of floral resources in roadside habitats would benefit pollinators.

4.1.3 Introduction

Insect species are in worldwide decline (Hallmann et al., 2017; Wagner, 2020). These declines have been reported in many species of North American bumble bees (Cameron et al., 2011; Colla et al., 2012) and butterflies (Schultz et al., 2017; Wepprich et al., 2019). This trend is concerning because both insect groups are important parts of the ecosystem; they pollinate plants and are a source of food for other animals.

While many factors are contributed to the decline of pollinators, habitat loss and fragmentation are key contributors (Potts et al., 2010). One solution to combat pollinator habitat loss is to convert land currently unsuitable for pollinators, such as farmland or degraded land, into more suitable habitat such as prairie or savannah. Much is known about how the plant communities in prairie restorations influence their pollinator communities. Increased floral diversity promotes increased pollinator diversity (Kelleher and Choi, 2020; Lane et al., 2020). A greater quantity of flowers is associated with a greater abundance of native bees (Paterson et al., 2019). Additionally, in restorations with ongoing management, pollinator communities become more similar to those in remnant prairies with age (Griffin et al., 2017; Summerville et al., 2007; Tonietto et al., 2017).

Roadsides present a relatively unexploited opportunity for pollinator habitat restoration. With over 10 million acres in the United States alone, roadsides have the potential to combat pollinator habitat loss (Forman et al., 2003). Roadsides are unique in that they are composed of thin strips of habitat exposed to traffic and anthropogenic chemicals including road salts and heavy metals. In addition, roadside vegetation is primarily managed to maintain an unobstructed view for drivers. Therefore, our understanding of traditional prairie restorations may not necessarily transfer to roadside restorations. Previous studies have shown that increased forb richness and floral abundance in roadsides increase the species richness and abundance for both bee and butterfly communities (Hopwood, 2008; Ries et al., 2001; Saarinen et al., 2005). However, more work needs to be done to investigate the impact of native versus non-native floral resources on roadside bumble bees and butterflies and if non-native pollinator species are influenced by the floral community in the same way as native pollinator species. Additionally, more work needs to be done on how roadside restoration age influences pollinator communities, especially since roadsides often receive less management than prairie restorations. Furthermore, studies rarely compare how the bee and butterfly communities differ in their response to the floral community.

Bumble bees and butterflies differ fundamentally in their life history, which translates into different habitat conditions in roadsides. Bumble bees require bare ground for nesting as well as flowers to provide nectar and pollen as food sources. Butterflies require species-specific hostplants on which to lay their eggs as well as flowers to provide nectar for adults. However, while butterfly larvae are often specialists, adult butterflies and bumble bees are foraging generalists, meaning they are not restricted to specific species of flowers on which to feed.

In this study, we quantified the floral, bumble bee, butterfly, and other insect communities in roadsides in central and southern Minnesota. Because bumble bees and butterflies use roadsides in different ways, we analyzed how the floral community impacted them separately. Specifically, this study provides descriptive data on the bumble bee, butterfly, and other insect communities occurring in roadside habitats and addresses several research questions. 1) Does roadside seeding treatment affect bumble bee, butterfly, and other insect diversity? 2) Do more diverse roadside floral communities support more diverse bumble bee, butterfly, and other insect communities? 3) Is the quantity of floral resources in roadsides able to predict the diversity of pollinators present? 4) Does the proportion of the floral community which is native matter to native pollinators? 5) Does site age (time since reseeding) affect pollinator diversity? Together, answers to these questions provide insights into how we can improve management of roadsides for pollinators.

4.2 METHODS

4.2.1 Surveying methods

We conducted bumble bee and butterfly surveys within the same sites as the plant surveys in Chapter 3. For methods on site selection criteria and plant survey methods see section 3.2.1 and 3.2.2. Briefly, we visited 57 sites six times each over a three-month survey period in summer 2021.

We conducted independent bumble bee and butterfly surveys concurrently with the plant surveys. One person (Luke Tonsfeldt) surveyed bumble bees while another (Ashley Darst) surveyed butterflies simultaneously. At the start of each pollinator survey, we recorded the percent cloud cover, temperature, and wind speed. Surveys consisted of a modified Pollard walk (Pollard, 1977). We started a timer and began walking at a rate of approximately 0.5 m/s in a zig-zag pattern of transects from one end of the site to the other. We counted the butterflies and bumble bees within ~1m to either side of the surveyor's path, recording each individual species and the species of flower they were foraging on, if any. For the butterfly surveys, the surveyor paused at the middle of each transect, looked left and right across the entire site, and counted any large, flying butterflies present that were not previously counted. Large butterflies such as monarchs and swallowtails often flush beyond the ~1m search radius and so this modification is necessary to account for them as well. We netted and photographed butterflies that could not be reliably identified without capture. For the bumble bee survey, we netted the first individual we encountered of each species and transferred it into vials in a cooler with ice, and any other individuals which we were not confident of its identification. We photographed and released them at the end of the survey. We paused the timer during identification and photographing such that our surveys only included active search time for insects. After 15 minutes, or after the surveyor reached the end of the site, the survey ended. We did not restrict our surveys to certain weather conditions or times of day, with the exception that we did not survey during hard rains.

At each site we also performed sweep netting for other arthropods. Using a sweep net, we took 15 sweeps through the base up to the top of vegetation and transferred the contents into Ziploc bags which were frozen. We processed samples by removing the arthropods from the vegetation in the bag, and then identifying insects and spiders to order. We refer to the insects and spiders collected from sweep netting as "other insects" in this report.

4.2.2 Statistical methods

In Table 1, total abundance is the total number of each species/species group we observed during all surveys regardless of site seeding. We calculated the relative abundance of each species/species group by dividing the total abundance of each species/species group by the total abundance for all species/species groups. We calculated relative abundance separately for bumble bees and butterflies. In

Table 3, we used rank abundance to represent the presence of forb species in roadsides by listing the 15 most observed forb species across all sites in order of abundance. The rank abundance for foraging choice represents the 15 most observed forb species that either bumble bees, native butterflies, and non-native butterflies were seen foraging on, respectively. Abundance is the total number of quadrats containing each forb species. Number of sightings is the total number of times either a bumble bee or a butterfly was observed foraging on that forb species. We calculated the percent occurrence by dividing the abundance of each forb species by the total abundance of all forb species. We calculated the relative foragings by dividing the number of sightings on each forb species by the total number of foraging sightings on all forb species separately for bumble bees, native butterflies, and non-native butterflies.

All analyses were performed in R version 4.1.2. The Shannon diversity index for forb species, bumble bees, butterflies, and other insects was calculated using the vegan package. Shannon diversity is calculated by the following formula:

$$H = -\sum_{i=1}^{s} p_i \ln p_i$$

Where H is the diversity index, s is the number of species, and p_i is the proportion of individuals of each species belonging to the ith species of the total number of individuals. While forb, bumble bee, and butterfly Shannon diversity was calculated at the species level, other insect diversity was calculated at the order level. We used Shannon diversity to represent diversity in this study because it is equally sensitive to rare and abundant species (Morris et al., 2014).

We had five research questions, all related to how the plant community influenced bumble bee, butterfly, and other insect diversity which we addressed with similar statistical models. While we initially performed statistical models analyzing only native butterfly Shannon diversity as a response variable for all of the questions, the trends and significance of results were no different from the same analyses including both native and non-native butterfly species. Therefore, we included all species of butterflies in our analyses, except in Q4, which focused on the value of native vegetation for native butterflies.

Q1) To address whether seeding treatment (native seed mix, non-native seed mix, and "typical") affected bumble bee, butterfly, and other insect diversity, we used linear mixed effects models with bumble bee, butterfly, or other insect Shannon diversity as the response variable, seeding treatment as a fixed effect, and site nested within project as a random effect. For bumble bee and butterfly models, survey time was also included as a fixed effect. Additionally, linear mixed effects models were fitted for each arthropod order with abundance as the response variable, seeding treatment as a fixed effect, and site nested within project as a random effect.

Q2) To address whether sites with more floral diversity had more bumble bee, butterfly, and other insect diversity, we used linear mixed effects models with bumble bee, butterfly, or other insect Shannon diversity as the response variable, floral Shannon diversity (modeled as a continuous variable) as a fixed effect, and site nested within project as a random effect. For bumble bee and butterfly models, survey time was also included as a fixed effect.

Q3) To address whether sites with more floral abundance had more bumble bee and butterfly diversity, we used linear mixed effects models with bumble bee or butterfly Shannon diversity as the response variable, the proportion of quadrats containing at least one flower (modeled as a continuous variable) and survey time as fixed effects, and site nested within project as a random effect.

Q4) To address the importance of native flowers in attracting native bumble bees and butterflies, we calculated what proportion of flower-containing quadrats contained native flowers. For example, if a site had ten total quadrats, four of which contained at least one flower, but only two of which contained native flowers, the relevant proportion would be 0.5. Accordingly, we used linear mixed effects models with bumble bee or butterfly Shannon diversity as the response variable, proportion of flower-containing quadrats which contained native flowers (modeled as a continuous variable) and survey time as fixed effects, and site nested within project as a random effect.

Q5) To address whether site age influenced bumble bee and butterfly diversity, we used linear mixed effects models with bumble bee or butterfly Shannon diversity as the response variable; seeding treatment, site age, the interaction between seeding treatment and age, and survey time as fixed effects; and site nested within project as a random effect. This analysis was restricted to native and non-native seeded sites because typical sites were of unknown age.

4.3 RESULTS

4.3.1 Bumble bee and butterfly community composition

We observed a total of 993 bumble bees and 3,720 butterflies during roadside surveys (Table 1). We identified bumble bees and butterflies to the lowest possible taxonomic unit, which was usually but not always to the species level. We observed 10 different bumble bee species/species groups and 33 different butterfly species/species groups. Bumble bees and butterflies observed on the wing and unable to be captured for further identification were placed into “unknown” categories of the lowest identifiable taxonomic group. We had a taxonomic expert (Elaine Evans) verify our bumble bee voucher photographs, and we initially identified 96.0% of photographed bumble bees correctly. The bumble bees observed are all native. Two species of butterflies encountered are non-native; the European skipper (*Thymelicus lineola*) and cabbage white butterfly (*Pieris rapae*) are both native to Eurasia and North Africa. Together, they comprised 57.2% of our butterfly observations (Table 1). We ran the statistical tests associated with questions 1, 2, 3, and 5 both with and without these two introduced species in the dataset (Table C1). Inclusion of these two species did not qualitatively influence our results, and the results presented do include the two non-native butterflies (with the exception of question 4).

Table 4.1. Bumble bees and butterflies recorded during roadside surveys from May 28, 2021 to August 24, 2021. The total abundance is the total number of each species or species group we observed during all surveys regardless of site seeding. The relative abundance is the proportion of each species or species group relative to the total abundance of its respective group (bumble bees or butterflies). Abundance is

shown separately for the number of species and species groups we observed in sites planted with native seed mixes and those planted with non-native seed mixes. Non-native pollinators are denoted by (*) and endangered/threatened pollinators are bolded.

Species	**Total Abundance**	**Relative Abundance**	**Abundance in roadsides planted with native seed mixes**	**Abundance in roadsides planted with non-native seed mixes**
Bumble bee (*Bombus*)				
B. impatiens	**401**	0.404	165	119
B. rufocinctus	**210**	0.211	62	121
B. bimaculatus	**120**	0.121	77	36
B. vagans	**55**	0.055	23	17
B. auricomus/pensylvanicus	**31**	0.031	8	22
B. griseocollis	**22**	0.022	13	5
B. citrinus	**4**	0.004	0	1
B. affinis	**3**	0.003	1	2
B. fervidus	**2**	0.002	1	0
B. borealis	**1**	0.001	0	1
Unknown *Bombus sp.*	**144**	0.145	53	61
Total	**993**	**1.000**	**403**	**385**
Butterfly (Papilionoidea)				
*Thymelicus lineola**	**1655**	0.445	377	418
*Pieris rapae**	**472**	0.127	174	158
Everes comyntas	**394**	0.106	195	129
Coenonympha tullia	**371**	0.100	87	75

Ancyloxypha numitor	**176**	0.047	75	60
Colias philodice	**164**	0.044	50	91
Danaus plexippus	**91**	0.024	40	32
Polites peckius	**89**	0.024	32	33
Anatrytone logan	**55**	0.015	16	20
Phyciodes selenis/tharos	**42**	0.011	18	13
Satyrodes eurydice	**29**	0.008	13	7
Asterocampa celtis	**22**	0.006	0	0
Speyeria cybele	**17**	0.005	7	5
Colias eurytheme	**12**	0.003	6	4
Celastrina ladon	**11**	0.003	2	1
Cercyonis pegala	**9**	0.002	4	4
Papilio glaucus/canadensis	**8**	0.002	4	1
Lycaena hyllus	**7**	0.002	0	4
Satyrium acadica	**7**	0.002	6	1
Limenitis archippus	**6**	0.002	3	2
Polites mystic	**6**	0.002	3	2
Lycaena dione	**5**	0.001	0	5
Boloria bellona	**4**	0.001	3	0
Enodia anthedon	**3**	0.001	3	0
Atalopedes campestris	**2**	0.001	0	2
Lycaena phlaeas	**2**	0.001	0	1
Pompeius verna	**2**	0.001	0	1
Vanessa atalanta	**2**	0.001	0	1
Nymphalis antiopa	**1**	0.0003	1	0
Epargyreus clarus	**1**	0.0003	0	1
Polites themistocles	**1**	0.0003	0	1
Papilio glaucus/polyxenes	**1**	0.0003	0	0
Satyrium caryaevorum	**1**	0.0003	0	0
Unknown skipper sp.	**38**	0.0102	19	10
Unknown sulphur sp.	**7**	0.0019	4	1
Unknown fritillary sp.	**4**	0.0011	1	1
Unknown blue sp.	**3**	0.0008	1	1
Total	**3720**	**1.0000**	**1144**	**1085**

We encountered the endangered rusty patched bumble bee (*B. affinis*) six times throughout the summer, but only three times during official surveying (Table 2). The other three individuals were encountered either before or after the 15-minute surveying period and not included in analyses.

Table 4.2. Occurrences of the endangered rusty patched bumble bee (*Bombus affinis*). Asterisks denote individuals that we observed outside of the official 15-minute surveying time. If the individual was foraging, the plant it was foraging on was recorded as the forage plant species. All the plants *B. affinis* individuals were observed foraging on are non-native to Minnesota.

Date	**County**	**Site**	**Latitude**	**Longitude**	**Sex**	**Forage plant species**
7/8/2021*	Scott	2nat	44.689218	-93.489646	female	
7/9/2021	Carver	7non	44.791242	-93.634736	female	*Medicago sativa*
7/12/2021	Washington	16nat	44.887906	-92.924699	female	*Hypericum perforatum*
7/13/2021*	Washington	13non	45.05713	-92.832987	female	
7/14/2021*	Washington	15nat	45.008425	-92.863173	female	*Melilotus alba*
8/9/2021	Washington	16non	44.890692	-92.924188	queen	*Medicago sativa*

89% of bumble bees and 16% of butterflies were foraging on flowers (Table 3). We highlight what plant species were most common in our vegetation surveys, and what plant species were most commonly used for foraging by bumble bee and butterflies in Table 3.

Table 4.3. Foraging observations for bumble bees and butterflies during roadside surveys from May 28, 2021 to August 24, 2021. Rank abundance was used to represent the presence of forb species in roadsides by listing the 15 most observed forb species across all sites in order of abundance. The rank abundance for foraging choice represents the 15 most observed forb species that either bumble bees or butterflies were seen foraging on, respectively. Abundance is the total number of quadrats containing each forb species. Number of sightings is the total number of times either a bumble bee or a butterfly was observed foraging on that forb species. Percent occurrence is the proportion of the abundance of each forb species relative to the total abundance of all forb species. Relative foragings is the proportion of the number of foraging sightings on each forb species relative to the total number of foraging sightings for either bumble bees or butterflies.

	Presence of Forb			***Bombus* foraging choice**			**Native butterfly foraging choice**			**Non-native butterfly foraging choice**		
Rank Abundance	**Species**	**Abundance**	**Percent occurrence**	**Forage plant species**	**# sightings**	**Relative foragings**	**Forage plant species**	**# sightings**	**Relative foragings**	**Forage plant species**	**# sightings**	**Relative foragings**
1	*Lotus corniculatus*	1149	0.19	*Centaurea stoebe*	203	0.23	*Lotus corniculatus*	45	0.31	*Lotus corniculatus*	247	0.54
2	*Medicago sativa*	536	0.09	*Monarda fistulosa**	132	0.15	*Medicago sativa*	12	0.08	*Leucanthemum vulgare*	86	0.19
3	*Melilotus alba*	365	0.06	*Lotus corniculatus*	87	0.10	*Centaurea stoebe*	12	0.08	*Medicago sativa*	42	0.09
4	*Cirsium arvense*	354	0.06	*Securigera varia*	87	0.10	*Trifolium pratense*	10	0.07	*Trifolium pratense*	13	0.03
5	*Rudbeckia hirta**	316	0.05	*Melilotus alba*	77	0.09	*Cirsium vulgare*	8	0.06	*Berteroa incana*	12	0.03
6	*Securigera varia*	315	0.05	*Cirsium arvense*	76	0.09	*Cirsium arvense*	7	0.05	*Cirsium arvense*	11	0.02
7	*Melilotus officinalis*	257	0.04	*Hypericum perforatum*	43	0.05	*Monarda fistulosa**	6	0.04	*Centaurea stoebe*	7	0.02
8	*Erigeron annuus**	240	0.04	*Sonchus arvensis*	31	0.03	*Trifolium hybridum*	5	0.03	*Asclepias syriaca**	5	0.01
9	*Berteroa incana*	196	0.03	*Astragalus canadensis**	24	0.03	*Echinacea angustifolia / purpurea**	5	0.03	*Astragalus canadensis**	5	0.01
10	*Centaurea stoebe*	188	0.03	*Lythrum salicaria*	21	0.02	*Leucanthemum vulgare*	4	0.03	*Securigera varia*	5	0.01
11	*Trifolium pratense*	178	0.03	*Cirsium vulgare*	18	0.02	*Berteroa incana*	4	0.03	*Trifolium hybridum*	5	0.01
12	*Trifolium repens*	163	0.03	*Solidago spp.**	14	0.02	*Melilotus alba*	4	0.03	*Euphorbia virgata*	3	0.01
13	*Solidago spp.**	160	0.03	*Medicago sativa*	13	0.01	*Melilotus officinalis*	3	0.02	*Erigeron strigosus**	2	0.004
14	*Leucanthemum vulgare*	109	0.02	*Agastache foeniculum**	10	0.01	*Apocynum cannabinum**	3	0.02	*Lythrum salicaria*	2	0.004
15	*Potentilla norvegica**	83	0.01	*Trifolium pratense*	7	0.01	*Trifolium repens*	2	0.01	*Melilotus alba*	2	0.004

Other	1324	0.22	Other	44	0.05	Other	13	0.09	Other	11	0.02
Total	**5933**	**1.00**	**Total**	**887**	**1.00**	**Total**	**143**	**1.00**	**Total**	**458**	**1.00**

*native forb

4.3.2 Influence of the forb community on bumble bee and butterfly communities

Q1) The seeding treatment planted in roadsides did not affect the diversity of bumble bees, butterflies, nor other insects (Figures 1 & 2). There was no difference in the Shannon diversity index for bumble bees, butterflies, nor other insects between sites planted with native seed mixes and those planted with non-native seed mixes (bumble bees: P = 0.5191; butterflies: P = 0.6923; other insects: P = 0.5259; Table C1-3). However, the Shannon diversity index for both bumble bees and butterflies was lower in typical roadsides, although this trend was insignificant in butterflies (bumble bee: P = 0.0073; butterflies: P = 0.0946; Table C1-2).

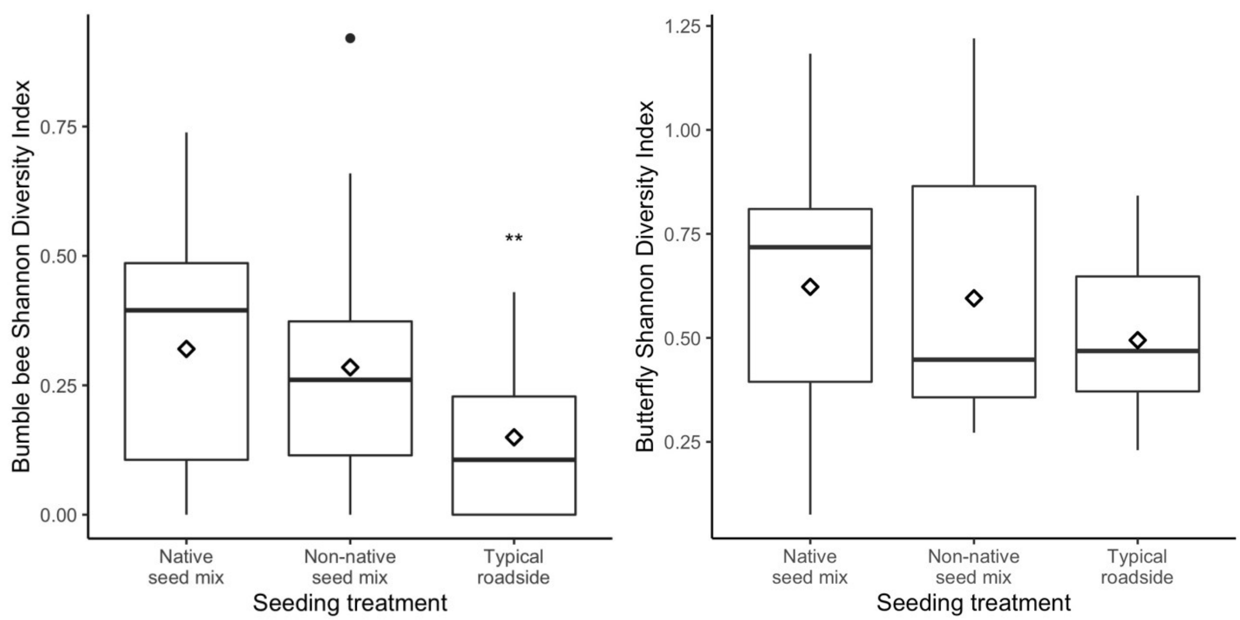

Figure 4.1. Diversity of bumble bees and butterflies in roadside sites planted with native seed mixes, non-native seed mixes, and typical roadsides. Open diamonds represent mean Shannon diversity index.

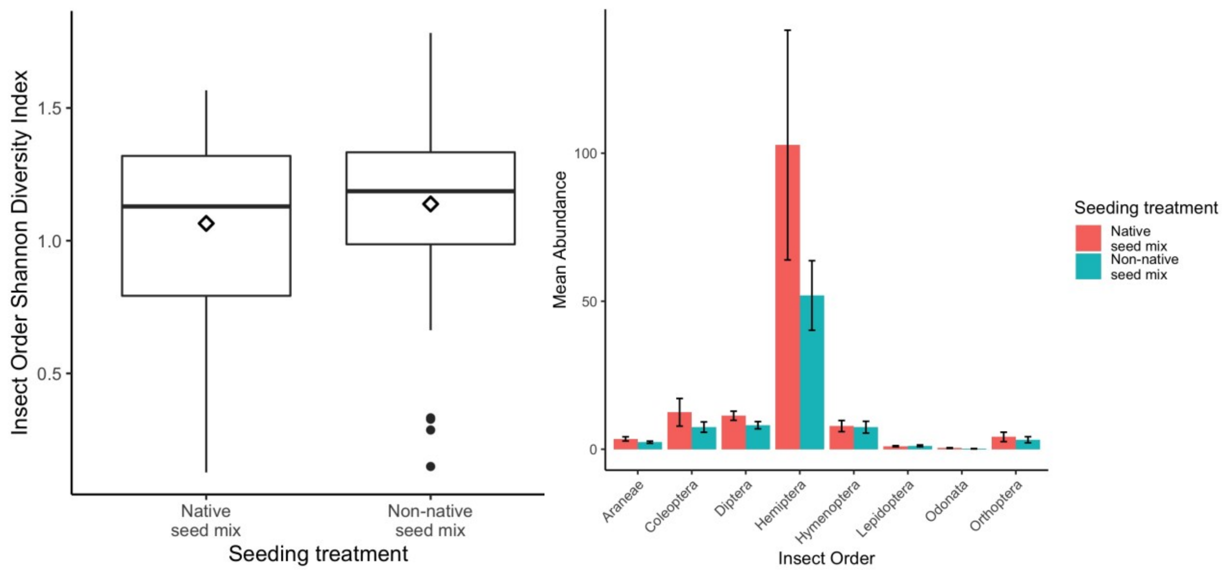

Figure 4.2. Diversity and abundance broken down by order of insects in roadside sites planted with native seed mixes and non-native seed mixes. Shannon diversity was calculated using arthropods identified to order which were collected from sweep netting. Open diamonds represent mean Shannon diversity index. Mean abundance represents the mean abundance of insects or spiders in each order over all three sweep netting surveys. Error bars show standard error.

Q2) Roadsides with greater floral diversity had greater bumble bee and butterfly diversity (Figure 3), but other insect diversity did not depend on plant diversity (Figure 4). The Shannon diversity index for bumble bees and butterflies both increased with the Shannon diversity index for floral plant species (bumble bees: slope±SE = 0.21±0.04, P = 0.0000; butterflies: slope±SE = 0.15±0.04, P = 0.0012; Table C1-2). However, the Shannon diversity index for other insects did not significantly change with the Shannon diversity index for forb and grass species (slope±SE = -0.002±0.02, P = 0. 9260; Table C3).

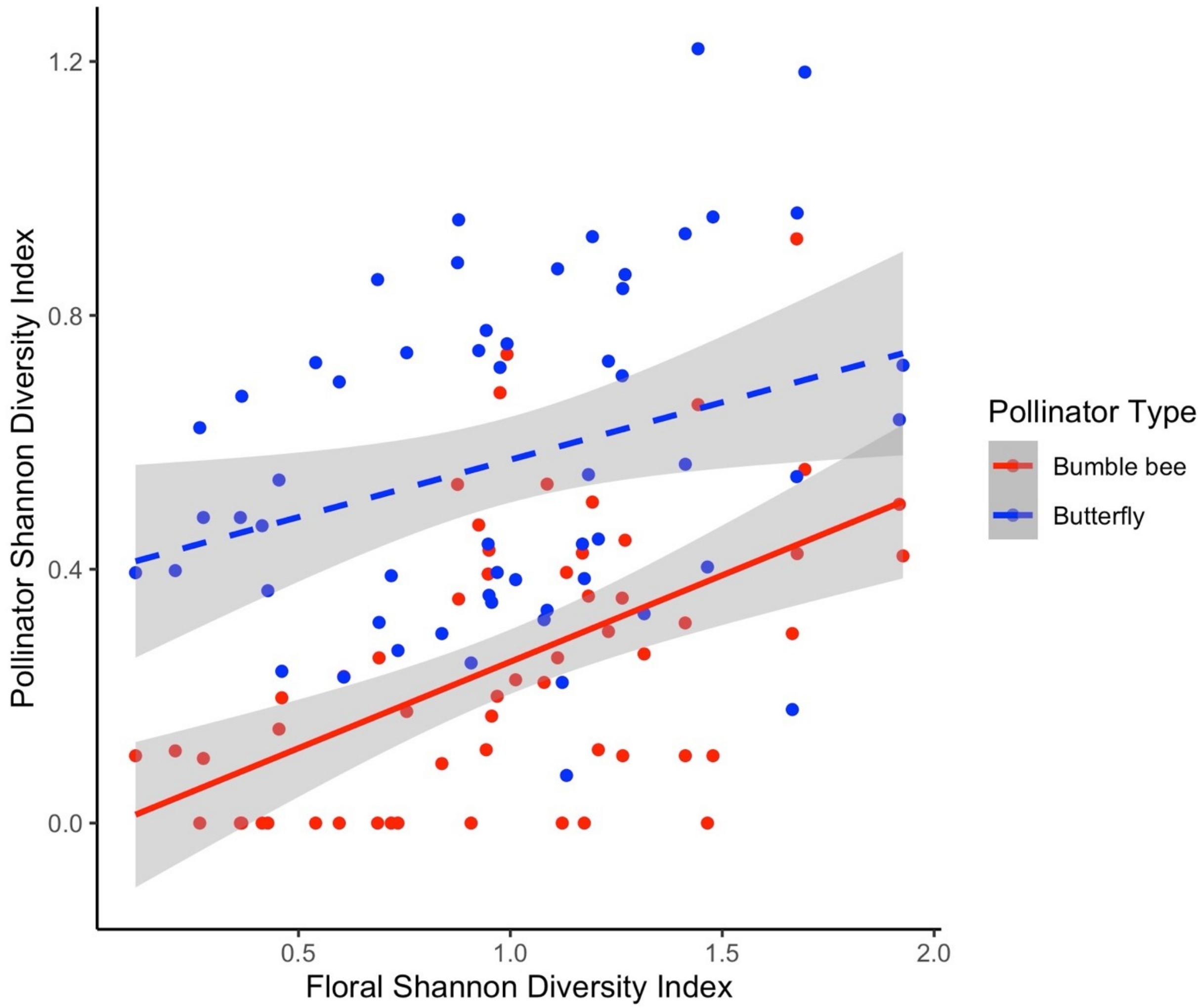

Figure 4.3. Bumble bee and butterfly with increasing roadside floral diversity. Diversity was measured using Shannon's diversity index. Each point represents the mean pollinator and floral diversity measured at one site over all six surveys. Each line is a simple linear trendline and the shaded area is a 95% confidence interval. Both bumble bee (red) and butterfly (blue) diversity increased with floral diversity.

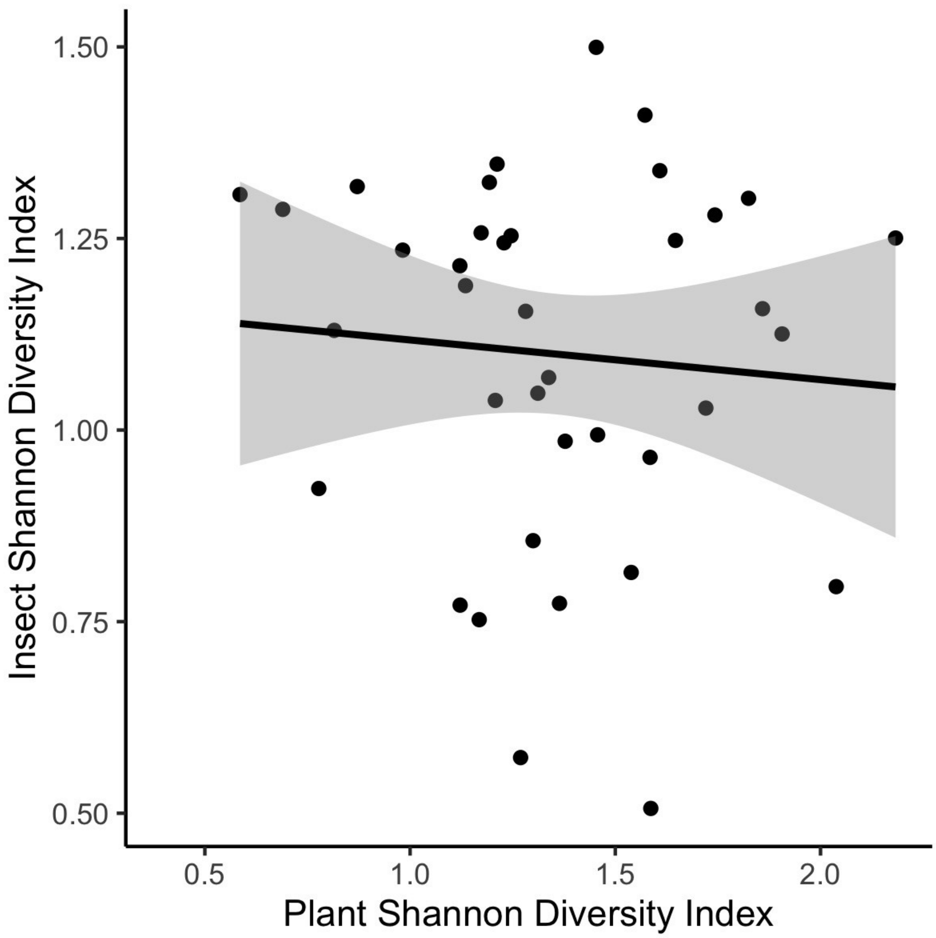

Figure 4.4. Insect diversity with increasing roadside plant diversity. Diversity was measured using Shannon's diversity index at the species level for plants and the order level for arthropods. Each point represents the mean insect and plant diversity measured at one site over three surveys. Each line is a simple linear trendline and the shaded area is a 95% confidence interval. Insect diversity did not significantly change with plant diversity.

Q3) Likewise, roadsides with more blooming flowers had greater bumble bee and butterfly diversity (Figure 5). The mean Shannon diversity index for both bumble bees and butterflies increased with the mean proportion of quadrats containing flowers (bumble bees: slope±SE = 0.58±0.07, P = 0.0000 butterflies: slope±SE = 0.23±0.10, P = 0.0207; Table C1-2).

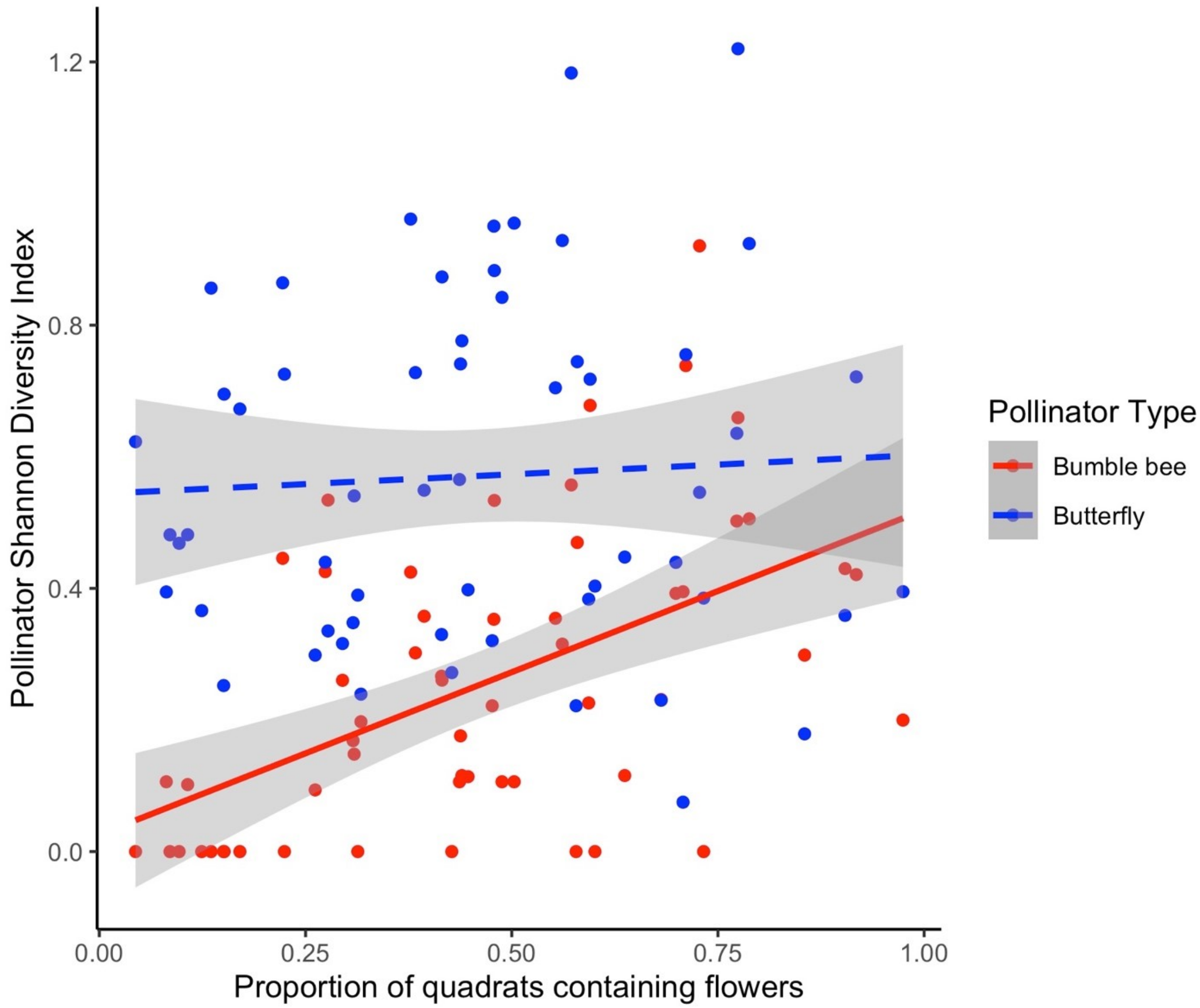

Figure 4.5. Bumble bee and butterfly diversity with increasing available floral resources. Diversity was measured using Shannon's diversity index. Each point represents the mean pollinator diversity and mean proportion of quadrats containing blooming flowers measured at one site over all six surveys. Each line is a simple linear trendline and the shaded area is a 95% confidence interval. Both bumble bee (solid red) and butterfly (dashed blue) diversity increased with increasing availability of floral resources.

Q4) Though roads with more blooming flowers attracted more bumble bee and butterfly species, it did not matter whether those flowers were native (Figure 6). Our analysis showed roadsides with a greater proportion of native blooming flowers did not support greater bumble bee nor native butterfly diversity (bumble bees: slope±SE = 0.09±0.08, P = 0.2437; butterflies: slope±SE = 0.05±0.09, P = 0.6062; Table C1-2).

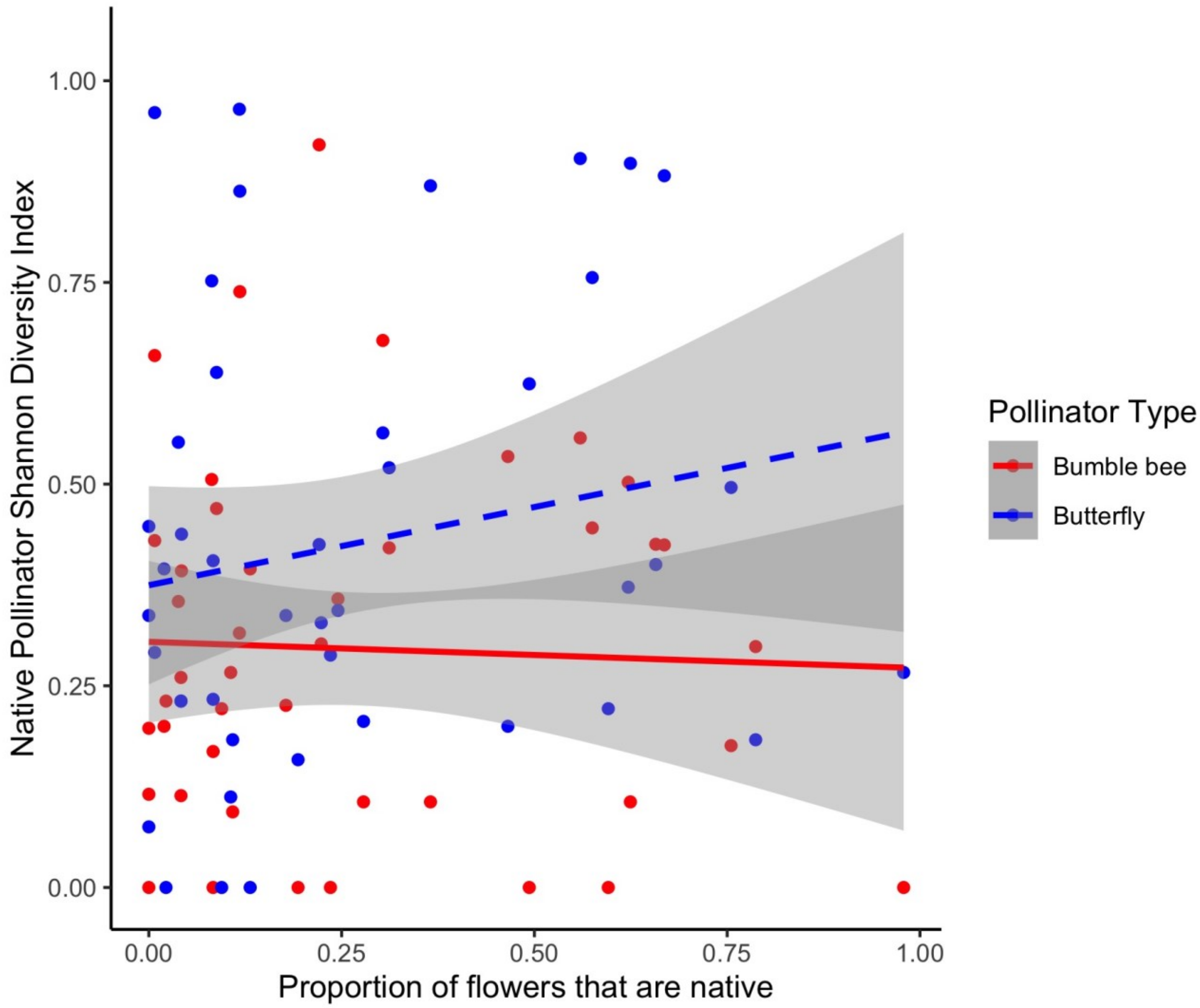

Figure 4.6. Native bumble bee and butterfly diversity with an increasing availability of native floral resources. Diversity was measured using Shannon's diversity index. Each point represents the mean pollinator diversity and mean proportion of quadrats with blooming flowers that contain natives measured at one site over all six surveys. Each line is a simple linear trendline and the shaded area is a 95% confidence interval. Neither bumble bee (solid red) nor butterfly (dashed blue) diversity changed with increasing availability of native floral resources.

Q5) The age of roadsides since seeding did not affect the diversity of the bumble bees and butterflies present (Figure 7). The Shannon diversity index for bumble bees (slope±SE = -0.007±0.009, P = 0.4672; Table C2) and butterflies (slope±SE = 0.005±0.01, P = 0.6347; Table C1) was not affected by site age, and the relationship between age and diversity was not dependent on treatment (bumble bees = slope±SE = -0.005±0.01, P = 0.6246; butterflies = slope±SE = 0.002±0.01, P = 0.8851, Table C1-2).

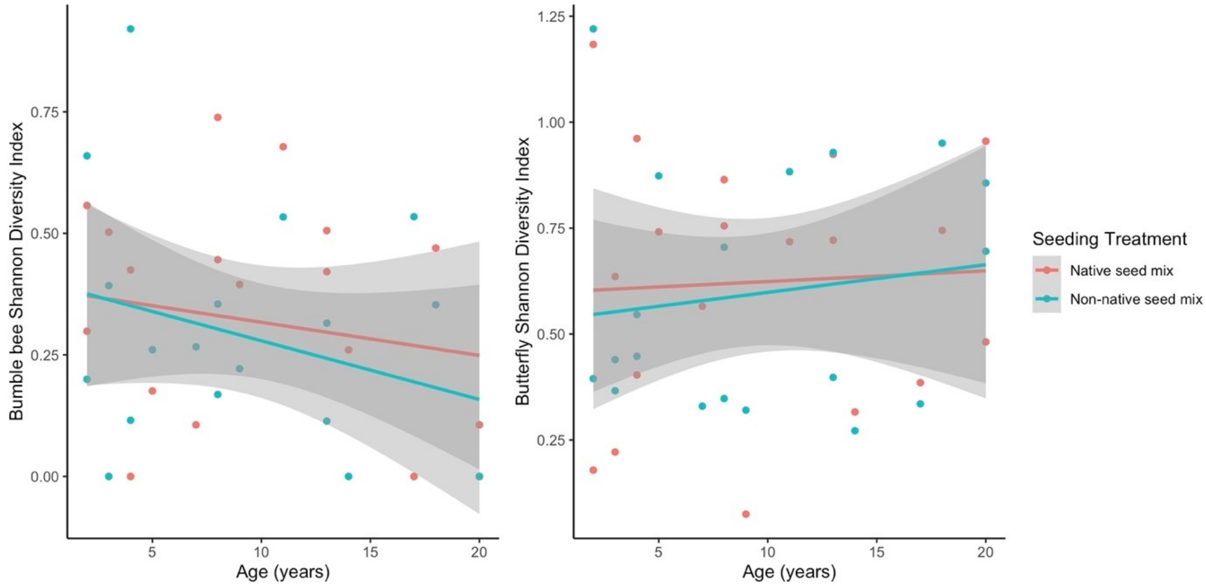

Figure 4.7. Bumble bee and butterfly diversity with roadside site age since seeding. Diversity was measured using Shannon's diversity index. Each point represents the mean pollinator diversity at one site over all six surveys. Each line is a simple linear trendline and the shaded area is a 95% confidence interval. Because typical sites have unknown history (and accordingly, no known "age") they are not included in this plot.

4.4 DISCUSSION

Roadsides host diverse bumble bee and butterfly communities and provide floral resources

A diversity of bumble bee and butterfly species are using roadsides in Minnesota (Table 1), including species of conservation concern. While we only encountered the rusty patched bumble bee six times throughout the summer (Table 2), monarch butterflies were within the top ten most common butterflies. However, the roadside butterfly community is dominated by two non-native species. The non-native European skipper (*Thymelicus lineola*) and cabbage white butterfly (*Pieris rapae*) accounted for 57.2% of all butterflies observed. As larvae, they are economically important pests of Timothy grass (*Phleum*) and plants in the mustard family (Brassicaceae) such as rapeseed, respectively (Scott, 1986). Additionally, these non-native species may compete with native species for resources such as nectar and host plants, or spread disease (Chew, 1981; Mallinger et al., 2017). Though they are non-native, these species may also provide pollination services and be important prey for other animals.

Roadsides are also supporting many native butterfly species, including several of conservation concern. The declining monarch was the fifth most abundant species along roadsides, which is not surprising given that their host plant, milkweeds, can reach high densities along Minnesota roadsides (Cariveau et al., 2019; Kasten et al., 2016). Roadsides in this study also supported individuals of several other native butterfly species that are suffering significant declines in the Midwest, including the American copper (*Lycaena phlaeas*), common wood nymph (*Cercyonis pegala*), tawny-edged skipper (*Polites themistocles*), meadow fritillary (*Boloria bellona*), orange sulphur (*Colias eurytheme*), long dash (*Polites mystic*), great spangled fritillary (*Speyeria cybele*), viceroy (*Limenitis archippus*), and hackberry emperor (*Asterocampa celtis*) (Table 2, Wepprich et al., 2019)). It is evident that bumble bees and butterflies are present in roadsides, and it is therefore important to understand how they are using them (Scott, 1986).

Bumble bees and butterflies use a diversity of forbs in roadsides as food sources. We observed more bumble bees (89.3%) than butterflies (16.2%) foraging (Table 3), suggesting that while bumble bees predominately use roadsides to forage, butterflies may use roadsides for other reasons, such as mating, laying eggs on hostplants, and shelter. As foraging generalists, the bumble bees and butterflies in this study do not require specific flowers on which to feed. Therefore, it is not surprising that the bumble bees and butterflies in this study foraged on forbs approximately proportional to their abundance in roadsides. However, there were some notable exceptions. While non-native spotted knapweed (*Centaurea stoebe*) only occurred in 3% of quadrats, it accounted for 23% of foragings by bumble bees and 8% of foragings by native butterflies. Additionally, native wild bergamot (*Monarda fistulosa*) only occurred in 1% of quadrats but accounted for 15% of foragings by bumble bees and 4% of foragings by native butterflies. Therefore, wild bergamot is a good candidate to include in native seed mixes if the goal is to attract and provide floral resources for bumble bees. However, the majority of forage plant species used by bumble bees and butterflies are non-native, suggesting that non-native forbs can provide nectar and pollen resources for generalist pollinators in roadsides. This includes planted non-native forbs such as alfalfa (*Medicago sativa*) as well as colonizing forbs such as bird's foot trefoil (*Lotus corniculatus*).

More blooming flowers and greater floral diversity supports greater bumble bee and butterfly diversity in roadsides.

Floral resources in roadsides are important for bumble bees and butterflies. Roadsides with a greater proportion of blooming flowers and greater floral diversity supported more diverse bumble bee and butterfly communities (Figures 3 & 5). Both effects were stronger in bumble bees than butterflies. This is likely due to bumble bees using roadsides predominantly to forage while butterflies may use roadsides for other reasons, as discussed previously. Therefore, we expect butterfly diversity to be dependent not only on the proportion and diversity of blooming flowers, but on the diversity of non-blooming host plants as well as the quality of habitat for mating and resting, such as protection from predation and adverse environmental conditions. However, since we only surveyed blooming forbs and common grasses with seed heads, our dataset is not suitable to answer these questions. Bumble bees may also use roadsides for nesting in addition to foraging, however we were not searching for these belowground bumble bee nests in our study. Regardless of other factors that may drive bumble bee and butterfly diversity in roadsides, roadsides with more blooming flowers and a greater diversity of flowers support a greater diversity of both bumble bees and butterflies.

Native flowers (and native seeded sites) do not attract a greater diversity of generalist bumble bees and butterflies than non-native flowers (and non-native sites). However, native flowers may be important for specialist pollinator species not examined in this study.

There are two lines of evidence from our results that suggest roadsides with more native flowers do not support a greater diversity of bumble bees and butterflies. First, we show that bumble bee and butterfly diversity were not different between native and non-native seeded sites (Figure 1), even though native sites have a greater proportion of native flowers (Chapter 3: Figure 2). Second, we ignored the sites' seeding history, and simply asked whether there is a relationship between bumble bee and native butterfly diversity and the proportion of flowers that were native. This analysis showed that bumble bee and native butterfly diversity were not affected by the proportion of flowers that were native (Figure 6). In other words, "more native" sites did not support more diverse bumble bee and native butterfly communities. Therefore, whether flowers in roadsides are native does not appear to be important for the native generalist bumble bee and butterfly species in this study. However, as previously noted, certain native species of flowers are important forage species, such as wild bergamot. Additionally, native species may be important as host plants for native butterflies. Furthermore, the diversity of specialist pollinator species, which require specific forage species, in roadsides is likely dependent on specific native forbs. Previous studies have shown that while generalist pollinator species, such as those in our study, use non-native forbs, specialist pollinators are associated with native forbs (Lopezaraiza–Mikel et al., 2007; Seitz et al., 2020). Since our study only surveyed foraging generalist bumble bee and butterfly species, we cannot conclude that native forbs are unimportant for all pollinator species.

The diversity of bumble bees and butterflies is not affected by roadside age since seeding.

The age of roadsides since seeding did not significantly affect the diversity of bumble bees nor butterflies. Older roadsides contained less flowers and less diversity of forbs (Chapter 3: Figures 3 & B4), and bumble bee and butterfly diversity increased with the proportion of flowers present and floral diversity (Figures 3 & 5). Therefore, we expected to see bumble bee and butterfly diversity decrease with roadside restoration age. However, the majority of the sites in this study were less than 10 years old, reducing the statistical power of analyses that included site age. Our results contrast studies in prairie restorations in which pollinator communities increase in diversity with restoration age (Griffin et al., 2017; Summerville et al., 2007). This may be due to management differences. Prairie restorations are often managed by removing non-native plants, occasional burning and/or grazing, and reseeding when needed. In contrast, management of roadsides, including those in our study, is often limited to annual mowing. Therefore, continual management of roadside restorations as is done in prairie restorations may be beneficial to maintaining and increasing bumble bee and butterfly diversity in older roadside restorations.

Limitations

There are limitations to this study. We did not limit bumble bee and butterfly surveys to ideal weather conditions nor time of day. However, flower detection is not weather nor time-of-day dependent. Therefore, we may not have observed any bumble bees nor butterflies in a site with abundant floral resources due to weather conditions. Because of this, we cannot analyze the data on a fine-scale level in which we look at a particular site on a particular date. Instead, we averaged across all six surveys conducted at each site. Additionally, we only surveyed bumble bee and butterfly species. Other important pollinators, such as other types of bees, wasps, moths, flies, and ants, were only analyzed at the order level in this study and certain species may have been missed in sweep netting altogether. Additionally, species within these orders may exhibit different trends in response to the plant community that we were unable to detect. Particularly, the bumble bees and butterflies in this study are foraging generalists. Specialist pollinator species require specific forage species and are therefore likely more dependent on native forbs than the pollinators in this study. We also only surveyed plants with blooming flowers; therefore, we are unable to determine how host plants affect butterfly diversity, since while butterflies are foraging generalists, they often require specific host plants on which to lay their eggs. In conclusion, this study is designed to assess how the overall floral community in roadsides affects the overall bumble bee and butterfly communities and is not conclusive of the pollinator community as a whole.

In summary, many bumble bee and butterfly species are present in roadsides and foraging on roadside flowers. Whether these flowers are native or non-native is unimportant for the generalist species in this study. Despite this, a greater proportion of blooming flowers and greater floral diversity supports greater bumble bee and butterfly diversity in roadsides. Additionally, older roadside restorations do not have less bumble bee nor butterfly diversity than younger restorations. We suggest that future seed mixes contain a diversity of forbs and that roadside restorations are maintained to ensure the establishment and maintenance of these forbs if the goal is to increase bumble bee and butterfly diversity within roadsides.

Management suggestions

- Plant native forage species important to bumble bees and butterflies, such as wild bergamot (*Monarda fistulosa*).
- When native seeding is not an option, plant readily-establishing non-native forage species used by bumble bees and butterflies, such as alfalfa (*Medicago sativa*) and red clover (*Trifolium pratense*).
- Plant seed mixes containing a diversity of forbs.
- Manage roadside restorations to ensure the establishment and maintenance of diverse forbs.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

Across our two studies, we see several overarching conclusions. First, bumble bee and butterfly communities are more abundant and diverse in roadsides with abundant and diverse floral resources. Bumble bee communities are also more abundant and diverse in roadsides with nearby pollinator and bumble bee-friendly habitat designations. Second, seeding roadsides with native plants increases the representation of native flowering plants, although their abundance declines over time. There is variation across species in terms of establishment in roadsides, with some having a relatively high payoff for inclusion in seed mixes, and others rarely if ever showing up, suggesting possible revisions to roadside seed mixes to reduce their cost. Finally, roadsides were particularly vulnerable to invasion by non-native plants, and management for native plant communities will likely require more active interventions such as mowing, nitrogen reduction, or selective herbicide treatment.

5.2 RECOMMENDATIONS

(1) Pollinator and bumble bee-friendly land cover designations can help to prioritize areas for restoration (Ch. 2). We found that habitat designated as pollinator friendly was positively associated with overall bumble bee abundance, and with refinement of the land cover designations to include habitats previously identified as being associated with bumble bees, we found a positive association with bumble bee richness as well. However, we found no association between either land cover designation or estimated abundances for individual bumble bee species. More refinement of land cover designations to include species-specific habitat requirements, particularly nesting habitat, could be needed to make predictions for particular bumble bee species.

(2) Across Chapter 2 and 4, we see that a primary predictor of pollinator abundance and diversity is the abundance of diversity of flowering plants (regardless of whether they are native). This suggests a strategy of “more flowers everywhere” could benefit pollinators without raising costs. Seed mixes that contain mostly grasses or non-native species could be bolstered with flowering forbs that thrive in roadsides (see point 3)

(3) Results on plant establishment and pollinator use can be combined to refine seed mixes for roadsides. Bumble bees and butterflies often use a wide variety of seeded forbs, some of which established well after seeding. Native plants such as wild bergamot (*Monarda fistulosa),* milkweed (*Asclepias syriaca*) and goldenrod (*Solidago* spp.), and non-natives such as alfalfa (*Medicago sativa*) and clovers (*Trifolium* spp.) generally responded well to seeding and were used by pollinators.

(4) If roadside managers want native plant communities along roadsides, they need to be planted (and managed (see point 5). Native plants are more likely to be found in areas where they were seeded, suggesting a payoff of seeding during revegetation. However, all seeded species were not equally likely to establish in roadsides, suggesting refinement of seed mix composition could maximize benefits of planting. Native species such as prairie clover (*Dalea* spp.), anise hyssop (*Agastache foeniculum*), and showy tick trefoil (*Desmodium canadense*) were especially enriched in native roadside restorations. Several species included in roadside mixes seemed to fail to establish, including stiff sunflower (*Helianthus pauciflorus*), blazing star (*Liatris* spp.), large-flowered beard tongue (*Penstemon grandifloras*), and obedient plant (*Physostegia virginiana*). However, it is worth noting that several plants on our "failure list" were potentially missed because they bloom late (e.g., *Symphyotrichum* spp., which blooms in early fall).

(5) The "set it and forget it" approach to native roadside revegetation efforts is insufficient if long-term establishment of native plants is the goal. Native sites are rapidly overwhelmed by introduced species such as Canada thistle, birds foot trefoil, and reed canary grass. Sustained management efforts to control invasive species will be required to establish and maintain native plant communities. Much of the broader restoration ecology literature shows continued targeted management is important if native plant communities are the goal. With respect to roadside restoration, future research efforts might consider the most effective management strategies for prioritizing native species over non-natives, whether that is reduced nitrogen inputs, mowing regime, herbicide use, or controlled burns.

(6) Our time series of roadside sites showed older sites had fewer flowering plants. Despite this, pollinator communities were not affected by site age. Future research could consider how roadside management tools such as mowing or re-seeding promotes flowering plant diversity past the first decade of planting.

Our results overall suggest roadside management for natives and pollinators requires some discussions within agencies and management organizations about primary goals. If the primary goal is supporting pollinator communities, planting and managing for diverse flowering plant communities should be a primary target. If restoration funds are limited in availability, placing roadside pollinator plantings within landscapes containing pollinator habitat, and refining seed mixes to species that establish well may maximize restoration benefits. However, our data call into question whether supporting bumble bees and butterflies requires plantings of native plants. Other benefits of native plant communities, such as reducing soil erosion, increasing carbon capture, improving water filtration, and supporting species of conservation concern, such as specialist pollinators and herbivores, may be more relevant justification for restoration of roadside native plant communities. Better record keeping during establishment and management coupled with monitoring can help elucidate how interventions along roadsides influence plant communities and resources for pollinators.

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APPENDIX A

SUPPLEMENT TO CHAPTER 2

Table A1. Summary of land cover across 27 sites for pollinator-friendly and bumble-bee friendly land cover maps.

	Not pollinator friendly	Pollinator friendly	Not bumble bee friendly	Bumble bee friendly
Grassland	**11.229%**	**23.185%**	**0.965%**	**33.448%**
11% to 25% impervious cover with perennial grasses	0.000%	1.108%	0.000%	1.108%
11% to 25% impervious with grasses and sparse trees	0.372%	0.000%	0.000%	0.372%
4% to 10% impervious with perennial grasses,sparse trees	0.338%	0.000%	0.000%	0.338%
4% to 10% impervious cover with perennial grasses	0.000%	0.166%	0.000%	0.166%
Altered/non-native grassland with sparse trees - saturated	0.008%	0.000%	0.000%	0.008%
Non-native grassland with sparse trees - temporarily flooded	0.000%	0.060%	0.000%	0.060%
Dry oak savanna	0.000%	0.009%	0.000%	0.009%
Dry oak savanna sand-gravel subtype	0.000%	0.119%	0.000%	0.119%
Dry prairie bedrock bluff subtype	0.000%	0.032%	0.000%	0.032%
Dry prairie hill subtype	0.000%	0.007%	0.000%	0.007%
Dry prairie sand-gravel subtype	0.000%	0.069%	0.000%	0.069%
Grassland w sparse mixed trees - altered/non-native	0.000%	3.285%	0.000%	3.285%
Grassland with sparse trees - non-native dominated veg	0.000%	3.565%	0.000%	3.565%
Intermittently exposed non-native dominated vegetation	0.050%	0.000%	0.000%	0.050%
Long grasses and forbs on upland soils	0.000%	0.073%	0.000%	0.073%
Long grasses and mixed trees with 11-25% impervious cover	0.000%	0.041%	0.000%	0.041%
Long grasses and mixed trees with 4-10% impervious cover	0.000%	0.292%	0.000%	0.292%
Long grasses and mixed trees with 51-75% impervious cover	0.007%	0.000%	0.000%	0.007%
Long grasses on hydric soils	0.181%	0.000%	0.000%	0.181%
Long grasses on upland soils	2.148%	0.000%	0.000%	2.148%
Long grasses with sparse tree cover on hydric soils	0.024%	0.000%	0.000%	0.024%
Long grasses with sparse tree cover on upland soils	1.540%	0.000%	0.000%	1.540%

Medium-tall grass altered/non-native dominated grassland	4.332%	0.000%	0.000%	4.332%
Mesic prairie	0.000%	0.092%	0.000%	0.092%
Non-native long grasses with 11-25% impervious cover	0.054%	0.000%	0.000%	0.054%
Non-native long grasses with 4-10% impervious cover	0.027%	0.000%	0.000%	0.027%
Saturated altered/non-native dominated graminoid vegetation	0.964%	0.000%	0.964%	0.000%
Seasonally flooded non-native dominated emergent veg	1.006%	0.000%	0.000%	1.006%
Seepage meadow	0.000%	0.015%	0.000%	0.015%
Short grasses and forbs on upland soils	0.000%	0.206%	0.000%	0.206%
Short grasses and mixed trees with 11-25% impervious cover	0.000%	6.002%	0.000%	6.002%
Short grasses and mixed trees with 4-10% impervious cover	0.000%	2.876%	0.000%	2.876%
Short grasses on hydric soils	0.091%	0.000%	0.000%	0.091%
Short grasses on upland soils	0.000%	1.948%	0.000%	1.948%
Short grasses with 11-25% impervious cover	0.000%	0.285%	0.000%	0.285%
Short grasses with 4-10% impervious cover	0.000%	0.411%	0.000%	0.411%
Short grasses with 51-75% impervious cover	0.044%	0.000%	0.000%	0.044%
Short grasses with sparse tree cover on hydric soils	0.000%	0.003%	0.000%	0.003%
Short grasses with sparse tree cover on upland soils	0.000%	2.119%	0.000%	2.119%
Tall grass altered/non-native dominated grassland	0.043%	0.000%	0.000%	0.043%
Temporarily flooded altered/non-native dominated grassland	0.000%	0.382%	0.000%	0.382%
Temporarily flooded graminoid vegetation	0.001%	0.000%	0.001%	0.000%
Wet prairie	0.000%	0.019%	0.000%	0.019%
Wooded	**13.589%**	**10.091%**	**0.828%**	**22.852%**
11% to 25% impervious cover with coniferous and/or deciduous shrubs	0.000%	0.002%	0.000%	0.002%
11% to 25% impervious cover with deciduous trees	0.357%	0.000%	0.000%	0.357%
11% to 25% impervious cover with mixed trees	0.337%	0.000%	0.000%	0.337%
4% to 10% impervious cover with mixed shrubs and trees	0.000%	0.010%	0.000%	0.010%
4% to 10% impervious cover with coniferous trees	0.044%	0.000%	0.000%	0.044%
4% to 10% impervious cover with deciduous trees	0.275%	0.000%	0.000%	0.275%
4% to 10% impervious cover with mixed trees	0.000%	0.132%	0.000%	0.132%

Alder swamp	0.000%	0.767%	0.000%	0.767%
Alder swamp - saturated soils	0.000%	0.002%	0.000%	0.002%
Altered/non-native deciduous forest	1.608%	0.000%	0.000%	1.608%
Altered/non-native deciduous woodland	2.861%	0.000%	0.000%	2.861%
Altered/non-native deciduous woodland - saturated	0.030%	0.000%	0.000%	0.030%
Altered/non-native deciduous woodland - seasonally flooded	0.015%	0.000%	0.000%	0.015%
Altered/non-native deciduous woodland - temporarily flooded	0.016%	0.000%	0.000%	0.016%
Altered/non-native dominated saturated shrubland	0.000%	0.032%	0.000%	0.032%
Altered/non-native dominated seasonally flooded shrubland	0.014%	0.000%	0.000%	0.014%
Altered/non-native semipermanently flooded shrubland	0.000%	0.009%	0.000%	0.009%
Altered/non-native dominated temporarily flooded shrubland	0.006%	0.000%	0.000%	0.006%
Altered/non-native dominated upland shrubland	0.125%	0.000%	0.000%	0.125%
Altered/non-native mixed woodland	0.086%	0.000%	0.000%	0.086%
Altered/non-native seasonally flooded deciduous forest	0.002%	0.000%	0.000%	0.002%
Aspen (forest, woodland) with 4-10% impervious cover	0.000%	0.004%	0.000%	0.004%
Aspen forest	0.000%	0.045%	0.000%	0.045%
Aspen forest - saturated soils	0.007%	0.000%	0.000%	0.007%
Aspen forest - temporaily flooded	0.000%	0.012%	0.000%	0.012%
Aspen woodland	0.000%	0.252%	0.000%	0.252%
Aspen-birch forest northern hardwoods subtype	0.000%	0.022%	0.000%	0.022%
Black ash swamp	0.000%	0.033%	0.000%	0.033%
Black ash swamp - seasonally flooded	0.075%	0.000%	0.000%	0.075%
Boxelder-green ash (forest) with 4-10% impervious cover	0.000%	0.082%	0.000%	0.082%
Coniferous trees on upland soils (nursery stock)	0.828%	0.000%	0.828%	0.000%
Deciduous trees on upland soils	0.000%	0.038%	0.000%	0.038%
Eastern Red Cedar woodland	0.000%	0.003%	0.000%	0.003%
Floodplain forest	0.121%	0.000%	0.000%	0.121%
Floodplain forest silver maple subtype	0.189%	0.000%	0.000%	0.189%
Jack pine barrens with 51-75% impervious cover	0.008%	0.000%	0.000%	0.008%

Jack pine forest jack pine-fir subtype	0.004%	0.000%	0.000%	0.004%
Lowland hardwood forest	0.000%	0.408%	0.000%	0.408%
Maple-basswood (forest) with 11- 25% impervious cover	0.000%	0.004%	0.000%	0.004%
Maple-basswood (forest) with 4-10% impervious cover	0.000%	0.050%	0.000%	0.050%
Maple-basswood forest	0.000%	0.528%	0.000%	0.528%
Mixed hardwood swamp	0.000%	0.214%	0.000%	0.214%
Mixed hardwood swamp seepage subtype	0.041%	0.000%	0.000%	0.041%
Mixed pine-hardwood forest	0.000%	0.064%	0.000%	0.064%
Native dominated disturbed upland shrubland	0.015%	0.000%	0.000%	0.015%
Native dominated temporarily flooded shrubland	0.000%	0.009%	0.000%	0.009%
Northern hardwood (forest) with 11- 25% impervious cover	0.000%	0.053%	0.000%	0.053%
Northern hardwood (forest) with 4-10% impervious cover	0.008%	0.000%	0.000%	0.008%
Northern hardwood forest	0.000%	0.056%	0.000%	0.056%
Oak (forest or woodland) with 11- 25% impervious cover	0.370%	0.000%	0.000%	0.370%
Oak (forest or woodland) with 4-10% impervious cover	0.566%	0.000%	0.000%	0.566%
Oak forest	0.000%	0.651%	0.000%	0.651%
Oak forest dry subtype	0.000%	1.763%	0.000%	1.763%
Oak forest mesic subtype	0.000%	4.121%	0.000%	4.121%
Oak forest red maple subtype	0.000%	0.398%	0.000%	0.398%
Oak woodland-brushland	1.620%	0.000%	0.000%	1.620%
Other deciduous trees with 11- 25% impervious cover	0.063%	0.000%	0.000%	0.063%
Other deciduous trees with 4-10% impervious cover	0.129%	0.000%	0.000%	0.129%
Other planted conifers with 11- 25% impervious cover	0.198%	0.000%	0.000%	0.198%
Planted mixed coniferous/deciduous trees with 11-25% impervious cover	0.542%	0.000%	0.000%	0.542%
Planted mixed coniferous/deciduous trees with 4-10% impervious cover	0.707%	0.000%	0.000%	0.707%
Red pine trees on upland soils	0.214%	0.000%	0.000%	0.214%
Spruce/fir trees on upland soils	0.178%	0.000%	0.000%	0.178%
Tamarack swamp	0.008%	0.000%	0.000%	0.008%

Tamarack swamp minerotrophic subtype	0.270%	0.000%	0.000%	0.270%
Tamarack swamp seepage subtype	0.055%	0.000%	0.000%	0.055%
Tamarack swamp sphagnum subtype	0.049%	0.000%	0.000%	0.049%
Temporarily flooded deciduous woodland	0.000%	0.023%	0.000%	0.023%
Upland soils with planted, maintained or cultivated deciduous trees	0.029%	0.000%	0.000%	0.029%
Upland soils w maintained mixed trees	0.239%	0.000%	0.000%	0.239%
Upland soils w maintained or cultivated mixed shrub/vine	0.043%	0.000%	0.000%	0.043%
Upland soils with maintained, or cultivated coniferous trees	1.230%	0.000%	0.000%	1.230%
White pine trees on upland soils	0.008%	0.000%	0.000%	0.008%
White pine-hardwood (forest) with 4-10% impervious cover	0.000%	0.022%	0.000%	0.022%
Willow swamp	0.000%	0.224%	0.000%	0.224%
Willow swamp - saturated soils	0.000%	0.060%	0.000%	0.060%
Crops	**20.378%**	**0.000%**	**18.617%**	**1.761%**
All other close grown cropland on upland soils	0.312%	0.000%	0.312%	0.000%
Corn	4.583%	0.000%	4.583%	0.000%
Fallow	0.206%	0.000%	0.206%	0.000%
Hayfield	1.720%	0.000%	0.000%	1.720%
Hydric soils - close grown cropland	0.026%	0.000%	0.026%	0.000%
Hydric soils - row cropland	0.275%	0.000%	0.275%	0.000%
Oats	0.016%	0.000%	0.016%	0.000%
Other vegetable and truck crops	0.041%	0.000%	0.000%	0.041%
Soybeans	0.458%	0.000%	0.458%	0.000%
Upland soils - close grown cropland	0.770%	0.000%	0.770%	0.000%
Upland soils - cropland	11.897%	0.000%	11.897%	0.000%
Wheat	0.073%	0.000%	0.073%	0.000%
Developed	**14.045%**	**0.000%**	**5.191%**	**8.854%**
0% to 10% impervious cover-exposed earth	0.090%	0.000%	0.090%	0.000%
11% to 25% impervious cover-exposed earth	0.099%	0.000%	0.099%	0.000%
26% to 50% impervious with mixed shrubs	0.021%	0.000%	0.000%	0.021%

26% to 50% impervious cover with mixed shrubs,sparse trees	0.011%	0.000%	0.000%	0.011%
26% to 50% impervious cover with deciduous trees	0.143%	0.000%	0.000%	0.143%
26% to 50% impervious cover with mixed trees	0.659%	0.000%	0.000%	0.659%
26% to 50% impervious cover with perennial grasses	0.134%	0.000%	0.000%	0.134%
26% to 50% impervious with perennial grasses,sparse trees	0.359%	0.000%	0.000%	0.359%
26% to 50% impervious cover-exposed earth	0.187%	0.000%	0.187%	0.000%
51% to 75% impervious cover with deciduous trees	0.031%	0.000%	0.000%	0.031%
51% to 75% impervious cover with perennial grasses	0.154%	0.000%	0.000%	0.154%
51% to 75% impervious with perennial grasses ,sparse trees	0.010%	0.000%	0.000%	0.010%
76% to 90% impervious cover	0.040%	0.000%	0.040%	0.000%
91% to 100% impervious cover	0.023%	0.000%	0.023%	0.000%
Boxelder-green ash (forest) with 26-50% impervious cover	0.001%	0.000%	0.000%	0.001%
Buildings and pavement with 76-90% impervious cover	0.299%	0.000%	0.299%	0.000%
Buildings and pavement with 91-100% impervious cover	1.027%	0.000%	1.027%	0.000%
Buildings with 76-90% impervious cover	0.027%	0.000%	0.027%	0.000%
Buildings with 91-100% impervious cover	0.062%	0.000%	0.062%	0.000%
Exposed earth	0.000%	0.000%	0.000%	0.000%
Hydric soils with maintained grasses and sparse tree cover	0.018%	0.000%	0.000%	0.018%
Oak (forest or woodland) with 26-50% impervious cover	0.078%	0.000%	0.000%	0.078%
Other deciduous trees with 26-50% impervious cover	0.053%	0.000%	0.000%	0.053%
Other exposed/transitional land with 0-10% impervious cover	0.454%	0.000%	0.000%	0.454%
Other exposed/transitional land with 11-25% impervious	0.112%	0.000%	0.000%	0.112%
Other exposed/transitional land with 26-50% impervious.	0.272%	0.000%	0.000%	0.272%
Pavement with 76-90% impervious cover	0.740%	0.000%	0.740%	0.000%
Pavement with 91-100% impervious cover	1.977%	0.000%	1.977%	0.000%
Planted mixed trees with 26-50% impervious	0.038%	0.000%	0.000%	0.038%
Planted or maintained grasses	0.048%	0.000%	0.048%	0.000%
Sand and gravel pits with 0-10% impervious cover	0.567%	0.000%	0.567%	0.000%
Sand flats temporarily flooded	0.005%	0.000%	0.005%	0.000%

Short grasses and mixed trees with 26-50% impervious cover	3.288%	0.000%	0.000%	3.288%
Short grasses and mixed trees with 51-75% impervious cover	1.993%	0.000%	0.000%	1.993%
Short grasses with 26-50% impervious cover	0.060%	0.000%	0.000%	0.060%
Upland soils with planted or maintained grasses	0.343%	0.000%	0.000%	0.343%
Upland soils with planted or maintained grasses and forbs	0.209%	0.000%	0.000%	0.209%
Upland soils with maintained grasses and sparse tree cover	0.413%	0.000%	0.000%	0.413%
Wetland	**6.529%**	**0.909%**	**4.165%**	**3.274%**
Cattail marsh - intermittently exposed	0.007%	0.000%	0.000%	0.007%
Cattail marsh - permanently flooded	0.124%	0.000%	0.000%	0.124%
Cattail marsh - saturated soils	0.101%	0.000%	0.000%	0.101%
Cattail marsh - seasonally flooded	0.207%	0.000%	0.000%	0.207%
Cattail marsh - semipermanently flooded	0.745%	0.000%	0.000%	0.745%
Floating algae	0.025%	0.000%	0.025%	0.000%
Floating algae - intermittently exposed aquatic bed	0.007%	0.000%	0.007%	0.000%
Floating vascular vegetation	0.159%	0.000%	0.000%	0.159%
Floating vascular veg - intermittently exposed aquatic bed	0.278%	0.000%	0.000%	0.278%
Floating vascular veg - intermittently exposed littoral bed	0.033%	0.000%	0.000%	0.033%
Intermittently exposed aquatic bed	0.017%	0.000%	0.000%	0.017%
Lake (lacustrine)	0.116%	0.000%	0.116%	0.000%
Limnetic open water	2.009%	0.000%	2.009%	0.000%
Littoral open water	0.152%	0.000%	0.152%	0.000%
Midwest pondweed submerged aquatic wetland	0.078%	0.000%	0.000%	0.078%
Mixed emergent marsh	0.085%	0.000%	0.000%	0.085%
Mixed emergent marsh - intermittently exposed	0.026%	0.000%	0.000%	0.026%
Mixed emergent marsh - permanently flooded	0.009%	0.000%	0.000%	0.009%
Mixed emergent marsh - seasonally flooded	0.305%	0.000%	0.000%	0.305%
Northern water lily aquatic wetland	0.011%	0.000%	0.000%	0.011%
Open sphagnum bog intermediate subtype	0.000%	0.004%	0.000%	0.004%
Palustrine open water	1.273%	0.000%	1.273%	0.000%

Permanently flooded altered/non-native dominated vegetation	0.031%	0.000%	0.000%	0.031%
Permanently flooded aquatic bed	0.006%	0.000%	0.000%	0.006%
Rich fen	0.000%	0.026%	0.000%	0.026%
Rich fen floating-mat subtype - permanently flooded	0.032%	0.000%	0.000%	0.032%
Rich fen floating-mat subtype - saturated soils	0.000%	0.008%	0.000%	0.008%
Rich fen sedge subtype	0.000%	0.497%	0.000%	0.497%
Rich fen shrub subtype	0.000%	0.155%	0.000%	0.155%
Semipermanently flooded altered/non-native dominated veg	0.107%	0.000%	0.000%	0.107%
Slow moving linear open water habitat	0.584%	0.000%	0.584%	0.000%
Standing water hydromorphic rooted vegetation	0.003%	0.000%	0.000%	0.003%
Wet meadow	0.000%	0.127%	0.000%	0.127%
Wet meadow - seasonally flooded	0.000%	0.049%	0.000%	0.049%
Wet meadow shrub subtype	0.000%	0.042%	0.000%	0.042%
Wetland-open water (palustrine)	0.045%	0.000%	0.000%	0.045%

Table A2. Plant species blooming in transects at 30 study sites. Ana= Anacardiaceae, Api=Apiaceae, Apo=Apocynaceae, Ast=Asteraceae, Bra=Brassicaceae, Car=Caryophylaceae, Cor=Cornaceae, Con=Convolvulaceae, Eup=Euphorbiaceae, Fab=Fabaceae, Hyp=Hyperiaceae, Lam=Lamiaceae, Lyt=Lythraceae, Ona=Onagraceae, Oxa=Oxalidaceae, Pla=Plantagiaceae, Pol=Polygoniaceae, Prim=Primulaceae, Ranunculaceae=Ran, Ros=Rosaceae, Rub=Rubiaceae, Scr=Scrophulaceae, Sol=Solanaceae, Ver=Verbenaceae

Family	Plant species	Family	Plant species	Family	Plant species
Ana	*Rhus glabra*	Ast	*Sonchus arvensis*	Lam	*Lycopus americanus*
Api	*Cicuta maculata*	Ast	*Symphyotrichum latifolia*	Lam	*Mentha arvensis*
Api	*Daucus carota*	Ast	*Symphyotrichum* sp.	Lam	*Monarda fistulosa*
Api	*Pastinaca sativa*	Ast	*Tanacetum vulgare*	Lam	*Nepeta cataria*
Api	*Torilis arvensis*	Ast	*Taraxacum officinale*	Lam	*Origanum vulgare*
Api	*Torilis japonica*	Bra	*Barbarea* sp.	Lam	*Prunella vulgaris*
Apo	*Apocynum androsaemifolium*	Bra	*Berteroa incana*	Ona	*Oenothera biennis*
Apo	*Apocynum cannabinum*	Bra	*Brassica nigra*	Oxa	*Oxalis dillenii*
Apo	*Asclepias incarnata*	Bra	*Thlaspi arvense*	Oxa	*Oxalis stricta*
Apo	*Asclepias syriaca*	Car	*Dianthus armeria*	Pla	*Digitalis lanata*
Apo	*Asclepias verticillata*	Car	*Silene latifolia*	Pol	*Persicaria pensylvanica*
Ast	*Achillea millefolium*	Car	*Myosoton aquaticum*	Pol	*Rumex crispus*
Ast	*Arctium minus*	Car	*Stellaria aquatica*	Pri	*Lysimachia terrestris*
Ast	*Carduus acanthoides*	Con	*Convolvulus arvensis*	Ran	*Ranunculus acris*
Ast	*Carduus nutans*	Cor	*Cornus racemosa*	Ran	*Thalictrum dasycarpum*
Ast	*Centaurea stoebe*	Eup	*Euphorbia corollata*	Ros	*Geum aleppicum*
Ast	*Cirsium arvense*	Eup	*Euphorbia esula*	Ros	*Potentilla norvegica*
Ast	*Cirsium discolor*	Fab	*Astragalus canadensis*	Ros	*Rosa* sp.
Ast	*Cirsium vulgare*	Fab	*Dalea purpurea*	Rub	*Galium boreale*
Ast	*Crepis tectorum*	Fab	*Desmodium canadense*	Scr	*Verbascum thapsus*
Ast	*Echinocystis lobata*	Fab	*Lathyrus sylvestris*	Scr	*Veronicastrum virginicum*
Ast	*Erigeron annuus*	Fab	*Linaria vulgaris*	Sol	*Solanum canadensis*
Ast	*Erigeron canadensis*	Fab	*Lotus corniculatus*	Sol	*Solanum dulcamara*
Ast	*Erigeron strigosus*	Fab	*Medicago lupulina*	Ver	*Verbena hastata*
Ast	*Grindelia squarrosa*	Fab	*Medicago sativa*	Ver	*Verbena stricta*
Ast	*Helianthus pauciflorus*	Fab	*Melilotus alba*		
Ast	*Heliopsis helianthoides*	Fab	*Melilotus officinalis*		
Ast	*Heliopsis pauciflorus*	Fab	*Securigera varia*		
Ast	*Lactuca canadensis*	Fab	*Trifolium arvense*		
Ast	*Lactuca serriola*	Fab	*Trifolium hybridum*		
Ast	*Leucanthemum vulgare*	Fab	*Trifolium pratense*		
Ast	*Matricaria discoidea*	Fab	*Trifolium repens*		
Ast	*Ratibida pinnata*	Fab	*Vicia villosa*		
Ast	*Rudbeckia hirta*	Hyp	*Hypericum perforatum*		

Ast	*Solidago canadensis*	Lyt	*Lythrum salicaria*

APPENDIX B
SUPPLEMENT TO CHAPTER 3

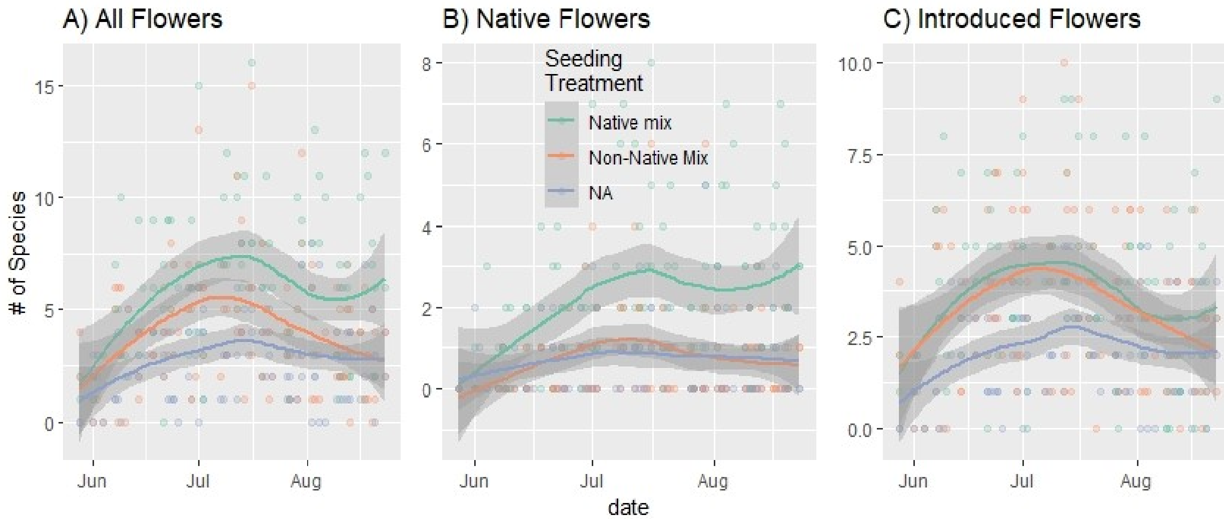

Figure B1: Seasonal change in the number of species (richness) of A) all flowering forbs, B) native flowering forbs, and C) introduced flowering forbs, broken down by seeding treatment. Lines represent an average computed by a Loess smoother and shaded area is the 95% confidence interval.

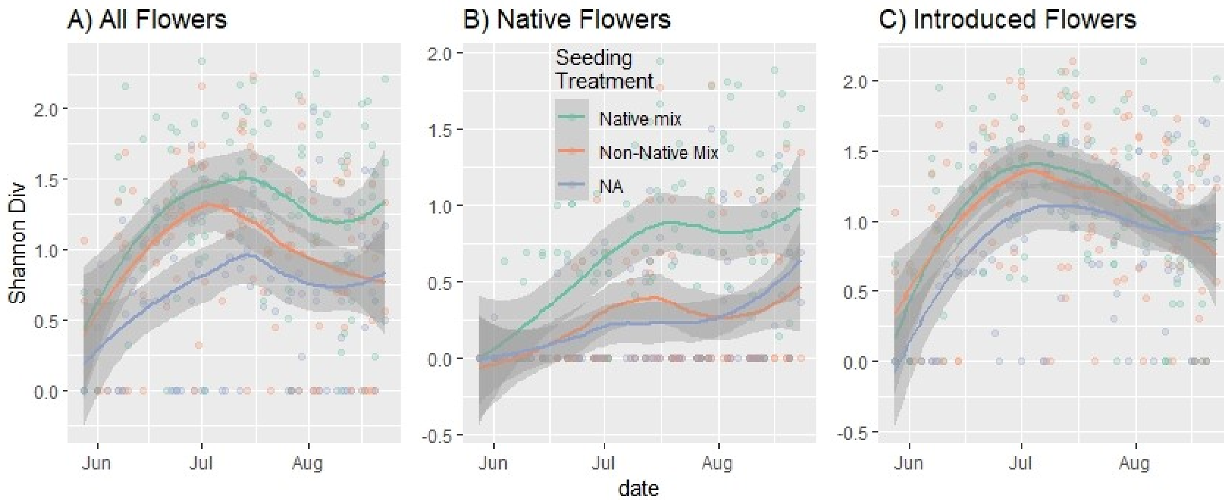

Figure B2. Seasonal change in the Shannon Diversity Index of A) all flowers, B) native flowers, and C) introduced flowers. Lines represent an average computed by a Loess smoother and shaded area is the 95% confidence interval (CI).

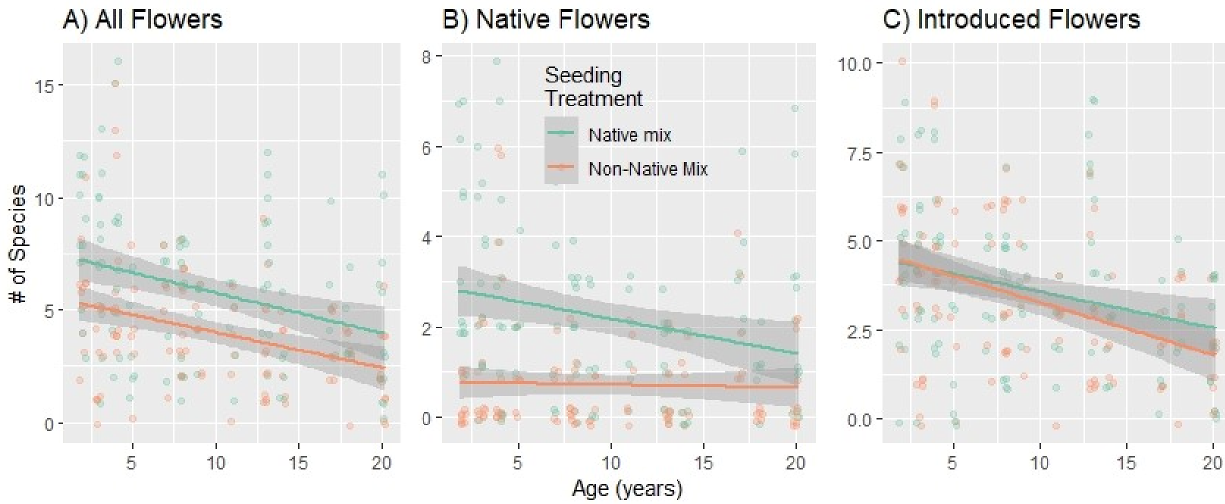

Figure B3: Change in the Richness of sites displayed as time since seeding (e.g site age, in years). We show the richness of A) all flowers, B) native flowers, and C) introduced flowers, broken down by seeding treatment. The line is a simple linear trendline and the shaded area is a 95% confidence interval. Because typical sites have unknown history (and accordingly, no known "age") they are not included in this plot.

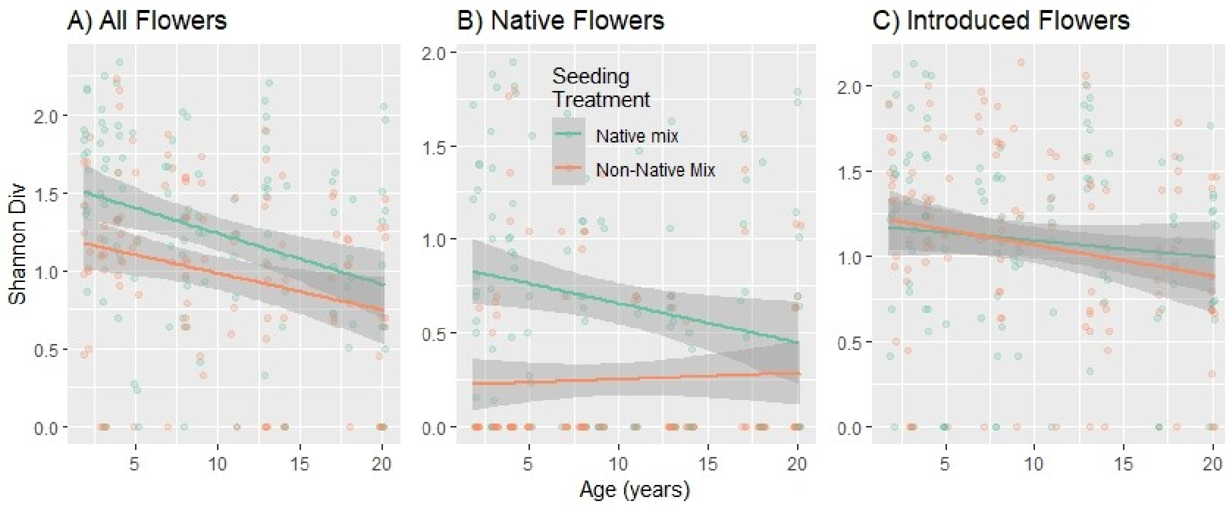

Figure B4. Change in the Shannon Diversity of sites displayed as time since seeding (e.g site age, in years). We show the Shannon Diversity Index of A) all flowers, B) native flowers, and C) introduced flowers, broken down by seeding treatment. The line is a simple linear trendline and the shaded area is a 95% confidence interval. Because typical sites have unknown history (and accordingly, no known "age") they are not included in this plot.

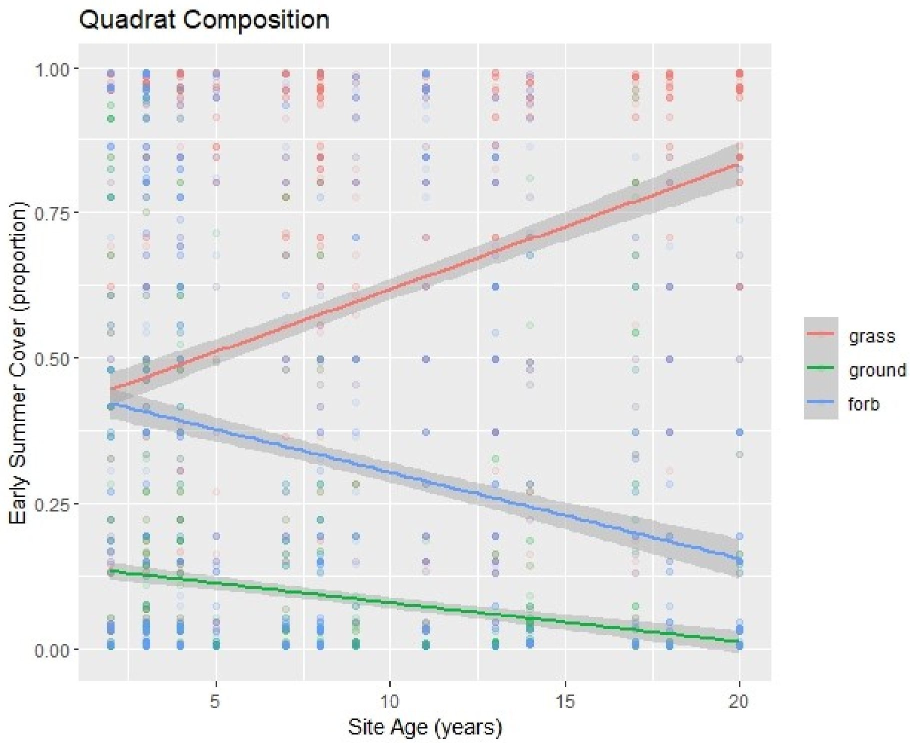

Figure B5. The relationship between site age and proportion of early season cover of grass, bare ground and forb. The line is a linear approximation and the shaded area is a 95% confidence interval. This figure includes all the native and non-native seeded sites. Because typical sites have unknown history (and accordingly, no known “age”) they are not included in this plot

Table B1. Background information on sites included in study. Seed mix composition is available in the Minnesota DOT seeding manual (MnDOT, 2014). Seed mix cost was evaluated from Turf Establishment Price Estimates worksheet 2019, and thus represent the costs that the seed mix used would have cost in 2019. GIS layers with site plans overlaid onto maps which delineate site areas is available upon request.

Prj	Year Est.	County	Project Code	Trt	Lat	Lon	Seed_mix	Cost/acre ($)
1	2017	Brown	0804-81	nat	45.065423	-92.862848	35-241	558
1	2017	Brown	0804-82	non	45.049679	-92863259	25-151	480
1		Brown		typ	44.946489	-92.984573		
2	2018	Scott	7001-115	nat	44.930095	-92.984825	35-221	735
2	2018	Scott	7001-116	non	44.92884	-92.984858	25-121	246
2		Scott		typ	45.050026	-92.863259		
3	2013	Washington	8204-55	nat	43.92395898	-92.6985013	35-221	735
3	2013	Washington	8204-56	non	43.922036	-92.698504	25-141	199
3		Washington		typ	43.92102865	-92.6859302		
4	2018	Wright	8604-42	nat	45.01112258	-92.79546883	35-221	735
4	2018	Wright	8604-43	non	45.03010954	-92.79122008	25-121	246
4		Wright		typ	45.03010954	-92.79122008		
6	2012	Ramsey	6280-308	nat	45.26989403	-93.0264595	35-241	558
6	2012	Ramsey	6280-309	non	45.26867446	-93.01179744	25-141	199
6		Ramsey		typ	45.26872633	-93.01108988		
7	2019	Carver	1017-108	nat	44.84766288	-92.96913887	35-241	558
7	2019	Carver	1017-109	non	44.85226902	-92.99012675	25-141	199
7		Carver		typ	44.85197025	-92.99000344		
8	2001	Washington	01-236102	nat	44.88369346	-92.94424318	33-262	660
8	2001	Washington	01-236103	non	44.86898298	-92.94430311	25-141	199
8		Washington		typ	44.86862169	-92.94453644		
9	2007	Washington	07-211802	nat	44.890941	-92.910633	35-241	558
9	2007	Washington	07-211803	non	44.890692	-92.924188	25-141	199
9		Washington		typ	44.887906	-92.924699		
13	2016	Washington	082-612-017	nat	44.997127	-92.863112	33-261	934
13	2016	Washington	082-612-018	non	45.007427	-92.858396	25-141	199
13		Washington		typ	45.008425	-92.863173		
14	2017	Washington	082-613-033	nat	45.001805	-92.945192	35-221	735
14	2017	Washington	082-613-034	non	45.011976	-92.944343	25-151	480
14		Washington		typ	45.012154	-92.9441		
16	2019	Washington	082-618-022	nat	44.933701	-92.771284	33-261	934
16	2019	Washington	082-618-023	non	44.920719	-92.773669	25-141	199
16		Washington		typ	44.921344	-92.773471		

19	2003	Washington	82-613-07	nat	45.091705	-92.833483	35-241	558
19	2003	Washington	82-613-07	non	45.121077	-92.852184	25-141	199
19		Washington		typ	45.121744	-92.852315		
20	2004	Washington	82-622-07	nat	44.790836	-93.656249	35-221	735
20	2004	Washington	82-622-07	non	44.791242	-93.634736	25-141	199
20		Washington		typ	44.790497	-93.623355		
23	2010	Washington	82-596-04	nat	44.992011	-93.089824	35-241	558
23	2010	Washington	82-596-04	non	44.961833	-93.091098	25-141	199
23		Washington		typ	44.965372	-93.090645		
25	2013	Washington	82-604-010	nat	45.064807	-93.101518	33-261	934
25	2013	Washington	82-604-010	non	45.042284	-93.106293	25-141	199
25		Washington		typ	45.045188	-93.105706		
26	2014	Washington	82-621-028	nat	45.065185	-93.88506	35-221	735
26	2014	Washington	82-621-028	non	45.089073	-93.868996	25-121	246
26		Washington		typ	45.076948	-93.86902		
27	2001	Dodge	GuyK	nat	45.035924	-92.922036	35-221	735
27	2001	Dodge	GuyK	non	45.035841	-92.951003	22-112	139
27		Dodge		typ	45.036222	-92.950222		
28	2008	Washington	82-615-20	nat	44.698673	-93.49668	33-361	1094
28	2008	Washington	82-615-20	non	44.693879	-93.461665	25-141	199
28		Washington		typ	44.689218	-93.489646		
29	2008	Washington	82-625-02	nat	44.313519	-94.431671	33-261	934
29	2008	Washington	82-625-02	non	44.320949	-94.463569	25-141	199
29		Washington		typ	44.324425	-94.455262		

APPENDIX C
SUPPLEMENT TO CHAPTER 4

Table C1. Statistical outputs from the models for Q1–5 described in section 4.3.2 in which butterfly Shannon diversity (*H*) was the response variable. Butterfly *H* is the Shannon diversity including both native and non-native butterflies. Native butterfly *H* is the Shannon diversity for only native butterflies. For Q4 only native butterfly species were expected to be attracted to native forbs; therefore, butterfly Shannon diversity including both native and non-native species was not analyzed. Because the trends and significance of results using only native butterfly Shannon diversity were no different from the same analyses including both native and non-native butterfly species, we only discuss analyses using the Shannon diversity for all species of butterflies in this report, with the exception of Q4. This table highlights these similarities. Site nested within project was a random effect in all models. Significant ($P < 0.05$) parameters are bolded.

Response	Question	Parameter	Estimate	Standard Error	*df*	*t-value*	*P*
Butterfly *H*	Q1	(Intercept)	0.19201	0.24623	280	0.77981	0.4362
		Non-native seed mix	-0.02907	0.07287	36	-0.39898	0.6923
		Typical roadside	-0.12630	0.07357	36	-1.71681	0.0946
		Survey time (min)	0.03042	0.01690	280	1.80012	0.0729
	Q2	(Intercept)	0.01371	0.24338	279	0.05633	0.9551
		Floral *H*	**0.15057**	**0.04589**	**279**	**3.28126**	**0.0012**
		Survey time (min)	0.02884	0.01656	279	1.74155	0.0827
	Q3	(Intercept)	0.07413	0.24882	279	0.29792	0.7660
		Proportion flowers	**0.23183**	**0.09966**	**279**	**2.32619**	**0.0207**
		Survey time (min)	0.02768	0.01697	279	1.63127	0.1040
	Q5	(Intercept)	0.05885	0.31324	189	0.18788	0.8512
		Age	0.00542	0.01121	17	0.48380	0.6347
		Treatment (Non-native seed mix)	-0.04417	0.11889	17	-0.37155	0.7148
		Age*treatment	0.00155	0.01059	17	0.14669	0.8851
		Survey time (min)	0.03618	0.01925	189	1.87902	0.0618
Native butterfly *H*	Q1	(Intercept)	0.11182	0.23242	280	0.48113	0.6308
		Non-native seed mix	-0.03715	0.06834	36	-0.54369	0.5900
		Typical roadside	-0.11157	0.06900	36	-1.61693	0.1146
		Survey time (min)	0.02693	0.01594	280	1.68954	0.0922
	Q2	(Intercept)	-0.03412	0.22897	279	-0.14903	0.8816
		Floral *H*	**0.12773**	**0.04291**	**279**	**2.97674**	**0.0032**
		Survey time (min)	0.02484	0.01556	279	1.59621	0.1116
	Q3	(Intercept)	0.00224	0.23396	279	0.00956	0.9924
		Proportion flowers	**0.22001**	**0.09352**	**279**	**2.35263**	**0.0193**
		Survey time (min)	0.02413	0.01593	279	1.51500	0.1309
	Q4	(Intercept)	0.02487	0.23735	261	0.10479	0.9166

		Proportion native flowers	0.04647	0.09004	261	0.51612	0.6062
		Survey time (min)	0.02969	0.01618	261	1.83500	0.0676
	Q5	(Intercept)	0.01325	0.29933	189	0.04425	0.9647
		Age	0.00639	0.01059	17	0.60338	0.5542
		Treatment (Non-native seed mix)	0.02072	0.11413	17	0.18157	0.8581
		Age*treatment	-0.00609	0.01017	17	-0.59909	0.5570
		Survey time (min)	0.02959	0.01844	189	1.60493	0.1102

Table C2. Statistical outputs from the models for questions 1-5 described in section 4.3.2 in which bumble bee Shannon diversity was the response variable. Bumble bee *H* is the Shannon diversity for all bumble bees surveyed, all of which are native. Site nested within project was a random effect in all models. Significant (P < 0.05) parameters are bolded.

Response	Question	Parameter	Estimate	Standard Error	*df*	*t-value*	*P*
Bumble bee *H*	Q1	(Intercept)	0.32264	0.17287	279	1.86632	0.0630
		Non-native seed mix	-0.03922	0.06024	36	-0.65111	0.5191
		Typical roadside	**-0.17213**	**0.06056**	**36**	**-2.84223**	**0.0073**
		Survey time (min)	0.00009	0.01175	279	0.00800	0.9936
	Q2	(Intercept)	-0.00934	0.16118	278	-0.05795	0.9538
		Floral *H*	**0.20742**	**0.03511**	**278**	**5.90790**	**0.0000**
		Survey time (min)	0.00413	0.01098	278	0.37565	0.7075
	Q3	(Intercept)	-0.04405	0.15735	278	-0.27996	0.7797
		Proportion flowers	**0.58394**	**0.07315**	**278**	**7.98322**	**0.0000**
		Survey time (min)	0.00228	0.01073	278	0.21249	0.8319
	Q4	(Intercept)	0.21945	0.17887	260	1.22690	0.2210
		Proportion native flowers	0.09293	0.07953	260	1.16853	0.2437
		Survey time (min)	0.00160	0.01231	260	0.13031	0.8964
	Q5	(Intercept)	0.27721	0.24326	188	1.13957	0.2559
		Age	-0.00689	0.00927	17	-0.74368	0.4672
		Treatment (Non-native seed mix)	0.01059	0.11287	17	0.09381	0.9264
		Age*treatment	-0.00497	0.00997	17	-0.49839	0.6246
		Survey time (min)	0.00801	0.01480	188	0.54124	0.5890

Table C3. Statistical outputs from the models in which insect and spider Shannon diversity index (Insect *H*) was the response variables. Site nested within project was a random effect in all models. Significant (P < 0.05) parameters are bolded, with the exception of the intercept and survey time.

Response	Parameter	Estimate	Standard Error	*df*	*t-value*	*P*
Insect *H*	(Intercept)	1.06458	0.05423	73	19.62937	0.0000
	Non-native seed mix	0.07277	0.07693	18	0.94585	0.3567
	(Intercept)	1.10200	0.04745	627	23.22668	0.0000
	Plant *H*	-0.00191	0.02058	627	-0.09295	0.9260

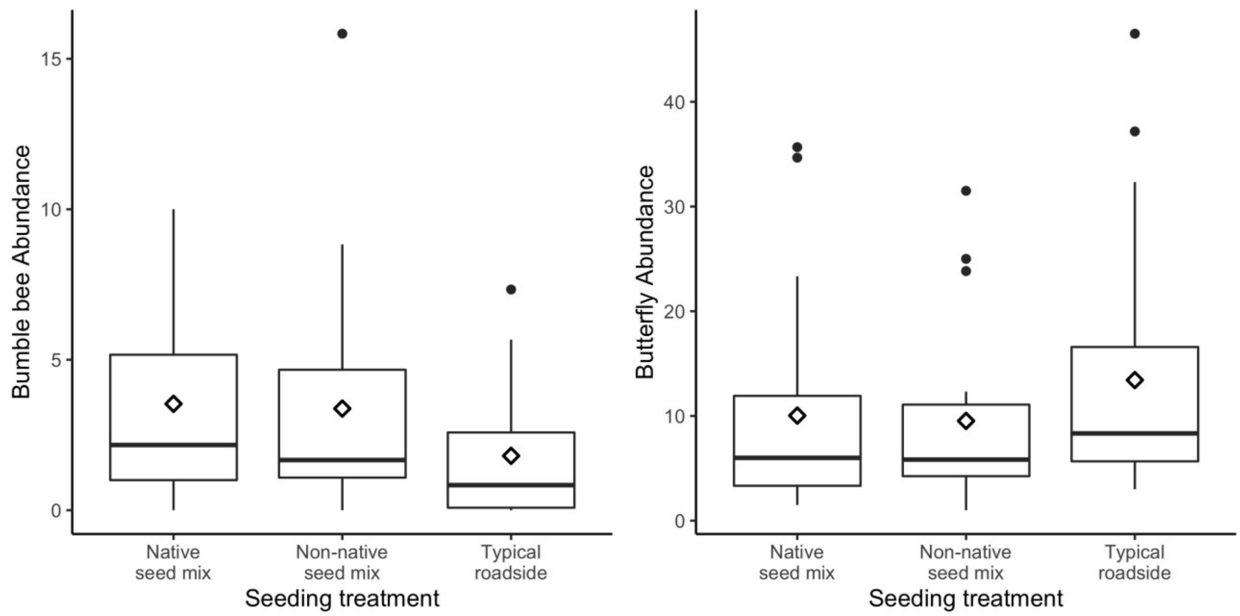

Figure C1. Abundance of bumble bees and butterflies in roadside sites planted with native seed mixes, non-native seed mixes, and typical roadsides. Open diamonds represent mean abundance.

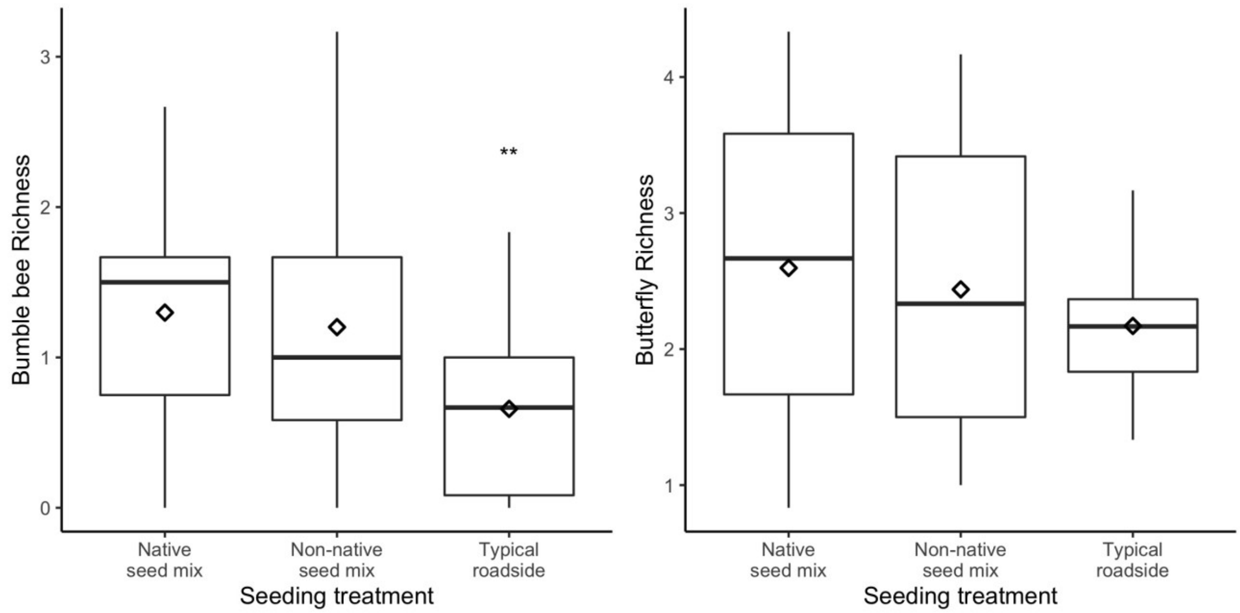

Figure C2. Richness of bumble bees and butterflies in roadside sites planted with native seed mixes, non-native seed mixes, and typical roadsides. Open diamonds represent mean richness.

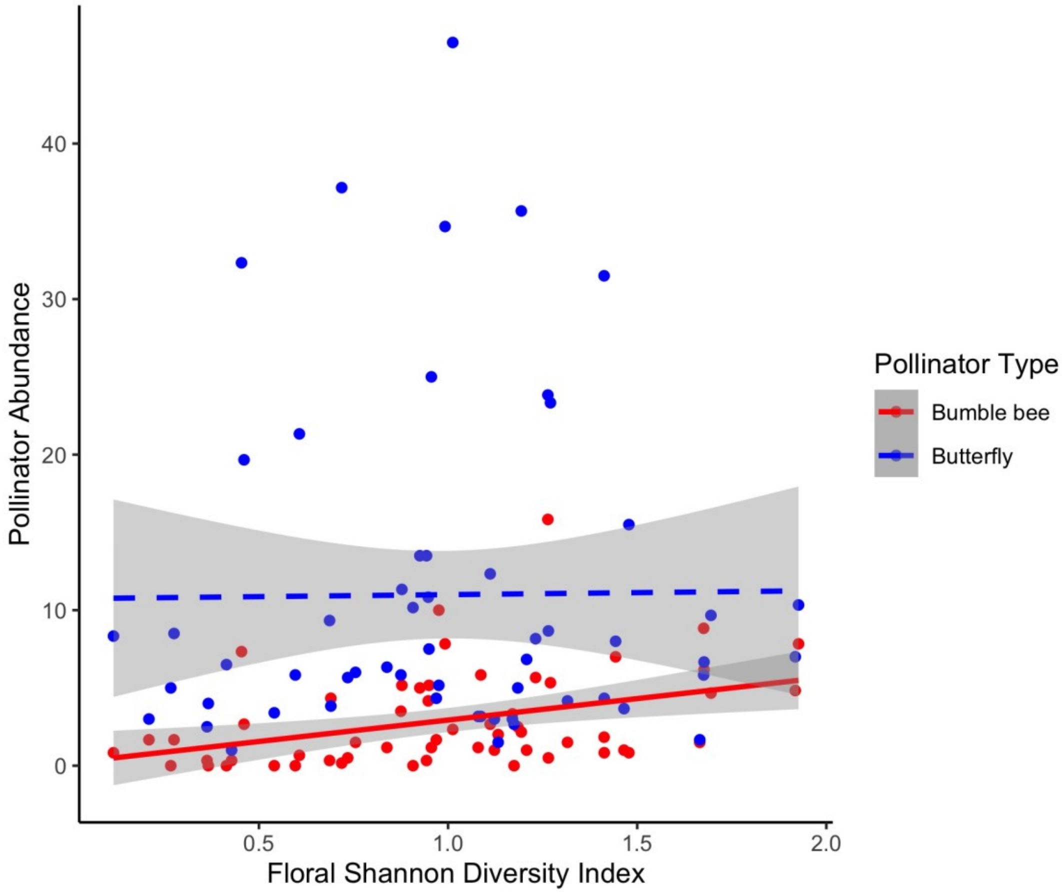

Figure C3. Bumble bee and butterfly abundance with increasing roadside floral diversity. Each point represents the mean pollinator abundance and mean floral Shannon's diversity index measured at one site over all six surveys. Each line is a simple linear trendline and the shaded area is a 95% confidence interval. Butterflies are represented by the blue dashed line while bumble bees are represented by the red solid line.

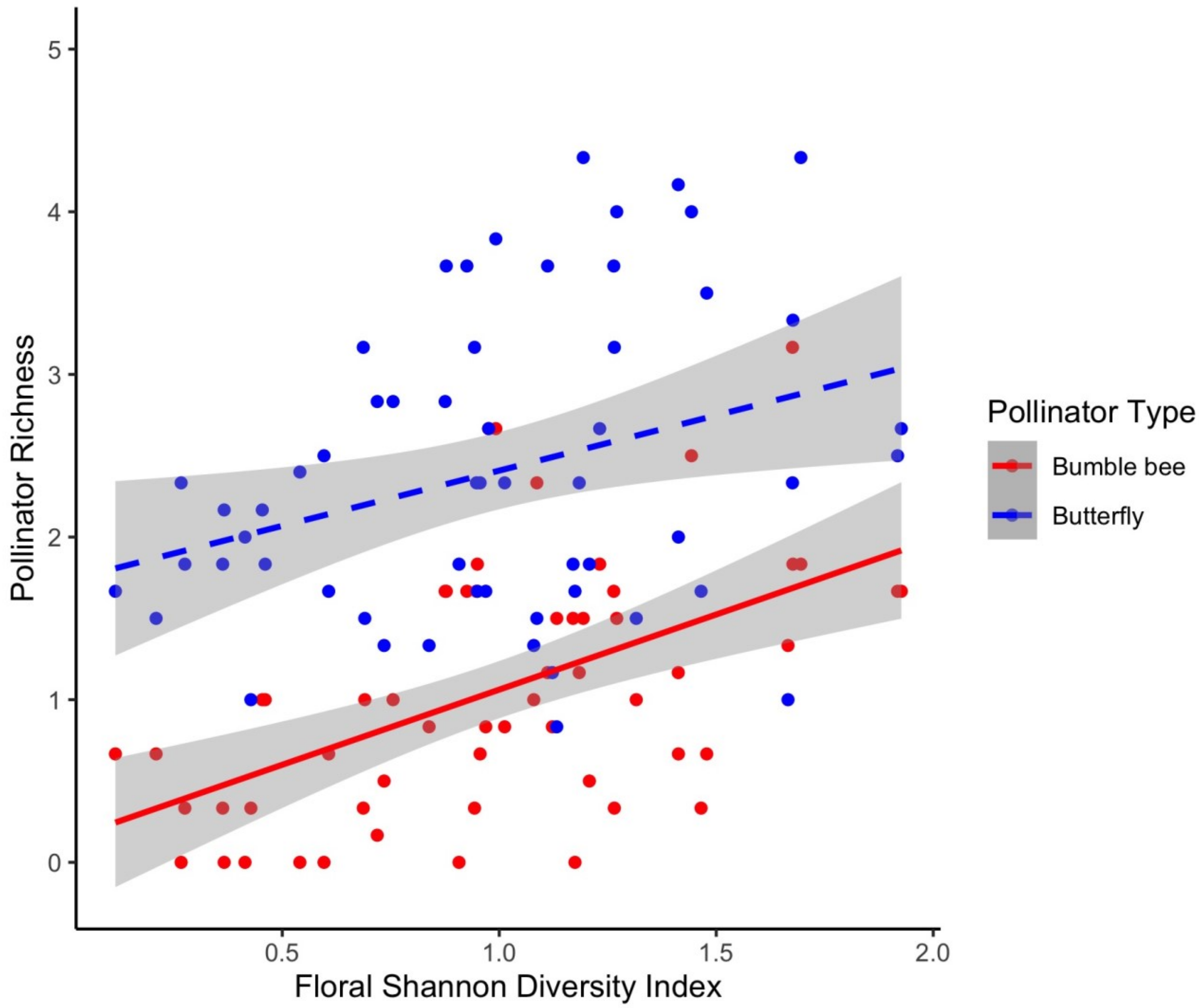

Figure C4. Bumble bee and butterfly species richness with increasing roadside floral diversity. Each point represents the mean pollinator species richness and mean floral Shannon's diversity index measured at one site over all six surveys. Each line is a simple linear trendline and the shaded area is a 95% confidence interval. Butterflies are represented by the blue dashed line while bumble bees are represented by the red solid line.

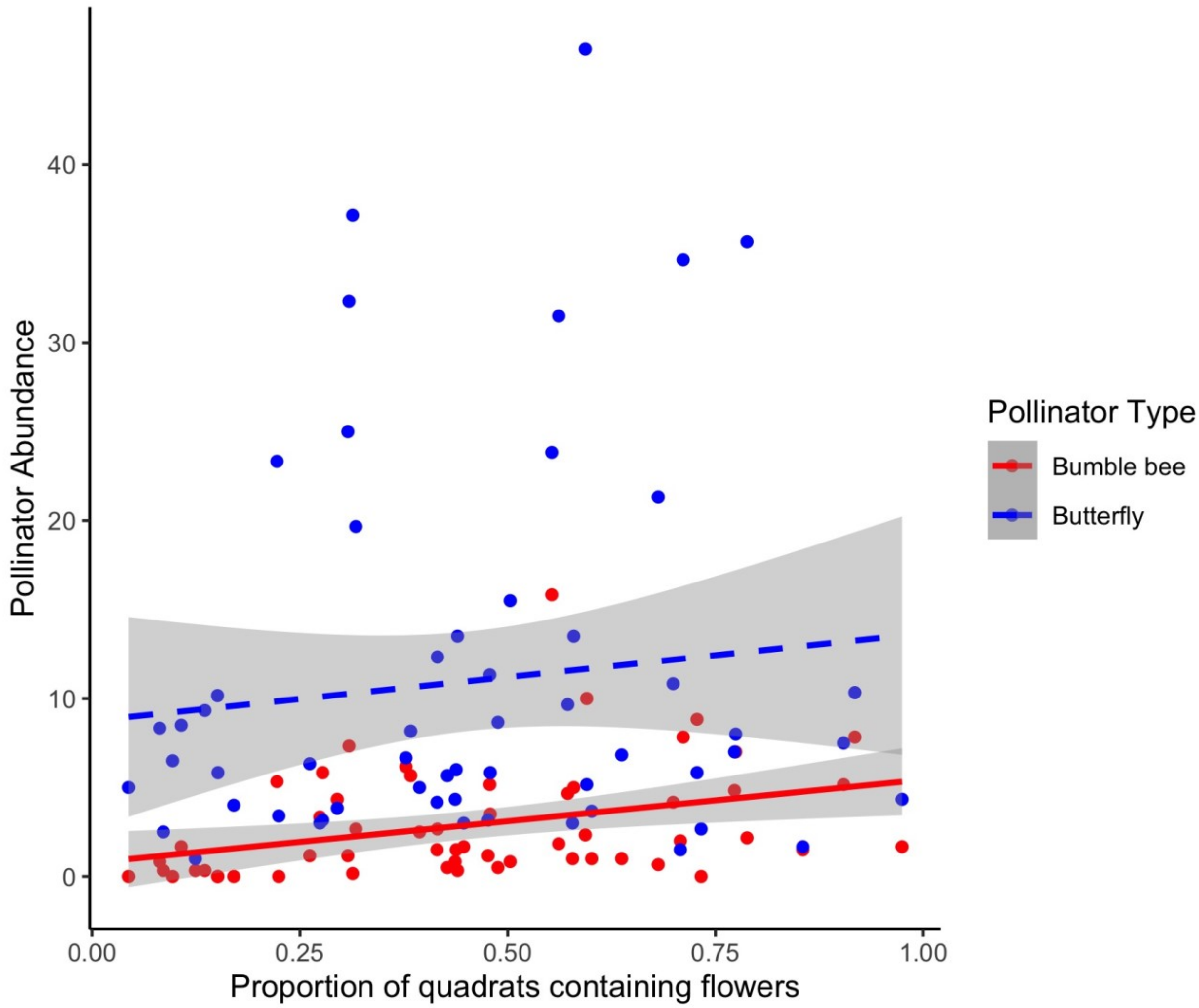

Figure C5. Bumble bee and butterfly abundance with increasing available floral resources. Each point represents the mean pollinator abundance and mean proportion of quadrats containing blooming flowers measured at one site over all six surveys. Each line is a simple linear trendline and the shaded area is a 95% confidence interval. Butterflies are represented by the blue dashed line while bumble bees are represented by the red solid line.

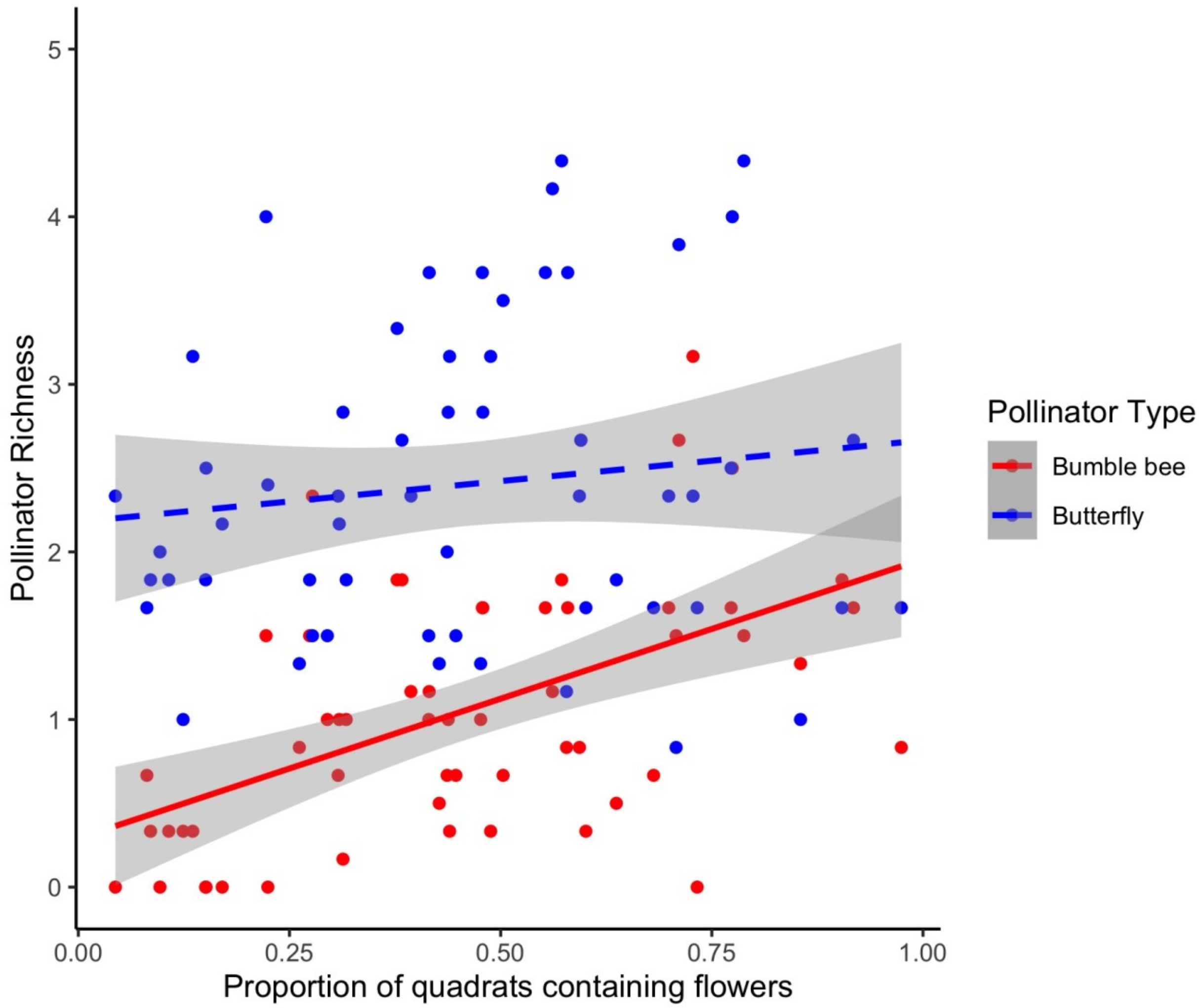

Figure C6. Bumble bee and butterfly species richness with increasing available floral resources. Each point represents the mean pollinator species richness and mean proportion of quadrats containing blooming flowers measured at one site over all six surveys. Each line is a simple linear trendline and the shaded area is a 95% confidence interval. Butterflies are represented by the blue dashed line while bumble bees are represented by the red solid line.

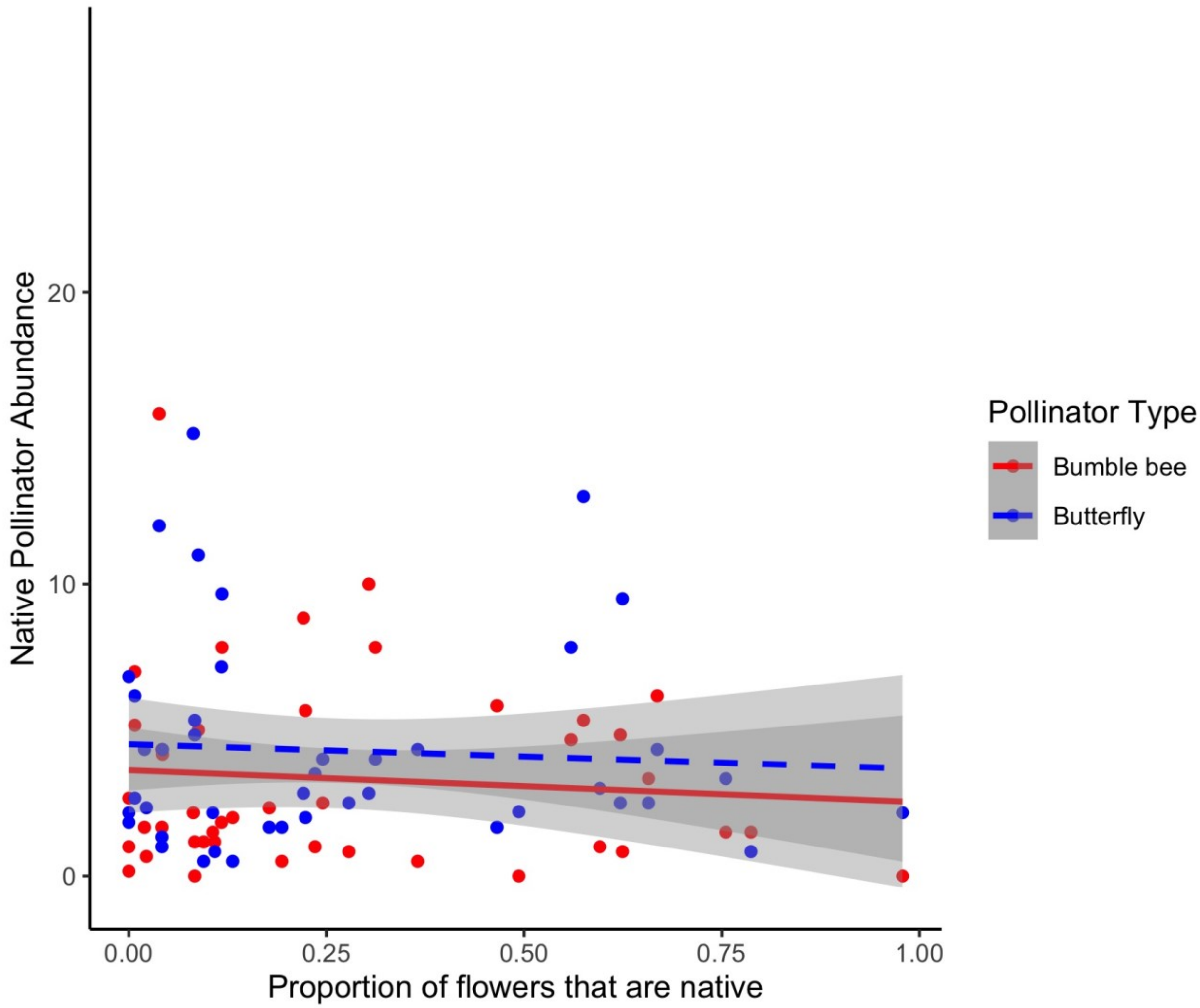

Figure C7. Native bumble bee and butterfly abundance with an increasing availability of native floral resources. Each point represents the mean pollinator abundance and mean proportion of quadrats with blooming flowers that contain natives measured at one site over all six surveys. Each line is a simple linear trendline and the shaded area is a 95% confidence interval. Butterflies are represented by the blue dashed line while bumble bees are represented by the red solid line.

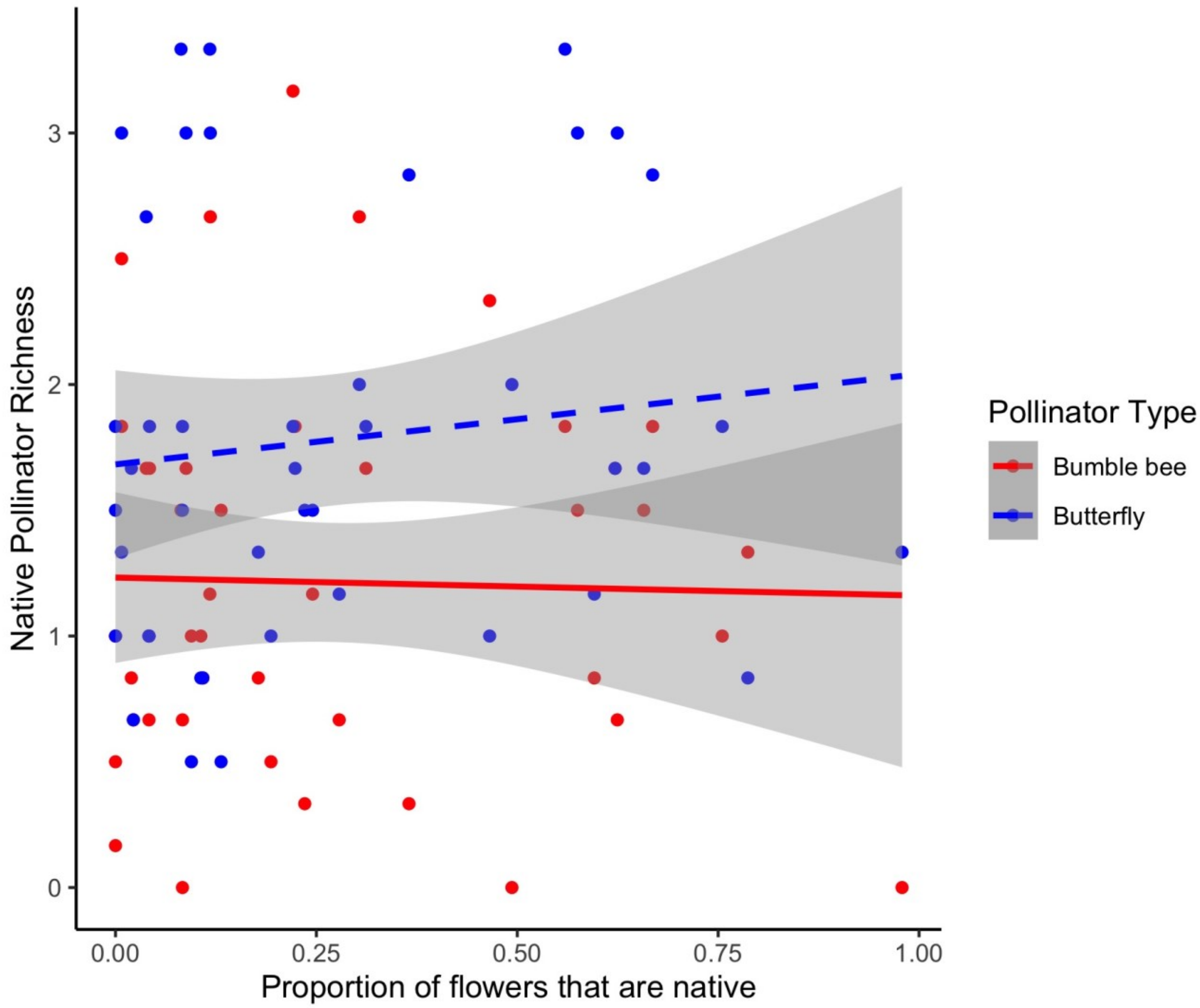

Figure C8. Native bumble bee and butterfly species richness with an increasing availability of native floral resources. Each point represents the mean pollinator species richness and mean proportion of quadrats with blooming flowers that contain natives measured at one site over all six surveys. Each line is a simple linear trendline and the shaded area is a 95% confidence interval. Butterflies are represented by the blue dashed line while bumble bees are represented by the red solid line.

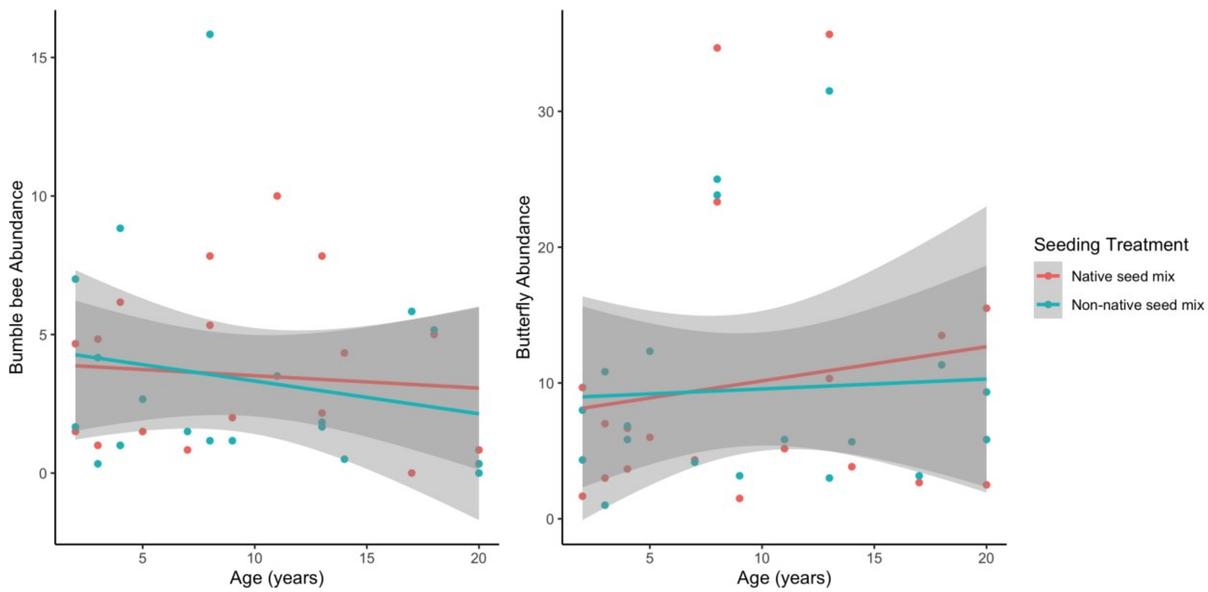

Figure C9. Bumble bee and butterfly abundance with roadside site age since seeding. Each point represents the mean pollinator abundance at one site over all six surveys. Each line is a simple linear trendline and the shaded area is a 95% confidence interval. Because typical sites have unknown history (and accordingly, no known "age") they are not included in this plot.

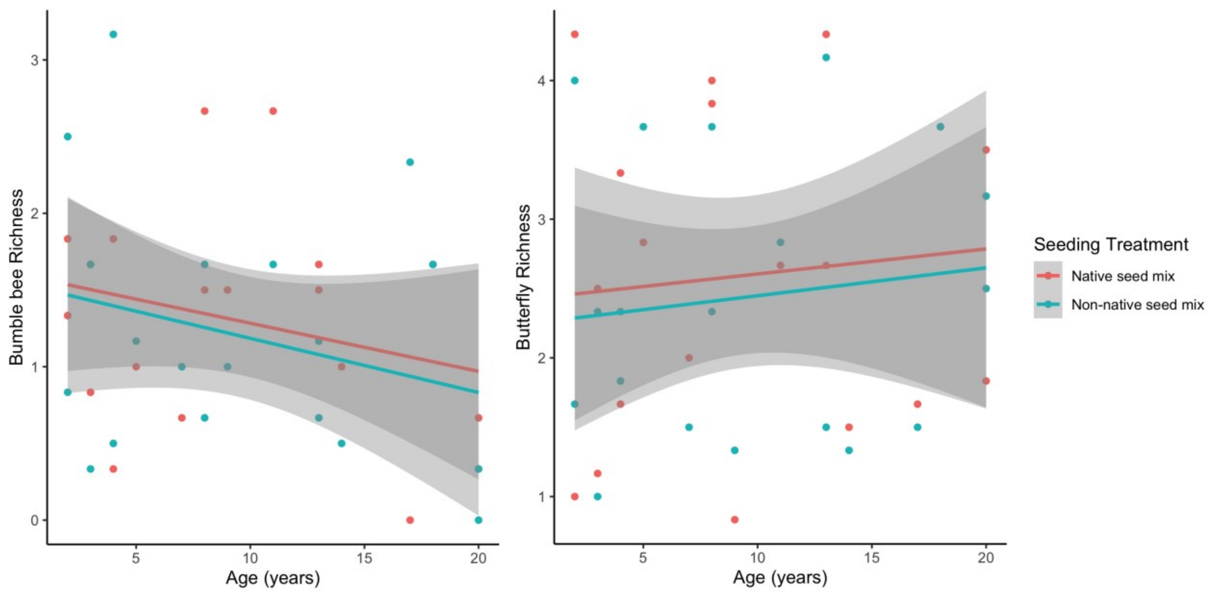

Figure C10. Bumble bee and butterfly species richness with roadside site age since seeding. Each point represents the mean pollinator species richness at one site over all six surveys. Each line is a simple linear trendline and the shaded area is a 95% confidence interval. Because typical sites have unknown history (and accordingly, no known "age") they are not included in this plot.

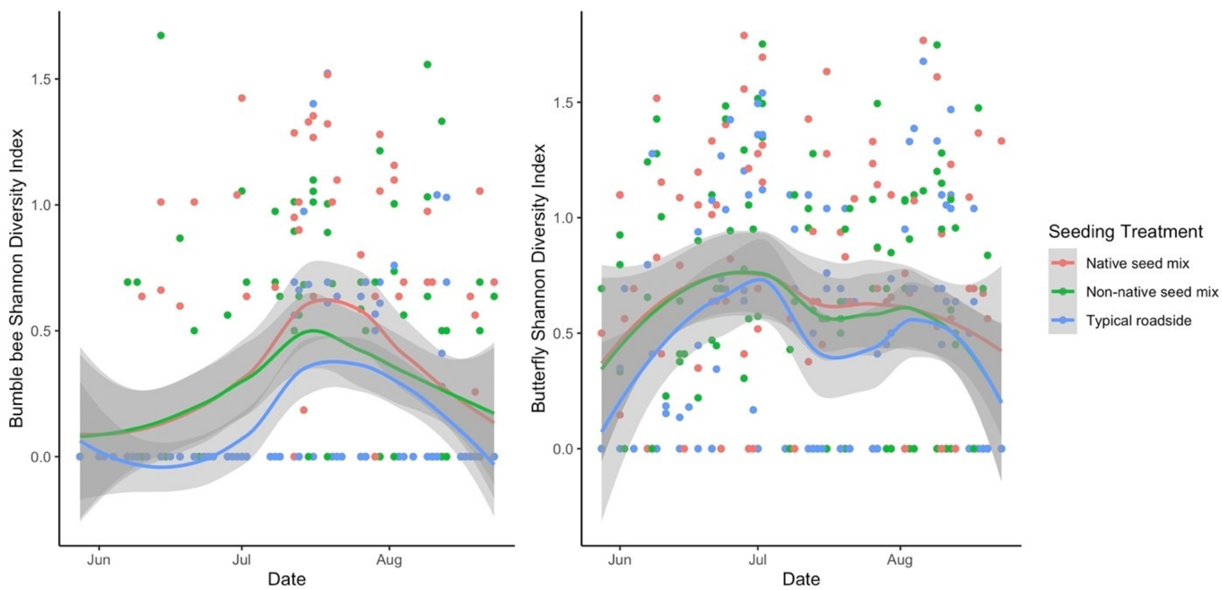

Figure C11. Seasonal diversity of bumble bees and butterflies. Diversity was measured using Shannon's diversity index. Each point represents the pollinator diversity during one survey at one site. Lines represent an average computed by a Loess smoother and shaded area is the 95% confidence interval.