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RESEARCH REPORT

Bees and Butterflies in Roadside Habitats: Identifying Patterns, Protecting Monarchs, and Informing Management

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16. Abstract

Pollinating insects provide vital ecosystem services and are facing global declines and habitat loss. Roadsides are increasingly regarded as important potential areas for enhancing pollinator habitat. Understanding which roadsides best support pollinators—and why—is essential to helping locate and prioritize pollinator conservation efforts across roadside networks. To support this effort, we assessed butterfly, bee, and flowering plant species richness and abundance on a set of 63 stratified randomized roadside transects in State-managed rights-of-way in SE Idaho. Our research evaluated pollinator diversity as a function of highway class (interstate, U.S., and state highways), remotely sensed NDVI values (a measure of vegetation greenness), and floral resources. We found that smaller highways and lower (less green) maximum NDVI values were associated with significantly more bee species and total bees. Roadsides bordering sagebrush habitats typically had low NDVI values and higher bee and butterfly species richness, potentially contributing to this observed pattern. Butterfly richness increased in association with higher floral abundance in roadsides. Additionally, we identified and mapped 1,363 roadside patches of milkweed (*Asclepias speciosa*), larval host plant for the imperiled monarch butterfly (*Danaus plexippus*), in a survey of over 900 miles of southern Idaho highways. Based on these results and a literature review, we recommend management strategies to promote the health of pollinator populations in Idaho's rights-of-way and provide data to help ITD prioritize areas for pollinator-friendly management practices and habitat restoration within their highway system.

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All photos are credited to Thomas Meinzen. All figures and maps were created by Thomas Meinzen.

Technical Advisory Committee

Each research project is overseen by a Technical Advisory Committee (TAC), which is led by an ITD project sponsor and project manager. The TAC is responsible for monitoring project progress, reviewing deliverables, ensuring that study objectives are met, and facilitating implementation of research recommendations, as appropriate. ITD's Research Program Manager appreciates the work of the following TAC members in guiding this research study.

- Project Sponsor: Steve Spoor (Idaho Transportation Department (SPR) Research Program)

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Table of Contents

List of Tables

List of Figures

List of Abbreviations and Acronyms

ANOVA .... Analysis of Variance

CCAA .... Candidate Conservation Agreement with Assurances

FHWA .... Federal Highway Administration

FWS .... U.S. Fish and Wildlife Service

GLMM .... Generalized Linear Mixed Model

IDFG .... Idaho Department of Fish and Game

IN .... Interstate Highway

ITD .... Idaho Transportation Department

NDVI .... Normal Difference Vegetation Index (a remotely sensed measure of greenness)

NMDS .... Nonmetric Multidimensional Scaling

ROW .... Right-of-way zone

SH .... State Highway

TAC .... Technical Advisory Committee

US .... U.S. Highway

USDA-ARS .... United States Department of Agriculture—Agriculture Research Service

Executive Summary

This research project had three major objectives: 1) to aid the Idaho Transportation Department (ITD) in their monarch butterfly ("monarch") conservation efforts on the Snake River Plain by locating milkweed populations and documenting monarch activity within their right-of-way (ROW) network; 2) to identify patterns of bee and butterfly diversity (as measured by species richness) and abundance in ROWs as they relate to differences in highway type and NDVI; and 3) to synthesize these patterns, along with the increasing body of scientific literature on roadside pollinators, in order to recommend ITD ROW management strategies and priorities that maximize benefit for pollinators, including monarchs.

To assess the presence of milkweeds (*Asclepias* spp*.*, the obligate host plants of monarch larvae) and monarch butterflies (*Danaus plexippus*) in Idaho ROWs, we surveyed 1,465 km (910 miles) of highways in both directions (thus 2,930 km or 1,820 survey miles) across southern Idaho in July 2021. We located 1,363 patches of *Asclepias speciosa* (showy milkweed), the largest of which contained over 7,000 milkweed stems. Monarchs bred on milkweed in Idaho ROWs in 2021 (eggs and ovipositing observed) and 2022 (larvae observed), although they were very rare during our 2021 surveys. Distance to perennial water was an important factor in determining milkweed distribution across the Snake River Plain, and milkweed was especially abundant in ROWs bordering irrigated fields and crops. Existing statewide milkweed models (Svancara, Abatzoglou, and Waterbury 2019) did not predict milkweed distribution in ROWs, suggesting the importance of roadside-specific factors, such as ROW management and disturbance history.

To identify patterns of bee and butterfly richness and abundance in Idaho ROWs, we established a stratified-randomized set of 63 100-meter (328-foot) transects in southeastern Idaho ROWs, stratified by highway class (State, U.S., and Interstate) and NDVI (a satellite imagery-derived measure of greenness) category (low, medium, high). At these transects, we surveyed butterflies and floral resources in 2021 and 2022 and sampled bees using pan traps at a subset of transects in 2021. Our study documented a wide diversity of pollinators using Idaho ROWs—38 species of butterflies and at least 67 species of bees in our surveys. Lower values of NDVI (less green areas), smaller, less-trafficked highways, and transects with more species of flowers were each associated with higher numbers of bees and more bee species. Butterfly species richness was higher at transects with more abundant flowers, but neither butterfly species richness nor abundance varied significantly with NDVI, highway class, or floral species richness. Low NDVI sites were often characterized by native sagebrush plant communities, while sites of high NDVI were associated with high proportions of non-native species, suggesting that NDVI might be useful both in predicting bee richness and abundance (low values) and locating developing noxious weed patches (high values).

NDVI, highway class, and floral species richness were associated with differences in both bee and butterfly species composition, suggesting that maintaining pollinator habitat across a range of NDVI and highway types is important for conserving the full complement of roadside pollinator species. Smaller highways and lower NDVI sites may be important priorities for targeted pollinator conservation

activities. We highlight particular ROW sections as hotspots of milkweed density for monarch habitat and recommend ROW sections (e.g., SH-37, US-26) with diverse pollinator populations and limited floral resources for restoration and native plantings. Finally, drawing from a detailed literature review, we provide recommendations for ROW management strategies that will minimize harm to pollinators and improve ITD's capacity to support monarchs and other pollinators within their ROW lands.

1. Literature Review: The Risks and Opportunities of Roadsides for Pollinators

Overview

Pollinators, which provide vital services to wild ecosystems and agricultural crops, are facing global population declines and habitat loss. As undeveloped land becomes increasingly scarce, much focus has been directed recently to roadsides as potential target zones for providing floral resources to pollinators. Roadsides, however, are risky places for pollinators, with threats from vehicle collisions, toxic pollutants, mowing, herbicides, and more. Although these threats have been investigated, studies have yet to quantify the costs and benefits of roadsides to pollinators, and thus do not address whether costs outweigh benefits for pollinator populations using roadside habitats. To provide context and background for our report, this general literature review addresses how, when, and under what conditions roadsides may benefit or harm pollinators, reviews existing knowledge, and recommends practical questions managers should consider when planning pollinator-focused roadside restoration projects.

Highlights

- As pollinator habitat loss and population declines accelerate, road edges and right-of-way zones are receiving increasing attention as potential target areas for creating and maintaining pollinator habitat.
- However, roads pose an array of threats to pollinating insects, including collision with vehicles, exposure to heavy metals, salts, and herbicides, mowing, and other potential sources of mortality.
- Road right-of-way zones are widely considered to be potential habitat corridors and beneficial to pollinator populations; however, data on pollinator reproduction and dispersal in roadsides is largely lacking to verify those assumptions.
- In cultivating roadside pollinator habitat, it is critical to consider how threats to pollinators can be mitigated, and under what conditions threats may outweigh the potential benefits of roadside habitat for pollinators.
- While further research is needed to accurately assess the overall impact of roadsides to pollinators, we summarize ways that roadside management can improve the ratio of benefits to costs for pollinator populations, based on available research.

Glossary

- **ROWs:** Right-of-way zones bordering roads; typically land owned by counties or state transportation departments between the edge of pavement and the adjacent property. ROWs vary in size and vegetation cover, often including a close-cropped mow zone or clear zone (defined below) and a buffer zone; terminology varies regionally. In this review, *ROW* is used interchangeably with *roadside*.
- **Ecological traps**: Situations in which organisms select habitat that results in poor rates of survival and/or reproduction, negatively impacting the population. Rapid environmental change, human impacts, and other causes can misalign an organism's behavioral cues with actual habitat

value. For example, roadsides may lure pollinators with vegetation that typically serves as food or shelter, but actually ends up being toxic or puts them in high danger of collision with traffic.

- **Collision mortality:** Death caused by collision with moving vehicles, also known as roadkill; a common source of pollinator mortality.
- **Holometabolous:** Insects that undergo complete metamorphosis, with markedly different immature stages (larvae) and a transition to adult form through a resting stage called a pupa. Bees, butterflies, flies, and beetles are all holometabolous insects.
- **Barrier effect:** Reduction or complete obstruction of movement between members of a population (and thus, gene flow) caused by a physical barrier or gap in suitable habitat, with negative implications for genetic diversity and population viability.
- **Road salt:** Halite, the natural mineral form of sodium chloride (table salt), typically with chemical impurities and additives, applied to roads to lower the freezing point of water and make roads less icy. Also known as rock salt or deicing salt.
- **Spot-treat:** Application of a pesticide to a small, distinct target area, such as an individual plant or patch of weeds.
- **Mow zone:** The unobstructed area bordering the traveled edge of a road, typically consisting of close-cropped vegetation/grass free of obstacles, intended for visibility and to allow vehicles that have left the roadway to safely regain control. This zone is typically referred to as the "clear zone" by many DOTs and road ecologists; in the ITD Operations Manual, this zone is referred to as Zone 1 or the Mow Zone.

Ubiquitous roads, declining pollinators

Roads have become a ubiquitous part of terrestrial environments: the U.S. alone has paved enough roads to circle the equator 168 times (L. Brown 2001; Federal Highway Administration 2017). Although roadways themselves occupy a small percentage of land area, their ecological impacts extend far beyond their physical dimensions; researchers estimate that roads affect about 20 times the area actually covered by pavement (Forman et al. 2003). The ecological impacts of existing roads on animals include mortality from collision with vehicles, toxicity from traffic, hydrologic disturbance, soil erosion, fragmentation of habitat and populations, and the expansion of artificial habitat edges and their effects, among others (Forman and Alexander 1998; Coffin 2007). Nonetheless, vegetated roadsides may provide habitat for pollinators, plants, and other wildlife, and corridors for animal feeding, breeding, and movement (Villemey et al. 2018).

For most vertebrates, the negative effects of roads—particularly habitat fragmentation and vehicle collisions—outweigh the potential benefits roadside habitats may offer, and the expanding global network of roads poses a serious threat to many vertebrate species (Benítez-López, Alkemade, and Verweij 2010; Laurance and Balmford 2013; Ouédraogo et al. 2020). For invertebrates like pollinating insects, however, the impact of roads and roadsides is more nuanced because roadsides can provide significant habitat for small insects. While vehicle collisions may cause similar—or higher—levels of mortality for mobile insects as for vertebrates, roadsides often harbor floral and vegetative resources in landscapes that otherwise lack these resources (Muñoz, Torres, and Megías 2015; Seibert and Conover 1991). As land becomes increasingly developed and homogenized for urban and agricultural uses, the

narrow but extensive bands of habitat available alongside roads in **ROWs** (see Glossary) may provide important refuges for pollinating insects (hereafter, *pollinators*).

Pollinator populations and habitats are facing steep global declines (Zattara and Aizen 2021). This is especially alarming because three quarters of global human food production and the function of most wild plant communities depend on pollinators (Tylianakis 2013). As such, supporting and increasing pollinator populations through strategic use of remaining land unused for agriculture or development is of paramount importance for both humans and natural systems. Many transportation departments and road managers are thus considering measures to support pollinators in road ROWs, utilizing these relatively undeveloped zones to benefit pollinator populations (Hopwood et al. 2015; Cariveau et al. 2019).

ROWs have several potential advantages for pollinators: they can connect habitat patches by providing bands of vegetation alongside roads and diverse floral resources in otherwise homogenous landscapes (e.g. crop monocultures), their management often allows forbs to thrive (Smallidge, Leopold, and Allen 1996), and they receive additional moisture from runoff in arid landscapes, resulting in enhanced resources for pollinators (Holzapfel and Schmidt 1990; Wojcik and Buchmann 2012). However, ROWs also come with potential risks; for example, runoff may contain pollutants that result in chemically-contaminated roadside plants (Mitchell et al. 2020). Some researchers express concern that road ROWs may be **ecological traps** for pollinators (Berenbaum 2015; Teixeira et al. 2017; Keilsohn, Narango, and Tallamy 2018), while others conclude that road ROW restoration is an important way to enhance pollinator populations (Ries, Debinski, and Wieland 2001; Raemakers et al. 2001; Hanley and Wilkins 2015). Such conflicts among researchers highlight the need to thoroughly review the costs and benefits of road ROW use for pollinators, and attempt to answer whether—and under what conditions—road ROW restoration for pollinators is a good conservation choice. Although reviews of road impacts on insects exist (Muñoz, Torres, and Megías 2015; Villemey et al. 2018; Phillips et al. 2020), these critical questions remain largely unanswered. This review aims to present what is known about the costs and benefits of roadsides to pollinators and summarize the factors that influence how roadside habitat affects pollinator populations, with recommendations for future research and management.

Roadside attraction: life and death for pollinators

Assessing roadsides' impacts on pollinator populations requires weighing opposing positive and negative impacts. For example: roads provide habitat where pollinators can breed, but traffic kills a portion of the pollinators that breed there (Phillips et al. 2019). Keilsohn and colleagues (2018) frame the question well: "How extensive does a [roadside] restoration planting have to be before it produces more insects than it kills?" (Keilsohn, Narango, and Tallamy 2018). The answer to that question depends on many factors, including the density of nectar and pollen resources and nest sites in the planting, the proportion of pollinators killed by vehicles, and the relative fidelity and survivorship of pollinators on roadsides compared to other available habitats. All those factors likely vary among roadsides and among pollinator species, making this question a difficult one.

Even if measured, the most direct population effects of roads—birth and death—can be difficult to compare, since pollinators that breed in road ROWs might also have bred successfully elsewhere, and individuals killed by roads may be transient and not breeding in roadside habitat. Nonetheless, a few studies have found that the community profile of road-killed pollinators more closely matched the community using roadside habitats than habitats away from roads, suggesting that roadside breeders likely represent a high proportion of road-killed pollinators (Skórka et al. 2013; Skórka, Lenda, and Moroń 2018).

Insect population sizes and birth rates are very difficult to estimate, and data on reproductive output along roadsides is lacking. A few studies document that butterflies and bees do reproduce and nest in roadside habitats, but reproduction rates are not quantified (Munguira and Thomas 1992; Heneberg, Bogusch, and Řezáč 2017). Comparison studies suggest that insects breeding in road ROWs may have a lower chance of survival and undergo higher stress than those breeding away from roads; for example, monarchs developing on roadsides have lower survivorship as larvae, even before potentially colliding with traffic as adults (Davis et al., 2018; Snell-Rood et al. 2014). Given that pollinators using roadside habitat are more likely killed by traffic (Skórka et al. 2013) or poisoned by pollutants (see pp. 19-21), breeding habitat along roadsides might not produce a net benefit for pollinator populations. However, without studies quantifying pollinator reproductive output along roadsides (and comparing this to non-roadside habitat), we cannot conclude whether roadside birth rates exceed mortality rates.

Collision mortality is estimated to kill billions of insects annually (Baxter-Gilbert et al. 2015). However, studies investigating its relative impact suggest that traffic kills a small proportion of the insects using roadside habitat: estimates of butterfly mortality by vehicle collision in three studies are between 0.6% and 7% of adults surveyed in roadside habitat, which researchers suggest is a much lower proportion than mortality caused by natural factors (Munguira and Thomas 1992; Skórka et al. 2013; Skórka, Lenda, and Moroń 2018). Road-crossing frequency and collision mortality varies by species; studies found greater mortality for butterfly species that are smaller (Skórka et al. 2013), fly below 2 m (Shyama Prasad Rao and Saptha Girish 2007), and are more mobile (de la Puente Ranea, Hueso, and Montesinos 2008; Remon et al. 2018). Flight style and speed vary greatly between taxa and likely impact an insect's susceptibility to vehicle collisions (Cant et al. 2005). Most studies estimating insect vehicle-collision mortality focus on butterflies, dragonflies, or carabid beetles; data on bees and pollinating flies are rare (Muñoz, Torres, and Megías 2015; Phillips et al. 2020). Some bees exhibit high site fidelity and may therefore be less likely to cross roads and be killed (Bhattacharya, Primack, and Gerwein 2002; Hopwood et al. 2010). However, a study in Ontario, Canada found higher collision mortality in bees (26.8/km/day) and flies (202.3/km/day) than in butterflies (10.1/km/day) (Baxter-Gilbert et al. 2015). This matches a correlation between smaller size and higher collision mortality (Skórka et al. 2013); smaller species may take more time to cross roads, be less able to avoid traffic, or simply be more common. Additionally, the small size and relative density of bees and flies mean they are more likely to remain stuck to vehicles and be overlooked and undercounted in roadkill surveys (Rao and Girish 2007).

Overall, data on the relative or absolute cost of collision mortality to pollinator populations are limited. Studies suggest higher traffic and medium-high speed roads may be especially lethal (McKenna et al.

2001). Roads appear to kill <10% of adult butterflies using roadsides habitats; percentages are lacking for other taxa, but may be higher (Baxter-Gilbert et al. 2015; Munguira and Thomas 1992; Skórka et al. 2013; Skórka, Lenda, and Moroń 2018). Given that the vast majority of insects die before reaching maturity, <10% mortality may not reduce populations considerably (Peterson et al. 2009). However, **holometabolous** insects (including most pollinators) exhibit very high mortality as eggs and first-instar larvae, and again as pupae; thus pre-breeding collision mortality of the relatively small proportion of individuals that reach adulthood could have an outsized impact on pollinator populations (Ito 1959). Also, collision mortality may compound other causes of mortality in roadsides, such as herbicide application, mowing, and chemical toxin exposure (Peterson et al. 2009).

While available evidence suggests that roads kill less than 1 in 10 butterflies using roadside habitats, the long-term impact of collision mortality on pollinator populations is unknown, and its potential impacts are often understated (Teixeira, Rytwinski, and Fahrig 2020). Further research is required, and many questions remain: for example, besides population reduction, collision mortality may also influence morphology by selecting for certain attributes, as recently noted in roadside-nesting cliff swallows, which exhibited selection for shorter wings and more maneuverability to avoid traffic (Brown and Brown 2013). In addition to impacting populations, could the ubiquity of roads be altering pollinator morphology or behavior (Taylor and Merriam 1995; Fahrig 2007), or selecting for a novel community of pollinators with particular morphologies and behaviors (Rebrina et al. 2022)?

Road impacts: habitat fragmentation or habitat corridor?

In addition to affecting reproduction and mortality rates, roadsides alter pollinator dispersal. Roadsides may connect pollinator metapopulations by providing habitat corridors parallel to roads. Alternatively, roads can fragment pollinator populations by limiting dispersal between habitats across roads. How do these opposing effects on dispersal balance out, and what influences that balance? Advances in fine-scale remote sensing, particularly harmonic radar, as well as the application of fluorescent powered dyes, may help address this question by enabling researchers to follow the paths of numerous individual insects over time (Osborne, Loxdale, and Woiwod 2002; Rhodes et al. 2022; Dániel-Ferreira et al. 2022). Comparing the extent to which pollinators travel alongside and across roads with their movements in the broader landscape can illuminate the influence of roads on pollinator dispersal and population connectivity.

As of yet, however, there is very little definitive evidence that roadsides serve as a habitat corridor for insects. Insect dispersal is difficult to study, and effective studies must compare dispersal rates of roadside insects with those away from roadsides (Villemey et al. 2018). A recent study showed that indeed, pollinators tended to move along vegetated roadsides more frequently than into adjacent habitat, suggesting a corridor effect (Dániel-Ferreira et al. 2022). This and other studies have also shown that improving roadside floral resources and habitat quality may reduce the frequency that butterflies leave the roadside and cross the road, suggesting that high-quality roadside habitat might promote corridor-like movement parallel to roads and reduce the proportion of pollinators killed by crossing roads (Ries, Debinski, and Wieland 2001; van Rossum et al. 2011; Skórka et al. 2013).

Ample evidence exists that roads can fragment species' populations, resulting in reduced gene flow, potential genetic bottlenecks, and threatened population viability for many imperiled species, including some invertebrates (Keller, Nentwig, and Largiader 2004; Shepard et al. 2008; Diffendorfer et al. 2020). Even mobile insect species that could easily cross roads may avoid doing so, resulting in an effective loss of gene flow (Holderegger and Di Giulio 2010). For example, researchers found that bumblebees avoided crossing roads even when adequate or better floral resources were present across the road, and when their favored plants were moved, they switched to plants on the same side of the road rather than cross it (Bhattacharya, Primack, and Gerwein 2002). Despite this, bumblebees that were captured and moved to flower patches across the road readily crossed it to return to their original patch, suggesting that tendencies toward site fidelity may work in concert with roads to fragment populations (Bhattacharya, Primack, and Gerwein 2002; Hopwood et al. 2010). This result is corroborated by a study which found substantially different bee and wasp communities on opposite sides of a large highway, suggesting again that bees were not dispersing across the road despite their physical capacity to cross it (Andersson et al. 2017). Butterflies, by contrast, show limited or variable **barrier effects**; mark-recapture studies suggest that roads partially restrict movement of some butterfly species, but not many others (Fry and Robson 1994; Askling and Bergman 2003; Munguira and Thomas 1992). This likely reflects that butterfly species vary from very local habitat specialists to long-distance migrants. However, the increasing prevalence of roads could alter these movement patterns, too; a landscape-scale of study of the bog fritillary butterfly, for example, found that individuals were more reluctant to cross habitat patch boundaries as fragmentation increased (Schtickzelle, Mennechez, and Baguette 2006).

Fragmentation by roads may reduce insect genetic diversity, especially for species with limited dispersal capacity (Tepedino 2016). Metapopulation research shows that limiting dispersal can threaten population viability even without removing habitat (Levins 1969; Templeton, Brazeal, and Neuwald 2011); roads can do both. Fortunately, most pollinators are capable of crossing even large roads at least sometimes (Jackson and Fahrig 2011).

Similar to collision mortality and roadside reproduction, far more evidence exists of roads' potential negative role in reducing dispersal than their positive role in facilitating it. To what extent this matches the actual balance of dispersal impacts is an important question. To address this, studies are needed that compare insect dispersal rates along roadsides with those in other habitats. In the meantime, limited available data suggest that native roadside plantings that connect with habitat on adjacent lands are most likely to benefit pollinators, reducing road collision mortality and fragmentation effects (Ries, Debinski, and Wieland 2001; Hopwood et al. 2015; Hopwood 2008).

Chemical costs of roadside life

Although roadsides provide floral resources, nest and oviposition sites, and host plants to pollinators, they also harbor high levels of toxins from vehicle emissions, exhaust fumes, tire and road wear, herbicide applications, and road salts. These toxins provoke concern that the roadside environment might harm or poison the pollinators that use it (Mitchell et al. 2020).

Vehicle emissions, exhaust, tire and road wear all release toxic heavy metals into the local environment. As expected, these toxins show a declining concentration gradient away from roads, with highest concentrations in the 0-50 m closest to the road (Van Bohemen and Van de Laak 2003). Most research on the impacts of vehicular toxins on insects occurred in the 1970's-90's, before the banning of lead from gasoline in 1996. In these decades, studies found that some insect families, including butterflies, had fewer individuals in areas of higher exhaust closer to roads, while others, such as hemipterans, increased in population size in proximity to roads, possibly due to lower predator density (Przybylski 1979; Muskett and Jones 1980). Higher concentrations of lead and other toxic heavy metals were incorporated into the tissues of butterfly larvae, beetles, true bugs, and bees closer to roads (Beyer and Moore 1980; Price, Rathcke, and Gentry 1974; Udevitz et al. 1980). One study found that lead in insects and other invertebrates decreased 64 % from 2 to 150 m from the road (Wade et al. 1980).

Decades after leaded gas was banned in most countries, lead concentrations in roadside plants and animals still exceed limits recommended by the World Health Organization, while new, potentially toxic elements have been recently introduced in vehicle emissions, including antimony from brake linings, platinum group elements from abraded catalytic converters, and manganese, which replaced lead as an anti-knock additive in gasoline (De Silva et al. 2021; WHO 2019). The impacts of pollutants on invertebrates depend largely on their bioavailability, and the bioavailability of most of these newer pollutants is unknown (De Silva et al. 2021). However, manganese contamination is now common along roadsides in soils and plants, often exceeds levels known to cause toxicity (Lytle, Smith, and McKinnon 1995), and has been shown to negatively affect honeybee foraging behavior by altering insect brain chemistry (Søvik et al. 2015). Pollinators including honeybees and bumblebees also may respond to heavy metals by reducing foraging time at contaminated flowers (Xun et al. 2018; Phillips, Bullock, Gaston, Hudson-Edwards, et al. 2021). Perhaps due to these avoidance behaviors, or to toxin-induced mortality, researchers have found a strong negative correlation between heavy metal concentrations in the environment (measured in pollen) and solitary bee diversity and abundance (Moroń et al. 2012).

Sodium poses an additional threat to roadside pollinators. In cold regions, salt is applied to reduce ice on roads, often in significant amounts (e.g.,300,000 tons per winter in one metropolitan area) (Sander et al. 2007). Running off into roadsides, **road salt** increases sodium concentration in soils and roadside plants, affecting pollinators (Mitchell et al. 2020). Road salt accumulates most in poorly-drained, clay soils and can result in high mortality of milkweeds and other nectar-providing forbs in those habitats (Haan, Hunter, and Hunter 2012; Malcolm 2018; Hintz and Relyea 2019). Where roads were salted in winter, monarchs that developed on roadside milkweed plants had significantly higher tissue sodium concentrations than monarchs that developed away from roads; as sodium is an essential insect micronutrient, this increased concentration appeared (up to a point) to actually benefit monarchs, causing increased muscle mass in males and larger eyes in females (Snell-Rood et al. 2014). Road salt runoff can lead to elevated salt concentrations in soil extending over 10 meters (33 feet) from the roadway, and much farther in waterways and streams (Findlay and Kelly 2011; Lundmark and Olofsson 2007). As with other pollutants, sodium concentrations are highest in plants closest to the road, and milkweed sodium may reach toxic levels for monarchs in <10% of plants (Mitchell et al. 2020).

Experimental manipulations show that egg-laying monarchs do not avoid plants with toxic levels of sodium (Mitchell et al. 2019). Road salt thus exemplifies another risky tradeoff of roadside use. Despite the potential fitness benefits (stronger flight, better vision) of sodium for monarchs, monarch larval survivorship was lower on sodium-rich roadside milkweed than on milkweed grown away from roads, suggesting that road salt, as with other pollutants, likely does more harm than good for roadsides pollinators (Mitchell et al. 2020).

In addition to sodium, heavy metals, and other contaminants, roadside pollinators also face chemical exposure from pesticides, most often herbicides, which are commonly applied to right-of-way zones to maintain visibility, discourage vegetative growth, and control noxious weeds and pests. Herbicide use can result in weed reduction, which may benefit pollinators by favoring native floral diversity (Ries, Debinski, and Wieland 2001; Valtonen, Jantunen, and Saarinen 2006); however, herbicides are often broadly applied and are sometimes misapplied, resulting in the killing of insect host plants at critical developmental periods, loss of floral resources, and direct death of pollinators (Hopwood et al. 2015; Pleasants and Oberhauser 2013). Herbicide use has been identified as a key threat to roadside monarch and milkweed populations in the western U.S. (Waterbury, Potter, and Svancara 2019). Although some studies have found that the benefits of reducing weeds through targeted herbicide use may outweigh negative impacts of herbicides on butterflies (Yahner 2004), most studies find that broad-spectrum herbicides increase pollinator mortality and can cause sublethal negative effects on foraging ability and disease resistance, and thus should be used sparingly (Larsen 2010; Prosser et al. 2016; Cullen et al. 2019). Both active and inert ingredients (e.g., adjuvants or surfactants) in common herbicides harm bees by damaging their gut microbiomes (Motta, Raymann, and Moran 2018), impairing their navigation abilities (Hahn et al. 2015), and reducing sperm counts and survival rates (Belsky and Joshi 2020), among other mechanisms (Mullin et al. 2016).

Whether herbicide application benefits pollinators (by reducing unpreferred weeds) or harms them (directly or by killing host plants and nectar-providing forbs) depends on when, where, and how herbicide is applied. Blanket herbicide application and herbicide application in peak flowering season, both common practices in roadside management, are likely to adversely impact pollinators, and tip roadside habitats toward becoming an **ecological trap**. Still, because roadsides are not cultivated for crops, they often still receive far less herbicide and pesticide input than surrounding agricultural land, making them a relatively safe option for pollinators in many areas. For example, a study showed that bumblebees preferred road margins over similar crop-facing margins by a factor of two (Hanley and Wilkins 2015). In some cases, agrochemical inputs may be more detrimental to pollinators than roadside herbicides and vehicle pollutants. In some parts of the U.S., roadside managers place "No Spray Zone" signs in roadways to protect valuable pollinator habitat that may be either native or planted.

Less lawn, longer pastures for pollinators

As with herbicides, mowing ROWs poses a major threat to roadside pollinators, yet may also provide them with benefits—in this case, by instilling regrowth and extending the bloom period of certain plants. First-instar monarch survival more than doubled on milkweed stems that regenerated after

mowing compared with undisturbed controls, for example, even though mowing reduced floral resource abundance for adult pollinators for 3-5 weeks (Haan and Landis 2020). Although mowing can promote beneficial regrowth in certain plants, it temporarily reduces floral resource availability and can change roadside vegetation composition, typically benefiting disturbance-prone exotics over native forbs (Phillips et al. 2019; Prevéy, Knochel, and Seastedt 2014). Most critically, mowing entire ROWs destroys pollinator habitat and larval host plants and can kill nesting adult pollinators, eggs, and larvae, presenting a significant—and unstudied—mortality risk to roadside pollinators. Mowing may also compound other negative effects; for example, one study found mowing ROWs increased butterfly collision mortality, likely by prompting dispersal across the road (Skórka et al. 2013).

As with herbicide application, timing and extent of mowing is critical: mowing entire ROWs during peak growing season can kill pollinator larvae and eggs and diminish essential floral resources. However, as discussed previously, vegetation immediately bordering well-trafficked roads may be toxic to pollinators due to accumulated pollutants and salts. Thus, regularly mowing a close-cropped **mow zone** of about 2 meters next to road pavement is generally recommended for the safety of both drivers and pollinators, while the rest of the right-of-way zone should be mown as infrequently as possible—at most once or twice a year, very early and late in the growing season (Hopwood et al. 2015). When mowing occurs, the use of arthropod-friendly mowing heads and flushing bars is recommended and can substantially reduce mortality of some insect groups, including butterflies (Humbert et al. 2010; Steidle et al. 2022).

Concluding remarks

As pollinator populations and undeveloped habitat decline, understanding the potential impacts of road ROW habitat on pollinator populations is increasingly important. Roads and roadsides present many risks to pollinating insects, and whether their potential benefits outweigh their harms likely depends on many factors, including herbicide use, mowing regime, traffic levels, and habitat quality (see Outstanding Questions). Under strategic management, many road ROWs have the potential to benefit pollinators. Locally, numbers of butterflies observed in roadside habitats appear to substantially exceed (by 10 to 30 times) numbers of butterflies killed by vehicles (Munguira and Thomas 1992; McKenna et al. 2001; Skórka et al. 2013), and although data comparing roadside mortality and birth rates is lacking, the increasing scarcity of quality pollinator habitat in many landscapes likely necessitates pollinators' reliance on roadsides.

With land-use change and habitat loss identified as the primary causes of global declines in bees and butterflies (Sánchez-Bayo and Wyckhuys 2019; Zattara and Aizen 2021) and over 50% of Earth's terrestrial surface now used by humans (Hooke, Martín-Duque, and Pedraza 2012), the provision of undeveloped land and floral resources in roadsides may be critical. Hearteningly, enhancing pollinator habitat along roadsides does not appear to increase per-individual road mortality of insects, although more research into this question is needed (Ries, Debinski, and Wieland 2001). However, the threats of collisions, pollutants, salts, mowing, and herbicide use still make roadsides a relatively poor choice for pollinator-focused restoration; where land is available that is not subject to more severe disturbance or pesticides, prioritizing the creation of pollinator habitat away from roads is recommended. In addition to

protected sites like parks, refuges, and reserves, powerline and pipeline right-of-way corridors, railway embankments, and buffer strips along waterways and streams represent good alternatives for cultivating pollinator habitat that may have fewer risks to pollinators than roadsides (Wojcik and Buchmann 2012; Villemey et al. 2018).

Evidence for the role of roads in restricting pollinator movement is mixed, and the effect is taxon-specific, with bumblebees and other pollinators with high site fidelity likely more impacted than wide-ranging species like some butterflies (Bhattacharya, Primack, and Gerwein 2002; Munguira and Thomas 1992). In general, roads do not present an absolute barrier to pollinator movement and gene flow as they do for some vertebrates, and models suggest roads' impacts on population genetics may arise more from collision mortality than from barrier effects of roads (Jackson and Fahrig 2011).

Most risks of roadside habitat are associated with roads themselves (e.g., collision with vehicles, pollutants) and thus decrease from higher to lower risk as organisms move further from roads. Thus, managers should prioritize cultivating pollinator habitat in wider road ROWs, and in parts of ROWs farthest from the road (never in medians) (Keilsohn, Narango, and Tallamy 2018). For most highways, a mow zone is recommended of 1.5-3 meters, as plants growing this close to the road are likely to be toxic for pollinators (De Silva et al. 2021; Hintz and Relyea 2019; Moroń et al. 2012). Mow zones are especially important for pollinators on roads with higher traffic and those that are de-iced in winter, since traffic increases deposition of toxins and de-icing salt can result in toxic sodium concentrations in roadside plants (Lytle, Smith, and McKinnon 1995; Mitchell et al. 2020). Additionally, providing diverse floral resources in the edges of road ROWs farthest from the road may draw pollinators away from the road, toward less toxic plants (Xun et al. 2018; Phillips, Bullock, Gaston, Hudson-Edwards, et al. 2021), potentially benefiting pollinator health and reducing collision mortality.

Mowing and herbicides are frequently employed to control weeds, maintain visibility, and prevent woody growth in road ROWs. Mowing typically reduces floral resource availability, may disturb or kill nesting and developing pollinators, and can benefit disturbance-prone species (often invasive species, but also native plants like milkweed) (Phillips et al. 2019). Outside of mow zones immediately adjacent to roadways, which should be kept short, road ROWs should generally be mown as little as possible—at most once or twice a year, early and/or late in the growing season, when mowing is less likely to impact flowers or developing pollinators. Even when no flowers are present, mowing can destroy overwintering pollinator eggs and larvae, so leaving grass and forbs standing—and, when mowing is necessary, using flushing bars and mowing heads designed to reduce arthropod mortality (such as the Eco 1200 mowing head from MULAG)—is recommended (Humbert et al. 2010; Steidle et al. 2022). In specific cases, well-timed mowing (typically in mid-July) may benefit milkweed, monarchs, or other target species, but timing mowing effectively requires location-specific and species-specific knowledge of phenology (Phillips et al. 2020). Herbicide use, on the other hand, is almost always detrimental to pollinators, except when it is applied very selectively to control invasive species and foster higher native plant diversity (Hopwood et al. 2015). Weeds should thus be **spot-treated** early or very late in the growing

season on non-windy days, to minimize detrimental impacts to pollinators and native forbs (Hopwood et al. 2015). Insecticide use should generally be avoided in roadside management.

Even under best management practices, road ROWs vary considerably in their safety and potential benefit for pollinators. Based on available evidence, pollinator habitat restoration should be prioritized along roads with less traffic, less intensive management regimes (mowing/grading/herbicides), wider ROWs, and adjacency with native habitat (McKenna et al. 2001; Skórka et al. 2013; Villemey et al. 2018).

Further research opportunities and outstanding questions

Further research is required to identify thresholds of traffic, pollutants, and disturbance from maintenance activities that create overall negative effects for pollinator populations, and these responses are likely to be species-specific even within one group of insects based upon morphology, life history, and dispersal behavior. Studies that compare pollinator birth and development rates in roadside habitat with mortality rates are needed. Additionally, mark-recapture studies that compare pollinator dispersal along roadsides with other habitats are required to test the common but weakly supported notion that roads serve as pollinator habitat corridors. While roadside pollinator ecology has rapidly expanded in recent years, many questions remain unanswered (see below), and new studies must assess both roadside pollinator reproduction and mortality if they are to successfully evaluate the impacts of roadside restoration on pollinators.

- Across various pollinator taxa, how does pollinator fecundity along roadsides compare with pollinator mortality caused by collisions, toxins, mowing, and other road-related impacts? Do changes in management practices and restoration plantings that might attract pollinators to roadsides increase or decrease pollinator mortality?

- Under what circumstances and for which taxa do roadside habitats benefit pollinator populations? What factors are most important in tipping the scales?

- Can well-timed mowing of roadsides promote regrowth and benefit other pollinators, as it does for monarchs? If so, what is the ideal time to mow in the growing season to produce such benefits?

- How far from a typical road edge do the negative effects of heavy metals and pollutants outweigh the benefits of habitat? In other words, how wide should the ideal mow zone be for pollinators?

- How do traffic levels and distance-from-road (ROW width) interplay to determine toxicity? For example, are narrow ROWs on less trafficked roads better suited for pollinators than wider ROWs on more trafficked roads, or vice versa?

- Can roadsides serve as a habitat corridor for pollinators? How do roads influence pollinator movements? Could roads link habitat fragments in an increasingly patchy landscape, or are they more likely to exacerbate habitat fragmentation?

2. Project Overview & Introduction

Insect pollinators serve a critical role in our natural and agricultural ecosystems, and their global declines have inspired increasing concern in recent years. Pollinators face many threats, including habitat loss, pesticides, landscape fragmentation, agricultural intensification, and invasive species. In recent years, much attention has turned to roadside right-of-way lands (ROWs) for their potential value in supporting pollinator populations. If managed effectively, these undeveloped lands can support a diverse array of flowering plants, providing food and potential nesting sites for pollinators.

Most insect pollinator species are understudied, with little known about their populations, habitat preferences, or distributions. This research project aims to begin filling this data gap for roadsides of Idaho's Snake River Plain and to provide useful information to the Idaho Transportation Department (ITD) regarding the relative diversity and abundance of pollinators in their roadsides. Additionally, through this project, we have identified the roadside locations of milkweed (*Asclepias* spp.), host for the imperiled monarch butterfly (*Danaus plexippus*). Finally, we offer recommendations for management strategies to promote the health of pollinator populations in Idaho's ROWs and provide data to help ITD prioritize areas for pollinator-friendly management practices and restoration plantings within their highway system.

Our research project consists of two main parts: a rapid assessment of roadside milkweed and monarch butterfly populations that spanned southern Idaho from Nampa to Island Park; and a series of surveys of butterflies, flowering plants, and bees at roadside transects in southeastern Idaho. Because these two parts have separate foci, protocols, and datasets, we have given them separate sections in each part of this report, including in our overview and introduction.

2.1 Introduction: Milkweed and Monarchs

The monarch (*Danaus plexippus*), an emblematic butterfly known for its charismatic coloration and remarkable long-distance migrations, is the official State Insect of Idaho. Its larval host plant, milkweed (*Asclepias spp.),* is a common roadside plant that grows abundantly in ROWs in Idaho's Snake River Plain. Because of this connection, roadsides are of special importance to the conservation of the monarch in Idaho.

While most species of insect pollinators have unknown population sizes or population trends, the monarch butterfly is a well-studied exception. Monarchs have two main migratory populations in North America, one that breeds east of the Rockies and winters in Mexico (eastern), and the other that breeds west of the Rockies and winters in coastal California (western). Thanks to work by the Xerces Society and volunteer groups, population surveys for western monarch butterflies have been conducted annually on their wintering grounds for the past 25 years (Xerces Society 2023). These and other data reveal a decline in the western U.S. monarch population by over 90 percent since the 1980s (Xerces Society 2023). The winters of 2018 & 2019 saw especially drastic declines in the annual overwintering western

monarch population, prompting the monarch butterfly to be petitioned for listing under the U.S. Endangered Species Act of 1973 (ESA).

While this petition decision is still pending and the western monarch population rebounded in 2021 and 2022, both the eastern and western monarch populations are still at a fraction of their historic levels, and the species is vulnerable to extinction (Pelton et al. 2019; Thogmartin et al. 2017; Semmens et al. 2016). The monarch was listed in 2022 as endangered by the International Union for the Conservation of Nature (IUCN 2022). It is also listed as a Species of Greatest Conservation Need by the Idaho Department of Fish and Game.

In March 2020, the U.S. Fish and Wildlife Service (FWS) issued its "Nationwide Candidate Conservation Agreement on Energy and Transportation Lands" which is "[a]n integrated Candidate Conservation Agreement with Assurances (CCAA) and Candidate Conservation Agreement (CCA)" supporting proactive conservation measures for the species. Lands owned by the Idaho Transportation Department (ITD) can be enrolled under the CCAA and CCA until the date of the monarch butterfly's listing as threatened or endangered under the ESA is signed by the FWS, if that occurs. If the monarch butterfly is listed under the ESA, there are provisions in the CCAA and CCA for the potential enrollment of additional lands after the FWS listing decision has been made.

Many factors have contributed to the monarch's decline, but habitat loss and pesticide use are considered the most detrimental (Pelton et al. 2019; Malcolm 2018; Thogmartin et al. 2017). Threats identified on energy and transportation lands include habitat loss resulting from mowing, herbicide use and land conversion (Cariveau et al. 2019). Transportation ROWs are present throughout the western U.S. landscape, occupying every type of land use and habitat, from mountains to the lower elevation deserts. While there are many types of ROWs in the western U.S., transportation and energy/utility ROWs are among the largest and most extensive networks (Villemey et al. 2018). Preserving and protecting existing habitat makes a difference and can increase habitat connectivity across the landscape; any amount of habitat ITD can preserve, enhance, or restore could help the monarch butterfly and other pollinators.

Immediate action is needed to avoid the loss of the western migratory monarch butterfly population and to conserve waypoints that provide nectar and milkweed host plants along its migration routes to and from California. In addition to protecting California overwintering sites, it is essential to protect and restore breeding and migration habitats outside of California, especially in areas that are suitable for monarchs or those that are lacking habitat. Researchers have identified the Snake River Plain, Columbia Plateau, and riparian areas as key locations for conservation and restoration actions for monarch butterflies outside of California wintering and migration habitat (Pelton et al. 2019).

ITD manages approximately 13,898 highway lane miles across the state, comprising an estimated total of 82,000 acres of ROW land. Much of this is in the Snake River Plain. Idaho Department of Fish and Game has joined other western states to conserve the western Monarch population (WAFWA 2019) and their initial surveys indicated that monarch butterflies and milkweed plants are present in various parts

of Idaho. Prior to this project, however, very little had been surveyed to indicate the acreage and locations in ITD ROWs that support robust pollinator populations or monarch breeding habitat.

Using existing milkweed and monarch observations in concert with climate, topography and soil data, researchers at the Idaho Department of Fish and Game (IDFG) and the University of Idaho developed statewide models of the current and potential future distributions of milkweed and monarchs in Idaho (Svancara, Abatzoglou, and Waterbury 2019). Their models highlight the importance of elevation, distance to water, soil depth, mean diurnal temperature range, and precipitation of the wettest month for predicting showy milkweed distribution; monarch distribution naturally followed the distribution of its host plant (Svancara, Abatzoglou, and Waterbury 2019). These models accurately predicted the distributions of showy milkweed (0.899 AUC—area under receiver operating characteristic curve) and monarchs (0.929 AUC) based on available data (Svancara, Abatzoglou, and Waterbury 2019).

By surveying nearly 3,000 km (over 1,800 miles) of ROWs for milkweed and using these data for comparison, we evaluated whether the showy milkweed model published in Svancara *et al.* 2019 (here after, "Svancara's model") would also accurately predict showy milkweed distribution in roadside ROWs. Because of the modified and disturbed character of roadsides, which could alter milkweed distributions from those of the statewide landscape, we hypothesized that Svancara's model would not accurately predict roadside milkweed distribution. Specifically, we predicted that Svancara's model would be an inadequate predictor of ITD ROW monarch and milkweed habitat in the Snake River Plain because ROW lands are often highly modified during construction and reconstruction and because their subsequent management (mowing, spraying, etc.) may complicate predictions based only on climate, topography, and soil depth.

In addition to testing the accuracy of Svancara's model in predicting milkweed distribution in Idaho roadsides, we located areas of high roadside milkweed density that can provide critical monarch habitat if managed effectively. Based on these data, we identified milkweed hotspot areas to enroll as potential adopted conservation acreage within an ITD Candidate Conservation Agreement with Assurances for the monarch butterfly. We hope that our surveys will help ITD fulfill their objectives to conserve and protect Idaho's State Insect and the milkweed on which it depends.

2.2 Introduction: Butterflies, Bees, and Floral Resources

The diversity and abundance of pollinators varies greatly across roadsides; some ROWs support high numbers of bees and butterflies, while others are largely depauperate (Du Clos et al. 2021). Many factors can drive these differences, including spatial variation in plant communities and floral resources, the availability of bee nesting sites and butterfly host plants, water and mineral resources, patterns of disturbance, ROW management activities like mowing and herbicide application, traffic impacts, toxins from roadways, and connectivity with habitat on adjacent lands and existing pollinator populations (Hopwood et al. 2015; Phillips et al. 2020; Du Clos et al. 2021; Wojcik and Buchmann 2012). The most important factors in determining roadside pollinator richness and abundance likely vary by region (Villemey et al. 2018).

In the arid Great Basin, the ecoregion of our study, water is an important limiting factor that drives the distribution of plant communities and the various animals that depend on them, including pollinators (Bailey et al. 2004). Areas of higher water availability also typically have higher rates of photosynthesis and plant growth; they show up as greener areas in satellite imagery. These greener, more productive areas for plants have higher values of the normal difference vegetation index, or NDVI, a remotely sensed measure of landscape greenness (Xu et al. 2012). Pollinator communities respond to plant communities, and thus may be associated with differences in NDVI (Basu et al. 2016; Leong and Roderick 2015).

We hypothesized that ROWs with higher values of maximum NDVI (i.e., the site's maximum NDVI value through the year) would be positively associated with higher butterfly species richness, as found in other studies (Seto et al. 2004; Bailey et al. 2004). In the Great Basin, locations with higher maximum NDVI were correlated with both higher butterfly species richness and higher water availability, a limiting factor in arid Great Basin ecosystems (Bailey et al. 2004; Seto et al. 2004). In natural habitats in California, NDVI was positively correlated with bee abundance, although not significantly associated with bee richness (Leong and Roderick 2015), while in heterogeneous agricultural landscapes in India, NDVI was a strong positive driver of bee species diversity (Basu et al. 2016). We thus predicted that roadside transects in areas with higher maximum NDVI would support higher pollinator richness and/or abundance, and that NDVI could be used to help identify patterns of pollinator diversity across Idaho ROWs.

In addition to varying in NDVI, the ROW network of southern Idaho varies in its highway type. ITD manages three main classes of highways: large, high-traffic, multi-lane *Interstates* which connect across multiple states; medium-sized, medium-traffic two- to four-lane *U.S. highways*, and small two-lane *State highways* with limited traffic and lower speeds. These highway types vary in their size (both pavement and ROW width), traffic levels, and management, with Interstates generally having the largest overall size, highest traffic levels, and largest managed area (i.e., most area mowed and sprayed), and State highways the least. As with differences in plant growth and greenness measured by NDVI, differences in highway size, traffic volume, and ROW management area related to highway type can affect pollinator populations, influencing their patterns of richness and abundance across the ROW network (Martin et al. 2018; Fitch and Vaidya 2021; Phillips et al. 2019).

We hypothesized that highway type would be associated with differences in pollinator richness and abundance, with larger, more highly trafficked highways having lower bee and butterfly richness and abundance than smaller highways with lower traffic levels. Traffic can directly cause pollinator mortality through vehicle collisions (Keilsohn, Narango, and Tallamy 2018; Baxter-Gilbert et al. 2015) and may disturb pollinators through dust, noise, and turbulence (Phillips, Bullock, Gaston, Hudson-Edwards, et al. 2021; Bunkley et al. 2017); it can also influence the nutritional quality of roadside plants (Mitchell et al. 2020) and increase rates of toxins in roadside soil, plants, and nectar, impacting pollinators (Bernardino et al. 2019; Xun et al. 2018). Larger highways may also present barriers to the movement of many pollinator species, separating their populations to some extent (Andersson et al. 2017; Dániel-Ferreira et al. 2022); the more extensive management areas of larger highways may also disturb pollinators and

encourage non-native, disturbance-tolerant plant communities in favor of the native forbs preferred by many pollinator species (Hopwood, Black, and Fleury 2015; Prevéy, Knochel, and Seastedt 2014; Steidle et al. 2022). We thus hypothesized that, after accounting for NDVI, Interstate transects would have the smallest and least diverse bee and butterfly communities, while State highway transects would have the most abundant and diverse communities.

Finally, floral resources, which we surveyed along with bees and butterflies at our transects, are another important driver of pollinator habitat quality. Because bees and most butterflies rely entirely on flowers for energy as adults, and because different pollinator species prefer to feed on different floral species (Cane 2021; Fründ, Linsenmair, and Blüthgen 2010), we predicted that higher floral richness and abundance would be associated with higher richness and abundance of bees and butterflies.

Using two years of transect surveys at 63 sites located in southeastern Idaho, we assessed how bee and butterfly species richness and abundance varied with respect to floral species richness, floral abundance, highway class (State, U.S., or Interstate), and category of maximum annual NDVI (low, medium, or high). By equally distributing our transects among highway classes, we could potentially inform the allocation of pollinator conservation resources by ITD and other transportation departments between their highway systems. Additionally, stratifying our transect locations by NDVI category ensured that a variety of roadside habitat types and vegetation patterns were surveyed, and enabled us to evaluate the utility of this widely accessible remotely sensed dataset in predicting roadside pollinator richness and abundance. If bee and/or butterfly richness varied significantly with NDVI, for example, NDVI could be a useful management tool to identify habitat of high pollinator potential and direct management priorities across Idaho's network of ROWs. Finally, comparing our data on pollinators with local floral resources helped identify species of flowering plants favored by pollinators in southern Idaho ROWs and aided in assessing the importance of floral resources in understanding patterns in roadside pollinator richness and abundance.

3. Methodology

3.1 Milkweed and Monarch Rapid Assessment

This assessment surveyed and marked *Asclepias* spp*.* (milkweed) locations occurring in right-of-way (ROW) zones on both sides of 1465 km (910 miles) of highways across southern Idaho, for a total of 2,930 survey km (1,820 survey miles) (shown in **Error! Reference source not found.**). ITD staff selected the highway sections to survey. These included the 555-km (345-mile) stretch of interstate highways I-15, I-86, and I-84 from Dubois to Nampa, ID, and a series of shorter highway sections totaling 910 km (565 miles), as follows:

    a.   US 20   Island Park to Idaho Falls
    b.   US 26   Blackfoot to Arco
    c.   US 91   Blackfoot to Idaho Falls
    d.   SH 33   Sage Junction to Rexburg
    e.   SH 48   Roberts to Lorenzo
    f.   SH 75   Shoshone to Stanton Crossing
    g.   US 26   Bliss to Carey
    h.   US 93   Jerome to Shoshone
    i.   SH 67   Mountain Home to Grand View
    j.   SH-48   Ririe to Roberts
    k.   SH-39   Blackfoot to American Falls
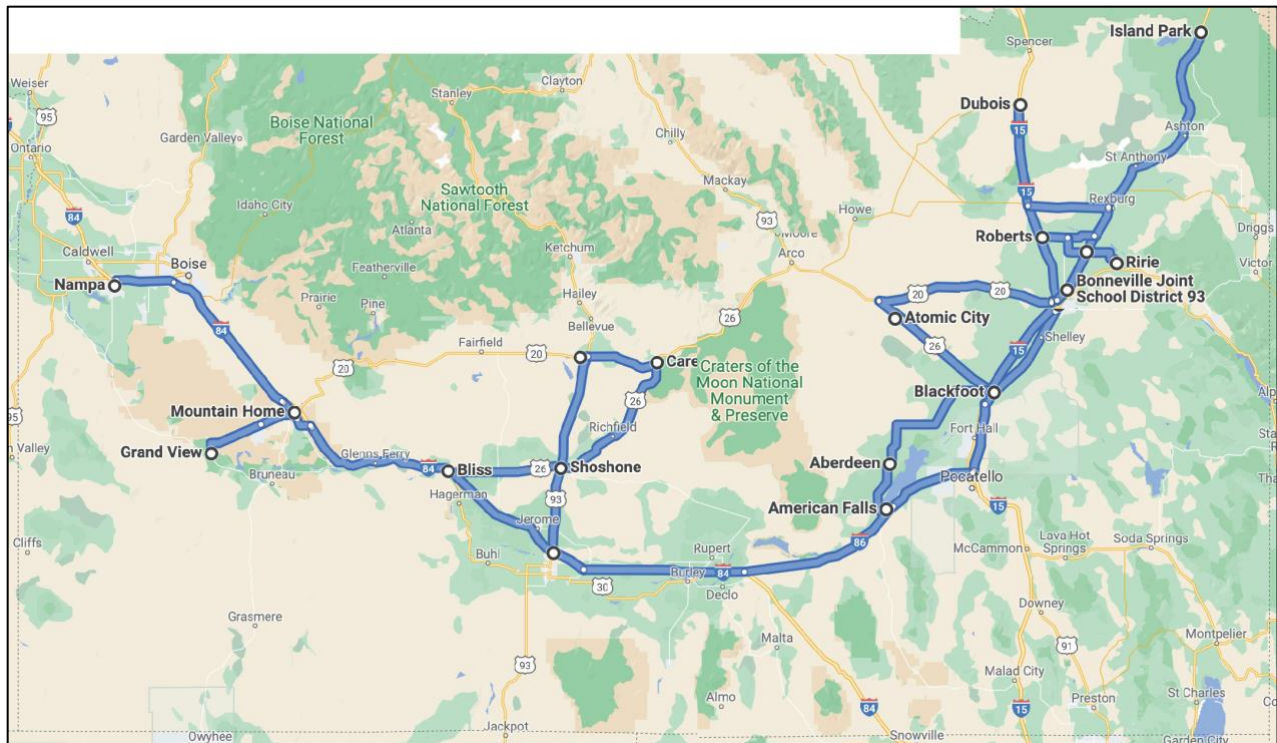
Figure 1 - Highway sections surveyed in the milkweed and monarch rapid assessment. Milkweed plants in ROWs along both sides of these highways were recorded and mapped. All highway sections were surveyed in both directions to record milkweed locations on both sides of the roadway.

This assessment was completed by two researchers July 1-13, 2021, during peak *Asclepias speciosa* (showy milkweed) flowering, following the Idaho Dept. of Fish and Game milkweed rapid assessment protocol (Waterbury, Potter, and Svancara 2019). Only one species of milkweed, *A. speciosa* (showy milkweed), was observed across all ROWs surveyed; we did not observe any of the other milkweed species found in the region (e.g., *A. incarnata*—swamp milkweed, *A. fascicularis*—narrowleaf milkweed, or *A. cryptoceras*—pallid milkweed). Surveys were completed while the temperature was at least 21°C (70°F) to increase the chance of observing active adult monarchs. One researcher drove at 80 to 110 km/hr (50 to 68 mph) while the other searched for milkweed in the ROW. Because of milkweed's distinctive form (stalks of long, arrow-shaped leaves), the plant could be effectively distinguished and identified at highway speeds, as noted by Waterbury et al. (2019) and tested by the research team.

The location of small clusters of milkweed plants with fewer than 50 stems together in one patch was marked with a GPS but plants were not individually counted. Locations of larger patches of greater than 50 stems were marked with a GPS and further measured, after stopping the survey vehicle along the road shoulder. Measurements including an estimated count of the number of milkweed stems, size and density of the patch (taken by measuring its length and width), physiognomic category of ROW and adjacent land (e.g. grassland, steppe, etc.), terrain type (flat, ditch, slope), distance from the patch to the edge of pavement, presence or absence of a canal adjacent to the ROW, and estimates of total non-milkweed floral abundance and species richness within 2 m (6.6 ft.) of the edge of the patch. Each

milkweed patch was assigned a location number in the Garmin eTrex GPS and additional information on patches over 50 stems was recorded in association with this location number.

While stopped at patches, one researcher spent 2 minutes searching patches exceeding 50 milkweed stems for monarch adults, caterpillars, and/or eggs on and around the plants. Monarch sightings were recorded with number and life stage. In case of frequent large milkweed patches in one section of highway ROW, milkweed patches over 50 stems were assessed only once in each 1.6 km (1 mile) of ROW in each direction; all patch locations were marked using a GPS. Milkweed stems separated by a gap of 20 m (65 ft.) or more were considered to be separate patches.

To select "hotspot zones" of high milkweed abundance for suggested inclusion as habitat conservation zones ("adopted acres") in an ITD Candidate Conservation Agreement with Assurances (CCAA) for the monarch butterfly, we used two methods. First, we calculated milkweed density by number of patches per mile using the Point Density tool in ArcGIS Pro to identify highway sections with patch densities of >5 and >10 patches per mile. Second, we identified statistically significant spatial clusters of milkweed patches using the Getis-Ord Gi* statistic (ArcGIS Pro Hot Spot Analysis tool), which produces z-values and p-values that identify spatial clustering at various confidence levels (ESRI 2021). The milkweed hotspot zones identified through these two methods are shown in our Results section.

Finally, to test the accuracy of the statewide showy milkweed model published in Svancara *et al.* 2019 ("Svancara's model") for predicting roadside milkweed populations on the Snake River Plain, we assigned each of our milkweed patch locations a suitability value by overlaying our milkweed data on the milkweed suitability map produced by Svancara's model. This allowed us to assess how well Svancara's model predicted the locations of the milkweed patches we observed in ROWs in our rapid assessment; higher average suitability values would indicate a more accurate model.

Using GIS layers generously provided by Dr. Leona Svancara, we also assessed the distribution of values of our roadside milkweed patches for four of the five factors identified as most influential in Svancara's model—soil depth, distance to perennial water (including all streams and perennial canals longer than one mile, as sourced from the National Hydrography Dataset, at https://viewer.nationalmap.gov/), precipitation of wettest month, and mean diurnal temperature range. We did not assess the fifth factor, elevation, because of the relatively limited elevational variation of the Snake River Plain.

3.2 Stratified Transect Surveys

3.2.1 Site Selection and Stratification

To best assess how and where pollinator communities are using ROWs, we selected 63 sites at which to conduct butterfly, bee, and floral resource roadside transects in SE Idaho.

We selected a study area for our sites that included a 175 km (110 mile) stretch of interstate across a 175 m (575 ft.) elevation gradient, with a similar number of miles of U.S. and State Highways within a

relatively compact area, which helped minimize driving time and allowed more sites to be surveyed. This study area ranged between Rexburg and Ririe to the north and east and near Roy and Raft River to the south and east, respectively (map in Figure 2). An early finding of our research was that SE Idaho really loves its R's.

Within our study area, we selected sites by organizing our study area into categories (stratifying) based on two variables, highway class and NDVI (normal difference vegetation index, a remotely sensed measure of greenness) (Seto et al. 2004), and randomly selecting sites within each of these categories. This stratified random selection process helped ensure that our survey sites included a variety of different habitat types and levels of aridity and allowed us to compare different highway types (state, U.S., and interstate) and NDVI categories (low, medium, and high) in a balanced framework, giving optimal statistical strength. Comparing different highway classes in this way enabled us to assess whether some highway types are associated with higher pollinator abundance or diversity than others, which could inform pollinator habitat enhancement funding allocations among highway systems.

Although there is considerable variation in traffic levels within highway classes (and some overlap between classes), State highways generally have the lowest traffic and the narrowest roadways and ROWs, while Interstate highways have the most traffic and widest roadways and ROWs (Nikolaus Sterbentz pers. comm.). Averaging daily traffic volume based on a subset of ITD Automatic Traffic Recorders in our study area, we found that State Highways averaged 1,034 vehicles daily (only one counter available), U.S. highways averaged 9,659 vehicles daily and Interstate highways averaged 25,568 vehicles daily (average of three counters for each highway class) ([ITD Road Data](#)). Our on-the-ground estimated measurements of ROW width during our transect surveys revealed similar average ROW widths between Interstate and U.S. highways (19.7 m/65 ft and 19.0/62 ft, respectively) and considerably smaller average ROW widths along State highways (11 m/36 ft.), with substantial variation in ROW width within each highway class.

Stratifying by different NDVI categories enabled a comparison of how peak greenness, which is associated with plant growth and soil moisture, related to pollinator abundance and diversity (Raynolds, Walker, and Maier 2006; Chen et al. 2014). If we found that NDVI was associated with differences in pollinator communities, this may help inform future use of satellite imagery to predict roadside habitat quality for pollinators.

For transect site selection, a random sample stratified across NDVI and highway classes was created through a multi-step process, as summarized in the following. First, using Google Earth Engine, we pulled Sentinel-2 MultiSpectral Instrument Level-1C data and filtered it from June 1, 2020 to August 15, 2020 (the season of peak plant growth and pollinator activity from the most recent year). Next, we masked for clouds (i.e., removed areas covered by clouds in each image) and calculated NDVI for each cloud-masked image in the collection. We then calculated the maximum value of NDVI for these data (i.e., the highest NDVI value reached by each point across the 2020 pollinator survey season). We used the maximum NDVI value in order to assess peak greenness, which we predicted would indicate high

plant productivity at some point during the months of high pollinator activity. We exported this result from Google Earth Engine at a 10 m (33 ft.) resolution and imported it into ArcGIS Pro.

In ArcGIS Pro, we imported road median shapefiles for the Idaho State Highway System, sourced from the ITD database, and separated out each road type (U.S. Hwy, State Hwy, and Interstate) using the *Route ID*—a metadata field which specified the highway type of each road within the ITD database—by applying the *Select Layer by Attribute* and *Contains within* functions (ESRI 2021). Then, we created a polygon buffer around the median road lines according to road class: 41 m (135 ft.) on either side of the median for Interstates, 24 m (80 ft.) for U.S. highways, and 15 m (50 ft.) for State highways. These numbers were derived from recommendations by the ITD GIS team, notes from the Federal Highway Administration and ITD Road Design Manual (see Appendix D1), and direct measurements of right-of-way width using ArcGIS. For the interstate buffer, initial recommendations from ITD were to use 61 m (200 ft.) buffers, but we found that this size of buffer was frequently catching irrigated agricultural fields outside the ROW, so a new buffer width was calculated: measurements of Interstate ROW width were taken from 20 random locations in ArcGIS, then averaged (41.5 m or 136 ft.) and rounded to the nearest 5 ft. (135 ft.).

Next, continuing in ArcGIS Pro, we created a second set of buffer polygons based on the *Lane Width* field multiplied by two to create equal buffers the width of the lane for all highways. This action created a polygon of the roads' pavement area. Using symmetrical difference analysis, we excised the second set of buffer polygons (paved portion of the road) from the first set (road area plus right-of-way) by clipping out where the two buffers overlap. By creating a buffer from this excision, we excluded pavement from the sample area, ensuring that pavement does not skew the NDVI distribution and is not included as potential area for the placement of stratified sample transects.

In R (R Core Team 2022), we clipped the 10 m (33 ft.) NDVI Sentinel data processed in Google Earth Engine to the final excised buffer layer produced in ArcGIS Pro. Then, we created a random sample of 10,000 georeferenced points within the clipped buffer layer in our study area, with each point having a value from the 10 m NDVI layer. We stratified NDVI only within the clipped buffer layer to analyze roadsides specifically, rather than the general landscape. Because unique patterns of disturbance, maintenance activities, and pollution occur in roadsides, their plant and pollinator communities are not likely to reflect the general trend of the landscape. Thus, a stratification of NDVI across the landscape would not reflect the distribution of NDVI in the roadsides themselves, which was our interest.

After creating the random sample of points, we aggregated the 10 m (33 ft.) resolution data to 100 m (328 ft.) resolution Sentinel data, creating 100 m grid cells from the average of all 10 m cells within the 100 m cell. Based on these aggregated values, we created 3 equal interval bins of NDVI values. Three intervals of NDVI gives us robust numbers in each category (NDVI x road class), even with less than 100 transects; using equal intervals (as opposed to quartiles, for example) ensures that we assess some greener (higher NDVI) sites, which tend to be relatively uncommon in roadsides. The exact NDVI values of each of the three bins and the nomenclature for our nine transect categories are given in Table 1.

Table 1 - Transect categories (white boxes) by NDVI class (rows) and highway class (columns). NDVI classes determined by equal interval; ranges of exact NDVI values at the 100 m resolution given in the leftmost column. Each transect category contains 7 transects in our design. For example, SH.1.3 is in the State Highway, low (1) NDVI category, and is the 3rd randomly selected transect site out of 7 in this category.

100 m Resolution NDVI Value Range	NDVI Class	State Highway	U.S. Highway	Interstate Highway
0.15 – 0.35	Low (1)	SH.1.	US.1.	IN.1.
0.351 – 0.58	Medium (2)	SH.2.	US.2.	IN.2.
0.581 – 0.84	High (3)	SH.3.	US.3.	IN.3.

Once the data were binned into 3 equal intervals, we classified the 100 m resolution NDVI raster into the bins (low, med, and high). We then extracted the new binned raster to the 10,000 random points distributed across the highway system ROWs of our study area (roughly between Rexburg, Ririe, Roy, and Raft River, ID, as previously defined), each point now reading the raster beneath and adding that raster information to the vector data. By assigning the 100 m resolution NDVI values to the 10 m resolution points, we ensured that the points themselves fell within the 10 m zone, and thus were within the ROW, while the NDVI associated with them was derived from the 100 m average, thereby calculating greenness at the scale of our 100 m transects rather than at the 10 m scale. This process helped us avoid placing high-NDVI transects at, say, a tiny patch of greenery or tree in an otherwise dry and brown landscape, and vice versa.

Continuing in R, we then created a stratified random sample by taking an equal number of sample points from each NDVI bin (low, med, high) and each road class (State, U.S., Interstate). We took 20 random sample points from each of 9 (3x3) unique categories, totaling 180 points, and exported our sample points to a shapefile with the Viewer SRS background map ESG:8826 – NAD83 / IDTM83 for compatibility with Street Smart (Hepp 2018).

We used Street Smart Online (streetsmart.cyclomedia.com) by CycloMedia to digitally "ground-truth" points by double-checking whether our points fell within the right-of-way or on pavement (Hepp 2018). Street Smart acts similarly to Google StreetView, allowing a close-up, on-the-ground view on points as well as a satellite view. Using Street Smart, we checked each of the 180 samples individually, marking any points that were outside the right-of-way on private property, directly in front of a building, within 100 m (328 ft.) of another transect point (in this case, one point was randomly chosen to be removed), in the road median, or in a ROW with more than 10% pavement or trees, which could block visibility in pollinator surveys. We recorded rejected points with their cause for rejection and captured screenshots of suitable points in Street Smart for reference.

Returning to R, we trimmed the 180 samples to exclude points rejected in the Street Smart screening process. After this trimming, at least 9 samples remained in each of the 9 highway class x NDVI categories; thus, we trimmed the point sample randomly down to 9 samples in each category. In this

set, we assigned a random ordinal value to the samples within each category (1 through 9). Then, back in ArcGIS, we created a field value in the sample point metadata combining the road class (IN, US, or SH), the NDVI bin (1, 2, or 3, corresponding to low, medium, or high), and this random ordinal value. This gave us our transect identifiers; for example: SH.1.3 represented our 3rd transect along a State highway with class 1 (low) NDVI. We then exported our 81 samples (9 in each of 9 categories) to Keyhole Markup Language (.kmz) for viewing in Google Earth and in the field, in the Gaia GPS app and on our Garmin eTrex GPS.

Once in the field, we found that a few of our 81 sites—up to two in each category—were not suitable for surveying due to roadside construction, unsafe access, or intersecting streets. Thus, we eliminated 2 transects from each of our 9 categories to end up with 63 random-stratified transects (7 in each highway class x NDVI category) to survey in our research project, as shown in Figure 2 (satellite map in Appendix D2). In 2022, one transect site surveyed in 2021 (IN.3.7) was no longer accessible due to a new road construction project; thus, we replaced it with one of the other 9 sites from that category.
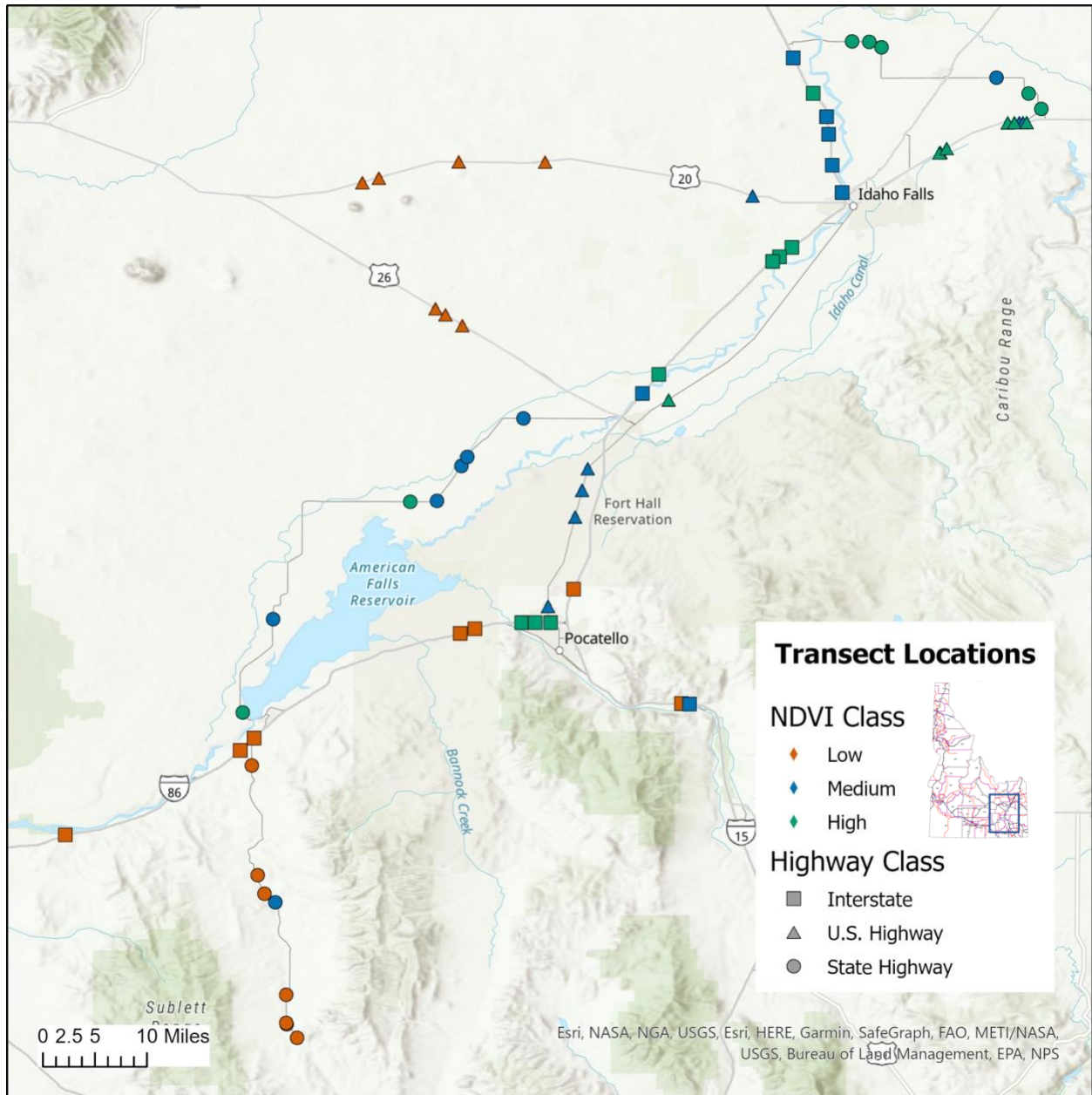
Figure 2 - **The locations of the study's 63 stratified random transects in SE Idaho, showing equal distribution across three NDVI classes (colors) and three highway classes (shapes). Although points were selected randomly, stratification by NDVI and highway class resulted in some clustering of transects in the same categories due to the NDVI characteristics of the ROW network. Map is centered approximately on Blackfoot, ID.**

3.2.2 Transect Surveys—Floral Resources

The 63 transect sites described in the former section were surveyed for floral resources in early June and in late June to early July 2021 and 2022 (four replicates). A selection of the transects (18 sites) were identified as containing late-season blooming plants (mostly *Chrysothamnus viscidiflorus* (yellow rabbitbrush), *Ericameria nauseosa* (rubber rabbitbrush), and *Helianthus annuus* (sunflower)) by researchers in July 2021 and by Cathy Ford, ITD Roadside Program Manager, in early August 2021. These 18 sites were surveyed in mid-August 2021 and 2022 to assess late-season floral resources, butterflies, and use by migrating monarchs (two additional replicates, using the same methodology). This selection allowed us to investigate late-season floral resources and butterflies without spending time at sites that were by August entirely dead, dry, and/or lacking in flowers, as were the majority of our transects by late summer. Because the subset of August-surveyed transects was not randomly selected, we did not include these survey data in our analyses or models of pollinator abundance and diversity (Section 4.3 Transect Surveys—Butterflies). These data are included, however, in our summaries of floral resources (Section 4.2 Transect Surveys—Floral Resources) and the plant and butterfly species lists in the appendices.

Using stakes and flags, we established 100 m (328 ft.) transects parallel to the road and fence, starting at the plotted point and walking south/west parallel to road. If the road was more north/south, we surveyed to the south, and if the road was more east/west, we surveyed to the west. The same 100 m (328 ft.) staked transects were used to survey floral resources, butterflies, and to place pan traps for bees (at a subset of sites), as described in further sections.

We surveyed floral resources by adapting the methods of Moranz et al. (2012), with recommendations from Dr. Diane Debinski and collaborator Nick Lyon. We counted all nectar plant inflorescences to species within 1.4 meters (4.6 ft.) on the side of the transect facing away from the road, safety and fences permitting (for a total area of 457 m^2 (1500 ft.^{2}), to match the Pollinator Scorecard nationwide dataset) (Rights-of-Way as Habitat Working Group 2019). This was accomplished by walking along the transect while holding a 1.4-meter (4.6-foot) stick and counting only flowers that are within that length. Inflorescences were characterized as the largest consistent floral unit among individuals within a species (e.g., compound umbels in Apiaceae and capitulums in Asteraceae); generally, if flowers were big enough to count individually, we counted them that way. The floral unit for each species is given in Appendix A. Plant Species List.

The researcher counting floral resources also collected a variety of transect metadata, including ROW width, distance from pavement to the transect, percentage bare ground (2022 only), presence of milkweed and late-season-blooming plants in the ROW, dominant species in the ROW, slope of ROW, presence of irrigation in adjacent land, and adjacent land use category.

3.2.3 Transect Surveys—Butterflies

Our 63 transect sites were surveyed for butterflies in conjunction with floral resources (all in June and July 2021 and 2022, and a selection in August 2021, 2022). Sites were surveyed for both butterflies and floral resources simultaneously or within no more than 2 days of each other, depending upon weather. To ensure we surveyed when butterflies were actively flying, we surveyed only between 21°C (70 °F) and 36°C (97 °F), when sustained winds were below 16 km/hour (10 mph), and when the sun was not fully obscured by clouds (Moranz et al. 2012).

Following previous methods, we surveyed by walking along the transect at a rate of 10 m (33 ft.) per 1 minute (Moranz et al. 2012). With flags placed every 20 m (66 ft.), we reached the 20 m flag at 2 minutes, the 40 m flag at 4 minutes, and so forth, using a stopwatch to record time and pausing it each time a butterfly is observed. When a butterfly was observed, we recorded the butterfly's species, number of individuals, sex (if possible), distance from transect (with the aid of a rangefinder), activity type (flying, nectaring, resting, interacting, and/or ovipositing), and if nectaring or ovipositing, which species of plant it is visiting—all while the timer was paused. Then, the stopwatch was resumed and the transect walk continued. In this way, sampling of a given transect generally took longer than 10 minutes (unless no butterflies were seen), even though the stopwatch showed 10 minutes. In order to ensure that each transect had an equal sampling effort, butterflies observed while the stopwatch was stopped, or at the site before or after the stopwatch was started, were noted with 0:00 and *not* counted, although these species were noted to contribute to our total butterfly list for the study area and to provide additional presence/absence data.

Butterfly distance was measured by a combination of unlimited distance sampling and 5 m x 5 m square sampling (Thomas 1983). Butterflies were observed in the 180° field of view ahead of the researcher, regardless of how far away they were, except for those that occurred past the end of the transect. Distances were measured using a rangefinder and rounded down to the nearest meter. Most butterflies could be identified in the field from the transect by eye or using butterfly binoculars; however, if closer inspection was necessary for identification, the researcher paused the stopwatch and attempted to capture the butterfly using a butterfly net. After butterflies were inspected and photographed, they were released back into the field unharmed. Butterflies were neither harmed nor collected in our study.

3.2.4 Transect Surveys—Bees

The diversity and minute characteristics distinguishing bee species preclude most bees from being reliably identified in the field. As such, we collected bees using pan traps: small blue, yellow, and white plastic cups filled with soapy water which attract bees by imitating flowers. These pan traps are commonly used in bee field studies and are more efficient and cost-effective than other sampling methods; they also help reduce observer bias (Westphal et al. 2008; Wilson, Griswold, and Messinger 2008). They do, however, catch large numbers of bees, especially halictids (sweat bees) (Roulston, Smith, and Brewster 2007). To minimize impacts to bee populations, we placed pan traps at a selection

of 27 of our 63 transect sites, with 3 transects sampled in each highway class x NDVI category, on one day in June 2021 and one day in July 2021 only.

Pan traps were only placed on sunny days with temperatures between 21°C (70 °F) and 35°C (95 °F) and sustained winds below 16 km/hour (10 mph). Six pan traps (two of each color) were placed at each transect (Figure 3), with one pan trap placed every 20 m (66 ft.) along the 100 m (328 ft.) transects; these locations were already flagged for our butterfly and floral resource surveys. Traps were placed in the morning between 07:30 and 09:30, and retrieved between 14:15 and 18:30, with the exact time of placement and retrieval recorded and attempts made to place and retrieve traps in a similar order so that traps would be out similar lengths of time. Our goal was that each pan trap be out for 5 to 6 hours of prime bee activity time. At retrieval, pan traps were strained of fluid and collected insects were bagged by transect and date, placed into a cooler of dry ice, and preserved for processing and pinning in the lab.

In the lab, we blow-dried (to align bee hair properly for specimen identification) and pinned all bee specimens and attached labels to them according to entomological convention, including coordinates of location collected, county and state, date, transect ID, and specimen number. After all specimens were pinned, labeled, and prepared, we delivered them to Skyler Burrows of the USDA-ARS Bee Lab in Logan, UT, an expert in bee identification for Western North America. Skyler identified the bees over the course of six months, and we retrieved them in October 2022.

Figure 3 - Pan traps brought together for collection, June 2021. These traps were collected from our most productive transect in terms of bee abundance and species richness: US.1.5 along US-26 between Blackfoot and Arco, Idaho.

3.3 Statistical Analysis

We conducted statistical analyses to assess the effects of highway class (State, U.S., or Interstate highway), NDVI class (low, medium, or high), floral richness (number of flowering plant species on transect) and floral abundance (number of individual flowers on transect) on butterfly and bee species richness and abundance. For butterflies, we ran all of our statistical analyses on data from our four complete rounds of surveys of 63 transects in June and July 2021 and 2022 (two rounds of surveys per year), excluding our data from August which contained a subset of only 18 surveys. Butterfly richness and abundance values were thus calculated using four temporal sampling replicates (i.e., two years) for each transect site. For bees, we ran our analyses on all of our bee data, which consisted of two rounds of pan-trapping at 27 transects in June and July 2021. Thus, bee richness and abundance were calculated using two temporal sampling replicates (one year) for each site. Both our butterfly and bee analyses were balanced with respect to highway class and NDVI, with equal numbers of transects in each category. To assess the impact of floral richness and abundance, we modeled our pollinator data with floral richness and abundance data from the corresponding transects and survey dates.

We used generalized linear mixed-effect effects models to test for the effect of highway class, NDVI, floral richness, and floral abundance on our pollinator response variables, with transect (site) and sampling date as crossed random effects to account for multiple surveys of the same transect over time and different transects being surveyed on different dates. Floral richness and floral abundance were log-transformed in our models to account for their highly left-skewed distributions and to enable clearer ecological interpretation of model results, given the log link functions used in our generalized models. We elected not to include the interaction between highway class and NDVI owing to convergence errors (for our butterfly models) and overfit errors (for our bee models) when the interaction was added; furthermore, when we assessed our models with the interaction term (using the BOBYQA optimizer (Powell 2009) and removing select random effects, respectively, for our butterfly and bee models), the interaction was non-significant in all of our models. Sampling date was removed as a random effect from our model of bee abundance because it resulted in an overfit (near singular) model.

Generalized linear mixed-effects models with Poisson distributions were fit for bee and butterfly richness data to account for left-skewed distributions in pollinator richness and generalized linear mixed-effects models with a negative binomial distribution were used to account for overdispersion in bee and butterfly abundance data. Dispersion was tested using the *dispersion_glmer* function from the blmeco package (Korner-Nievergelt et al. 2015). Our analyses were evaluated at a 0.05 significance level, where $p < 0.05$ indicates significance, $0.05 < p < 0.1$ indicates marginal significance, and $p > 0.1$ indicates no significance.

Mixed-effects models were developed using the *lmer* and *glmer.nb* functions in the lme4 package (Bates et al. 2015); p values for fixed effects were obtained from these models using the likelihood ratio test (LRT) method in the *mixed* function of the afex package (Singmann, Bolker, and Westfall 2017; Luke 2017). We produced estimated marginal mean pairwise comparisons using the *emmeans* function in the emmeans package (Lenth et al. 2020) in R to identify significant differences between NDVI and highway classes. Multiple comparisons of means using a Tukey's contrast were conducted for each mixed-effects model using the *glht* function in the multcomp package (Hothorn, Bretz, and Westfall 2008) in R to determine relative effects of treatment. Figures of these models and the raw data were created using the base R, effects (Fox et al. 2022), and ggplot2 packages (Wickham and Chang 2014) in R (R Core Team 2022).

Spatial correlation was evaluated for average transect (site) values of bee and butterfly richness and abundance using Moran's I in the ape package in R (Paradis et al. 2023). Spatial correlation was higher (lower Moran's I p-value) in NDVI, one of our predictor variables than in our response variables of pollinator abundance and richness, as shown in Table 2. We expect that non-random spatial patterns in soil type, moisture availability, and plant growth (as measured by NDVI), along with differences in highway type, drive the spatial patterns in butterfly richness and bee richness and abundance we observed.

Table 2 - Moran's I p-values indicate significant spatial correlation in butterfly richness and bee richness and abundance, with more significant spatial correlation in NDVI. Highway class is necessarily spatially correlated as well, since multiple transects exist along the same roads, causing relative clustering of transects of the same highway class.

Moran's I p-values	Butterflies	Bees
Average Richness	0.003	0.009
Average Abundance	0.411	0.013
NDVI	<0.001 (6.27 e-12)	0.008

To assess the effects of NDVI, highway class, floral richness, and floral abundance on butterfly and bee community composition and dispersion (beta-diversity, or among-transect variation in species composition), we used PERMANOVAs with *adonis2* (Oksanen 2015) and distance-to-centroid tests (betadisper) based on Bray-Curtis dissimilarities. Ordination (NMDS) plots were created to visualize compositional differences using non-metric multidimensional scaling. We used similarity percentage analysis (*simper*) to identify which species contributed most strongly to observed compositional differences between NDVI and highway classes (Oksanen 2015).

4. Results

4.1 Milkweed and Monarch Rapid Assessment

4.1.1 Milkweed Data

We located a total of 1,363 milkweed patches along 1,465 km (910 miles) miles of highway surveyed in our milkweed and monarch rapid assessment, as shown in Figure 4. Only *Asclepias speciosa* (showy milkweed) was observed. All milkweed patches located in our rapid assessment have been incorporated into the interactive online ITD Roadside Vegetation Guide, available at https://arcg.is/1iDH9a0.

Milkweed patches varied in stem number from as few as two stems to as many as 7,075 stems. While most patches (89%) had fewer than 50 stems, we assessed 15 patches with over 1,000 stems and 88 patches with over 100 stems. Milkweed was generally, though not always, found in areas with nearby water sources, including in association with canals, irrigated crops, and riparian zones. The following three paragraphs refer only to milkweed patches over 50 stems, which we assessed in detail.
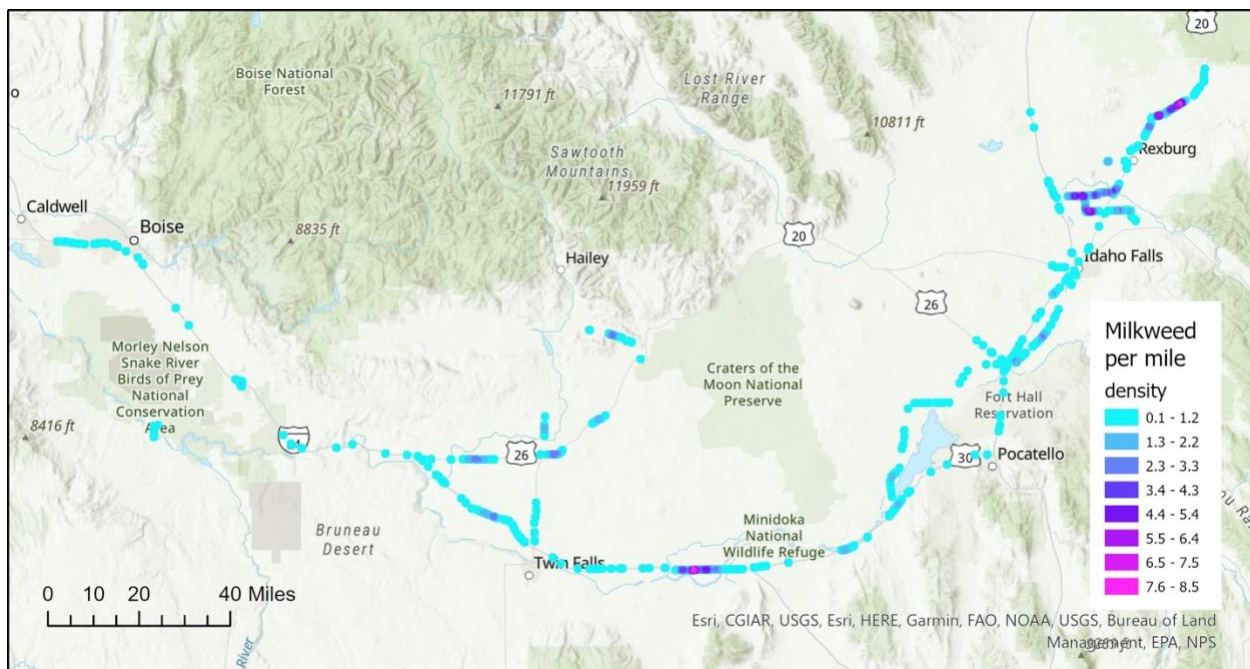

Figure 4 - Roadside milkweed patches located on 2021 rapid assessment. Patches colored by density (number of milkweed patches per mile).

Milkweed was typically found in grassy and grass-dominated ROWs, often in company with other forbs and non-native weeds. Milkweed was frequently the only native plant species evident in the ROW, as it often flourishes in disturbed areas that otherwise harbored mostly non-native plants. Common

dominant plant species in milkweed patches included *Agropyron cristatum* (crested wheatgrass) and *Thinopyrum intermedium* (intermediate wheatgrass), *Bassia scoparia* (kochia), *Equisetum spp.* (horsetails), *Carex* spp. (sedges), and *Sisymbrium altissimum* (tall tumblemustard). None of these species appeared to have a consistent or close relationship with milkweed, however, but merely shared the same preferred habitat—disturbed areas with access to water. Milkweed was typically found in association with other flowering plants, with 93% of milkweed patches over 50 stems found within < 10 m of at least one other flowering plant. Excluding milkweed, the average floral diversity of milkweed patches was 2.67 species, with a maximum of 11 other flowering plant species. Milkweed patch sizes and densities were variable; the average size of patches over 50 stems was 292.2 m^2 (3,145.2 ft.^{2}), and the average density was 3.9 stems/m^2 (0.4 stems/ft^2). Some very large and dense patches skewed average values higher than medians. Distributions of these metrics are shown in Figure 5.
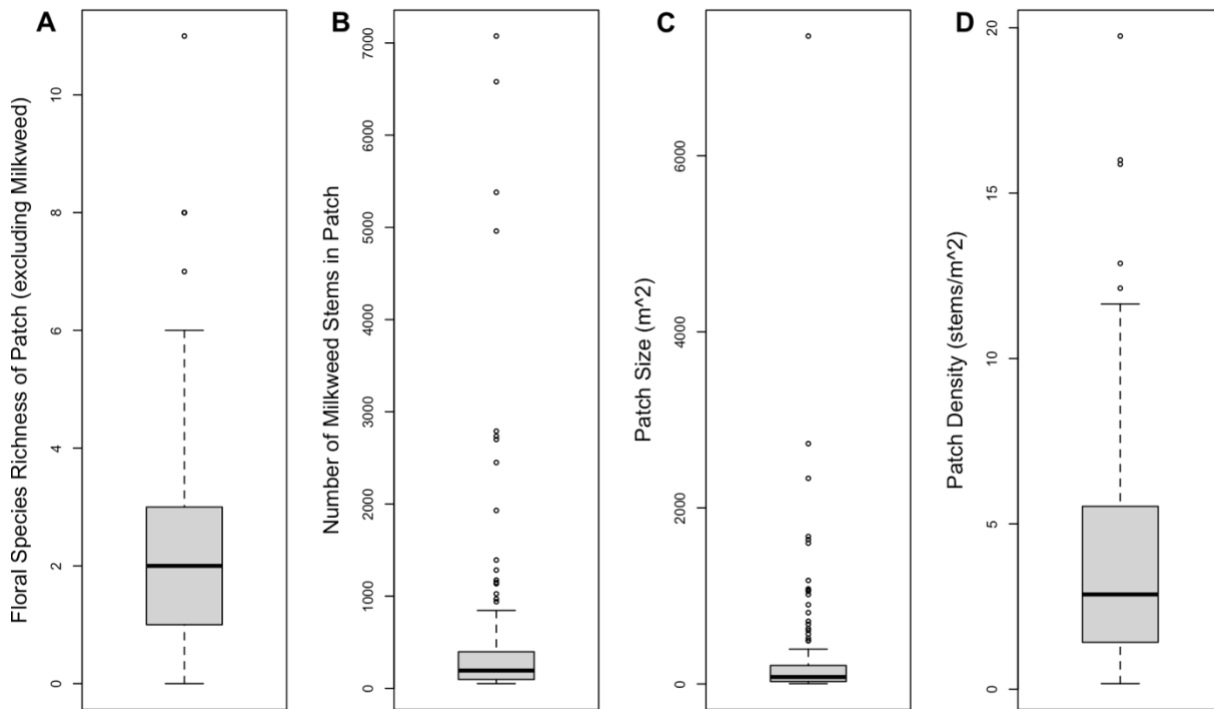
Figure 5 - Distributions of four metrics for roadside milkweed patches over 50 stems, showing spread of values for: (A) floral species richness of patch (excluding milkweed), (B) number of milkweed stems in patch, (C) patch size in m^2, (D) patch density in stems/m^2.
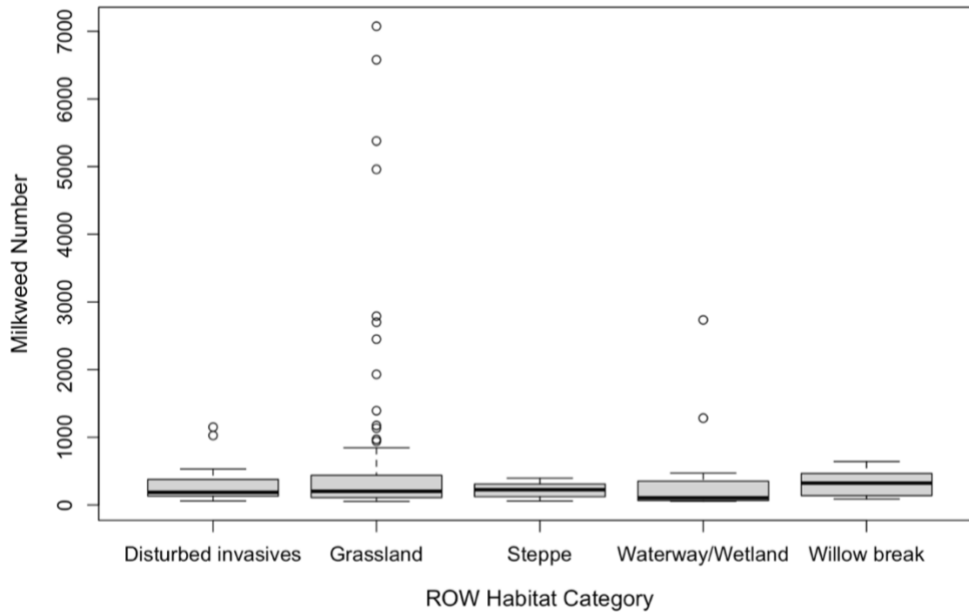
Figure 6 - Number of stems per milkweed patch (over 50 stems) by ROW habitat category.
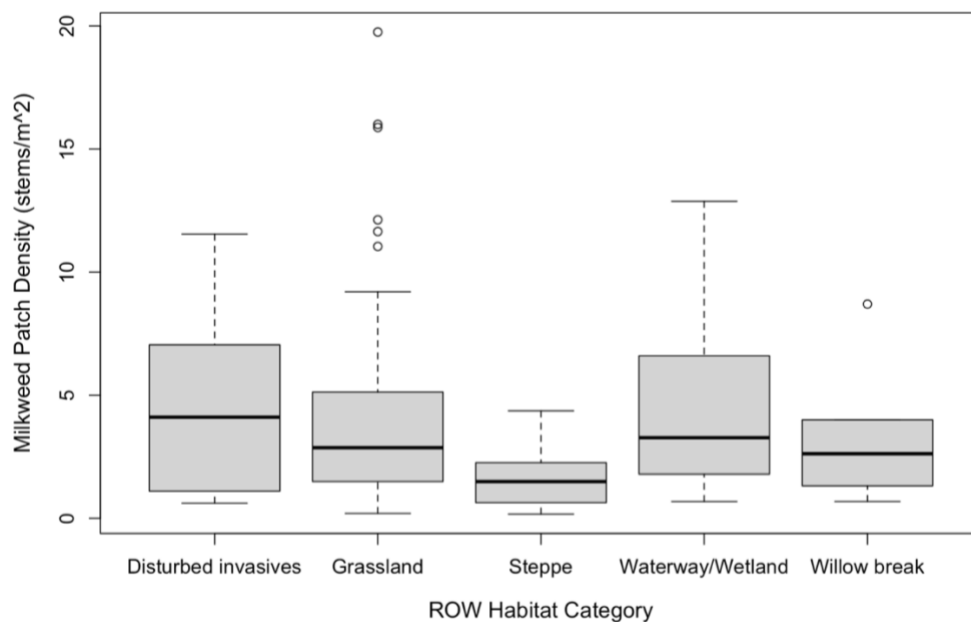

Figure 7 - Density of milkweed stems in patches over 50 stems by ROW habitat category.

Based on our physiognomic characterization of ROW habitat and adjacent land (cross-the-fence) habitat for milkweed patches over 50 stems, grassland (a grass-dominated ROW) was the most common ROW habitat for large milkweed patches, followed by waterway/wetland. Most of the largest milkweed patches (by stem count) also occurred in grassland ROW habitats, as shown in Figure 6. Grassland, waterway/wetland, and disturbed invasives (areas dominated by non-native weedy forbs) also had the

highest milkweed densities within patches (Figure 7). Milkweed patches most commonly occurred adjacent to plant or animal agriculture (Figure 8); these categories along with waterway/wetland/willows had the highest milkweed density within patches, while patches with disturbed invasives (weedy areas) across the fence were of the largest median size and stem number.

Our assessment of terrain category—flat, ditch, or slope—revealed that most milkweed patches grew in flat areas or in ditches, with relatively few (11.6%) growing on slopes. This makes intuitive sense given that water runs off rather than collects on slopes, and water availability seems to be a key factor influencing milkweed distribution in roadsides. Although large patches sometimes extended across multiple terrain categories, the nine largest milkweed patches we surveyed were all in the flat terrain category. As with habitat, however, we could not assess the proportion of ROW area occupying each terrain category, so milkweed occurrence may simply reflect the distribution of different terrain (or habitat) types in the ROWs, and not a specific preference of the plant.

One of our goals with the milkweed rapid assessment was to determine "hotspot zones" of high milkweed abundance for suggested inclusion as habitat conservation zones ("adopted acres") in an ITD Candidate Conservation Agreement with Assurances (CCAA) for the monarch butterfly. Hotspot zones identified by milkweed patch density per mile are shown in Figure 9, while hotspot zones identified using the Getis-Ord Gi* statistic (statistically significant spatial clusters of patches) are shown in Figure 10. Both these selection methods identified three major zones of highest milkweed density and clustering, which we propose are maximally important for careful management and protection in support of monarchs. These three road segments each contained sections exceeding 10 milkweed patches per mile of highway as well as statistically significant clusters of milkweed patches at the $p = 0.01$ (99% confidence) level, and are designated as "Tier 1" milkweed hotspots in Table 3. Road segments containing sections exceeding 5 milkweed patches per mile are designated as "Tier 2" milkweed hotspots in Table 3. Tier 1 and Tier 2 hotspots are the highest priority ROW segments for milkweed protection and focused monarch-friendly management.
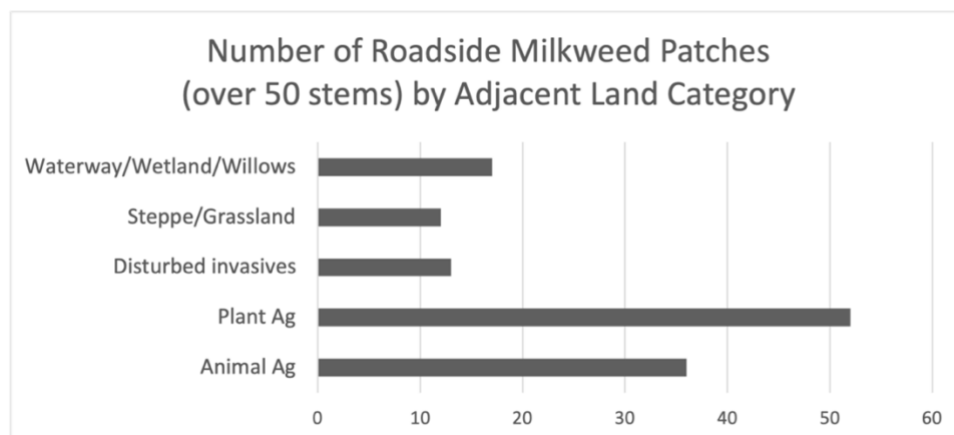
Figure 8 - Number of milkweed patches over 50 stems by adjacent (across the fence) land category, showing that most large milkweed patches occurred in ROWs bordering agricultural land, including crop fields (plant ag) and pasture and feedlots (animal ag).
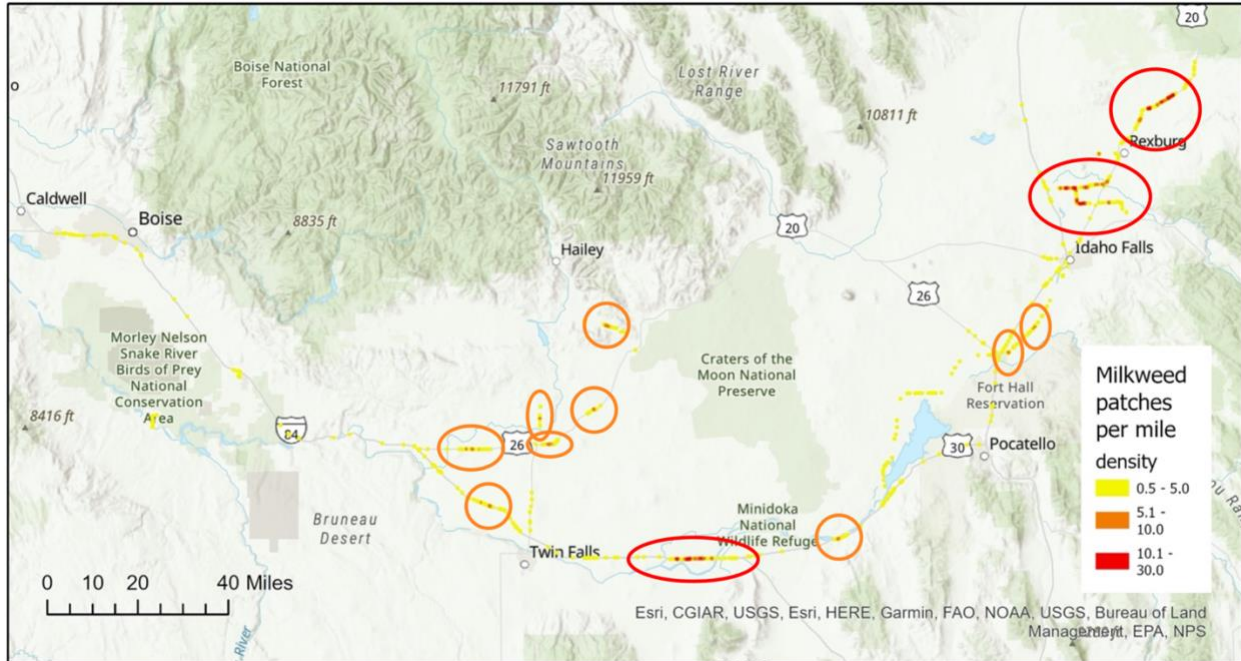
Figure 9 - Roadside milkweed hotspots based on patch density per mile, showing areas with greater than 10 patches per mile (red, Tier 1 hotspots) and greater than 5 patches per mile (orange, Tier 2 hotspots). Coordinates and mileposts for these hotspots are given in Table 3, below.

Table 3 - Coordinates and mile markers for roadside milkweed hotspots, based on milkweed patch density and spatial clustering. Tier 1 hotspots (bold) include sections of >10 milkweed patches/mile and statistically significant spatial clustering of milkweed (Getis-Ord Gi* p < 0.01). Tier 2 hotspots are shorter segments that include sections of >5 milkweed patches/mile.

Tier	Start Coordinates	End Coordinates	Route	Start Milepost	End Milepost	County
1	42.568837, -113.834089	42.568780, -113.638644	I-84	205.652	215.699	Minidoka
1	43.721781, -112.080981	43.661079, -111.804454	SH-48	2.998	20.883	Jefferson
1	43.899581, -111.732265	44.008213, -111.542002	US-20	340.331	354.270	Fremont
2	42.761255, -114.680591	42.743324, -114.622226	I-84	157.821	161.039	Gooding
2	42.923835, -114.732435	42.927339, -114.638319	US-26	149.107	153.898	Gooding
2	42.938025, -114.388263	42.952274, -114.317661	US-26	166.914	170.871	Lincoln
2	43.034872, -114.186578	43.049297, -114.153462	US-26	180.132	182.083	Lincoln
2	43.318539, -114.11006	43.308448, -114.069718	US-20	186.903	189.054	Blaine
2	43.015014, -114.395446	43.030636, -114.395482	SH-75	79.201	80.274	Lincoln
2	42.62261, -113.109836	42.632129, -113.069342	I-86	21.184	23.365	Power
2	43.199918, -112.332208	43.206307, -112.325145	US-91	101.713	102.281	Bingham
2	43.272301, -112.2214	43.28668, -112.203955	US-91	109.224	110.552	Bingham

Figure 10 - Roadside milkweed hotspots based on the Getis-Ord Gi* statistic, showing statistically significant spatial clustering of milkweed patches by confidence interval in red.

4.1.2 Monarch Data

Monarchs were rarely found in our 2021 rapid assessment, despite widespread searching (two minutes of dedicated searching at each patch over 50 stems per one mile of road). Throughout our assessment, we detected just one monarch egg (NE of Rexburg), zero larvae, and two adult monarch butterflies. Both adult butterflies were visiting roadside milkweed, one north of American Falls and one NE of Rexburg. The latter individual was a female ovipositing on roadside milkweed; she appeared to lay 14 eggs on roadside milkweed plants while we observed her. This occurred at a diverse patch of 770 milkweed stems with 9 species of flowering plants adjacent to a willow break and wetland (photo in Figure 11). After ovipositing, the female monarch flew out across the highway and was immediately hit by a passing truck, falling to the pavement. We collected this individual's body and found her abdomen still full of unlaid eggs—a testament to the risks of roadside ROWs for breeding monarchs.

In addition to monarchs, we observed 12 other butterfly species visiting milkweed patches during our rapid assessment, as listed in Table 4. We also observed a variety of bees feeding on milkweed flowers and three species of beetles, *Lygaeus kalmia, Tetraopes femoratus,* and *Chrysochus cobaltinus*, that rely specifically on milkweed as their host plant, as the monarch does (Figure 12). These observations suggest that milkweed is supporting not only the imperiled monarch, but also a diverse insect community.

Figure 11 - Diverse milkweed patch along US-20 NE of Rexburg with ovipositing female monarch (circled in red), Milkweed Rapid Assessment, July 13, 2021.

Table 4 - Butterfly species observed at milkweed patches of at least 50 stems during the 2021 rapid assessment, listed in order of number of milkweed patches at which the species was sighted. # indicates the single non-native species.

Butterfly Species Common Name	Butterfly Species Scientific Name	Number of Patches
Cabbage White[#]	*Pieris rapae*[#]	25
Clouded Sulphur	*Colias philodice*	8
Common Wood-Nymph	*Cercyonis pegala*	7
Becker's White	*Pontia beckerii*	4
Common Checkered-Skipper	*Burnsius communis*	4
Mylitta Crescent	*Phyciodes mylitta*	4
Melissa Blue	*Plebejus melissa*	3
Monarch Butterfly	*Danaus plexippus*	2
Field Crescent	*Phyciodes pulchella*	2
Small Wood-Nymph	*Cercyonis oetus*	1
Two-tailed Swallowtail	*Papilio multicaudata*	1
Checkered White	*Pontia protodice*	1
Ruddy Copper	*Lycaena rubidus*	1

Figure 12 - 5th-instar monarch larva and *Tetraopes femoratus*, red-femured milkweed borer, feed on their shared host plant, *Asclepias speciosa,* showy milkweed.
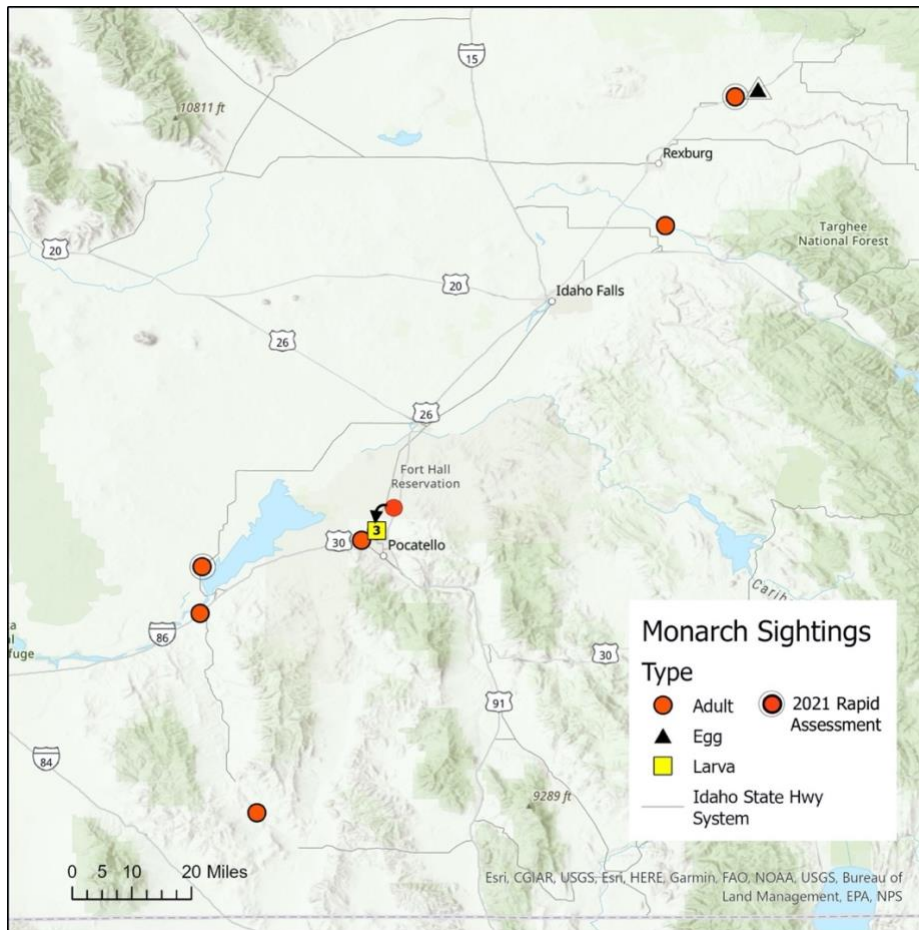

Figure 13 - All monarch sightings that occurred during the project (2021 and 2022). Double rings on symbols indicate sightings that occurred as part of the 2021 Milkweed & Monarch Rapid Assessment. Symbols without a double ring indicate 2022 sightings, which included observations at transects and incidental observations while camping for field work.

By late November 2021, the western monarch population had undergone a remarkable recovery, increasing over 100-fold from less than 2,000 to more than 247,000 individuals at its wintering roost sites in coastal California (Xerces Society 2023). As such, we were pleased but not surprised to see more monarchs in Idaho during our 2022 field season, despite not explicitly searching milkweed patches. In 2022, we observed adult monarchs at three of our stratified survey plots (compared with zero in 2021), and documented evidence of breeding in one of our plots, finding one third-instar and two fifth-instar monarch caterpillars feeding on roadside milkweed just north of Pocatello. All of our monarch observations from both years are shown in Figure 13, and coordinates, dates, and details of sightings have been recorded and are available in supplementary files.

### 4.1.3 Comparison with Model of Svancara *et al.* (2019)

As concern over monarch populations increases, it is particularly useful to understand and predict where milkweed, the butterfly's host plant, occurs across landscapes, and what factors determine its distribution. If models can accurately predict where milkweed occurs, then these models can be used in place of intensive survey efforts like our milkweed rapid assessment to forecast and conserve important areas for milkweed growth and monarch breeding. In Idaho, we are fortunate to have the work of Leona Svancara and her colleagues, who used a variety of climatic, edaphic, geographic, and topographic factors to model both current and future distributions of milkweed and monarchs in the state (Svancara, Abatzoglou, and Waterbury 2019). They developed a statewide model of suitability for showy and swamp milkweed and monarchs, which predicted current distributions of these species with high accuracy (0.899 AUC—area under the receiver operating characteristic curve—for showy milkweed).

We compared our milkweed rapid assessment data with the models published in Svancara *et al.* 2019 (hereafter, "Svancara's model") to determine whether these models could accurately predict milkweed distribution in ROWs specifically. While some milkweed patches had high suitability values (i.e., their locations were predicted by Svancara's model), many did not, and the average modeled suitability value for our roadside milkweed patches was 0.53, just over chance. This confirmed our hypothesis that Svancara's model does not accurately predict milkweed distribution in Snake River Plain roadsides.

In addition to location, we did not find any relationships between the size (number of stems) of milkweed patches and the modeled suitability of their associated patch. These results are shown in the histogram and scatter plot in Figure 14, and graphically in Figure 15, where patch size is indicated by circle size and suitability indicated by color (red more suitable, white less), overlaid over a landscape-wide suitability map generated using Svancara's model (dark less suitable, light more suitable).

Among the top five variables influencing statewide milkweed distribution identified in Svancara's model, distance to perennial water was the only variable with a distinct directional distribution in our roadside milkweed dataset. Roadside milkweed patches generally occurred closer to perennial water sources, and the number of milkweed patches tapered off at approximately 1.5 km from perennial water sources (Figure 16A). The other top variables in Svancara's model predicted that milkweed would occur in deeper soils in areas with lower precipitation in the wettest month and a larger diurnal temperature

range. Our roadside milkweed patches show approximately normal distributions (with outliers and irregularity) in these variables, rather than the directional trends of Svancara's model (Figure 16B-D).
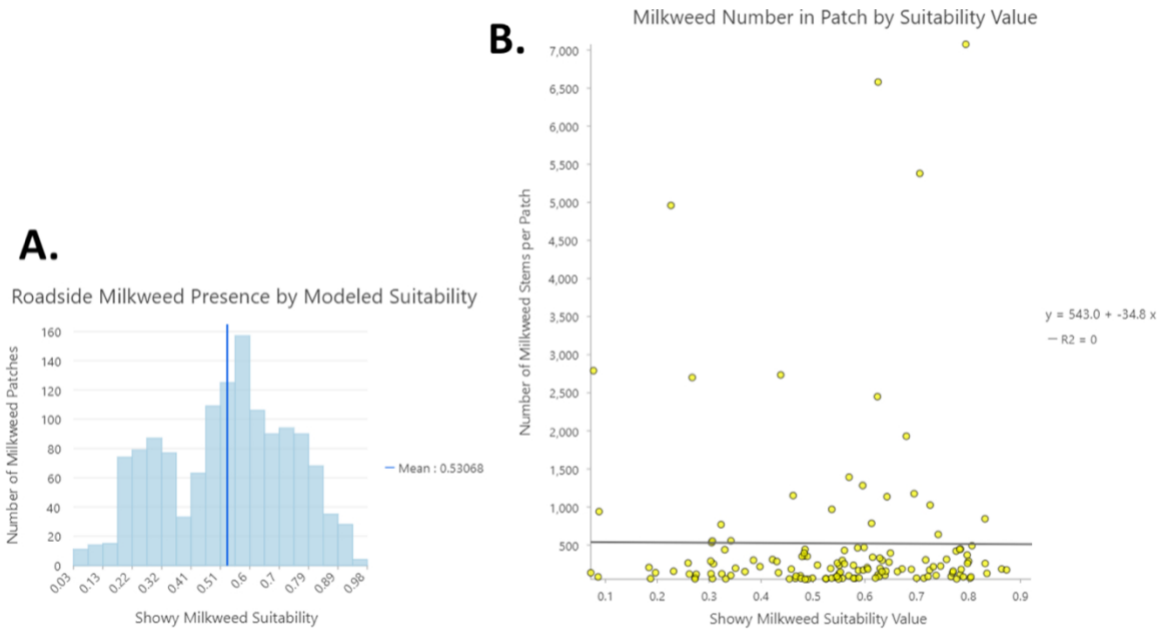
Figure 14 - A. Suitability values, derived from Svancara, Abatzoglou, and Waterbury 2019, of roadside milkweed patches identified on rapid assessment (mean = 0.53). B. Suitability values by number of milkweed stems per patch, showing flat regression line (no relationship, R^2 = 0).
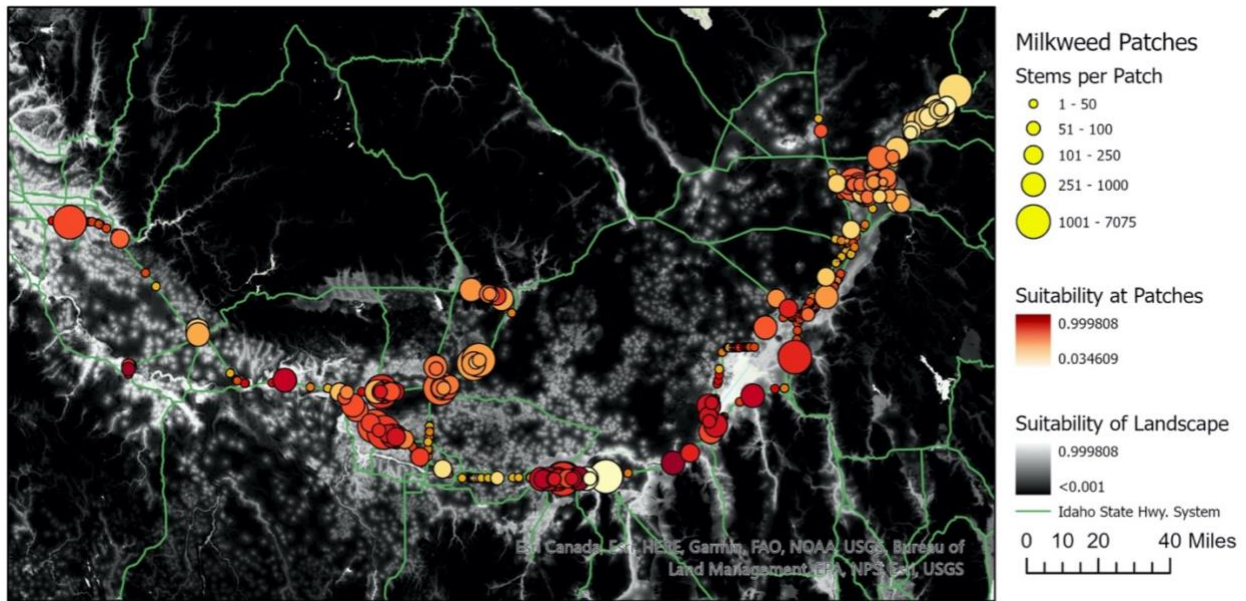
Figure 15 - Milkweed patches by modeled suitability. Size of circles indicates size (number of stems) of milkweed patch; circle color indicates suitability value of patch from low (white) to high (dark red). Landscape gradient indicates suitability from low (dark) to high (light). Suitability values derived from Svancara, Abatzoglou, and Waterbury 2019.

*Response curves below reproduced from Svancara et al. (2019):*
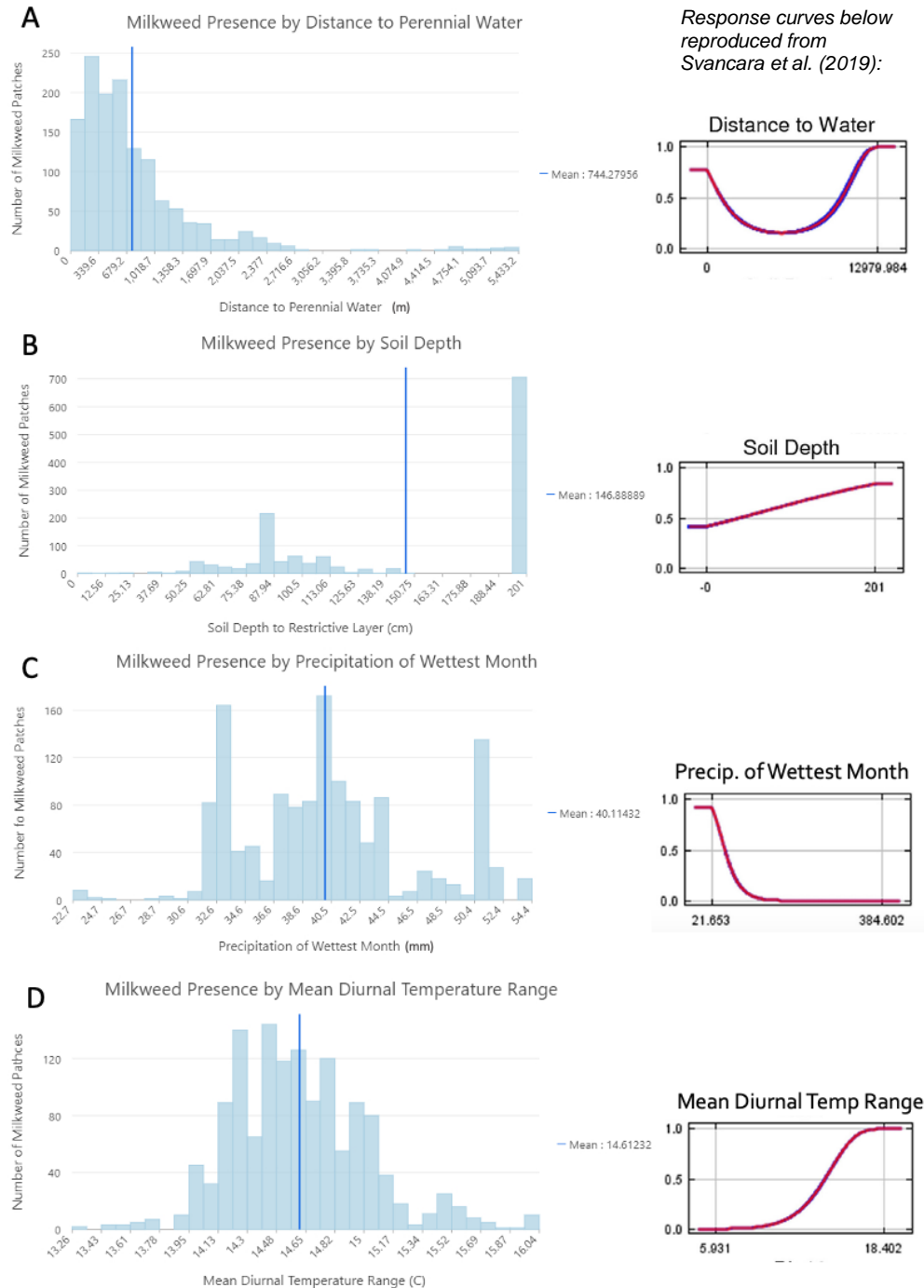
Figure 16 - A comparison of the distribution of roadside milkweed with modeled milkweed suitability across four top factors identified in the milkweed suitability model of Svancara, Abatzoglou, and Waterbury 2019. Histograms (left column) show the distribution of roadside milkweed patches relative to (A) Distance to perennial water, (B) Soil depth to restrictive layer, (C) precipitation of wettest month, and (D) mean diurnal temperature range. Response curves (right column), reproduced with permission from Svancara et al. (2019), show the relationship between showy milkweed suitability (vertical axis) and the same four factors.

4.2 Transect Surveys—Floral Resources

Across all floral resource surveys of all transects in both years, we identified 87 flowering plant species, listed in Appendix A. Because of the study's focus on pollinators and floral resources to provision them with nectar and pollen, only species with open flowers at the time of survey were counted, potentially excluding plants that flowered at our sites early or very late in the growing season. Our results are by no means a complete account of roadside flora at our transect sites or within our study area, but they do provide a general understanding of patterns of floral composition, diversity, and abundance in the roadsides of SE Idaho.

We recorded 58 flowering plant species in summer 2021 and 64 species in summer 2022 (Appendix A). Most of the Snake River Plain was in a moderate to extreme drought in summer 2021, and a moderate drought in summer 2022, as shown in Figure 17 (U.S. Drought Monitor). We observed noticeable increases in floral richness and phenological shifts in flowering during the relatively cool, wet spring of 2022 after the especially hot, dry spring and summer of 2021. These increases in floral richness were especially evident in our transects in the low NDVI category. Areas with the highest aridity, not supported by irrigation runoff or perennial water, seemed to have responded most to the added moisture, as shown by increases in total floral richness from 2021 to 2022 (Figure 18).

Among the 87 flowering plant species we recorded, 45 (52%) were native to Idaho and 41 (48%) were non-native. While some species, such as the disturbance-loving invasive mustards *Sisymbrium altissimum* (tall tumblemustard) and *Descurainia sophia* (fixweed), were widespread and abundant across the study area, many species were uncommon and found at only one or a few of our sites. In fact, nearly half of the plant species we recorded (43 of 87, or 49%) were detected at only one of our 63 transects (Appendix A).
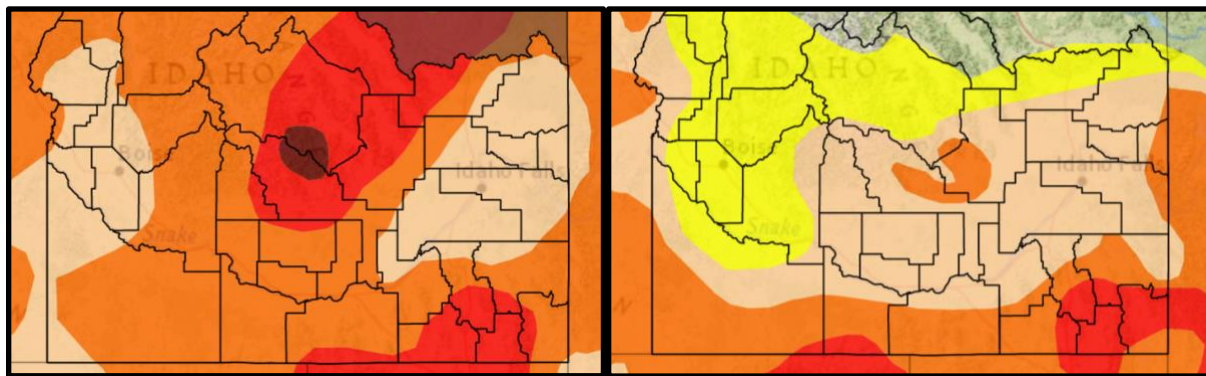
Figure 17 - Drought levels in southern Idaho, July 1st 2021 (left) and July 1st 2022 (right). From most to least severe, red indicates extreme drought, orange severe drought, beige moderate drought, and yellow abnormally dry. Source: U.S. Drought Monitor
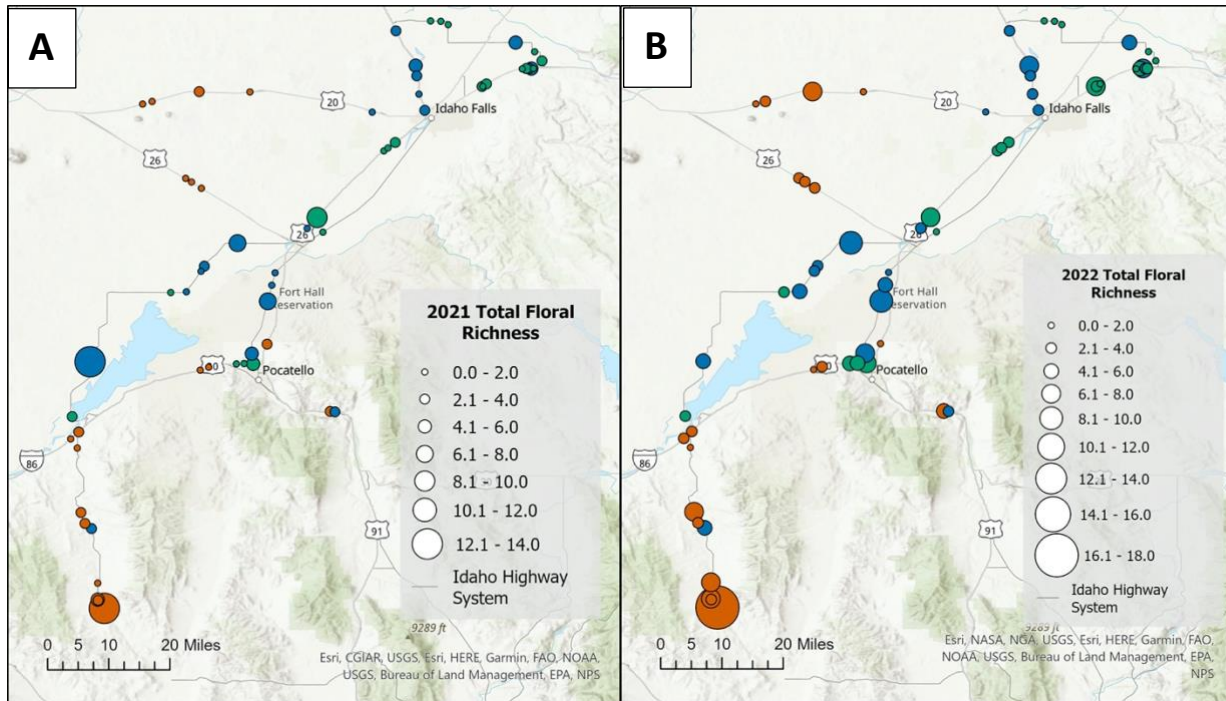
Figure 18 - Floral species richness of transects in 2021 (left) and 2022 (right), showing increases in floral richness following the cooler, wetter spring of 2022. Color of dots signifies NDVI class, from low (orange) to medium (blue) to high (green).

As expected, given the ubiquity of disturbance in roadsides, weedy non-native species predominated in terms of floral abundance. Among our ten most abundant floral resources (based on total number of flowers in the study), only two were native species (Table 5). Both native and non-native floral resources varied between species in their attractiveness to butterflies and bees. Among our most abundant roadside flowers, for example, *Medicago sativa* (alfalfa), *Melilotus officinalis* (yellow sweet clover), and *Chrysothamnus viscidiflorus* (yellow rabbitbrush) were frequently visited by butterflies and some bee species, while other flowers, such as *Asperugo procumbens* (German madwort), *Convolvulus arvensis* (field bindweed), and *Lappula occidentalis* (western stickseed) were rarely visited (Meinzen, pers. obs.).

In addition to being most abundant across all survey transects, non-native species were also typically more widespread across our study area than native species. Our ten most widespread floral species— those detected on the most transects—included nine non-native and one native species (Table 6). *Sisymbrium altissimum* (tall tumblemustard) was found at a remarkable 83% of our transects, while another non-native mustard, *Descurainia sophia* (fixweed), was found at 73% of transects. *Lappula occidentalis* (western stickseed) was the most widespread native species, found at 24% of our transects, followed by *Chrysothamnus viscidiflorus* (yellow rabbitbrush) and *Helianthus annuus* (annual sunflower). *Asclepias speciosa* (showy milkweed) was also fairly widespread, found at about 10% (6 of 63) of our transects—all but one of these in the medium NDVI category. Among our 288 surveys of 63 transects, 53 surveys (18.4%) detected no flowers blooming within the transect.

Table 5 - Top ten floral resources at roadside pollinator transects by total number of flowers, summed across all surveys from 2021 and 2022. Floral units (e.g., ramets, heads, etc.) for each species are listed in Appendix A. * indicates native species.

Abundance Rank	Floral Code	Floral Species Scientific Name	Floral Species Common Name	Total Number of Flowers
1	SIAL	*Sisymbrium altissimum*	tall tumblemustard	35,637
2	DESO	*Descurainia sophia*	fixweed	15,680
3	MESA	*Medicago sativa*	alfalfa	13,685
4	CHVI*	*Chrysothamnus viscidiflorus*	yellow rabbitbrush*	10,925
5	BRNI	*Brassica nigra*	black mustard	6,533
6	ASPR	*Asperugo procumbens*	German madwort	4,697
7	MEOF	*Melilotus officinalis*	yellow sweet clover	4,289
8	COAR	*Convolvulus arvensis*	field bindweed	3,756
9	LAOC*	*Lappula occidentalis*	western stickseed*	2,991
10	SILO	*Sisymbrium loeselii*	small tumblemustard	2,233

Table 6 - Top ten floral resources at roadside pollinator transects by number of transects detected, including all survey data from 2021 and 2022. Columns indicate how many transects (out of 63) and what percentage of transects the species was detected at, and number of surveys in which the species was detected (each transect was surveyed 4 or 6 times). * indicates native species.

| Freq. Rank	Floral Code	Floral Species Scientific Name	Floral Species Common Name	Number of Transects (out of 63)	% of Transects	Number of Surveys (out of 288)
1	SIAL	*Sisymbrium altissimum*	tall tumblemustard	52	83%	152
2	DESO	*Descurainia sophia*	fixweed	46	73%	88
3	LAOC	*Lappula occidentalis*	western stickseed*	15	24%	20
4	MESA	*Medicago sativa*	alfalfa	13	21%	32
5	COAR	*Convolvulus arvensis*	field bindweed	12	19%	25
6	MEOF	*Melilotus officinalis*	yellow sweet clover	11	17%	23
7	CANU	*Carduus nutans*	musk thistle	11	17%	19
8	LEPE	*Lepidium perfoliatum*	clasping pepperweed	11	17%	15
9	LASQ	*Lappula squarrosa*	European stickseed	11	17%	14
10	ASPR	*Asperugo procumbens*	German madwort	10	16%	12

During our August subset of surveys, we observed that most non-native plants had concluded flowering by mid-August, while a few common native species began flowering during this time. Two species of rabbitbrush, *Chrysothamnus viscidiflorus* and *Ericameria nauseosa*, and sunflower, *Helianthus annuus,* offered abundant floral resources at our transects in August. Anecdotally, outside of our surveys, we observed rabbitbrush flowering along roadsides well into October in Idaho*.*

We did not find any plant species in our transects that are currently considered Sensitive or Species of Conservation Concern, as tracked by the Idaho Fish and Wildlife Information System (Idaho Department of Fish and Game n.d.). IDFG's published list of Species of Greatest Conservation Need does not include any plant species (Idaho Department of Fish and Game 2016). However, painted milkvetch (*Astragalus ceramicus* var. *apus*), found on one of our transects, is endemic to a 60-mile-diameter area of the Snake River Plain (Barneby 1964) and was not previously known from the area where we located it, along State Highway 37 south of Rockland, ID. This uncommon endemic is known for its wiry foliage and large, inflated, red-and-yellow mottled seed pods.

Also along State Highway 37, we identified native *Penstemon* species favored by *Bombus* spp. (bumblebees), as well as *Sphaeralcea munroana* (orange globemallow) and *Opuntia polyacantha* (plains prickly-pear), preferred flowers of native *Diadasia* and *Agapostemon* bees*.* Our southernmost transect, SH.1.8 located along SH-37 near Roy, stood out as particularly floristically diverse, with 14 flowering plant species recorded in 2021 and 18 species in 2022, among them several native species found at no other transect in our study. This transect, which bordered BLM land with sagebrush steppe and included a small drainage, was also host to a diverse butterfly community.

With sampling date and transect as random effects, we used our complete survey rounds (June and July 2021 and 2022) to model how floral species richness (number of flowering plant species) related to NDVI and highway class and found that floral richness differed marginally between NDVI categories (Generalized Linear Mixed Model, Likelihood Ratio Test ANOVA p = 0.052). As shown in Figure 19, the highest floral richness tended to occur at transects that were neither extremely green nor extremely brown (medium NDVI). We observed that higher NDVI sites were more often dominated a lush, green monoculture species (e.g., *Bassia scoparia*, *Sisymbrium* spp*.*) to the exclusion of other plant species, while some low NDVI sites appeared to lack sufficient moisture for many different plant species to grow. There were exceptions to this; for example, SH.1.8, discussed in the previous paragraph, was a low NDVI transect with high plant diversity. Highway classes were not significantly different from each other in terms of floral richness (GLMM, LRT ANOVA p = 0.254).
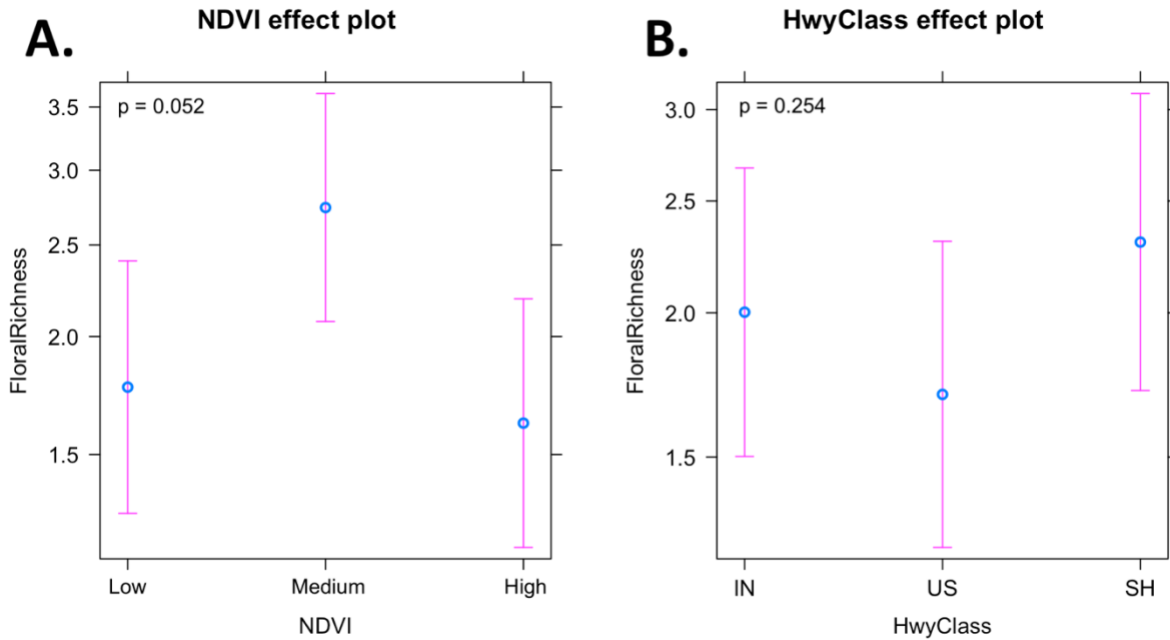
Figure 19 - Effect plots of two variables on floral richness from a Generalized Linear Mixed Model with Poisson distribution. A.) NDVI categories range from low (less green) to high (greener). B.) Highway classes are interstate (IN), U.S. highways (US), and State highways (SH), in order of decreasing size and traffic intensity. *p*-**values, calculated from an ANOVA on the model using the Likelihood Ratio Test, indicate a marginally significant (p = 0.052) difference in floral richness between NDVI classes.**

We observed in the field that low and medium NDVI transects, which tended to be drier and frequently associated with sagebrush steppe environments, had far more native flowering plants than high NDVI transects, which were typically dominated by non-native species. When we separated out native and non-native plants in our dataset, we found this pattern to be even more extreme than expected, with only 4 native plant species appearing in all of our high NDVI transects combined, compared with 23 in the medium NDVI class and 34 in the low NDVI class (Figure 20). By comparison, non-native species were distributed more evenly among NDVI classes, with the fewest (21) in low NDVI sites and the most (30) in medium sites (Figure 20). Thus, it appears that together, low NDVI sites had the highest native plant species richness, medium NDVI sites had high richness of both native and non-native plants, and high NDVI sites had almost exclusively non-native flowering plants. This may be because the deep green that causes these transects to have higher NDVI values is produced by highly successful invasive species, which exclude native flowering plants.
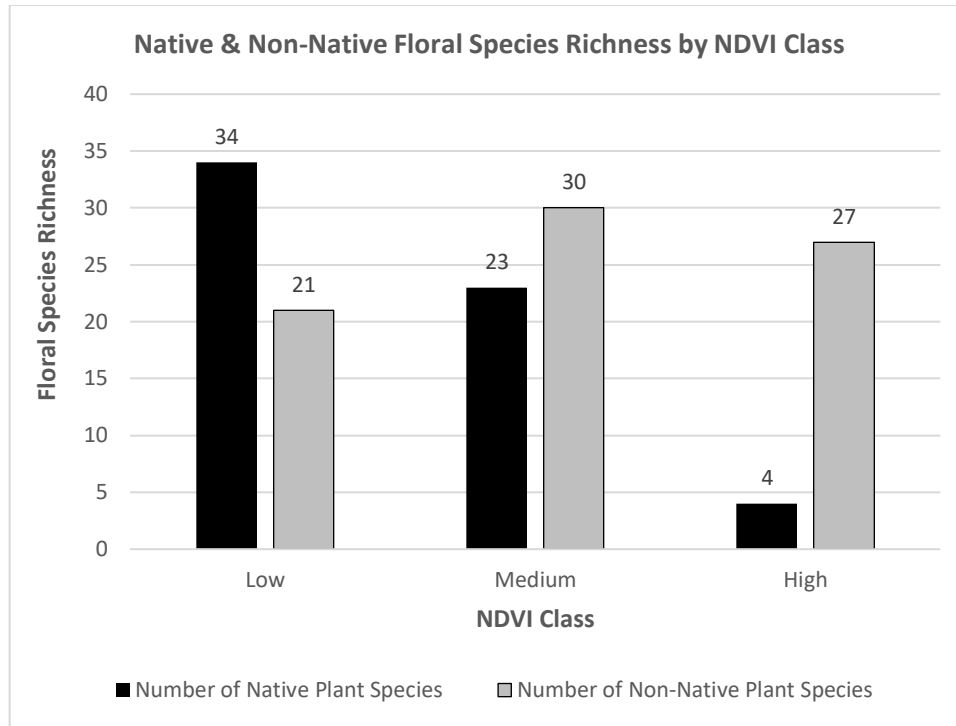
Figure 20 - Total native (left, black columns) and non-native (right, gray columns) floral species richness by NDVI class (all transect survey data summed).

In addition to understanding patterns of floral richness and identifying roadside floral species composition, we aimed to link floral resources to pollinators. Our models in the following sections assess relationships between floral richness and abundance and butterfly and bee richness and abundance. Additionally, when nectaring was observed during our surveys, we noted on which specific flowering species butterflies were feeding. Figure 21 lists these species by most to fewest observations of butterfly nectaring behavior. *Medicago sativa* (alfalfa) was the most visited species, with 74 nectaring observations by 12 butterfly species. Most nectaring observations on alfalfa were of *Colias philodice* (Clouded sulphur) butterflies, which lay their eggs on alfalfa and other legumes. Alfalfa mostly occurred in roadsides as spillover from adjacent agricultural alfalfa fields, and the combination of abundant blooming alfalfa and a variety of plant species in ROWs attracted many butterfly species.

Following *Medicago sativa* (alfalfa) in number of nectaring butterfly observations were *Sisymbrium altissimum* and *S. loeselii* (tall and small tumblemustard), which were abundant in roadsides and used by a variety of butterfly species (8 and 5 species, respectively). *Chrysothamnus viscidiflorus* and *Ericameria nauseosa* (yellow and rubber rabbitbrush) and *Helianthus annuus* (sunflower) had the most nectaring observations among native flora. In addition to attracting butterflies, we noted that sunflower was particularly popular with bumblebees late in the summer.

Number of Nectaring Butterflies by Plant Species
Includes all plants with at least one nectaring butterfly observation (all data included)

| Plant Species	Count
Medicago sativa	74
Sisymbrium altissimum	42
Sisymbrium loeselii	17
Chrysothamnus viscidiflorus	9
Rhaponticum repens	6
Carduus nutans	6
Brassica nigra	6
Helianthus annuus	5
Ericameria nauseosa	5
Cirsium arvense	4
Taraxacum officinale	3
Grindelia squarrosa	3
Convolvulus arvensis	3
Crepis acuminata	2
Achillea millefolium	2
Tragopogon dubius	1
Sinapis arvensis	1
Melilotus officinalis	1
Mentha arvensis	1
Lepidium draba	1
Elaeagnus angustifolia	1
Cynoglossum officinale	1
Asclepias speciosa	1

Figure 21 – Total number of nectaring butterfly observations at transects by plant species. Includes all transect survey data summed from both 2021 and 2022.

## 4.3 Transect Surveys—Butterflies

Our butterfly transect surveys identified 38 butterfly species of five butterfly families (Hesperiidae, Lycaenidae, Nymphalidae, Papilionidae, and Pieridae) at 63 sites in SE Idaho in 2021 and 2022. The single butterfly species in our study on the list of Idaho's Species of Greatest Conservation Need was *Danaus plexippus* (monarch butterfly) (Idaho Department of Fish and Game 2016). We found three adult monarch butterflies and three monarch larvae on our surveys, all in 2022. We also observed two relatively uncommon sagebrush specialists, *Satyrium behrii* (Behr's hairstreak) and *Satyrium semiluna* (Sagebrush sooty hairstreak), and a rare stray to Idaho, *Euptoieta claudia* (Variegated fritillary). The full butterfly species list is available in Appendix B.

Across all surveys (including our subset rounds of surveys in August), we observed a total of 1,482 individual butterflies over two years. The bulk of these observations (73.8%) were of Pierids (whites and sulphurs); these butterflies use several species of abundant roadside plants (Brassicaceae (mustards) and Fabaceae (legumes), respectively) as their host plants for reproduction (Brock and Kaufman 2003). The most abundant butterfly, *Pieres rapae* (Cabbage white), was also the only non-native butterfly species we observed, and constituted 31.7% of our butterfly observations. Other common roadside species included *Colias philodice* (Clouded sulphur, 27.9% of observations), *Pontia beckerii* (Becker's white, 9.1%), and *Coenonympha tullia* (Common ringlet, 7.8%). The total numbers observed of each butterfly species are shown in Appendix D**Error! Reference source not found.**.

Butterfly diversity and abundance was generally low along roadsides in our study area. At 68 (26.6%) of our 252 plot surveys in June and July, we detected no butterflies within the 100-meter, 10-minute survey. Overall, these surveys averaged less than one (0.9) butterfly species and less than three (2.4) individuals per survey. There were some notable exceptions, including consistently high butterfly richness at a transect along I-86 near American Falls, and high numbers of butterflies along I-15 near Blackfoot, as shown in Figure 22. Many transects had high butterfly richness and/or abundance on certain surveys, but lower levels on other sampling dates.
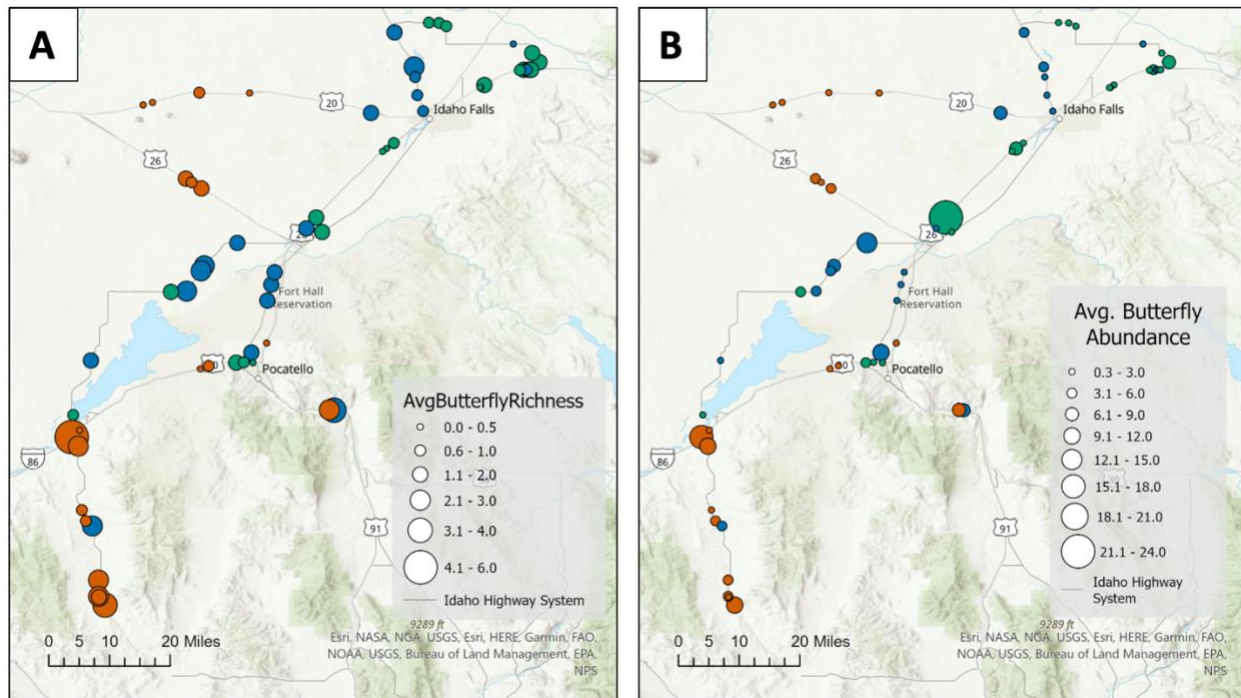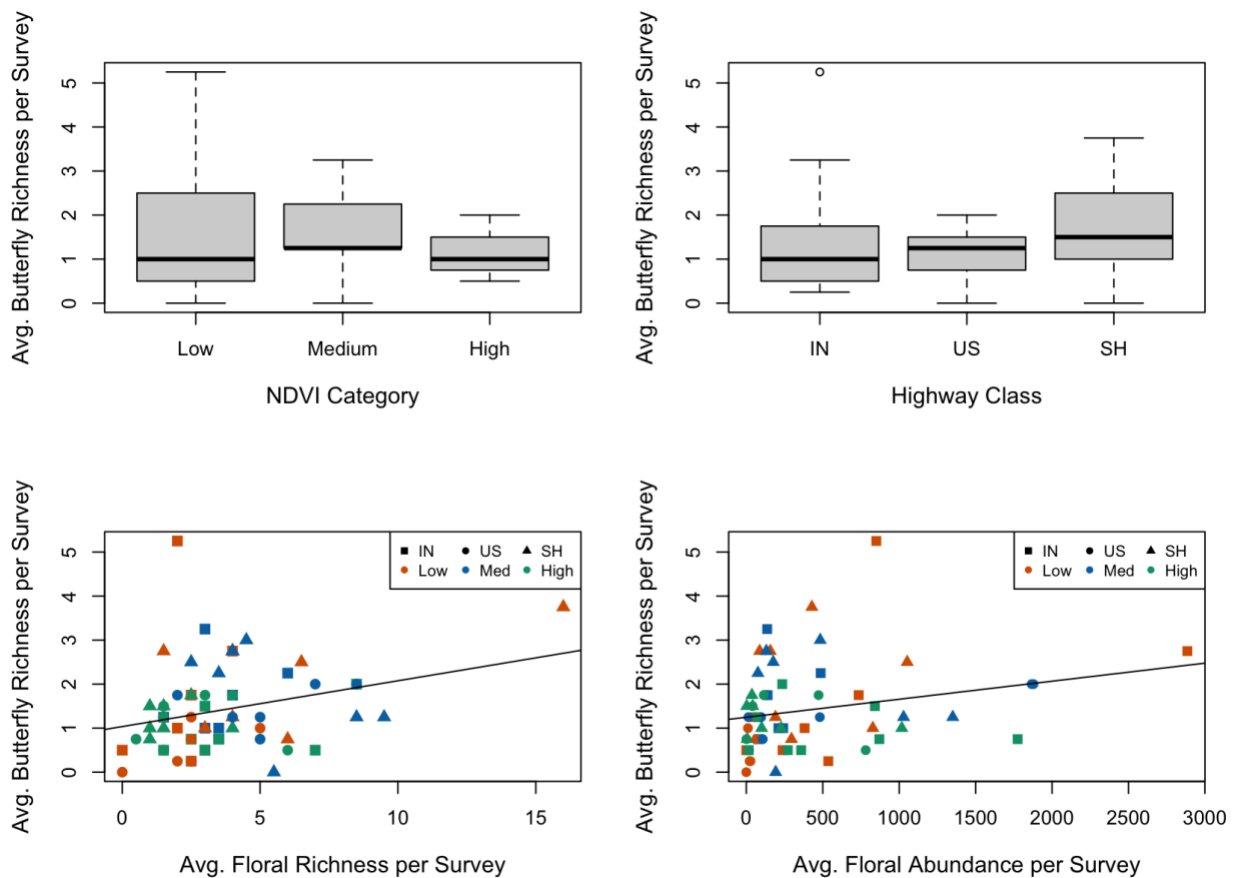

Figure 22 - Average butterfly richness (A) and abundance (B) of transects across study area in 2021 and 2022. Size of dots represents the average number of butterfly species (A) and average number of individual butterflies (B) recorded on surveys of that transect; color of dots signifies NDVI class, from low (orange) to medium (blue) to high (green).
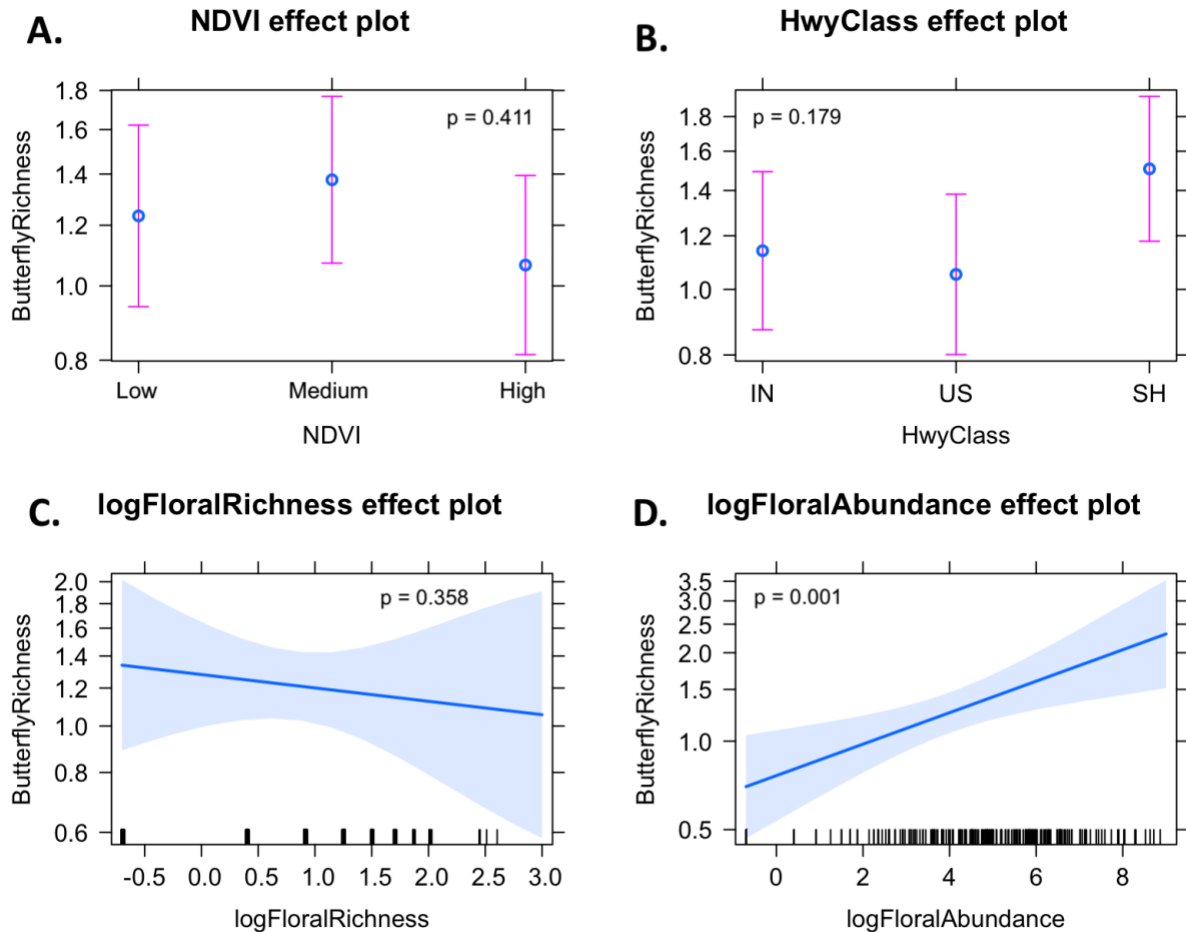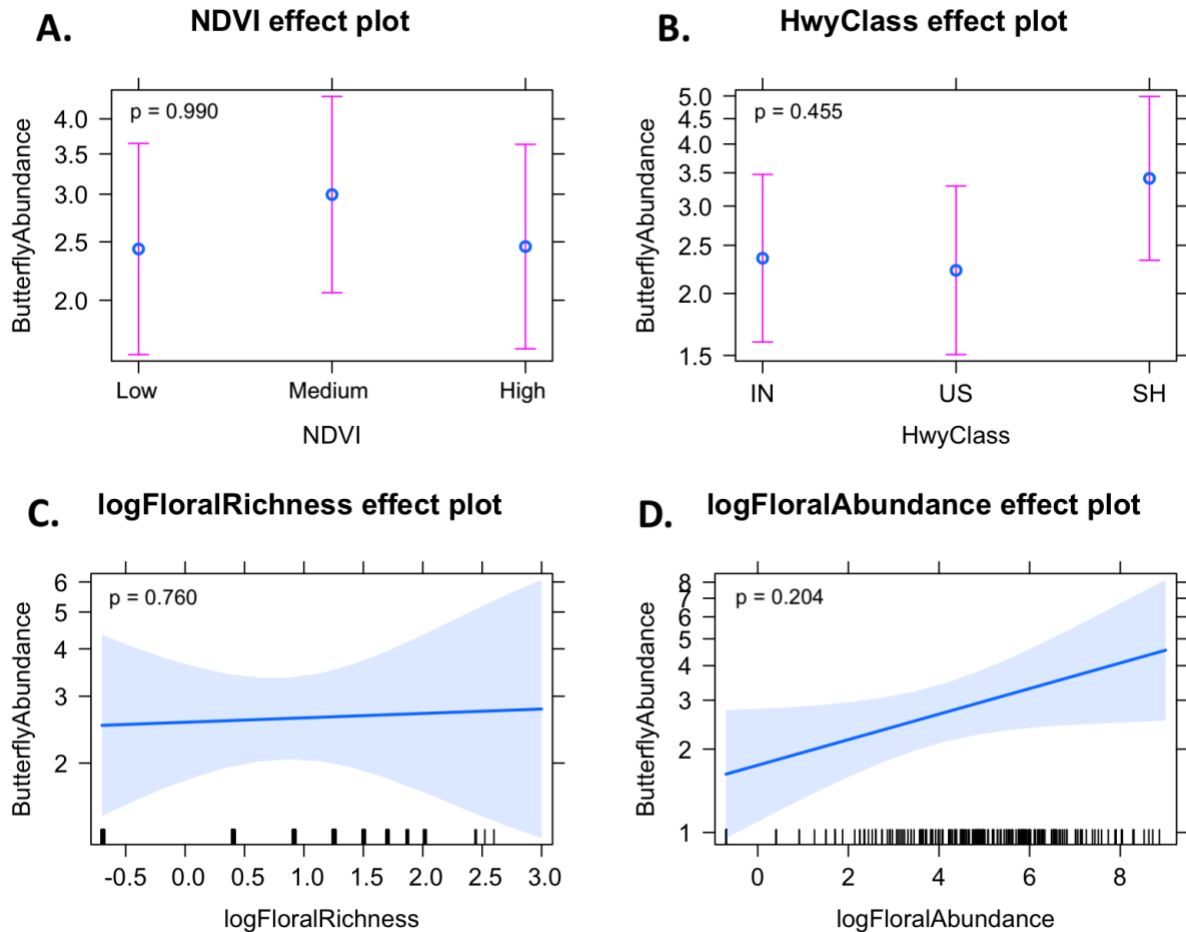
Figure 23 – Effect plots of four variables on <u>butterfly species richness</u> from a Generalized Linear Mixed Model with Poisson distribution. A.) NDVI categories range from low (less green) to high (greener). B.) Highway classes are interstate (IN), U.S. highways (US), and State highways (SH), in order of decreasing size and traffic intensity. C.) Floral richness and (D.) floral abundance are log-transformed. *p*-values, calculated from an ANOVA on the model using the Likelihood Ratio Test, indicate a significant log-log relationship between floral abundance and butterfly richness.

Using data from our four complete survey replicates (June/July 2021 and 2022) to model species richness (hereafter, richness) and abundance of butterflies in relation to NDVI class, highway class, floral richness, and floral abundance, we found that butterfly richness did not vary significantly between NDVI (Figure 23A) or highway classes (Figure 23B) (Generalized Linear Mixed Model (GLMM) with Poisson distribution, ANOVA p = 0.411 for NDVI, p = 0.179 for highway class). Butterfly richness also did not vary significantly with floral species richness (GLMM with Poisson, ANOVA p = 0.358; Figure 23C). Butterfly richness did increase significantly with increasing floral abundance (GLMM with Poisson, ANOVA p = 0.001; Figure 23D). With all other variables kept constant, each doubling of floral abundance would be expected to lead to an 8.9% increase in butterfly richness.
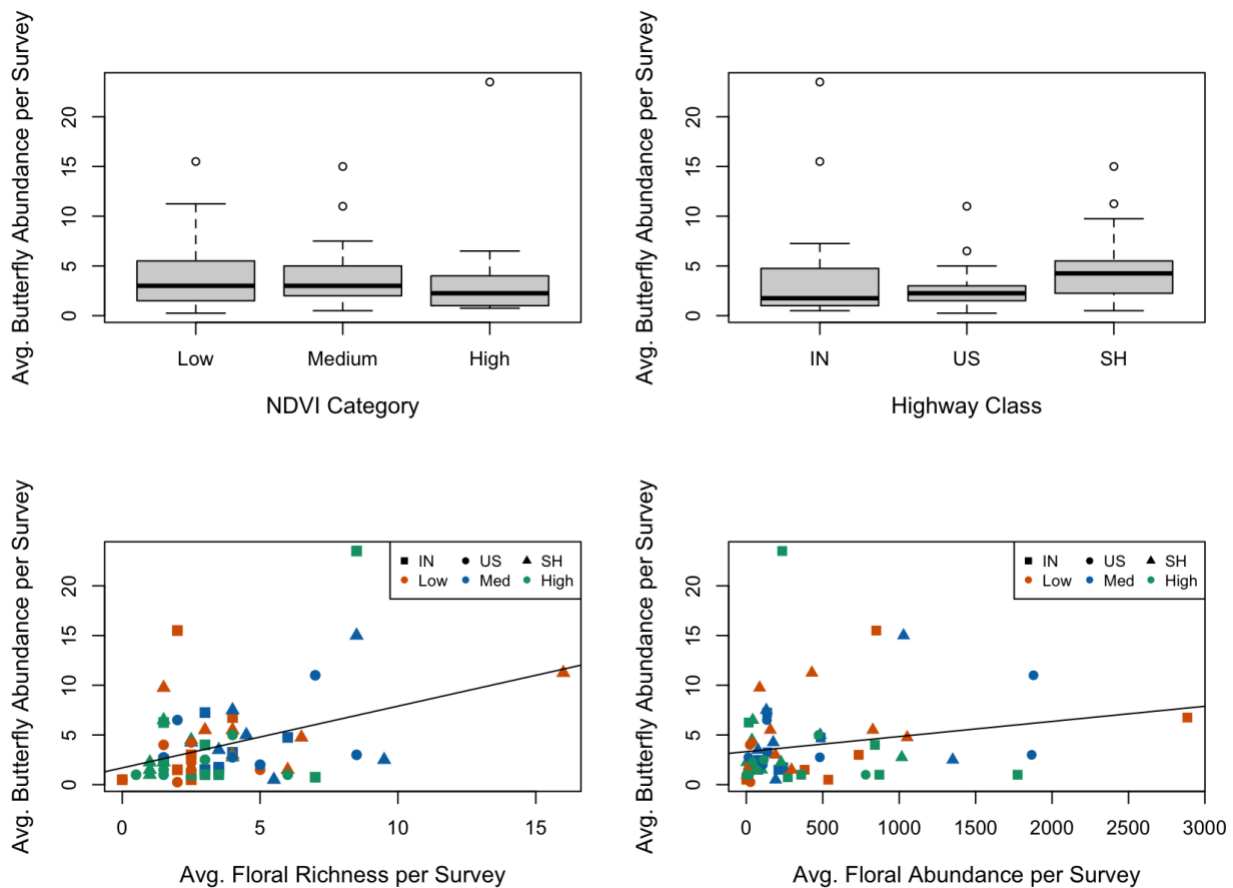
Figure 24 – Average <u>butterfly richness</u> by NDVI class, highway class, floral richness, and floral abundance. Each point represents the average value from four surveys of a single transect. Shapes and colors in the scatterplots indicate highway class and NDVI, respectively. This figure does not show the effects of variables in our model or any model results, but instead shows the distributions of our (averaged) untransformed data.

Although butterfly richness did not vary significantly with NDVI class in our models, averaging richness across surveys for each transect did reveal trends in NDVI classes, as shown with untransformed, averaged data in Figure 24. The 12 transects with highest average butterfly richness occurred in the low and medium NDVI classes, with the highest average butterfly richness values occurring in the low NDVI class. Transects in the high NDVI class, by contrast, generally had low butterfly richness, never exceeding an average of two butterfly species per survey.

Figure 25 - Effect plots of four variables on <u>butterfly abundance</u> **from a Generalized Linear Mixed Model with negative binomial distribution. (A) NDVI categories range from low (less green) to high (greener). (B) Highway classes are interstate (IN), U.S. highways (US), and State highways (SH), in order of decreasing size and traffic intensity. (C) Floral richness and (D) floral abundance are log-transformed.** *p***-values, calculated from an ANOVA on the model using the Likelihood Ratio Test method, indicate no significant relationships.**

Butterfly abundance did not vary significantly between NDVI or highway classes, or with changes in floral richness or floral abundance (GLMM with negative binomial distribution, ANOVA $p = 0.990$ for NDVI, p = 0.455 for highway class, $p = 0.760$ for log floral richness, $p = 0.204$ for log floral abundance). These results are shown in Figure 25. The distributions of the untransformed data averaged for each transect are shown in Figure 26.

Figure 26 - Average <u>butterfly abundance</u> by NDVI class, highway class, floral richness, and floral abundance. Each point represents the average value from four surveys of a single transect. Shapes and colors in the scatterplots indicate highway class and NDVI, respectively. This figure does not show the effects of variables in our model or any model results, but instead shows the distributions of our (averaged) untransformed data.

Butterfly species composition varied significantly with NDVI class, highway class, and floral richness (Table 7). NDVI classes varied statistically in their dispersion (i.e., beta-diversity, or among-transect variation in species composition) (ANOVA p < 0.001), while highway classes did not (ANOVA p = 0.478). Low NDVI plots had more transect-to-transect variation (beta-diversity) in butterfly species composition than high NDVI plots (Figure 27). While many species contributed small amounts to this variation in composition, over 50% of the variation between each pair of NDVI classes was driven by three common Pierid butterflies: *Pontia beckerii* (Becker's white), *Pieris rapae* (Cabbage white), and *Colias philiodice* (Clouded sulphur). Among these, *Pontia beckerii* was most common at low NDVI transects, while *Pieris rapae* and *Colias philodice* were more common at medium and high NDVI transects, respectively.

Table 7 - Results of a PERMANOVA (*adonis2*) test assessing the contributions of four factors to variation in <u>butterfly species composition</u>.

PERMANOVA results	Df	F	*p*
NDVI Class	2	2.799	**0.001**
Highway Class	2	6.721	**0.001**
Floral Richness	1	2.104	**0.032**
Floral Abundance	1	1.481	0.139
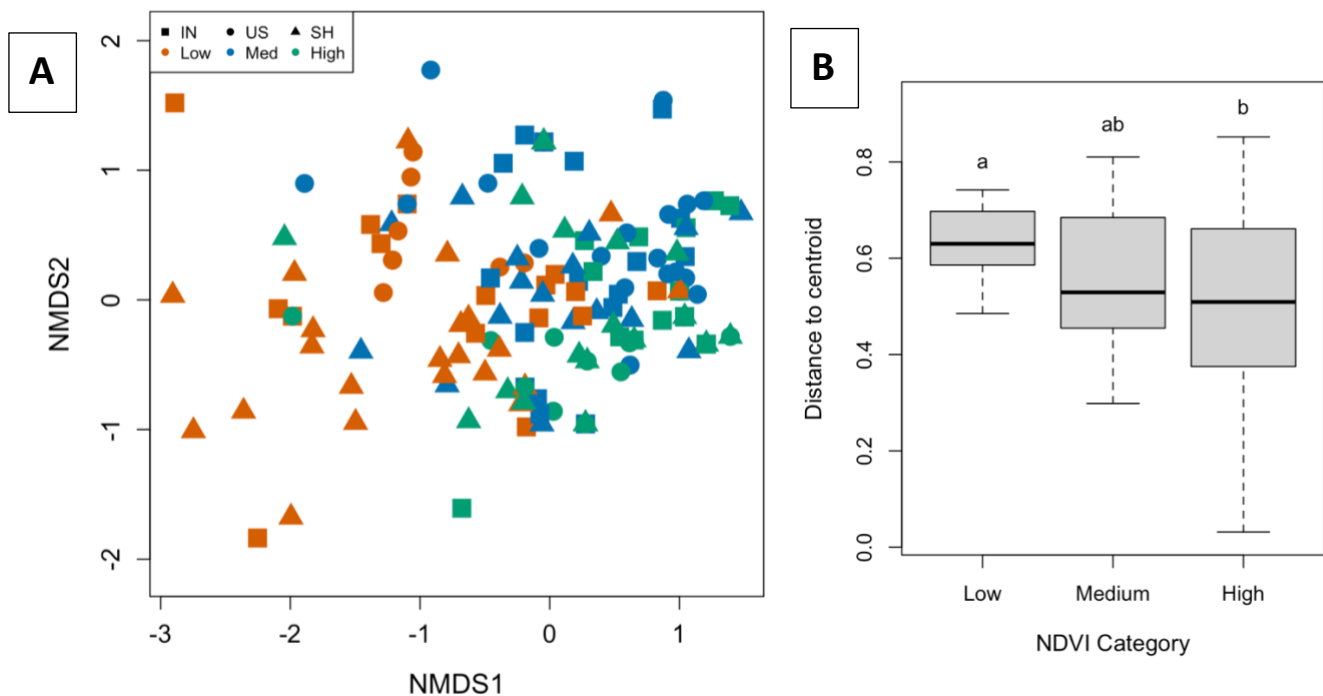
Figure 27 - A. Ordination (NMDS) of <u>butterfly species composition</u> by highway class (shape) and NDVI (color). Each point represents a single survey with at least one butterfly observation. B. Boxplots of community dispersion (distance-to-centroid) of butterflies in low, medium, and high NDVI classes. Letters indicate significant differences in community dispersion (i.e., beta-diversity) (P < 0.05) between NDVI classes based on Tukey's tests. Distance-to-centroids were calculated using Bray-Curtis dissimilarities.

4.4 Transect Surveys—Bees

Our pan-trapping bee surveys identified 70 bee taxa representing five bee families (Apidae, Megachilidae, Halictidae, Andrenidae, and Colletidae) at 27 sites in SE Idaho during 2021, as listed in Appendix C. Among these taxa, 61 were identified to species, 6 were identified to species group (indicating one or more related species within a genus, mostly within the taxonomically difficult and speciose genus *Lasioglossum*), and 3 were identified to genus. Thus, the total number of bee species in our study was at least 67 and likely over 70, given that some groups (e.g., *Lasioglossum—Dialictus*) with multiple specimens probably contained multiple species, although taxonomy and identification of species within these groups is extremely difficult and ever-evolving (Gibbs 2011). We treated the 70 identified taxa as distinct for analyses of transect bee species richness, whether they were identified to species, subgroup, or genus. This treatment was appropriate because taxa identified only to genus did not occur on the same transect with any specific species of that genus (e.g., *Melissodes* sp. and *Melissodes saponellus*).

We collected 1,485 individual bees. Halictids (sweat bees) were our most abundant family, representing 1,253 bees (84.4% of our sample), all in the tribe Halictini. This was expected, as the group is especially attracted to pan traps (Roulston, Smith, and Brewster 2007; Droege et al. 2010). *Lasioglossum, Halictus,* and *Agapostemon* were our most abundant genera sampled. *Lasioglossum* was also represented by the most species in our study, with 16 different taxa (including species and species groups) represented; *Osmia* (mason bees) was also diversely represented, with 10 species, and *Melissodes,* with 5 species. *Diadasia,* a genus of bees that specialize on specific flowers, especially Malvaceae (mallows) and Cactaceae (cacti), was surprisingly widespread in our study. One species, *Diadasia diminuta,* a specialist bee on *Sphaeralcea spp.* (globemallow), was collected on 19 of our 27 transects, even though we recorded globemallow at none of these transects (and only observed it nearby at two sites).

93.6% of the bees we collected were ground-nesters (Wilson and Carril 2016). We also collected 27 species of bees (in smaller numbers) that nest in plant stems, wood, or use pre-existing cavities. We collected 3 bee species that lay their eggs in the nest cells of other bees (kleptoparasites)—*Dioxys pomonae, Sphecodes* sp., and *Nomad* sp.—and one non-native bee, *Apis mellifera,* the European honeybee, each in very small numbers (one or two individuals).

Honeybees, like bumblebees, generally avoid pan traps and are undersampled using this collection method; many more honeybees and bumblebees were observed in the field than caught in our traps (Roulston, Smith, and Brewster 2007). Nonetheless, we did collect three bumblebee species (*Bombus griseocollis, B. huntii,* and *B. rufocinctus*) and observed a fourth (*B. nevadensis*) in the field. One of these species, *Bombus huntii* (Hunt's bumblebee), is on the list of Idaho's Species of Greatest Conservation Need (Idaho Department of Fish and Game 2016). As discussed later, most bee populations are poorly known and more bee species are inevitably deserving of this conservation status than are currently listed (Lebuhn et al. 2013).
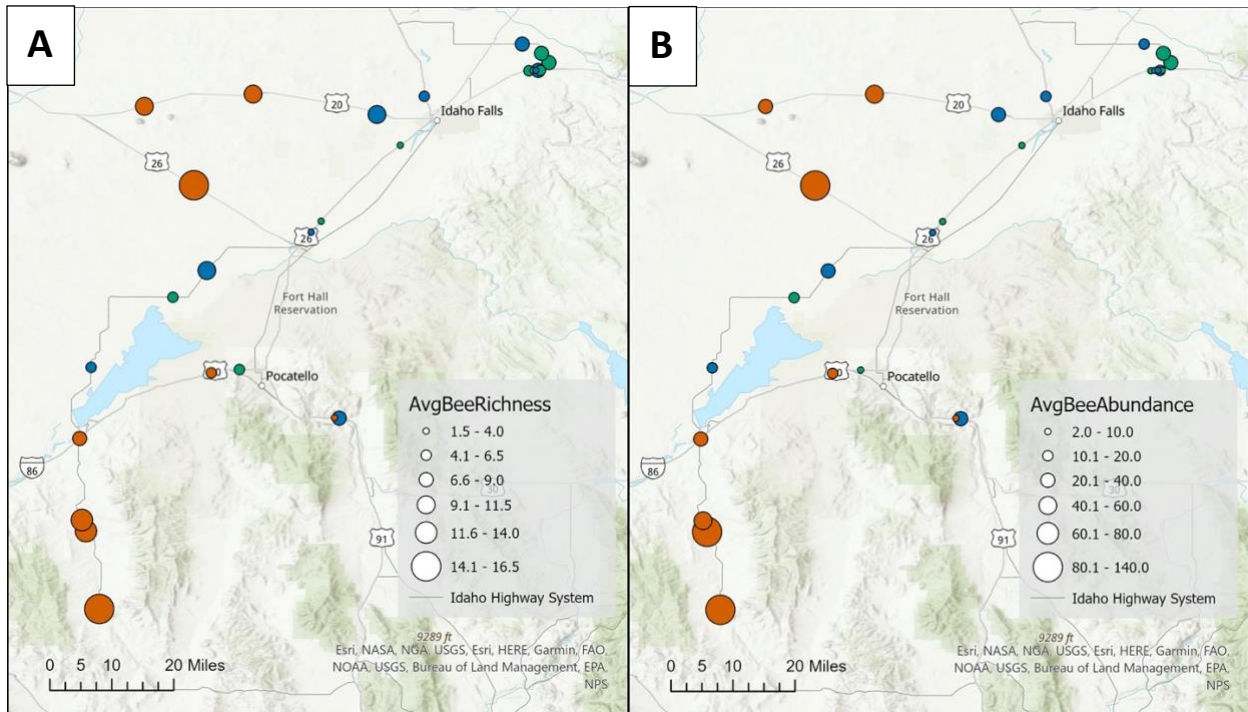
Figure 28 – A. Average bee richness and (B.) abundance of transects across study area. Size of dots represents (A) average number of bee species and (B) average number of individual bees recorded per survey of that transect; color of dots signifies NDVI class, from low (orange) to medium (blue) to high (green).

We observed high numbers and diversity of bees at some sites, even in the absence of roadside flowers. The presence of sagebrush steppe, one of the few relatively intact native habitats in our study area, was often associated with high numbers and diversity of bees. These productive sites mostly occurred along State Highway 37 (south from American Falls) and U.S. Highways 26 and 20 (from Blackfoot and Idaho Falls, respectively, toward Arco), as shown in Figure 28. Transects that were in ROWs adjacent to properties with sagebrush steppe averaged over twice as many bee species and more than four times as many individual bees per survey as transects that were in ROWs adjacent to other land cover types (Figure 29).
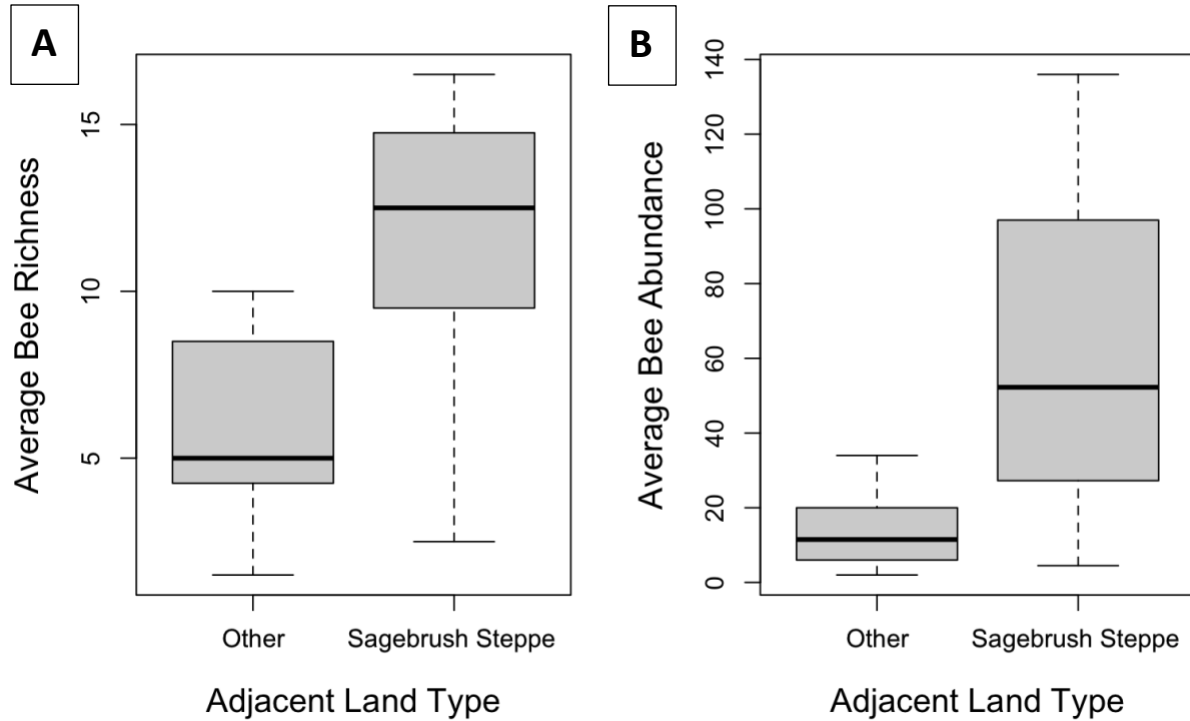
Figure 29 – Distribution of values for (A.) average bee richness and (B.) average bee abundance per survey for transects in ROWs bordering sagebrush steppe versus other land cover types. Transects in ROWs adjacent to sagebrush steppe (n = 8) had higher (A.) average bee richness (mean = 14.03) and (B.) abundance (mean = 61.69) than transects adjacent to any other land cover types, including pasture, agricultural lands, and developed areas (n = 19; mean richness = 5.95, mean abundance = 11.56).

Bee species richness increased as NDVI decreased; low NDVI (less green) sites had significantly more bee species than medium and high NDVI (greener) sites (GLMM with Poisson, ANOVA $p < 0.001$, Figure 30A). Bee richness also increased between highway classes of decreasing road size and traffic levels; state highways had the highest mean bee richness, with U.S. highways next, and interstates the least. This relationship was also significant in the model ($p = 0.001$, Figure 30B). In addition, bee richness showed significant positive and negative log-log relationships with floral richness ($p < 0.001$, Figure 30C) and floral abundance ($p < 0.001$, Figure 30D), respectively. More flowering plant species were associated with more bee species, but more individual flowers were associated with fewer bee species. The untransformed average bee richness values for each transect are shown in Figure 31.
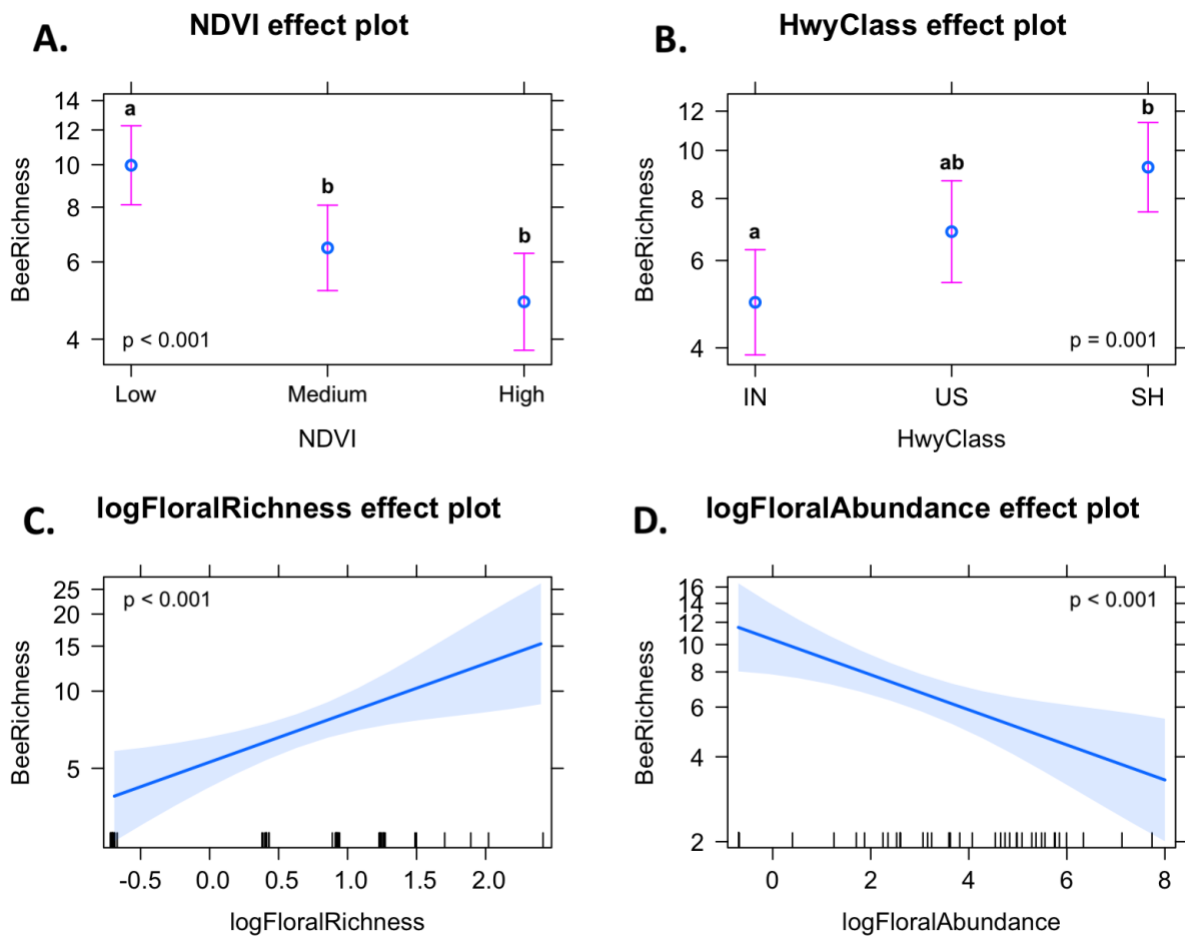
Figure 30 – Effect plots of four variables on <u>bee richness</u> from a Generalized Linear Mixed Model with Poisson distribution. Floral richness and floral abundance are log-transformed. p-values, calculated from an ANOVA on the model using the Likelihood Ratio Test method, indicate that all four variables included in the model are significant in explaining variation in bee richness. Letters indicate significant differences between NDVI and highway classes (p < 0.05) based on Tukey's tests.
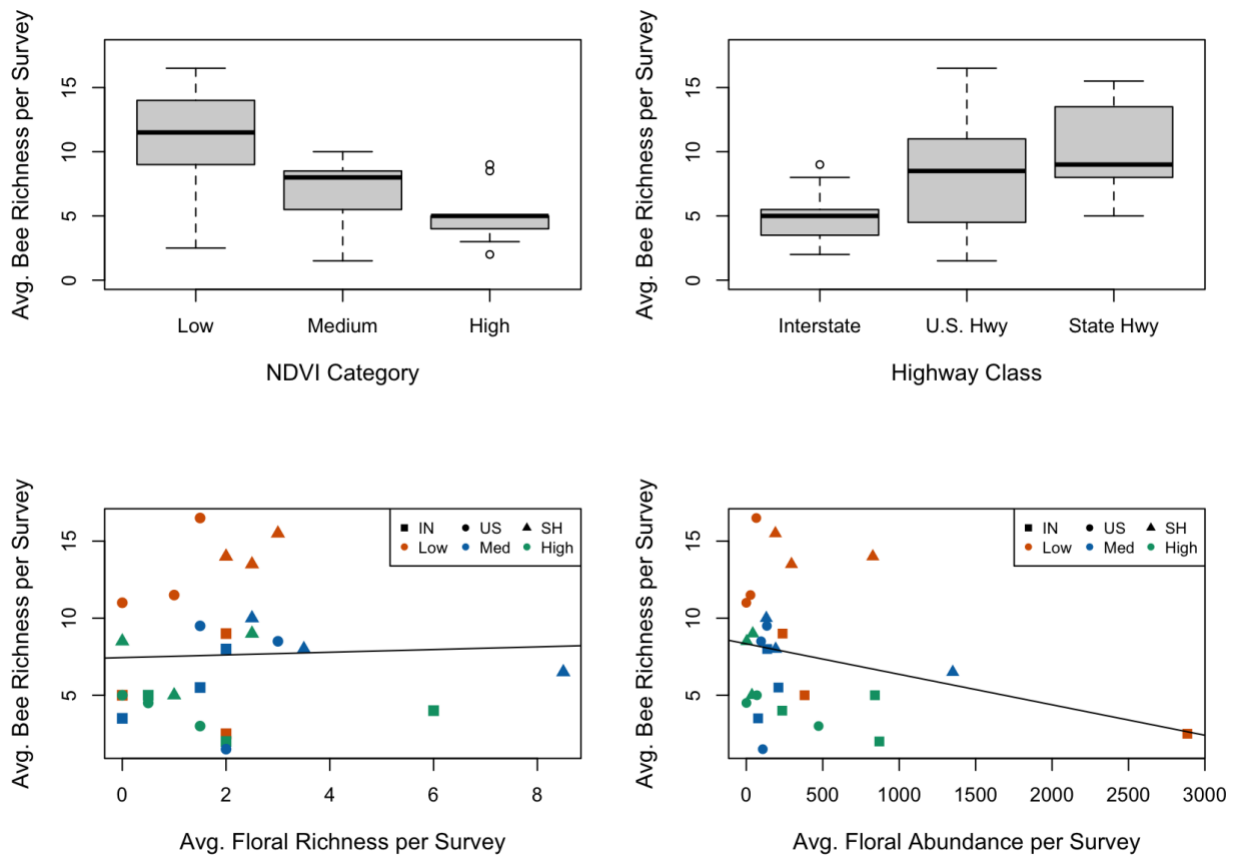
Figure 31 - Average <u>bee richness</u> by NDVI class, highway class, floral richness, and floral abundance. Each point represents the average value from four surveys of a single transect. Shapes and colors in the scatterplots indicate highway class and NDVI, respectively. This figure does not show the effects of variables in our model or any model results, but instead shows the distributions of averages of untransformed data.

Bee abundance showed similar patterns to bee richness, and the two were highly correlated (Pearson's r = 0.89, $p < 0.001$). Bee abundance increased as NDVI decreased; low NDVI (less green) sites had significantly more individual bees per survey than medium and high NDVI (greener) sites (GLMM with Poisson, ANOVA $p < 0.001$, Figure 32A). Bee abundance also increased between highway classes of decreasing road size and traffic levels; state highways had the highest mean bee richness, with U.S. highways next, and interstates the least, but this variable was only marginally significant in the model ($p = 0.084$, Figure 32B). Bee richness showed significant positive and negative log-log relationships with floral richness ($p < 0.001$, Figure 32C) and floral abundance ($p < 0.001$, Figure 32D), respectively. More flowering plant species were associated with more individual bees, but more individual flowers were associated with fewer bees. The untransformed average bee abundance values for each transect are shown in Figure 33.
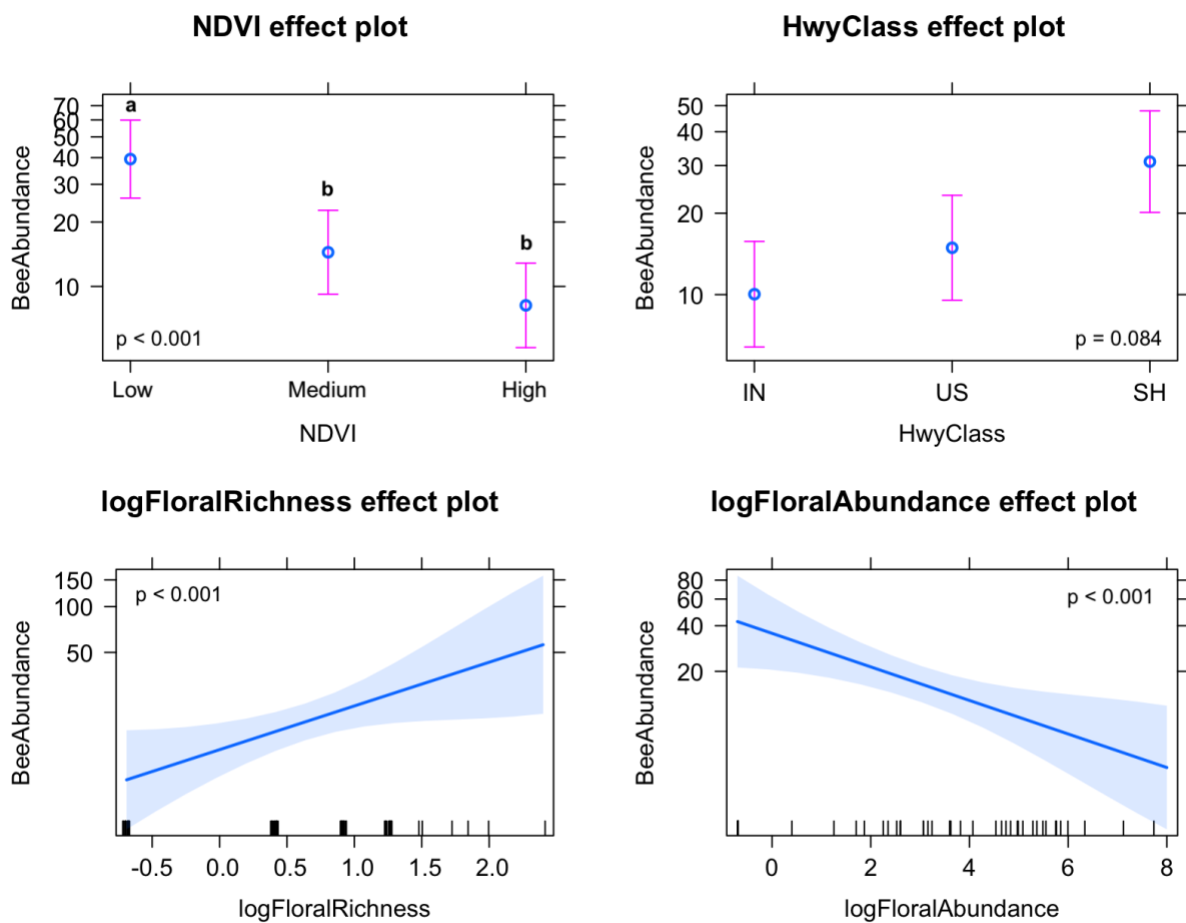
Figure 32 - Effect of four variables on <u>bee abundance</u> from a Generalized Linear Mixed Model with negative binomial distribution. Floral richness and floral abundance are log-transformed. p-values, calculated from an ANOVA on the model using the Likelihood Ratio Test method, indicate that NDVI, floral richness, and floral abundance are significant in explaining variation in bee abundance. Letters indicate significant differences between NDVI classes (p < 0.05) based on Tukey's tests.
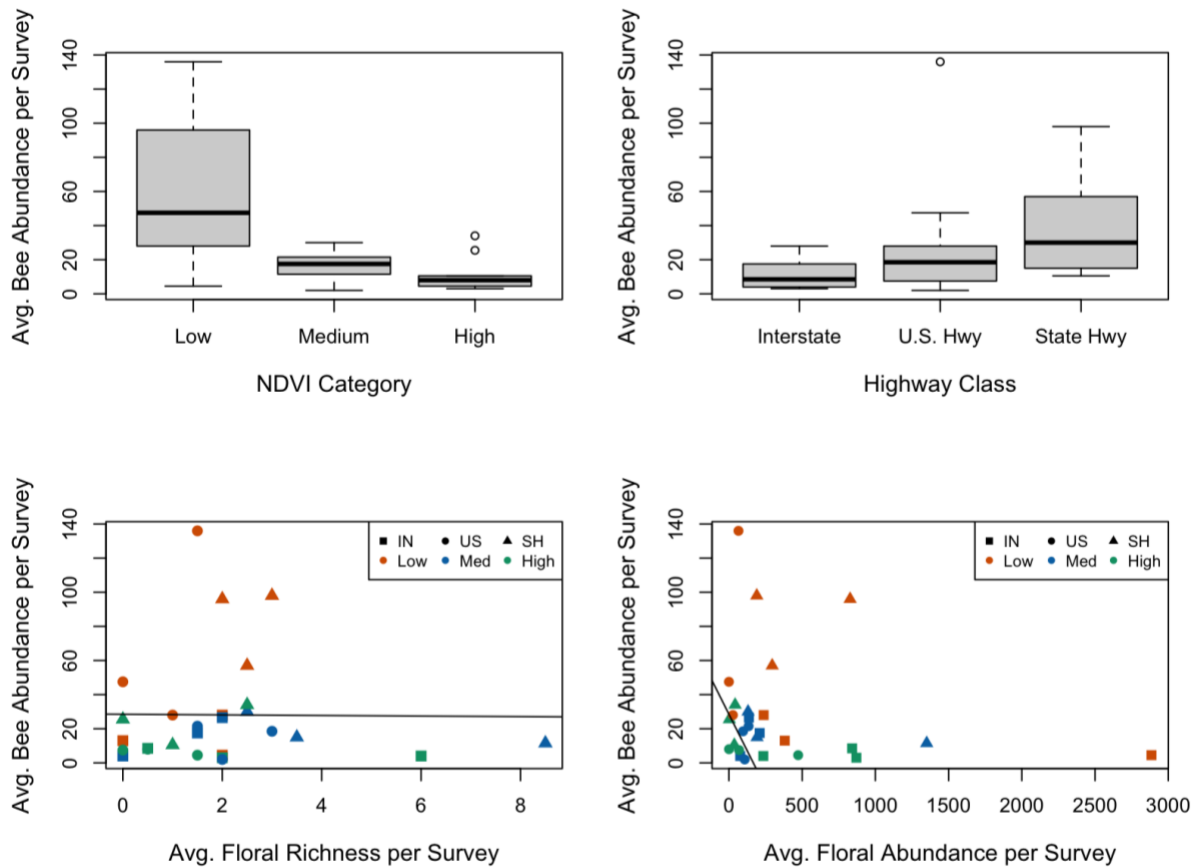
Figure 33 - Average <u>bee abundance</u> by NDVI class, highway class, floral richness, and floral abundance. Each point represents the average value from four surveys of a single transect. Shapes and colors in the scatterplots indicate highway class and NDVI, respectively. This figure does not show the effects of variables in our model or any model results, but instead shows the distributions of our (averaged) untransformed data.

Bee species composition varied significantly with NDVI class, highway class, and floral abundance (Table 8). Many bee species contributed to this compositional variation. The largest contributors to variation between highway classes were *Halictus tripartitus* and *Agapostemon angelicus/texanus,* species which were abundant along State highway transects but not along Interstates; *A. angelicus/texanus* was also fairly common along U.S. highway transects, but *H. tripartitus* was not. *Lasioglossum brunneiventre* and *Lasioglossum incompletum* were also key contributors; the former species was mostly found along U.S. highway transects, while the latter was found more along State highway transects. These species were also important contributors to variation between NDVI classes; *H. tripartitus, A. angelicus/texanus, L. brunneiventre,* and *L. incompletum* were all much more common along low NDVI transects than medium or high NDVI transects. They were also less common along medium than along high NDVI transects, although *Halictus ligatus* had a greater role in explaining compositional variation between these classes than did the *Lasioglossum* species. Also of note were three species, *Agapostemon virescens, Andrena*

scurra, and *Lasioglossum hudsionellum,* which were absent from high NDVI transects and very rare at medium NDVI transects, yet totaled over 55 individuals in our study, mostly from low NDVI transects.

Figure 34 shows that low NDVI sites, particularly those along State and US highways, were relatively similar to each other but differed in their composition from other sites. Neither NDVI nor highway classes varied significantly in their community dispersion (beta-diversity) ($p = 0.051$ for NDVI; $p = 0.083$ for highway class).

Table 8 - Results of a PERMANOVA (adonis2) test assessing the contributions of four factors to <u>bee species composition</u>.

PERMANOVA results	Df	F	*p*
NDVI Class	2	2.409	**0.002**
Highway Class	2	1.865	**0.001**
Floral Richness	1	1.096	**0.027**
Floral Abundance	1	1.730	**0.046**
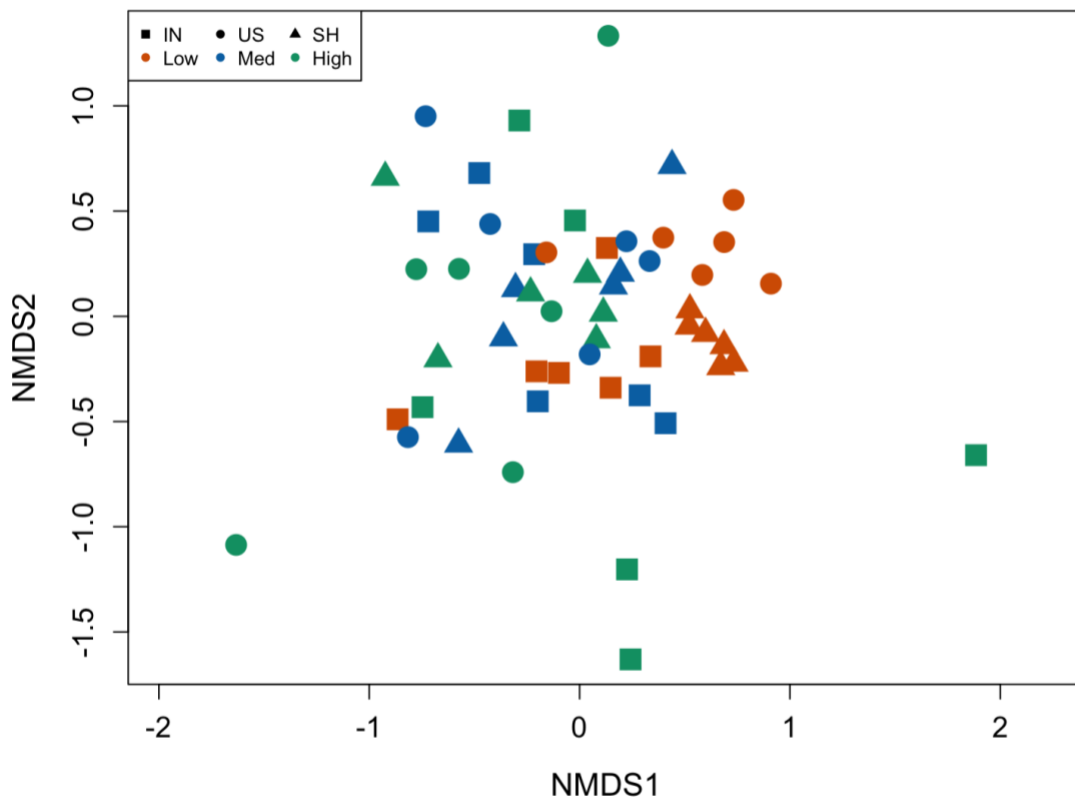
Figure 34 - Ordination (NMDS) of <u>bee species composition</u> by highway class (shape) and NDVI (color). Each point represents a single pan-trapping survey (all surveys collected at least one bee).

5. Discussion

5.1 Milkweed and Monarchs in Idaho Roadsides

Asclepias spp*.* (milkweeds) are important native plants in our wild and cultivated ecosystems: in addition to attracting beneficial insects and pollinators to agricultural systems (Fiedler, Landis, and Wratten 2008; James et al. 2016), milkweeds serve as the larval food source for monarch butterflies, which require the plants to complete their life cycle.

Asclepias speciosa (showy milkweed) favors disturbed areas, often growing abundantly along roads and canals, at field edges, or amid crops (Borders et al. 2013). This preference opens an extensive array of habitats for the plant, but it also exposes milkweed and its insect associates to dangers. With the intensification of agriculture and development of herbicide-resistant crops in recent decades, herbicide application has increased, substantially reducing the amount of milkweed growing in cropfields (Pleasants and Oberhauser 2013). Many of milkweed's preferred edge habitats have also been lost in the conversion to huge single-crop monocultures maintained by pesticides. These changes have not only affected the populations of various milkweed species (*Asclepias* sp.), but also impact the variety of insects that rely on this unique group of plants for forage and reproduction (Pleasants and Oberhauser 2013). In many parts of the United States, milkweeds have become not only scarcer outside of protected areas, but riskier host plants, because the life cycles of insects that depend on them are more frequently cut short by mowing, spraying, or development (Myers et al. 2019). In the eastern United States, declines in *Asclepias syriaca* (common milkweed) have been attributed to steep losses of monarch butterflies over the past several decades (Malcolm 2018). As agricultural intensification accelerates, many scientists and conservationists have turned to remaining undeveloped areas as refuges of milkweed habitat, among them roadsides and right-of-way lands, where milkweeds often thrive (Knight et al. 2019). Although roadsides and other undeveloped edges are marginal when taken individually, in sum these lands total over 15 million acres of potential milkweed habitat, making them critical in the efforts to recover monarch populations (Hopwood, Black, and Fleury 2015).

Danaus plexippus (the monarch butterfly) has two main populations in North America, distinguished by their wintering sites and, generally, by their breeding grounds. Monarchs that breed east of the Rockies congregate in winter in the montane oyamel fir forests of south-central Mexico. Monarchs that breed west of the Rockies winter in groves of eucalyptus, Monterey pine, cypress, and redwood along the California coast (Griffiths and Villablanca 2015; Lyons et al. 2012). Genetic and population time series evidence suggests mixture between the two populations, with some Idaho breeders possibly arriving from the eastern wintering grounds in Mexico (Vandenbosch 2007). However, the eastern and western populations remain morphologically distinct when raised together in the same location, and the extent of crossover between populations is not yet understood (Davis, Farrey, and Altizer 2005; Altizer and Davis 2010). While the eastern population has experienced precipitous declines of more than 80% since the mid-1990's (Semmens et al. 2016), the western population has experienced even steeper losses, with a population reduction of over 95% since the 1980's (Pelton et al. 2019; Xerces Society 2023).

Although there is vigorous debate over the causes of western monarchs' dramatic decline (and its remarkable, if tenuous, 100-fold rebound from November 2020 to November 2021), scientists agree that milkweed is an essential requirement for monarchs to complete their life cycle (Xerces Society 2023). As such, we were encouraged to find that *Asclepias speciosa* (showy milkweed) is generally common and widespread in roadsides across the southern Idaho highway system. We found the plant growing in particular abundance in the vicinity of Heyburn in south-central Idaho and Menan and Chester in southwest Idaho. We identified these locations as our "Tier 1" milkweed hotspots, as their especially high density of milkweed patches make them ideal areas for targeted monarch-friendly management, including reducing or eliminating spraying and mowing activities during the growing season. We also identified nine additional "Tier 2" hotspots, stretches of highway that also contained relatively high milkweed patch density. Together, these hotspot zones (detailed in Section 4.1.1, Table 3) are the principal areas we recommended enrolling as "adopted acres" for conservation protection with a Candidate Conservation Agreement with Assurance for the monarch butterfly. However, these zones contain only a fraction of all the milkweed growing along southern Idaho roadsides; as such, we strongly recommend that the location of milkweed patches across the entire Snake River Plain be considered when managing Idaho ROWs, and milkweed be managed with care wherever it occurs to ensure that monarchs are not incidentally killed.

Landscape factors can help managers predict milkweed occurrence in ROWs and assess the potential for ROWs to host milkweed populations. We observed that showy milkweed was particularly common in proximity to irrigated crops, canals, and riparian zones. Our analyses and comparison with the existing statewide milkweed suitability model (hereafter, "Svancara's model") suggest that milkweed typically occurs within 1.5 km of a perennial water source (Svancara, Abatzoglou, and Waterbury 2019). Growing a kilometer away from a perennial water source may not directly affect milkweed's access to water; however, it likely increases the plant's chances of accessing water above ambient rates through agricultural irrigation and near-surface groundwater availability. Milkweed patches most frequently occurred in ROWs adjacent to agriculture, which may be because agriculture increases water availability through irrigation spillover, or because agricultural activities are more common in areas with available water access and higher soil moisture. In some cases, agricultural activities might also create disturbance and edge habitat beneficial to milkweed colonization and maintain open landscapes favorable to milkweed seed dispersal (which occurs via wind in most species, include *A. speciosa*) (Wilbur 1976). However, we expect that disturbance and wind dispersal potential are high across the southern Idaho ROW system, regardless of adjacency to agriculture; water availability is thus the most likely driver of a relationship between roadside milkweed presence and adjacent agricultural land.

Along with distance to perennial water, Svancara's model identified four other factors as most influential in determining showy milkweed presence in the state: elevation, soil depth, precipitation of wettest month, and mean diurnal temperature range. Because our rapid assessment area was low in elevation compared with the rest of the state, we captured only a limited extent of the model's variation in our rapid assessment area (the Snake River Plain), thereby reducing the power of elevation as a variable to explain milkweed occurrence. We suspect that a similar phenomenon may have occurred

with some of Svancara's model's other predictor variables as well; they may have been important in excluding some parts of Idaho (mountain ranges, example), but when these parts were already excluded, their value in explaining variation in milkweed occurrence became far more limited.

The normal distributions (rather than directional trends) of our study's data in regard to the key variables identified by Svancara's model may result from capturing data only from the relatively arid, low-lying Snake River Plain as well as from capturing data only from roadsides, which have their own distinct soil depth characteristics and relationships with precipitation through runoff (Xu et al. 2009). These variables can help us understand why Svancara's model did not accurately predict the distribution of roadside milkweed patches in our study. While the climatic, geographic, and soil variables considered in the model do eliminate parts of the state not suitable for milkweed, such variables may not provide enough information to determine milkweed suitability in heavily modified areas (roadsides) across the relatively climatically similar Snake River Plain. Additionally, management regimes, which vary from roadside to roadside, may cause milkweed distribution in ROWs to differ from that of the overall landscape.

Svancara's model comparison and our adjacent land category data suggest that water availability may be the most important limiting factor for roadside milkweed growth in the arid Snake River Plain. This finding is supported by showy milkweed's known preference for moist soils (Borders et al. 2013). ROWs adjacent to streams, rivers, wetlands, canals, and irrigated land, which can often be easily differentiated in satellite imagery, are thus generally good candidates for milkweed habitat; we observed that the majority of large milkweed patches occurred adjacent to agricultural land (Figure 8).

Although milkweed's abundance in well-watered roadsides is encouraging, these habitats also put milkweed and monarchs at risk. Our research found that milkweed often occurs in small patches and in proximity to nonnative species, increasing its chance of being sprayed or mowed as a non-target species when managing roadside noxious weeds. This risk makes knowledge of milkweed locations particularly valuable for efforts to protect monarchs. Outside of ROWs, milkweed is also often treated as an agricultural weed and sprayed or mown, especially when it grows near or among crops (Pleasants and Oberhauser 2013, Waterbury, Potter, and Svancara 2019).

Although roadside monarchs may be relatively scarce compared with roadside milkweed, it is difficult to predict which milkweed plants are being utilized by monarchs, and thus any destruction—spraying or mowing—of milkweed plants may cause the accidental killing of monarchs. As such, conserving the existing showy milkweed community growing in Idaho's ROWs using the maps and locations identified in this project will serve to benefit monarchs, limit accidental destruction of monarch eggs and caterpillars, and increase the species' chances of successful breeding. Furthermore, the western monarch population will only be able to recover fully if a sufficient stock of milkweed for future growth is available on their breeding grounds; thus, having more than enough milkweed for the current population is essential to enable monarch recovery.

Monarchs were quite rare during our 2021 rapid assessment, likely reflecting the tiny population of migratory western monarchs that year (only 1,914 monarchs were counted at wintering sites in California in winter of 2020-21) (Xerces Society 2023). This scarcity made it difficult to make predictions about which roadside milkweed patches might be preferred by monarchs for breeding. Furthermore, given the abundance of milkweed relative to monarchs and the wide-ranging habits of the species, there is likely considerable stochasticity as to which milkweed patches are selected by ovipositing female monarchs. We were, however, able to document monarch breeding (ovipositing and the presence of eggs and larvae) on roadside milkweed in both 2021 and 2022, in southeastern Idaho near Chester (2021) and Pocatello (2022, plot US.2.2).

In our study, milkweed patches used by breeding monarchs varied in size from a few to 770 stems, but generally tended to be very floristically diverse, with each patch including at least 8 species of other plants in flower when evidence of monarch breeding was observed (compared with an average floral richness for roadside milkweed patches of 2.67 species). Although our sample size is too small to draw conclusions, this suggests that monarchs may prefer breeding in diverse floral communities (Pocius et al. 2018; Nestle, Daniels, and Dale 2020), which naturally might be more attractive to nectar-seeking adults. However, it should be noted that most floral species in roadsides—especially those in the wetter areas where milkweed tends to grow—are non-native; many are considered noxious weeds. The milkweed patch containing a patrolling adult male monarch and three larvae in 2022, for example, contained two species of *Sisymbrium* (tumble mustard) and two thistle species (*Cirsium arvense* and *Carduus nutans*), on which we observed the monarch nectaring. This raises a concern that roadside milkweed growing among noxious weeds is potentially preferred—or at least, used—by monarchs, putting developing monarchs in danger if these areas are treated with herbicides or mowed to control noxious weeds.

While the small sample size of monarchs found in our study provides only anecdotal evidence concerning monarch breeding preferences, in years like 2021, when the migratory western monarch population was fewer than 2,000 individuals, every monarch adult, egg, and larva counts towards the survival and continuation of the population. Given that each monarch butterfly has the potential to lay over 700 eggs (Oberhauser 1997), the mowing or spraying of even one roadside milkweed patch—for example, the patch in which we observed a female monarch ovipositing 14 eggs—may have a substantial negative impact on the local monarch population (Oberhauser and Solensky 2004). As such, we emphasize the importance of protecting existing milkweed plants in ROWs and avoiding blanket-spraying or mowing milkweed. While our study did not address the potential mortality of monarchs using ROW milkweeds, we encourage that further research also evaluate this risk, which has important implications for roadside milkweed management (Mitchell et al. 2020).

Spatial modeling can aid in guiding milkweed management by focusing conservation efforts on areas likely to harbor target milkweed presently or in the future. In Idaho, models developed by Leona Svancara and colleagues at IDFG offer a useful tool for predicting milkweed and monarch distribution across the state (Svancara, Abatzoglou, and Waterbury 2019). However, we hypothesized that these models, based on statewide data, would not successfully predict milkweed distributions in roadsides due to the modification and heavy human influence on plant communities of roadside lands. This

hypothesis was supported; the average suitability value of roadside milkweed patch locations from Svancara's model was only 0.53, making it an ineffective predictor. As is often the case, caution should be exercised when using landscape models at broad scales to direct conservation at fine scales without field validation. We hope that our survey of milkweed in Idaho roadsides fills an important data gap in this respect, allowing future models to better predict milkweed distribution in both natural and modified landscapes. Human modification and management often alter distributions from that predicted by climatic, topographical, and soil variables alone (Benítez-López, Alkemade, and Verweij 2010; Hooke, Martín-Duque, and Pedraza 2012), and ROW management can play a valuable role in making these lands either better or worse than expected for milkweed establishment and growth.

5.2 Flowering Plants in Idaho Roadsides

We recorded 87 flowering plant species within our 63 transects. Just over half of these species were native to the state, but these native species were far less common overall than the 41 non-native species. This is typical of roadsides, which are often dominated by non-native species that disperse and flourish in response to anthropogenic disturbance along roads (Kalwij, Milton, and McGeoch 2008; Pollnac et al. 2012). Overall, our data support the notion that abundant non-native plants likely provide the majority of nectar available to native butterflies and bees in many roadsides in our study area.

Non-native plants were particularly dominant at transects in the high NDVI class (with annual maximum NDVI values of 0.58 and above), which had very few native forbs; only four native flowering plants were observed in high NDVI transects, and these plants were rarely recorded therein, only appearing on 6 of 48 total surveys of high NDVI transects. These high NDVI transects also averaged the lowest floral richness, averaging 61% fewer flowering plant species per survey than medium NDVI transects. Since high NDVI transects are thus designated because they are extremely green, these areas are not lacking in plants, but rather appear to typically be dominated by one or a few prolific invasive species. For land managers seeking to identify priority areas for invasive species control from the office, our results suggest that 100 m (328 ft.) resolution annual maximum NDVI values above ~0.58 may represent a useful benchmark for identifying such areas.

Nonetheless, these high NDVI sites are not mere "sacrifice zones" to be sprayed and mowed without consideration. They were still extensively used by butterflies (see Figure 21 for butterfly nectaring observations). They also hosted a variety of bees (although fewer, on average, than low or medium NDVI sites). One high NDVI transect contained blooming *Asclepias speciosa* (showy milkweed). At high NDVI sites, techniques to manage invasive species before and after flowering begins could be effective, and many non-native flowering plants not considered noxious weeds can be left to provide for pollinators. Although native forbs may struggle to compete in these spaces, native pollinators still use the non-native flora for food and in some cases, for reproduction (nest sites for bees, host plants for butterflies). For example, *Colias philodice* (Clouded sulphur) reproduces on legumes (Fabaceae) including *Medicago sativa* (alfalfa) and *Melilotus spp.* (sweet clover), while *Pontia protodice* (Checkered white) and *Pontia beckerii* (Becker's white) both reproduce on mustards (Brassicaceae), including *Sisymbrium spp.*

(tumblemustard) common in ROWs (Brock & Kaufman, 2003). These native butterfly species in the Pieridae family, the most common family along Idaho roadsides, have adapted to using non-native host plants for reproduction, and many butterflies will feed on the nectar of non-native plants if available. Thus, we encourage management of invasive species that minimizes the destruction of pollinators by avoiding spraying or mowing during flowering times and applying herbicides only on state-listed noxious weeds, as detailed in our Recommendations section.

High maximum NDVI values represent areas that become very green at some point in the growing season due to plant growth. Although we did not measure overall plant cover by species, the near absence of native flowers in areas of high NDVI suggests that this green plant growth likely consists of dense grasses and/or non-native forbs. In the ROWs of southeastern Idaho, native flowering plants seem to struggle to compete in these high NDVI areas. We thus recommend focusing native plantings on sites with lower maximum NDVI values (< 0.5), where native forbs may stand a better chance of becoming established within the existing plant community.

A final takeaway from our plant data is that significant changes can occur in the roadside flora of Idaho from year to year, especially with changes in climate and water availability. Floral species were surveyed in 2022 that were not detected in 2021, and vice versa. Floral composition also varied across the summer, as expected, with different groups predominating in the early (mustards: *Sisymbrium, Descurainia, Brassica*), mid (thistles: *Cirsium, Carduus, Rhaponticum*), and late summer (rabbitbrush—*Chrysothamnus, Ericameria*, sunflower—*Helianthus*) in our study area. This seasonal variation in flowering is important to provision pollinators across the growing season; for example, late-blooming rabbitbrush and sunflowers may provide important nectar and pollen resources to support monarch butterflies on their southward migration as well as other late-season pollinators. As such, it is important to consider plant composition across months and years when making management decisions. Important plant species may not be visible in some years or times of year, and indiscriminate application of herbicides could have unintended negative impacts.

5.3 Bee and Butterfly Populations in Idaho Roadsides

Our results show strikingly different patterns of species richness and abundance between bees and butterflies across the roadside landscapes of southeastern Idaho. Bee richness varied significantly between NDVI and highway classes and with changes in floral richness and abundance, suggesting that these variables were indeed important factors in explaining how many bee species occur across different roadsides. Butterfly richness, on the other hand, was not significantly different between NDVI or highway classes, and only increased significantly with increasing floral abundance (bee richness, by contrast, declined as floral abundance increased). Pollinator abundance followed similar patterns; bee abundance differed significantly in association with all variables but highway class (and this association was nearly significant), while butterfly abundance varied significantly with none of the variables we studied.

These diverging patterns likely reflect differences in the ecology and natural history of bees and butterflies, including in their ranges, habitat needs, and relationships with plants, as well as the different methods used to sample them. Compared with bees, most butterflies are relatively wide-ranging, regularly covering several hundred meters in a day (Ovaskainen et al. 2008; Nowicki et al. 2014; Jones et al. 1980); by contrast, most bees are smaller, have smaller home ranges, and forage out and back from nests ("central-place foraging"), unlike butterflies (Greenleaf et al. 2007; Bell 1990). Butterflies may therefore be less constrained to the qualities of local habitat for reproduction and can forage more widely for nectar, pollen, and mates. Butterfly life cycles are tied to specific plants through their larval stage, when they feed on only a few plant species or groups (Brock and Kaufman 2003). Several of our most common roadside butterfly species use common, disturbance-tolerant roadside plants (e.g., mustards, grasses) or crop species (e.g., alfalfa) as their host plants; the presence of these butterflies—mostly pierids—might be driven by the availability of their host plants, which were widespread and often abundant along Interstates and in greener (higher NDVI) areas. Conversely, a host of relatively uncommon roadside butterfly species—for example, *Lycaena* spp. (coppers), *Satyrium* spp. (hairstreaks), and *Speyeria* spp. (fritillaries)—tend to prefer native, relatively disturbance-intolerant floral species, which were more often associated with lower NDVI classes and smaller highways. These contrasting preferences may balance each other, smoothing out differences in overall butterfly richness and abundance between NDVI and highway classes. This pattern was supported by the differences in butterfly species composition we found between highway and NDVI classes, with butterfly communities being more similar (i.e., composed of more widespread species) among high NDVI classes and more unique (i.e., composed of more local species) among low NDVI classes. Varying assemblages of uncommon, local butterflies at lower NDVI sites and similar assemblages of common generalists at high NDVI sites can create similar per-site species richness among NDVI classes.

Butterflies may also be cueing in to patterns of floral and host plant abundance, as well as sources of water and nutrients, that simply were not correlated to NDVI or highway class (Menéndez et al. 2007). We found more butterfly species at transects with more flowers; this was the only predictor in our butterfly richness models that was significant, and suggests that to find hotspots of butterfly diversity, we ought to look for areas with lots of flowers. Likewise, planting more flowers in ROWs may increase roadside butterfly diversity.

Unlike butterflies, most of which are generalist foragers as adults, many species of bees specialize in pollinating just a few species or genera as adults (Cane 2021). This can link floral species richness to bee species richness, since more forb species can support more species of specialist bees. Even generalist bee species, such as the halictids common in our study, may prefer habitats with a variety of floral species to ensure they get necessary nutrients and can find forage throughout the breeding season (Mallinger, Gibbs, and Gratton 2016; Ogilvie and Forrest 2017). Native bees do not use specific host plants for reproduction as do butterflies; rather, they rely on nesting sites in the soil, in plant stems, wood, or other material (Wilson and Carril 2016). This nesting requirement may tie bees more closely to habitat characteristics such as the availability of bare ground, uncompacted soils, woody plants, or standing stems, which in turn may be related to NDVI; in the field, we observed that low NDVI sites

more commonly included bare ground and native shrubs such as sagebrush, while high NDVI sites were usually covered in grass or weedy forbs, so had little bare ground. A global review of urban bee assemblages found that in *every case,* less groundcover was associated with higher bee species richness (Prendergast, Dixon, and Bateman 2022). Because bare ground has a low NDVI and plant cover has a high NDVI, NDVI may decrease in proportion to the amount of bare ground (Raynolds, Walker, and Maier 2006) and in correlation, increases in roadside bee richness.

Nearby sagebrush steppe was a common denominator in our sites of high bee richness and abundance, and sagebrush was almost always found in proximity to low NDVI sites. The sagebrush ecosystem is adapted to the arid conditions of the Great Basin ecoregion and to conserve water and protect limited plant resources, sagebrush and its associated forbs generally lack the large, bright green (high NDVI) profusions of foliage found in riparian and weedy species (Kremer and Running 1993). The sparser vegetation, higher percentage of bare ground, and less bright green foliage of typical sagebrush steppe all results in lower NDVI values for this ecosystem.

Because the NDVI categories in our study were determined by maximum NDVI across the growing season, extensive green plant cover could have led to high NDVI values even if these plants were short-lived and dead for much of the summer. This extensive green-up is common for roadside invasive plants, which can both cover all bare ground and outcompete native plants. While some invasives (e.g. *Sisymbrium altissimum, Rhaponticum repens*) provide abundant nectar sources that may partially compensate for these impacts—especially for nectar-reliant butterflies—others (e.g. *Bassia scoparia, Bromus tectorum*) provide pollinators little to nothing. Maximum annual NDVI may thus be useful in locating large patches of invasive plants (high values), and areas with fewer invasives and more native flora used by native bees (low values) (Madonsela et al. 2018; Liu et al. 2020).

Our data revealed dramatically different proportions of native to non-native plants across NDVI classes, offering another reason why bees might favor low NDVI transects, but not butterflies. Low NDVI classes included far more native species, while high NDVI transects had virtually zero native flowering plants and were dominated only by non-natives. Bees, which gather nectar and pollen to provision their young, may be more selective, choosing native species with preferred pollen lipid macronutrient ratios (Vaudo et al. 2020), while most adult butterflies, which only feed themselves and consume nectar, can afford to be less choosy and rely on abundant non-native nectar sources such as mustards, thistles, and other non-native weeds common in roadsides (Slansky et al. 1985). These species may provide abundant nectar sources but lack the pollen preferred by brood-rearing bees (Roulston and Cane 2000; Vaudo et al. 2020). Many studies have found increases in native bee richness and abundance in response to higher proportions of native flora (Prendergast, Dixon, and Bateman 2022; Fründ, Linsenmair, and Blüthgen 2010), as found in our low NDVI sites.

To summarize, maximum annual NDVI can provide useful information about roadside bee species richness and abundance in our study area by differentiating between native sagebrush steppe environments (which often contain more bare ground) and roadsides dominated by invasive species, and by locating areas with higher proportions of native flora. Butterflies, which do not depend on bare

ground or woody plants for nesting sites, feed mostly on nectar rather than pollen as adults, and were frequently observed visiting invasive roadside flowers, may thus not vary significantly with NDVI in their species richness or abundance, as do bees. Contrary to our original predictions that greener (higher NDVI) areas might harbor more pollinators, our results indicate that less green (lower NDVI) areas generally had higher bee richness and abundance.

These NDVI patterns may differ, or at least be less significant, in wetter ecoregions farther north in Idaho, where the native vegetation is typically denser and greener. Here, high NDVI areas may frequently be forested areas (which may also have fewer pollinators, but for different reasons). While we expect that patches of invasive species would still typically fall into the high NDVI category, with similar results for pollinators, there may be little difference in NDVI between these patches and native plant communities, and thus NDVI may be less useful a planning tool in Idaho's wetter ecoregions (Chen et al. 2014).

Interestingly, bee—but again, not butterfly—species richness also varied with highway class. The three classes of highways in our study varied in their pavement width, ROW width, and traffic intensity, as well as, to some extent, in the frequency and extent of their ROW management, with interstates having the highest widths and extents and state highways the lowest (Idaho Transportation Dept., pers. comm.). Highway class may mediate bee richness (and abundance) by creating barriers between bee populations which vary in permeability by highway size (Bhattacharya, Primack, and Gerwein 2002; Andersson et al. 2017; Dániel-Ferreira et al. 2022); for example, bees may be less common or diverse at the edges of population barriers created by large interstates (Fitch and Vaidya 2021, J. Brown et al. 1995).

Highway class may also impact bees through traffic collision mortality (Jackson and Fahrig 2011; Keilsohn, Narango, and Tallamy 2018; Baxter-Gilbert et al. 2015), and through toxins from vehicle emissions, exhaust, and tire dust (Xun et al. 2018; De Silva et al. 2021; Udevitz et al. 1980), all of which generally occur at higher levels on larger, more heavily trafficked highways (Bernardino et al. 2019). Bees nesting in roadside soils might be particularly susceptible to toxins and heavy metals from highways (De Silva et al. 2021; Van Bohemen and Van de Laak 2003); increasing vehicle use could increase roadside soil contamination and reduce numbers of nesting bees. These toxins also enter plant nectar and may dissuade foraging bees from visiting flowers along highly trafficked roadways (Meindl and Ashman 2013; Sivakoff and Gardiner 2017; Phillips, Bullock, Gaston, Hudson-Edwards, et al. 2021). Traffic also creates turbulence, which can disturb pollinators and reduce their floral visitation rates (Phillips, Bullock, Gaston, Hudson-Edwards, et al. 2021). Noise and dust from traffic can further deter insects (Bunkley et al. 2017; Łukowski et al. 2018), although a recent study found no apparent effect of these disturbances on pollinators (Phillips, Bullock, Gaston, Hudson-Edwards, et al. 2021). Given all these potential mechanisms, it is not surprising that increasing traffic has been associated with decreasing flying insect abundance (Martin et al. 2018), or that traffic levels may help drive the differences in bee richness and abundance between highway classes.

Another potentially important difference between highway classes—albeit a difficult one to measure or generalize–lies in their ROW management regimes. Although ROW management in Idaho is carried out

by individual foremen and is not standardized by highway class, we observed that larger interstates may be managed at higher intensities, with more frequent and extensive mowing and spraying than smaller highways (Idaho Transportation Dept. pers. comm.). If and when this is the case, frequent mowing and spraying can reduce pollinator populations along more intensively managed roadways, leading to differences that may align with highway type (Hopwood, Black, and Fleury 2015). These differences do not depend upon characteristics of the roads themselves, but of allocations of management attention and activities to different highway systems.

A final consideration regarding the impact of highway class is a geospatial one: highways of various types are not placed randomly across a landscape. Interstates, in particular, generally connect major cities, which are frequently concentrated, as in southern Idaho, along large river systems. Smaller U.S. and state highways, by contrast, more often connect small towns, departing from major cities, economic centers, and (in association) river floodplains into areas with lower rates of human development (Federal Highway Administration 2017). Our study area includes state and U.S. highway segments that offer alternate routes to interstates and some which roughly parallel them, but it also includes US-26, US-20, and SH-37, which depart from the major developed and agricultural corridor of the Snake River to connect to smaller towns off the major interstate corridor. Although these roads are still often bordered by agriculture, they are also bordered in stretches by sagebrush steppe, an arid-adapted native habitat that has remained relatively intact in southern Idaho compared with the major Snake river corridor, which has been extensively converted to agriculture and urban development (Miller and Eddleman 2000). Tellingly, transects with the highest bee richness and abundance were located along these "offshoot" highways, which don't exist among interstates. Smaller highways may not only support more bees through reduced rates of collision mortality, toxin spillover, turbulence, and barrier effects; they may also tend to cross areas that have been less impacted by humans.

While median butterfly richness and abundance trended in the same direction as that of bees—higher values for smaller highways—there were some notable exceptions (e.g., interstate transects with very high butterfly richness and abundance) and the relationship was much weaker. Most butterfly species in our study were larger and more wide-ranging than most bee species, so larger roads might present less of a barrier to butterflies; butterflies also do not nest in the soil, as do many bee species, and so might be less affected by higher toxin levels therein (although a few butterfly species do pupate in the soil). Butterflies, at least of some species, might also be more tolerant of human disturbance and capable of subsisting in non-native plant communities than most bees. A final possibility is that native bees might be more competitively excluded than butterflies by European honeybees, which are likely to occur more commonly near cities and major agricultural areas along interstates, where they are farmed for pollination services and honey production. Honeybees have been shown to substantially depress native bee populations, with which they compete for pollen and nectar resources and to which they can spread diseases (Torné-Noguera et al. 2016; Mallinger, Gaines-Day, and Gratton 2017). Further research examining the impact of honeybee hives on native bees in ROWs could provide useful insights about this potential cause.

Although our findings suggest that NDVI and highway class offer useful geospatial tools for managers seeking to predict and assess roadside pollinator diversity and abundance, the relationship of NDVI and highway class to pollinators may be driven by a variety of possible mechanisms. Floral richness and abundance, on the other hand, impact pollinators directly by providing them food. While difficult to map or predict geospatially, floral resources offer possibilities for enhancing pollinator habitat, if pollinators respond to increases in floral species richness or abundance. Our study shows that in general, they do, although these relationships are complex. While butterfly species richness increased in response to increasing floral abundance, bee species richness and abundance decreased. This may be an artifact of our survey method: pan traps have been found to catch more bees when fewer flowers are immediately available and catch fewer bees when floral resources are abundant and bees have other options (Baum and Wallen 2011; Roulston, Smith, and Brewster 2007).

Unlike floral abundance, floral species richness was significantly positively associated with bee species richness and abundance. Despite the potential undersampling of pan traps in floral-rich areas, we collected more species and higher numbers of bees at transects with more species of flowers. This relationship between floral diversity and bee diversity has been well documented in other habitats (Fründ, Linsenmair, and Blüthgen 2010; Potts et al. 2003; Mallinger, Gibbs, and Gratton 2016). While we did not separate native and non-native flowers in our models, bee richness and abundance were generally higher at transects which had a higher proportion of native flowers.

Our analyses of bee and butterfly species composition found that composition varied with both NDVI and highway class, suggesting that in our study area, different pollinator species tend to use environments of different greenness and live alongside different classes of highways. For example, in our study, the bees *Agapostemon virescens* and *Andrena scurra* and butterflies *Plebejus melissa* (Melissa blue) and *Speyeria callippe* (Callippe fritillary) were found almost exclusively at low NDVI transects, while the butterflies *Lycaena rubidus* (Ruddy copper) was found exclusively at medium NDVI transects. Its congener *Lycaena helloides* (Purplish copper) was mostly found at medium NDVI transects as well. Among highway classes, the bees *Agapostemon virescens* and *Lasioglossum tegulare* and butterflies *Burnsius communis* (Common checkered-skipper)*, Cercyonis oetus* (Small wood-nymph)*,* and *Speyeria callippe* (Callippe fritillary) were found almost entirely along State highways. In general, transects in the low NDVI and State highway classes hosted higher numbers of species restricted to just these classes. Higher transect-to-transect variation (i.e., beta-diversity) in butterfly species composition in low NDVI transects suggests that each low NDVI site is important for the high cumulative species richness across the ROW network, and also suggests that habitat specialization might occur at higher rates in the low NDVI environments in our study area. By contrast, most species that were found at high NDVI transects and Interstate transects were also found at transects of other NDVI and highway classes, suggesting that high NDVI sites and Interstates may host a higher proportion of widespread, generalist species.

These results suggest that smaller highways and lower NDVI roadsides may support pollinator species not found in other ROWs, thereby increasing the pollinator diversity of the entire roadside system. In bees, this corresponded with higher species richness at low NDVI sites and along State highways. In butterflies, although species richness per survey did not vary significantly with NDVI or highway class,

some butterfly species (such as those with more specific habitat requirements or higher sensitivity to disturbance) may nonetheless favor roadsides with lower NDVI and along smaller highways.

Overall, our results indicate that NDVI and highway class are important factors associated with differences in bee species richness and abundance, and in the species composition of bee and butterfly communities. These factors provide easy tools to help managers identify roadsides of higher potential for bee diversity and abundance, and to ensure they are supporting the full range of bee and butterfly species using roadsides. According to our results, roadsides along smaller State highways and in areas of lower NDVI generally support higher numbers and diversity of bees; bee populations may thus especially benefit from pollinator-friendly management strategies applied in these areas, including reducing mowing and spraying, spot-treatment herbicide application, and planting native forbs. Our results also show a positive relationship between floral richness and bee richness, and between floral abundance and butterfly richness. This suggests that planting more species of forbs along roads may support more species of bees, while increasing the number of flowers in ROWs may foster higher roadside butterfly diversity.

6. Recommendations for ITD ROW Management to Benefit Pollinators

ROW management plays a critical role in shaping the positive and negative effects ROWs have on pollinator populations. ROW management is essential for driver safety and visibility, the reduction of noxious weeds, and the improvement of public natural resources and ecosystem services, including those provided by pollinators and native plants. In the following sections, we focus on three main ROW management activities that affect pollinators: mowing, herbicide application, and restoration/planting. By adjusting the extent, timing, location, and frequency of these three management activities, ITD ROW managers can effectively support pollinator populations in ROWs while still meeting safety and vegetation management objectives.

Table 9 - A summary of recommendations for roadside best management to support pollinators. The recommendations listed in the first row are illustrated on the following page in Figure 35.

Mowing	Revegetation	Herbicide Application
(Fig. 35, 1) Maintain a close-cropped mow zone of 1.5 to 3 m bordering the pavement (narrower on lower traffic roads), reducing pollinator exposure to roadway toxins.	(Fig. 35, 2) Plant diverse, native wildflowers in areas farthest from the road, away from the zone of toxicity. These areas should not typically be mown, especially in the growing season.	(Fig. 35, 3) Noxious weeds should be spot-treated with herbicides rather than blanket-treating ROWs. Treatment should not occur while weeds are in flower.
Limit mowing to the close-cropped mow zone beside the roadway. Selective mowing of noxious weeds beyond this zone may be appropriate.	Plant hardy, fast-growing native grasses and forbs after construction projects and in disturbed areas to compete against invasive species.	Spot-spray noxious weeds at the rosette stage. NDVI may be useful to predict where invasive species patches occur and treat them early in the growing season.
Avoid mowing while plants are in flower; mow early in the growing season to control noxious weeds.	Prioritize planting diverse native forb communities at the periphery of existing intact native plant communities, helping them expand.	Use the minimum effective amount of herbicides to protect pollinators and native plants.
Mow during warmer times of day (when pollinators are more active and can flee) and use flushing bars and arthropod-friendly mowing heads to minimize harm to insects.	Plant more species of flowers to support bees and more total flowers to support butterflies. Minimize ground disturbance and leave woody stems and bare patches for nesting bees.	Avoid spraying on windy days to reduce pesticide drift and take special care to avoid spraying milkweed.
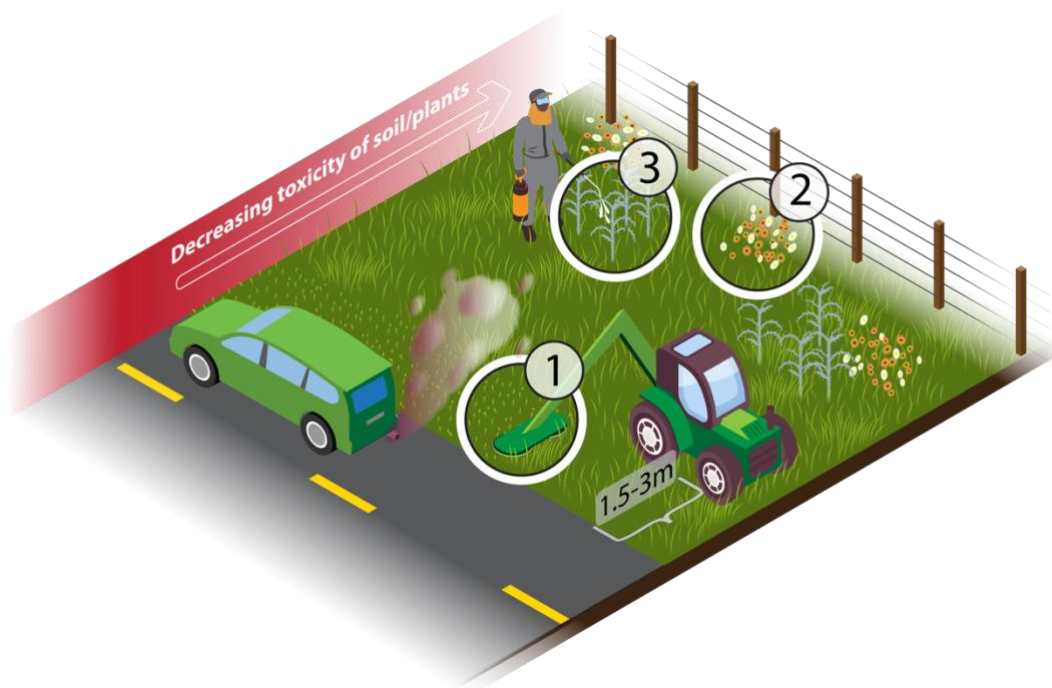
Figure 35 - Three roadside management best practices to support pollinators, as described on the previous page in Table 9 (first row, columns 1, 2, 3).

6.1 Mowing

ROWs are mown to maintain visibility for drivers, increase safety in case of run-off-road accidents, control populations of noxious weeds, and reduce roadside fire risks. While some ROW mowing is necessary for safety, mowing can have many negative impacts on pollinators, including killing nesting and developing bees and butterflies, reducing floral resource availability, and displacing native perennial forbs and shrubs in favor of disturbance-tolerant, non-native annuals (Phillips et al. 2019). A recent study found that conventional mowing of roadside grassland caused losses of over 50% of Hymenoptera (the order that includes bees) and 87% of Lepidoptera (butterflies and moths) (Steidle et al. 2022). Because of these impacts, how, when, wherein, and at what frequency ROWs are mown can dramatically influence their capacity to support pollinators. Fortunately, there are many ways that mowing can occur to meet safety needs while minimizing negative effects for pollinators.

First and perhaps most importantly, the 1.5 to 3 meters (5 to 10 feet) closest to the pavement—the "mow zone" (also referred to by some DOTs as the "clear zone")—should be frequently mown and kept short year-round. This zone is first and foremost critical for human safety, enhancing visibility and reducing accidents and fires. However, keeping it close-cropped also benefits pollinators, as plants in this zone are likely to be toxic for pollinators due to the accumulation of toxins from vehicles and

roadways (De Silva et al. 2021; Hintz and Relyea 2019; Moroń et al. 2012). To protect pollinators, this mow zone should be somewhat wider (although it need not exceed 3-4 meters) on roads with higher traffic and on those that are de-iced in winter, since traffic increases toxin deposition and de-icing salt can result in toxic sodium concentrations in roadside plants (Lytle, Smith, and McKinnon 1995; Mitchell et al. 2020). For smaller, low-traffic highways, a narrower mow zone is more appropriate. We also recommend that highway medians be managed like mow zones and kept short year-round, since attracting pollinators or other animals to medians is likely to result in high rates of collision mortality and toxin exposure (Keilsohn, Narango, and Tallamy 2018).

Beyond mow zones adjacent to roadways and in medians, ROWs should generally be mown minimally, and only when required to control invasive species or address safety and fire concerns. Mowing the entire ROW ("fenceline-to-fenceline") is highly discouraged, as this practice destroys all pollinator habitat simultaneously and leaves no refuge for pollinators to escape to or find food within the ROW. ROWs contain the only floral resources available at some times of year in many agricultural or semi-urban landscapes, so mowing their entirety can dramatically affect the survival of any pollinators relying on them (Hopwood, Black, and Fleury 2015; Phillips et al. 2019). If ROWs must be mown to address fire risks or other concerns, we recommend staggering mowing by zone and leaving strips of vegetation near the fenceline. Mowing close to the roadway while leaving ROW habitat intact near the fenceline provides pollinator habitat and resources in ROW areas farthest from traffic, drawing pollinators away from more hazardous areas adjacent to roadways. Beyond mow zones, some ROWs may not require mowing at all, especially those with intact native habitat, such as sagebrush steppe, and low levels of traffic. In our study area, we observed many such ROWs along SH-37, US-20, and US-26, which we would not recommend mowing beyond a 1 to 2 m (3.3 to 6.6 ft.) mow zone adjacent to the roadway.

Timing as well as frequency of mowing is important. Generally, mowing very early or late in the growing season is less likely to abruptly reduce floral resources or harm developing pollinators. Avoiding mowing ROWs while flowers are in bloom is critical; as our study has shown, even non-native weeds are frequently visited by native butterflies, which feed on their nectar and, in some cases, develop as larvae on their foliage. By mowing non-native weeds shortly after they have sprouted in spring (i.e., April in the Snake River Plain), managers can better control weed populations while minimizing harm to pollinators. This timing also gives native plant communities an early-season opportunity to fill in the gaps left by non-natives, providing an alternative (and potentially superior) source of nectar and pollen for roadside pollinators (Roulston and Cane 2000; Vaudo et al. 2020). Removing cuttings from mowing (rather than leaving them on top of the cut area) has also been shown to increase plant diversity, and in response, may support higher pollinator diversity (Roberts and Phillips 2019).

Some research on *Asclepias syriaca* (common milkweed), an eastern U.S. milkweed species, has shown that well-timed, mid-season mowing of milkweed can be beneficial, increasing monarch oviposition in response to resprouting (Knight et al. 2019; Fischer et al. 2015). However, timing mowing to achieve such results requires location- and species-specific phenological information for both milkweeds and monarchs (Fischer et al. 2015; Phillips et al. 2020). Because monarchs potentially use roadside milkweed in Idaho for breeding across the growing season, we generally do not recommend mowing milkweed. If

areas with milkweed require mowing (for example, milkweed is growing within an emergent patch of noxious weeds), we recommend that mowing activities are completed before May 15th in southern Idaho to prevent accidental destruction of monarch eggs or larvae. With small numbers of western monarchs, such destruction could have a severe negative impact on the Idaho breeding population. We hope that our survey data locating milkweed patches and milkweed hotspot zones will assist managers in these protection efforts.

Although mowing outside the growing season can reduce harm to pollinators, this practice can still destroy overwintering pollinator eggs, larvae, and pupae. In our surveys, we collected 27 species of bees in ROWs that nest in plant stems, woody plants, or pre-existing cavities; these bees overwinter as newly hatched adults or pupae and may do so in roadside vegetation, such as dry weed stems or cavities in shrubs, making them vulnerable to mowing activities in the fall, winter, and spring. Similarly, many temperate butterflies overwinter as pupae in leaf litter or at the base of plants, especially woody shrubs (Douglas 1987; Hayes 1982). Thus, we recommend that perennial shrubs and standing woody stems be left in ROWs for nesting bees rather than mowed, even at season's end. At our transects, we often observed high bee diversity at transects with perennial shrubs like *Chrysothamnus viscidiflorus* (yellow rabbitbrush) and *Artemesia tridentata* (sagebrush), highlighting the potential importance of these plants for supporting roadside bee species. Unfortunately, in some ROWs in our study area, we observed *A. tridentata* (sagebrush) near ROW fencelines mown down, likely by private landowners haying the ROW (Cathy Ford pers. comm.). These native shrubs can live over 100 years and provide habitat for numerous native speciesClick or tap here to enter text., including forbs (by bringing water from deeper in the soil for shallower-rooted plants to access) and pollinators (Barbour and Billings 2000, Cardon et al. 2013); they are, for example, the favored perching substrate for *Lycaeana rubidus,* the ruddy copper butterfly (Brock and Kaufman 2003). We strongly encourage the preservation of sagebrush beyond the mow zone in ITD ROWs.

When mowing is necessary, we recommend that it be done with methods and equipment that minimize harm to pollinators and other beneficial insects. Mowing during warmer times of day, when pollinators are active and can flee the approaching mower, is recommended instead of mowing during the colder morning hours, when pollinators may be too cold and slow to escape (Hopwood, Black, and Fleury 2015). While studies on pollinators are limited, raising the height of the mower blade may reduce mortality of a variety of animals (Humbert, Ghazoul, and Walter 2009); cutting at least 25 cm (10 inches) from the soil is recommended to promote plant recovery and reduce disturbance to ground-nesting bees or butterfly larvae at the base of plants (Hopwood, Black, and Fleury 2015). Cutter bar mowers are recommended over rotary or flail mowers, as they cause half as much insect mortality, according to one study (Humbert, Ghazoul, and Walter 2009). Flushing bars are a simple innovation that can be added to mowers and substantially reduce mortality from mowing by prompting insects (as well as birds and other wildlife) to escape ahead of the mower (Humbert et al. 2010). Additionally, arthropod-friendly mowing heads (e.g., Eco 1200 mowing head from MULAG) have been designed in recent years that have been shown to "fully offset losses" of Lepidoptera (butterflies and moths) and reduce losses of Hymenoptera (bees, wasps, ants) by 15% (Steidle et al. 2022). These mowing heads kill far fewer

arthropods by utilizing a scraper device that flushes insects ahead of the mower and small blades designed so that no suction effect is created from below or in front, by cutting at heights of 10-15 cm, and by rolling on smaller sensing rollers which protect ground-dwelling organisms and reduce ground contact and soil compaction (MULAG 2023). While the prevalence of these technologies is still limited in the U.S. (most products have been developed in Germany), we recommend that ITD considers investing in arthropod-friendly mowing heads and techniques in their ROWs, especially given the abundance of pollinators using them.

To summarize, mowing ROWs can have a major negative impact on roadside pollinators, but this can be mitigated by limiting the extent (only mowing the mow zone, 1.5 to 3 m (5 to 10 ft.) from the pavement) and frequency (0 to 2 times per year) of ROW mowing, adjusting the timing (only before and after the flowering season), and using cutter bar mowers, flushing bars, and insect-friendly mowing heads when mowing ROWs. Leaving stems and shrubs standing through the winter beyond the mow zone, minimizing mowing of native habitat such as sagebrush steppe, and avoiding ever mowing fenceline-to-fenceline are important steps that ITD vegetation managers can take to boost Idaho's pollinator populations and make ITD ROWs a more hospitable place for a wide variety of beneficial insects.

6.2 Herbicide Application

Transportation departments are mandated to control plant species in ROWs designated by the State as noxious weeds. This control is usually enacted through mowing and the use of a variety of herbicides. Herbicides, especially when used selectively on only noxious weeds, can result in the reduction of these dominant species, which may in some circumstances benefit pollinators by favoring native floral diversity (Lyon et al. 2021; Coon et al. 2021). Among our transects, ROWs of the high NDVI category were largely rated as such due to the rich green (high NDVI) of these non-native weeds; these transects had significantly fewer bee species and fewer numbers of bees than transects of lower NDVI values. This demonstrates the potential value of reducing noxious weed monocultures (single-species patches) in ROWs for pollinators. By making space for native floral diversity, managers can also improve habitat for native bees. On the other hand, butterflies were commonly observed nectaring on noxious weeds, and were statistically no less common in high NDVI transects than in others. Despite their negative effects on plant diversity and potentially on local bee diversity, these noxious weeds still support native pollinators, which underscores the importance of controlling them strategically.

Using herbicides in ROWs can come at a serious cost for pollinators and other invertebrates. Herbicides can kill butterfly host plants at critical developmental periods, reduce floral resources for native pollinators, and kill or weaken pollinators directly (Hopwood et al. 2015; Pleasants and Oberhauser 2013). Herbicides can also cause sublethal negative effects on insect foraging ability and disease resistance (Prosser et al. 2016; Cullen et al. 2019). Both active and inert ingredients (e.g., adjuvants or surfactants) in common herbicides harm bees by damaging their gut microbiomes (Motta, Raymann, and Moran 2018), impairing their navigation abilities (Hahn et al. 2015), reducing sperm counts and survival rates (Belsky and Joshi 2020), and slowing development (Mullin et al. 2016). Herbicides persist

in the environment for months to years—and many, particularly pre-emergent herbicides (e.g indaziflam, prodiamine) are designed to do so, meaning that the negative impact to roadside pollinators and ecosystems can extend far beyond their initial application (Arias-Estévez et al. 2008). On the Snake River Plain, roads and their ROWs often immediately border waterways and canals, increasing the chances of herbicides sprayed on ROWs leaching into the water system (Arias-Estévez et al. 2008). This is a widespread concern for both ecological and human health; as far back as 1999, a US Geological Survey study found at least one pesticide in 95% of stream samples and nearly 50% of well samples (Stone, Gilliom, and Ryberg 2014). Pesticide contamination in soil, groundwater, surface water, and drinking water has only increased since then (Mahler et al. 2021).

In addition to negatively affecting bees and posing risks to ecosystem and human health, herbicide use has been identified as a key threat to roadside monarch and milkweed populations in the western U.S. (Waterbury, Potter, and Svancara 2019). Milkweed losses due to increased use of glyphosate have been identified as a primary driver of the >80% decline of the eastern U.S. monarch population (Pleasants and Oberhauser 2013). Outside of ROWs, milkweed, which often grows at the margins of crop fields and in other agricultural settings, is subject to a variety of pesticides, including insecticides, herbicides, and fungicides, several of which have been shown to have sublethal effects on monarchs (Olaya-Arenas and Kaplan 2019). Milkweed in ROWs is generally protected from direct application of insecticides and fungicides, which have been implicated as a likely cause of the western monarch's decline, and should not be applied to ROWs unless mandated (Pelton et al. 2019). However, herbicides still represent a major risk for roadside milkweed and monarchs. In the field in southern Idaho, we observed multiple patches of milkweed in ROWs that had been sprayed with herbicides (Figure 36). This is concerning, especially because any one of those patches may have contained monarch eggs or larvae, which could fail to develop as a result of the spraying.

As an example of this concern, in 2021, after the wintering monarch population was censused at less than 2,000 individuals, we observed a female monarch lay more than a dozen eggs in one roadside milkweed patch. If this patch had been sprayed or mown, the consequences for monarchs may have been significant. Since monarch eggs are very difficult to locate and one cannot visually distinguish which patches have been selected by breeding monarchs, the precautionary principle advises that every roadside milkweed patch be treated as though it contains monarch eggs, and we recommend that ITD managers take special care not to spray (or mow) milkweed in ROWs, especially after May 15th. Placing no-spray signage to flag established milkweed patches and milkweed hotspot zones may assist vegetation managers in protecting this important plant.

Figure 36 - Milkweed sprayed with herbicides in ITD ROWs, June 2021. Milkweed plants shrivel and curl in response to herbicide application, as shown on the right-hand side of this figure, endangering monarch eggs and larvae that may be using these plants.

While monarchs have received considerable conservation attention in recent years, they are but one of many native pollinator species that are negatively affected by herbicides. We strongly recommend that managers attempt to avoid spraying any native plants in ROWs and avoid blanket herbicide treatments and broad-spectrum herbicides wherever possible. Spot treatments with selective herbicides that target specific weeds minimize herbicide use and damage to non-target plants; this is a much better option economically and for pollinators and ecosystem health (Hopwood, Black, and Fleury 2015). We also encourage ITD to avoid treating weeds while they are in bloom or growing adjacent to blooming flowers. As with mowing, spraying plants in bloom, whether native or non-native, can have severe negative consequences for pollinators. It is best to treat noxious weeds at the seedling or rosette stage, when they are more vulnerable; this timing can reduce herbicide use, costs, and negative impacts to pollinators (Hopwood, Black, and Fleury 2015). While pre-emergent herbicides may be useful in some circumstances, their long persistence in the soil can negatively impact native plants and their seed sets along with target species (Clark et al. 2019), so their use should be limited.

To treat weeds early in their growth, advance information about noxious weed distribution in ROWs is especially useful. Our study shows that maximum NDVI values above 0.58 may be a useful indicator for identifying areas with non-native weed infestations; maximum NDVI data from the previous season may be useful in forecasting these areas and treating emergent weeds in the spring before they bloom.

Weather and time of day are also important factors that affect the effects of herbicide applications on pollinators. We recommend that herbicides be applied in colder conditions (the opposite of mowing), including early mornings, when pollinators are less likely to be active and present on treated plants; herbicides should also be applied in still conditions with minimal wind to reduce herbicide drift (Hopwood, Black, and Fleury 2015). In addition to limiting drift, using herbicides with shorter residuals that will break down sooner can help reduce damage to butterfly caterpillars and minimize the unintended negative effects of herbicides on non-target flowering plants. Finally, we encourage ITD to consider alternative weed control options to synthetic herbicides if feasible, such as acetic acid, clove or pine oil, wood chips, and mowing (Barker and Prostak 2014).

Herbicides are a necessary tool for reducing noxious weed populations in ROWs. However, they have complex and unintended negative consequences for pollinators and native plants, so they should always be used conservatively and efficiently on focused plant targets rather than in a routine or broad manner. Minimizing herbicide use in ROWs can help maximize their potential as healthy pollinator habitat.

6.3 Restoration and Revegetation

Highway construction projects provide impetus and funding for restoration and revegetation in Idaho ROWs. Restoration efforts are an opportunity to improve ROW habitat for pollinators and native plants, but they also face many challenges. Many ROWs have vigorous seed banks of non-native weeds, which thrive after the disturbance created during construction projects and can quickly overtake restoration plantings. We observed this in the field along I-15 north of Idaho Falls, where ROW embankment construction occurred in 2021; in 2022, a long, dense field of *Bassia scoparia* (kochia) and *Sisymbrium altissimum* (tall tumblemustard) had colonized the former construction site. Even without the disturbance of construction activities, most ROWs are historically disturbed environments with many non-native plant species. In the Snake River Plain, there is often a difficult tradeoff: ROWs with more water availability are often dominated by non-native plants, increasing competition for native plantings, while ROWs farther from water sources are very arid, making plant propagation challenging (Svejcar et al. 2017).

In response to these challenges, many agencies have historically turned to hardy non-native grasses like *Agropyron cristatum* (crested wheatgrass) or *Bromus inermis* (smooth brome) in an attempt to occupy the soil, edge out potential noxious weed infestations, and control erosion on steeper slopes (Ament et al. 2014). However, these perennial grasses are a poor choice for enhancing pollinator habitats and may even be more detrimental than some noxious weeds because they provide neither food nor nesting resources to pollinators, occupy soils to the exclusion of many native plants, and persist as the dominant species in ROWs for decades to centuries (Henderson and Naeth 2005; Christian and Wilson 1999). At the majority of our ROW transects, for example, non-native European grasses *Agropyron cristatum* (crested wheatgrass), *Thinopyrum intermedium* (intermediate wheatgrass), and *Poa compressa* (Canada bluegrass) were identified as the dominant plant species. To improve native plant diversity and support

pollinators, we recommend that these non-native grasses *not* be seeded in future restoration mixes, but instead be replaced by native grasses and forbs wherever possible.

Native grasses and forbs have many advantages for ROWs: they provide food and nesting resources to native pollinators and other invertebrates (Ries, Debinski, and Wieland 2001; Hopwood 2008); they can be less expensive to maintain by requiring less mowing and herbicide application; and once established, they can reduce erosion and resist weed invasions (Ament et al. 2014; Hopwood, Black, and Fleury 2015; Quarles 2003). Although native forbs can be difficult to establish in southern Idaho ROWs, native grasses (especially bunchgrasses) can leave space for native forbs and make it easier for them to colonize ROWs after restoration occurs (Ament et al. 2014; Ament, Pokorny, and Mangold 2017). *Pseudoroegneria spicata* (bluebunch wheatgrass) and *Festuca idahoensis* (Idaho fescue) were the most successful native grasses in previous restoration experiments in Idaho ROWs (Ament, Pokorny, and Mangold 2017)*.* We recommend using restoration seed mixes that include these and other native grasses along with a variety of native forbs (see Ament, Pokorny, and Mangold 2017 for recommendations).

In our study, bee species richness and abundance were positively associated with floral species richness, so the higher the diversity of native forbs established in restoration plantings, the better for bees. Additionally, planting native forbs that flower at different times, including early in spring and late in fall, will better provide for bees and butterflies throughout the growing season (Hopwood, Black, and Fleury 2015). Several regional guides are available to assist in selecting forbs preferred by pollinators (Pollinator Partnership and NAPPC, n.d.; Ogle et al. 2017; 2011).

Current standard practice for ITD is to attempt to revegetate ROWs at major construction projects after their completion, using mitigation and restoration funding directly onsite (Cathy Ford pers. comm.). However, these revegetation efforts often fail to successfully restore native plant communities or prevent the development of invasive monocultures, as the disturbance caused by construction greatly advantages invasive plants; many highway construction sites also have dense invasive plant seed banks in the soil and adjacent land (Meinzen pers. obs., Cathy Ford pers. comm.). In such areas, restoring native plant diversity typically requires sustained, targeted management efforts across multiple years to control invasive species. Seeding the most successful native grasses and forbs based on previous studies (e.g., Ament, Pokorny, and Mangold 2017) and continually monitoring for and reducing non-native plant encroachment are recommended strategies (Mitchell et al. 2022). At these challenging sites, investing more in relatively inexpensive grass cover (again, native species preferred) may be warranted, while remaining restoration funding could be more effectively spent at other ROW locations where diverse native forb communities stand a better chance of establishment. This "mitigation banking" approach, in which impacts in one place are mitigated through restoration elsewhere, is commonly practiced in stream and wetland restoration (P. H. Brown and Lant 1999; Theis and Poesch 2022). This approach could allow ITD to better use pollinator and plant data to prioritize restoration sites and maximize their overall positive ecological impact.

Hardy native grasses and relatively aggressive native forbs (e.g. yarrow, flax, some penstemon) are best suited to revegetation efforts among stands of invasive plants or into freshly disturbed soil with invasive

plant seed sets, as is often the situation following road construction projects. However, we recommend prioritizing habitat enhancement at the periphery of relatively intact native plant communities; these areas should be planted with a diverse array of native plantings, including less aggressive species. This will give native plantings a better chance of success and may better serve existing diverse pollinator populations. This strategy can help native plant communities expand into semi-disturbed habitat and provides additional resources for pollinators already using these areas. Additionally, we recommend prioritizing habitat enhancement with native plants along smaller roads with less traffic; these roads are safer for pollinators, with typically lower rates of collision mortality (Martin et al. 2018; Phillips et al. 2019) and toxins (Bernardino et al. 2019; Mitchell et al. 2020), and our study suggests that they also tend to harbor higher bee species richness and abundance.

Based on the results in our study area, we recommend sections of State highway 37 and U.S. highway 26 as prime locations for native plantings, as shown in Figure 37. These ROWs have diverse bee and butterfly populations, relatively low levels of traffic, and tended to have relatively low numbers of flowers. For example, at a transect along US-20, we collected 191 bees of 21 species in one day in June 2021, with only 6 open flowers present in our floral survey and virtually no flowers apparent elsewhere in the ROW or adjacent land. This would be a great site to plant native forbs, as they would serve a diverse and abundant bee fauna that may be lacking nectar and pollen resources, especially during drought years like 2021. Additionally, because these ROW sections represent the transition zones from more disturbed and degraded agricultural roadsides to more intact sagebrush steppe, planting native forbs here may help native bees and butterflies expand further into agricultural environments, potentially increasing crop pollination. These are also areas where we especially recommend avoiding mowing and spraying, given their relative lack of noxious weeds and existing diverse pollinator populations.
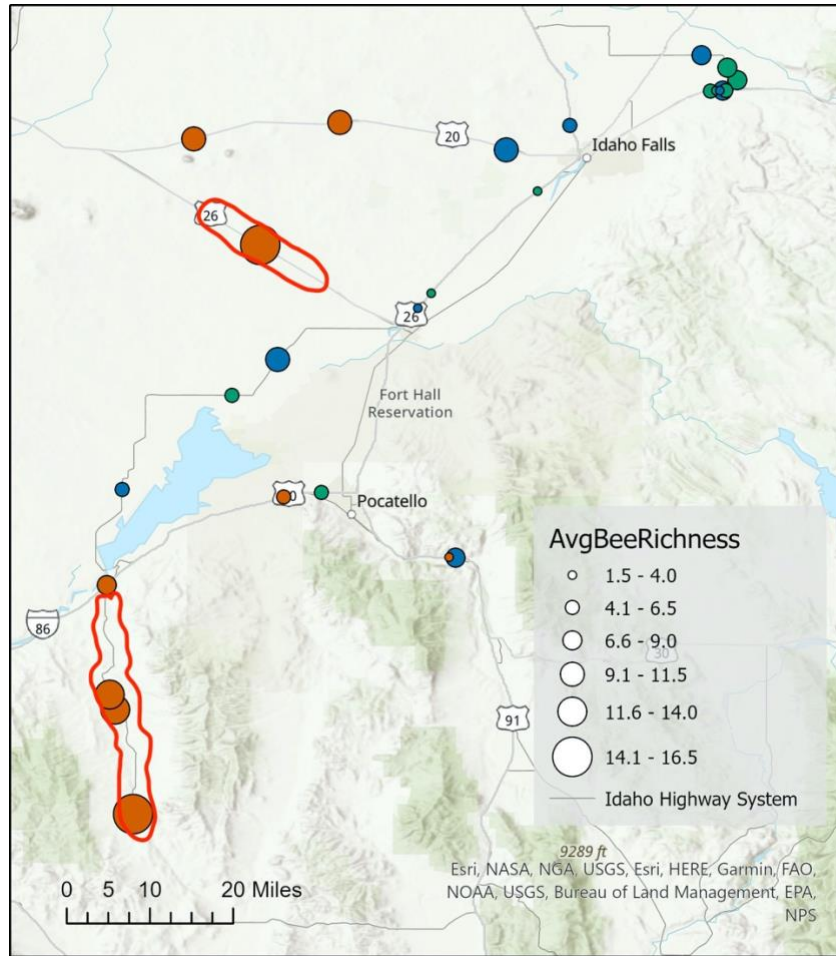
Figure 37 – Recommended zones for restoration and planting native forbs, outlined in red. These transition zones from agriculture to sagebrush steppe host high numbers of bee species, as shown by the larger circles, and may be limited by floral resource availability. Plantings here could help expand native habitat in ROWs and support crop pollination services.

Regardless of which ROWs are restored, flowering plants should be planted in ROW zones farthest from the paved road area to reduce pollinator collision mortality and toxin exposure (Wojcik and Buchmann 2012; De Silva et al. 2021). Although aesthetic goals might encourage planting flowers in road medians, along highly trafficked roads, or near highway lanes, these sites can be ecological traps for pollinators, luring them into dangerous areas (Muñoz, Torres, and Megías 2015; Phillips et al. 2020). Instead, we recommend planting flowers farther from traffic and as close to the outer fenceline as possible. Improving floral resources in ROW habitats away from traffic can reduce the likelihood of pollinators colliding with vehicles, potentially making ROWs both safer and more productive for pollinators (Ries, Debinski, and Wieland 2001; van Rossum et al. 2011; Skórka et al. 2013).

In addition to restoring ROWs and planting native forbs, protecting existing ROW habitat from disturbance (particularly mowing and herbicide treatment) is of critical importance. Our bee data highlights the value of sagebrush steppe habitat, the predominant native habitat in ROWs in our study area; we found higher average bee species richness and abundance in ROWs adjacent to sagebrush steppe. The sagebrush steppe ecosystem is home to a variety of native forbs, provides bare ground and woody stems for nesting bees, and can provide nectar and pollen resources throughout the summer, making it a better habitat for pollinators than large, homogenous stands of flowers, which provide only a short pulse of food. Sagebrush steppe also hosts several habitat-specific butterfly species, including *Satyrium behrii* (Behr's hairstreak) and *Satyrium semiluna* (Sagebrush sooty hairstreak), both found on our surveys. We thus suggest that the protection of *Artemesia tridentata* (sagebrush), *Chrysothamnus viscidiflorus* (yellow rabbitbrush), and their associated plant communities be considered a priority in ROW management, and that restoration efforts work to expand and enhance this habitat in ROWs for the benefit of pollinators.

In summary, we recommend that ROW restoration activities always prioritize the use of native plants, and that plantings for pollinators are sited farther from the pavement, along smaller, less-trafficked roads, and at the edges of more intact native habitats, with a particular emphasis on the value of expanding and protecting sagebrush steppe habitats in the ITD ROW system of southern Idaho.

7. Conclusions

As discussed in our literature review at the beginning of this report, roadsides present a challenging conservation dilemma. In many areas, they constitute nearly the only undeveloped land and can provide a variety of floral resources to pollinators. Our study has shown that roadside ROWs are clearly used by pollinators, including at least 38 species of butterflies and 67 species of bees in southeastern Idaho alone. Across the state, they provide strips of grassland and flowering forbs across a range of elevations and habitat types, potentially providing corridors for pollinator movement (Dániel-Ferreira et al. 2022). Idaho ROWs also host large populations of *Asclepias speciosa* (showy milkweed), supporting *Danaus plexippus* (monarch butterflies), which we documented breeding in ROWs in 2021 and 2022.

However, as we've described, pollinators in ROWs also face many threats, including collision with vehicles, toxins from roadways, and disturbances caused by ROW management activities, particularly mowing and herbicide application. These dangers have led to concerns that ROWs could become ecological traps or population sinks for pollinators, luring them into environments where death rates exceed birth rates (Milton et al. 2015; Keilsohn, Narango, and Tallamy 2018). Such concerns are difficult to address because scientists have very little quantitative data about pollinator population sizes or population dynamics, with the exception of a few well-studied species like monarchs (Archer et al. 2014). We cannot compare birth and death rates between ROWs and other habitats, for example, because we lack data on those rates for any habitat. What we do know is that as a group, pollinators and other insects are declining globally and precipitously, and that habitat loss and pesticide use are two major causes of these declines (Potts et al. 2010; Sánchez-Bayo and Wyckhuys 2019). We also know that prudent roadside management strategies can improve conditions for pollinators in ROWs by reducing threats, improving habitat quality, and increasing the availability of food and breeding sites. Although ROWs can be dangerous places for pollinators, ITD management decisions have the potential to mitigate some of these dangers, and in doing so, play a part in addressing the global crisis of pollinator decline.

The first objective of this project was to aid ITD in their monarch conservation efforts on the Snake River Plain by locating milkweed populations and documenting monarch activity within the ROW network. Our milkweed and monarch data, now incorporated into ITD's geospatial database (IPLAN), establish not only the distribution and abundance of potential monarch habitat across southern Idaho, but also the scarcity of monarchs within the State in 2021, when our milkweed surveys were conducted and when the western monarch population was at an all-time low. The protection of existing patches is invaluable because of the uncertainty as to which patches monarchs will use for breeding. Although the monarch's rarity and the associated small sample sizes prohibited us from statistically testing for preferences in their choice of patches, and there is likely stochasticity involved in breeding site selection, we did observe monarch breeding at milkweed patches within especially diverse roadside floral communities relative to other patches recorded in our study. To aid in monarch conservation, we encourage the careful protection of all roadside milkweed from mowing and herbicide application, and recommend enrolling the hotspot zones of high milkweed density identified in this report as adopted conservation

acres for monarch protection (as described in the Monarch Candidate Conservation Agreement with Assurances).

Our project's second objective was to identify patterns of bee and butterfly abundance and diversity (as measured by species richness—hereafter, "richness") in ROWs as they relate to differences in floral richness and abundance, highway class, and NDVI class. These patterns can help ITD prioritize highway systems for restoration activities and targeted management, support efforts to increase native plant diversity, and use satellite data to predict bee richness—and potentially invasive weed abundance— within the ROW network.

In our study, bee richness was significantly higher in lower NDVI classes and along smaller, lower-traffic highways (State > U.S. > Interstate), and bee abundance was also significantly higher in lower NDVI classes. Higher floral richness also related to higher bee richness and abundance, although the opposite was true for floral abundance—perhaps an artefact of the pan-trapping bias, as discussed, and the relatively higher numbers of flowers produced by non-native weeds compared with native flora. These patterns confirmed our hypothesis that larger highways would have lower bee richness, potentially because of their higher traffic levels and concomitant collision and toxin risks, larger spans and associated barrier effects, and more intensive ROW management. However, we did not initially expect that lower NDVI would be associated with higher bee richness and abundance, because we presumed that higher greenness would relate to more plants, higher floral productivity, and thus more pollinators. Instead, we found that higher maximum NDVI values in Snake River Plain ROWs were associated with predominantly non-native plant communities, with one or a few dominant plant species, while bees instead favored more diverse native plant communities with less plant cover and lower greenness values. This interesting result showed that NDVI could not only help predict bee richness and abundance in our study area, but also might help identify ROWs dominated by invasive species for early-response control efforts.

In contrast to bees, butterflies did not respond significantly to differences in NDVI or highway class, and butterfly richness only increased in response to higher floral abundance. This may be because ROW butterfly communities were dominated by several widespread, disturbance-adapted species, some of which readily use non-native host plants (which predominated in high NDVI areas). Furthermore, butterflies are generally more wide-ranging than bees, and may be less influenced by the local habitat quality of ROWs, the availability of nesting sites, or the barrier effect of larger highways. Butterfly species composition, however, did vary with NDVI, highway class, and floral richness, and low NDVI transects had more transect-to-transect variation in composition than high NDVI transects. This suggests that while overall butterfly richness and abundance might not vary with NDVI or highway class, maintaining ROW pollinator habitat across various road types and NDVI values will increase the overall butterfly diversity of the ROW network, with low NDVI sites more often hosting specific butterfly communities not found elsewhere.

Our third objective was to synthesize the results from our study together with the increasing body of scientific literature on roadside pollinators to recommend ITD ROW management strategies and

priorities that maximize benefit for pollinators, including monarchs. As detailed in our recommendations section, we advise limiting the extent and frequency of ROW mowing, cutting only the mow zone regularly and otherwise mowing only before and after the flowering season, and using arthropod-friendly mowing methods and equipment. We also recommend minimizing herbicide use, spot-treating specific weeds instead of blanket-treating ROWs, and spraying early in weed development, never after flowering. To restore ROWs, we suggest prioritizing the use of native plants and planting flowers farther from road pavement, along smaller, less-trafficked roads, and at the peripheries of relatively intact native habitat. We also emphasize the value of expanding and protecting sagebrush steppe habitats in ITD ROWs.

We hope to have provided useful information that will help ITD better understand, conserve, and promote roadside pollinators and their habitat. Although we have addressed our project's objectives, many research opportunities and questions remain. We recommend 1) further monitoring and support of research projects that test the effects of specific ROW management practices on pollinators, 2) further investigation of pollinator use of ROW resources for both feeding and reproduction, and 3) assessment of the costs and benefits of roadsides for pollinator populations of selected focal species. Because there are so many species of both bees and butterflies, it would be impossible to conduct population-level assessments of all species in Idaho ROWs. As such, selecting one or more focal species and attempting to quantify rates of reproduction (e.g., by locating bee nests or butterfly larvae), immigration and emigration (e.g., by mark-recapture methods) and mortality across ROWs would provide opportunities to analyze population dynamics and begin to address whether (and under what circumstances) ROWs benefit that species or are a population sink. For example, research quantifying monarch mortality rates and reproduction on roadside milkweed could help determine whether and where monarch reproduction on roadside milkweed outweighs the risks of collision mortality and toxin exposure for the species, thereby informing roadside milkweed management. Future research could also specifically compare ROW widths and traffic levels at specific survey locations with local pollinator and flowering plant diversity and abundance, further exploring those relationships. Focal species could be habitat specialists or generalists, and either rare or common pollinator species could be used to address research questions, depending upon the goals. Such future research could help answer questions about the most effective restoration placements, mow zone widths, and management timings to benefit pollinator populations in Idaho ROWs.

Fortunately, concern and funding for pollinator protection is increasing, and thanks to this project and others, ITD has a baseline for understanding pollinator populations in its ROWs and important information about how to best conserve and protect them. We look forward to seeing how ITD continues to manage and enhance its ROW habitat for pollinators and express appreciation for the opportunity to assist in this important goal as researchers and concerned scientists.

8. References

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Appendix A. Plant Species List

Scientific Name	Common Name	Native to Idaho?	Floral Units (Count By)	2021 Present?	2022 Present?	Total Number of Floral Units	Total Number of Surveys Detected (out of 288)	Total Number of Transects Detected (out of 63)
Achillea millefolium	Yarrow	Y	Umbels	X	X	188	12	6
Allium acuminatum	tapertip onion	Y	umbels		X	4	1	1
Alyssum alyssoides	pale alyssum, pale madwort	N	Heads	X		1335	1	1
Amsinckia menziesii	Menzie's fiddleneck	Y	flowers		X	201	2	1
Astragalus ceramicus	Painted milkvetch	Y	flowers		X	9	1	1
Asparagus officinalis	garden asparagus	N	Flowers	X		29	2	2
Asperugo procumbens	German madwort	N	flowers		X	4697	12	10
Asclepias speciosa	showy milkweed	Y	Heads (flower clusters)	X	X	218	7	6
Balsamorhiza sagittata	Arrowleaf balsamroot	Y	flowers		X	4	1	1
Boechera pauciflora	hairystem rockcress, fuzzy suncress	Y	Heads (flowing stems)	X		1	1	1
Brassica nigra	black mustard	N	Branches from main stem with flowers	X		6533	11	7
Castilleja angustifolia	northwestern paintbrush	Y	Stems	X	X	5	2	1
Calochortus bruneaunis	Bruneau mariposa lily	Y	flowers		X	2	1	1
Capsella bursa-pastoris	Shepherd's purse	N	stems		X	192	6	6
Calochortus eurycarpus	white mariposa lily	Y	Flowers	X		8	1	1
Camelina microcarpa	False flax	N	Branches from main stem with flowers		X	184	6	6
Carduus nutans	musk thistle, nodding thistle	N	Flowers	X	X	385	19	11
Chaenactis douglasii	Douglas' dustymaiden	Y	umbel		X	3	1	1
Chondrilla juncea	Rush skeleton weed	N	flowers		X	12	2	2

Scientific Name	Common Name	Native to Idaho?	Floral Units (Count By)	2021 Present?	2022 Present?	Total Number of Floral Units	Total Number of Surveys Detected (out of 288)	Total Number of Transects Detected (out of 63)
Chorispora tenella	Purple mustard	N	Branches from main stem with flowers		X	546	6	6
Chrysothamnus viscidiflorus	yellow rabbitbrush	Y	Terminal branches with flowers	X	X	10925	12	7
Cirsium arvense	Canada thistle, lettuce-from-hell thistle	N	Flowers	X	X	1952	10	7
Cirsium vulgare	bull thistle, spear thistle	N	Flowers	X	X	111	2	1
Convolvulus arvensis	field bindweed	N	Flowers	X	X	3756	25	12
Crepis acuminata	tapertip hawksbeard, long leaved hawksbeard	Y	Flowers	X	X	125	5	2
Cynoglossum officinale	hound's tongue	N	Flowers	X	X	376	3	1
Descurainia sophia	fixweed, not much flower	N	Branches from main stem with flowers	X	X	15680	88	46
Dieteria canescens	hoary-aster	Y	Flowers	X		3	1	1
Elaeagnus angustifolia	Russian-olive, oleaster	N	Flower estimate	X	X	676	5	3
Epilobium lactiflorum	milk-flower willowherb, whiteflower willowherb	Y	Flowers	X		42	1	1
Erodium cicutarium	Redstem storksbill	N	flowers		X	428	14	7
Erigeron corymbosus	longleaf fleabane, foothill daisy	Y	Flowers	X		58	2	2
Ericameria nauseosa	rubber rabbitbrush, chamisa	Y	Terminal branches with flowers	X	X	1557	7	5
Erigeron pumilus	Shaggy fleabane	Y	flowers		X	13	5	4
Eriogonum umbellatum	Sulphurflower buckwheat	Y	umbels		X	13	2	1
Euphorbia esula	leafy spurge, wolf's milk	N	Raceme	X	X	844	6	2
Grindelia squarrosa	curlycup gumweed	Y	Flowers	X	X	10	3	3

Scientific Name	Common Name	Native to Idaho?	Floral Units (Count By)	2021 Present?	2022 Present?	Total Number of Floral Units	Total Number of Surveys Detected (out of 288)	Total Number of Transects Detected (out of 63)
Gutierrezia sarothrae	broom snakeweed, perennial matchweed	Y	Stems with flowers from a basal rosette	X	X	590	2	1
Helianthus annuus	sunflower	Y	Flowers	X	X	835	10	7
Hymenoxys cooperi	Cooper's rubberweed	Y	flowers		X	11	1	1
Ipomopsis congesta	ballhead ipomopsis	Y	Head	X		2	1	1
Bassia scoparia	kochia, ragweed	N	Flowers	X	X	225	3	3
Lappula occidentalis	western stickseed	Y	Flowers	X	X	2991	20	15
Lappula squarrosa	European stickseed	N	flowering stems		X	1100	14	11
Lactuca serriola	prickly lettuce	N	Flowers	X		564	4	4
Lepidium draba	whitetop, hoary cress	N	Heads	X	X	999	10	6
Lepidium perfoliatum	clasping pepperweed	N	Clusters of small flowers	X	X	1778	15	11
Linum lewisii	Lewis flax, blue flax	Y	Flowers	X	X	100	4	3
Lonicera tatarica l.	Tatarian honeysuckle	N	flowers		X	50	1	1
Lupinus argenteus	Silvery lupine	Y	stems		X	92	2	1
Malva neglecta	common mallow, dwarf mallow	N	Flower	X	X	285	7	3
Melilotus albus	white sweet clover, white melilot	N	Head	X		220	3	2
Mentzelia albicaulis	whitestem blazing star, small flowered blazing star	Y	Flowers	X		134	1	1
Mentha arvensis	wild mint, field mint	Y	Flowering stems	X		246	1	1
Melilotus officinalis	yellow sweet clover, yellow melilot	N	Heads	X	X	4289	23	11
Medicago sativa	alfalfa	N	Heads	X	X	13698	32	13
Nepeta cataria	catnip, catswort	N	Heads	X		9	1	1
Oenothera villosa	hairy evening-primrose	Y	Flowers	X		54	3	2

Scientific Name	Common Name	Native to Idaho?	Floral Units (Count By)	2021 Present?	2022 Present?	Total Number of Floral Units	Total Number of Surveys Detected (out of 288)	Total Number of Transects Detected (out of 63)
Opuntia polyacantha	plains prickly pear, hair-spine prickly pear	Y	Flowers	X	X	12	8	5
Penstemon cyaneus	Blue penstemon	Y	stems		X	5	1	1
Penstemon glaber	western smooth beardtongue, sawsepal penstemon	Y	Stems	X		3	1	1
Phacelia hastata	silverleaf phacelia	Y	Heads	X	X	151	3	1
Phlox hoodii	Spiny phlox	Y	flowers		X	10	1	1
Phlox longifolia	Longleaf phlox	Y	flowers		X	2	1	1
Potentilla norvegica	Norwegian cinquefoil	Y	Flowers	X		135	1	1
Potentilla pensylvanica	Pennsylvania cinquefoil, prairie cinquefoil	Y	Flowers	X		24	1	1
Purshia tridentata	bitterbrush, antelope brush	Y	Terminal branches with flowers	X	X	210	6	3
Ranunculus macounii	Macoun's buttercup	Y	Flowers	X	X	858	3	1
Rhaponticum repens	Russian knapweed	N	Flowers	X		654	1	1
Rhus typhina	staghorn sumac	N	Heads	X		86	1	1
Rosa sp. (cultivated)	ornamental yellow rose	N	Flowers	X	X	127	2	1
Sisymbrium altissimum	tall tumblemustard	N	Branches from main stem with flowers	X	X	35637	152	52
Sinapis arvensis	charlock mustard, field mustard	N	Branches from main stem with flowers	X	X	195	2	2
Sisymbrium loeselii	small tumblemustard	N	Branches from main stem with flowers	X	X	2233	18	9
Sphaeralcea munroana	orange globe mallow, Munro's globemallow	Y	Stem flowering stems from ground	X	X	11	2	1

Scientific Name	Common Name	Native to Idaho?	Floral Units (Count By)	2021 Present?	2022 Present?	Total Number of Floral Units	Total Number of Surveys Detected (out of 288)	Total Number of Transects Detected (out of 63)
Stenotus lanuginosus	woolly mock goldenweed	Y	Flowers	X		2	1	1
Taraxacum officinale	common dandelion, dandelion	N	Flowers	X	X	32	7	4
Tetradymia canescens	spineless horsebrush, gray horsebrush	Y	Terminal branches with flowers from last woody stem	X	X	427	4	3
Thlaspi arvense	Field penny-cress	N	Branches from main stem with flowers		X	8	1	1
Toxicodendron rydbergii	Western poison ivy	Y	Branches from main stem with flowers		X	21	1	1
Tragopogon dubius	yellow salsify, western salsify	N	Flowers	X	X	209	12	7
Tripleurospermum inodorum	Scentless false mayweed	N	flowers		X	7	1	1
Trifolium pratense	red clover	N	Heads	X		15	1	1
Tribulus terrestris	Puncture vine	N	flowers		X	1	1	1
Verbascum thapsus	common mullein	N	Stems	X		2	1	1
Verbascum virgatum	Twiggy mullein	N	flowers		X	8	2	2
Vicia americana	American vetch	Y	flowers		X	9	2	1

Appendix B. Butterfly Species List

*Note: "Total Number Observed" sums all butterfly observations from our transect surveys (both 2021 and 2022), including data from our subset of August surveys and butterflies observed at transect sites before the 10-min timer began, after it ended, or while the timer was stopped—data which were not included in our statistical analyses.

Common Name	Scientific Name	Total Number Observed*	Family
Anise Swallowtail	*Papilio zelicaon*	2	Papilionidae
Becker's White	*Pontia beckerii*	135	Pieridae
Behr's Hairstreak	*Satyrium behrii*	1	Lycaenidae
Blue Copper	*Lycaena heteronea*	2	Lycaenidae
Cabbage White	*Pieris rapae*	4	Pieridae
Boisduval's Blue	*Plebejus icarioides*	470	Lycaenidae
California Hairstreak	*Satyrium californica*	7	Lycaenidae
California Tortoiseshell	*Nymphalis californica*	1	Nymphalidae
Callippe Fritillary	*Speyeria callippe*	18	Nymphalidae
Checkered White	*Pontia protodice*	60	Pieridae
Clouded Sulphur	*Colias philodice*	414	Pieridae
Common Checkered-Skipper	*Burnsius communis*	28	Hesperiidae
Common Ringlet	*Coenonympha tullia*	115	Nymphalidae
Common Sootywing	*Pholisora catullus*	3	Hesperiidae
Common Wood-Nymph	*Cercyonis pegala*	8	Nymphalidae
Field Crescent	*Phyciodes pulchella*	12	Nymphalidae
Gray Hairstreak	*Strymon melinus*	3	Lycaenidae
Juba Skipper	*Hesperia juba*	5	Hesperiidae
Juniper Hairstreak	*Callophrys gryneus*	1	Lycaenidae
Melissa Blue	*Plebejus melissa*	36	Lycaenidae
Milbert's Tortoiseshell	*Aglais milberti*	1	Nymphalidae
Monarch Butterfly	*Danaus plexippus*	3	Nymphalidae
Mourning Cloak	*Nymphalis antiopa*	2	Nymphalidae
Mylitta Crescent	*Phyciodes mylitta*	3	Nymphalidae
Orange Sulphur	*Colias eurytheme*	2	Pieridae
Painted Lady	*Vanessa cardui*	2	Nymphalidae
Purplish Copper	*Lycaena helloides*	37	Lycaenidae
Ruddy Copper	*Lycaena rubidus*	3	Lycaenidae
Sagebrush Sooty Hairstreak	*Satyrium semiluna*	1	Lycaenidae
Sandhill Skipper	*Polites sabuleti*	2	Hesperiidae

Common Name	Scientific Name	Total Number Observed*	Family
Small Wood-Nymph	*Cercyonis oetus*	14	Nymphalidae
Two-tailed Swallowtail	*Papilio multicaudata*	9	Papilionidae
Variegated Fritillary	*Euptoieta claudia*	1	Nymphalidae
Weidemeyer's Admiral	*Limenitis weidemeyerii*	2	Nymphalidae
Western Branded Skipper	*Hesperia colorado*	16	Hesperiidae
Western Tiger Swallowtail	*Papilio rutulus*	5	Papilionidae
Woodland Skipper	*Ochlodes sylvanoides*	3	Hesperiidae

Appendix C. Bee Species List

Scientific Name	Total Sampled	Family	Nest Type
Agapostemon angelicus/texanus	276	Halictidae	Ground
Agapostemon femoratus	6	Halictidae	Ground
Agapostemon virescens	60	Halictidae	Ground
Andrena medionitens	2	Andrenidae	Ground
Andrena prunorum	1	Andrenidae	Ground
Andrena scurra	62	Andrenidae	Ground
Anthidium utahensis	1	Megachilidae	Pre-existing Cavity
Anthophora bomboides	1	Apidae	Wood, Plant Stems
Anthophora urbana	4	Apidae	Wood, Plant Stems
Apis mellifera	2	Apidae	Cavity/Artificial Hive
Bombus griseocollis	1	Apidae	Ground
Bombus huntii	1	Apidae	Ground
Bombus rufocinctus	1	Apidae	Ground
Ceratina acantha	3	Apidae	Wood, Plant Stems
Ceratina nanula	9	Apidae	Wood, Plant Stems
Ceratina pacifica	3	Apidae	Wood, Plant Stems
Colletes fulgidus	2	Colletidae	Ground
Diadasia diminuta	40	Apidae	Ground
Diadasia enavata	1	Apidae	Ground
Diadasia rinconis	4	Apidae	Ground
Dianthidium pudicum	5	Megachilidae	Pre-existing Cavity
Dianthidium subparvum	1	Megachilidae	Pre-existing Cavity
Dioxys pomonae	1	Megachilidae	Kleptoparistic
Eucera edwardsii	4	Apidae	Ground
Halictus confusus	7	Halictidae	Ground
Halictus ligatus	95	Halictidae	Ground
Halictus rubicundus	8	Halictidae	Ground
Halictus tripartitus	327	Halictidae	Ground
Heriades variolosa	2	Megachilidae	Pre-existing Cavity
Hoplitis albifrons	1	Megachilidae	Wood, Plant Stems
Hoplitis hypocrita	1	Megachilidae	Wood, Plant Stems
Hylaeus mesilla	1	Colletidae	Pre-existing Cavity
Lasioglossum (Dialictus) spp.	15	Halictidae	Ground

Lasioglossum (Hemihalictus) sp. 1	20	Halictidae	Ground
Lasioglossum (Hemihalictus) spp.	14	Halictidae	Ground
Lasioglossum brunneiventre	105	Halictidae	Ground
Lasioglossum cooleyi	10	Halictidae	Ground
Lasioglossum glabriventre	3	Halictidae	Ground
Lasioglossum hudsoniellum	57	Halictidae	Ground
Lasioglossum incompletum	99	Halictidae	Ground
Lasioglossum kincaidii	4	Halictidae	Ground
Lasioglossum liliputense	7	Halictidae	Ground
Lasioglossum nevadense	7	Halictidae	Ground
Lasioglossum prasinogaster	15	Halictidae	Ground
Lasioglossum pruinosum	100	Halictidae	Ground
Lasioglossum ruidosense	6	Halictidae	Ground
Lasioglossum tegulare group	9	Halictidae	Ground
Lasioglossum trizonatum	2	Halictidae	Ground
Megachile montivaga	1	Megachilidae	Pre-existing Cavity
Megachile onobrychidis	6	Megachilidae	Pre-existing Cavity
Megachile rotundata	1	Megachilidae	Pre-existing Cavity
Melissodes agilis	1	Apidae	Ground
Melissodes lupinus	12	Apidae	Ground
Melissodes microstictus	3	Apidae	Ground
Melissodes rivalis	2	Apidae	Ground
Melissodes saponellus	1	Apidae	Ground
Melissodes sp.	1	Apidae	Ground
Nomada sp.	2	Apidae	Kleptoparistic
Osmia "acanthosmioides" sp. 1	1	Megachilidae	Pre-existing Cavity
Osmia "acanthosmioides" sp. A	1	Megachilidae	Pre-existing Cavity
Osmia albolateralis	1	Megachilidae	Pre-existing Cavity
Osmia brevis	4	Megachilidae	Pre-existing Cavity
Osmia bruneri	3	Megachilidae	Pre-existing Cavity
Osmia calla	5	Megachilidae	Pre-existing Cavity
Osmia ednae	1	Megachilidae	Pre-existing Cavity
Osmia longula	1	Megachilidae	Pre-existing Cavity
Osmia nemoris	17	Megachilidae	Pre-existing Cavity
Osmia texana	3	Megachilidae	Pre-existing Cavity
Osmia trevoris	11	Megachilidae	Pre-existing Cavity
Sphecodes sp.	1	Halictidae	Kleptoparistic

Appendix D. Supplementary Information

1. Notes on highway and right-of-way design standards for study design:

Note from the [Federal Highway Administration Website](#):

*"*Examples of design standards for the Interstate System include full control of access, design speeds of [80 to 113 km/hr] 50 to 70 miles per hour (depending on type of terrain), a minimum of two travel lanes in each direction, [3.7 m] 12-foot lane widths, [3 m] 10-foot right paved shoulder, and [1.2 m] 4-foot left paved shoulder."

Note from [ITD Road Design Manual](#):

"Non-NHS Rural Roads: [6.7-10.4 m] 22-34 ft. minimum, [7.3-11 m] 24-36 ft. desirable, depending upon traffic volume (over 2,000 per day is [10.4 m] 34 ft.); this includes both lanes and shoulders."

"Clear zones are between [2.1 and 9.1 m] 7 and 30 ft., depending upon traffic and slope."

[*Metric conversions added in brackets by the author.*]

2. Transect locations on satellite imagery, showing general pattern of agriculture and associated greenness in the Snake River corridor.
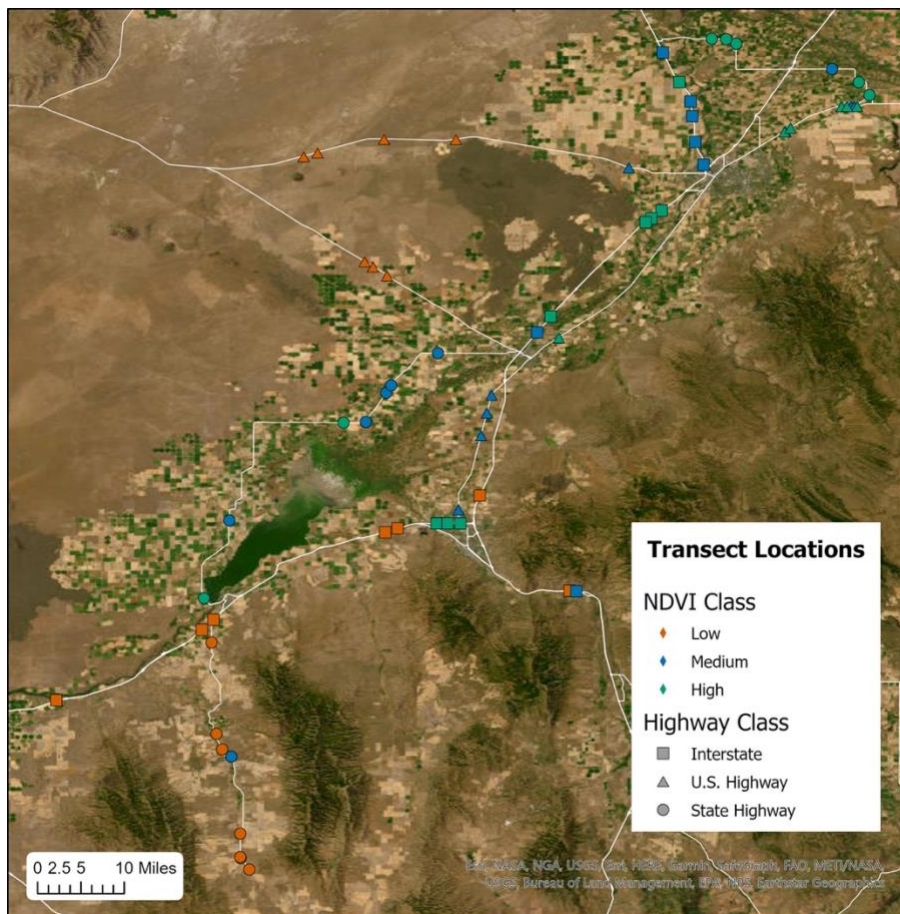
3. Butterfly species ranked in order of abundance. Totals sum all observations across the study, including those observed at transects outside of the 10-minute window (not included in analyses).

Number of Butterflies by Species
from all butterfly data collected at plots, including August & before/after/time-stopped data

Species	Count
Cabbage White	470
Clouded Sulphur	414
Becker's White	135
Common Ringlet	115
Checkered White	60
Purplish Copper	37
Melissa Blue	36
Common Checkered-Skipper	28
Callippe Fritillary	18
Western Branded Skipper	16
Small Wood-Nymph	14
Field Crescent	12
Two-tailed Swallowtail	9
Common Wood-Nymph	8
California Hairstreak	7
Western Tiger Swallowtail	5
Juba Skipper	5
Boisduval's Blue	4
Woodland Skipper	3
Ruddy Copper	3
Mylitta Crescent	3
Monarch Butterfly	3
Gray Hairstreak	3
Common Sootywing	3
Weidemeyer's Admiral	2
Sandhill Skipper	2
Painted Lady	2
Orange Sulphur	2
Mourning Cloak	2
Blue Copper	2
Anise Swallowtail	2
Variegated Fritillary	1
Sagebrush Sooty Hairstreak	1
Milbert's Tortoiseshell	1
Juniper Hairstreak	1
California Tortoiseshell	1
Behr's Hairstreak	1