# Literature Review: Conservation Value of Monarch Roadside Habitat & Associated Risks



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# I. Conservation Value of Roadside Habitat

Various studies have documented the value of roadside habitat for monarchs and pollinators. Roadsides provide host plant and nectar resources for breeding and migratory monarchs, and studies have demonstrated significant use of these areas by pollinators (Ries et al., 2001; Hopwood 2008; Kasten et al., 2016; Webb 2017; Cariveau et al., 2019; Kaul & Wilsey, 2019; Phillips et al., 2019; Dietzel et al., 2023). However, a 2023 study by Garfinkel et al. suggests that more information than floral and nesting resource availability may be needed to predict the abundance of pollinators in Midwestern ROW sites. High densities of milkweed have been documented in the Upper Midwest, and rare species of interest have been documented along roadways in Florida and the Chihuahuan Desert (Daniels et al., 2018; McCoshum & Agrawal, 2021). A study of rural roadsides in Poland suggests that maintaining high-quality pollinator habitat may reduce the effect of car strikes on butterfly populations (Skórka et al., 2013)

Roadsides play significant roles in sustaining biodiversity - ROW corridors may provide the only resources in heavily developed and agricultural regions and connect habitat patches across the landscape (Schact & Wu-Smart, 2019; Ding & Eldridge, 2022). Maintaining high quality habitat along roadsides has been shown to reduce road crossings by pollinators in Sweden, though lateral movement along roadways was not affected (Dániel-Ferreira et al., 2021). Lastly, Thogmartin et al., 2017's analysis of milkweed restoration in the eastern US suggests that roadside habitat is essential to reaching monarch butterfly habitat targets (establishing 1.3-1.8 billion milkweed stems).

Additional Supporting Information (Details by Paper)		
Author, Yr	Title	Key Takeaways
Ries et al., 2001	Conservation Value of Roadside Prairie Restoration to Butterfly Communities	<u>BUTTERFLIES</u> : Documented benefits of roadside restorations on butterfly populations. Overall, prairie/restored roadsides are beneficial for pollinators and have lower mortality risk than grass.
Hopwood 2008	The contribution of roadside grassland restorations to native bee conservation	<u>BEES</u> : Restored roadsides supported greater bee abundances and higher species richness compared to weedy roadsides. Floral richness, floral abundance, and % bare ground resulted in greater bee abundance and bee sp. richness along restored roadsides. Traffic and ROW width did not significantly influence bees - narrow verges near heavy traffic can provide valuable habitat to bees. Restored and weedy roadside bee communities were similar to the prairie remnant, but the remnant was more similar in bee richness and abundance to restored roadsides.
Kasten et al., 2016	Can roadside habitat lead monarchs on a route to recovery?	<u>MILKWEED &amp; MONARCHS</u> : Milkweeds found on ~60% of roadside transects in Minnesota. Immature monarchs were observed in roadsides but in lower densities than other habitats during the same time period. MN roadsides have conservation potential for monarchs, especially when other habitat is scarce and if wildlife-friendly management practices are enacted.
Webb 2017	Roadside Environments	MILKWEED - OKLAHOMA: A. viridis and A. asperula densities were

Native habitat in roadsides not only benefits pollinators; it also improves soil and water quality, infiltration, carbon sequestration, and more (McLaughlin et al., 2020).

	and the Effects of Roadside Management Practices on Milkweeds and Monarchs	higher on roadsides than adjacent lands in Oklahoma; mowing roadsides lowers counts of <i>A. viridis</i> milkweed but those lower counts are limited to the actual times of mowing; monarch mortality during fall migration did not differ with highway orientation. Roadsides have the potential to provide monarch butterfly habitat and should be considered when assessing conservation strategies.
Thogmartin et al., 2017	Restoring monarch butterfly habitat in the Midwestern US: 'all hands on deck'	<u>MILKWEED &amp; MONARCHS</u> : Examined scenarios for restoring milkweeds in the eastern US. Result = all land use/cover sectors must contribute to reach the goal of establishing 1.3-1.8 billion stems, including roadside rights-of-way.
Daniels et al., 2018	Better Understanding the Potential Importance of Florida Roadside Breeding Habitat for the Monarch	<u>MILKWEED</u> : Documented extensive populations of <i>A. tuberosa</i> and <i>A. humistrata</i> on Florida roadsides (important species for early season monarch recolonization). Majority of plants occurred on back slope of verge. Alterations to current roadside mowing frequency/scope are needed to conserve these populations & ensure they are available to monarchs.
Phillips et al., 2019	Road verges support pollinators in agricultural landscapes, but are diminished by heavy traffic and summer cutting	<u>FLOWERS &amp; POLLINATORS</u> : Road verges and hedges had greater flower abundance, flower sp. richness and pollinator abundance than field interiors. Verge hedges had less woody cover but greater flower species richness. Fewer pollinators along verge edges/next to roads than along verge centers (2–11 m from roads) and fewer pollinators in road verges next to busier roads. Road verges were generally cut once (in summer), and cuttings were never removed. Substantially fewer flowers and pollinators in road verges that had been cut, even though surveys often took place many weeks after cutting. Road verges and hedges can provide habitat hotspots for pollinators in ag landscapes, but their capacity to do so is reduced by heavy traffic and summer verge cutting. Beneficial mgmt for pollinators should prioritize wider road verges (>2 m), roads with less traffic, areas away from the immediate vicinity of the road, and NO mowing during peak flowering period.
Kaul & Wilsey, 2019	Monarch butterfly host plant (milkweed Asclepias spp.) abundance varies by habitat type across 98 prairies	<u>MILKWEED</u> : Documented milkweed in Iowa roadsides (mean = 1,274 plants/ac, mostly <i>A. syriaca</i> ). Density was positively correlated with higher soil pH, greater site age, and lower bulk density soil. Rural roadside sites had higher milkweed density than non-roadside conservation plantings.
Cariveau et al., 2019	Rapid assessment of roadsides as potential habitat for monarchs and other pollinators	MILKWEED & MONARCHS: Documented milkweed populations and monarch use on Minnesota roadsides. Observed high habitat values in roadsides that confirm the potential of ROW habitat for monarch conservation.
McLaughlin et al., 2020	Stormwater Infiltration and Pollinator Habitat Zones Along Highways	Wildflowers as a substitute for grass can provide greater infiltration potential, in part because mowing traffic is reduced from four times per year to one. Among the many wildflowers that were planted as a mix, very few were present in our plots. However, those perennials that dominated (Lanceleaf coreopsis and blanketflower) were quite resilient in both field plots and under different soil conditions in the greenhouse tests, and would be highly recommended based on their ability to grow and develop robust root systems
McCoshum & Agrawal,	Ecology of <i>Asclepias</i> brachystephana: a plant for	RARE SPECIES: MILKWEED: Bract milkweed (Asclepias brachystephana Engelm. ex Torr.) plant populations were observed occurring on roadsides,

2021	roadside and right-of-way management	rarely spreading into neighboring habitats. Study documents a variety of native pollinators and herbivores utilizing floral resources and plant tissue. Data suggest <i>A. brachystephana</i> should be considered for ROW plantings, restoration projects, or seeding in the Chihuahuan Desert and adjoining ecoregions.
Ding & Eldridge, 2022	Roadside verges support greater ecosystem functions than adjacent agricultural land in a grassy woodland	<u>NATIVE PLANTS - AUSTRALIA</u> : Compared ecological functions of roadside verges and adjacent ag land. Road verges supported greater carbon stocks, veg coverage, plant diversity, habitat complexity, and tree recruitment, and were subject to less modification and erosion. These effects strengthened with increasing roadside verge width, particularly for plant cover and diversity, proportion of native plant species and habitat complexity. Management practices were major regulators of roadside functions, with verge width and site modification negatively associated with tree recruitment and soil organic carbon pool. Study provides empirical evidence of the ecological importance of roadside verges in maintaining ecosystem functions and the sustainability of native plant communities in peri-agricultural landscapes.
Garfinkel et al., 2023	Testing the accuracy of a Rights-of-Way pollinator habitat scoring system	<u>POLLINATOR ABUNDANCE</u> : Compared results of a biodiversity survey of pollinator species (lepidopteran and bee hymenopteran) to an evaluation under a pollinator habitat scorecard (developed by the Rights of Way as Habitat Working Group) at 12 sites on Illinois ROW land. A site's pollinator habitat score did not meaningfully predict the observed diversity of pollinators present; However, the authors suggest that this may be due to various limitations of the study itself, as well as the pollinator habitat scorecard. For example, many factors considered important to pollinator establishment, such as soil conditions, surrounding landscape characteristics, site age, and disturbance history, were not accounted for by either metric. This study suggests that an annual evaluation of floral/nesting resources does not provide a complete picture of a site's potential as pollinator habitat but may still play an important role in site evaluation and long-term monitoring.
Dietzel et al., 2023	Enhanced urban roadside vegetation increases pollinator abundance whereas landscape characteristics drive pollination	<u>ROADSIDE HABITAT - GERMANY:</u> Investigated the factors driving the relationship between the availability of flowering plants, pollinators, and the characteristics of an urban landscape. Increasing the richness of local flowering plants along roadsides was correlated with higher pollinator abundance. However, rates of pollination did not follow the same trend. Pollination of phytometric plant species was most comprehensive in urban areas with large proportions of impervious surfaces (ie. roads and sidewalks) as well as lower edge density. The authors suggest that this is due to a concentration effect; in those areas there is less overall habitat available for pollinators, resulting in the few remaining plants being highly visited. The authors recommend more research on the effects of urbanization on plant/pollinator communities, as the relationship is complex and still poorly understood.
Skórka et al., 2013	Factors affecting road mortality and the suitability of road verges for butterflies	<u>ROADSIDE HABITAT - POLAND</u> : Evaluated the value of rural roadside habitat for lepidopterans, with special consideration to roadkill mortality. The authors note that the most suitable habitat for butterflies suffered the least proportionally from road-related mortality. To create suitable habitat, the authors recommend planting flowering and host plants, mowing

		infrequently or partially (ie. one side of the road at a time), maintaining wide road verges, and maintaining habitat in areas with grasslands in the surrounding landscape.
Western Riverside County Regional Conservatio n Authority	Western Riverside County Multiple Species Conservation Plan	<u>ROADSIDE HABITAT - CALIFORNIA</u> : This habitat conservation plan from Riverside County, California, features an annual report on the status of a overcrossing of the Clinton Kieth highway as habitat for the endangered Quino Checkerspot butterfly ( <i>Euphydryas editha quino</i> ). Common host and nectar plants for the Quino were observed on the overcross, but no butterflies were observed directly using it to avoid crossing the highway directly. The surveyors suggest that as host, nectar, and shelter-providing plants continue to grow on the overcrossing, its value as habitat for the Quino and other butterflies will increase.
Dániel- Ferreira et al., 2021	Road verges are corridors and roads barriers for the movement of flower- visiting insects	<u>ROADSIDE HABITAT - SWEDEN</u> : Examined the impacts of traffic and habitat quality affects pollinator behavior on Swedish roadsides. Roads were found to be barriers for pollinator movement, regardless of traffic level. High quality habitat along roadsides (i.e., higher number of flowering plant species/floral abundance) were associated with a reduction in road crossing by pollinators but did not induce higher movement lateral to roads. This lateral movement is seen as desirable, because it increases connectivity between patches of fragmented habitat.
Schact & Wu-Smart, 2019	Establishment of Wildflower Islands to Enhance Roadside Health and Aesthetics	<u>ROADSIDE MANAGEMENT - NEBRASKA</u> : Compared alternate methods of establishing wildflowers along roadsides, utilizing varying sizes of flower patches rather than an even mix of forb/grass seed. Although only covering a few years, the authors suggest initial improvements in forb establishment using the patch system, with higher forb and bee richness observed in patch plantings during the first year.

## II. Roadside Mortality & Risks

#### Vehicle Collisions

Current research suggests that monarch butterfly roadside mortality is greatest during the fall migration, especially along migration corridors in Oklahoma and Texas, and at highway crossing "hotspots" in Mexico (Kantola et al., 2019; Alvarez et al., 2019). Mortality during this time may be significant - two existing studies estimate losses of up to 3-4% of the overwintering population. Mortality rates likely vary by year, population size, weather conditions, and other factors. An additional study estimated 500,000 monarchs killed in a 1-week period during the fall migration in Illinois. This pattern of seasonal variation in mortality is also observed in other species: a 2022 paper found that queen bumblebee mortality along Swedish roadsides is highest in the spring, when individual queens are searching for nest sites (Dániel-Ferreira et al., 2022).

Breeding season vehicle collisions also occur but mortality rates appear to be much lower (though large-scale studies are lacking). Ries et al., 2001 found that butterfly mortality is higher on grassy roadsides than those with native wildflower plantings (butterflies were less likely to exit prairie roadsides). Keilsohn et al., 2018 found that insect mortality is greatest on high-speed roads and

those bordered by lawns or meadows, particularly in medians. A 2018 paper noted that road developments in Washington state may increase mortality rates for the select native bee species which currently bolster the yields of alfalfa crops (Vinchesi et al 2018).

Pitman et al., 2018 found lower egg densities on roadsides than other habitat types in Ontario. The authors suggest that lower egg densities may be caused by adult mortality from vehicle collisions (though this is not something they measured) and that habitat restoration should be prioritized in other land use types.

Additional Supporting Information (Details by Paper)		
Author, Yr	Title	Key Takeaways
McKenna et al., 2001	Mortality of Lepidoptera Along Roadways in Central Illinois	MONARCHS: Estimated 500,000 monarchs were killed in 1 week in Illinois during fall migration.
Ries et al., 2001	Conservation Value of Roadside Prairie Restoration to Butterfly Communities	<u>BUTTERFLIES, IOWA</u> : Compared butterfly responses to planted vs weedy roadsides in Iowa. Although there were higher concentrations of road-killed butterflies near weedy and prairie roadsides than on grassy roadsides, relative numbers indicated that mortality risk was more than twice as high on grassy roadsides. Tracking studies showed that butterflies were less likely to exit prairie roadsides - mortality rates may be lower. Results suggest that roadsides have the potential to be used as corridors.
Keilsohn et al. 2018	Roadside habitat impacts insect traffic mortality	<u>INSECTS</u> : Studied insect mortality on high-speed roads (70–90 kmh). Mortality was lower when roadsides were bordered by woodlots compared to lawn/meadows. Butterfly & dragonfly mortality was highest on roads with meadow medians; bee mortality highest on roadsides with lawn medians. Vegetated medians significantly elevated fatal insect-vehicle strikes. Authors state that regardless of the habitat bordering roadsides, mortality was unacceptably high for areas being considered for conservation.
Pitman et al., 2018	Patterns and causes of oviposition in monarch butterflies: Implications for milkweed restoration	<u>MONARCHS</u> : Found higher egg densities in ag/non-ag lands compared to ROWs in Ontario. Authors suggest it may be due to vehicle mortality (adults getting hit upon finding ROW milkweeds). Because of lower egg density in ROWs, authors recommend prioritizing habitat in ag and non-ag lands.
Alvarez et al., 2019	Mortality of Monarch Butterflies at Two Highway Crossing "Hotspots" During Autumn Migration in Northeast Mexico	<u>MONARCHS</u> : Significant mortality occurred at 2 highway crossings where migrating monarchs are typically concentrated in NE MX because of canyons (196,500 monarchs during 10/15 - 11/11/18). Mortality during autumn migration may be of the order of 2 million per year or ~3% of the population.
Kantola et al., 2019	Spatial risk assessment of eastern monarch butterfly road	MONARCHS: Estimated 3.6 and 1.1 million monarchs were killed along roads in OK & TX during the 2016 & 2017 fall

	mortality during autumn migration within the southern corridor	migrations (approx. 4% and 2% of the overwintering population in Mexico in those years).
Campioni et al., 2022	Mud-puddling on roadsides: a potential ecological trap for butterflies	BUTTERFLIES, SPAIN: Documents butterflies killed by farm vehicles in Spain (not monarch specific).
Skórka et al., 2013	Factors affecting road mortality and the suitability of road verges for butterflies	<u>BUTTERFLIES, POLAND</u> : Butterfly road mortality was positively correlated with traffic volume, road width, and frequency of mowing on rural Polish roadways. Authors noted that the proportion of roadkill butterflies did not change with overall butterfly abundance along roadways, suggesting that high quality roadside habitat (ie. high forb abundance, well- timed mowing, wide width of roadside plantings) may reduce the impact of road mortality on local butterfly populations.
Vinchesi et al., 2018	Assessing Transportation Impacts to Alkali Bees (Hymenoptera: Halictidae) and Alfalfa Seed Production in Southeastern Washington State	<u>BEES, UNITED STATES</u> : In Washington state, a few native bee species are used to enhance the yields of alfalfa crops. Researchers found that these bees tend to fly low to the ground when crossing roads, meaning they are likely to suffer mortality from vehicle strikes as new road development bisects their feeding range.
Tracy et al., 2019	Modeling fall migration pathways and spatially identifying potential migratory hazards for the eastern monarch butterfly	<u>MONARCHS, UNITED STATES</u> : Using data from density estimation and ecological niche models, researchers were able to characterize the hazards monarchs may face during their migration. The southern central flyway was identified with roadkill hotspots, while coastlines along the eastern flyway brought the largest risk of non-target effects from mosquito spraying. In the core of the central flyway, loss of nectar resources and herbicide/neonicotinoid contamination were identified as central threats.
Dániel- Ferreira et al., 2022	Bumblebee queen mortality along roads increase with traffic	<u>BUMBLEBEES, SWEDEN</u> : A study was conducted on the effects of roadside flowering plant diversity and traffic intensity on bumblebee queen behavior and mortality. The study noted a higher probability of finding dead bumblebees on high-traffic roads compared to low, with the diversity of forbs along the roadway having little impact on mortality. Bumblebee queens tend to be most susceptible to collisions with cars when they search for nesting habitat in the spring. With these findings in mind, the authors recommend establishing pollinator habitat along low-traffic roads, and maintaining a buffer of low-quality habitat adjacent to roads to reduce the risk of collisions.

#### Road Noise

Two studies identified potential negative effects of noise on developing monarch larvae. When exposed to noise for a 2-hour period, developing larvae experienced elevated heart rates (Davis et al., 2018). However, the same effect was not observed when larvae were exposed to continuous noise for 7-12 days, suggesting that they became accustomed to it. The authors argue that this habituation to noise may impact monarchs' reaction to real-world stressors, such as their ability to ward off predators and parasitoids.

Another study found that monarch larvae exposed to wasp buzzing experience a shorter development time and pupal weight (Lee et al., 2021). Authors suggest that larvae developing along ROWs or near airports may be negatively impacted, as wasp buzzing is similar to noise experienced in those land use types.

Additional Supporting Information (Details by Paper)		
Author, Yr	Title	Key Takeaways
Davis et al., 2018	Effects of simulated highway noise on heart rates of larval monarch butterflies, Danaus plexippus: implications for roadside habitat suitability	<u>MONARCHS</u> : Examined if simulated highway noise stresses monarch larvae by monitoring heart rates. Larvae exposed for 2 h experienced a significant increase in heart rate but those exposed for 7 or 12 days to continuous noise did not. Authors suggest that habituation to stress may impair reactions to real-world stressors as adults, which could be problematic for undertaking an annual migration that is fraught with dangers.
Lee et al., 2021	Auditory predator cues affect monarch (Danaus plexippus; Lepidoptera: Nymphalidae) development time and pupal weight	<u>MONARCHS</u> : Exposure to wasp buzzing reduced monarch larval development time and pupal weight. Authors suggest there may be implications on monarch fitness/survival when living near airports/ROWs - shorter development time leads to caterpillars pupating at a suboptimal weight, potentially reducing future fecundity and lowering overall fitness.

#### Road Salts & Heavy Metals

Findings suggest that overall, roadsides are suitable for pollinator habitat, but some studies recommend prioritizing areas >2m from the road edge and those with lower traffic volume for pollinator conservation management and restoration (especially in snowy areas with road salt applications) (Phillips et al., 2021; Shepherd et al., 2021).

Snell-Rood Lab research has documented elevated sodium and zinc concentrations (but not nickel) in roadside milkweeds (Snell-Rood et al., 2014; Mitchell et al., 2020). Nitrogen concentrations were affected by adjacent agricultural use but not by traffic volume or proximity to road. The effects of road salts and heavy metals on butterflies has been shown to vary by species, sex, and macronutrient levels in diets (Shephard et al., 2020; Shephard et al., 2021; Shepherd et al., 2022). In monarchs, elevated sodium in roadside milkweeds resulted in increased muscle mass in males and neural investment in females. A follow-up study found no evidence for negative impacts on survival when exposed to the elevated levels of sodium and zinc seen in roadside habitat. The authors state that plant toxicity is typically not lethal, that most plants are suitable for developing monarchs, and that roadside habitat provides a net benefit for pollinators. To minimize risk, restoration in snowy regions could be prioritized in ROWs with lower traffic volume and away from the road edge.

Phillips et al., 2021 suggest that threats to pollinators are greatest within the habitat adjacent to roadways (within the first 2m). They state that roadsides are suitable for pollinator habitat overall but recommend prioritizing areas >2m from the road edge and those with lower traffic volume.

Future work should characterize the nutritional quality of nectar, pollen, and other species of host plants in roadside habitats.

Additional Supporting Information (Details by Paper)		
Author, Yr	Title	Key Takeaways
Snell-Rood et al., 2014	Anthropogenic changes in sodium affect neural and muscle development in butterflies	<u>BUTTERFLIES</u> : Road salt runoff affects sodium concentrations of roadside plants and can affect neural and muscular development of herbivores. Roadside milkweeds contained elevated concentrations of sodium (1.5-30x). When fed to monarchs, effects differed based on sex (and species); increased muscle mass in males and increased neural investment in females.
Mitchell et al., 2020	Traffic influences nutritional quality of roadside plants for monarch caterpillars	<u>MILKWEED:</u> Sodium and zinc concentrations were highest in <i>A. syriaca</i> milkweed leaves that were in close proximity to busy roads. Nickel was not elevated in soil or plants. Nitrogen concentrations were affected by adjacent agricultural use but not by traffic volume or proximity to road. Authors suggest most plants are still suitable for developing monarchs but that restoration in snowy regions should be prioritized in ROWs with lower traffic volume and away from the road edge to minimize toxic impacts of high sodium. Future work should characterize the nutritional quality of nectar, pollen, and other species of host-plants in roadside habitats.
Shephard et al., 2020	Assessing zinc tolerance in two butterfly species: consequences for conservation in polluted environments	BUTTERFLIES (MONARCHS & CABBAGE WHITES): Elevated zinc reduced monarch survival but increased cabbage white (CW) survival. CWs displayed prolonged development time, smaller adult body size, and slower growth rate, consistent with the possibility that zinc tolerance had fitness costs. Results suggest that zinc pollution alone is unlikely a risk in developing roadside habitat for monarchs.
Shephard et al., 2021	Monarch caterpillars are robust to combined exposure to the roadside micronutrients sodium and zinc	<u>MONARCHS:</u> A study of the migratory generation showed that while high levels of sodium exposure can slightly prolong larval development, there was no evidence for negative impacts on survival when exposed to the elevated levels of sodium and zinc seen in roadside habitat. Sodium, more than zinc, is likely influencing phenotypic development and performance of insect pollinators in roadside habitats. In contrast to previous work, experiment suggests that the highest levels of sodium found along roads are not always harmful for developing monarchs.
Phillips et al., 2021	Impacts of multiple pollutants on pollinator activity in road verges	POLLINATORS & HABITAT, ENGLAND: Exposure to noise, turbulence, dust, and metals decreased with distance from road edge except for turbulence. Pollinator densities were lower closer to road edge (particularly within 2 m (55% lower than at 7–9 m) where pollution is greatest), despite a similar flower density and species richness. Simulated turbulence reduced pollinator visit duration by up to 54%. Some pollinator taxa preferentially avoided concentrations of metals that were more frequently found in flowers within 2m of roads, while noise and dust had no apparent effect. Authors suggest that roadsides are suitable for pollinator habitat

		overall but recommend prioritizing areas >2m from the road edge and those with lower traffic volume.
Shephard et al., 2022	Anthropogenic Zinc Exposure Increases Mortality and Antioxidant Gene Expression in Monarch Butterflies with Low Access to Dietary Macronutrients	<u>MONARCHS</u> : Exposure to elevated zinc reduced survival only in monarchs developing on low macronutrient diet (tended to increase survival when fed high macronutrient diet). Monarchs exposed to elevated zinc displayed higher expression of antioxidant genes when developing on the low-macronutrient diet but lower expression when developing on the high-macronutient diet. Impacts of zinc contamination depends on availability of macronutrient resources in the developmental environment.

#### Parasites/Parasitoids

There is no evidence that OE parasitism differs in roadsides compared to other habitat types (Mueller & Baum, 2014).

Additional Supporting Information (Details by Paper)		
Author, Yr	Title	Key Takeaways
Mueller & Baum, 2014	Monarch–parasite interactions in managed and roadside prairies	<u>MONARCHS</u> : The proportion of OE infected monarchs does not differ between roadside prairies and managed prairies. Roadsides may provide habitat for monarchs that is similar in quality (at least in terms of parasitism rates) to managed prairies. The role of roadsides as habitat for monarchs should be considered when developing roadside management strategies.

#### Light Pollution

Parlin et al., 2022 highlight the negative consequences of nighttime light pollution on diurnal animals, including migratory monarch butterflies. Monarchs roosting along roadways could be negatively impacted by light from cars passing by.

Additional Supporting Information (Details by Paper)		
Author, Yr	Title	Key Takeaways
Parlin et al., 2022	Oriented migratory flight at night: consequences of nighttime light pollution for monarch butterflies	Study highlights the negative consequences of nighttime light pollution on diurnal animals. Nighttime light pollution can interfere with their circadian clocks; monarchs were triggered into directional flight when exposed to a full-spectrum light source at night. Authors suggest that conservation practitioners consider proximity to artificial light and avoid creating habitat in areas with nighttime light pollution.

#### **Pesticides**

Multiple studies (Pecenka & Lundgren, 2015, Botias et al., 2016) show evidence that neonicotinoids used to seed-treat crop species may be taken up by the tissues of flowering plants adjacent to crop fields, ultimately exposing monarchs and other insects to harmful concentrations. These "non-target effects" of neonicotinoids have also been observed through widespread declines in butterfly populations after neonicotinoids began to be used in agricultural fields in lowland Northern California (Forister et al., 2016). Modeling and toxicity data from Krishnan et al., 2020 shows that neonicotinoid and chlorpyrifos-based insecticide exposure can vary for larval monarch populations living close to fields, with exposure and mortality being highest for populations located downwind of the field at time of exposure. A figure from the same paper also shows that the highest rates of pesticide spraying tend to occur during the largest abundances of monarch larvae on host plants.

A laboratory study of monarchs suggested that acute exposure to neonicotinoids at levels observed in non-target wildflowers increased mortality rates and induced symptoms of poisoning (James 2019). A longer-term study by Olaya-Arenas et al., 2020 found that the exposure levels of multiple pesticides observed in the field did not significantly affect larval mortality in lab conditions, but did lead to reductions in wing size in the adults, suggesting that sublethal pesticide exposure may still reduce monarch success in the long term. However, modeling work suggests that the benefits of increasing monarch habitat to include field margins will produce more monarchs as a whole, while accounting for increased mortality due to pesticide exposure (Grant et al., 2022, Krishnan et al., 2021).

Additional Supporting Information (Details by Paper)		
Author, Yr	Title	Key Takeaways
Pecenka & Lundgren, 2015	Non-target effects of clothianidin on monarch butterflies	NEONICOTINOIDS & MONARCHS: This study combined a field survey of the levels of the neonicotinoid clothianidin on milkweed plants adjacent to South Dakota cornfields with a lab experiment, designed to find the toxicological effects of clothianidin on larval monarchs. At levels close to the mean observed concentration of clothianidin in field samples, larval monarchs in the lab experiment suffered reduced size and growth rate. The authors suggest that crops seed-treated with neonicotinoids may negatively impact monarchs living nearby.
Botias et al., 2016	Contamination of wild plants near neonicotinoid seed- treated crops, and implications for non-target insects	<u>NEONICOTINOIDS &amp; INSECTS</u> : Investigated the distribution of multiple pesticides in both seed-treated crop plants and in non-treated plants growing adjacent to crop fields. Bioaccumulation was variable depending on the pesticide and non-crop plant in question, but frequently resulted in insect species (including <i>D. plexippus</i> ) being chronically exposed to sublethal concentrations of pesticides.
Forister et al., 2016	Increasing neonicotinoid use and the declining butterfly fauna of lowland California	<u>NEONICOTINOIDS &amp; BUTTERFLIES:</u> A long-term survey of the diversity and abundance of butterfly species in Northern California found a broad decline in butterfly abundance after the introduction of neonicotinoids as an agricultural pesticide in the late 1990s. This association remained after controlling for land use and climatic change, and the most negatively affected species were reported to have reduced body sizes and fewer generations per season.
James 2019	A neonicotinoid insecticide at a rate found in nectar	NEONICOTINOIDS & MONARCHS: A small laboratory study exposed a treatment group of monarch butterflies to the pesticide imidacloprid at

	reduces longevity but not oogenesis in monarch butterflies, Danaus plexippus (L.)	levels previously recorded in wildflower nectar. This exposure induced similar symptoms to those seen in honeybee neonicotinoid poisoning, and increased mortality of treatment group butterflies to 80% by 22 days post-eclosion.
Krishnan et al., 2020	Assessing Field-Scale Risks of Foliar Insecticide Applications to Monarch Butterfly (Danaus plexippus) Larvae	<u>PESTICIDES &amp; MONARCHS</u> : Investigated the impact of insecticide exposure on monarch larvae as a result of its application to nearby crop fields. Toxicity data were established for five active ingredients in insecticides, via both cuticular and dietary exposure. Notably, the study documented interruption of ecdysis in fifth-instar monarchs following cuticular exposure of both neonicotinoid and chlorpyrifos-based insecticides. Larval mortality following pesticide exposure was predicted to vary greatly depending on the proximity to the field where it was applied, as well as the current wind conditions.
Olaya- Arenas et al., 2020	Larval pesticide exposure impacts monarch butterfly performance	<u>PESTICIDES &amp; MONARCHS</u> : Investigated the impact of six pesticides, including a neonicotinoid (clothianidin), two herbicides, and three fungicides, on adult monarch survival following chronic larval exposure at field recorded levels. While weak effects were observed regarding larval survival and development time, two of the fungicides (azoxystrobin and trifloxystrobin) reduced wing size in adults, suggesting that sub-lethal larval exposure may reduce adult survival and reproductive success.
Krishnan et al., 2021	Monarch Butterfly (Danaus plexippus) Life-Stage Risks from Foliar and Seed- Treatment Insecticides	PESTICIDES & MONARCHS: Conducted a toxicological study of monarch larvae and adults, across a range of insecticides and routes of exposure. Combined with field data from other papers on observed insecticide exposures, as well as modeling on aerial exposure and drift due to wind. They concluded that seed treatment of crop species does not have the potential to damage monarch populations residing on nearby host plants. While aerial application does cause monarch mortality, the authors conclude that the use of land proximal to agricultural fields for milkweed establishment is a net positive for monarchs.
Prouty et al., 2021	Host plant species mediates impact of neonicotinoid exposure to Monarch butterflies	PESTICIDES AND HOST PLANTS: This study investigated a potential relationship between the toxicity of a milkweed host plant species (measured by the concentration of cardenolides each species produces) and the response of monarch larvae to neonicotinoids. A significant association between cardenolide content of host plants and susceptibility to pesticide was noted, with the larvae exposed to low cardenolide and high pesticide exposure experiencing the highest proportion of visual deformity and failed ecdysis. Adult performance was also affected for these groups. The authors note that only the highest range of experimentally recorded pesticide concentrations on host plants were associated with negative effects on monarch survival.
Grant et al., 2022	Monarch Butterfly Ecology, Behavior, and Vulnerabilities in North Central United States Agricultural Landscapes	PESTICIDES AND MONARCHS: Produced multiple models of monarch behavior, including adult migration, oviposition, and habitat selection, as well as larval behavior. When coupled with existing studies on milkweed susceptibility to herbicides, monarch susceptibility to pesticides, and current regulations on milkweed planting within field margins, the authors suggest that the increased habitat and subsequent production of monarchs resulting from planting milkweed near agricultural fields

### III. Roadside Habitat Management

#### <u>Mowing</u>

Monarch egg abundance and survival increased after mid-summer mowing in Michigan (Knight et al., 2019; Haan & Landis, 2020). However, frequent mowing can create ecological traps for monarchs (and other species), killing immature monarchs as they are developing on milkweed plants and potentially interfering with reproductive diapause (Alcock et al., 2016). Halbritter et al., 2015 found that reduced mowing during peak seasonal butterfly activity can result in increased butterfly numbers, while Dee & Baum, 2019 recommend only mowing after the peak of milkweed reproduction to maintain resources for monarchs. Multiple authors found that mowing of milkweed in mid-summer can result in increased growth of new leaves and higher rates of oviposition, but warned that this effect is region-specific and depends on local phenology (Baum & Mueler, 2015, Fischer et al., 2015). Further research is necessary to understand effects of mowing on floral resource availability and host plants for other species. Phillips et al., 2019 recommends that pollinator habitat in roadsides not mowed during peak flowering period.

Multiple studies have documented significant benefits of reduced mowing, including improved safety and cost saving (Storey et al., 2020; Van Dyke et al., 2021). Van Dkye et al., 2021 found that the KY Transportation Cabinet could save between \$9-\$24 million over 5 years through mowing cutbacks. Entsminger et al., 2019 also documents reductions in long-term maintenance costs associated with reduced mowing. They suggest that mowing once per year is necessary in Mississippi to control tree and shrub species, but that mowing 2-3 times per year is no different (in terms of plant community composition) than mowing once.

Additionally, McLaughlin et al., 2020 states that traffic from mowers can reduce or eliminate infiltration benefits in roadsides, so reducing mowing may also improve infiltration.

In a study on mowing in Oklahoma, Baum & Mueler, 2015 hypothesized that mowing may impact the establishment of monarch parasites, such as *O. elektroscirrha*. Transmission of the parasite's spores may be reduced by removing contaminated plant tissue, but the subsequent regrowth of highly attractive tender leaves may concentrate spores for monarchs who visit after the regrowth has occurred. Further research is needed to understand the impact of mowing on monarch migration and rates of parasitization.

Additional Supporting Information (Details by Paper)		
Author, Yr	Title	Key Takeaways
Knight et al., 2019	Strategic mowing of roadside milkweeds	At a 43°N latitude (NY, southern WI, WY), mowing roadsides in the 2nd & 3rd weeks of July resulted in higher egg abundance per milkweed plant compared

	increases monarch butterfly oviposition	to unmowed controls & other mowing treatments. Mowed plots had higher egg abundance than unmowed controls. Note that this study did not find mowing to extend the egg laying season, though other studies have found this. Need for studying impacts on floral availability and other host plants. Follow up research: are monarchs shifting their laying preference from nearby older milkweeds to the cut milkweed, potentially pulling monarchs from the safety of a nearby prairie to the more dangerous roadside ROW?
Haan & Landis, 2020	Grassland disturbance effects on first-instar monarch butterfly survival, floral resources, and flower-visiting insects	<u>MOWING:</u> First instar monarch survival was more than twice as high on regenerating milkweed in MI (after mid-summer mowing) than on older stems. Authors caution that frequently mowing milkweeds can create ecological traps for monarchs initial disturbances may attract ovipositing adults, but continued mowing can kill large numbers of immature monarchs as they develop. It is important to consider the frequency of disturbance events and to further study the effects on pollinator communities as a whole.
Phillips et al., 2019	Road verges support pollinators in agricultural landscapes, but are diminished by heavy traffic and summer cutting	Road verges had greater flower abundance, flower sp. richness and pollinator abundance than field interiors. Verge hedges had less woody cover but greater flower species richness. Fewer pollinators along verge edges/next to roads than along verge centers (2–11 m from roads) and fewer pollinators in road verges next to busier roads. Road verges were generally cut once (in summer), and cuttings were never removed. Substantially fewer flowers and pollinators in road verges that had been cut, even though surveys often took place many weeks after cutting. Road verges and hedges can provide habitat hotspots for pollinators in ag landscapes, but their capacity to do so is reduced by heavy traffic and summer verge cutting. Beneficial management for pollinators should prioritize wider road verges (>2 m), roads with less traffic, areas away from the immediate vicinity of the road, and should not be mowed during peak flowering period.
Storey et al., 2020	Comparison of Cost, Safety, and Environmental Benefits of Routine Mowing and Managed Succession of Roadside Vegetation	The ecosystem services provided by minimizing roadside vegetation maintenance practices can include ecosystem diversity, stormwater quantity and quality management, carbon sequestration, conservation and/or restoration of pollinator and/or wildlife habitat, and aesthetics. A key safety benefit comes from reducing maintenance personnel exposure to traffic hazards, equipment, and chemical treatments. This provides both short-term and long-term cost savings and benefits. Overall, managing the roadside as a valued transportation asset consists of taking advantage of the natural ecosystem services that modified mowing regimes and/or managed succession can provide to see the return in cost/benefit.
Halbritter et al., 2015	Reducing mowing frequency increases floral resource and butterfly (Lepidoptera: Hesperioidea and Papilionoidea) abundance in managed roadside margins	Studied whether changes in mowing frequency affects abundance and mortality of butterflies and abundance and sp. richness of blooming plants along highway margins. 3 mowing treatments applied to sections of highway margin in Florida: no mowing, mowing every 6 week, and mowing every 3 week. Mowing treatment had significant effects on floral resources; 3 week treatment = lowest floral abundance and sp. richness. Mowing treatment alone did not have significant effects on any butterfly variable, but the mowing treatment*time interaction had a significant effect on butterfly abundance. No-mow treatment resulted in greatest numbers of butterflies from late summer into early fall. Reducing mowing during peak seasonal butterfly activity can increase butterfly numbers. Future studies in different regions would benefit from considering the effects of time of year of mowing in addition to the frequency.

Van Dyke et al., 2021	Economic and Environmental Benefits of a Reduced Roadside Mowing Program for Kentucky Highways	Provides best conservation mowing practices based on a review of landscape management policies, programs, and procedures in use at 15 state DOTs. Most agencies divide the roadside into discrete management zones. While they continue to mow regularly in the areas closest to roadways, beyond this mowing is done less often and is combined with selective herbicide use to facilitate plant species favored by pollinators. An economic analysis of different mowing strategies found that KYTC can save between \$9 million and \$24 million over a five-year period through mowing cutbacks. Eliminating a single litter cycle can generate an additional \$5 million in savings over the same period.
McLaughlin et al., 2020	Stormwater Infiltration and Pollinator Habitat Zones Along Highways	This project explored the possibilities of managing soils on new and existing roadside areas to reduce runoff through increased infiltration. This was pursued through several greenhouse studies, controlled field plots at three sites monitored for three years, and several installations on existing roadside areas. Tillage was very beneficial for improving infiltration in compacted soil, often by a factor of 3X or more. Incorporating compost at the rate tested, 5cm incorporated into 15cm of soil, often had additional benefits but not always. Improved vegetation establishment and resistance to compaction may result from the compost treatment. Traffic from tractor mowers can reduce or eliminate the infiltration benefits, however. Wildflowers as a substitute for grass can provide greater infiltration potential, in part because mowing traffic is reduced from 4 times per year to 1. Among the many wildflowers that were planted as a mix, very few were present in our plots. Coreopsis and blanketflower were resilient in field plots and under different soil conditions in greenhouse tests - recommended for planting based on their ability to grow and develop robust root systems.
Entsminger et al., 2019	Mowing effects on woody stem density and woody and herbaceous vegetation heights along Mississippi highway right-of- ways	Investigated effects of ROW mowing frequency on native and nonnative herbaceous and woody plant vertical height coverage and native and nonnative woody stem density within plant communities along highway ROWs. Recommends 1 mowing per year to control tree/shrub species for visibility along roadside ROWs. 2-y mowing regimen was no different from mowing once annually and/or more than three times annually in the plant communities in east- central Mississippi. However, 1 mowing/y retained agronomic plant coverage, useful for erosion control and soil stabilization during roadside maintenance. Proactive management implementations can include native plantings, selective herbicide use to decrease nonnatives, continual mowing from roadside edge to 10 m, and only 1 mowing in late fall with an extension of the boundary to reach beyond 10 m from the roadside edge to suppress invasion of woody plants. Adopting this less-frequent mowing regimen can reduce long-term maintenance costs.
Fischer et al., 2015	Enhancing Monarch Butterfly Reproduction by Mowing Fields of Common Milkweed	Conducted a study comparing the growth, senescence, and egg density of milkweed ramets cut at different times throughout the growing season. Mowing towards the middle of summer resulted in milkweed regrowth, with a resulting increase in the number of monarch eggs and larvae on cut ramets due to a preference for younger leaves. The authors point out that mowing too late in the season can prevent proper regrowth, and potentially cause eggs to be laid which cannot develop in time to set out on the fall migration. Other forms of disturbance than mowing can also achieve the same effect, but the authors warn that the timing of any disturbance should be set specifically for the region it is implemented in.

Baum & Mueler, 2015	Grassland and roadside management practices affect milkweed abundance and opportunities for monarch recruitment.	In the south central US, some of the monarchs traveling south during the fall migration are still reproductively active. To investigate the possibility of creating additional habitat for this subset of monarchs, the authors conducted surveys of milkweed abundance in prairies, rangelands, and roadsides, both with and without disturbance (ie. fire, mowing, or grazing). Undisturbed sites saw senescence of the most commonly found milkweed species ( <i>A. viridis</i> ) by the start of fall, while milkweed populations that experienced disturbance persisted longer and showed more new growth favorable to reproductively active monarchs. The authors mention the sensitive timing required to maximize the benefits of mowing, and also propose disturbance regimes as a way to manage predator/parasite activity for migratory monarch populations. However, they also mention the possibility that spores may be concentrated in small groups of highly attractive regrown plants.
Alcock et al., 2016	Monarch Butterflies Use Regenerating Milkweeds for Reproduction in Mowed Hayfields in Northern Virginia	Conducted a similar study to Fisher et al. 2015, in which counts of monarch eggs and larvae were conducted on both senesced milkweed and milkweed induced to regenerate via mowing. More eggs and larvae were found on regenerated milkweed than unmowed in the weeks after mowing, which was conducted later in the growing season than Fisher et al because of the lower latitude of Alcock et al.'s study system. However, the authors did not take into account the monarch larvae lost in the mowing process, or the effect unseasonably young host plant may have on reproductive diapause, or the ability of the later-developing monarchs to successfully migrate. Increased exposure to parasites is also a possibility, meaning that mowing could create an ecological trap for monarchs if not timed appropriately.
Dee & Baum, 2019	Mowing frequency influences number of flowering stems but not population age structure of Asclepias viridis, an important monarch host plant.	Examined the impact of mowing regimen on the age structure and floral resources of <i>Asclepias viridis</i> in Oklahoma. Age structure of <i>A. viridis</i> individuals was not affected by different mowing regimes (ie. mowing did not create increased mortality), but the number of observed stems and flowers were reduced in highly mowed populations. Cessation of mowing until after the peak flowering season of <i>A. viridis</i> was recommended, not only to maximize the floral and hosting resources available for monarchs, but also to increase the quantity of host plants available early in the growing season, and to reduce larval mortality due to mowing.
Jakobsson et al., 2018	How does roadside vegetation management affect the diversity of vascular plants and invertebrates? A systematic review	This review synthesized ~50 papers to evaluate the impacts of different roadside management methods (with mowing being the most common) on roadside plant communities. While no statistical difference was observed between mowed and unmowed sites, mowing twice a year and removing the cut plant material (hay) resulted in higher plant species richness. The authors note that the impacts of different mowing regimes may vary based on a multitude of factors, including the type of road along which the habitat is situated, surrounding land usage, and base productivity of the habitat in question. Ultimately, it is asserted that roadsides require unique management regimens to grasslands, and that more research on the impact of mowing is necessary to understand its relationship to plant diversity.

#### **Native Plant Establishment**

Soper et al., 2019 suggests that less diverse seed mixes could be planted in roadsides adjacent to high quality habitat whereas diverse mixes should be planted at sites next to ag fields and areas with

less diverse habitat. Schact & Wu-Smart, 2019 suggest that planting wildflowers in strips or islands may be a cost-effective method of improving establishment and persistence in diverse roadside mixtures. McLaughlin et al., 2020 recommends planting coreopsis and blanketflower because of their ability to grow and develop robust root systems in ROWs. Mitchell et al. 2022 found that seeding resulted in different levels of establishment success for different native forb species, and noted a low level of colonization of roadside habitats by native species from adjacent ecosystems. Askew et al., 2023 asserts that native plants are often slower to establish than invasives, and require different regimens of mowing, alongside pre-seeding practices like cold stratification. The same paper also noted a considerable difference in cost between expensive native seed and cheaper introduced species.

Additional Supporting Information (Details by Paper)		
Author, Yr	Title	Key Takeaways
Schact & Wu- Smart, 2019	Establishment of Wildflower Islands to Enhance Roadside Health and Aesthetics	Explored ways to improve wildflower establishment by separating wildflower seeds from the conventional seed mixture which includes both wildflower and grass seeds. Wildflower plots were seeded at different patch or island sizes to assess cost-effective ways of reducing competition by nonnative weeds and enhancing the longevity of roadside habitat. Conventional roadside seeding methods yielded plots with lower abundance and richness of forbs and bees compared to plots seeded with wildflowers only but only in the first year. Bee richness was highest in the late season, while forb abundance and richness were highest in midseason. No differences were observed across differently sized wildflower-only patches likely because of the recent establishment of plots. In fact, only ~50% of seeded forbs had established and roughly 14 plants out of the 40 species in the seed mixture did not establish in either survey years and may therefore be replaced in future seed mixtures. Wildflower segregation in strips or islands may be a cost-effective method of improving wildflower establishment and persistence in diverse roadside mixtures.
Soper et al., 2019	Evaluating Composition and Conservation Value of Roadside Plant Communities in a Grassland Biome	<u>ADJACENT HABITAT, NEBRASKA</u> : Found that roadside vegetation is influenced by adjacent land use type - neighboring plant communities influence the composition of ROW vegetation. The authors suggest that less diverse mixes could be planted in ROWs if adjacent to diverse habitat (e.g., Sandhills rangeland). Diverse mixes are likely necessary in ROWs surrounded by cropland or plant communities with many non-native, weedy species. The persistence of many seeded, native species is minimal (mostly forbs) because of the competitiveness of both seeded and invasive grasses.

McLaughlin et al., 2020	Stormwater Infiltration and Pollinator Habitat Zones Along Highways	Project explored the possibilities of managing soils on new and existing roadside areas to reduce runoff through increased infiltration via greenhouse studies, controlled field plots, and several installations on existing roadsides. Tillage was beneficial for improving infiltration in compacted soil. Incorporating compost at the rate tested often had additional benefits but not always. Traffic from tractor mowers can reduce or eliminate the infiltration benefits, however. Wildflowers as a substitute for grass can provide greater infiltration potential, in part because mowing traffic is reduced from four times per year to one. Among the many wildflowers that were planted as a mix, very few were present in our plots. Coreopsis and blanketflower were resilient in field plots and under different soil conditions in greenhouse tests - recommended for planting based on their ability to grow and develop robust root systems.
Askew et al., 2023	Promoting Native Roadside Plant Communities and Ensuring Successful Vegetation Establishment Practices	Conducted a review of the Virginia Department of Transportation's vegetation establishment practices along roadways. Despite the use of largely introduced species in state-planned seed mixes, native species were observed frequently on roadsides, with the proportion of natives positively related to the distance from a roadside due to different environmental and management conditions. In general, native species were reported to be slower to establish than introduced or invasive species, which means they are a less effective means of controlling erosion than faster-growing introduced species. To promote the establishment of native grass species, cold stratification before planting was recommended, as well as mowing in the spring one year after establishment. While there was opportunity for improvement within the current policy on plant establishment, the slow rate of native plant establishment and high cost of seeds mean that native seed blends will not entirely replace the current blends.
Blader, 2018	Milkweed patch size effects on Monarch butterfly oviposition within lowa prairies and roadsides	This master's thesis project attempted to understand if a preference exists for ovipositing monarchs based on milkweed patch size. Interpretation of trends in the data depended on the data analysis used, but the author suggested that other drivers of monarch oviposition may not have been captured by this study. The author suggested that larval mortality may fluctuate as a result of patch size, and indicated that increased oviposition does not necessarily lead to increased numbers of adult monarchs.

Mitchell et al.	Cost-Effective Roadside	This report contained multiple studies of roadside habitat and native
2022	Vegetation Methods to	plants. The first used mapping to demonstrate increased bumblebee
	Support Insect	presence at sites with higher floral area. The second study consisted of
	Pollinators	surveys of roadside habitats, seeded in different years with both native and
		non-native seed mixes. Diverse insect communities were observed at both
		native an non-native seeded sites with high floral diversity, however the
		authors acknowledge that native species may be necessary for specialist
		insects. Only introduced species were able to spontaneously colonize
		roadside sites: native plants require seeding to establish, and are gradually
		replaced by grasses and invasive forbs over time. Seeding resulted in
		more successful establishment of some native species compared to
		others.

#### **Roadside Habitat Prioritization**

Phillips et al., 2019 and 2021 recommend prioritizing road verges wider than 2 meters for pollinator habitat, along with roads with less traffic, areas away from the immediate vicinity of the road, and areas that are not mowed during peak flowering period. Hopwood 2008 suggests that narrow verges near heavy traffic can still provide valuable habitat to bees. Keilsohn et al. 2018 found elevated insect-vehicle mortality within vegetated medians.

Additional Supporting Information (Details by Paper)		
Author, Yr	Title	Key Takeaways
Hopwood 2008	The contribution of roadside grassland restorations to native bee conservation	<u>BEES</u> : Traffic and ROW width did not significantly influence bees - narrow verges near heavy traffic can provide valuable habitat to bees. Restored and weedy roadside bee communities were similar to the prairie remnant, but the remnant was more similar in bee richness and abundance to restored roadsides.
Keilsohn et al. 2018	Roadside habitat impacts insect traffic mortality	<u>INSECTS</u> : Studied insect mortality on high speed roads (70–90 kmh). Mortality was lower when roadsides were bordered by woodlots compared to lawn/meadows. Butterfly & dragonfly mortality was highest on roads with meadow medians; bee mortality highest on roadsides with lawn medians. Vegetated medians significantly elevated fatal insect- vehicle strikes. Authors state that regardless of the habitat bordering roadsides, mortality was unacceptably high for areas being considered for conservation.
Phillips et al., 2019	Road verges support pollinators in agricultural landscapes, but are diminished by heavy traffic and summer cutting	<u>POLLINATORS</u> : Road verges had greater flower abundance, flower sp. richness and pollinator abundance than field interiors. Verge hedges had less woody cover but greater flower species richness. Fewer pollinators along verge edges/next to roads than along verge centers (2–11 m from roads) and fewer pollinators in road verges next to busier roads. Road verges were generally cut once (in summer), and cuttings were never removed. Substantially fewer flowers and pollinators in road verges that had been cut, even though surveys often took place many weeks after cutting. Road verges and hedges can provide habitat hotspots for pollinators in ag landscapes, but their capacity to do so is reduced by heavy traffic and summer verge cutting. Beneficial mgmt for pollinators

		should prioritize wider road verges (>2 m), roads with less traffic, areas away from the immediate vicinity of the road, and should not be mowed during peak flowering period.
Phillips et al., 2021	Impacts of multiple pollutants on pollinator activity in road verges	POLLINATORS & HABITAT, ENGLAND: Exposure to noise, turbulence, dust, and metals decreased with distance from road edge except for turbulence. Pollinator densities were lower closer to road edge, despite a similar flower density and species richness. Simulated turbulence reduced pollinator visit duration by up to 54%. Some pollinator taxa preferentially avoided concentrations of metals that were more frequently found in flowers within 2m of roads, while noise and dust had no apparent effect. Authors suggest that roadsides are suitable for pollinator habitat overall but recommend prioritizing areas >2m from the road edge and those with lower traffic volume.
Fitch & Vaidya, 2021	Roads pose a significant barrier to bee movement, mediated by road size, traffic and bee identity	<u>BEES &amp; ROAD SIZE:</u> Investigated the influence of roads on pollinator movement and pollination by examining patterns of pigment transfer between focal plants of two species, <i>Coreopsis verticillata</i> and <i>Monarda</i> <i>fistulosa</i> . Medium and large roads may impede movement of bees which may impact foraging and pollination (and consequently, successful reproduction of plants). Recommend evaluation and implementation of strategies to make roads less of a barrier to pollinators such as habitat corridors and designs that do not encourage pollinators to cross roads.

## References

- Alcock, J., Brower, L. P., & Williams Jr, E. H. 2016. Monarch butterflies use regenerating milkweeds for reproduction in mowed hayfields in northern Virginia. The Journal of the Lepidopterists' Society, 70(3), 177-181. doi.org/10.18473/107.070.0302
- Alvarez, B. X. M., Carrera-Treviño, R., & Hobson, K. A. 2019. Mortality of monarch butterflies (Danaus plexippus) at two highway crossing "Hotspots" during autumn migration in Northeast Mexico. Front. Ecol. Evol. 7. Biological Conservation, 231(150-160), ISSN 0006-3207. <u>doi.org/10.1016/j.biocon.2019.01.008</u>.
- Askew, S. D., Goatley, J. M., & Gonçalves, C. 2023. Promoting Native Roadside Plant Communities and Ensuring Successful Vegetation Establishment Practices. Virginia Transportation Research Council (VTRC). No. FHWA/VTRC 23-R14
- Baum, K. A., & Mueller, E. K. 2015. Grassland and roadside management practices affect milkweed abundance and opportunities for monarch recruitment. Monarchs in a changing world: Biology and conservation of an iconic butterfly, 197-202.ISBN: 9780801453151
- Blader, T. 2018. Milkweed patch size effects on monarch butterfly oviposition within Iowa prairies and roadsides (Doctoral dissertation, Iowa State University).

- Botías, C., David, A., Hill, E. M., & Goulson, D. 2016. Contamination of wild plants near neonicotinoid seed-treated crops, and implications for non-target insects. Science of the Total Environment, 566, 269-278. doi.org/10.1016/j.scitotenv.2016.05.065
- Campioni, L., Marengo, I., Román, J. et al. 2022. Mud-puddling on roadsides: a potential ecological trap for butterflies. J Insect Conserv 26, 131–134. https://doi.org/10.1007/s10841-021-00367-y
- Cariveau AB, Anderson E, Baum KA, Hopwood J, Lonsdorf E, Nootenboom C, Tuerk K, Oberhauser K and Snell-Rood E. 2019. Rapid Assessment of Roadsides as Potential Habitat for Monarchs and Other Pollinators. Front. Ecol. Evol. 7:386. doi: 10.3389/fevo.2019.00386
- Daniels, J., Kimmel, C., McClung, S., Epstein, S., Bremer, J., Rossetti, K. 2018. Better Understanding the Potential Importance of Florida Roadside Breeding Habitat for the Monarch. Insects 9, no. 4: 137. <u>doi.org/10.3390/insects9040137</u>
- Dániel-Ferreira, J., Berggren, Å., Bommarco, R., Wissman, J., & Öckinger, E. (2022). Bumblebee queen mortality along roads increase with traffic. *Biological Conservation*, 272, 109643. <u>doi.org/10.1016/j.biocon.2022.109643</u>
- Dániel-Ferreira, J., Berggren, Å., Wissman, J., & Öckinger, E. 2022. Road verges are corridors and roads barriers for the movement of flower-visiting insects. Ecography, 2022(2). doi.org/10.1111/ecog.05847
- Davis, A.K., Schroeder, H., Yeager, I., Pearce, J. 2018. Effects of simulated highway noise on heart rates of larval monarch butterflies, Danaus plexippus: implications for roadside habitat suitability Biol. Lett.142018001820180018
- Dee, J. R., & Baum, K. A. 2019. Mowing frequency influences number of flowering stems but not population age structure of Asclepias viridis, an important monarch host plant. The American Midland Naturalist, 182(1), 27-35. doi.org/10.1674/0003-0031-182.1.27
- Dietzel, S., Rojas-Botero, S., Kollmann, J., & Fischer, C. 2023. Enhanced urban roadside vegetation increases pollinator abundance whereas landscape characteristics drive pollination. Ecological Indicators, 147, 109980. doi.org/10.1016/j.ecolind.2023.109980
- Ding, J., Eldridge, D.J. 2022. Roadside verges support greater ecosystem functions than adjacent agricultural land in a grassy woodland. Journal of Environmental Management, 308(114625). ISSN 0301-4797. https://doi.org/10.1016/j.jenvman.2022.114625.
- Entsminger, E.D., Jones, J.C., Guyton, J.W., Leopold, B.D., Strickland, B.K. 2019. Mowing Effects on Woody Stem Density and Woody and Herbaceous Vegetation Heights Along Mississippi Highway Right-of-Ways. Journal of Fish and Wildlife Management, 10 (1): 19–37.
- Fischer, S. J., Williams, E. H., Brower, L. P., Palmiotto, P. A. 2015. Enhancing monarch butterfly reproduction by mowing fields of common milkweed. The American Midland Naturalist, 173(2), 229-240.
- Fitch, G, Vaidya, C. 2021. Roads pose a significant barrier to bee movement, mediated by road size, traffic and bee identity. J Appl Ecol.; 58: 1177–1186. doi.org/10.1111/1365-2664.13884

- Forister, M. L., Cousens, B., Harrison, J. G., Anderson, K., Thorne, J. H., Waetjen, D., Nice, C.C., De Parsia, M., Hladik, M.L., Meese, R., van Vliet, H., Shapiro, A. M. 2016. Increasing neonicotinoid use and the declining butterfly fauna of lowland California. Biology letters, 12(8), 20160475. https://doi.org/10.1098/rsbl.2016.0475
- Garfinkel, M., Yakandawala, K., Hosler, S., Roberts, M., Whelan, C., & Minor, E. (2023). Testing the accuracy of a Rights-of-Way pollinator habitat scoring system. *Ecological Indicators*, *148*, 110062. doi.org/10.1016/j.ecolind.2023.110062
- Grant, T.J., Bradbury, S.P. 2019. The Role of Modeling in Monarch Butterfly Research and Conservation. Front. Ecol. Evol. 7:197. doi: 10.3389/fevo.2019.00197
- Grant, T. J., Fisher, K. E., Krishnan, N., Mullins, A. N., Hellmich, R. L., Sappington, T. W., ... & Bradbury,
  S. P. 2022. Monarch Butterfly Ecology, Behavior, and Vulnerabilities in North Central United States
  Agricultural Landscapes. BioScience, 72(12), 1176-1203. doi.org/10.1093/biosci/biac094
- Grant, T.J., D.T.T. Flockhart, T.R. Blader, R.L. Hellmich, G.M. Piman, S. Tyner, D.R. Norris, and S.P. Bradbury. 2020. Estimating arthropod survival probability from field counts: a case study with monarch butterflies. Ecosphere. 11 (4).
- Haan, N. L., and Landis, D. A. 2020. Grassland disturbance effects on first-instar monarch butterfly survival, floral resources, and flower-visiting insects. Biol. Conserv. 243, 108492.
- Halbritter, D.A., Daniels, J.C., Whitaker, D.C., Huang, L. 2015. Reducing Mowing Frequency Increases Floral Resource and Butterfly (Lepidoptera: Hesperioidea and Papilionoidea) Abundance in Managed Roadside Margins. Florida Entomologist, 98(4), 1081-1092.
- Hopwood, J.L. 2008. The contribution of roadside grassland restorations to native bee conservation. Biological Conservation, 141(10): 2632-2640. ISSN 0006-3207. doi.org/10.1016/j.biocon.2008.07.026.
- Jakobsson, S., Bernes, C., Bullock, J. M., Verheyen, K., & Lindborg, R. 2018. How does roadside vegetation management affect the diversity of vascular plants and invertebrates? A systematic review. Environmental Evidence, 7, 1-14. doi.org/10.1186/s13750-018-0129-z
- James, D. G. 2019. A neonicotinoid insecticide at a rate found in nectar reduces longevity but not oogenesis in monarch butterflies, Danaus plexippus (L.).(Lepidoptera: Nymphalidae). Insects, 10(9), 276. doi.org/10.3390/insects10090276
- Kantola, T., Tracy, J.L., Baum, K.A., Quinn, M.A., Coulson, R.N. 2019. Spatial risk assessment of eastern monarch butterfly road mortality during autumn migration within the southern corridor,
- Kasten, K., Stenoien, C., Caldwell, W. et al. 2016. Can roadside habitat lead monarchs on a route to recovery?. J Insect Conserv 20, 1047–1057. doi.org/10.1007/s10841-016-9938-y
- Kaul, A.D. and Wilsey, B.J. 2019. Monarch butterfly host plant (milkweed Asclepias spp.) abundance varies by habitat type across 98 prairie. Restoration Ecology. 27, 1274-1281.

- Keilsohn, W., Narango, D.L. & Tallamy, D.W. 2018. Roadside habitat impacts insect traffic mortality. J Insect Conserv 22, 183–188. doi.org/10.1007/s10841-018-0051-2
- Knight, S.M., Norris, R.D., Derbyshire, R., Flockhart, D.T.T. 2019. Strategic mowing of roadside milkweeds increases monarch butterfly oviposition. Global Ecology and Conservation 19(e00678).
- Krishnan, N., Zhang, Y., Bidne, K. G., Hellmich, R. L., Coats, J. R., & Bradbury, S. P. 2020. Assessing field-scale risks of foliar insecticide applications to monarch butterfly (Danaus plexippus) larvae. *Environmental toxicology and chemistry*, 39(4), 923-941. doi.org/10.1002/etc.4672
- Krishnan, N., Zhang, Y., Aust, M. E., Hellmich, R. L., Coats, J. R., & Bradbury, S. P. 2021. Monarch Butterfly (Danaus plexippus) Life-Stage Risks from Foliar and Seed-Treatment Insecticides. Environmental Toxicology and Chemistry, 40(6), 1761-1777. doi.org/10.1002/etc.5016
- Lalonde, S., McCune, J.L., Rivest, S.A., Kharouba, H.M. 2022. Decline in common milkweed along roadsides around Ottawa, Canada, Écoscience, 29:1, 25-37, DOI: 10.1080/11956860.2021.1943930
- Lee ZA, Baranowski AK, Preisser EL. 2021. Auditory predator cues affect monarch (Danaus plexippus; Lepidoptera: Nymphalidae) development time and pupal weight. Acta Oecologica 111: 103740.
- Martini, M. 2022. Pollinator biodiversity and interaction networks in anthropogenic systems roadside verges and transmission line easements as pollinator habitat in Manitoba, Canada. [Master's thesis, University of Manitoba].
- McCoshum, S., Agrawal, A.A. 2021. Ecology of Asclepias brachystephana: a plant for roadside and rightof-way management. Native Plants 22(3): 256-267.
- Mckenna, D., McKenna, K.M., Malcom, S.B., Berenbaum, M.R. 2001. Mortality of lepidoptera along roadways in Central Illinois. Journal of the Lepidopterists' Society. 55. 63-68.
- McLaughlin, R.A., Ashraah, S.H., Haselton, A.M., Heitman, J.L. 2020. Department of Crop and Soil SciencesStorm Water Infiltration and Pollinator Habitat Zones Along Highways. North Carolina Department of Transportation Research and Development Unit. Report Number FHWA/NC/2017-27. Accessed at <u>rosap.ntl.bts.gov/view/dot/60604</u>.
- Mitchell, T.S., Agnew, L., Meyer, R., Sikkink, K.L., Oberhauser, K.S., Borer, E.T., Snell-Rood, E.C. 2020. Traffic influences nutritional quality of roadside plants for monarch caterpillars. Science Direct 724(138045).
- Mitchell, T., Verhoeven, M., Darst, A., Evans, E., Cariveau, D., & Snell-Rood, E. (2022). Cost-effective roadside revegetation methods to support insect pollinators (No. MN 2022-30). Minnesota. Department of Transportation. Office of Research & Innovation.
- Mody, K., Lerch, D., Müller, A.K., Simons, N.K., Blüthgen, N., Harnisch, M. 2020. Flower power in the city: Replacing roadside shrubs by wildflower meadows increases insect numbers and reduces maintenance costs. PLoS ONE 15(6): e0234327. <u>doi.org/10.1371/journal.pone.0234327</u>
- Mueller, E.K., Baum, K.A. Monarch–parasite interactions in managed and roadside prairies. J Insect Conserv 18, 847–853 (2014). doi.org/10.1007/s10841-014-9691-z

- Nemec, K., Stephenson, A., Gonzalez, E.A. et al. 2021. Local Decision-makers' Perspectives on Roadside Revegetation and Management in Iowa, USA. Environmental Management 67, 1060–1074. doi.org/10.1007/s00267-021-01448-z
- O'Brien, J.E. 2016. The Contribution of Roadside Wildflower Plantings for Enhancing Pollinator Habitat in the Piedmont of North Carolina. [Master's Thesis, North Carolina State University.]
- Olaya-Arenas, P., Hauri, K., Scharf, M. E., & Kaplan, I. 2020. Larval pesticide exposure impacts monarch butterfly performance. Scientific reports, 10(1), 14490. doi.org/10.1038/s41598-020-71211-7
- Parlin AF, Stratton SM, Guerra PA. Oriented migratory flight at night: Consequences of nighttime light pollution for monarch butterflies. iScience. 2022 Apr 27;25(5):104310. doi: 10.1016/j.isci.2022.104310. PMID: 35573206; PMCID: PMC9097705.
- Pecenka, J. R., & Lundgren, J. G. 2015. Non-target effects of clothianidin on monarch butterflies. The Science of Nature, 102, 1-4. doi.org/10.1007/s00114-015-1270-y
- Phillips, B.B., Bullock, J.M., Gaston, K.J., et al. 2021. Impacts of multiple pollutants on pollinator activity in road verges. J Appl Ecol. 58: 1017–1029.
- Phillips, B.B., Wallace, C., Roberts, B.R., et al. 2020. Enhancing road verges to aid pollinator conservation: A review. 250:208687.
- Phillips, B.B., Bullock, J.M., Gaston, K.J., et al. Impacts of multiple pollutants on pollinator activity in road verges. J Appl Ecol. 2021; 58: 1017–1029. doi.org/10.1111/1365-2664.13844
- Pitman, G.M, Flockhart, D.T.T., Norris, R.D. 2018. Patterns and causes of oviposition in monarch butterflies: Implications for milkweed restoration, Biological Conservation, 217: 54-65, ISSN 0006-3207. doi.org/10.1016/j.biocon.2017.10.019.
- Prouty, C., Barriga, P., Davis, A. K., Krischik, V., & Altizer, S. 2021. Host plant species mediates impact of neonicotinoid exposure to Monarch butterflies. Insects, 12(11), 999. doi.org/10.3390/insects12110999
- Ries, L., Debinski, D. M., Wieland, M. L. 2001. Conservation Value of Roadside Prairie Restoration to Butterfly Communities. Conservation Biology, 15(2), 401–411. jstor.org/stable/2641838
- Schacht, W. and Wu-Smart, J. 2019. Establishment of Wildflower Islands to Enhance Roadside Health and Aesthetics. NDOT Research Report SPR-1(17) M058.
- Shephard A.M., Mitchell, T.S., Snell-Rood, E.C. 2021. Monarch caterpillars are robust to combined exposure to the roadside micronutrients sodium and zinc. Conservation Physiology, 9(1). doi.org/10.1093/conphys/coab061 Open Source
- Shephard, A.M., Brown, N.S., Snell-Rood, E.C. 2022. Anthropogenic Zinc Exposure Increases Mortality and Antioxidant Gene Expression in Monarch Butterflies with Low Access to Dietary Macronutrients. Environmental Toxicology and Chemistry 00:1-11.

- Shephard, A.M., Mitchell, T.S., Henry, S.B., Oberhauser, K.S., Kobiela, M.E. Snell-Rood, E.C. 2020, Assessing zinc tolerance in two butterfly species: consequences for conservation in polluted environments. Insect Conserv Divers, 13: 201-210.
- Skórka, P., Lenda, M., Moroń, D., Kalarus, K., & Tryjanowski, P. 2013. Factors affecting road mortality and the suitability of road verges for butterflies. Biological Conservation, 1*59*, 148-157. doi.org/10.1016/j.biocon.2012.12.028
- Snell-Rood, E., Espeset, A., Boser, C.J., Smykalski, R. 2014. Anthropogenic changes in sodium affect neural and muscle development in butterflies. Biological Sciences 111 (28) 10221-10226. <u>doi.org/10.1073/pnas.1323607111</u>
- Soper, J.M., Raynor, E.J., Wienhold, C. et al. Evaluating Composition and Conservation Value of Roadside Plant Communities in a Grassland Biome. Environmental Management 63, 789–803 (2019). <u>doi.org/10.1007/s00267-019-01154-x</u>
- Storey, B., Das, S., McFalls, J., Avelar Moran, R., Dadashova, B. 2020. Comparison of Cost, Safety, and Environmental Benefits of Routine Mowing and Managed Succession of Roadside Vegetation. Final Report: National Cooperative Highway Research Program. https://onlinepubs.trb.org/Onlinepubs/nchrp/docs/NCHRP14-40FinalReport.pdf
- Thogmartin, W.E., López-Hoffman, L., Rohweder, J., Diffendorfer, J., Drum, R., et al. 2017. Restoring monarch butterfly habitat in the Midwestern US: 'all hands on deck'. Environ. Res. Lett. 12 074005. DOI 10.1088/1748-9326/aa7637
- Tracy, J. L., Kantola, T., Baum, K. A., & Coulson, R. N. 2019. Modeling fall migration pathways and spatially identifying potential migratory hazards for the eastern monarch butterfly. *Landscape ecology*, *34*, 443-458. doi.org/10.1007/s10980-019-00776-0
- Tracy, JL., Birt, A., McFalls, J., Prozzi, J., Coulson, R. 2022. Evaluating Fall Monarch Butterfly Roadkill Hotspot Incidence and Potential Roadkill Mitigation. Texas Department of Transportation [Project Report]. Retrieved from <u>static.tti.tamu.edu/tti.tamu.edu/documents/0-7022-PSR.pdf</u>
- Trauth, K.M, Aloysius, N.R., Brown, H., Sullivan, L.L. 2021. Pollinator Habitat Along Highway Right of Way. Missouri Department of Transportation Construction and Materials Division, Report Number: cmr 21-005. Available at: https://rosap.ntl.bts.gov/view/dot/58945
- Van Dyke, C., Wallace, C., Kreis, D. 2021. Economic and Environmental Benefits of a Reduced Roadside Mowing Program for Kentucky Highways. Kentucky Transportation Center Research Report. 1724. <u>uknowledge.uky.edu/ktc\_researchreports/1724</u>
- Vinchesi, A., Walsh, D., & Broadhead, C. 2018. Assessing transportation impacts to alkali bees (Hymenoptera: Halictidae) and alfalfa seed production in southeastern Washington State. American Entomologist, 64(1), 52-58. doi.org/10.1093/ae/tmy011
- Wang, Y., Jia, S., Wang, Z. et al. 2021. Planning considerations of green corridors for the improvement of biodiversity resilience in suburban areas. J Infrastruct Preserv Resil 2, 6. <u>doi.org/10.1186/s43065-021-00023-4</u>

- Webb, M.A. 2017. Roadside Environments and the Effects of Roadside Management Practices on Milkweeds and Monarchs. [Master's Thesis, Oklahoma State University.]
- Western Riverside County Regional Conservation Authority. 2021. Multiple Species Habitat Conservation Plan. [Annual Report] Retrieved from <u>https://www.wrc-rca.org/wp-</u> <u>content/uploads/2022/11/RCA\_2021\_Annual\_Report.pdf</u>