Effects of traffic and flower abundance on pollinators in road verges

Luca Mannella

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Swedish University of Agricultural Sciences, SLU
Faculty of Natural Resources and Agricultural Sciences • Department of Ecology
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Luca Giovanni Mannella

Supervisor:	**Erik Öckinger, SLU, Department of Ecology**
Assistant supervisor:	Sofia Blomqvist, Lund University
Examiner:	Ola Lundin, SLU, Department of Ecology

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Swedish University of Agricultural Sciences
Faculty of Natural Resources and Agricultural Sciences
Department of Ecology

Abstract

Pollinator populations across the globe are rapidly declining. One reason for this is habitat loss. To mitigate this effect, road verges could serve as new habitats, similar to the species-rich semi-natural grasslands that are among the most rapidly declining habitats. I examined road verges of streets with three different traffic densities for the number and diversity of butterflies and bees, and mortality from direct collisions with cars. The results showed a negative effect of traffic on the number of pollinator species and individuals in road verges, and a lower mortality rate per car the higher the traffic density was. These results were independent of the number of flowers in road verges. Either road verges along busy roads act as an ecological trap, attracting pollinators to these habitats even though the risk of mortality is higher than reproductive success, or they actively avoid these habitats. In either case, traffic appears to be an important factor determining the number and diversity of pollinators in road verges.

Keywords: pollinators, road verges, traffic, semi-natural grasslands, Lepidoptera, Anthophila

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Abbreviations

SLU	Swedish University of Agricultural Sciences
GLM	General Linear Model
df	Degrees of Freedom
sd	Standard Deviation
se	Standard error

Introduction

Science agrees that humans depend on healthy ecosystems which provide life-sustaining functions such as nutrient cycling, maintaining biodiversity, waste assimilation or climate regulation (Chee 2004). These functions that benefit humans are called “ecosystem services” (Daily 1997; Norberg 1999). The underlying ecological processes are often very complex and therefore susceptible to change. To prevent serious consequences, it is therefore necessary to investigate these processes with the help of studies.

1.1 Pollination

One example of a key ecosystem service that humans rely on is pollination (Klein *et al.* 2007). The reproductive success of almost 90 % of the world's terrestrial flowering plant species relies, at least in part, on animals that distribute their pollen between individuals (Kearns *et al.* 1998; IPBES 2016). This process is referred to as pollination and the animals associated with it are called pollinators. The primary pollinating animal group are insects (Watanabe 1994) out of which bees are considered the most important. There has also been increasing evidence for the importance of other pollinators in recent years. Non-bee pollinators significantly contribute to global crop pollination, visiting around 39% of flowering crop species (Rader *et al.* 2016). Other pollinating invertebrate species include beetles, butterflies and hoverflies, but also vertebrate species like birds, bats, and some lizards (IPBES 2016).

*Figure 1. The western honey bee (*Apis mellifera*) collecting nectar and pollen. The pollen is seen on her hint leg.*

Many plant and pollinator species are co-dependent on each other (Biesmeijer *et al.* 2006). Nectar and pollen from flowers nourishes pollinators and their ability to move through their environment ensures pollen dispersal for reproduction and genetic variability. If one would cease to exist, many other organisms would also become extinct.

Apart from preserving biodiversity, there is also a substantial economic value to pollination. It is estimated that five to eight per cent of current global crop production is attributable to animal pollination, which has an annual market value of $235 billion - $577 billion Dollars (IPBES 2016). Many livelihoods in rural environments or low-income countries depend on beekeeping for its monetary value, as it can generate products like beeswax or honey with relatively little capital investment (Bradbear 2009).

1.2 Pollinator decline

In recent decades, the biomass of insects has steadily declined not only regionally, but also worldwide (Hallmann *et al.* 2017; Forister *et al.* 2019). This includes important pollinator species like wild bee populations, with numerous studies showing a rapid decline in Europe (Banaszak 1995; Kosior *et al.* 2007). The reasons for this are complex and often depend on several interlinked factors. Recent studies also show that the causes of insect declines are very individually dependent on the environment, and in most studies it is probably not so much one factor but several that add up (Hallmann *et al.* 2017; Wagner *et al.* 2021).

Land-use change, agricultural intensification, and climate change are among the most important drivers of the decline in insect biomass (Forister *et al.* 2019; Dicks *et al.* 2021; Wagner *et al.* 2021). Land-use changes can result in the degradation and fragmentation of vital habitats or their conversion to forests, farmland or urban landscapes, making resources for food and nesting increasingly scarce (Klein *et al.* 2007). Especially semi-natural grasslands, a highly diverse and species-rich habitat, rapidly decreased over the last decades in Europe (Öckinger & Smith 2006). These grasslands are being defined as usually either intensively mowed or grazed by livestock without external fertilization and can host a large number of flowering plants. Agricultural intensification is also driving the loss of pollinating insects. More monocultures and extensive usage of pesticides and fertilizers lead to a deterioration in habitat quality (Potts *et al.* 2016; Raven & Wagner 2021). The changes in precipitation and temperature as well as the associated extreme weather events make climate change another important driver of the insect decline (Ewald *et al.* 2015; Raven & Wagner 2021). In a lot of tropical habitats, insects are facing increased periods of drought that they have not adapted to (Wagner 2020). Higher fluctuations in temperatures have been shown to drive insects away from their habitats (Colwell *et al.* 2008).

Furthermore, introduced non-native species are often competing for resources and nesting opportunities or a direct threat to the native wildlife. One example is the parasitic varroa mite (*Varroa destructor*) which lives on fat reserves of bees and can weaken as well as kill a hive. Originally from Asia it became a global threat when it was introduced in other countries. Even though it is mainly a threat to domestic honey bees (*Fig. 1*), it can also influence viral prevalence in wild bee populations (Piot *et al.* 2022).

A decline in insect pollinators can also have an effect on wild plants that are dependent on them for sexual reproduction, that is producing seeds and fruits (Ashman *et al.* 2004). There is already evidence for parallel declines in insect and plant populations in western Europe (Biesmeijer *et al.* 2006). This could further limit food resources creating a negative feedback loop which could result in devastating effects on both plant and insect populations.

1.3 A possible mitigation: road verges

Land-use change, one of the main causes of the recent insect decline, is unlikely to stagnate in the future. When looking at global infrastructure projections alone it becomes apparent that the problem of habitat degradation is only getting bigger. It is predicted that the total length of paved roads is going to increase by 25 million kilometres until 2050 (Laurance *et al.* 2014), which is more than 155 times the distance from earth to the moon. While this is likely to cause destruction, it will also create new habitats that border these roads (‘road verges’).

Road verges are linear strips of land along roads that are mowed regularly to keep the road clear and can therefore resemble traditionally managed semi-natural grasslands (Gardiner *et al.* 2018). Similar to semi-natural grasslands they also host various plant and animal species (Gardiner *et al.* 2018). They could also act as migration corridors and mitigate the barrier effect that roads can have, especially for animals that are flightless or small (Muñoz *et al.* 2014). Well managed road verges could enable species to spread throughout the landscape and establish in new habitats.

It is not yet known which factors influence the abundance and diversity of animals most within road verges. Flower abundance and diversity is likely to play a key role, as they attract pollinating insects with their pollen and nectar. In a study researching the combined effects of traffic intensity and flower diversity in road verges on bumblebee queens (Dániel-Ferreira *et al.* 2022), their mortality through collisions with cars tended to be lower on roads with a high flowering plant diversity in road verges compared to roads with low flowering plant diversity. A high flowering plant diversity could therefore decrease bumblebee mortality through collisions. However, an attractive flower-rich road verge could also act as an ecological trap, as they could attract insects from the surrounding landscapes but increase the mortality rate through collisions with traffic (Battin *et al.* 2004; Dániel-Ferreira *et al.* 2022), possibly causing a net negative impact on populations. Road verges are also exposed to various forms of pollution from roads and traffic, such as noise, turbulence, dust and metals, which may affect pollinator activity and survival (IPBES 2016; Phillips *et al.* 2020). Therefore, it is important to understand the factors involved in promoting animal biodiversity and abundance in these strips of land and the effect of different traffic intensities and surrounding landscapes. Tailoring management of road verges could offer an opportunity to conserve and enhance pollinator biodiversity, especially in degraded or intensely managed homogenous landscapes.

1.4 Aim of the study

To decipher the effects of roads on pollinators and tie into existing studies, I studied roads with different traffic intensities for insect mortality through collisions with cars and pollinator diversity and abundance in road verges. I expected lower pollinator abundance or diversity in road verges with high traffic intensity caused by negative impacts of traffic, and lower mortality from collisions on roads with high traffic volume due to the lower baseline numbers of insects on these roads. I also used floral cover data from road verges to test for an effect. I hypothesized that flower cover would correlate with the abundance and diversity of pollinators and insects.

Material & Methods

2.1 Study species

Pollinators included in this study were solitary bee species (Anthophila), the western honey bee (*Apis mellifera*), and butterflies (Rhopalocera), as they are among the most important group of pollinating animals (IPBES 2016). Arguments for the inclusion and exclusion of pollinators were difficulty of identification both in the field and from photos, and their relevance as flying insects and pollinators. Bumblebee species were not included in the study because a parallel study by Sofia Blomqvist (Lund University), using the same sites, already focused on bumblebees. As butterflies and bees go through several changes in their life cycle, each species has different requirements in these phases, which allows conclusions to be drawn about the quality and heterogeneity of habitats. In their first stage of life, many butterflies are specialists and rely on specific plants as a food source, while as adults they are generalists and feed on the nectar of different types of flowers (Altermatt & Pearse 2011). Bees collect both pollen and nectar and are often specialized on certain flowering plant species (Westrich 1989). Combining both in a study gives insight about plant species composition.

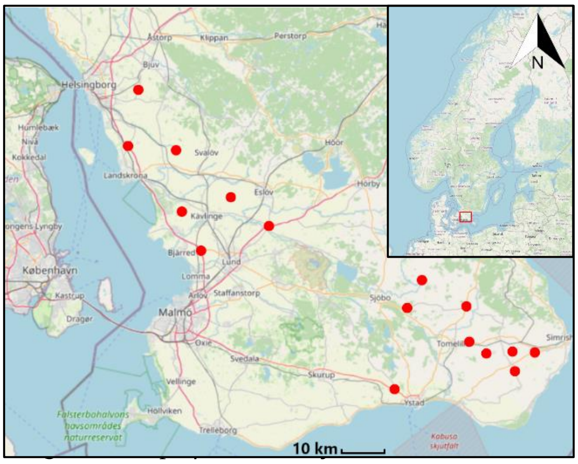

Figure 2. Map of the 16 study sites. The individual sites are marked as red dots. On the top right is a reference map of Scandinavia.

2.2 Study sites

All landscapes are located in Skåne, southern Sweden (*Fig. 2*). Each landscape consisted of three different road sections within a 2 km radius: high traffic density, low traffic density and a control road which was usually a gravel road with no constant traffic. This design was chosen to eliminate effects of surrounding landscapes, as the different road segments now had similar surrounding landscapes. Data on the average number of vehicles per day was obtained from the Swedish traffic agency (Trafikverket). The classification of high and low traffic densities was seen as relative to each other and not classified with a fixed number of cars per day. Therefore, there was quite a wide range in the number of cars: low density roads had 60 to 2000 cars and high density roads had 260 to 50,000 cars per day. All roads were adjacent to fields, except for some of the control sections where some were surrounded by more heterogenous landscapes.

2.3 Data collection

2.3.1 Insects

Fieldwork was conducted three times; in late spring (18.05. – 21.05.), early summer (12.06. – 15.06.), and mid-summer (17.07. – 20.07.) of 2023. The first data collection in May was mostly sunny with temperatures ranging from 12°C to 25°C and strong gusts of wind. During the second data collection, temperatures ranged from 15°C to 27°C with sunny conditions. During the third data collection, the weather was more mixed with temperatures ranging from 11°C to 22°C as well as partly cloudy conditions.

The order of sites was randomized in the three fieldwork periods to get data from the locations during different times of day to control for differences in pollinator activity. Data collection took place between 9:30 am and 5 pm.

Figure 3. Sticky traps mounted on a car. The sticky traps (yellow) are mounted to a hard cardboard plate (brown) with metal clippers. The cardboard is mounted to the air intake of the vehicle with wire.

To obtain data on insect mortality, two sticky insect traps were mounted to the front of a car (*Fig. 3*). There were two parts to a trap: the first was a mounting plate made out of hard cardboard which is fixed to the car with wire, and the second was the sticky trap sheet which can be taken on and off the mounting plate with metal clips. One individual sticky trap sheet had a size of 10 x 25 cm which results in a total trap area of 500 cm². The car was then driven a total distance of 2.5 km, split up into two 1.25 km sections. Because some roads sections were too short, especially some of the control roads, the total distance was not driven in two 1.25 km sections, but in more and smaller sections to still cover the same distance. The aim was to drive with a consistent speed of 60 km/h. On some of the gravel roads it was not possible to drive with that speed for safety reasons. Afterwards, photos were taken of both traps and the sheets were removed. Each insect on the sticky traps was counted later. Using all insects to get reliable data on mortality was necessary, as there were hardly any butterflies or bees stuck to the sticky traps in test drives.

To determine the number of bees and butterflies in the road verges, a 200 m transect was walked down the middle of the road verges. The width of the transect corresponded to the width of the road verge. Species were photographed when possible to help with identification of unknown species, and the number of individuals was noted. A net was used to catch unknown pollinators or ones that were hard to identify. The weather, temperature and time was also noted.

2.3.2 Flowers

Data on flower coverage in the road verges was collected from 26th of June to 7th of July by the research team of Sofia Blomqvist (Lund University). For each road verge, four transects with a length of 250 meters were established (1000 meters in total); two on each side of the road and each in the middle of the road verge. The different flower species and both the size and number of flowers were noted. To calculate the flower coverage, the number of flowers in all four transects was multiplied with their size. It was also noted if a road verge was freshly mowed.

2.4 Statistical analyses

All statistical analyses were done with RStudio (Version 2023.06.1). Data from the individual sample rounds was summed for each site. To test if traffic density had an effect on the number of bee and butterfly species and individuals I used an analysis of variance (ANOVA). To test if the different landscapes in combination with traffic density had an effect on number of species and individuals, I used a two-way ANOVA. To assess the significance of pairwise contrast I used Tukey’s adjustment for multiple comparisons (Tukey test). To test for a combined effect of flower coverage and traffic density on pollinator species and individuals, I used a linear regression model (lme4 package). I also tested for an effect of landscape on flowering plant species with a two-way ANOVA. For testing the effect of traffic density and landscape on insect mortality, I also used a two-way ANOVA. To get the number of insects killed by collisions with cars per day, I multiplied the number of insects killed with one car with the average number of cars per day on each road. For the control roads, I assumed one car per day. To visualize the data, I used the ggplot2 package.

Results

3.1 General findings

A total of 182 butterflies from 19 different species and 121 bees from 12 different species were found (*Fig. 4*). The most frequently observed butterfly species were the small tortoiseshell (*Aglais urticae*), meadow brown (*Maniola jurtina*), large skipper (*Ochlodes sylvanus*), and the cabbage white butterfly (*Pieris brassicae*).

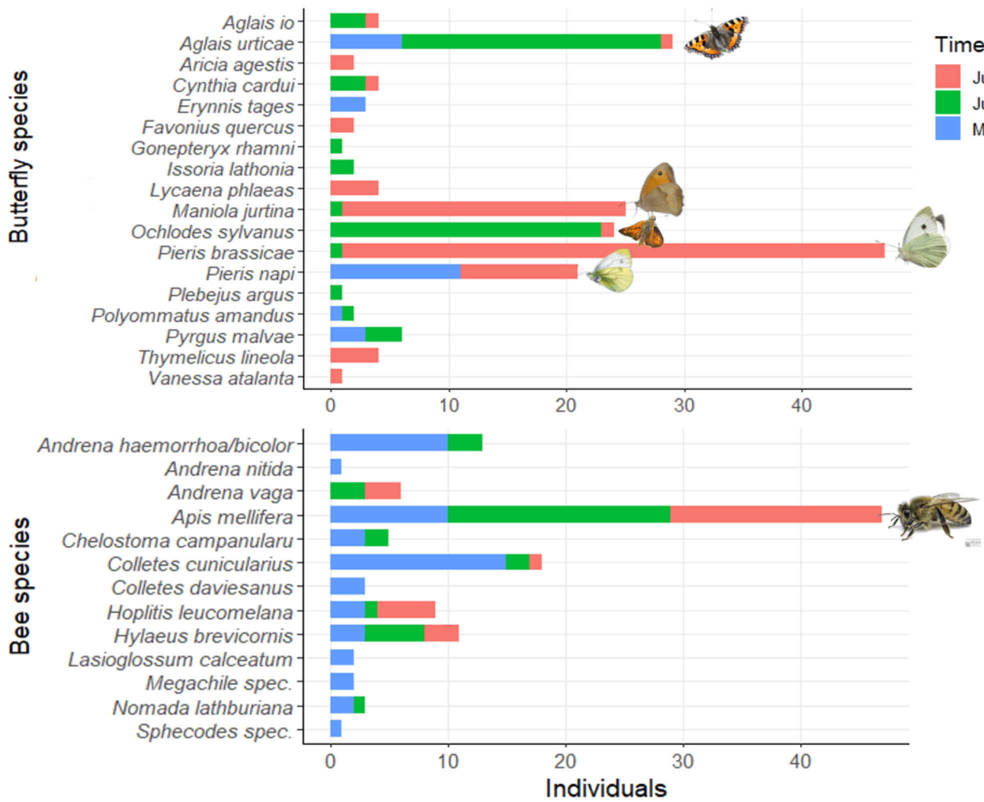

Figure 4. Sightings of butterfly and bee species in May, June, and July in alphabetical order. The length of the bars correspond to how many individuals were seen.

The number of butterfly individuals varied strongly between the three summer months. During the first sampling round in May I found the least butterfly individuals, much less than compared to the two other sampling rounds. In June and July the number of butterfly individuals was similar. In total I found five butterfly species during May, eleven in June and twelve in July. The most frequently observed bee species was the western honey bee (*Apis* mellifera) with more evenly distributed sightings between May and July. In total I found twelve bee species during May, eight in June and five in July.

3.2 Pollinators within road verges

The total number of insect individuals (ANOVA, df = 2, F = 14.15, p < 0.0001) and the number of species (ANOVA, df = 2, F = 12.47, p < 0.0001) were negatively related to traffic intensity. There was no detectable interaction between traffic intensity and landscape, neither on total number of species (Two-way ANOVA, df = 15, F = 13.082, p = 0.362) nor on total number of individuals (Two-way ANOVA, df = 15, F = 13.775, p = 0.553).

Table 1. Testing the effect of traffic on bees, butterflies, and both added together (One-way ANOVA).

Testing effect of traffic on	Total # of species	Total # of individuals	Bee species	Bee individuals	Butterfly species	Butterfly individuals
Df	2	2	2	2	2	2
F-value	12.47	14.15	6.205	5.275	8.646	11.98
p-value (ANOVA)	< 0.0001	< 0.0001	0.0042	0.0088	0.0007	< 0.0001

When looking at both bees and butterflies added together, there was a significant difference in the number of species and individuals between the control roads and the other two traffic densities (*Fig. 5,* ***a*** *&* ***c****; Table 2*) but no significant difference between roads with low and high traffic density, both with number of individuals (Tukey-test, p = 0.159) and number of species (Tukey-test, p = 0.326). The results for butterfly individuals and species were similar, as there was a significant difference between control roads and the other two traffic densities, but not between roads with low and high traffic density (*Fig. 5,* ***b*** *&* ***e****; Table 2*). The number of bee species and bee individuals (*Fig. 5,* ***c*** *&* ***f***) were significantly different between high traffic intensities and the control roads (*Table 2*), but not between low traffic intensities and control roads. The number of bee individuals also did not significantly differ between roads with low and high traffic densities (*Table 2*, p = 0.1336).

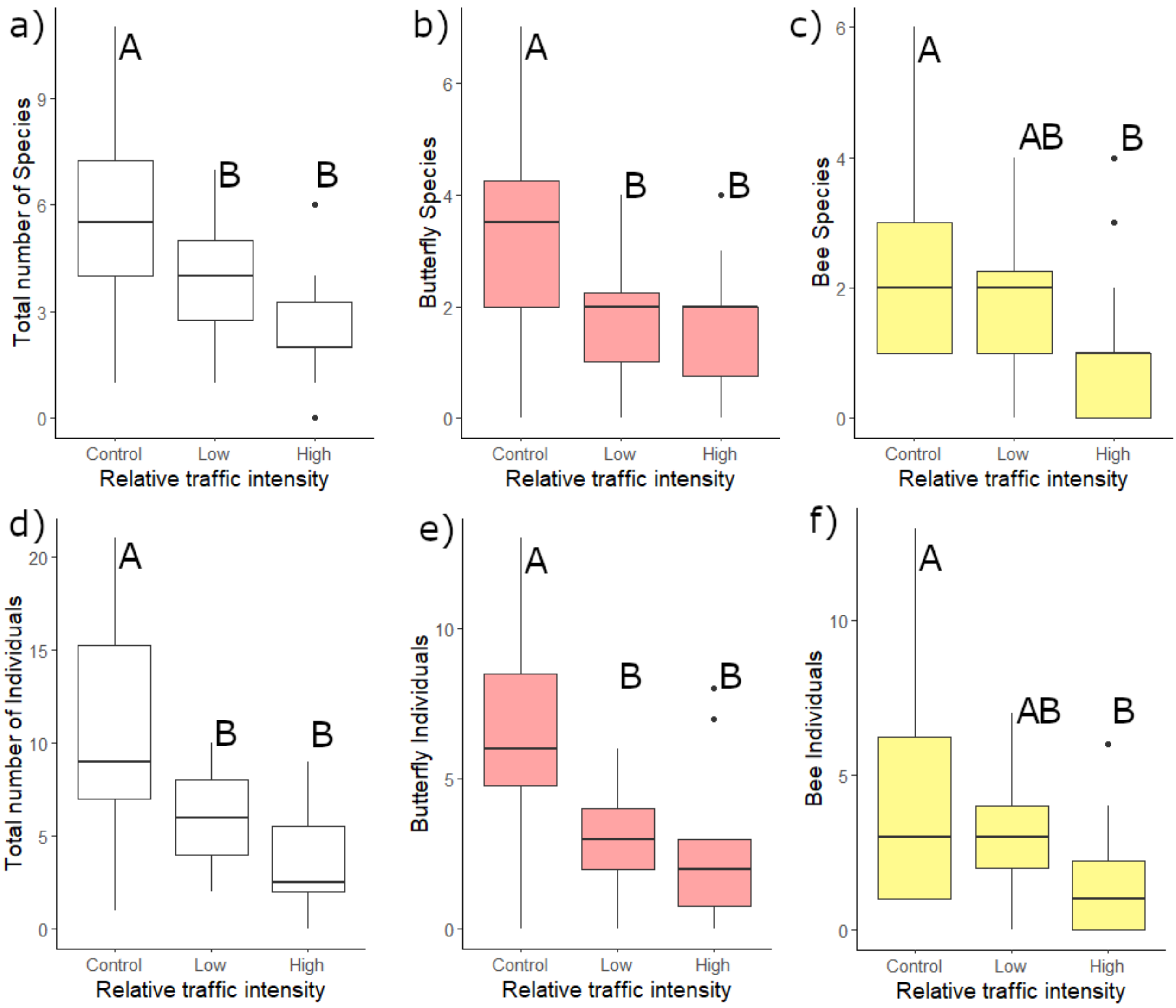

Figure 5. Visualization of number of species and number of individuals compared to traffic intensity. The capital letters represent statistical differences within each graph. The graphs a) – c) show the number of species added together and of butterflies and bees separately, d) – f) shows the number of individuals added together and for butterflies and bees separately.

Table 2. P-values (Tukey-test) of comparisons between different traffic intensities. Non-significant p-values are red.

p-values of comparing traffic intensities	Tot. # of Individuals	Tot. # of Species	# of bee individuals	# of bee species	# of butterfly individuals	# of butterfly species
control-high	< 0.0001	< 0.0001	0.0066	0.0012	0.0001	0.0011
low-high	0.159	0.326	0.1336	0.0669	0.7779	0.8666
low-control	0.0053	0.0068	0.9026	0.287	0.001	0.005

3.3 Flower coverage

In total 101 different flowering plant species were recorded along the road verges. The most observed plant species were *Achillea millefolium* (145 times), *Convolvulus arvensis* (126 times), *Potentilla spec.* (116), and *Matricaria spec.* (112 times).

There was no detectable effect of traffic on flower diversity (ANOVA, df = 2, F = 0.623, p = 0.54) or on abundance (ANOVA, df = 2, F = 0.821, p = 0.44, *fig. 6*). When combining the flower data with traffic and pollinators through a linear regression model, there was only an effect of traffic but not of flowers both on pollinator species an individuals (*Table 3*).

Table 3. Linear regression model output of the effect of traffic and flower coverage on number of pollinator species and individuals.

	Pollinator species			Pollinator individuals		
	Variable estimate (+/-se)	t-value	p-value	Variable estimate (+/-se)	t-value	p-value
(Intercept)	5.1 (0.978	5.223	<0.0001	10.34 (1.944)	5.321	<0.0001

High traffic	-3.47 (0.695)	-4.994	<0.0001	-7.106 (1.382)	-5.142	<0.0001
Low traffic	-2.28 (0.694)	-3.283	0.002	-4.729 (1.381)	-3.425	0.00134
Flower coverage	0.03 (0.035)	0.906	0.37	0.014 (0.07)	0.205	0.83886

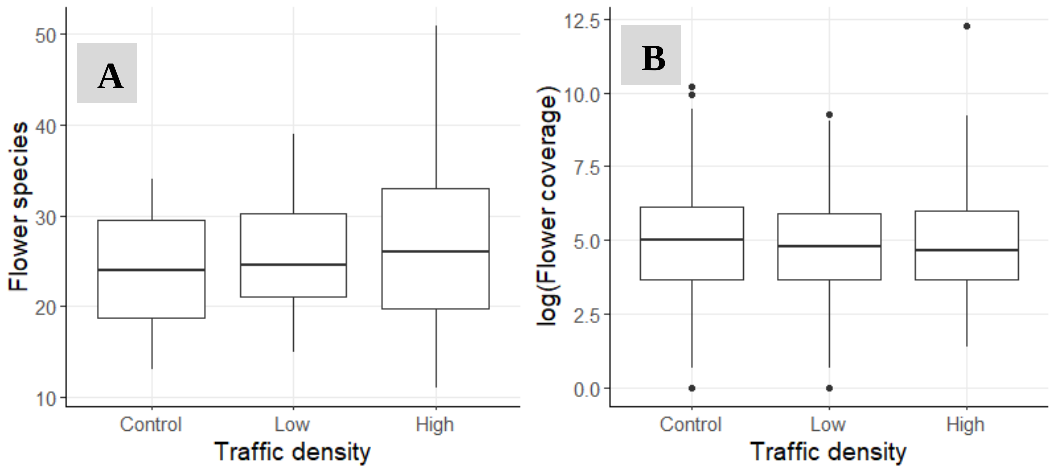

*Figure 6. The number of flower species in road verges of roads with three different traffic densities (**A**) and the flower coverage in road verges of roads with three different traffic densities (**B**).*

However, there was a significant effect of the landscape on the number of flowering plant species when combined with the traffic intensity (Two-way ANOVA, df = 19, F = 3.181, p = 0.00117).

3.4 Insect mortality through collisions

The number of insects killed through collisions with one car decreased with increasing traffic. Within a distance of 2.5 km, 2.15 +/- 2.5 (mean +/- sd) insects were killed on roads with high traffic density, 3.02 +/- 2.71 on roads with low traffic density, and 6.21 +/- 5.73 insects were killed per car on the control roads (*fig. 7, **A***). An analysis of variance showed that both traffic density (ANOVA, df = 2, F = 18.217, p < 0.0001) and location (ANOVA, df = 15, F = 3.692, p < 0.0001) had a significant effect on insect mortality. Pairwise comparisons showed that insect mortality in control roads was significantly different to roads with high traffic densities (Tukey-test, p < 0.0001) and roads with low traffic densities (Tukey-test,

p = 0.0002). Insect mortality in roads with high and low traffic densities did not differ significantly from each other (Tukey-test, p = 0.6015).
The number of insects killed by collisions per car decreased with increasing traffic (*Fig. 7, **A***), but increased when the number per day was considered (*Fig. 7, **B***).

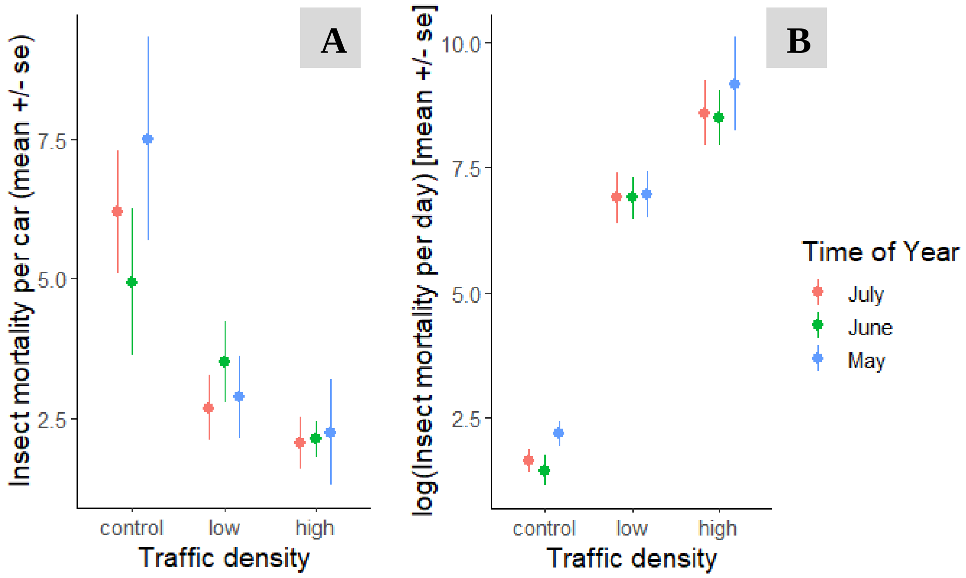

*Figure 7. Insect mortality in relation to traffic density. **A** shows the mean number +/- se of insects killed by one car on roads with three different traffic densities (control, low, high traffic density). **B** shows the same data multiplied with the number of cars per day on a logarithmic scale. The data is color-coded and split up into the three different months in the summer 2023.*

Discussion

The aim of this study was to find out if traffic would have an effect on pollinator populations within road verges and on mortality through collisions. To test this, I categorized roads by traffic density and surveyed road verges for butterfly, bee, and flowering plant species, as well as tested the insect mortality through collisions with sticky traps.

The highest abundance and diversity of pollinators included in this study was found in the control roads with almost no traffic (*Fig. 5*). Insect mortality per car was also highest in these roads (*Fig. 6*). Consequently there are two main results of this study:

1) The more traffic on a road, the fewer bee and butterfly species and individuals in the road verges.
2) The more traffic on a road, the fewer insects die per car, but the more insects die overall from collisions.

Even though there was no significant difference in the number of species and insect individuals between low and high traffic density, there was still a trend visible (*Fig. 5*). Longer periods of fieldwork or additional data collection could have improved the significance of the statistical models, as a similar study had two to three week long surveys per visit compared to my three to four day surveys per visit, leading to more replicates of each treatment (Phillips *et al.* 2019). Because of its statistical significance, I will focus on the effect that roads with high traffic volume had on insects.

There are two possible explanations why I found fewer pollinating insects along roads with high traffic density: either they are actively avoiding roads with higher traffic, or the traffic already reduced their numbers.

If it is a behavioural response and the insects avoid proximity to busy roads, the next step would be to determine what causes this behaviour. Based on previous studies, it is likely that this avoidance behaviour is largely influenced by turbulence, heavy metal concentrations in flowers, and collisions with vehicles (Muñoz *et al.* 2014; Phillips *et al.* 2020; Phillips *et al.* 2021).

If the traffic already reduced the number of pollinators, road verges of roads with high traffic densities would act as a population sink and could potentially be an ecological trap. An ecological trap is a scenario in which an animal chooses a low-quality habitat over other habitats of higher quality in the surrounding landscapes (Robertson & Hutto 2006). The reason is usually disrupting disturbances that change a habitat, which leads to animals having a harder time assessing the true quality of a habitat when the usual cues are no longer present. In the example of road verges, the traffic itself could be a disrupting disturbance. As there is no such disturbance in semi-natural grasslands, which the road verge resembles, both habitats could have similar attractiveness to pollinators regardless of the mortality risk through collisions while looking for nectar and pollen. Another disturbance changing the quality of habitat is frequent mowing. A road verge at a given time could seem like an attractive habitat for insects to reproduce and produce offspring, even though the mowing at a later time could potentially cause high mortality in eggs or larvae that rely on plant foliage as a food source. The timing of mowing greatly influences insect populations and optimizing mowing regimes can have a positive effect on pollinators in road verges (Phillips *et al.* 2020). Further studies researching population trends over longer periods of time are needed to clarify if and to what extend road verges could act as an ecological trap.

Contrary to my expectations, flower coverage did not have any effect on pollinators and was similar in road verges of all three traffic densities. The significant effect of the location on flower coverage makes sense, as plant species composition is likely to be similar in close proximity (between the three road verges of different traffic

Figure 8. One landscape at two different times. The left picture shows a freshly mowed road verge in June. The right picture shows the same road verge a month later.

intensities within one landscape). Surveying flowers only once and at a different time than the pollinators could be the reason for not finding an effect. If the surveyed road verge was mowed between the individual surveys for pollinators and plants, the resulting data would be incorrect as freshly mowed road verges had no or very few flowering plants (*Fig. 8*). Because only a few of the inventoried road verges were mowed between the surveys and the flower survey only happened once per site whereas there were three pollinator surveys, the effect would have been likely to cancel itself out or still show an effect on pollinators in these road verges. In addition, certain flower species differ in how frequently they are visited by pollinators. This depends partly on the general attractiveness of the flower, which depends on visual and olfactory factors (Flacher *et al.* 2020). A study of pollinators in road verges in Cornwall, UK, (Phillips et al. 2019), which examined plant visitation by pollinators, found that the flowers of only five plant species were visited by sixty percent of pollinators, suggesting a key role for certain flowering plants in supporting pollinators. It seemed obvious during fieldwork, that pollinators had certain flower species to target. Some examples of these preferred species would be the spear thistle (*Cirsium vulgare*), white and red clover (*Trifolium repens/pratense*), or the dandelion (*Taraxacum officinale*), which was one of the five plant species most visited by pollinators in the previously mentioned study. As I did not survey plant-pollinator interactions, this observation cannot be backed up with data.

While the general attractiveness of a flower plays a role in how frequently they are visited by pollinators, it also depends on the specialization of pollinator species. In particular, bee species are often specialized in collecting pollen and nectar from certain types of flowers and therefore prefer flowers of certain closely related plant species (Müller 1996).

Additionally, my results show that it is unlikely that flowering plant diversity or abundance has an effect on insect mortality through collisions with cars. This aligns with a similar study done with bumblebees in the same area (Dániel-Ferreira *et al.* 2022) which found no effect influencing the mortality of bumblebee queens.

Overall, categorizing roads by total number of cars per time could be a better option than categorizing them by comparison, because the number of cars differed a lot within categories. This would give a clearer image on the effect on pollinators in road verges, and if the effect is directly correlated to the number of cars. The low numbers of both species and individuals overall could also contribute to making the model inaccurate. More rounds of fieldwork would have likely been beneficial for producing representative numbers of pollinator populations.

The data on how many insects are killed per day (*Fig.* 7) is also simplified, as the number of vehicles per day show the average per year and my data was confined to three individual days, one each in May, June, and July. Especially on roads with high traffic densities, a difference of just one insect on the sticky traps had a very big effect on the number of insects killed daily. The varying speed of the vehicle during sampling the insect mortality due to road quality could also have resulted in a difference in insect mortality. Using sticky traps for measuring how many insects are getting killed by car could also seem unfitting, as the area and surface of the traps is different from a vehicle. Because the goal in this study was to compare mortalities per vehicle at different traffic densities and not to find out the total number of insects killed per vehicle, using sticky traps made sense. It also makes it reproducible and comparable to similar future studies.

My results show a negative effect of roads with traffic on pollinator populations. They are in line with previous studies suggesting road verge management to focus on roads with low traffic densities (Phillips *et al.* 2019). It is necessary to get a clearer image of factors involved in reducing pollinators in road verges though, as my results did not show a significant difference in pollinator populations between roads with low and high traffic densities. Future studies should focus on population trends over longer periods of time to clarify whether the positive effects of road verges outweigh the negative, and how this balance shifts with varying traffic densities.

References

Altermatt, F. & Pearse, I.S. (2011). Similarity and specialization of the larval versus adult diet of European butterflies and moths. *Am Nat*, 178(3), 372-82. https://doi.org/10.1086/661248

Banaszak, J. (1995). International-Colloquium on the Trends of Changes in Fauna of Wild Bees in Europe. *Bee World*, 76(2), 97-97. <Go to ISI>://WOS:A1995RC88100010

Biesmeijer, J.C., Roberts, S.P., Reemer, M., Ohlemuller, R., Edwards, M., Peeters, T., Schaffers, A.P., Potts, S.G., Kleukers, R., Thomas, C.D., Settele, J. & Kunin, W.E. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*, 313(5785), 351-4. https://doi.org/10.1126/science.1127863

Bradbear, N. (2009). Bees and their role in forest livelihoods: a guide to the services provided by bees and the sustainable harvesting, processing and marketing of their products. *Non-wood Forest Products*(19).

Chee, Y.E. (2004). An ecological perspective on the valuation of ecosystem services. *Biological Conservation*, 120(4), 549-565. https://doi.org/10.1016/j.biocon.2004.03.028

Colwell, R.K., Brehm, G., Cardelús, C.L., Gilman, A.C. & Longino, J.T. (2008). Global Warming, Elevational Range Shifts, and Lowland Biotic Attrition in the Wet Tropics. *Science*, 322(5899), 258-261. https://doi.org/doi:10.1126/science.1162547

Daily, G.C. (1997). *Introduction: what are ecosystem services*. (Nature's services: Societal dependence on natural ecosystems).

Dániel-Ferreira, J., Berggren, Å., Bommarco, R., Wissman, J. & Öckinger, E. (2022). Bumblebee queen mortality along roads increase with traffic. *Biological Conservation*, 272. https://doi.org/10.1016/j.biocon.2022.109643

Dicks, L.V., Breeze, T.D., Ngo, H.T., Senapathi, D., An, J., Aizen, M.A., Basu, P., Buchori, D., Galetto, L., Garibaldi, L.A., Gemmill-Herren, B., Howlett, B.G., Imperatriz-Fonseca, V.L., Johnson, S.D., Kovacs-Hostyanszki, A., Kwon, Y.J., Lattorff, H.M.G., Lungharwo, T., Seymour, C.L., Vanbergen, A.J. & Potts, S.G. (2021). A global-scale expert assessment of drivers and risks associated with pollinator decline. *Nat Ecol Evol*, 5(10), 1453-1461. https://doi.org/10.1038/s41559-021-01534-9

Ewald, J.A., Wheatley, C.J., Aebischer, N.J., Moreby, S.J., Duffield, S.J., Crick, H.Q. & Morecroft, M.B. (2015). Influences of extreme weather, climate and pesticide use on invertebrates in cereal fields over 42 years. *Glob Chang Biol*, 21(11), 3931-50. https://doi.org/10.1111/gcb.13026

Flacher, F., Raynaud, X., Hansart, A., Geslin, B., Motard, E., Verstraet, S., Bataille, M. & Dajoz, I. (2020). Below-ground competition alters attractiveness of an insect-pollinated plant to pollinators. *AoB Plants,* 12(4), plaa022. https://doi.org/10.1093/aobpla/plaa022

Forister, M.L., Pelton, E.M. & Black, S.H. (2019). Declines in insect abundance and diversity: We know enough to act now. *Conservation Science and Practice,* 1(8), e80.

Gardiner, M.M., Riley, C.B., Bommarco, R. & Öckinger, E. (2018). Rights-of-way: a potential conservation resource. *Frontiers in Ecology and the Environment,* 16(3), 149-158. https://doi.org/10.1002/fee.1778

Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Muller, A., Sumser, H., Horren, T., Goulson, D. & de Kroon, H. (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS One,* 12(10), e0185809. https://doi.org/10.1371/journal.pone.0185809

IPBES (2016). Summary for policymakers of the assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination and food production. *Zenodo*. https://doi.org/https://doi.org/10.5281/zenodo.2616458

Kearns, C.A., Inouye, D.W. & Waser, N.M. (1998). Endangered mutualisms: The conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics,* 29, 83-112. https://doi.org/DOI 10.1146/annurev.ecolsys.29.1.83

Klein, A.M., Vaissiere, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C. & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proc Biol Sci,* 274(1608), 303-13. https://doi.org/10.1098/rspb.2006.3721

Kosior, A., Celary, W., Olejniczak, P., Fijał, J., Król, W., Solarz, W. & Płonka, P. (2007). The decline of the bumble bees and cuckoo bees (Hymenoptera: Apidae: Bombini) of Western and Central Europe. *Oryx,* 41(1), 79-88. https://doi.org/10.1017/s0030605307001597

Laurance, W.F., Clements, G.R., Sloan, S., O'connell, C.S., Mueller, N.D., Goosem, M., Venter, O., Edwards, D.P., Phalan, B. & Balmford, A. (2014). A global strategy for road building. *Nature,* 513(7517), 229-232.

Müller, A. (1996). Host-plant specialization in western palearctic anthidiine bees (Hymenoptera: Apoidea: Megachilidae). *Ecological Monographs,* 66, 235-257. https://doi.org/https://doi.org/10.2307/2963476

Muñoz, P.T., Torres, F.P. & Megías, A.G. (2014). Effects of roads on insects: a review. *Biodiversity and Conservation,* 24(3), 659-682. https://doi.org/10.1007/s10531-014-0831-2

Norberg, J. (1999). Linking Nature's services to ecosystems: some general ecological concepts. *Ecological Economics,* 29(2), 183-202. https://doi.org/Doi 10.1016/S0921-8009(99)00011-7

Öckinger, E. & Smith, H.G. (2006). Semi-natural grasslands as population sources for pollinating insects in agricultural landscapes. *Journal of Applied Ecology,* 44(1), 50-59. https://doi.org/10.1111/j.1365-2664.2006.01250.x

Phillips, B.B., Bullock, J.M., Gaston, K.J., Hudson-Edwards, K.A., Bamford, M., Cruse, D., Dicks, L.V., Falagan, C., Wallace, C., Osborne, J.L. & Stanley,

M. (2021). Impacts of multiple pollutants on pollinator activity in road verges. *Journal of Applied Ecology*, 58(5), 1017-1029. https://doi.org/10.1111/1365-2664.13844

Phillips, B.B., Gaston, K.J., Bullock, J.M. & Osborne, J.L. (2019). Road verges support pollinators in agricultural landscapes, but are diminished by heavy traffic and summer cutting. *Journal of Applied Ecology*, 56(10), 2316-2327. https://doi.org/10.1111/1365-2664.13470

Phillips, B.B., Wallace, C., Roberts, B.R., Whitehouse, A.T., Gaston, K.J., Bullock, J.M., Dicks, L.V. & Osborne, J.L. (2020). Enhancing road verges to aid pollinator conservation: A review. *Biological Conservation*, 250. https://doi.org/10.1016/j.biocon.2020.108687

Piot, N., Schweiger, O., Meeus, I., Yanez, O., Straub, L., Villamar-Bouza, L., De la Rua, P., Jara, L., Ruiz, C., Malmstrom, M., Mustafa, S., Nielsen, A., Mand, M., Karise, R., Tlak-Gajger, I., Ozgor, E., Keskin, N., Dievart, V., Dalmon, A., Gajda, A., Neumann, P., Smagghe, G., Graystock, P., Radzeviciute, R., Paxton, R.J. & de Miranda, J.R. (2022). Honey bees and climate explain viral prevalence in wild bee communities on a continental scale. *Sci Rep*, 12(1), 1904. https://doi.org/10.1038/s41598-022-05603-2

Potts, S.G., Imperatriz-Fonseca, V., Ngo, H.T., Aizen, M.A., Biesmeijer, J.C., Breeze, T.D., Dicks, L.V., Garibaldi, L.A., Hill, R., Settele, J. & Vanbergen, A.J. (2016). Safeguarding pollinators and their values to human well-being. *Nature*, 540(7632), 220-229. https://doi.org/10.1038/nature20588

Rader, R., Bartomeus, I., Garibaldi, L.A., Garratt, M.P., Howlett, B.G., Winfree, R., Cunningham, S.A., Mayfield, M.M., Arthur, A.D., Andersson, G.K., Bommarco, R., Brittain, C., Carvalheiro, L.G., Chacoff, N.P., Entling, M.H., Foully, B., Freitas, B.M., Gemmill-Herren, B., Ghazoul, J., Griffin, S.R., Gross, C.L., Herbertsson, L., Herzog, F., Hipolito, J., Jaggar, S., Jauker, F., Klein, A.M., Kleijn, D., Krishnan, S., Lemos, C.Q., Lindstrom, S.A., Mandelik, Y., Monteiro, V.M., Nelson, W., Nilsson, L., Pattemore, D.E., Pereira Nde, O., Pisanty, G., Potts, S.G., Reemer, M., Rundlof, M., Sheffield, C.S., Scheper, J., Schuepp, C., Smith, H.G., Stanley, D.A., Stout, J.C., Szentgyorgyi, H., Taki, H., Vergara, C.H., Viana, B.F. & Woyciechowski, M. (2016). Non-bee insects are important contributors to global crop pollination. *Proc Natl Acad Sci U S A*, 113(1), 146-51. https://doi.org/10.1073/pnas.1517092112

Raven, P.H. & Wagner, D.L. (2021). Agricultural intensification and climate change are rapidly decreasing insect biodiversity. *Proc Natl Acad Sci U S A*, 118(2). https://doi.org/10.1073/pnas.2002548117

Robertson, B.A. & Hutto, R.L. (2006). A Framework for Understanding Ecological Traps and an Evaluation of Existing Evidence. *Ecology*, 87(5), 1075-1085. https://doi.org/10.1890/0012-9658(2006)87[1075:Affuet]2.0.Co;2

Wagner, D.L. (2020). Insect Declines in the Anthropocene. *Annu Rev Entomol*, 65, 457-480. https://doi.org/10.1146/annurev-ento-011019-025151

Wagner, D.L., Grames, E.M., Forister, M.L., Berenbaum, M.R. & Stopak, D. (2021). Insect decline in the Anthropocene: Death by a thousand cuts. *Proc Natl Acad Sci U S A*, 118(2). https://doi.org/10.1073/pnas.2023989118

Watanabe, M.E. (1994). Pollination worries rise as honey bees decline. *Science*, 265(5176), 1170. https://doi.org/10.1126/science.265.5176.1170

Westrich, P. (1989). Die wildbienen baden-württembergs. *(No Title)*.

Popular science summary

More traffic is linked to a decline in road verge pollinators!

It is no longer a secret that every year there are fewer and fewer insects buzzing around. I remember the long car rides to Italy with my family, where we had to stop every two hours to clear the windshield of all the unfortunate insects. Nowadays, that's no longer necessary.

There are many reasons for the worldwide insect die-off. Besides the use of pesticides in agriculture, the scarcity of suitable habitats is a major threat. Semi-natural grassland in particular, which is home to an enormous variety of species, is becoming increasingly rare. These grasslands are usually either grazed or mowed regularly, thus preventing the growth of trees and the emergence of forest.

An example for a road verge in Skåne.

A habitat that closely resembles semi-natural grasslands is often found on the side of the road. These green strips, or road verges, are also mowed regularly and could have the potential to host species usually found in semi-natural grasslands.

I wanted to investigate road verges as a habitat for pollinators further, so during three summer months, I photographed and counted the number and diversity of bees and butterflies in different road verges in Skåne, southern Sweden. I looked at road verges along roads with no traffic, low traffic, or heavy traffic, to see if there was a difference and if the traffic would have an effect on the observed species. What I found was that there were fewer pollinators in road verges of roads with high traffic, compared to road verges of roads with no traffic, even though the number and diversity of flowers were similar. This led me to conclude that traffic has in fact a strong negative impact on bees and butterflies. However, it is not clear what causes this negative effect. It could be the often fatal collisions with cars, but also secondary effects such as noise, air pollution, or an increased concentration of heavy metals in plants near the road. So for road verges to act as habitat substitutes and antidotes to the decline of semi-natural habitats, it is first necessary to clarify how and whether the negative effect of traffic can be prevented.

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Appendix

Table 4. The mean and standard error of the number of species and individuals at different traffic densities.

	Means (+/- se) of					
Traffic density	Total number of species	Total number of individuals	Butterfly species	Butterfly individuals	Bee species	Bee individuals
Control	5.56 (0.54)	9.81 (1.21)	3.31 (0.44)	5.94 (0.85)	2.25 (0.27)	3.88 (0.82)
Low	3.81 (0.47)	6.38 (0.65)	1.81 (0.29)	2.88 (0.43)	2 (0.32)	3.5 (0.52)
High	2.56 (0.32)	4.12 (1.03)	1.69 (0.27)	2.75 (0.66)	0.88 (0.26)	1.375 (0.45)