SPR-Part B
MD-19-SHA/UM/4-38

MARYLAND DEPARTMENT OF TRANSPORTATION
STATE HIGHWAY ADMINISTRATION

Larry Hogan
Governor

Boyd K. Rutherford
Lt. Governor

Pete K. Rahn
Secretary

Gregory Slater
Administrator

MARYLAND DEPARTMENT OF TRANSPORTATION STATE HIGHWAY ADMINISTRATION

RESEARCH REPORT

EVALUATING INTEGRATED ROADSIDE VEGETATION MANAGEMENT (IRVM) TECHNIQUES TO IMPROVE POLLINATOR HABITAT

LISA KUDER
DEPARTMENT OF ENTOMOLOGY
UNIVERSITY OF MARYLAND

FINAL REPORT

August 2019

This material is based upon work supported by the Federal Highway Administration under the State Planning and Research program. Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of the Federal Highway Administration or the Maryland Department of Transportation. This report does not constitute a standard, specification, or regulation.

Technical Report Documentation Page

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
MD-19-SHA/UM/4-38		

4. Title and Subtitle Evaluating Integrated Roadside Vegetation Management (IRVM) Techniques to Improve Pollinator Habitat	**5. Report Date** August 2019 **6. Performing Organization Code**
7. Author(s) Lisa Kuder, PhD candidate	**8. Performing Organization Report No.**
9. Performing Organization Name and Address University of Maryland Department of Entomology 4291 Fieldhouse Dr., College Park, MD 20742	**10. Work Unit No.** **11. Contract or Grant No.** SHA/UM/4-38
12. Sponsoring Agency Name and Address Maryland Department of Transportation (SPR) State Highway Administration Office of Policy & Research 707 North Calvert Street Baltimore MD 21202	**13. Type of Report and Period Covered** SPR-B Final Report (December 2016-June 2019) **14. Sponsoring Agency Code** (7120) STMD - MDOT/SHA

15. Supplementary Notes

16. Abstract

In an effort to improve roadside habitat for pollinators this three-year field study had two main goals: to determine which vegetation management tactics best maximize quality floral resources for pollinators in the Northeast, and to assess how those different regimes affect regional bee populations. The findings show that managing roadsides via selective herbicide use (SH) and annual fall mow (fall mow) can significantly increase floral diversity and bee abundance compared to a traditional frequent mowing (turf) regime. While differences between treatments – SH and fall mow – were detected, they were not significant. Bee diversity, which accounts for both abundance and the evenness of species in a given area, was mainly determined by site/surrounding landscape not treatment and was the sole significant factor. Given that floral abundance and diversity, as well as bee abundance, were increased under SH and fall mow compared to turf plots, both Integrated Roadside Vegetation Management (IRVM) practices have shown great potential in supporting pollinators.

This report also discusses some of the potential benefits and challenges associated with Maryland Department of Transportation State Highway Administration's transition to meadow management. The pollinator friendly vegetation management guidelines in this report are timely and practical to implement on a landscape scale.

17. Key Words	18. Distribution Statement
Environment, Pollinators, Bees, Roadside, Mowing, IRVM, Integrated Roadside Vegetation Management.	This document is available from the Research Division upon request.

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
None	None	47	

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

Table of Contents

Summary

Bees provide irreplaceable ecosystem services as the primary pollinators of economically important food crops and an estimated 88% of natural flora [1]. Their contribution to global food production is significant, valued at $235 - 577 billion annually [2]. Thus the severity and extent of recent bee declines can have profound consequences on food security, sustainability of agriculture, and the health of the environment. While multiple factors contribute to bee losses, the primary drivers are the combined stress of pathogens, pesticides and lack of flowers [3]. Reducing or removing stress, such as improving nutrition, can thus benefit pollinator health [3]. Recent national initiatives are addressing the nutritional component of the 'pollination crisis' by restoring or enhancing seven million acres of native meadows and grasslands by 2020 [4]. Subsequently there has been an increased interest in early successional landscapes created through management of transportation rights-of-way (ROW) [5].

National roadways in the U.S. have an estimated habitat potential of 10 million acres [5]. Roadsides not only cover extensive acreage but also provide connectivity in a fragmented landscape and traverse multiple habitats, making them particularly important for wildlife conservation [6-8]. A literature review on pollinator conservation and Best Management Practices for highway ROW by The Xerces Society, concluded roadsides can support insect pollinators by providing shelter, nesting sites and valuable sources of pollen and nectar [9]. Roadsides can be improved for pollinators in several ways: sowing native wildflower seeds, planting bee-friendly plants, and through minor adjustments to existing management strategies that promote natural regeneration of native grasses and wildflowers [5].

Cost effective techniques that promote floral resources are likely to receive wide acceptance and can be implemented on a landscape scale. While many state Departments of Transportation (DOTs) have voiced interest, vetted roadside management studies are limited [5]. Richard Forman known as the 'Father of Road Ecology' emphasized the need for "rigorous research in different regions and roadsides and . . . how little we know about the ecology of roadside vegetation considering the decades of mowing" [6]. Also, roadside landscapes have unique properties heavily shaped by human activities that set them apart from other landscape types. Thus current roadside pollinator habitat recommendations, largely based on semi-natural prairies, are likely not optimal [10].

In an effort to improve roadside habitat for pollinators this three-year field study had two main goals: to determine which vegetation management tactics best maximize quality floral resources for pollinators in the northeast, and to assess how those different regimes affect regional bee populations. The findings, some presented in this report, and the remainder to be shared with Maryland DOT State Highway Administration (MDOT SHA) via submitted publication(s) show that managing roadsides via selective herbicide use (SH) and annual fall mow (fall mow) can significantly increase floral diversity and bee abundance compared to a traditional frequent mowing (turf) regime. While differences between treatments – SH and fall mow – were detected, they were not significant. Bee diversity, which accounts for both abundance and the evenness of species in a given area, was mainly determined by

site/surrounding landscape not treatment and was the sole significant factor. Given that floral abundance and diversity, as well as bee abundance, were increased under SH and fall mow compared to turf plots, both Integrated Roadside Vegetation Management (IRVM) practices have shown great potential in supporting pollinators. This report also discusses some of the potential benefits and challenges associated with MDOT SHA's transition to meadow management.

Background

Pollinator conservation – why bees need a diversity and abundance of flowers

Bees (Order: Hymenoptera, Superfamily: Apoidea) belong to the Clade: Anthophila, a combination of Greek words that mean 'flower' + 'lover.' They are descendants of apoid wasps, whose foraging preferences over many generations changed from animal protein to an entirely vegetarian diet of nectar and pollen [11, 12]. The earliest bees date back to the Cretaceous Period (over 100 MYA) which coincides with the appearance of the first angiosperms or flowering plants [12]. Hence bees evolved alongside flowers, facilitating one another's rapid spread across the globe and extraordinary diversification [11]. Worldwide, over 20,000 bee species have been described from nearly every terrestrial habitat except Antarctica [11]. Similarly, angiosperms have flourished throughout the land and are now the dominant vegetation type with over 300,000 species, comprising more than 80% of all existing plants [13].

Among the world's flowering plants, there is vast variation in floral morphology, bloom time and rewards. Differences in floral traits are mirrored by different sizes, phenology and specialized structures of bees [11]. As a result, different groups of bees visit different types of flowers. For instance, the seven bee families are divided into three major groups based on tongue length: small- (Families: Andenidae, Colletidae and Stenotritidae), medium- (Families: Melittidae and Halictidae) and long- tongued bees (Families: Apidae and Megachilidae) [11]. Tongue-length is a good indicator from which flowers (shallow or deep tubular structures) bees can collect nectar and pollen [11]. Matching phenologies, or seasonal cycles, is another determining factor in bee-plant interactions. The majority of bee species in temperate zones are active as adults for only a brief time (~ 6 weeks), which typically parallels the bloom time of their floral hosts [14].

Bee-plant interactions are also shaped by the varied nutritional composition of floral rewards. Pollen grains, a rich source of protein necessary for larval bee development, include 10 essential amino acids with protein levels ranging from 2 – 60% [15]. They also contain varying amounts of carbohydrates, lipids, sterols and other micronutrients depending on the floral species [15]. Recent evidence by Danforth et al. suggests that ancestral bees were oligolectic or specialist feeders, provisioning their nests with pollen from a single plant species, genus or family [16]. While some bees such as honey bees (*Apis mellifera*) and bumble bees (Genus: *Bombus*), have evolved to be polylectic or generalist feeders, many bees (some mining bees, cellophane bees and resin bees) continue to have a narrow diet breadth [15]. The other major floral reward is nectar, a sugar-rich food that fuels adult bees. Nectar contains primarily water and the sugars fructose, glucose and sucrose, which range in concentrations from 10 – 70% depending on the plant species and abiotic factors (temperature, precipitation, humidity and time of day) [15].

Along with nutrients, pollen and nectar may also contain secondary metabolites, defense chemicals used by plants to deter herbivores [17, 18]. Secondary metabolites are generally broken up into three main categories: alkaloids, terpenoids and flavonoids. These chemicals vary widely among plant families with equally wide-ranging effects on different groups of pollinators from beneficial to toxic [17, 18]. Resulting in evolutionary adaptive responses from pollinators including avoidance, floral specificity and pollen-mixing to mitigate unfavorable chemical properties [18, 19]. Due to the complexities and spatial-temporal fluctuations of floral rewards, to thrive, bees need an abundance of flowers as well as heterogeneity. In fact, the number of floral species, density and quality of floral resources are the strongest factors structuring pollinator communities [20, 21]. Unfortunately, intensive agriculture and urbanization have led to large-scale habitat degradation and loss, creating a dearth of wildflowers and a florally homogenous landscape [3, 22, 23].

To improve floral resources for bees and butterflies, numerous pollinator-friendly plant lists have been compiled by government agencies and non-profit organizations. These generally comprise mostly native plant species with staggered bloom times for the entirety of the growing season (April – Oct in temperate zones). Temporal considerations will ensure adequate sustenance for solitary bees that generally forage for only short periods, as well as social bees that are active from early spring to late fall. In addition to plantings for farmland and gardens, there is an increasing focus on early successional landscapes created through management practices of transportation ROW.

From a practical standpoint, green infrastructure faces practical challenges, as it is a complex and interacting combination of social, cultural and economic factors with multiple and diverse stakeholders [24, 25]. Previous efforts to create pollinator habitat in both suburban and urban areas have been met with mixed results, eliciting both positive and negative feelings from those living nearby [24-26]. To achieve desired outcomes and public acceptance, transportation ROW must meet pollinators' needs in a way that is economically practical and respectful of societal norms and safety concerns [11, 12]. Lessons learned from numerous case studies, show that community engagement is a key component to successful public green space projects [25, 27, 28], such as roadsides that are in urban centers and/or are highly visible.

Social aspects of transitioning to sustainable roadside meadow management

Transitioning to meadow management presents numerous challenges for DOTs, which are tasked with multiple and sometimes competing land use objectives. Challenges including: limited funding for beautification projects, unfavorable public perception, internal and external resistance to change, complexities of multilevel communication, shortage of wildflower meadow management practitioners, unknown cost-to-benefit ratios, regulatory laws with a bias toward tree plantings, and knowledge gaps about the efficacy of different meadow management strategies (AASHTO survey, 2016). Combined, such obstacles if not addressed could impede local and regional progress towards broad adoption of sustainable, pollinator friendly vegetation management schemes.

To pave the way for sustainable verge management, the following questions should be considered: ***Who*** will be impacted by modifications to typical roadside maintenance? ***What*** obstacles hinder transitions to sustainable vegetation management? ***Where*** should meadow restoration occur (rural, residential and/or commercial zones)? ***Why*** do some roadside meadow programs succeed and others fail? ***How*** can impediments be overcome in a productive, equitable way for the various actors? Past and present practices and views of verge management can provide valuable insights. Further, viewing repercussions of bee declines and compromised pollination services through a socio-environmental (SE) lens can help identify critical societal and environmental interactions that ultimately determine outcomes of roadside habitat enhancement efforts. The overarching goal of this section is to elucidate how complex SE dynamics might drive and shape a transition to sustainable verge management for district shops throughout the state. Specifically, to 1) provide an historical and modern perspective of verge management and 2) reflect on the why and how questions raised through an SE system lens.

An overview of verge management

Historically, functionality has been the primary focus of verge management. Yet government institutions and the general public have long recognized the potential economic and ecological benefits of roadside vegetation, especially remnant indigenous flora. In 1965, president Lyndon Johnson laid the groundwork for vegetation enhancement by passing the Beautification Act, which encourages federal projects that enhance natural beauty and ecological functions [6, 29]. His wife 'Ladybird' Johnson also embraced these ideals, championing native wildflower conservation along roadsides. During the 80's – 90's a string of additional laws both pivotal and specific to the transportation sector were enacted, requiring incorporation of native wildflowers and control of noxious weeds [29].

In addition to being aesthetically appealing, roadside vegetation is often enhanced to provide a diverse array of functions that promote environmental and human well-being as outlined by Barton et al. (2005):

- Soil stabilization/erosion control
- Lessen damage by vehicle impacts
- Block or emphasize views
- Improve worker safety
- Greater carbon sequestration
- Control of snow drift/increased visibility
- Reduce maintenance inputs (costs and carbon emissions)
- Combat driver hypnosis/increased alertness
- Promote calmness/decrease road rage
- Act as a noise and glare buffer for adjacent land owners
- Improve water quality

- Increase wildlife/pollinator habitat

Despite the many benefits of naturalized roadside vegetation, regular mowing has been a cultural norm in many parts of the country since the 1930's as indicated by an important historical work titled: 'Roadsides: The Front Yard of the Nation' [30]. Frequent mowing can serve as an effective preventive safety measure along certain stretches of roadway by improving visibility. Yet millions of acres outside the required line of sight have also been traditionally maintained as a front lawn. Hence a tidy, orderly appearance has become expected and preferred by many [31]. However, effective outreach on the advantages of naturalized roadsides is helping shift public opinion.

A novel study conducted in Germany, assessed people's awareness of roadside vegetation beyond manicured parks and tree lined streets [32]. Respondents both perceived wild-grown roadside vegetation (green components other than trees) and were highly aware of its ecological importance. Overall, wild verges were met with wide approval despite preferences for more manicured vegetation [32]. Similar views are shared by some U.S. conservation groups who see roadsides as valuable habitat for native flora and fauna. Thus, making state DOTS' shift to a reduced mowing regime (FHA's 'Reduced Mowing' webinar 2015), to cut maintenance costs and be in compliance with pollinator initiatives, more socially accepted.

<u>Transition to sustainable verge management through a socio-economic lens</u>

Pollinator initiatives to mitigate bee losses by enhancing highway ROW, involve actors across various levels from individuals to state agencies. Viewing the ecological problem – bee declines and compromised pollination services – from a SE lens can help identify critical societal interactions that will ultimately determine outcomes of roadside habitat enhancement efforts. Answering key questions – who, what, where, why and how is used below to determine key components of the SE system: the actors (who) and details (what, when, why, where and how) of potential interactions.

Who will be impacted by modifications to typical roadside maintenance? The following are some of the key actors involved in the transition to sustainable verge management:

- <u>Landscape contractors</u> have invested in equipment and employee training for traditional mowing practices; reduced mowing regimes can jeopardize their profits if they are unable to adapt quickly to new landscape practices
- <u>Landscape employees</u> are hired seasonally as needed; meadow management over time reduces demand for mowing crews, potentially negatively impacting their livelihoods
- <u>Abutters</u> or adjacent land owners might feel that wild vegetation takes away from the value and appearance of their manicured yards [31]
- <u>Farmers</u> with livestock have concerns about toxic wildflowers such as certain milkweeds [33] and have valid concerns about wildflowers infiltrating their crops, a conundrum that is balanced with the need for pollination services

- State DOTs are urged to make significant changes to their usual vegetation regime which in many areas entails justifying their landscape design and maintenance practices to local businesses and residents [31]
- State Highway Administrations are requested to reevaluate budget and procedural guidelines in adherence with new regulatory legislation and voluntary initiatives
- Federal agencies initiate voluntary and mandatory initiatives that determine how federal funding can be spent on vegetation establishment and maintenance
- Commerce and tourism will be impacted by the visual appeal of surrounding areas
- Conservationists of flora and fauna will have more beneficial habitat to support their cause but might also have valid concerns about dangers inherent with roads
- Commuters and neighboring communities increased greenspace can mitigate stress and contribute to overall well-being [34]; some may view wild vegetation as an eye sore
- Pollinators can benefit from increased forage and nesting opportunities [35, 36] but may also face threats inherent to roadsides such as being struck by automobiles or experience reduced fitness due to toxins [37, 38]

What obstacles hinder transitions to sustainable vegetation management? One of the main hurdles the research team experienced over the last three years and which will likely at times impede MDOT SHA's efforts is – human resistance to change. On several occasions during the course of the study, both MDOT SHA maintenance crews and adjacent landowners ignored strategically placed 'do not mow' signs either mowing over the five foot metal stakes with large white signs or carefully mowing around them. One land owner's response to why he mowed despite the signage, he said, "I'm used to doing it. I've been mowing this land for many years and my neighbors like to see my well-manicured property when they drive by." Outreach and gentle reminders are key to overcoming this hurdle. For abutters, the research team shared a one-page update at the start and end of each season to notify land owners about the purpose and preliminary results of the ongoing study, which included a reminder to please not mow areas demarcated by signs. The research team similarly communicated with the two relevant district shops (districts 4 and 7) to minimize disruptions to the research plots.

When should meadow management be implemented? Ideally, meadow management should be implemented after adjacent land owners have been notified and given a chance to ask questions, express concerns, etc. Actor involvement at the local level will help ensure public support and possibly get them involved with the process. During this study, dozens of abutters and passers-by stopped to inquire about the visible changes to their roadsides. While one gentleman was upset and said the state of the unmown vegetation was a "disgrace," the majority expressed favorable attitudes. Initially, one store owner felt the state was neglecting the roadsides, after learning the reason for the changes, later said he thought it made sense and was happy to see flowers and butterflies return.

Where should meadow restoration occur (rural, residential and/or commercial zones)? Delaware has adopted a meadow tier approach making verge management appealing to even commercial zones. High profile areas have planted and manicured vegetation while more rural zones reduce mowing enabling the natural flora to return. Residential areas would be managed somewhere in between [29]. While this may not seem entirely fair, areas with plantings and higher maintenance requirements could contribute to premium landscaping either financially or by providing volunteer gardeners. A similar tier approach, if not already in place in Maryland, could similarly yield positive outcomes.

Why do some roadside meadow programs succeed and others fail? At the Transportation Research Board's 2016 annual meeting several state DOTS shared their success stories. Ohio DOT has actively sought management partnerships with local farmers, conservation groups and gardening clubs. Rather than enforcing a roadside vegetation blueprint, they encourage interested parties to take initiative and develop their own meadow management style tailored to their specific goals (i.e., Pheasants Forever plants grasses conducive to pheasant breeding). In essence partners have pride and a sense of ownership in their project at minimal cost to the state. Failures at sustained meadow management are most often attributed to poor site prep and long-term maintenance plans. As more state DOTS begin to transition to sustainable verge management, valuable lessons can be learned about what works and what doesn't from other state DOTS.

How can impediments be overcome in a productive, equitable way for the various actors? Forming partnerships with utility ROW, federal and state agencies, NGOs and researchers in conjunction with effective communication between all stakeholders can magnify the benefits of pollinator friendly IRVM management in a cost effective manner. Incorporating the socio-economic aspects of green infrastructure into MDOT SHA's pollinator protection plan will surely aid in achieving optimal social outcomes and lasting conservation value for bees and other pollinating insects.

In summary, while the social aspects of roadside vegetation management were not the focus of the present research, the research team was mindful of how the project activities might impact the locals and made an effort to engage with them when appropriate. The importance of the social/public aspect cannot be over emphasized in achieving desired outcomes.

Study Sites and Design Layout

Six roadside sites were established in Frederick and Carroll Counties of Maryland's Piedmont Plateau Province in spring 2016. The Piedmont, located in the central part of the state between the Blue Ridge Mountains and Atlantic coastal plain [39], is characterized by rolling hills and moderately fertile land that is generally clay-like (Ultisols) [40]. Sites 1 - 4 are located along US 15/Catoctin Mountain Highway, while sites 5 and 6 are on MD 194/Woodsboro Pike. Collectively, sites 1 - 6 cover an area with a radius of ~ 24 km, are in USDA plant hardiness zones 6a, 6b and 7a [41], and have an average annual precipitation of 103.1 cm [42].

Historically, this region of the Piedmont has been largely farmland (dairy, corn and soybeans) with minimal development, but in recent decades has become increasingly urban [39].

Design layout & site descriptions

The six sites were .8 km ≥ apart (Figure 1). Each is divided into 2 - 3 treatment plots (SH, fall mow, and turf) of approximately equal acreage, ranging from .6 – 1.8 acres (Figure 2). While all sites had SH and fall mow treatment plots, only half of the sites (sites 4 – 6) had turf treatments (grass height < 7.6 cm) due to logistical limitations. Turf plots are mowed by adjacent land owners, who maintain these state-owned strips as an extension of their well-manicured landscaping. Meadow restoration signs demarcated SH and fall mow plots. However, occasionally utility and highway maintenance crews had to weed whack patches in these zones (i.e., near utility boxes and road signs) for access and safety reasons. The surrounding landscapes at sites 1 – 3 and 5 are similar, predominantly conventional crops of corn and soybeans with borders of natural vegetation. Site 4 is adjacent to Catoctin Mountain Orchard, which grows multiple fruit and vegetable crops using an Integrated Pest Management approach, conventional corn fields and sparse natural vegetation. Lastly, site 6 borders a large swath managed as turf and a patch of woodland mixed with conifers and hardwoods.

Treatments

IVM Partners (Newark, DE), a non-profit organization that works with utility and transportation ROW, performed selective herbicide treatments because MDOT SHA did not have in-house expertise. Timing of herbicide applications varied, depending on when their crew was in Maryland, as they have contracts in numerous states. For the first two seasons, IVM Partners sprayed on September 25, 2016 and July 20, 2017. Applicators used backpack sprayers with a site specific blend of herbicides to treat target species (i.e., Johnson grass, Canada thistle and Callery pear). A team of 5 – 7 applicators methodically covered the entire area by foot, spraying only target species identified by either the foreman or me. Type and quantities of each chemical used were reported for each site. The late season annual mow treatment was handled by MDOT SHA's district shops for Frederick and Carroll Counties. During seasons one and two, they mowed November 16 – 17, 2016 and December 5 – 6, 2017. As mentioned above, turf plots were consistently maintained at < 7.6 cm by adjacent landowners.

Fixed quadrats

Wooden stakes (.05 m x .05 m x 1.2 m) were driven into the ground and marked with a unique, numbered metal ID tag (Figure 3). Stakes serve as fixed points for monitoring the same areas over the course of the study using a collapsible 2 m x 2 m quadrat made of PVC and rope. Fixed stakes were randomly placed along one of two transects or distances (~ 5 and 8 m) from the main highway. Each site had 24 fixed stakes, 12 for the selective herbicide plot and 12 for the late season annual mow plot. Since turf plots were mowed regularly and had been incorporated into the adjacent land owner's landscaping, we could not erect permanent stakes. Instead, twelve random numbers, which correspond to the number of steps in a linear transect, were used for every sampling event. GPS coordinates for each fixed point were recorded. Collectively, there were 180 fixed quadrats (15 treatment plots x 12 fixed points).

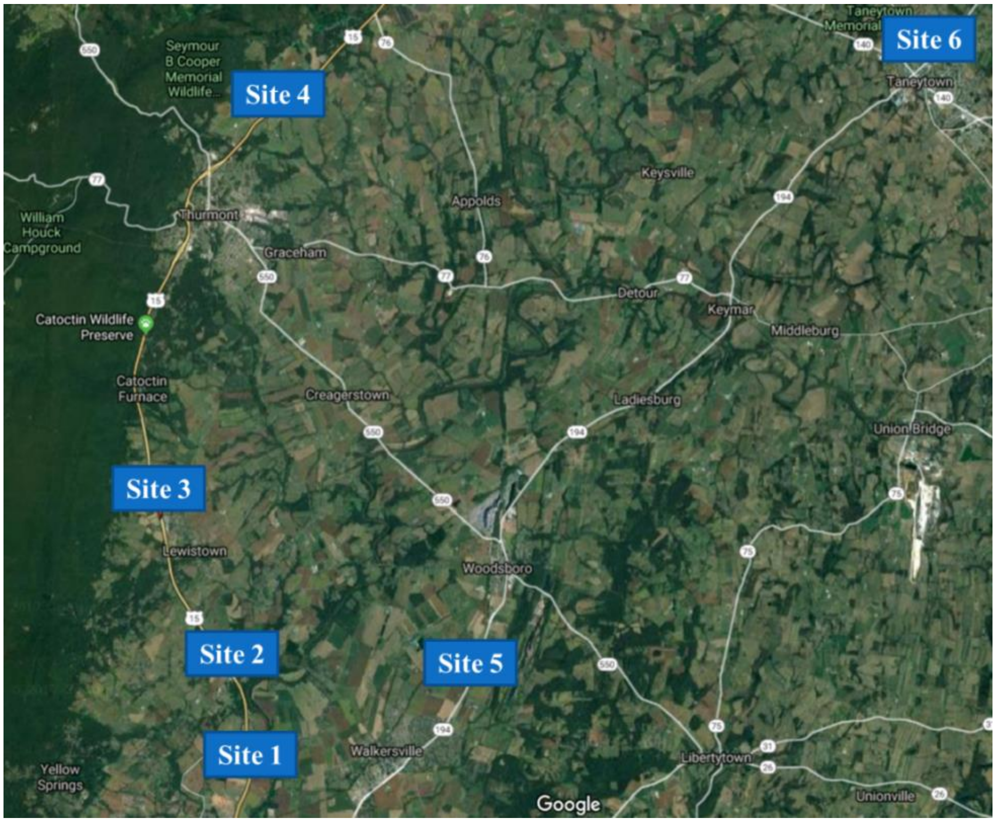


Figure 1: Map of sites 1 – 6 located in Central Maryland

Figure 2: Design layout at Site 4 with three different treatment plots (turf, SH and fall mow)

Figure 3: Fixed quadrat marker, each of which had a unique ID tag enabling us to better monitor changes to the vegetation over time

Measuring effects of three vegetation management regimes on floral resource availability for insect pollinators in highway rights-of-way

Rationale

Monitoring changes in floral resource availability is essential to effective pollinator conservation. Fixed quadrats, small semi-permanent sample plots for assessing the local distribution of plants or animals, are commonly used for detecting vegetational changes over time and are fitting for most plant communities including grasslands and meadows [43]. The research team used sampling techniques that would provide a detailed assessment of floral resources in plots under one of three management regimes – selective herbicide use (SH), annual fall mow (fall mow) and frequent mowing (turf) – by recording the number and abundance of species present. The aim was to answer an important question: which ROW management approach maximizes food resource availability?

Floral diversity and density are integral to bee health because nectar sugar and pollen rewards vary widely across species and fluctuate in space and time [44]. To establish the effectiveness of roadside vegetation management practices at improving habitat for pollinators**,** floral resource availability estimates are needed [44, 49]. Yet despite the vital role flowers play in sustaining bees [14], no generally accepted methodology for estimating floral resources for pollinators exists [45-47]. A recent pollination study review found large methodological differences for estimating food resources and insufficient vegetation sampling both spatially and temporally [44].

Szigeti et al. (2016) determined of the 158 studies reviewed that vegetation sampling (60.6% quadrats/ 33.8% transects) covers only a small proportion (median 0.69%) of the study site, with lengthy gaps between sampling events (median 30 days) and that most studies were short in duration (64% investigated a single year) [45]. Low sampling coverage, which is common with quadrat methods, might be fitting for a homogenous landscape (i.e. monoculture crops) but will likely be inadequate for more heterogeneous environments [45, 48]. The authors reasoned that in the latter case, not only rare but also abundant species can be overlooked if flowers are highly aggregated spatially [45], which is the case in our roadside plots. Long intervals between sampling (i.e. once/month) can yield inaccurate estimates because pollen and nectar stores change rapidly over the season [49] and even throughout the day [45, 50]. Flower compositions (and bee populations) also vary significantly among years [45, 51] so multi-year studies are required to detect treatment effects. The sample design challenges posed above are not unique to pollinator research but apply to the vast majority of ecological studies [52].

One practical solution is to combine different sampling methods that can provide high spatio-temporal resolution or coverage [44]. In a follow up study, Szigeti et al. (2016) compared two common sampling approaches: counting floral units in quadrats and recording the presence-absence of flowering species for the entire meadow plot with qualitative abundance categories, hereafter referred to as ‘scanning’[48]. Overall, they found that quadrat sampling provided higher resolution for abundance estimates, while scanning was better at detecting presence and timing of species [48]. Either method alone would have provided less accurate food resource estimates but when used simultaneously their research effort was optimized [48]. During our first field season, we noted many of the sampling design limitations described by Szigeti et al. Thus with the aim of providing appropriate spatio-temporal resolution and data coverage, Chapter 2 will combine two different sampling methods, quadrat sampling and scanning, for measuring floral resource availability.

Hypotheses

H1: Number of floral species and their relative abundances will be maximized in plots treated with selective herbicide and lowest in those maintained as turf.

H2: Number of floral species and their relative abundances will increase over time

Methods

Fixed quadrat protocol

Quadrat monitoring took place ~ every four weeks (May –September) for three field seasons (2016 – 2018). Quadrat set up: the outside of the quadrat's lower left hand corner is positioned at the fixed stake (from the observer's perspective as they face the centerline of the meadow that runs parallel to the highway), or in the case of turf plots fixed points are located by following the designated number of steps along a transect. After the first field season, we determined that more coverage was needed to more accurately reflect the heterogeneity of the sites. Lengyel et al. (2016) and Szigeti et al. (2016) suggest that a 2 m^2 quadrat is the minimal size that should be used for mowed meadows [48, 53]. Yet, Kearns and Inouye (1993) reason 2 m^2 should be the maximum size used because it is difficult to detect small or rare flowers in a larger quadrat without stepping on them [54], this will be important for Chapter 2. Thus in 2017 the research team increased the quadrats from 1 m^2 to a more appropriate size of 2 m^2.

According to Barbour et al. (1999) species density and frequency or percent cover can be accurately estimated by assessing as little as 1% of a floral community [43]. Acreage of each of the 15 treatment plots (6 selective herbicide, 6 annual mow and 3 turf), ranges from 0.56 – 1.7 acres. Using the 2 x 2 m quadrats, the proportion of site coverage ranges from 0.70 – 2.12%, with only one site having less than 1% coverage (Appendix, Table 1). A review of 158 pollination studies shows that sampling covered on average 0.69% of the study sites [45], so coverage for all of our treatment plots is above the median.

Defining the count variable, the unit of resource availability

Ideally nectar and pollen resources would be measured directly, but that is rarely feasible for many flower species, as collecting adequate samples for analysis is complicated and labor intensive [45, 55] . Thus most pollination studies use count variables that are easy to estimate such as the number of flowers or floral cover [45]. Several studies show that counting flowers can serve as reasonable proxies for floral resources [21, 56] whereas others show they yield fairly imprecise estimates [57]. Szigeti et al. (2016) suggests that a floral unit or visual display may be a "reasonably good choice" as long as the user provides a clear definition [45]. For the present study we chose the same definition for floral unit as Woodcock et al. (2014): pollinators should be able to walk and not have to fly when foraging [58]. The research team selected this description because it closely matches a pollinator's perspective of flowers (food resources) and accounts for floral structures of all sizes and shapes.

<u>Sampling floral resource availability</u>

The research team used two sampling methods, quadrat sampling and scanning, to measure floral resource availability for two field seasons (2017 and 2018). Flowering species are identified using multiple references including 'Wild Urban Plants of the Northeast: A Field Guide' [59], Peterson's Field Guide 'Wildflowers for the Northeastern/ North-central North America' [60], GoBotany.newenglandwild.org [61] and MarylandBiodiversity.com [62] . At least seven new county records were submitted to the MD Biodiversity Project.

Quadrat sampling: each treatment (selective herbicide use, late season annual mow and turf) has 12 – 2 x 2 m fixed quadrats. The research team used sampling protocols developed by Szigeti et al. (2016) although the sampling frequency was adjusted from every 3 to 30 days to allow time to sample six sites and to conduct other field experiments. Approximately every 4 weeks from May to September the research team recorded plant abundance for each flowering species by counting floral units as described above. Only open, non-wilting floral units were counted. Quadrat coverage varies per site, ranging from 0.70 – 2.12% (Appendix, Table 1).

Scanning: approximately every 2 weeks from May to September the research team scanned the field for flowering plants as described by Szigeti et al. (2016) by walking along the edge of the meadow and along the same paths each time to minimize damage to vegetation. Each flowering species was recorded and its abundance estimated based on our overall impression of the meadow's vegetation during our sampling period (30 – 60 minutes, depending on the size of the plot). Using the same protocols as Szigeti et al. (2016), the research team estimated the levels of flower abundance categories of each open, non-wilted flowering species with a slightly revised rank scale for the entire meadow plot: 1: very scarce (1 - 5); 2: scarce (6 – 10); 3: more or less scarce (11 – 100); 4: more less abundant (101 - 500) ; 5: abundant (501 – 1,000); 6: extremely abundant (> 1,000) [48]. The descriptors (i.e. 'very scarce'), while intuitive, were somewhat fuzzy and abstract. Thus in addition to the descriptor, each rank is also associated with a range of numbers. Stefanescu (1997) also assigned number categories to his rank scale [63].

<u>Data analysis and statistics</u>

Quadrat data: each site had 2 – 3 treatments, depending on whether there is a turf treatment, and each treatment plot had 12 fixed quadrats. Thus, only pseudo-replication (n = 12) was achieved so the quadrat data was summed (number and relative abundance of each flowering species) for each treatment and site. Relative abundance corresponds to the number of floral units. The Shannon biodiversity index, which accounts for both abundance and evenness of species present, was calculated and used as the response variable in a linear regression model. Site, treatment and year were treated as explanatory factors.

Scanning data: The relative abundance for each treatment plot was calculated as an arithmetic mean of the section abundances. The Shannon biodiversity index was calculated for each treatment plot and used as the response variable in a linear regression model. Statistical analyses for were done in the JMP statistical environment (JMP® Pro, Version 14.1. SAS Institute Inc., Cary, NC, 1989-2019).

Limitations

Due to time constraints and logistics, there were long intervals between quadrat sampling events, approximately 30 days. While once/month was the median interval for pollination studies [45], the rapid changes in floral composition means the research team likely overlooked or underestimated species that are rare or have short bloom times. Also, the relatively short duration of this study (3 seasons) may make it difficult to detect treatment changes, as meadow restoration can be a slow process. Therefore, the study results might most reflect the early stages of meadow restoration.

Results

Summary of all sites (1 – 6)

Across all sites and seasons, the research team detected a total of 145 different flowering plant species of which 68 are native to the state of Maryland and 77 are introduced or exotic. Table 1 lists all species in alphabetical order according to their common names. Also included are columns indicating species, family and native status (native to Maryland or not), where Y = yes and N = no.

Table 1: List of flowering plant species recorded across all sites

Common name	**Species**	**Family**	**Native to MD?**
Allegheny blackberry	*Rubus allegheniensis*	Rosaceae	Y
Allegheny monkeyflower	*Mimulus ringens*	Scrophulariaceae	Y
American germander	*Teucrium canadense*	Lamiaceae	Y
American pokeweed	*Phytolacca americana*	Phytolaccaceae	Y
Annual ragweed	*Ambrosia artemisiifolia*	Asteraceae	Y
Asian bush honeysuckle	*Lonicera maackii*	Caprifoliaceae	N
Beard-tongue	*Penstemon digitalis*	Plantaginaceae	Y
Biennial beeblossom	*Oenothera gaura*	Onagraceae	Y
Black bindweed (false buckwheat)	*Fallopia convolvulus*	Polygonaceae	N
Black medic	*Medicago lupulina*	Fabaceae	N

Common name	Species	Family	Native to MD?
Black-eyed Susan	*Rudbeckia hirta serotina*	Asteraceae	Y
Blue mistflower	*Conoclinium coelestinum*	Asteraceae	Y
Blue vervain	*Verbena hastata*	Verbenaceae	Y
Blue waxweed	*Cuphea viscosissima*	Lythraceae	Y
Blue-eyed grass	*Sisyrinchium atlanticum*	Iridaceae	Y
Bouncing bet (phlox)	*Saponaria officinalis*	Caryophyllaceae	N
Bull thistle	*Cirsium vulgare*	Asteraceae	N
Butter and eggs	*Linaria vulgaris*	Scrophulariaceae	N
Buttercup sp.	*Ranunculus sp.*	Ranunculaceae	N
Butterfly milkweed	*Asclepias tuberosa*	Asclepiadaceae	Y
Calico aster	*Symphyotrichum lateriflorum*	Asteraceae	Y
Canada goldenrod	*Solidago canadensis*	Asteraceae	Y
Canada thistle	*Cirsium arvense*	Asteraceae	N
Canadian horseweed	*Conyza canadensis*	Asteraceae	Y
Chicory	*Cichorium intybus*	Asteraceae	N
Climbing hempvine	*Mikania scandens*	Asteraceae	Y
Common boneset	*Eupatorium perfoliatum*	Asteraceae	Y
Common burdock	*Arctium minus*	Asteraceae	N
Common chickweed	*Stellaria media*	Caryophyllaceae	N
Common cinquefoil	*Potentilla simplex*	Rosaceae	Y
Common mallow	*Malva neglecta*	Malvaceae	N
Common milkweed	*Asclepias syriaca*	Asclepiadaceae	Y
Common mullein	*Verbascum thapsus*	Scrophularaiaceae	N
Common plantain (broadleafed)	*Plantago major*	Plantaginaceae	Y
Common sow-thistle	*Sonchus sp.*	Asteraceae	N
Common vetch	*Vicia sativa*	Fabaceae	N
Crown vetch	*Securigera varia*	Fabaceae	N
Curly or yellow dock	*Rumex crispus*	Polygonaceae	N
Curlytop knotweed/smartweed	*Persicaria lapathifolia*	Polygonaceae	Y
Daisy fleabane	*Erigeron annuus*	Asteraceae	Y
Dames rocket (purple rocket)	*Hesperis matronalis*	Brassicaceae	N
Dandelion	*Taraxacum officinale*	Asteraceae	N
Deptford pink (Dianthus)	*Dianthus armeria*	Caryophyllaceae	N
Desmodium sp.	*Desmodium sp.*	Fabaceae	Y
Dogbane, Indian hemp	*Apocynum cannabinum*	Apocynaceae	Y
Dotted smartweed	*Polygonum punctatum*	Polygonaceae	Y
Early goldenrod	*Solidago juncea*	Asteraceae	Y
English plantain	*Plantago lanceolata*	Plantaginaceae	N
Evening primrose	*Oenothera biennis*	Onagraceae	Y
False dandelion	*Hypochaeris radicata*	Asteraceae	N

Common name	Species	Family	Native to MD?
Field bindweed	*Convolvulus arvensis*	Convolvulaceae	N
Field mint	*Mentha arvensis*	Lamiaceae	Y
Field mustard	*Brassica rapa sp.*	Brassicaceae	N
Flat topped goldenrod	*Euthamia graminifolia*	Asteraceae	Y
Flower of an hour	*Hibiscus trionum*	Malvaceae	N
Flowering spurge	*Euphorbia corollata*	Euphorbiaceae	Y
Four o'clocks, heart-leaved	*Mirabilis nyctaginea*	Nyctaginaceae	N
Garlic mustard	*Alliaria petiolata*	Brassicaceae	N
Goldenrod sp.	*Solidago sp.*	Asteraceae	Y
Grass-like starwort	*Stellaria graminea*	Caryophyllaceae	N
Great blue lobelia	*Lobelia siphilitica*	Campanulaceae	Y
Great ragweed	*Ambrosia trifida*	Asteraceae	Y
Green milkweed	*Asclepias viridiflora*	Asclepiadaceae	Y
Green ponsettia	*Euphorbia dentata*	Euphorbiaceae	N
Ground ivy	*Glechoma hederacea*	Lamiaceae	N
Hairy jointed meadow parsnip	*Thaspium barbinode*	Apiaceae	N
Hairy vetch	*Vicia villosa*	Fabaceae	N
Hawkweed/wiry, yellow flwr	*Hieracium caespitosum*	Asteraceae	N
Heal-all	*Prunella vulgaris*	Lamiaceae	Y
Henbit	*Lamium amplexicaule*	Lamiaceae	N
Hollyhock	*Alcea rosea*	Malvaceae	N
Honeyvine	*Cynanchum laeve*	Asclepiadaceae	Y
Horsenettle	*Solanum carolinense*	Solanaceae	Y
Horseweed	*Conyza canadensis*	Asteraceae	Y
Indian tobacco	*Lobelia inflata*	Campanulaceae	Y
Iris sp.	*Iris sp.*	Iridaceae	N
Ivy leaved morning glory	*Ipomoea hederacea*	Convolvulaceae	N
Japanese honey-suckle	*Lonicera japonica*	Caprifoliaceae	N
King of the meadow	*Thalictrum pubescens*	Ranunculaceae	Y
Korean clover	*Kummerowia stipulacea*	Fabaceae	N
Lespedeza sp.	*Lespedeza cuneata*	Fabaceae	N
Maiden's tears	*Silene vulgaris*	Caryophyllaceae	N
Moth mullein	*Verbascum blattaria*	Scrophularaiaceae	N
Mugwort	*Artemisia vulgaris*	Asteraceae	N
Multiflora rose	*Rosa multiflora*	Rosaceae	N
Musk thistle	*Carduus nutans*	Asteraceae	N
Mustard sp.	*Barbarea sp.*	Brassicaceae	N
Narrowleaf mountain mint	*Pycnanthemum tenuifolium*	Lamiaceae	Y
New York ironweed	*Vernonia noveboracensis*	Asteraceae	Y

Common name	Species	Family	Native to MD?
Nightshade sp.	*Solanum sp.*	Solanaceae	N
Nodding plumeless thistle	*Carduus nutans*	Asteraceae	N
Oldfield aster	*Symphyotrichum pilosum*	Asteraceae	Y
Orange daylily	*Hemerocallis fulva*	Liliaceae	N
Orange jewelweed	*Impatiens capensis*	Balsaminaceae	Y
Orchard grass	*Dactylis glomerata*	Poaceae	N
Orchid (Ladies tresses)	*Spiranthes lacera*	Orchidaceae	Y
Oxeye daisy	*Chrysanthemum leucanthemum*	Asteraceae	N
Pepperweed sp.	*Lepidium campestre*	Brassicaceae	N
Pimpernel	*Lysimachia arvensis*	Primulaceae	N
Plumeless thistle	*Carduus acanthoides*	Asteraceae	N
Poison hemlock	*Conium maculatum*	Apiaceae	N
Prickly lettuce	*Lactuca serriola*	Asteraceae	Y
Purple deadnettle	*Lamium purpureum*	Lamiaceae	N
Purple stemmed aster	*Symphyotrichum puniceum*	Asteraceae	Y
Queen Anne's lace	*Daucus carota*	Apiaceae	N
Red clover	*Trifolium pratense*	Fabaceae	N
Rose bush, wild	*Rosa sp.*	Rosaceae	N
Rose of Sharon	*Hibiscus syriacus*	Malvaceae	N
Rough bugleweed	*Lycopus sp.*	Lamiaceae	Y
Rough cinquefoil	*Potentilla norvegica*	Rosaceae	Y
Small white morning glory	*Ipomoea lacunosa*	Convolvulaceae	Y
Smartweed, arrowleaf tearthumb	*Persicaria sagittata*	Polygonaceae	Y
Smartweed, Pennsylvania	*Persicaria pensylvanica*	Polygonaceae	Y
Sow thistle sp.	*Sonchus sp.*	Asteraceae	N
Spearmint	*Mentha spicata*	Lamiaceae	N
Speedwell, bird's eye	*Veronica persica*	Scrophulariaceae	N
Speedwell thyme	*Veronica serpyllifolia*	Plantaginaceae	Y
Spotted Joe Pye weed	*Eutrochium maculatum*	Asteraceae	Y
Spotted knapweed	*Centaurea maculosa*	Asteraceae	N
Spotted sandmat	*Euphorbia maculata*	Euphorbiaceae	Y
St. John’s wort	*Hypericum sp.*	Hypericaceae	N
Star of Bethlehem	*Ornithogalum umbellatum*	Liliaceae	N
Starwort	*Stellaria graminea*	Caryophyllaceae	N
Sulphur cinquefoil	*Potentilla recta*	Rosaceae	N
Swamp beggarsticks	*Bidens connata*	Asteraceae	Y
Swamp milkweed	*Asclepias incarnata*	Asclepiadaceae	Y
Teasal	*Dipsacus fullonum*	Dipsacaceae	N

Common name	Species	Family	Native to MD?
Velcro plant	*Galium aparine*	Rubiaceae	Y
Velvet weed	*Abutilon theophrasti*	Malvaceae	N
Viburnum	*Viburnum sp.*	Adoxoceae	Y
Virgin bower	*Clematis virginiana*	Ranunculaceae	Y
Virginia ground cherry	*Physalis virginiana*	Solanaceae	Y
Watercress	*Nasturtium officinale*	Brassicaceae	N
White clover	*Trifolium repens*	Fabaceae	N
White sweet clover	*Melilotus alba*	Fabaceae	N
White vervain	*Verbena urticifolia*	Verbenaceae	Y
Wild bergamot	*Monarda fistulosa*	Lamiaceae	Y
Wild garlic	*Allium vineale*	Amaryllidaceae	N
Wild geranium	*Geranium maculatum*	Geraniaceae	Y
Wood sorrel	*Oxalis stricta*	Oxalidaceae	Y
Yarrow	*Achillea millefolium*	Asteraceae	Y
Yellow rocket	*Barbarea vulgaris*	Brassicaceae	N
Yellow salsify	*Tragopogon dubius*	Asteraceae	N
Yellow sweet clover	*Melilotus officinalis*	Fabaceae	N
Yellow wingstem	*Verbesina alternifolia*	Asteraceae	Y
		Total native	68
		Total non-native	77
		Sum	145

Descriptive statistics of plant composition across all sites

Statistical package JMP® Pro, Version 14.1 was used for all statistics and graphs in the graphs and figures that follow. Figures 4 and 5 on the following page demonstrate stark differences in the total number of plant species of the control group (turf) from the two IRVM treatment groups (fall mow and SH). Figure 4 is a plot of the mean no. of plant species per treatment and illustrates changes from 2016 – 2018. Figure 5 parses out the pooled data in Figure 4 to examine the effects of site. Both bar graphs show detectable variation between the three explanatory factors: site, treatment and year.

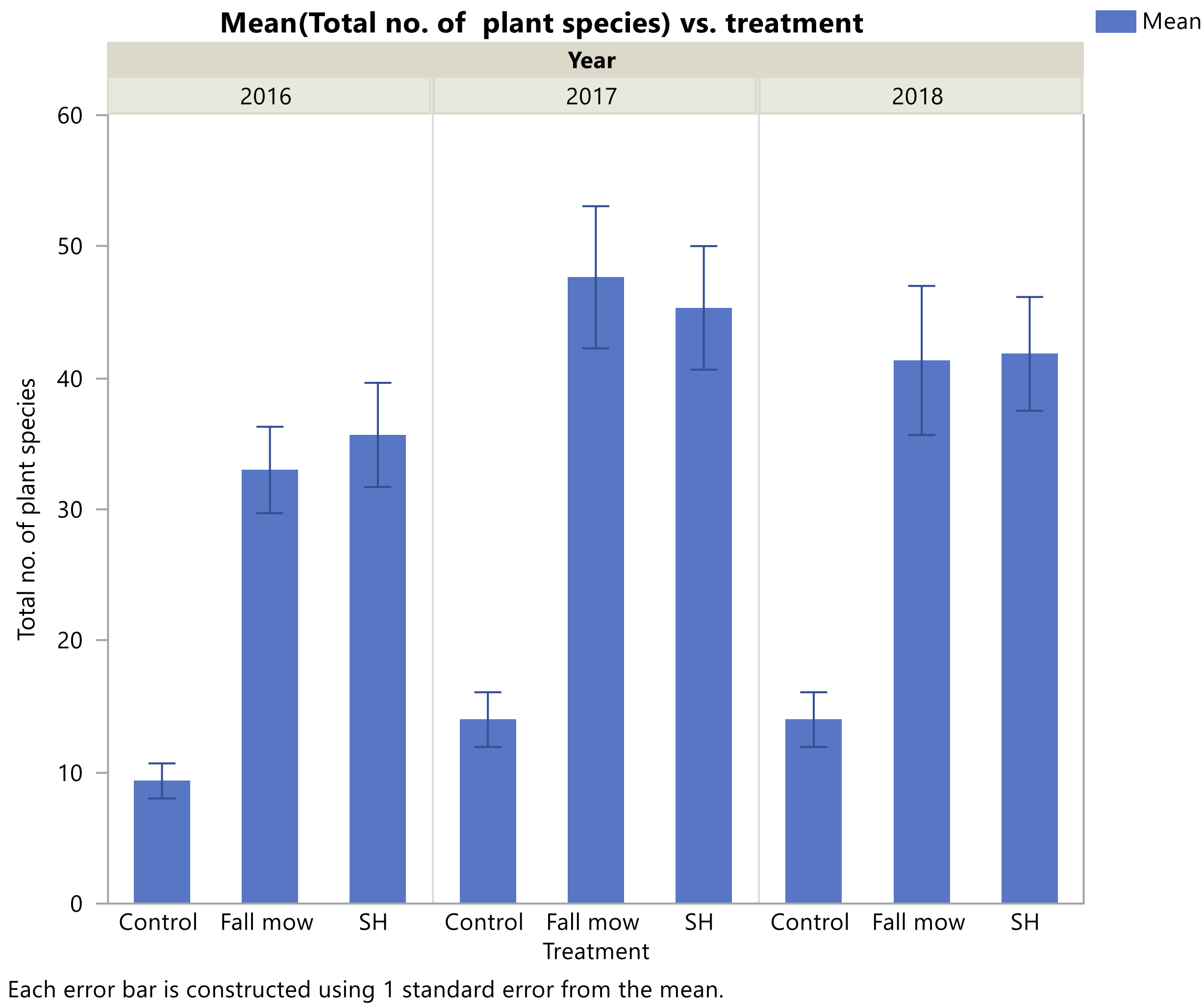


Figure 4: bar graph comparing the pooled means of total no. of plant species for each treatment. Data from all sites (1 – 6) are represented.

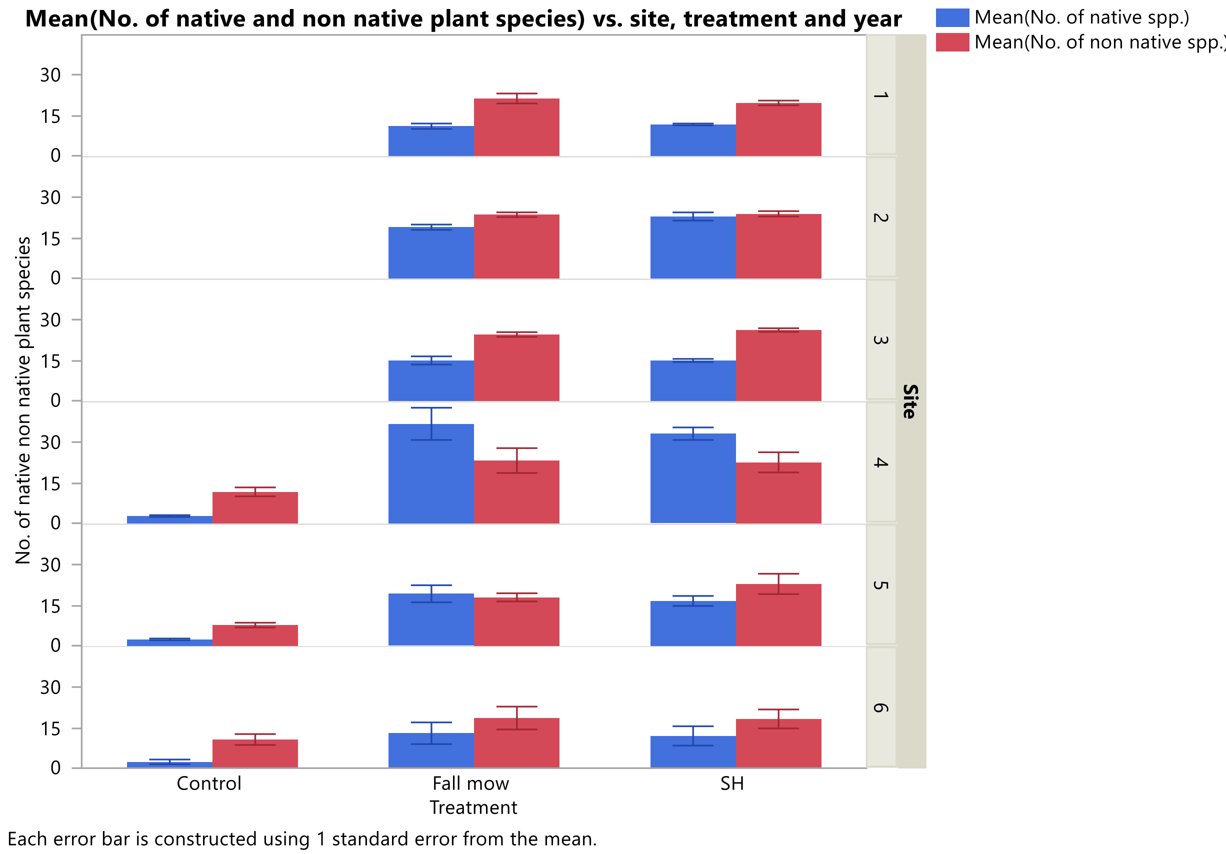
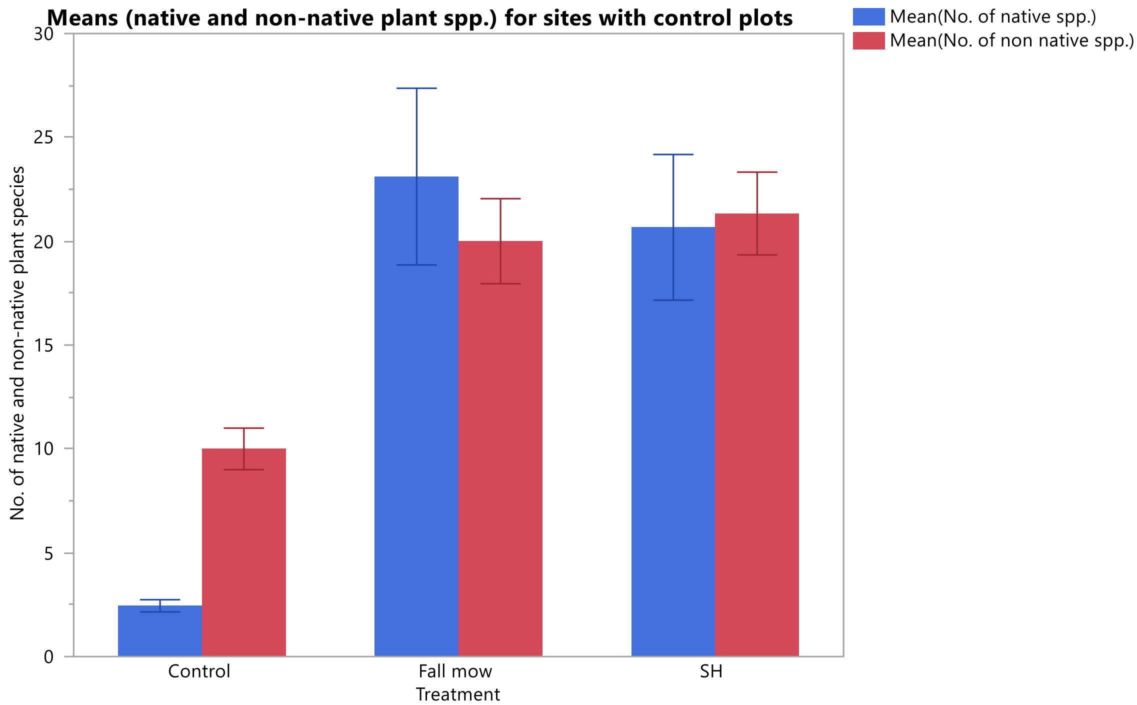


Figure 5: No. of floral species parsed out by site, treatment and year. The factor 'site' is on the left hand side of the bar graph. Control groups were not an option at sites 1 – 3 hence the no. of species for their controls are zero. Plant species fall into one of two categories, native or non-native, where blue = native and red = non-native.

<u>Comparisons of sites with control plots</u>

As noted earlier, control groups were limited to sites 4 – 6 because of logistical constraints. Given the complexities associated with unbalanced designs, we believe it's most useful to restrict the remainder of the plant data analyses for this report to sites with controls (sites 4 – 6). For my doctoral thesis and any resulting publications, data from the other three sites (1 - 3) will be included in a more exhaustive analysis and shared with MDOT SHA.

Figures 6 and 7 on the following page compare means of native and non-native plant species for sites with control plots, where blue bars represent native species and red non-native species. The means for each treatment in Figure 7 show that both fall mow and SH treatments had ~ 8x the number of plant species than control plots (turf maintained as lawn) with means similar to one another (fall mow 19.1 spp. and SH 18.6). Treatment was a significant predictor of total number of native vs. non-native species in a linear model (p value > .0001). Site (p value >.57) and year (p value > .09) were not significant predictors of the native/non-native ratio.



Where(18 rows excluded)
Each error bar is constructed using 1 standard error from the mean.

Figure 6: Bar graph comparing mean number of plant species (blue = native and red = non-native) by one of three treatments (control or turf, fall mow and SH for selective herbicide).

Linear model results

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	2150.7225	537.681	7.9822
Error	40	2694.3886	67.360	**Prob > F**
C. Total	44	4845.1111		<.0001 *

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	2	2	1831.3889	13.5941	<.0001 *
Site	1	1	22.5225	0.3344	0.5663
Year	1	1	192.5333	2.8583	0.0987

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
Control	1.903904	2.8910670	2.4444
Fall mow	19.190691	1.9485437	19.0556
SH	18.746246	1.9485437	18.6111

Figure 7: Least squares regression shows that treatment ($p < .0001$) was a significant predictor of plant species composition (native vs. non-native) in a linear model. Whereas, factors site (p value > 0.5) and year (p value >.1) were not significant. Both fall mow and SH had approximately 8x the mean number of plant species than control groups (grass that is maintained as turf).

Results for quadrat floral counts

As described earlier, each treatment plot had 12 fixed quadrats. Thus, the research team had pseudo-replication (n = 12) so summed the quadrat data (number and relative abundance of each flowering species) for each treatment and site. Relative abundance corresponds to the number of floral units. The Shannon biodiversity index, which accounts for both abundance and evenness of species present, was calculated and used as the response variable in a linear regression model. Site, treatment and year were treated as explanatory factors. Figure 8 sums quadrat floral counts for each treatment and compares the means. Figure 9 breaks the data down by year as well showing that floral counts decreased for all treatments from 2017 to 2018. Figure 10 provides the linear model results. Both treatment (p-value < .012) and year (p-value <.012) were significant predictors of plant biodiversity. Site (p-value >0.2) was not a significant factor.

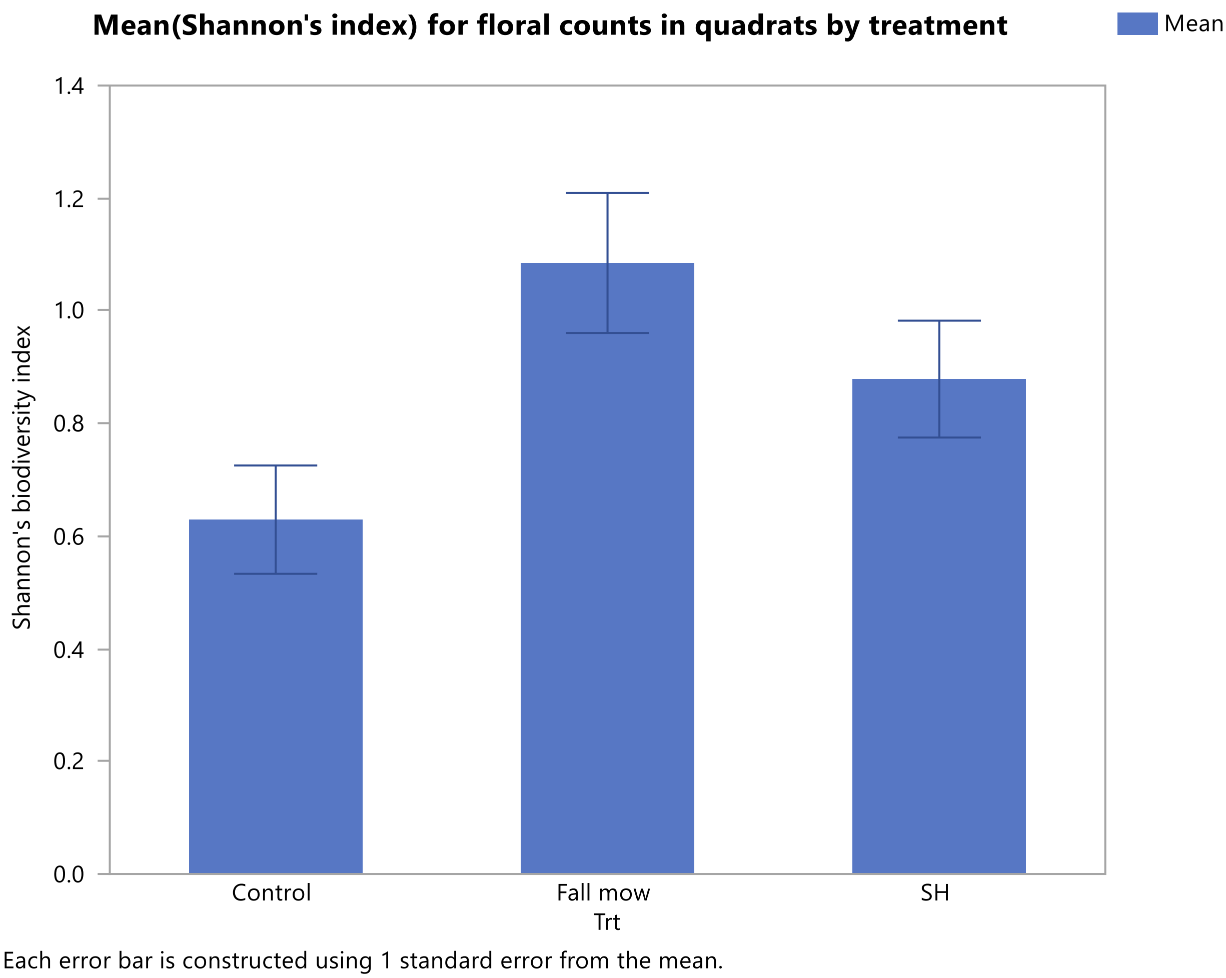
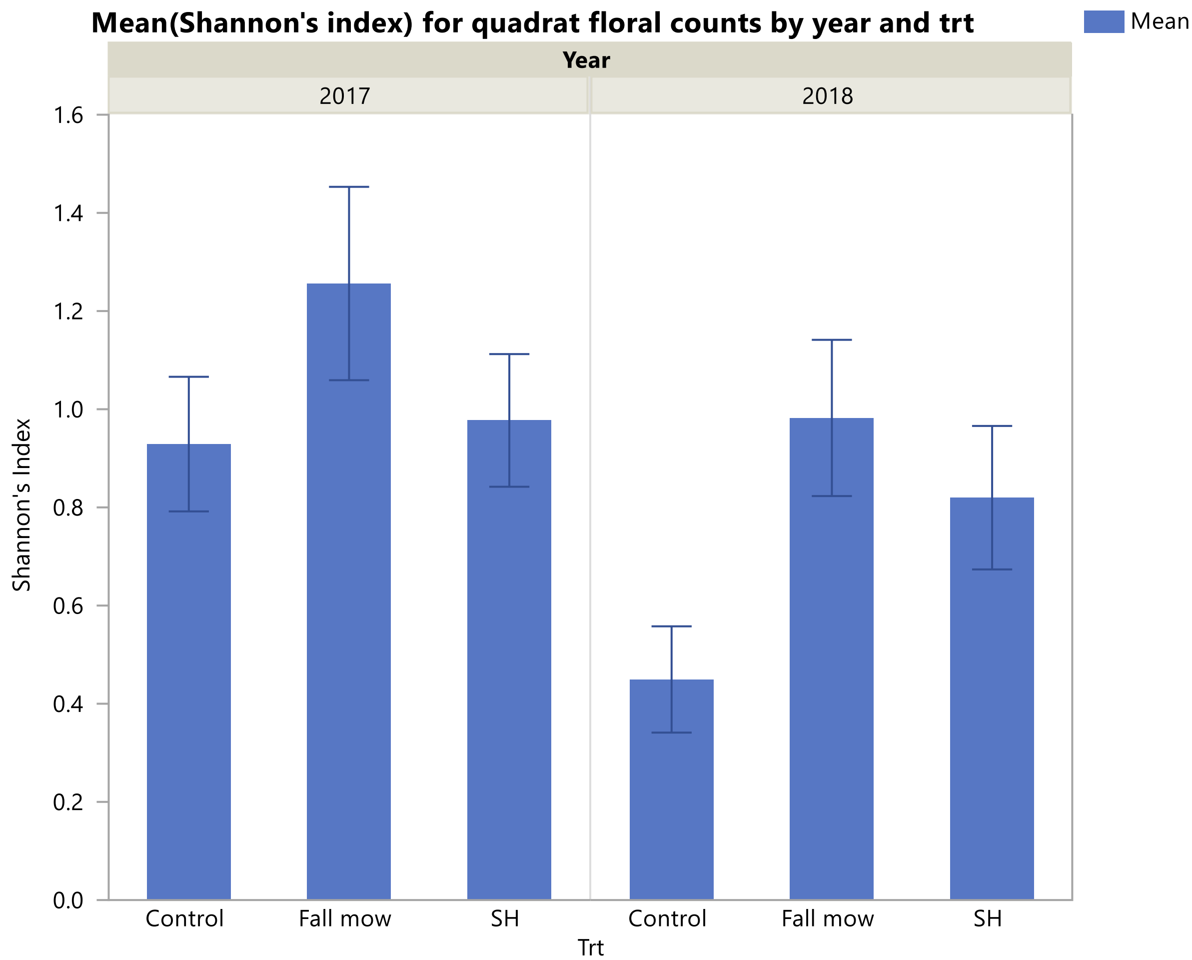


Figure 8: Bar graph comparing mean (Shannon's biodiversity index) for quadrat floral counts by treatment; Data were summed for each treatment.



Each error bar is constructed using 1 standard error from the mean.

Figure 9: Bar graph comparing mean (Shannon's biodiversity index) for quadrat floral counts by treatment and year; Data from sites with control groups were summed by treatment and plotted by year to detect annual fluctuations.

Quadrat floral counts – linear model predictors of Shannon's biodiversity index

Effect Summary

Source	LogWorth		PValue
Trt	1.927		0.01184
Year	1.750		0.01778
Site	0.629		0.23472

Summary of Fit

RSquare	0.200763
RSquare Adj	0.153048
Root Mean Square Error	0.513256
Mean of Response	0.864332
Observations (or Sum Wgts)	72

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	4.433549	1.10839	4.2075
Error	67	17.649900	0.26343	**Prob > F**
C. Total	71	22.083449		0.0042 *

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	613.891	252.0883	2.44	0.0176 *
Trt[Control]	-0.235075	0.085543	-2.75	0.0077 *
Trt[Fall mow]	0.2205998	0.085543	2.58	0.0121 *
Site	-0.08883	0.074082	-1.20	0.2347
Year	-0.303616	0.124943	-2.43	0.0178 *

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Trt	2	2	2.4992143	4.7436	0.0118 *
Site	1	1	0.3787553	1.4378	0.2347
Year	1	1	1.5555790	5.9051	0.0178 *

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
Control	0.6292575	0.10476787	0.62926
Fall mow	1.0849320	0.10476787	1.08493
SH	0.8788072	0.10476787	0.87881

Figure 10: Linear model results to determine the best predictors of Shannon's biodiversity indices from quadrat floral counts. Treatment and year were significant predictors while site was not.

Results for scanning data

The relative abundance for each treatment plot was calculated as an arithmetic mean of the section abundances. The Shannon biodiversity index was then calculated for each treatment plot and used as the response variable in a linear regression model. Site, treatment and year were treated as explanatory factors. Figure 11 sums scanning data for each treatment and compares the means. Figure 12 breaks the data down by year as well showing that floral counts decreased for all treatments from 2017 to 2018. Figure 13 provides the linear model results. Treatment (p-value $< .0001$), site (p-value $<.0001$) and year (p-value $<.0001$) were significant predictors of plant biodiversity.

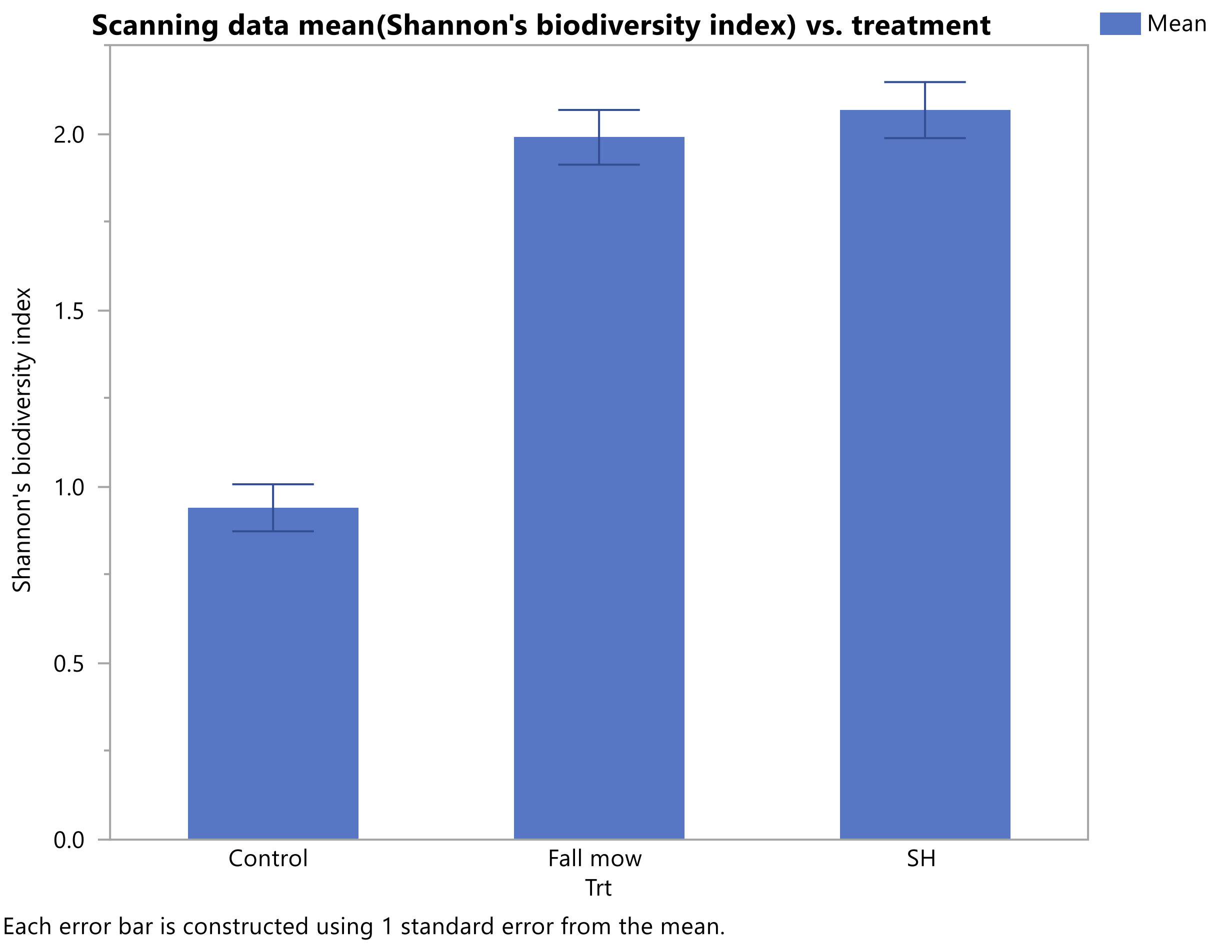


Figure 11: Bar graph comparing mean (Shannon's biodiversity index) for scanning floral counts by treatment; Data were summed for each treatment.

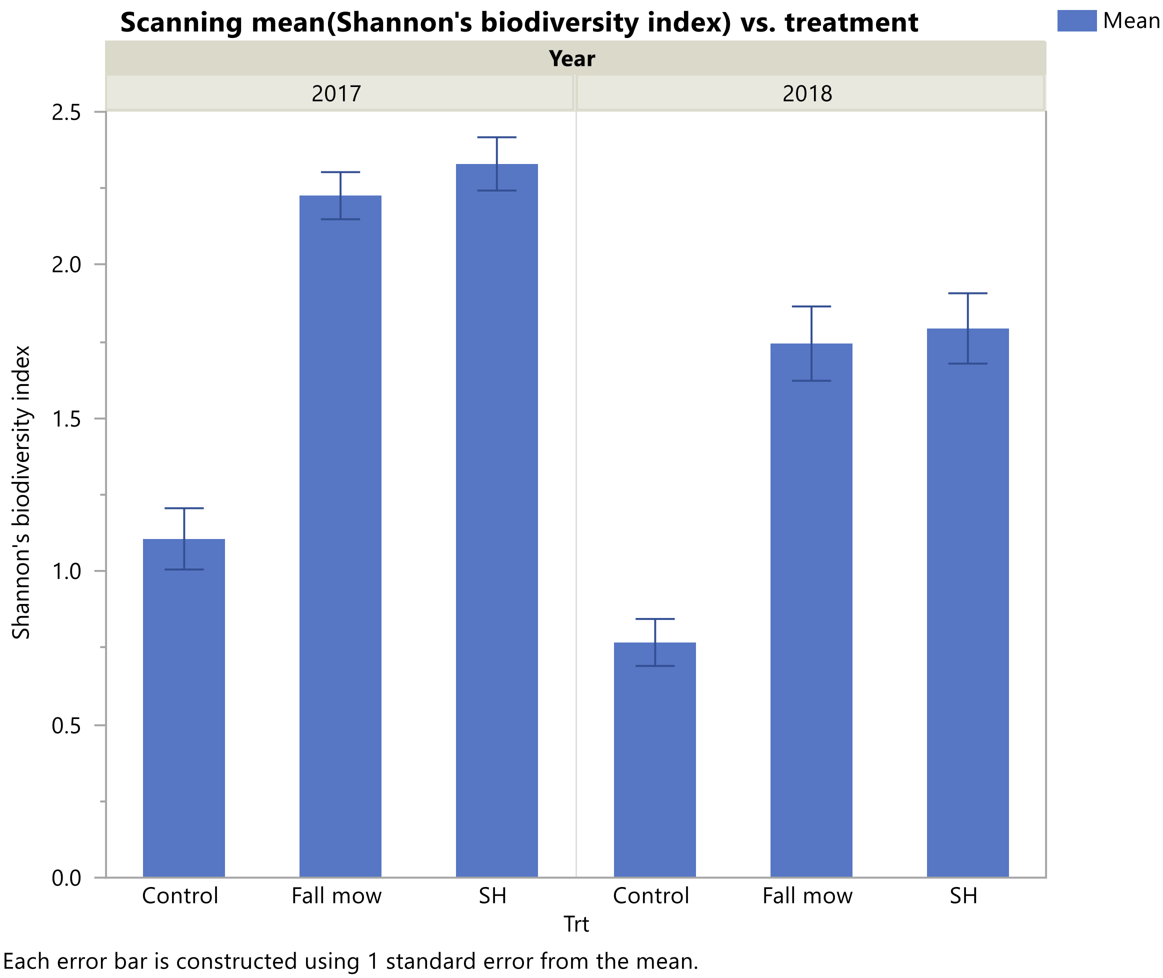


Figure 12: Bar graph comparing mean (Shannon's biodiversity index) for scanning by treatment and year; Data from sites with control groups were summed by treatment and plotted by year to detect annual fluctuations.

Scanning – linear model predictors of Shannon's biodiversity index

Effect Summary

Source	LogWorth	PValue
Trt	30.114	0.00000
Site	10.079	0.00000
Year	8.923	0.00000

Summary of Fit

RSquare	0.637549
RSquare Adj	0.62912
Root Mean Square Error	0.468051
Mean of Response	1.66491
Observations (or Sum Wgts)	177

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	66.27943	16.5699	75.6368
Error	172	37.68028	0.2191	**Prob > F**
C. Total	176	103.95971		<.0001 *

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	916.50323	141.9747	6.46	<.0001 *
Trt[Control]	-0.725114	0.049753	-14.57	<.0001 *
Trt[Fall mow]	0.3240137	0.049753	6.51	<.0001 *
Site	-0.295875	0.042727	-6.92	<.0001 *
Year	-0.45272	0.070372	-6.43	<.0001 *

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Trt	2	2	46.707711	106.6039	<.0001 *
Site	1	1	10.505047	47.9526	<.0001 *
Year	1	1	9.066675	41.3868	<.0001 *

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
Control	0.9397965	0.06093500	0.93980
Fall mow	1.9889240	0.06093500	1.98892
SH	2.0660103	0.06093500	2.06601

Figure 13: Linear model results to determine the best predictors of Shannon's biodiversity indices from scanning floral resources. Treatment, site and year were all significant predictors of Shannon's biodiversity index.

Discussion of vegetation monitoring results

During the 3-year field study, the research team identified 145 flowering plant species, 68 native and 77 introduced. Some of the sites had some real gems not commonly found in Central Maryland, including, green milkweed (*Asclepias viridis*) and orchids called ladies tresses (*Spiranthes lacera*). While meadows are known to take many years to establish, in the fall mow and SH treatments, we saw wildflower species regenerate naturally in many patches. As one MDOT SHA employee and plant expert John Krause said after a site visit to a few of the roadside trial plots, some of the areas were likely at some point seeded with wildflowers by MDOT SHA. If so, it stands to reason that viable seed is still in the soil column and will indeed germinate given the opportunity, such as under IRVM management.

At the onset of the study, we started with two the following two hypotheses: H1: Number of floral species and their relative abundances will be maximized in plots treated with selective herbicide and lowest in those maintained as turf, and H2: Number of floral species and their relative abundances will increase over time. Both vegetation monitoring methods, scanning the 'meadow' plots for and ranking floral species in addition to floral counts in fixed quadrats, found that treatment was a significant predictor of plant biodiversity, as measured by the Shannon-Weiner biodiversity index (Figures 10 and 13). Again, the Shannon biodiversity index (SI) is a quantitative measurement that reflects the number of different species in a given habitat and how evenly they are distributed. Thus, it is frequently used index in ecological studies to determine the health of an ecosystem. For the quadrat floral counts, which provided accurate counts for a small percentage of the entire plot, the SI range was 0.63 – 1.08 in the following order control<SH<fall mow. On the other hand, the scanning method provided an overview of the entire meadow mind you with less precision because floral estimates were used vs. direct counts. The SI range for each treatment ranged from 0.94 – 2.07 in the following order control<fall mow<selective herbicide. In both cases, there was a statistically significant difference between the mean (SI) of the IRVM treatments and the control but not between the two treatments. To detect a statistical difference between the two IRVM treatments, more sites and a longer term study would likely be needed.

Several factors potentially affected our results. The decreases observed in both abundance and number of species, was likely owing in part to record levels of rainfall during the summer of 2018 [64]. The study also experienced several unplanned mowing events. A more in-depth discussion about the role of natural variability due to climate, unplanned human activities, etc. can be found in the pollinator monitoring discussion section. Lastly, a few comments about the selective herbicide applications and how they might have shaped the floral outcomes in the SH plots. In the research team's opinion, spraying took place during non-optimal times (09/25/16 and 07/20/17). Those were the only dates that IVM Partners were available to spray, as they travel across the nation. SH likely has great potential as an alternative or in conjunction with a reduced mowing regime, but to optimize the tool, timing of applications will be key.

A comparison of roadside bee communities in Central MD under different vegetation management regimes

Rationale

While the first section of this research focused on how different management strategies affect floral resource availability, the second half focuses on pollinators, specifically wild and managed bees.

Despite the growing interest in managing highway rights-of-way (ROW) to promote pollinators, little is known about roadside bee communities. Wojcik and Buchmann's (2012) review of pollinator conservation and management of transportation ROW, found only a few roadside studies that assess bee populations [4]: one from Kansas where Hopwood (2008) showed native plantings had a strong positive effect on roadside bee diversity and abundance, and a second from the Netherlands where Noordijk et al. (2009) established that mowing twice per year plus thatch removal supported more bee groups than less frequent mowing [67].

More recently, Hatten et al. (2015) conducted a short-term survey of bumble bees (Genus: *Bombus*) along highway ROW in British Columbia and Yukon territories, recording 14 different species with varied geographical distributions [65]; and in England, Hanley and Wilkins (2015) found bumble bees preferred road-facing hedgerows over crop-facing margins [66]. While the available research is promising, we still have only a handful of studies on bee diversity and abundance in transportation ROW, with half focusing on only a single genus. Multiyear bee population data are needed to evaluate whether roadside restoration efforts are effective. Also, to my knowledge, no roadside pollinator studies for the Northeast Region of the U.S. exist. Regional bee monitoring is necessary for establishing best management practices for local roadside vegetation.

Common bee monitoring methods include pan traps, aerial netting and an observational approach. Colored pan traps are small bowls in hues known to attract bees (blue, white and yellow) that are filled with soapy water [67, 68]. Insects land on the water, then drown [68]. While particularly effective at catching smaller species such as sweat bees (Family: Halictidae) they have several known biases [68]. Toler et al. (2005) noted that pan traps catch bumble bees and honey bees (*Apis mellifera*) and some species from the genus Colletes much less frequently than expected based on field observations [69]. Also, flowers may compete with pan traps, particularly in floral-rich areas, but this dynamic is not well understood [68]. Thus pan traps are often supplemented by other methods such as aerial netting, a method of collecting insects at host plants, and observations of bees while visiting blooms.

Chapter 3 will thus use a combination of three sampling methods: pan traps, aerial netting and observations, to compare the influence of different vegetation management strategies (selective herbicide use, late season annual mow and turf) on bee diversity, abundance and general host-plant associations. Data from this study will help establish regional best management practices for promoting pollinators in transportation ROW.

Hypothesis

H1: Roadside bee communities will be more species rich and more abundant in plots treated with selective herbicides and least in those maintained as turf

H2: Roadside bee communities will be more species rich and more abundant over time as roadside plots transition from turf to naturally regenerating meadows

Methods

<u>Pan traps</u>

Collections were conducted at each of the treatment plots across six sites, which are described in detail in 'Study sites and design layout' of Chapter 1. Sampling began the last week of May and continued approximately every 4 weeks through the end of September 2016 -2017. The same procedures will be used in 2018. Three sampling methods were used, pan traps, aerial netting and observations. For the pan trapping we used 1 - 3 sampling transects, depending on the length and shape of the plots. Pan trap and netting collection procedures were similar to those used previously [70, 71]. Combined, transects for each treatment plot were made up of 27 pan traps (New Horizons Support Services, Inc.; 3.5 oz.) of three alternating colors: blue, white and yellow each spaced about 10 m apart. Permanent wooden stakes were erected in selective herbicide and reduced mow treatment plots to elevate pan traps to the height of the vegetation (Figure 14). Vegetation height changed drastically from month to month, so the research team adjusted the height of the pan traps as needed to ensure they were visible to foraging bees. In turf plots, pan traps were placed directly on the ground. Pans traps were filled with soapy water then left in place for ~ 24 hours. Bowl traps from a given site and treatment were combined into a single filter cone then placed in vials with 70% ethanol and a collection label.

Figure 14: Adjustable pan trap holders used to elevate bowls to the height of the vegetation

Netting/bagging

Bees were collected from flowering blooms in the same treatment plots as pan traps with an aerial net (Bioquip 38 cm net ring diameter, 850 x 780 micron mesh, 91.5 cm aluminum handle) and plastic bags (Ziploc gallon bags). The research team initially used aerial nets but discovered that they did not work well, as roadside vegetation is often comprised of prickly species that tear mesh netting. Thus the research team switched to clear storage bags, which allow one to avoid barbed plants and more easily collect foraging bees (Unpublished work of Olivia Bernauer). Sampling of treatment plots was carried out as two people walking simultaneously along different transects for a total of 30 minutes, searching blooms for ~ 30-second intervals on the same day as pan trapping. Sampling was performed during optimal foraging conditions from 0900 – 1700. The research team attempted to net all bees except honey bees and carpenter bees (*Xylocopa virginica*), which can be identified by sight. When copious amounts of poison ivy and dense vegetation made it difficult to effectively bag insects, an observational approach in lieu of netting was used. Observational data were largely recorded in more general terms, i.e. bee groups such as: bumble bees and halictid bees, as most bees cannot be identified to species on the wing. Specimens were placed in vials with 70% ethanol and a collection label that included the name of the host-plant.

Curation

Specimens were processed, pinned and labeled according to treatment, site, date, collection methods and floral-host where relevant and given a unique barcode and Discover Life (www.discoverlife.org) ID number. After the research team sorted specimens by genus using taxonomic guides in 'The Bees in Your Backyard: A Guide to North America's Bees' [72] Sam Droege from USGS – Bee Monitoring and Identification Lab identified them to species level.

Data analysis

Pan trap, observational and netting data will be pooled for each treatment plot. Then the Shannon biodiversity index, which accounts for abundance and evenness of the species at a given location, was calculated and used as the response variable in a linear regression model. Treatment, site and year were treated as explanatory variables. Statistics were performed in JMP® Pro, Version 14.1. SAS Institute Inc., Cary, NC, 1989-2019.

Limitations

Long intervals (~ 30 days) and timing of sampling (May – September) while common in pollination studies will increase the probability of overlooking rare species or species with a short foraging period. Many bees in Maryland are only active early spring [73], so those species might complete their life cycle before our sampling period. Yet, the pilot sampling from early spring yielded very few captures and floral resources were largely absent until late May. Regarding pan traps, Roulston et al. (2007) noted that caution must be used when comparing bee samples from flower-rich and flower poor sites, as pan traps may be more effective in areas where flowers are scarce [68]. Thus, that will be a factor when comparing bee samples from turf to those of the other two treatments, which have more diverse and abundant flora. Lastly, observational data lack the resolution of pan trapping and aerial netting, as bees on the wing can generally be identified to genus level at best.

Results for pollinator monitoring

Over the course of three growing seasons (May – October), a total of 5,159 bees and 83 different bee species were recorded, including seven new county records (marked with an asterisk). Table 2 provides the list of bee species, all of which were confirmed by taxonomist Sam Droege from the USGS Native Bee Monitoring Inventory Lab in Patuxent, MD.

Bee species from roadside plots in Frederick and Carroll Counties from 2016 - 2018		
Agapostemon texanus	*Halictus confusus*	*Megachile exilis*
Agapostemon virescens	*Halictus ligatus/poeyi*	*Megachile mendica*
Andrena commoda	*Halictus rubicundus*	*Megachile montivaga*
Andrena cressonii	*Heriades carinatus*	*Megachile rotundata*
Andrena erigeniae	*Heriades leavitti/variolosus*	*Megachile sculpturalis*
Andrena nasonii	*Hoplitis pilosifrons*	*Melissodes bimaculatus*
Andrena perplexa	*Hoplitis producta*	*Melissodes comptoides*
**Andrena personata*	*Hoplitis spoliata*	**Melissodes denticulatus*
Andrena vicina	*Hylaeus affinis/modestus*	*Melissodes desponsus*
Andrena violae	*Hylaeus mesillae*	*Melissodes trinodis*
Andrena wilkella	*Lasioglossum admirandum*	*Melitoma taurea*
Anthidium oblongatum	*Lasioglossum albipenne*	*Nomada bidentate_*
Apis mellifera	*Lasioglossum bruneri*	*Nomada pygmaea*
Augochlora pura	*Lasioglossum callidum*	*Osmia bucephala*
Augochlorella aurata	*Lasioglossum coriaceum*	**Osmia distincta*
Augochloropsis metallica_fulgida	*Lasioglossum cressonii*	*Osmia georgica*
Bombus bimaculatus	*Lasioglossum hitchensi*	*Osmia pumila*
**Bombus fervidus*	*Lasioglossum illinoense*	*Peponapis pruinosa*
Bombus griseocollis	*Lasioglossum imitatum*	*Pseudoanthidium nanum*
Bombus imitatum	**Lasioglossum nymphaearum*	**Ptilothrix bombiformis*
Bombus impatiens	*Lasioglossum obscurum*	*Svastra obliqua*
Bombus perplexus	*Lasioglossum pilosum*	*Triepeolus cressonii*
Calliopsis andreniformis	*Lasioglossum platyparium*	*Xylocopa virginica*
Calliopsis mikmaqi	*Lasioglossum tegulare*	
Ceratina calcarata	*Lasioglossum trigeminum*	
Ceratina dupla	*Lasioglossum versatum*	
Ceratina mikmaqi	*Lasioglossum weemsi*	
Ceratina strenua	*Lasioglossum zephyrum*	
Colletes latitarsis	*Megachile addenda*	
Eucera hamata	**Megachile brevis*	

Table 2: Bee species from all roadside plots (sites 1 – 6)

The statistical package JMP® Pro, Version 14.1 was used for all statistics and graphs in this report. Figure 15 below provides collective bee counts for all monitoring methods (hand net, observation and pan trapping) and all sites (1 – 6) across time (2016 – 2018). The highest bee count was in 2017. Bee counts for 2017 are 2x higher than for both 2016 and 2018. Bee abundances also varied by treatment and site (Figure 16). Figure 16 shows that sites 2 – 4 had the largest number of bees, particularly in 2017.

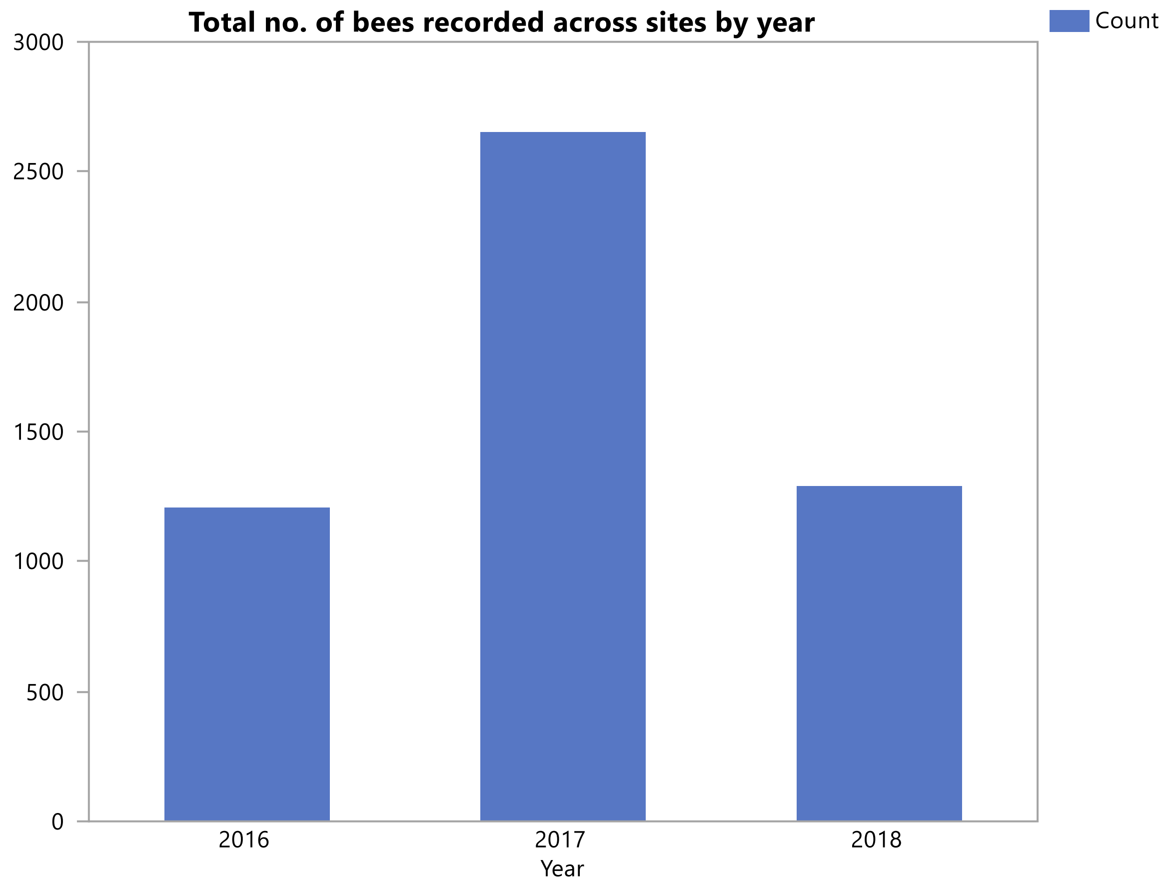


Figure 15: Bar graph of the total number of bees (N = 5,159) reported across all sites (1 – 6) for three growing seasons (2016 – 2018)

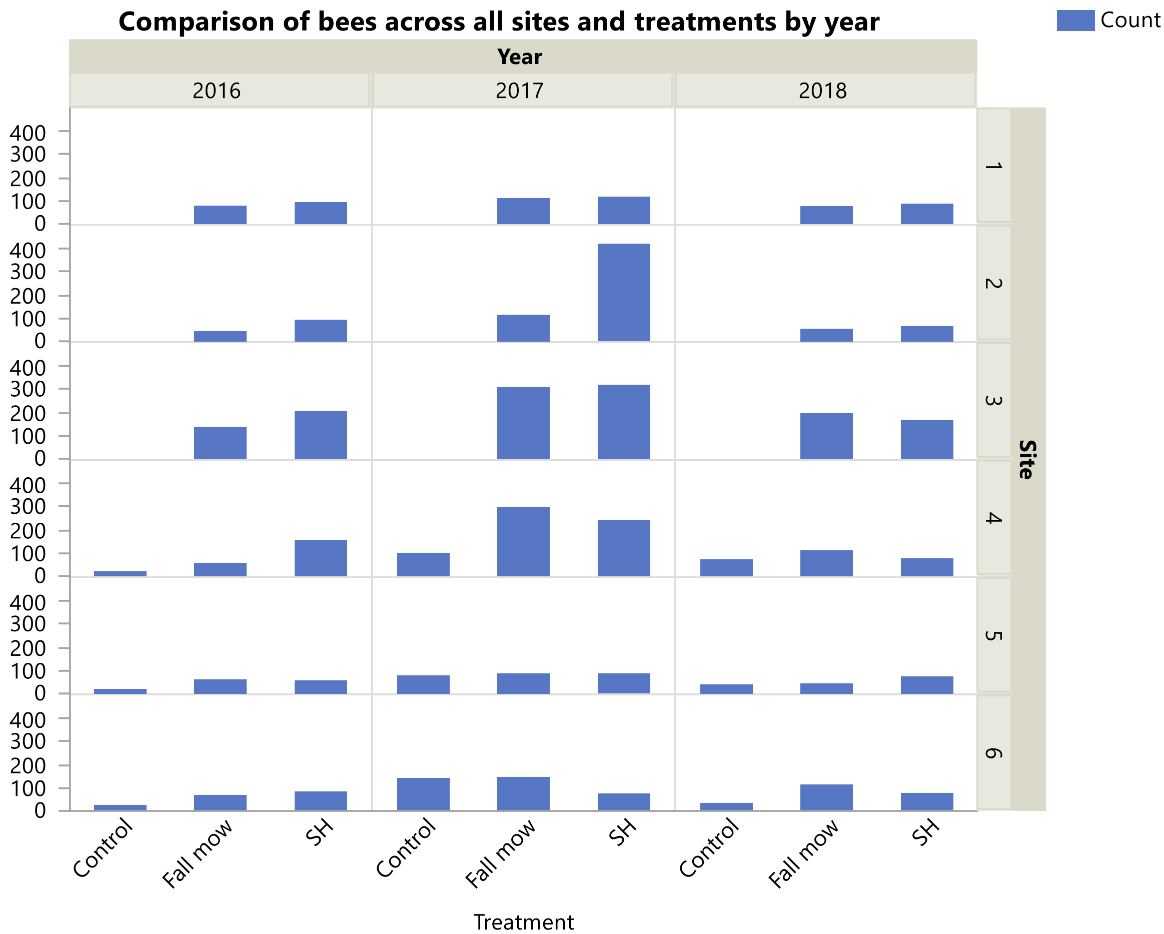


Figure 16: Bar graph of bee abundances recorded at each site (shown on the left hand side of the graph) and treatment. Sites 1 – 3 did not have a control group so have only two columns per year (fall mow and SH)

<u>Comparisons of sites with control plots</u>

As noted in the last section, control groups were limited to sites 4 – 6 because of logistical constraints. Given the complexities associated with unbalanced designs, the research team believes it is most useful to restrict the remainder of the plant data analyses for this report to sites with controls (sites 4 – 6). In future publications, data from the other three sites (1 - 3) will be included in a more exhaustive analysis and shared with MDOT SHA. Figure 17 shows the pooled number of bees per treatment at the three relevant sites, while Figure 18 breaks it down further to show the influence of two additional factors, site and year.

Figure 19 compares the mean abundance of bees (N) using a linear model, specifically least square regression. The three explanatory variables site (p-value <.02), treatment (p-value <.048) and year (p-value <.002) significantly predict the number of bees for a given treatment plot. Figure 20 compares the mean Shannon's biodiversity indices for the three different vegetation management treatments. Site (p-value <.0003) was a significant predictor of bee biodiversity, whereas, treatment (p-value >.24) and year (p-value >.58) were not significant predictors of bee biodiversity.

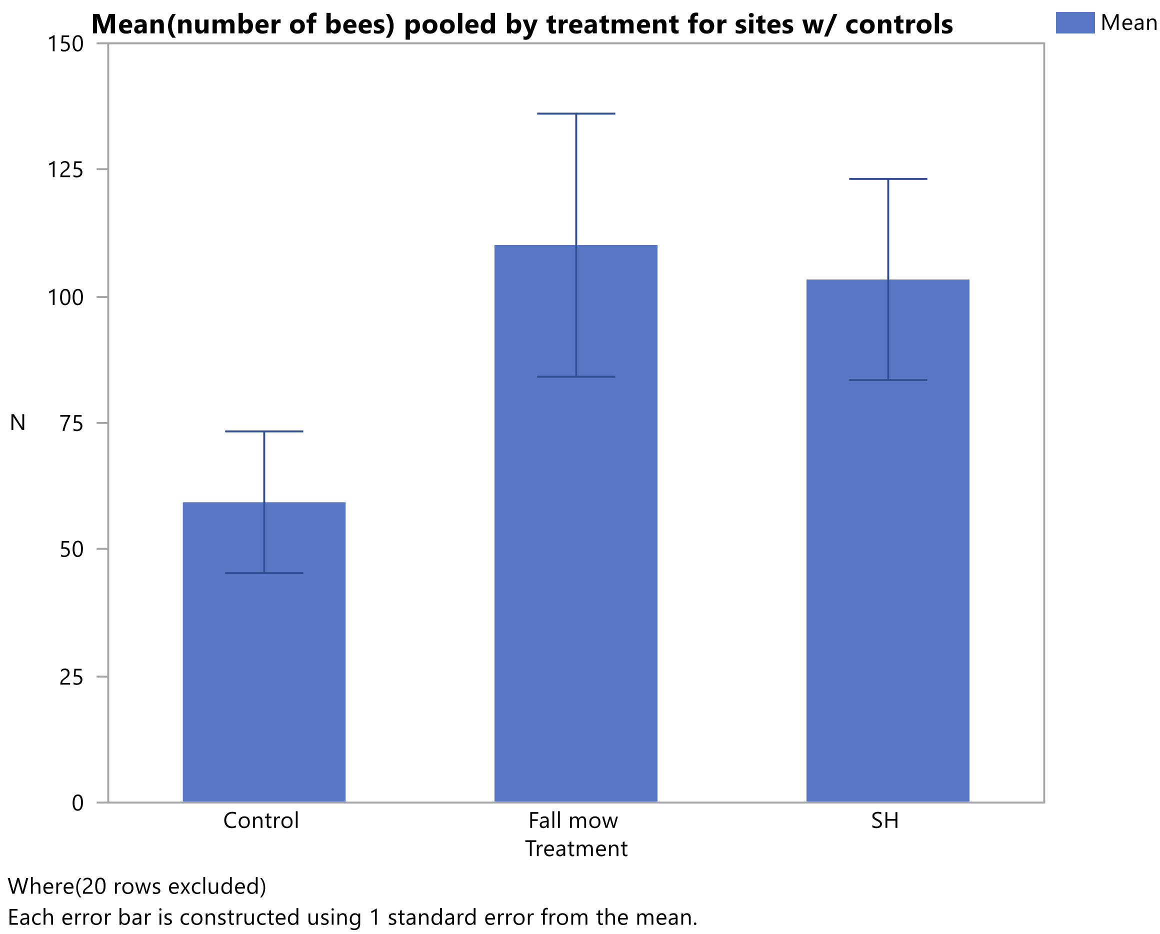


Figure 17: bar graph showing the mean number of bees per treatment for sites with control plots

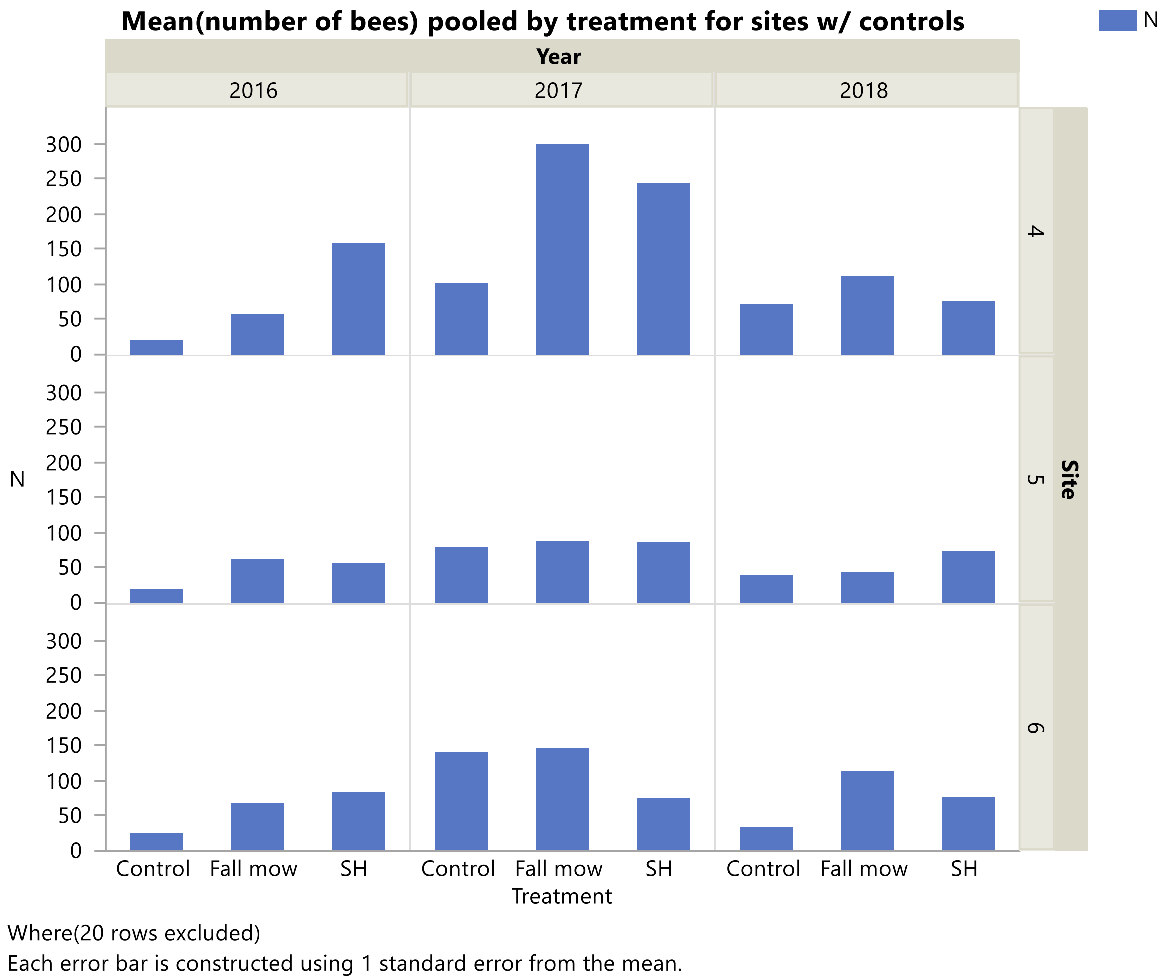


Figure 18: Bar graph of bee abundances recorded at sites with control plots. Sites 1 – 3 did not have a control group so have only two columns per year (fall mow and SH)

Least squares regression results for predictors of mean bee abundance (N)

Effect Summary

Source	LogWorth		PValue
Year	2.658		0.00220
Site	1.796		0.01601
Treatment	1.315		0.04844

Summary of Fit

RSquare	0.630943
RSquare Adj	0.520226
Root Mean Square Error	43.98527
Mean of Response	90.92593
Observations (or Sum Wgts)	27

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	6	66151.78	11025.3	5.6987
Error	20	38694.07	1934.7	**Prob > F**
C. Total	26	104845.85		0.0014 *

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	2	2	13680.963	3.5357	0.0484 *
Site	2	2	19812.963	5.1204	0.0160 *
Year	2	2	32657.852	8.4400	0.0022 *

Least Squares Means Table

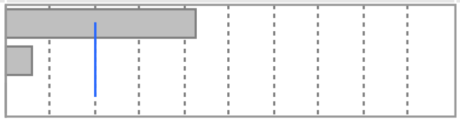
Level	Least Sq Mean	Std Error	Mean
Control	59.33333	14.661756	59.333
Fall mow	110.11111	14.661756	110.111
SH	103.33333	14.661756	103.333

Figure 19: Least squares regression results comparing bee abundance (N) from different treatment plots over time, with bee abundance as the response variable and treatment, site and year as explanatory variables. The means for all explanatory variables are statistically significant.

Linear regression results for predictors of bee biodiversity

Effect Summary

Source	LogWorth	PValue
Site	4.245	0.00006
Treatment	0.622	0.23856
Year	0.031	0.93213



Summary of Fit

RSquare	0.55782
RSquare Adj	0.477424
Root Mean Square Error	0.307688
Mean of Response	2.198178
Observations (or Sum Wgts)	27

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	2.6274717	0.656868	6.9384
Error	22	2.0827764	0.094672	**Prob > F**
C. Total	26	4.7102481		0.0009 *

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	2	2	0.2898282	1.5307	0.2386
Site	1	1	2.3369410	24.6847	<.0001 *
Year	1	1	0.0007026	0.0074	0.9321

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
Control	2.0522773	0.10256253	2.05228
Fall mow	2.2828011	0.10256253	2.28280
SH	2.2594564	0.10256253	2.25946

Figure 20: Linear model results are provided for each of the three explanatory variables (treatment, site and year) above. Treatment and year were not statistically significant with p-values above .05. Whereas, site was a major predictor of bee biodiversity as measured by the Shannon-Weiner biodiversity index.

Discussion of bee monitoring data

The state of Maryland is home to a known 428 different species of bees [73], while Frederick and Carroll counties have 155 and 124 species respectively (as of 06/31/19 per data from the Bee Monitoring Inventory Lab). Over the course of three field seasons (2016 – 2018), the research team recorded a total of 83 different bee species. At the Frederick County sites (N = 5) the research team recorded a total of 71 bee species, ~46% of the county's known species. While at the single site in Carroll County, 59 bee species were reported or ~48% of the county's records. There were at least seven new county records (Table 3), meaning those species had not formerly been reported for those counties. Thus, a wide range of bee species were found in central Maryland roadside verges.

Table 3: New county bee records

Bee species	County
Osmia distincta	Carroll
Andrena personata	Carroll
Ptilothrix bombiformis	Carroll
Melissodes denticulatus	Frederick
Megachile brevis	Frederick
Lasioglossum nymphaerum	Frederick
Bombus fervidus	Frederick

The total number of bees recorded for the entirety of the study was 5,159 (Figure 15). The first hypothesis was that roadside bee communities would be more species rich and abundant in plots treated with selective herbicides and least in those maintained as turf. However, the research team found that the fall mow treatment had the highest number of bees than both the control plots and the selective herbicide plots. Results from the linear regression test show that not only treatment but site and year were significant predictors of bee abundance (Figure 19). The second hypothesis was that both bee abundance and diversity would increase over time. While the research team saw a near doubling of bee abundance from 2016 to 2017, the numbers dramatically decreased in 2018 almost to 2016 levels. As for bee species richness, the research team found that treatment was not a significant predictor of bee biodiversity nor was year (Figure 20). However, site (i.e., the surrounding landscape) was a significant factor.

There are several factors that should be taken into account when interpreting our roadside data, which may have influenced the results. First, several large swaths of the plots at site 4 (both SH and fall mow treatments) were mistakenly mowed mid-July at the height of the growing season in 2018 by an MDOT SHA maintenance crew. This was unfortunate as it meant a good portion of floral resources (and likely foraging bees) were no longer present in those plots, thus negatively impacting both vegetation and bee surveys, at least in the short term. Also, Maryland experienced record levels of rainfall in 2018. In neighboring Baltimore County, from June – August, NOAA reported more than 23 inches of rain, a total of 250% more rain than normal [64]. While mostly anecdotal, it is generally believed that heavy and frequent rains negatively affect the ability of bees to forage and reproduce. The reproductive success of ground nesting bees is likely especially susceptible to heavy rains and flooding. Thus, unplanned mowing events and unpredictable weather, may have contributed to lower bee numbers and species richness in 2018. Arguably, natural annual fluctuations in bee and plant communities, as well as climate and human induced changes, points to the need for long-term data collection to fully understand roadside bee and plant population dynamics.

Regarding species diversity, again site was the only significant predictor of the Shannon-Weiner biodiversity index, which accounts for both abundance and evenness of species for a given location. Figure 18, illustrates that the highest number of bees was recorded at site 4 in 2017. Site 4 is next to Catoctin Mountain Orchard. In 2017 the orchard's owner Mr. Black had a honey bee hive approximately 40' from the roadside plots, which the research team believes was not there in 2016 or 2018. Interestingly, that year bee counts for site 4 nearly doubled and are predominantly honey bee observations. The honey bees were especially keen on a common roadside plant called dogbane (*Apocynum cannabinum*), which was particularly abundant that season. This example, demonstrates how site or surrounding landscape, can greatly shape roadside pollinator and plant communities. Roadsides are far from isolated patches but are part of a much larger and dynamic landscape.

Conclusions

The findings confirm that bees of Central Maryland are present and bountiful along roadsides with naturalized vegetation, and they forage on common roadside wildflowers, including a broad range of native and exotic species. The findings, some presented in this report, and the remainder to be shared with MDOT SHA via submitted publication(s) show that managing roadsides via SH and fall mow can significantly increase floral diversity and bee abundance compared to a turf regime. While minor differences between IRVM treatments were detected, they were not statistically significant. Bee diversity, which accounts for both abundance and the evenness of species in a given area, was mainly determined by site/surrounding landscape not treatment and was the sole significant factor. Data from this study supports the hypothesis that MDOT SHA's transition from frequent mowing to a fall mow and/or SH regime can benefit bees and other pollinators by increasing floral resources in terms of floral abundance and diversity.

References

1. Ollerton, J., R. Winfree, and S. Tarrant, *How many flowering plants are pollinated by animals?* Oikos, 2011. **120**(3): p. 321-326.
2. IPBES, *Summary for policymakers of the assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination and food production*, in *IPBES*. 2016. p. 1 - 28.
3. Goulson, D., et al., *Bee declines driven by combined stress from parasites, pesticides, and lack of flowers.* Science, 2015. **347**(6229): p. 1255957.
4. The_White_House, *Creating a Federal Strategy to Promote the Health of Honey Bees and Other Pollinators*, O.o.t.P. Secretary, Editor. 2014.
5. Wojcik, V.A. and S. Buchmann, *POLLINATOR CONSERVATION AND MANAGEMENT ON ELECTRICAL TRANSMISSION AND ROADSIDE RIGHTS-OF-WAY: A REVIEW.* Journal of Pollination Ecology, 2012. **7**.
6. Forman, R.T., *Road ecology: science and solutions*. 2003: Island Press.
7. Weber, T.C. and W.L. Allen, *Beyond on-site mitigation: An integrated, multi-scale approach to environmental mitigation and stewardship for transportation projects.* Landscape and Urban Planning, 2010. **96**(4): p. 240-256.
8. Hopwood, J., S. Black, and S. Fleury, *Roadside Best Management Practices that Benefit Pollinators: Handbook for Supporting Pollinators through Roadside Maintenance and Landscape Design.* 2015: p. 88.
9. Hopwood, J., et al., *Literature review: pollinator habitat enhancement and best management practices in highway rights-of-way.* 2015.
10. Bernes, C., et al., *How are biodiversity and dispersal of species affected by the management of roadsides? A systematic map protocol.* Environmental Evidence, 2016. **5**(1): p. 1.
11. Wilson-Rich, N., *The Bee: A Natural History*. 2014: Princeton University Press.
12. Poinar, G. and B. Danforth, *A fossil bee from Early Cretaceous Burmese amber.* Science, 2006. **314**(5799): p. 614-614.
13. David L. Dilcher, D.W.S.a.O.S.A.C. *Angiosperm*. 2017 [cited 2017 December 15, 2017]; Available from: https://www.britannica.com/plant/angiosperm.
14. Michener, C.D., *The bees of the world*. Vol. 1. 2000: JHU press.
15. Lee-Mäder, E., et al., *100 plants to feed the bees.* 2016.

16. Danforth, B.N., et al., *The history of early bee diversification based on five genes plus morphology.* Proceedings of the National Academy of Sciences, 2006. **103**(41): p. 15118-15123.
17. Arnold, S.E.J., et al., *Herbivore Defence Compounds Occur in Pollen and Reduce Bumblebee Colony Fitness.* Journal of Chemical Ecology, 2014. **40**(8): p. 878-881.
18. Stevenson, P.C., S.W. Nicolson, and G.A. Wright, *Plant secondary metabolites in nectar: impacts on pollinators and ecological functions.* Functional Ecology, 2017. **31**(1): p. 65-75.
19. Eckhardt, M., et al., *Pollen mixing in pollen generalist solitary bees: a possible strategy to complement or mitigate unfavourable pollen properties?* Journal of Animal Ecology, 2014. **83**(3): p. 588-597.
20. Potts, S.G., et al., *Linking bees and flowers: how do floral communities structure pollinator communities?* Ecology, 2003. **84**(10): p. 2628-2642.
21. Potts, S.G., et al., *Nectar resource diversity organises flower-visitor community structure.* Entomologia experimentalis et applicata, 2004. **113**(2): p. 103-107.
22. Potts, S.G., et al., *Global pollinator declines: trends, impacts and drivers.* Trends in ecology & evolution, 2010. **25**(6): p. 345-353.
23. Vanbergen, A.J., et al., *Threats to an ecosystem service: pressures on pollinators.* Frontiers in Ecology and the Environment, 2013. **11**: p. 251-259.
24. Aronson, M.F., et al., *Biodiversity in the city: key challenges for urban green space management.* Frontiers in Ecology and the Environment, 2017. **15**(4): p. 189-196.
25. Turo, K.J. and M.M. Gardiner, *From potential to practical: conserving bees in urban public green spaces.* Frontiers in Ecology and the Environment, 2019.
26. Hoyle, H., et al., *"Not in their front yard" The opportunities and challenges of introducing perennial urban meadows: A local authority stakeholder perspective.* Urban Forestry & Urban Greening, 2017. **25**: p. 139-149.
27. Hunter, M.R. and M.D. Hunter, *Designing for conservation of insects in the built environment.* Insect Conservation and Diversity, 2008. **1**(4): p. 189-196.
28. Green, O.O., et al., *Adaptive governance to promote ecosystem services in urban green spaces.* Urban Ecosystems, 2016. **19**(1): p. 77-93.
29. Barton, S., R. Darke, and G. Schwetz, *Enhancing Delaware highways: Roadside vegetation concept and planning manual.* 2005.

30. Bennett, J.M., *Roadsides: The Front Yard of the Nation*. 1936: Stratford Company.
31. Lucey, A. and S. Barton, *Public Perception and Sustainable Management Strategies for Roadside Vegetation.* Transportation Research Record: Journal of the Transportation Research Board, 2011(2262): p. 164-170.
32. Weber, F., I. Kowarik, and I. Säumel, *A walk on the wild side: perceptions of roadside vegetation beyond trees.* Urban Forestry & Urban Greening, 2014. **13**(2): p. 205-212.
33. Keeler, R.F., K.R. Van Kampen, and L.F. James, *Effects of poisonous plants on livestock*. 2013: Elsevier.
34. Ward Thompson, C., et al., *Mitigating Stress and Supporting Health in Deprived Urban Communities: The Importance of Green Space and the Social Environment.* International Journal of Environmental Research and Public Health, 2016. **13**(4): p. 440.
35. Hopwood, J.L. *Roadsides as habitat for pollinators: management to support bees and butterflies*. in *Proceedings of the 7th International Conference on Ecology and Transportation (IOECT): Canyons, Crossroads, Connections. Scottsdale, Arizona, USA*. 2013.
36. Halbritter, D.A., et al., *Reducing mowing frequency increases floral resource and butterfly (Lepidoptera: Hesperioidea and Papilionoidea) abundance in managed roadside margins.* 2015.
37. Snell-Rood, E.C., et al., *Anthropogenic changes in sodium affect neural and muscle development in butterflies.* Proceedings of the National Academy of Sciences, 2014. **111**(28): p. 10221-10226.
38. Baxter-Gilbert, J.H., et al., *Road mortality potentially responsible for billions of pollinating insect deaths annually.* Journal of Insect Conservation, 2015. **19**(5): p. 1029-1035.
39. Godfrey, M.A., *Field Guide to the Piedmont: The Natural Habitats of America's Most Lived-in Region, from New York City to Montgomery, Alabama*. 2012: UNC Press Books.
40. Markewich, H., M. Pavich, and G. Buell, *Contrasting soils and landscapes of the Piedmont and Coastal Plain, eastern United States.* Geomorphology, 1990. **3**(3-4): p. 417-447.
41. USDA.gov. *Plant hardiness zones interactive map*. 2017 [cited 2017 12/19/2017]; Available from: http://planthardiness.ars.usda.gov/phzmweb/interactivemap.aspx.
42. USClimateData.com. *Climate Frederick - Maryland*. 2017 [cited 2017 12/19/17]; Climate data for Frederick, MD - 21701 - 1961-1990 normals - weather]. Available from:

https://www.usclimatedata.com/climate/frederick/maryland/united-states/usmd0161.
43. Barbour, M., et al., *Methods of sampling the plant community*. Terrestrial Plant Ecology, 3rd Edition. Wesley Longman, Inc., Don Mills, ON. 1999.
44. Hicks, D.M., et al., *Food for pollinators: quantifying the nectar and pollen resources of urban flower meadows.* PLoS One, 2016. **11**(6): p. e0158117.
45. Szigeti, V., et al., *Measuring floral resource availability for insect pollinators in temperate grasslands–a review.* Ecological Entomology, 2016.
46. Frankl, R., S. Wanning, and R. Braun, *Quantitative floral phenology at the landscape scale: Is a comparative spatio-temporal description of "flowering landscapes"possible?* Journal for Nature Conservation, 2005. **13**(4): p. 219-229.
47. Hegland, S.J., et al., *How to monitor ecological communities cost-efficiently: the example of plant–pollinator networks.* Biological Conservation, 2010. **143**(9): p. 2092-2101.
48. Szigeti, V., et al. *Comparing two methods for estimating floral resource availability for insect pollinators in semi-natural habitats*. in *Annales de la Société entomologique de France (NS)*. 2016. Taylor & Francis.
49. Bagella, S., et al., *Effects of plant community composition and flowering phenology on honeybee foraging in Mediterranean sylvo-pastoral systems.* Applied Vegetation Science, 2013. **16**(4): p. 689-697.
50. Wyatt, R., S.B. Broyles, and G.S. Derda, *Environmental influences on nectar production in milkweeds (Asclepias syriaca and A. exaltata).* American Journal of Botany, 1992: p. 636-642.
51. Alarcón, R., N.M. Waser, and J. Ollerton, *Year-to-year variation in the topology of a plant–pollinator interaction network.* Oikos, 2008. **117**(12): p. 1796-1807.
52. Mortelliti, A., G. Amori, and L. Boitani, *The role of habitat quality in fragmented landscapes: a conceptual overview and prospectus for future research.* Oecologia, 2010. **163**(2): p. 535-547.
53. Lengyel, A., et al., *Classification and syntaxonomical revision of mesic and semi-dry grasslands in in Hungary= Klasifikace a syntaxonomická revize mezických a polosuchých trávníků v Maďarsku.* PRESLIA, 2016. **88**: p. 201-228.
54. Kearns, C.A. and D.W. Inouye, *Techniques for pollination biologists*. 1993: University press of Colorado.

55. Morrant, D.S., R. Schumann, and S. Petit, *Field methods for sampling and storing nectar from flowers with low nectar volumes.* Annals of botany, 2009. **103**(3): p. 533-542.
56. Torné-Noguera, A., et al., *Determinants of spatial distribution in a bee community: nesting resources, flower resources, and body size.* PloS One, 2014. **9**(5): p. e97255.
57. Benadi, G., et al., *Specialization and phenological synchrony of plant–pollinator interactions along an altitudinal gradient.* Journal of Animal Ecology, 2014. **83**(3): p. 639-650.
58. Woodcock, B., et al., *Enhancing floral resources for pollinators in productive agricultural grasslands.* Biological conservation, 2014. **171**: p. 44-51.
59. Del Tredici, P., *Wild urban plants of the northeast*. 2010: Cornell University Press.
60. Peterson, R.T. and M. McKenny, *A field guide to wildflowers: northeastern and north-central North America*. 1968: Houghton Mifflin Harcourt.
61. GoBotany.newenglandwild.org. *Simple ID Key*. 2017 [cited 2017 12/28/17]; Available from: https://gobotany.newenglandwild.org/.
62. marylandbiodiversity.com. *Maryland Biodiversity Project*. 2017 [cited 2017 12/29/17]; Available from: https://www.marylandbiodiversity.com/.
63. Stefanescu, C., *Migration patterns and feeding resources of the Painted Lady butterfly, Cynthia cardui (L.)(Lepidoptera, Nynphalidae) in the northeast of the Iberian peninsula.* Miscel· lània Zoològica, 1997. **20**(2): p. 31-48.
64. Di Liberto, T. *A soggy summer for the Mid-Atlantic in 2018*. 2018 [cited 2019 June 29]; Available from: https://www.climate.gov/news-features/event-tracker/soggy-summer-mid-atlantic-2018.
65. Hatten, T.D., J.P. Strange, and J.M. Maxwell, *Late-season survey of bumble bees along Canadian highways of British Columbia and Yukon Territories.* Western North American Naturalist, 2015. **75**(2): p. 170-180.
66. Hanley, M.E. and J.P. Wilkins, *On the verge? Preferential use of road-facing hedgerow margins by bumblebees in agro-ecosystems.* Journal of Insect Conservation, 2015. **19**(1): p. 67-74.
67. Droege, S., *The Very Handy Bee Manual*. 2016, Patuxent Wildlife Research Center: USGS.
68. Roulston, T.a.H., S.A. Smith, and A.L. Brewster, *A comparison of pan trap and intensive net sampling techniques for documenting a bee*

(Hymenoptera: Apiformes) fauna. Journal of the Kansas Entomological Society, 2007. **80**(2): p. 179-181.
69. ToLER, T.R., E.W. EvANs, and V.J. Tepedino, *Pan-trapping for bees (Hymenoptera: Apiformes) in Utah's west desert: the importance of color diversity.* Pan Pacific Entomologist, 2005. **81**(3-4): p. 103-113.
70. Tucker, E.M. and S.M. Rehan, *Wild bee pollination networks in northern New England.* Journal of insect conservation, 2016. **20**(2): p. 325-337.
71. Richards, M., et al., *Bee diversity in naturalizing patches of Carolinian grasslands in southern Ontario, Canada.* The Canadian Entomologist, 2011. **143**(3): p. 279-299.
72. Wilson, J.S. and O.J.M. Carril, *The Bees in Your Backyard: A Guide to North America's Bees*. 2015: Princeton University Press.
73. North_American_Native_Bee_Collaborative, *Bees of Maryland: A Field Guide*. 2017.

Appendix

Table 1: Site coverage

Site	Trt	Plot size (acres)	Plot size (m^2)	Proportion covered
1	SH	1.145	4633.65	1.04%
1	Mow	1.145	4633.65	1.04%
2	SH	1.7	6879.66	0.70%
2	Mow	1.7	6879.66	0.70%
3	SH	1.15	4653.89	1.03%
3	Mow	1.15	4653.89	1.03%
4	SH	0.905	3662.41	1.31%
4	Mow	0.905	3662.41	1.31%
4	Ctrl	0.905	3662.41	1.31%
5	SH	0.955	3864.75	1.24%
5	Mow	0.955	3864.75	1.24%
5	Ctrl	0.955	3864.75	1.24%
6	SH	0.56	2266.24	2.12%
6	Mow	0.56	2266.24	2.12%
6	Ctrl	0.56	2266.24	2.12%