Pollinator diversity on roadsides and traffic-related impacts

Master's thesis presented by **Sarah Lescot** to obtain the degree of

Master in Organisms and Ecology Biology

Conducted in the zoology laboratory at the University of Mons

Supervisor: **Pr. Denis Michez**

Co-Supervisor: **William Fiordaliso**

Academic year: 2023-2024

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Acknowledgments

I would like to start by thanking Denis Michez for allowing me to complete this thesis in ecology, a field that greatly interests me. Despite managing many students he has always been attentive to my work. He provides the perfect balance of autonomy and support and working in his department has been a pleasure. Thank you very much, Denis, for your support and guidance.

I also would like to thank the Safeguard team and, in particular, William Fiordaliso, who supervised me throughout my dissertation. His assistance with fieldwork, hoverfly identifications, statistics, and writing was invaluable. Beyond to this thesis, he sparked my interest in the fascinating world of insects during the entomology laboratory sessions in BAB 3. His availability and support have meant a great deal to me. I also would like to thank Simon Potts, Chris Wyver, and Tea Hasanbegovic for our discussions and their availability.

Special thanks to my fieldwork partner, Simon, for our days spent traversing the roads of Wallonia. Thank you to the entire zoology lab team for their warm welcome and good vibes. I appreciate Dimitri's logistical and field assistance and Kimberly's presentation advice. Thanks to the taxonomists for their help with bee identifications. For all these wonderful years and incredible courses, I would like to thank Pierre Rasmont. I would also like to thank Clément, who has always been there to listen and help, especially during my first year at UMONS. Thank you to the amazing team of Master's 2 and Master's 1 students of the zoology lab, without whom this year would not have been the same.

I am also grateful to the entire teaching staff of the biology department for providing us with a solid scientific, and linguistic education, always being attentive and available.

A heartfelt thank you to my family for their unwavering support throughout these years, especially my mom, who spent time on my dissertation. Additional thanks to those who took care of the household menagerie during my mobility out: Dad & Mom, Brigitte & Bernard, Patrick & Martine, David & Marie& Léa & Lucie, Martin & Thérèse-Marie & Lise & Emilie, Benjamin & Marie & Elliot & Marceau.

Thank you, Pauline, for your encouragement throughout my master's and your help with my thesis. Thank you, Thérèse-Marie, for the Instagram breaks, and thanks to Lindsay, Thomas, and Manon for listening to and encouraging me.

Lastly, but certainly not least, thanks to you, Thomas, for tuning my dreams into reality and always supporting me through all of life’s moments, especially during this wonderful adventure of Master, which has been the most wonderful experience of my life.

Résumé

La diversité et l'abondance des pollinisateurs sauvages sont en déclin. L'un des principaux facteurs de ce déclin est la fragmentation de leur habitat. Les routes contribuent largement à cette fragmentation, jouant le rôle de barrières physiques potentiellement mortelles. Notre recherche, menée dans le cadre du projet Safeguard, se concentre sur l'étude des effets de la circulation routière sur les pollinisateurs. En Europe de l'Ouest, les routes sont omniprésentes et la voiture reste le mode de transport privilégié des citoyens. Notre étude compare deux pays, la Belgique et la Serbie, qui présentent des densités routières différentes, la Belgique ayant un réseau routier huit fois plus dense que celui de la Serbie.

Dans un premier temps, notre étude se concentre sur la diversité des abeilles, des syrphes et des papillons le long des routes afin d'évaluer la qualité écologique des bords de route. L'attractivité des plantes pour ces groupes de pollinisateurs est également étudiée afin de conseiller des aménagements de territoire. Ensuite, nous évaluerons l'impact des bords de route fleuris sur la mortalité par collision des pollinisateurs au sens large. Enfin, nous analyserons les communautés d'abeilles, syrphes et papillons impactées par le risque de collision.

Pour répondre à ces questions, nous réalisons des échantillonnages en transect linéaire le long de routes à trafic dense et peu dense, dans des paysages agricoles et semi-naturels. Un total de 24 sites en Belgique et 24 sites en Serbie sont échantillonnés à trois reprises. Lors de chaque sortie, un échantillonnage au filet des abeilles, syrphes et papillons est effectué sur les bords de route. Un relevé des espèces florales et de leur couverture est réalisé à l'aide d'un quadrat de 1 mètre carré. De plus, un échantillonnage sur la route est effectué à l'aide d'un piège collant installé sous la plaque d'immatriculation du véhicule.

Notre étude permet d'arriver aux résultats suivants : la diversité des pollinisateurs, et principalement des abeilles, est faible le long des bords de route, ceux-ci pouvant malgré tout accueillir des espèces menacées. Un résultat intéressant de notre étude en Belgique révèle que la richesse spécifique des pollinisateurs est significativement plus élevée le long des routes mineures que le long des routes majeures, avec 66 % d'espèces d'abeilles, 76 % d'espèces de syrphes et 46 % d'espèces de papillons en plus dans les bords de route à faible trafic. Ce pattern n'est pas observé en Serbie, probablement en raison de la faible densité et utilisation du réseau routier serbe. Concernant l'attractivité des plantes, *Cirsium arvense* et *Carduus acanthoides* sont les fleurs accueillant la plus grande diversité d'abeilles à la fois en Belgique et en Serbie, et figurent également parmi les plantes préférées des syrphes belges et des papillons belges et serbes. Nous n'avons pas mis en évidence que les bords de route fleuris agissent comme piège à miel pour les pollinisateurs. Les insectes les plus fréquemment heurtés par les véhicules sont les thysanoptères, les diptères et les hyménoptères dans les deux pays. Parmi les principaux groupes de pollinisateurs, les abeilles, syrphes et papillons sont peu nombreux à entrer en collision avec les véhicules, tandis que les insectes plus petits ont été plus abondants.

Mots-clés : Pollinisateurs, abeilles, syrphes, papillons, fleurs, routes, berges, collisions, volume du trafic, richesse spécifique

Abstract

The diversity and abundance of wild pollinators are declining. One of the primary drivers behind this decline is the fragmentation of their habitat. Roads are significant to this fragmentation, acting as potentially lethal physical barriers. As part of the Safeguard project, our research focuses on the effects of road traffic on pollinators. In Western Europe, roads are omnipresent, and cars remain citizens' favored mode of transport. Our study compares two countries, Belgium and Serbia, each characterized by distinct road densities. Belgium boasts a road network eight times denser than that of Serbia.

Our study initially investigates the diversity of bees, hoverflies, and butterflies along roadsides to assess their ecological quality. We then examine the attractiveness of plants to these pollinator groups to provide recommendations for land-use planning. Additionally, we will evaluate whether flowering roadsides act as honey traps for pollinators in general. Finally, we will analyze the bee, hoverfly and butterfly communities impacted by the risk of collision.

We are conducting line-transect sampling along roads with heavy and light traffic in agricultural and semi-natural landscapes to answer these questions. A total of 24 sites in Belgium and 24 sites in Serbia are sampled three times. During each survey, bees, hoverflies, and butterflies are net-sampled along roadsides. Floral species and their coverage are assessed using a 1-square-meter quadrat. Additionally, roadside sampling is conducted using a sticky trap installed under the vehicle's license plate.

The results of our study are as follows: The diversity of pollinators, particularly bees, is low along roadsides, which can nevertheless host endangered species. An interesting result of our study in Belgium reveals that pollinator species richness is significantly higher along minor roads than along major roads, with 66% more bee species, 76% more hoverfly species and 46% more butterfly species on low-traffic roadsides. This pattern is not observed in Serbia, likely due to the lower density and use of the Serbian road network. Concerning plant attractiveness, *Cirsium arvense* and *Carduus acanthoides* are the most attractive flowers for bees in both Belgium and Serbia. They are also among the preferred plants for Belgian hoverflies and butterflies in Belgium and Serbia. We have not identified floral roadsides as honey traps for pollinators. Thysanoptera, Diptera, and Hymenoptera are the insect orders most frequently hit by vehicle collisions in both countries. Within the primary pollinator groups, bees, hoverflies, and butterflies were few killed by vehicle collisions, whereas smaller insects were more dominant.

Keywords: Pollinators, bees, hoverflies, butterflies, flowers, roads, roadsides, roadkills, traffic volume, species richness

1. Table

1. TABLE VII

2. ABBREVIATIONS XI

3. FOREWORK 1

4. INTRODUCTION 2

4.1. POLLINATION ECOLOGY 2

4.2. POLLINATING INSECTS 3

4.2.1. Bees 3

4.2.2. Hoverflies 6

4.2.3. Butterflies 8

4.2.4. Status and trends 9

4.3. ROAD NETWORK DEVELOPMENT 11

4.3.1. Europe 11

4.3.2. Belgium 12

4.3.3. Serbia 13

4.4. ROAD ISSUES FOR WILDLIFE 14

4.4.1. Habitat fragmentation 14

4.4.2. Collision 14

4.4.3. Pollution 15

4.5. ROADSIDE MANAGEMENT 15

4.5.1. Belgium 16

4.5.2. Serbia 16

4.6. BIOLOGICAL QUESTIONS 17

5. MATERIALS AND METHODS 19

5.1. STUDY AREAS 19
5.2. SITE SELECTION 20
5.2.1. Landscape context 20
5.2.2. Road type 20
5.2.3. Site replications 20
5.3. SAMPLING METHOD 22
5.3.1. Walking transect 23
5.3.1.1. Butterfly transect 23
5.3.1.2. Bee and hoverfly transect 24
5.3.1.3. Flower survey 24
5.3.2. Car transect 24
5.4. SPECIMEN PROCESSING 25
5.4.1. Walking transect 25
5.4.2. Car transect 25
5.5. STATISTICAL ANALYSES 27
5.5.1. Completeness analysis 27
5.5.2. Diversity indices 27
5.5.3. Statistical modeling 27
6. RESULTS 29
6.1. RELATIONSHIP OF PLANT DIVERSITY AND POLLINATOR DIVERSITY ALONG ROADSIDES 30
6.1.1. Belgium 30
6.1.2. Serbia 35
6.2. ATTRACTIVENESS OF FLOWERS FOR POLLINATORS ALONG ROADSIDES 39
6.2.1. Belgium 39
6.2.2. Serbia 40

6.3. Relationship between flower-rich roadsides and pollinator mortality by vehicle collisions 41

6.3.1. Belgium 41

6.3.2. Serbia 42

6.4. Impact of vehicle collisions on pollinator communities 44

7. DISCUSSION 46

7.1. Relationship of plant diversity and pollinator diversity along roadsides 46

7.1.1. Bees.. 46

7.1.2. Hoverflies 47

7.1.3. Butterflies... 49

7.2. Attractiveness of flowers for pollinators along roadsides 50

7.3. Relationship between flower-rich roadsides and pollinator mortality by vehicle collisions 53

7.4. Impact of vehicle collisions on pollinator communities 53

7.5. Conclusion and outlooks 56

8. BIBLIOGRAPHY ....A

9. ANNEX ....M

9.1. Poem « Le vieux canal » by Andre Lescot ....M

9.2. European transport networks..N

9.3. Field data sheet : butterflies ....O

9.4. Field data sheet : bees and hoverflies .... P

9.5. Field data sheet : Quadrats...Q

9.6. Field data sheet : Traffic survey .... R

9.7. Description of walking experiment data ....S

9.7.1. Belgium ....S

9.7.2. Serbia X

9.8. BELGIAN SITES AND ASSOCIATED VARIABLES .... AA

9.9. SERBIAN SITES AND ASSOCIATED VARIABLES .... AA

9.10. DESCRIPTION OF CAR TRANSECT DATA........ BB

9.10.1. Belgium .... BB

9.10.2. Serbia CC

10. ILLUSTRATION TABLE .... DD

2. Abbreviations

BE : Belgium

CiEi : Cellule interdépartementale Espèces invasives (i.e. Interdepartmental invasive species unit)

CR : Critically Endangered

eBMS : European Butterfly Monitoring Schem

EN : Endangered

IUCN : International Union for Conservation of Nature

RS : Republic of Serbia

SNH : Semi-natural habitats

SPW : Service public de Wallonie (i.e. Wallonia's public service)

VU : Vulnerable

3. Forework

This master's thesis is part of the Safeguard project, a study funded by the European Union that started in September 2021 and will finish in August 2025. The goal of the Safeguard project is to protect European wild pollinators. Specifically, the focus is on understanding the causes behind pollinator decline, examining the economic, environmental, and societal repercussions, and participating in political solutions.

One of this year's objectives, and the goal of this master's thesis, involves investigating the impact of traffic on a community of pollinators (bees, hoverflies, butterflies) across a network of six countries: Belgium, Germany, Romania, Sweden, Serbia, and the U.K. While the effects of roads on vertebrates are well documented, research into their impact on invertebrates has been less numerous in the scientific literature. In this study, we aim to fill this gap.

While writing this dissertation, I remembered my grandfather's thoughts on the canal connecting Mons to Condé. In 1975, as an amateur writer and member of the Art and Poetry Association of Saint-Ghislain, he wrote a poem about the canal published in the magazine « Poète de chez nous » *(cf. Annex 9.1)*. The once peaceful waters, frequented by boats, have succumbed to the advance of the asphalt, now drowned in the loud noise of passing cars. Despite being a man-made creation, the canal held memories of abundant nature for the older generation, with my family gathering there for fishing trips.

Home for boatmen's children in Saint-Ghislain in front the Mons-Condé canal, around 1930 © *Delcampe*

Now transformed into housing in front the highway E19-E42 (A7), picture from 2023 © *Google*

Figure 1 : Canal Mons-Condé buried under asphalt in Saint-Ghislain

Recently, and echoing the history of the Mons Condé canal, the construction of a new highway, the A69, linking Castres to Toulouse in France, has sparked lively controversies. In protest against this project, several environmental activists went on a hunger strike, denouncing ecocide. The destruction of semi-natural habitats to save motorists a few minutes is poorly perceived. This raises the question: Why are the authorities approving the destruction of semi-natural habitats to build more and more roads?

Because of my family history, my interest in pollinators, and the current context of reducing semi-natural habitats for road construction, the prospect of contributing to a project dedicated to studying pollinators along roadways immediately captivated my interest.

4. Introduction

4.1. Pollination ecology

In angiosperms, the process of pollination occurs when the female stigma receives male pollen grains (Michener, 2007). Biotic or abiotic agents can transport pollen. Abiotic factors are wind and water, while there are several biotic pollinators such as birds, lizards, bats and insects (Urry et al., 2017).

Pollinators seek pollen and/or nectar contained within flowers, representing high-quality resources (Roulston & Cane, 2000; Willmer, 2011). The pollen is a source of proteins and lipids required for egg production and larval growth. The nectar, the reward the flower offers to the pollen carriers, is rich in sugar, which is essential to cover energy expenditure (Michener, 2007; Westerkamp, 1996). In this way, insects visit flowers to feed themselves and reproduce rather than with the goal of pollination (Potts et al., 2016). Thus, pollination is a byproduct of their behaviors (Frame, 2003 in Wardhaugh, 2015).

Insects are the primary pollinators of wild flowering plants and most crops (Ollerton, 2017; Potts et al., 2010; Wardhaugh, 2015). Indeed, around 87,5 percent of wild flowering plants rely on pollinators (Potts et al., 2016; Urry et al., 2017; Wardhaugh, 2015). According to the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) 2016, animal pollination is crucial for three-quarters of the world’s food crops. It represents an annual market of 235 to 577 billion US dollars. Regarding human health, pollinators are essential for pollinating most vegetables, fruits, nuts, and seeds. These contain micro-nutrients essential to the human diet, such as folates, vitamins A and C and iron (Potts et al., 2016). Pollinator loss would increase the risk of lung and oesophageal cancer, cardiovascular disease and diabetes (Smith et al., 2015).

Pollinators are part of our cultural heritage and have inspired humankind for thousands of years. Through stories, myths, art, and music, they are part of human creativity and imagination. In Greek mythology, for example, bees are depicted alongside the goddesses Artemis and Demeter (Kievits, 2013). In music, Nikolai Rimsky-Korsakov wrote « Flight of the Bumblebee » which captures the bumblebee's flight and buzzing melodies.

Because pollinators provide so many services, it is vital to ensure their conservation and better understand the causes of their decline *(cf. Section 4.2.4).*

4.2. Pollinating insects

Pollinating insects are key players in preserving terrestrial biodiversity and human and animal feeding. As explained below, they constitute a diversified group.

When discussing pollinators, especially bees, the general public thinks of the honeybee, *Apis mellifera*. Humans have bred them for millennia in artificial hives, focusing mainly on honey production and crop fertilization. However, the bee diversity extends far beyond this species, and the world hosts over 20,000 wild bee species (Potts et al., 2016). Collectively, bees are recognized as the most crucial pollinator for food production and pollinate around 73 % of the world's crops (Potts et al., 2010). Compared to other taxonomic groups, bees pollinate more plant species and are the only group more or less dependent on floral resources in the larval and imago stages (Ollerton, 2017; Wardhaugh, 2015). Bees are not the only Hymenoptera capable of pollinating flowers. Among them, wasps also contribute significantly to pollination and play a role in pest control (Ollerton, 2017).

Among the « non-bee pollinators », some fly families are considered valuable pollination contributors (Doyle et al., 2020). Hoverflies are the most efficient and diverse family, with 6,000 species, and are documented to visit at least 72% of global food crops (Doyle et al., 2020; Ollerton, 2017; Wardhaugh, 2015). Moreover, their larval nutrition enables them to play a role in pest control *(cf. Section 4.2.2)*. Butterflies and moths are also significant contributors to pollination, comprising approximately 150.000 to 200.000 species, they represent the most diverse group of pollinators in the world (Menken et al., 2010; Ollerton, 2017).

Besides these three important groups, thrips and beetles contribute to global pollination (Wardhaugh, 2015). Following the Lepidoptera, Coleoptera counts 77.300 pollinating species of which 4000 are Cetoniidae (Wardhaugh, 2015). Often ignored in research on pollination and paradoxically considered valuable pollinator contributors, mainly in rainforests, the Thysanoptera order contains 6000 species (Mound, 2009; Ollerton, 2017). Half feed on fungi, and the other half on pollen or green leaves (Mound, 2009). Thysanoptera pollinator species reproduce on flowers (Wardhaugh, 2015). Winged and wingless individuals disperse with the wind (Mound, 2009).

This research will focus on bees, hoverflies, and butterflies, considered the most essential taxa for pollination. Indeed, the study by Rader et al. (2020) on 105 crops highlights the relative importance of different pollinators. Hymenopterans, primarily bees, visited 93% of the crops. Dipterans visited 72% of the crops, with hoverflies being the most prominent non-bee pollinators. Lepidopterans, such as butterflies, visited 54% of the crops (Rader et al., 2020). Thus, these three groups are the most effective pollinators, capable of pollinating a wide variety of flowers, which is why they are the focus of this research.

4.2.1. Bees

Bees or Antophila are members of the Hymenoptera order, having four wings, with the anterior and posterior wings coupled by hamuli (Michener, 2007). One of the distinctive features of bees is the ramified structure of their hair morphology and enlarged hind leg basitarsi (Michez et al., 2019).

According to the new annotated checklist of the wild bees of Europe (Hymenoptera: Anthophila) from Ghisbain (2023), the continent counts 2138 bee species distributed in six families as shown in Table 1 and illustrated in Figure 2:

Table 1 : Bee family diversity in Europe, adapted from (Ghisbain et al., 2023).

Class	Order	Family	Number of species
Insecta	Hymenoptera	Andrenidae	526
		Apidae	617
		Colletidae	154
		Halictidae	356
		Megachilidae	444
		Melittidae	41
Total			**2138**

Their ecology is highly variable and can be summarized based on their social structure, nesting requirements and dietary resources.

Bees exhibit varying degrees of sociality, as they can be solitary, social, or brood parasites. As *Osmia cornuta*, belonging to the Megachilidae family and well-known in insect houses, most bee species are solitary, building their nest alone and dying before their eggs hatch (Nieto et al., 2014). Honeybees, bumblebees, and some Halictidae species are eusocial bee species that live in groups, with a queen responsible for the birth of other individuals within the nest. They represent only 6% of the world's bee diversity (Danforth, 2007 in Michez et al., 2019). Each colony member is assigned specific tasks, such as resource collection, nest maintenance, or raising additional offspring which may change over time (Nieto et al., 2014). Finally, in Apidae, Halictidae and Megachilidae, certain species exhibit brood parasite behavior, utilizing the resources and nests of other bees. Consequently, they do not collect nectar and pollen themselves but consume the resources of other species (Drossart et al., 2019; Nieto et al., 2014).

Excluding kleptoparasites species, bees construct nests to deposit eggs. Depending on nesting requirements, bees are classed into two major groups: the ground-nesting digger species, as *Dasypoda hirtipes* from the Melitidae family, and the species that nest aboveground as *Osmia cornuta* from Megachilidae family (Drossart et al., 2019). Ground-nesting bee species have specific requirements for their installation, including soil texture, slope, and exposure to the sun (Potts et al., 2005). For example, *Dasypoda hirtipes* and *Andrena fuscipes* prefer well-exposed and sandy soils (Michez et al., 2019). Bees that breed above the ground utilize various cavities such as plants, wood, rock crevices, and abandoned nests, depending on the species (Nieto et al., 2014).

Concerning food resources, adult bees feed on nectar and gather pollen to feed their larvae. Bee species may exhibit specialization in their foraging habits: monolectic bees collect from only one plant species, while oligolectic and polylectic bees feed on one host plant family and more than one host plant family, respectively (Michener, 2007).

By building their nests and regularly supplying them with food, bees remain relatively faithful to their site. Certain bees, referred to as "social foragers," communicate the location of resources to their colony members. This behavior is well known to the general public through the Waggle dance of the honeybee (Tautz, 2023). Studies on the movement of bumblebees suggest that they tend to stay close to their colony to minimize energy and time expenditure, covering a few hundred meters or even 1 or 2 kilometers, and up to 4 kilometers if resources are scarce (Osborne et al., 2008). Smaller bees, such as the *Andrena* genus, only travel at distances of less than 100 meters or a few hundred meters (François & Le Féon, 2017).

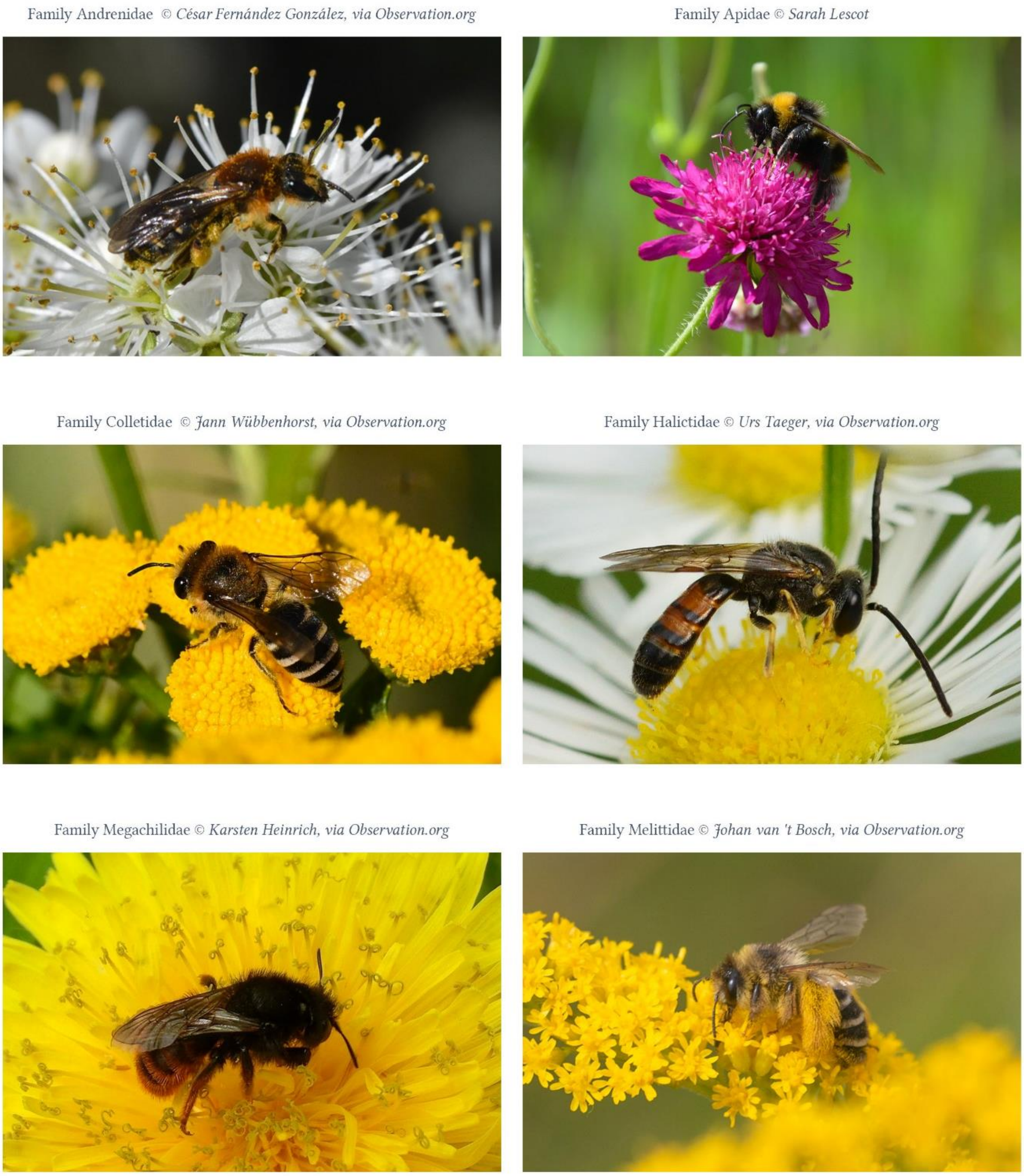


Figure 2 : Representation of the six European bee families

4.2.2. Hoverflies

Often known as a flower fly, the hoverfly is a family of the Diptera order that is notably characterized by a false wing vein called Vena spuria(Ball & Morris, 2015).

According to the European Red List of Hoverflies from 2022, the European continent counts 890 Syrphidae species. They are distributed in three subfamilies : Syrphinae, Eristalinae and Microdontinae (Doyle et al., 2020) and illustrated in Figure 3.

Hoverflies are not social animals and do not take care of their brood. Some species are parasitic, such as *Microdon mutabilis* and *Microdon myrmicae,* which parasitize ant colonies that feed their larvae (Bonelli et al., 2011; Elmes et al., 1999).

Hoverflies do not build nests (Rodríguez-Gasol et al., 2020a). The female deposits eggs, singly or in small batches, in a suitable location, ensuring that the larvae can readily find a food source(Ball & Morris, 2015). Unlike bees, where larvae are enclosed in a cell, hoverfly larvae are free-living. The eggs hatch a few days after being laid, and they go through three larval stages before forming a pupa and developing into an adult (Ball & Morris, 2015).

Adult hoverflies feed on nectar and pollen preferring usually umbellifers because resources are easily accessible and exposed (Ball & Morris, 2015). The larvae exhibit various dietary traits including saprophagy, phytophagy, mycophagy, entomophagy including aphidophagy, i.e. predation on aphids. This feeding habit enables them to play a crucial role in the biological control against crop pests and they are considered « gardener's friends » (Ball & Morris, 2015; Rodríguez-Gasol et al., 2020a). Concerning their habitat, hoverfly larvae are mainly terrestrial, but some groups can be aquatic, like *Chrysrogaster*, *Lejogaster* and *Eristalis*, also known as « rat-tailed » maggots (Ball & Morris, 2015).

Unlike bees, hoverflies are generally more nomadic, as they do not have a nest to return to regularly. Throughout their lifecycle, they require different habitats for feeding, mating, overwintering and larval life (Meyer et al., 2009). The degree of mobility varies among hoverfly species, with some staying relatively close to their larval habitat while others exhibit greater mobility, ranging from a few meters to several kilometers per day (Schönrogge et al. 2006; Schneider 1958 in Schweiger et al., 2010). Some individuals of *Episyrphus balteatus* can even migrate thousands of kilometers from northern to southern Europe to overwinter (Sahib et al., 2020; Wotton et al., 2019 in Doyle et al., 2020).

Family Eristalinae © *Johan Vekemans, via Observation.org*

Family Microdontinae © *Jeroen van Soolingen, via Observation.org*

Family Syrphinae © *Claudia, via Observation.org*

Figure 3 : Representation of the three European hoverfly subfamilies

4.2.3. Butterflies

Butterflies, belonging to the order Lepidoptera, are characterized by large wings and body covered by scales. They possess a long proboscis, part of their oral system, enabling them to extract nectar from flowers (Van Swaay et al., 2010).

According to the European Red List of Butterflies from 2010, the European continent counts 482 butterfly species, Rhopalocera, distributed in six families as shown in Table 2 and illustrated in Figure 4.

Table 2: Butterfly family diversity in the European continent, adapted from (Van Swaay et al., 2010).

Class	Order	Family	Number of species
Insecta	Lepidoptera	Hesperiidae	46
		Lycaenidae	129
		Nymphalidae	237
		Papilionidae	13
		Pieridae	56
		Riodinidae	1
Total			**482**

While most adult species are solitary, butterfly larvae can develop a social structure that includes defense signals and cohesion behaviors, as well as sharing information about the location of food resources (Costa & Pierce, 1997). In the Lycaenidae family, some butterfly species can realize mutualism or parasitism with ants (Fiedler, 2012).

Regarding food resources, adult butterflies feed themselves on nectar (Chinery, 1988). They are considered generalists who can switch between various flowers (Stefanescu & Traveset, 2009). Adult butterflies may expand their diet with wet sand, fruit, mud, dung, or carrion by sucking up the liquid (Bonebrake et al., 2010; Hardy et al., 2007). This behavior is called mud puddling. In contrast, larvae are mostly phytophagous, feeding on leaves and specialized to varying degrees. For example, *Pieris brassicae* feeds on specific *Brassicaceae* genera, while *Aglais io* feeds on *Urtica dioica*. (Chinery, 1988; Tolman & Lewington, 2015).

Butterflies do not construct nests; they deposit their eggs directly on a host plant. Most of the time, eggs hatch after a few weeks and the caterpillars develop inside or on the host plant. Following three or four larval stages, they form a chrysalis before emerging adults (Chinery, 1988).

Like hoverflies, butterflies do not return regularly to the nest, making them more nomadic than bees. Their habitats are also different, depending on their larval and imago stages and their feeding resources. Butterflies can display various movements depending on species, life stage, resources, and reproduction. There are highly sedentary species like *Euphydryas*, which remain in very localized colonies, and migratory species like the well-known monarch, *Danaus plexippus* which can travel up to 6,000 kilometers in 3 to 5 generations or *Vanessa cardui* migrating from northern Europe to west Africa in 6 to 7 generations (Bergerot et al., 2012; Chowdhury et al., 2021; Ohsaki, 1980).

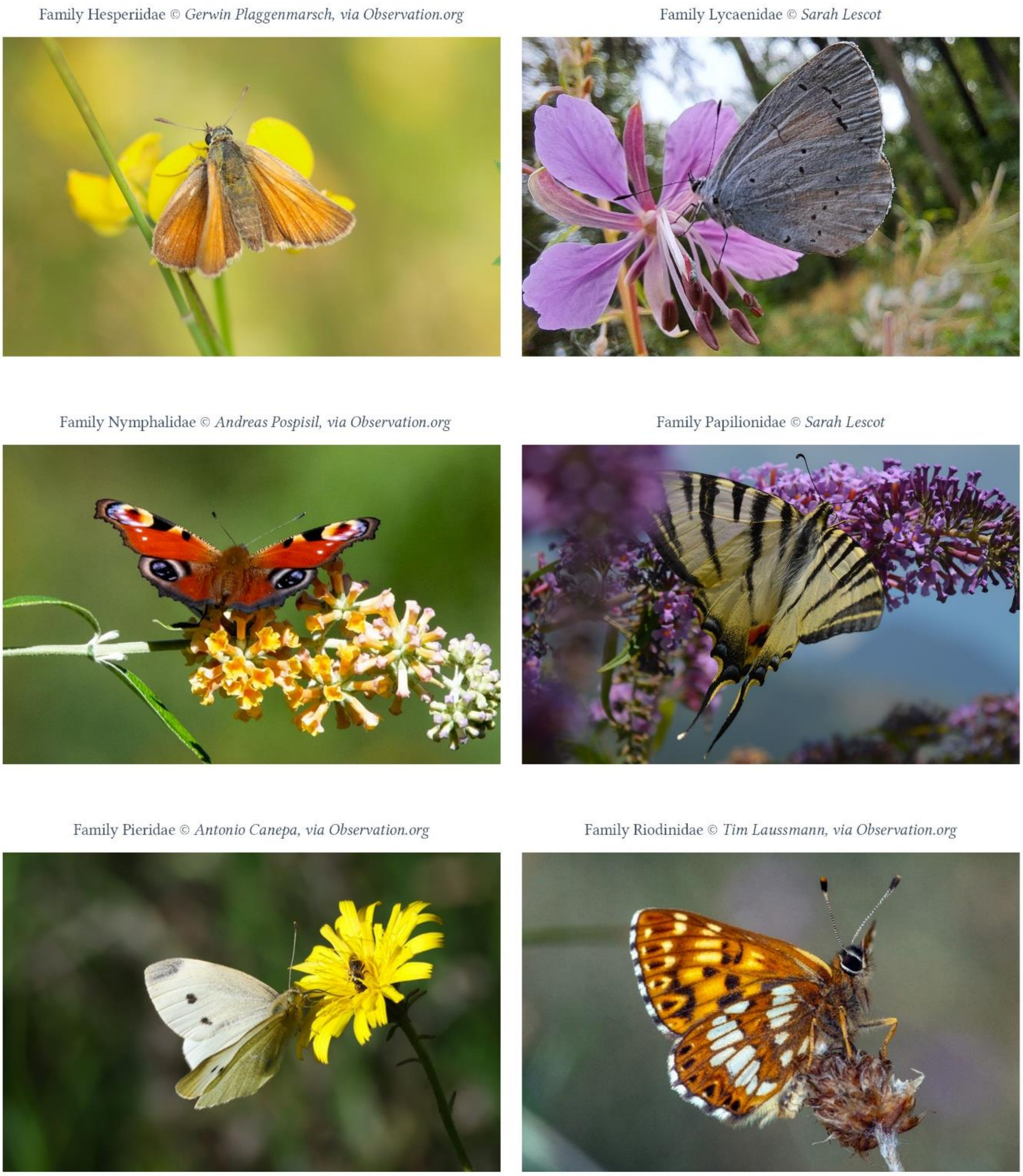


Figure 4: Representation of the six European butterfly families

4.2.4. Status and trends

These pollinators' presence, diversity and abundance are declining because of habitat fragmentation and loss, pesticides, alien species, pathogens and climate change (Potts et al., 2010, 2016). The European Red List of Bees 2014, Hoverflies 2022, and Butterflies 2010 report that 9.2 % of bee species, 37.2% of syrphidae species and 8.5 % of butterfly species are threatened[1] at the European scale.

Habitat fragmentation is a major cause of pollinator decline (Potts et al., 2010). Although habitat fragmentation can occur naturally through events like fire or windfall (Wright et al., 1974; Picket et al., 1978; Foster et al., 1980 in Andrén, 1994), the majority of instances are linked to human activity including agriculture, forestry, urbanization, railroad and road construction (Llausàs & Nogué, 2012). These physical barriers, obstructing animal movements, result in both the loss and isolation of the original habitat (natural or semi-natural) (Andrén, 1994; Muñoz et al., 2015). Isolation increases inbreeding within animal populations, leading to a reduction in genetics.

Currently, European agricultural landscapes predominantly feature intensive monocultures using synthetic nitrogen fertilizers and pesticides. In contrast, traditional farming practices involved periodic rotations with fallow periods, rich in leguminous (Rasmont, 2006). These were a food source for pollinators and helped to fix atmospheric nitrogen and release it into the soil so plants could assimilate (Rasmont, 2006). This traditional soil enrichment method has been replaced by nitrogen fertilizers to boost crop yields. However, these compounds can spread over a wide area, shaping landscapes for nitrophilous plant species such as *Urtica dioica*, *Arrhenatherum elatius* or *Dactylis glomerata*. This change can disadvantage oligotrophic species, depriving them of essential resources (Carvalheiro et al., 2020). In addition, the use of pesticides impacts our pollinator populations. They can be divided into three groups: insecticides, fungicides and herbicides (Rodríguez-Gasol et al., 2020b). While insecticides are often considered the most directly toxic to wild pollinators, herbicides and fungicides can also have significant indirect impacts. Indeed, herbicides and fungicides can reduce available floral resources and habitats and be stored in the tissues of insects in the larval and imago stages (Main et al., 2020). At lethal doses, insecticides paralyze insects, but at smaller doses and over the long term, they induce sublethal effects on learning, feeding behavior, orientation, mobility, fecundity, immunology, longevity, molting, larval development and neurophysiology (Desneux et al., 2007; Belzunces et al., 2012; de França et al., 2017; in Braak et al., 2018).

The presence of invasive alien species can also be a threat to pollinators. Invasive alien plants can be a danger to specialist species, who lose their native food resource through the presence of the invasive plant (Potts et al., 2016). For generalist species such as *Bombus terrestris* or *Bombus pascuorum*, invasive alien plants do not have strictly adverse effects, as they represent a source of nectar and pollen (Drossart et al., 2019). Eradication of these plants in forage-poor environments could even have an impact on these generalist species (e.g. Rasmont et al., 1990; Saad et al., 2009; Drossart et al., 2017; Davis et al., 2018; in Drossart et al., 2019). Invasive alien insects can compete with our native pollinators for food and nesting resources, transmit pathogens and hybridize with our native insects (Potts et al., 2010).

Not all species are impacted in the same way by global warming, and some may even benefit from it (Duchenne et al., 2020; Gérard et al., 2020). Global warming has altered the interactions between

[1] Species holding to Critically Endangered (CR), Endangered (EN) and Vulnerable (VU) categories in the International Union for Conservation of Nature (IUCN) red list

existing species, adding or removing species from local assemblages of plants and animals (Parmesan, 2006; Hegland et al., 2009; in Schweiger et al., 2007) In the long term, climate change causes pollinators to move toward the poles or higher altitudes (Potts et al., 2016). Some groups of pollinators, such as bumblebees, have limited capacity to adapt to climate change (Kerr et al., 2015; Schweiger et al., 2007). In the short term, the intensification in frequency and intensity of major climatic events such as excessive heat, fires, drought, and floods could exceed the adaptation threshold of native species (Nicholson & Egan, 2020).

Among all these decline factors, we can wonder about the impact of roads on pollinators. In our European societies, the landscape has significantly changed over the past centuries to support human activities. Urbanization and the development of transport networks have led to the ubiquity of roads, which aim to improve the quality of human life (Muñoz et al., 2015).

4.3. Road network development

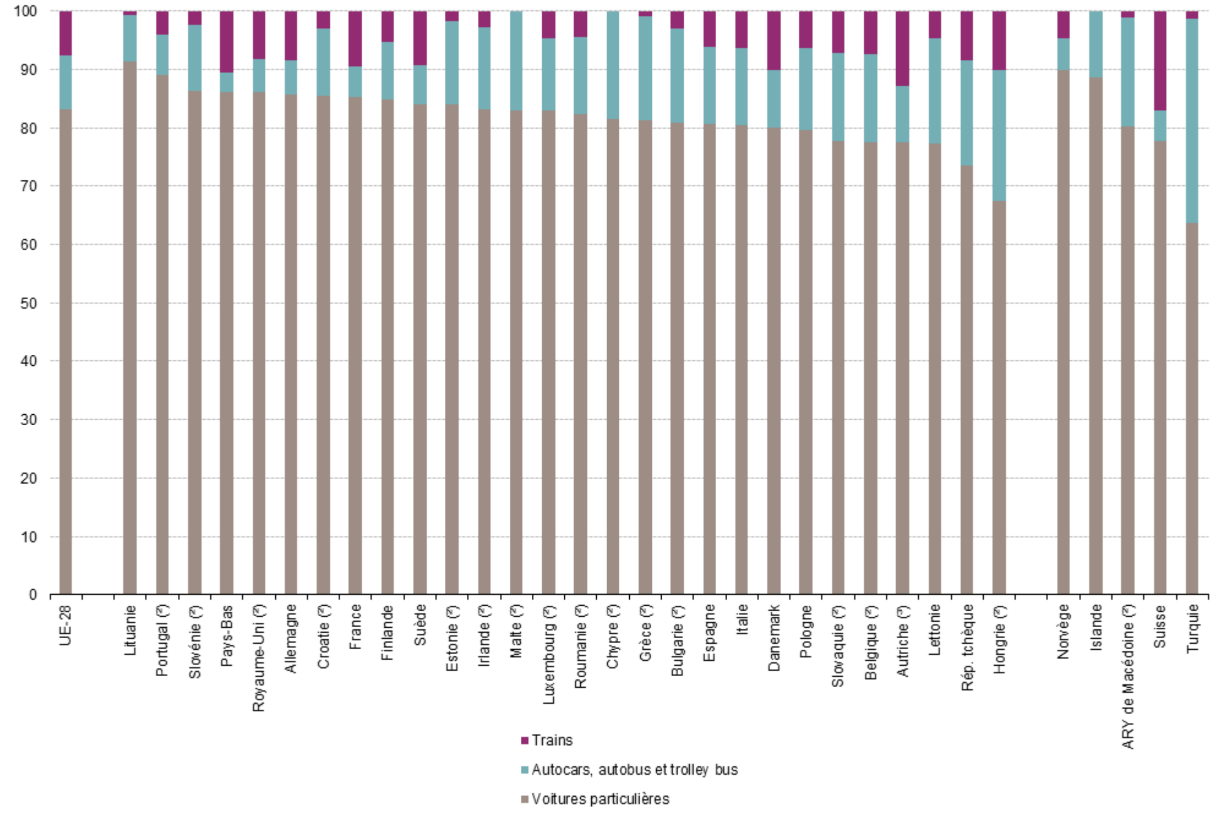
Economic, political and technological changes over the centuries mark the development of Europe's road networks. It stems from the primitive and eternal human need for mobility, trade and communication (Mouratidis & Kehagia, 2014). Currently, the global road network spans approximately 32 million kilometers (IRF, 2017 in Fitch & Vaidya, 2021). The projections for 2050 by Dulac (2013) indicate an expansion of an additional 25 million kilometers worldwide (Fitch & Vaidya, 2021). In this introduction, we will briefly explore the general evolution of road networks in Europe before looking more specifically at the cases of Belgium and Serbia.

4.3.1. Europe

Since the Industrial Revolution, our travel modes have changed considerably, intensified and accelerated (Bavoux et al., 2005). Before the 18th century, we traveled mainly on foot and horseback between dense towns linked by straight, paved Roman causeways (Mouratidis & Kehagia, 2014). Around cities, a few roads distributed the towns and villages, surrounded by forests, meadows, heaths and marshes. With the advent of coal mining and the steam engine, the development of the railroads made it possible to link large towns to industry, from raw materials to labor. In Europe, after the Second World War, the intensive use of the automobile brought about a second transformation of the countryside (Newman & Kenworthy, 1989). Mainly rural, open landscapes were fragmented by rail and asphalt motorway infrastructures. An increase in vehicle speeds has accompanied this construction through fields and forests (Bigo et al., 2022).

Today's transport networks for people and goods are multiplying, involving road, rail, river and air networks *(cf. Annex 9.2)* (Bigo et al., 2022). In 1800, the average person walked 4 to 5 kilometers a day, but today, they travel ten times more, ten times faster (Bigo et al., 2022). Road transport has maintained a predominant position among various modes of transportation (Bigo et al., 2022; Mouratidis & Kehagia, 2014). Urban and rural landscapes are crisscrossed by roads in an "all-car" world, reflecting a desire to go ever further, ever faster. Automobiles are the favored mode of transportation among European Union residents because of their flexibility. As illustrated in Figure

5, data from 2013 reveal that single-passenger cars constituted 83.2% of passenger transport in the European Union. Bus and train use accounted for 9.2% and 7.6% respectively (EXT-A-Redpath, 2017).



(1) À l'exclusion des deux-roues motorisés. Chypre, Malte et Islande: trains sans objet.
(2) Inclut des estimations ou des données provisoires.
(3) Le chemin de fer au Liechtenstein est détenu et exploité par la société autrichienne ÖBB et inclus dans ses statistiques.
Source: Eurostat (code des données en ligne: tran_hv_psmod)

Figure 5: Modal distribution of domestic passenger transport, 2013 (in % of total domestic transport, in passenger-kilometers)

4.3.2. Belgium

Situated in the heart of Europe, Belgium hosts numerous European institutions, including the European Parliament. Consequently, the country plays a crucial role in the European communications network. A part of its economy revolves around goods distribution, facilitated by the import and export activities through its three harbors: Antwerpen, Zeebrugge and Gent (Daubresse & Laine, 2017; Thomas & Verhetsel, 1999).

In a few numbers, and according to (Thomas & Verhetsel, 1999), Belgian roads ensure 78% of passenger transport and 72% of goods transport. In an area of 30 528 km^2, Belgium's infrastructure comprises:

- 1.700 km of highways
- 14.000 km of main roads (both regional and provincial)
- 130.000 km of communal road

The Belgian road network density is 4.77km/km² *(Cf. Figure 6)*. This small country is considered one of the highest highway densities globally (Thomas & Verhetsel, 1999).

4.3.3. Serbia

In southern Europe, Serbia has a superficial of 77 474 km². In 2022 and according to the Eurostat dataset (Eurostat, 2024b, 2024a), the road network spans over 45.963 km and is distributed as follows :

- 941 km of highways
- 45.022 km of both provincial and communal roads

The Serbian road network density is 0.59 km/km² *(Cf. Figure 6)*.

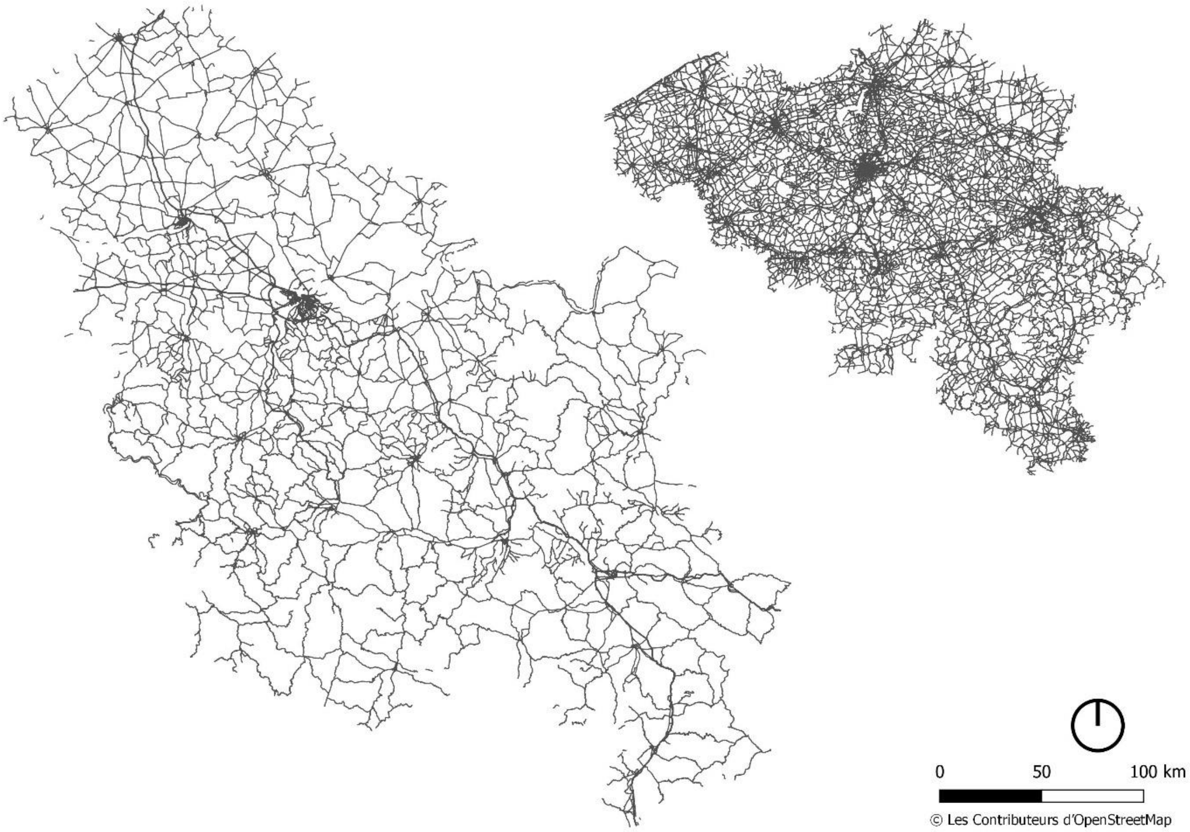


Figure 6: Map of the Belgian and Serbian road network (Credit : OpenStreetMap, 2024; Lescot, 2024)

Figure 6 displays motorways, national and local roads at the same scale and from the same OpenStreetMap dataset. It compares Belgian and Serbian road densities, highlighting the greater concentration of roads in Belgium, which is approximately eight times higher than that of Serbia.

4.4. Road issues for wildlife

4.4.1. Habitat fragmentation

In our European environments, roads are widespread and act as barriers, potentially obstructing the movement of pollinators (Muñoz et al., 2015; Phillips et al., 2020). In general, insects avoid crossing roads but it can vary depending on the taxonomic group (Muñoz et al., 2015). The study of Askling and Bergman (2003) highlights that roads may act as barriers for certain species. They captured and marked 8,415 butterflies of 55 species along a highway. While species such as *Pieris napi* and *Gonepteryx rhamni* from the Pieridae family frequently crossed the road, other species like *Coenonympha arcania*, *Aphantopus hyperantus* (Nymphalidae), and *Polyommatus semiargus* (Lycaenidae) rarely crossed the road (Askling & Bergman, 2003). A study on bumblebees highlights that they *« avoid crossing roads, and rather move to patches located on the same side of the road »* (Bhattacharya et al., 2003). Larger roads, owing to their large width, higher traffic volume and speed tend to form more substantial barriers compared to smaller roads (Dunn & Danoff-Burg, 2007; Fitch & Vaidya, 2021; Muñoz et al., 2015). In addition, this fragmentation by the road network is accentuated by the construction of housing around these roads, the ribbon urbanization, that reinforces this barrier (Sérusiaux et al., 2012).

4.4.2. Collision

As illustrated in Figure 7, vehicle strikes represent one of the most direct sources of mortality for both vertebrates and invertebrates (Keilsohn et al., 2018). In the U.K., the most affected groups are amphibians, barn owls, badgers, hedgehogs and foxes (English Nature, 1993; Slater et al., 1995 in Underhill & Angold, 2000).

Figure 7: Amphibian (Credit : Lescot, 2024) and butterfly (Credit : Oren Ravid, 2023) hit by a vehicle

Recent studies suggest a high level of insect mortality due to vehicle strikes (Baxter-Gilbert et al., 2015; Keilsohn et al., 2018) and mortality increase with traffic volume (Rao et al., 2007; McKenna et al., 2001; Seshadri et al., 2011; Soluk et al., 2011; Sko´rka et al., 2013 in Muñoz et al., 2015).

In few numbers, the mean roadkill rates for Lepidoptera spanning different road types is from 0.45 to 10.1 roadkills per kilometer per day (Baxter-Gilbert et al., 2015; Keilsohn et al., 2018). For Hymenoptera, the mean, only on highways, is from 21.31 to 26.8 roadkills per kilometer per day (Baxter-Gilbert et al., 2015; Keilsohn et al., 2018 in Phillips et al., 2020). Nevertheless, the consequences of roadkill at the population level remain uncertain (Phillips et al., 2020). However, a recent study on bumblebee queens showed that high traffic intensity could impact bumblebee populations, killing more queens (Dániel-Ferreira et al., 2022).

4.4.3. Pollution

The vulnerability of insects to roads may also be linked to their intolerance for road pollutant environments (Muñoz et al., 2015). Indeed, road maintenance and use contribute to air and soil pollution including heavy metals, nitrogen oxides and ozone (Ryalls et al., 2022; Trombulak & Frissell, 2000).

Aluminum, cadmium, iron, manganese, copper, nickel, titanium, boron and zinc are heavy metals, derived from road deicing salts and gasoline additives, present in roadside environments (Garcia-Miragaya et al., 1981; Clift et al., 1983; Gjessing et al., 1984; Oberts et al., 1986; Araratyan et al., 1988 in Trombulak & Frissell, 2000). They can accumulate in the tissues of plants and insects. These contaminants exhibit distinct patterns of distribution, correlating with vehicular traffic and decreasing with distance from the road (Goldsmith et al., 1976; Dale et al., 1982; Leharne et al., 1992; Quarles et al., 1974; Dale et al., 1982 in Trombulak & Frissell, 2000). A study in Poland and the UK indicates that the increase in heavy metals in the environment negatively impacts the abundance and diversity of solitary wild bees (Moroń et al., 2012). A study on *Osmia rufa* showed a decrease in the number of eggs laid by females and an increase in the mortality rate of larvae associated with an increase in the concentration of heavy metals (Moroń et al., 2014). Gekière's review (2023) also shows that trace metals and metalloids modify bee behavior by enhancing flight take-off and vertical flight activity and impairing the walking of some bees (Rodrigues et al., 2016; Bernardes et al., 2021; in Gekière et al., 2023). Foraging behavior is also disrupted with an increase or decrease in food collection (Milivojević et al., 2015; Rodrigues et al., 2016; Hladun et al., 2016; Di et al., 2016, 2020; Al Naggar et al., 2020; Monchanin et al., 2022; in Gekière et al., 2023).

Nitrogen oxides and ozone, emitted by vehicles, can alter flower odor plumes, a crucial stimulus for many pollinating insect species (Ryalls et al., 2022). Their laboratory studies highlight that even at levels deemed environmentally safe by legislation, NO_x and O_3 significantly decrease flower visitation by hoverflies, bees, butterflies, and moths.

4.5. Roadside management

Although roads have an ecological negative impact as described above, roadside can accommodate many plant and insect species (Fitch & Vaidya, 2021; Muñoz et al., 2015; Underhill & Angold, 2000). Phillips and colleagues (2020) define road verge as « *vegetated strips, generally consisting of grassland, shrubland, woodland or forest, which often form distinctly managed borders that separate roads from adjacent land* ». They have distinct management practices from the surrounding landscape (Underhill & Angold, 2000). They serve various practical functions including increasing user visibility, the accommodation of road infrastructure such as signs, pathways for pedestrians and when correctly managed, the habitats for wildlife (Gardiner et al., 2018 in Phillips et al., 2020). Road verges are considered an important extensive habitat in the context of reducing natural and semi-natural habitats (Underhill & Angold, 2000). In few numbers, roadsides represent 270.000 km^2 in the world (Phillips et al., 2020)

Road verges can offer habitats for various pollinator life cycle stages, including reproduction, nesting, feeding and overwintering. While the physical presence of roads may present semi or complete barriers to pollinator movements, their verges can function as navigational aids, creating corridors that facilitate the movement of wildlife through fragmented landscapes and increase connectivity (Cranmer et al., 2012; François & Le Féon, 2017; Phillips et al., 2020; Trombulak & Frissell, 2000).

4.5.1. Belgium

In Belgium, roadsides cover tens of thousands of hectares (Departement Leefmilieu, Natuur en Energie, 2015). Communes realize the management of communal road verges, while the responsibility for the main road and highways lies with the Flemish and Walloon governments.

A decision outlined in the Belgian Monitor in 1984 prohibited roadside biocides (BdM, 2023). Since 1985 in Flanders and 1995 in Wallonia, both regions have been implementing an ecologically oriented management program (BdM, 2023; Departement Leefmilieu, Natuur en Energie, 2015).

In Wallonia, half of the plant flora is found on roadsides. Within this territory, a practice known as "late mowing" is adopted by 226 communes (BdM, 2023). This technique, which constitutes a form of mosaic mowing, involves the regular mowing of a safety strip, approximately 1 meter closest to the road. In addition, beyond the safety band, a broader strip is mowed only once a year, and this is done later in the season, typically after August 1st or September 1st, depending on the presence of specific wild species (BdM, 2011). Since 2009, the « Interdepartmental Invasive Species Unit » (CiEi) managed the exotic invasive plant in Wallonia. The specific characteristics of each species are considered to kill them (CiEi, 2023).

In Flanders, 60 % of the plant flora is found on roadsides (Departement Leefmilieu, Natuur en Energie, 2015). The management program provides spring mowing from June 15 and autumn mowing from September 15 (*Beheer van de wegbermen in Vlaanderen*, 2023). High-value roadside can be included in a progressive mowing management system in consultation with the Nature and Forest Agency (*Beheer van de wegbermen in Vlaanderen*, 2023).

4.5.2. Serbia

Although there is no official source for road maintenance rules, a quick review of local press articles and personal comments from environmental researchers indicates a lack of specific guidelines for roadside management in Serbia. However, the management practices observed seem to give priority to the safety of road users and aim to prevent the creation of environments likely to attract animals, thereby reducing the risk of collision accidents (Javno Preduzece, 2009).

4.6. Biological questions

This study is structured into the following biological questions *(cf. Figure 8)* :

1. **Do flowering roadsides promote pollinator diversity?**

This biological question is divided into two sub-questions:

- Does the diversity of floral species increase the diversity of pollinator assemblages ? *(cf. Figure 8:* ***Q1a****)*
- Which flowers are most attractive to pollinators in the plant communities along roadsides? *(cf. Figure 8:* ***Q1b****)*

We will assess the floral diversity of each site, the diversity of bees, hoverflies, and butterflies, and the frequency of visits by each pollinator to specific plant species. We hypothesize that greater floral species richness supports a greater pollinator species richness (Hopwood, 2008; Phillips et al., 2020; Ries et al., 2001; Saarinen et al., 2005). We aim to establish a ranking of the plants most frequently visited by pollinators to investigate their equivalency. We hypothesize that (i) not all flowers are equal in their attractivity and (ii) the classification should differ for bees, hoverflies and butterflies (Rader et al., 2020; Warzecha et al., 2018). The results will help us to advise on good maintenance practices.

2. **Do rich-floral road verges act as honeytraps for pollinators?** *(cf. Figure 8:* ***Q2****)*

We will analyze the concentration of flowers at each site and its correlation with the number of fatal insect collisions (i.e. pollinators in a broad sense). Our hypothesis suggests that a greater abundance and diversity of flowers may be associated with a reduced risk of collision. (Dániel-Ferreira et al., 2022; Ries et al., 2001; Skórka et al., 2013).

3. **Are pollinator communities impacted by vehicle collisions?** *(cf. Figure 8:* ***Q3****)*

First, we will describe all the insects impacted by vehicle collisions. Then, the bee, hoverfly, and butterfly communities will be analyzed. Finally, sizes will be measured and analyzed. We hypothesize that smaller insects will be more numerous (Fitch & Vaidya, 2021; Martin et al., 2013).

Geographical context:

These biological questions will be asked in the Belgian and Serbian territories. The aim is to compare a highly developed road network with a less developed one on a European scale. We hypothesize that the effect will be more visible on a dense network.

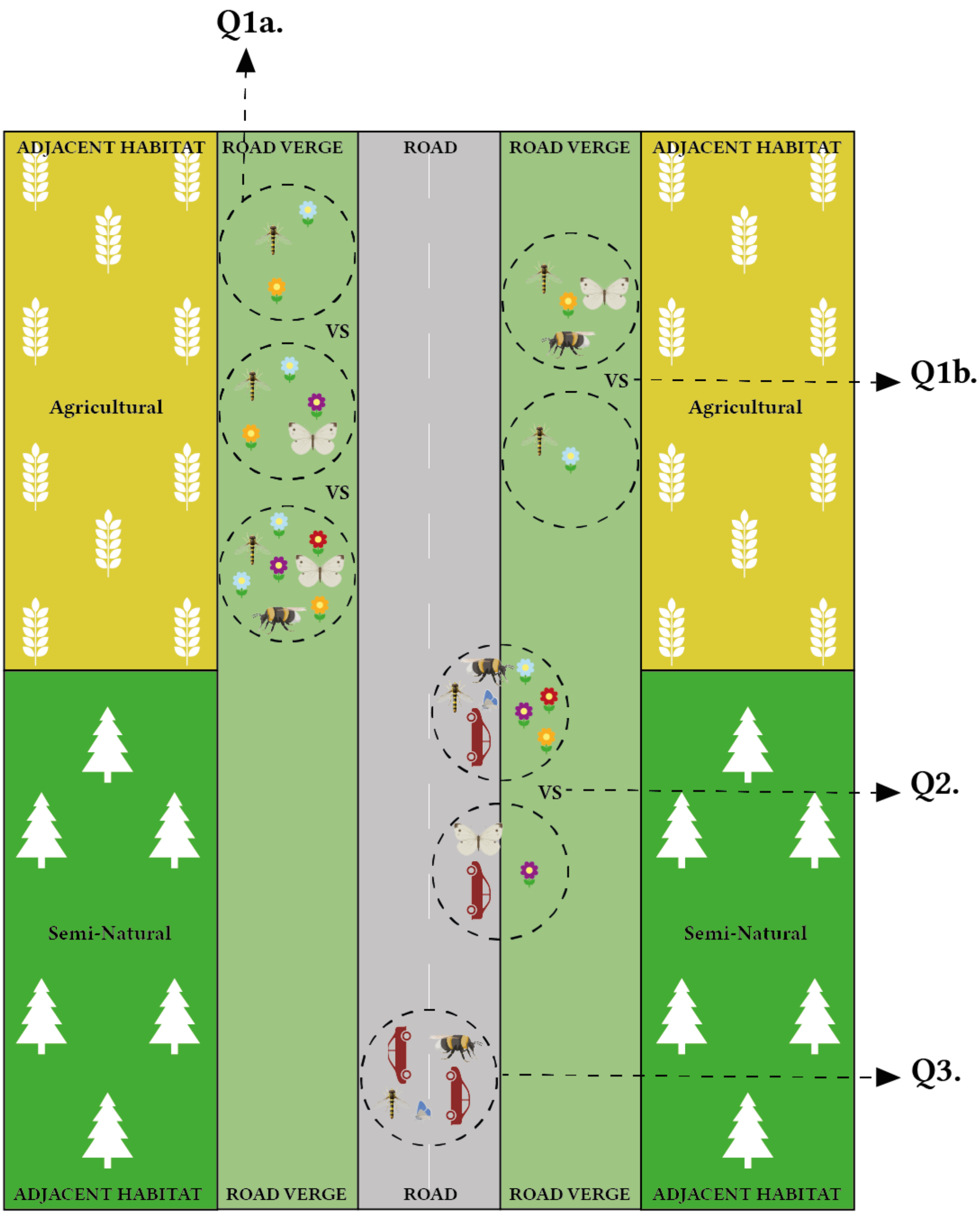


Figure 8: Illustration of biological questions; according to (Phillips et al., 2020) (Credit : Lescot, 2024). ***Q1a*** *: Does the diversity of floral species increase the diversity of pollinator assemblages ?* ***Q1b*** *: Which flowers are most attractive to pollinators in the plant communities along roadsides?* ***Q2*** *: Do rich-floral road verges act as honeytraps for pollinators?* ***Q3*** *: Are pollinator communities impacted by vehicle collisions?*

5. Materials and methods

5.1. Study areas

The first study area is located in Belgium's Hainaut and Namur Provinces. Twenty-four sites are selected and illustrated in Figure 9, detailed in Table 3.

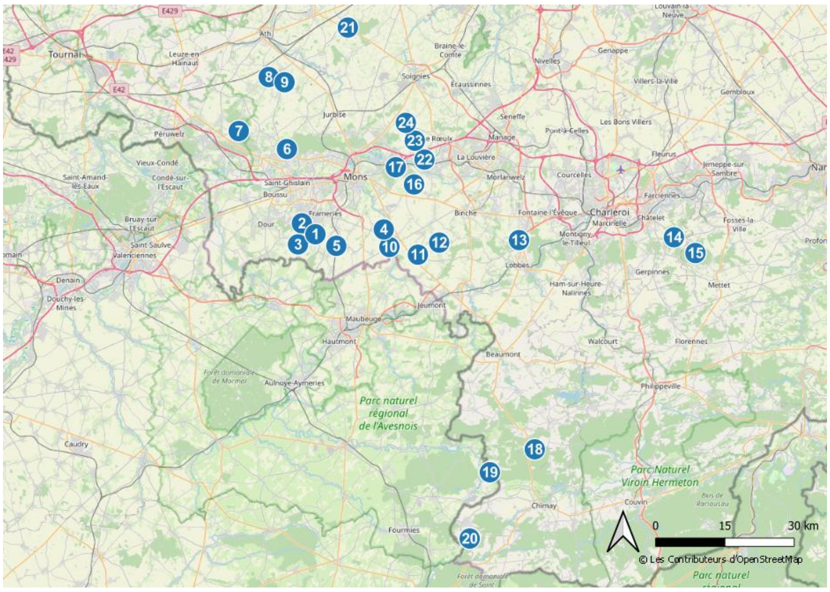


Figure 9: The Belgium study area (Credit : Lescot, 2023)

The second study area is situated in Vojvodina Province in Serbia. Twenty-four sites are selected and illustrated in Figure 10, detailed in Table 3.

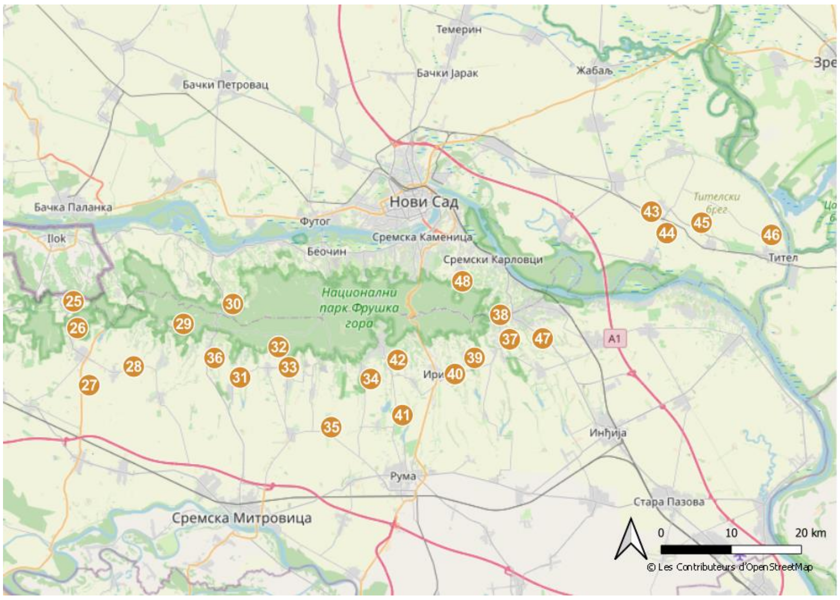


Figure 10: The Serbia study area (Credit : Lescot, 2023)

5.2. Site selection

Site selection and sampling *(cf. Section 5.3)* were carried out using a protocol developed as part of the Safeguard project and executed in different European countries (Safeguard Task 2.6 (ii) Traffic study).

The length of the roads selected must be at least 1.250 km *(cf. Section 5.3)*. The centers of each site must be at least 2 kilometers apart, to avoid spatial autocorrelation (Oliveau & Doignon, 2016).

The twenty-four sites in each country were selected based on landscape context, road type and verge management.

5.2.1. Landscape context

The aim is to control for the landscape context. For this purpose, we selected roads dominated by an intensive agricultural landscape and roads surrounded by a mosaic of semi-natural elements (mainly forests and extensive meadows). Urban roads are avoided because they do not have verges but pavements, parking spaces and houses.

5.2.2. Road type

After identifying the landscape context, we overlaid the road networks. The objective is to choose roads with strong contrasts in vehicle usage and classify them into two categories: major and minor roads. The criteria used to classify roads in these two categories are the management, the carriageway width, the number of lanes, and the presence of road markings.

Minor roads are mainly managed by the municipality, and have a road width of 6m or less, without road markings and facilities. Major roads are mainly managed by the region and are mostly over 6m wide with facilities, consistently equipped with two lanes. For safety reasons, the selection is limited to single-carriageway roads in both directions, excluding multi-lane roads like highways. In the field, a study of traffic is realized during 15 minutes. This metadata will be used for the discussion. The number and type of vehicles (cars, motorcycles, light goods vehicles, heavy goods vehicles) passing on the road in both directions are documented on a traffic sheet *(cf. Annex 9.6).*

5.2.3. Site replications

Six replicas are subsequently selected for the landscape and the road type, resulting in the following formula for each country: **2 landscapes x 2 road types x 6 replicate blocks = 24 sites.**

Table 3: Sites list including the country, the latitude and longitude of walking transect start (cf. Section 5.3) in CRS WG 84 Decimal degree and Safeguard Task 2.6 Traffic protocol road characterization

Site index	Country	Latitude	Longitude	Landscape	Road type	Site code
1	Belgium	50,380333	3,883366	Arable	Major	**BE5SLB01**
2	Belgium	50,38985	3,839333	Semi-natural	Major	**BE1PTG02**
3	Belgium	50,376933	3,85225	Semi-natural	Major	**BE1EUG03**
4	Belgium	50,385083	3,995583	Arable	Major	**BE7GVR04**
5	Belgium	50,3656	3,924483	Arable	Major	**BE5QLP05**
6	Belgium	50,485733	3,809916	Semi-natural	Major	**BE3BDR06**
7	Belgium	50,50505	3,73645	Semi-natural	Major	**BE3STB07**
8	Belgium	50,574116	3,793666	Arable	Major	**BE5LDZ08**
9	Belgium	50,568616	3,80605	Arable	Major	**BE7CVR09**
10	Belgium	50,36162	3,99947	Arable	Major	**BE7HLC10**
11	Belgium	50,352233	4,090766	Arable	Minor	**BE6ETN11**
12	Belgium	50,369516	4,132666	Arable	Minor	**BE6EAV12**
13	Belgium	50,369583	4,287366	Semi-natural	Minor	**BE4LBS13**
14	Belgium	50,372283	4,5548	Semi-natural	Minor	**BE4CTT14**
15	Belgium	50,353583	4,630966	Semi-natural	Minor	**BE2BSM15**
16	Belgium	50,441983	4,050383	Arable	Minor	**BE6EAV16**
17	Belgium	50,460816	4,011383	Semi-natural	Major	**BE1HVR17**
18	Belgium	50,113766	4,28425	Semi-natural	Major	**BE3CMY18**
19	Belgium	50,084816	4,229433	Semi-natural	Minor	**BE2BLV19**
20	Belgium	50,002166	4,190033	Semi-natural	Minor	**BE4MMG20**
21	Belgium	50,63555	3,954583	Semi-natural	Minor	**BE2SLY21**
22	Belgium	50,484133	4,102833	Arable	Minor	**BE8LRL22**
23	Belgium	50,501233	4,05145	Arable	Minor	**BE8GTG23**
24	Belgium	50,515483	4,031033	Arable	Minor	**BE8TUS24**
25	Serbia	45,161229	19,386721	Semi-natural	Minor	**RS1LJU01**
26	Serbia	45,137102	19,391852	Semi-natural	Major	**RS1ERD02**
27	Serbia	45,137102	19,391852	Semi-natural	Major	**RS1ERV03**
28	Serbia	45,102443	19,463752	Semi-natural	Minor	**RS1BIN04**
29	Serbia	45,14085	19,527566	Semi-natural	Minor	**RS2ROH05**
30	Serbia	45,158774	19,590858	Semi-natural	Major	**RS2SVI06**
31	Serbia	45,092558	19,599972	Semi-natural	Minor	**RS2MAN07**
32	Serbia	45,120003	19,649259	Arable	Minor	**RS3GRG08**
33	Serbia	45,101773	19,66242	Arable	Minor	**RS3SUL09**
34	Serbia	45,091212	19,767053	Arable	Major	**RS3JAZ10**
35	Serbia	45,047652	19,716659	Arable	Major	**RS3STE11**
36	Serbia	45,110152	19,567233	Semi-natural	Major	**RS2LEZ12**
37	Serbia	45,126983	19,945083	Semi-natural	Major	**RS4KRU13**
38	Serbia	45,149021	19,933059	Semi-natural	Minor	**RS4VRE14**
39	Serbia	45,110232	19,900029	Arable	Minor	**RS5NER15**
40	Serbia	45,09565	19,874958	Arable	Major	**RS5IRI16**
41	Serbia	45,058566	19,80779	Arable	Minor	**RS5PAV17**
42	Serbia	45,108323	19,801427	Arable	Major	**RS5VRD18**
43	Serbia	45,241913	20,126377	Arable	Major	**RS6VIL19**
44	Serbia	45,222644	20,146037	Arable	Minor	**RS6GAR20**
45	Serbia	45,231981	20,190539	Arable	Major	**RS6LOK21**
46	Serbia	45,22101	20,280121	Arable	Minor	**RS6TIT22**
47	Serbia	45,127722	19,987199	Semi-natural	Major	**RS4MAR23**
48	Serbia	45,179211	19,884527	Semi-natural	Minor	**RS4BUK24**

5.3. Sampling method

The protocol involves two sampling methods, each linked to distinct surveys within the Safeguard project. The first type of sampling is conducted on foot along a 250-meter transect to collect butterflies, bees, and hoverflies *(cf. Figure 11: blue and purple arrow)*, referred to as the « **Walking experiment** ». The second type involves sampling from a car, covering a 1,250-kilometer transect to collect all insects striking the vehicle *(cf. Figure 11: yellow arrow)*, referred to as the « **Car experiment** ».

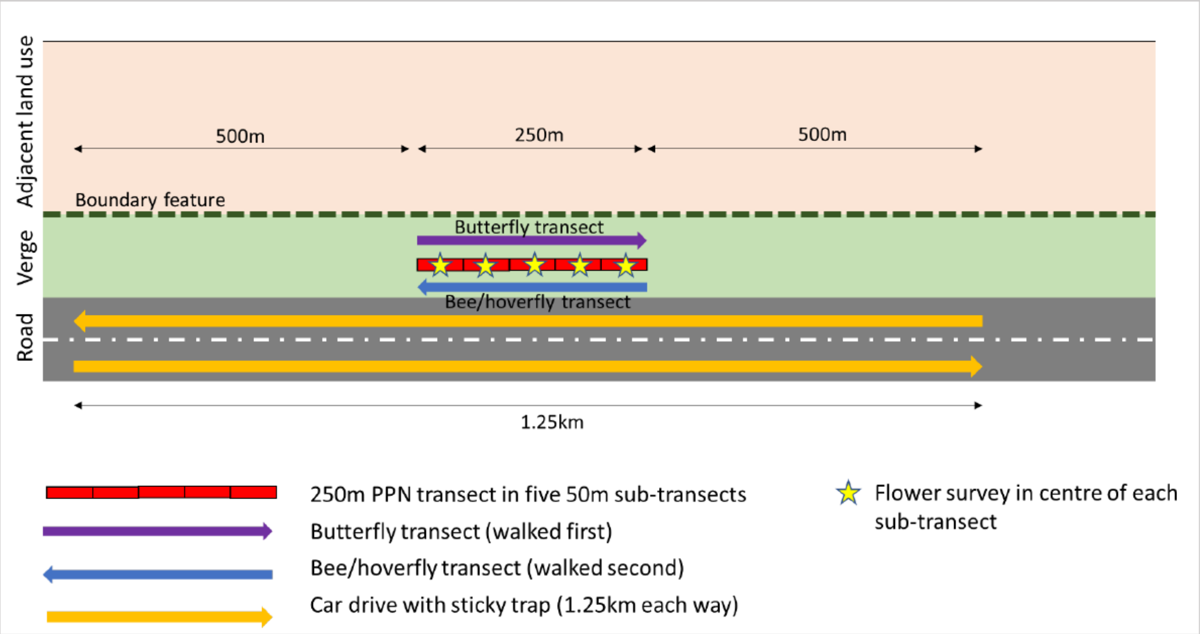


Figure 11: Schematic showing the location of the walking and car experiment, with verge, boundary feature and adjacent land use (Credit : Safeguard Task 2.6 (ii) Traffic study)

The 24 sites of each country were sampled three times to cover the evolution of communities during the pollinator activity period. In Belgium, we sampled each site every month, from June to September 2023 depending on the weather. Indeed, sites were sampled when minimum temperatures were 13°C on sunny days, and up to 17°C on cloudy days without rain. Moreover, field trips are not recommended when the wind is too strong[2] (Westphal et al., 2008). The transects are carried out between 9 am and 5 pm to adhere to the activity of pollinators (Westphal et al., 2008). These time and weather conditions are based on the European Butterfly Monitoring Scheme (eBMS) (Sevilleja et al., 2019) and also described in several publications (Van Swaay et al., 2008; Williams et al., 2001). The same methodology was used in Serbia, with the collection period from May to July.

[2] According to eBMS : *« it should be 5 or lower on the Beaufort scale (called a fresh breeze), which is when the branches of a moderate size move and small trees in leaf begin to sway »* (Sevilleja et al., 2020).

5.3.1. Walking transect

The transect is 250 meters in length and 2 meters in width, positioned at the center point between the road and the adjacent land use. The eBMS recommends that to allow maximum coverage of field variability (Sevilleja et al., 2019), the transect is divided into five sub-transects, each spanning 50 meters. A paint mark is placed on the road to ensure each sub-transect can easily be located in the next round *(cf. Figure 13.1)*.

The sampling starts with butterflies because they can be more easily scared away. Following this, we proceed with transect sampling of bees and hoverflies. The total time spent on the transect is 30 minutes per site (15 minutes for butterflies and 15 minutes for bees/hoverflies) to search and capture pollinators. The timer is stopped for all other activities such as taking notes, transferring specimens into a vial, and labeling.

The same methodology was used in Serbia, but the sampling was non-lethal for bees and hoverflies that can be easily identified in the field.

5.3.1.1. Butterfly transect

The methodology employed is derived from the « Pollard Walk » which implies a fixed itinerary visited several times and in favorable weather conditions (Pollard & Yates, 1993 in Sevilleja et al., 2019).

The transect is traveled at a steady and constant speed for 3 minutes by sub-transect. A large, black net is employed to capture day butterflies, Rhopalocera, observed within an imaginary box of 5 meters long and high and 2 meters wide (*cf Figure 12)*. Once caught, butterflies are identified using two reference manuals: Tolman et al. (2015) and Claerebout et al. (2014) *(cf. Figure 13.2)*. After identification, butterflies were released. In some cases, identification was limited to the genus level because the capture was unsuccessful. The identification is registered on a capture sheet *(cf. Annex 9.3)* with the butterfly micro-location (flying, on the ground or on a plant species).

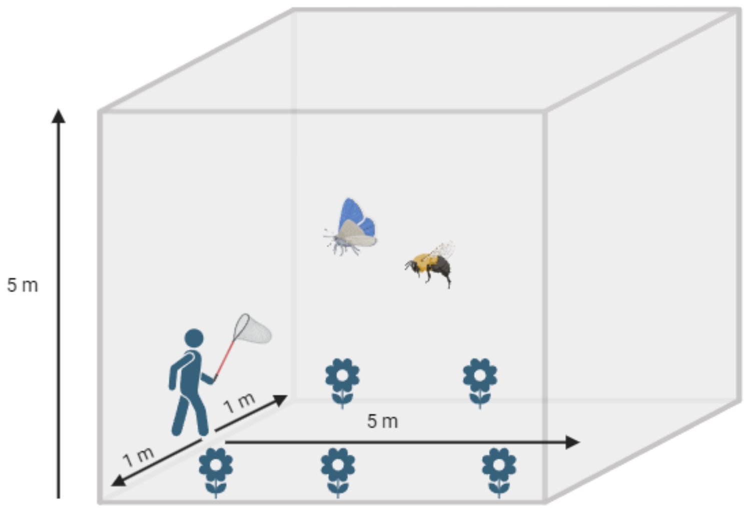


Figure 12: Capture imaginary box (Credit : Lescot, 2023)

5.3.1.2. Bee and hoverfly transect

The bee and hoverfly transect methodology is similar to the one used for butterflies *(cf. Figure 12)*.

Bees and hoverflies were captured in a smaller and white net. Due to the difficulty of identifying these specimens to the species level in the field, the individuals, except *Apis mellifera*[3], were transferred to a container filled with absorbent cotton soaked in ethyl acetate, which led to the animal's death. Each container is labeled in advance, and all the specimens collected on the same sub-transect and micro-location are put together. All the observations are registered on a capture sheet *(cf. Annex 9.4)*. In certain instances, when pollinators could not be successfully captured, they were documented with as much available taxonomic information as possible and marked as 'not caught' on the capture sheet. Otherwise, the data is considered lost.

5.3.1.3. Flower survey

Following the completion of the walking transect, a 1m² quadrat is positioned in the center of each sub-transect *(cf. Figure 13.3)*. Every plant within this quadrat was identified at the species level using a reference book (Steeter & Cole, 2017). In cases where precise identification could not be established, identification was recorded at the genus level. The data were registered in a quadrat sheet *(cf. Annex 9.5)*, including each flowering species' plant. Floral abundance was represented by flower cover, expressed as a percentage, reflecting the amount of shade created by each flower when the sun is at its zenith.

5.3.2. Car transect

The car transect is 1.250 km long and begins approximately 500 meters ahead of the starting point of the walking transect *(cf. Figure 11)*. As its name suggests, it is covered by a car and requires the installation of sticky traps.

The sticky trap fixation is performed at the onset of the car transect. For every site visit, two sticky traps measuring 10x25 cm each are glued to a plastic plate fixed on the car using cable ties below the license plate *(cf. Figure 13.4 and 13.5)*. Then, the protective film of the sticky trap is removed. Following the installation, the driver travels a distance of 1.250 km along the road adjacent to the walking transect *(cf. Figure 11)*, then turns around and returns to the starting point. The distance covered with the sticky trap is therefore 2.5 km. As much as possible, the car's speed is fixed at 60 km/h to respect speed limits outside built-up areas and to be able to identify the specimens collected. Once the car transect is finished, the sticky trap is marked with site index, round number and unique identifiers. It's removed in a box, and small plastic beads are glued to it to prevent them from sticking together *(cf. Figure 13.6)*. The plastic plate is also removed from the car and reused at the following site.

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[3] Their observation and abundance is noted in the capture sheet with the mention "not caught".

5.4. Specimen processing

5.4.1. Walking transect

At the end of the field day, collected bees and hoverflies are pinned *(cf. Figure 13.7)*. The male genitalia are extracted, as it is useful for identification. Two labels are then affixed to each specimen *(cf. Figure 13.7)*. The first label includes the collection date, the collection location, the specimen micro-location and the collector's name. The second label displays the specimen's unique identifier.

At the end of the fieldwork, all the specimens are pre-identified. Bees are pre-identified to the genus level using a reference manual (Michez et al., 2019). Bee identifications, at the species level, were a teamwork involving the following experts, using the following species keys :

- Thomas Wood for *Andrena* (Wood, 2023)
- Clément Tourbez for *Bombus* (Rasmont & Terzo, 2017)
- William Fiordaliso for *Colletes* (Amiet et al., 1999)
- Thomas Brau for *Halictus* (Pauly, 2015)
- Le Divelec Romain for *Hylaeus* (Amiet et al., 1999)
- Flaminio Simone for *Lasioglossum* and *Seladonia* (Pauly, 2015)

Hoverflies are identified to the species level using reference manuals (Ball & Morris, 2015), (Schulten, 2019) and confirmed by William Fiordaliso.

Once identification is finished, a third label is created indicating the species, sex, and identifier of the specimen *(cf. Figure 13.7)*. The specimens are stored in the UMONS collection within the zoology laboratory.

5.4.2. Car transect

At the end of the fieldwork, all the insects captured in the sticky trap were initially identified at the family level. William Fiordaliso verified the identification, and further identification of hoverflies was conducted up to the species level. Insect fragments or damaged specimens are documented to the highest possible taxonomic level or categorized as 'other' when specific identification is not feasible.

Each specimen is measured using a digital caliper from the head's tip to the abdomen's tip. The length of insects under 2 mm has been rounded to the nearest millimeter (1 or 2 mm).

Figure 13: Illustration of the various stages of the materials and methods (Credit : Lescot, 2023): ***1*** *: Paint mark on the road ;* ***2*** *: Butterfly identification ;* ***3*** *: 1m² quadrat ;* ***4*** *: Plastic plate fixed on the car ;* ***5*** *: One sticky trap glued on the plastic plate on the car ;* ***6*** *: Small plastic beads on a section of the sticky trap ;* ***7*** *: Specimen pinned and labeled*

5.5. Statistical analyses

The statistical analyses are realized on R version 4.3.2.

5.5.1. Completeness analysis

First, we analyze the completeness of the walking experiment, defined as the proportion of the total number of species at each site, which depends on sampling effort and quality. Completeness will be illustrated by an accumulation curve for each site. This curve tends toward an asymptote when all species at the site are collected (Gotelli & Colwell, 2001). We will then calculate the Jackknife 1, Jackknife 2, Chao, and Bootstrap estimators, which extrapolate the accumulation curve to estimate the total number of species. These estimators allow us to compare the number of species collected with the estimated number of species, thereby assessing completeness. This analysis will be performed using the specpool() function in the vegan package (Oksanen et al., 2007). The accumulation curve and these estimators will be applied to Belgian bees and hoverflies, as they were the only samples to quantify abundances.

5.5.2. Diversity indices

Standardization is necessary to ensure the same level of representativeness and quality across community samples. This is achieved through rarefaction, which involves reducing the sampling effort to a common level of representativeness. Sample representativeness was calculated using the concept of sample coverage as defined by Chao (Chao & Chiu, 2016). Sample coverage corresponds to the total proportion of individuals in an assemblage belonging to species discovered in its samples. Rarefaction will be based on coverage to account for differences in abundance between sites, preserving more data than rarefaction based solely on the number of individuals collected. Diversity metrics are carried out using the estimateD() function from the iNEXT package.

Raw species richness will be used to measure taxonomic diversity. It's the response variables in our statistical models for groups where a non-lethal method was used: Belgian and Serbian butterflies, Serbian bees and hoverflies. For Belgian bees and hoverflies, the species richness was standardized by coverage and used as response variables.

5.5.3. Statistical modeling

To address our biological questions, we will employ generalized linear models (GLMs) with a Poisson distribution appropriate for count data (Dunn & Danoff-Burg, 2007). The default link function associated with Poisson regression is the logarithm function:

$$log\,\mu = \beta 0 + \beta 1 x 1 + \beta 2 x 2 + \beta 3 x 3 + \ldots + \beta k\, xk.$$

Models be implemented using the glm() function in R. A key rule of the Poisson distribution is that the mean equals the variance. To verify this assumption, we will perform a dispersion test using the dispersiontest() function from the AER package. If the dispersion ratio is not equal to 1, indicating overdispersion or underdispersion, we will instead use a generalized Poisson distribution with the glmmTMB() function from the glmmTMB package. We analyze the residuals using the simulateResiduals() function from the DHARMa package to validate the model. This allows us to perform a Kolmogorov-Smirnov test to ensure that the chosen distribution matches the observed distribution and to identify any potential deviations.

In practice, models will be run separately for the walking experiment for different pollinator communities and countries. The response variables will be *(c.f figure 14)* :

- The rarefied species richness based on coverage for Belgian bees and hoverflies was sampled using a completely lethal method.
- The species richness for Belgian and Serbian butterflies as well as Serbian bees and hoverflies using a non-lethal method.

This approach provides a more precise model for Belgium and a less precise one for Serbia due to the inability to standardize since part of the data was not collected.

Concerning the car experiment, the response variable will be the number of collisions.

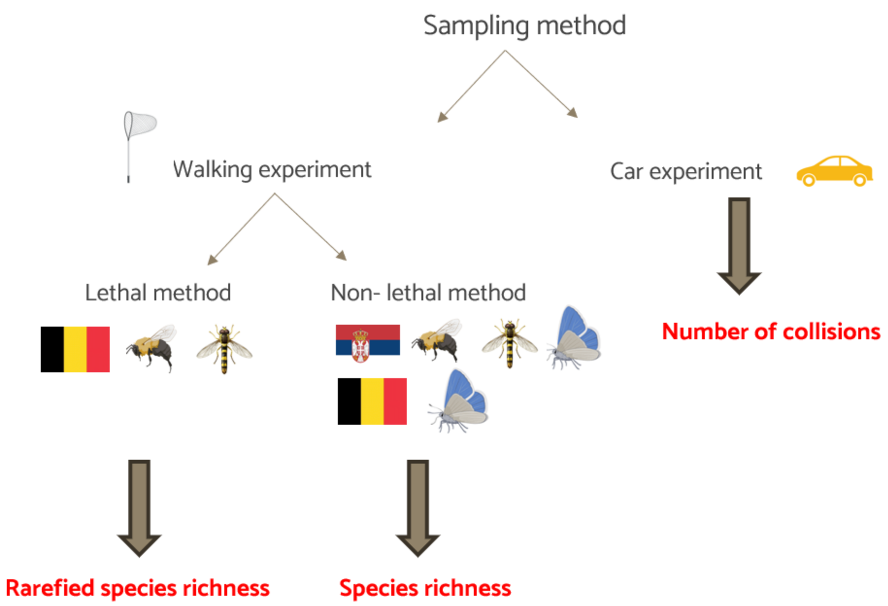

Figure 14: Choice of response variables depending on the sampling method

The explanatory variables will include the landscape context, road type, number of floral species, and floral coverage of each site. To answer our first biological question *(cf. Figure 8 Q1A)*, our model will be: glmmTMB (Pollinator species richness ~Flower species richness + Flower abundance + Landscape + Road type). To answer our second biological question *(cf. Figure 8 Q2)*, our model will be: glmmTMB (Number of collisions ~ Flower species richness + Flower abundance + Landscape + Road type).

6. Results

For the walking experiment in Belgium, we collected 197 bee specimens of 20 species, 759 hoverfly specimens of 30 species and 24 butterfly species. In Serbia, we collected 64 bee species, 30 hoverfly species and 39 butterfly species. For the car experiment in Belgium, we collected 691 insects including 4 hoverfly specimens. In Serbia, we collected 476 specimens, including 6 bee specimens, 4 hoverfly individuals, and 2 butterfly specimens.

Appendix *(cf. Annex 9.7)* describes the bee, hoverfly, and butterfly communities collected during the walking experiment, along with the species accumulation curves and species richness indicators. Appendices 9.8 and 9.9, respectively, provide the list of Belgian and Serbian sites and associated variables. Moreover, a detailed description of invertebrate roadkills from the car experiment is also available *(cf. Annex 9.10)*.

6.1. Relationship of plant diversity and pollinator diversity along roadsides

6.1.1. Belgium

Figure 15 illustrates the impact of floral species richness and abundance on pollinator species richness in Belgium. Figure 16 highlights the effect of landscape and road type on pollinator species richness in Belgium. Table 4 provides a summary of the generalized Poisson model in Belgium.

Statistical analysis (p.value = 0.00422) indicates that flower abundance significantly impacts the number of butterfly species. For a floral abundance value of 0.901, the average number of butterfly species is 3.86 (SE = 0.291, IC 95% = [3.33, 4.47]). A 1% increase in floral cover results in a 55% increase in the number of butterfly species.

We did not detect a significant effect of flower species richness on the species richness of wild bees (p.value = 0.3839), hoverflies (p.value = 0.381855), and butterflies (p.value = 0.81503). However, concerning bees, the standard error of the estimated effect is large (Estimate = 0.06267, SE=0.07196), indicating that our study may not have sufficient power to detect substantial effects. For instance, our model could not identify an effect where adding five flower species to the floral community would result in a 100% increase in bee richness. Concerning hoverflies, the model cannot identify an effect where adding five flower species to the floral community would result in a 60% increase in hoverfly richness (Estimate = 0.04213, SE=0.04818). Concerning butterflies, the model cannot identify an effect where adding five flower species to the floral community would result in a 55% increase in butterfly richness (Estimate = -0.01046, SE=0.04472).

We did not detect a significant effect of flower abundance on the species richness of wild bees (p.value = 0.6493) and hoverflies (p.value = 0.404408). However, concerning bees, the large standard error of the estimated effect indicates limited power to detect substantial effects (Estimate = 0.12149, SE=0.26717). For instance, our model would be unable to identify an effect where increasing flower coverage by 1% results in a 69% increase in bee richness. Concerning hoverflies, the model cannot identify an effect where adding 1% flower cover would result in a 43% increase in hoverfly species richness (Estimate = 0.15336, SE=0.18394).

We detect a significant effect of road type on the species richness of wild bee assemblages (p.value = 0.0241) with significantly higher species richness along minor roads than major roads. The average rarefied species richness is 3.33 (SE = 0.596, IC 95% = [2.35, 4.73]) along the minor roads and 1.86 (SE = 0.330, IC 95% = [1.31, 2.63]) along major roads. This means around 79% more bee species are along minor roads than major roads. Despite a large standard error, the effect is detected (Estimate = 0.58283, SE = 0.25841).

We detect a significant effect of road type on the species richness of hoverfly assemblages (p.value = 0.001194) with significantly higher species richness along minor roads than major roads. The average rarefied species richness is 7.55 (SE = 0.809, IC 95% = [6.12, 9.31]) along the minor roads and 4.28 (SE = 0.563, IC 95% = [3.31, 5.54]) along major roads. This means there are around 76% more hoverfly species along minor roads than along major roads.

We detect a significant effect of road type on the species richness of butterfly assemblages (p.value = 0.01556) with significantly higher species richness along minor roads than major roads. The average species richness is 4.66 (SE = 0.459, IC 95% = [3.84, 5.65]) along the minor roads and 3.19 (SE = 0.377, IC 95% = [2.53, 4.02]) along major roads. This means around 46% more butterfly species are along minor roads than major roads.

Statistical analysis (p.value = 0.000242) indicates that landscape significantly impacts the hoverfly species richness along the Belgian roadside. The rarefied species richness is significantly higher in SNH, with an average of 8.11 (SE = 0.906, IC 95% = [6.52, 10.10]) compared to 3.98 (SE = 0.561, IC 95% = [3.02, 5.25]) in the agricultural landscape. This means there are around 104% more hoverfly species in SNH than in farm habitats.

Statistical analysis (p.value = 7.93e-06) indicates that landscape significantly impacts the butterfly species richness along the Belgian roadside. The species richness is significantly higher in SNH, with an average of 5.49 (SE = 0.512, IC 95% = [4.57, 6.59]) compared to 2.71 (SE = 0.334, IC 95% = [2.13, 3.45]) in the agricultural landscape. This means there are around 103% more butterfly species in SNH than in agricultural habitats.

We did not detect a significant effect of landscape on the species richness of wild bee assemblages (p.value = 0.5343). However, the standard error of the estimated effect is large, indicating that our study may not have sufficient power to detect substantial effects (Estimate = 0.17529, SE=0.28209). Indeed, the smallest increase detected by the model is 74%.

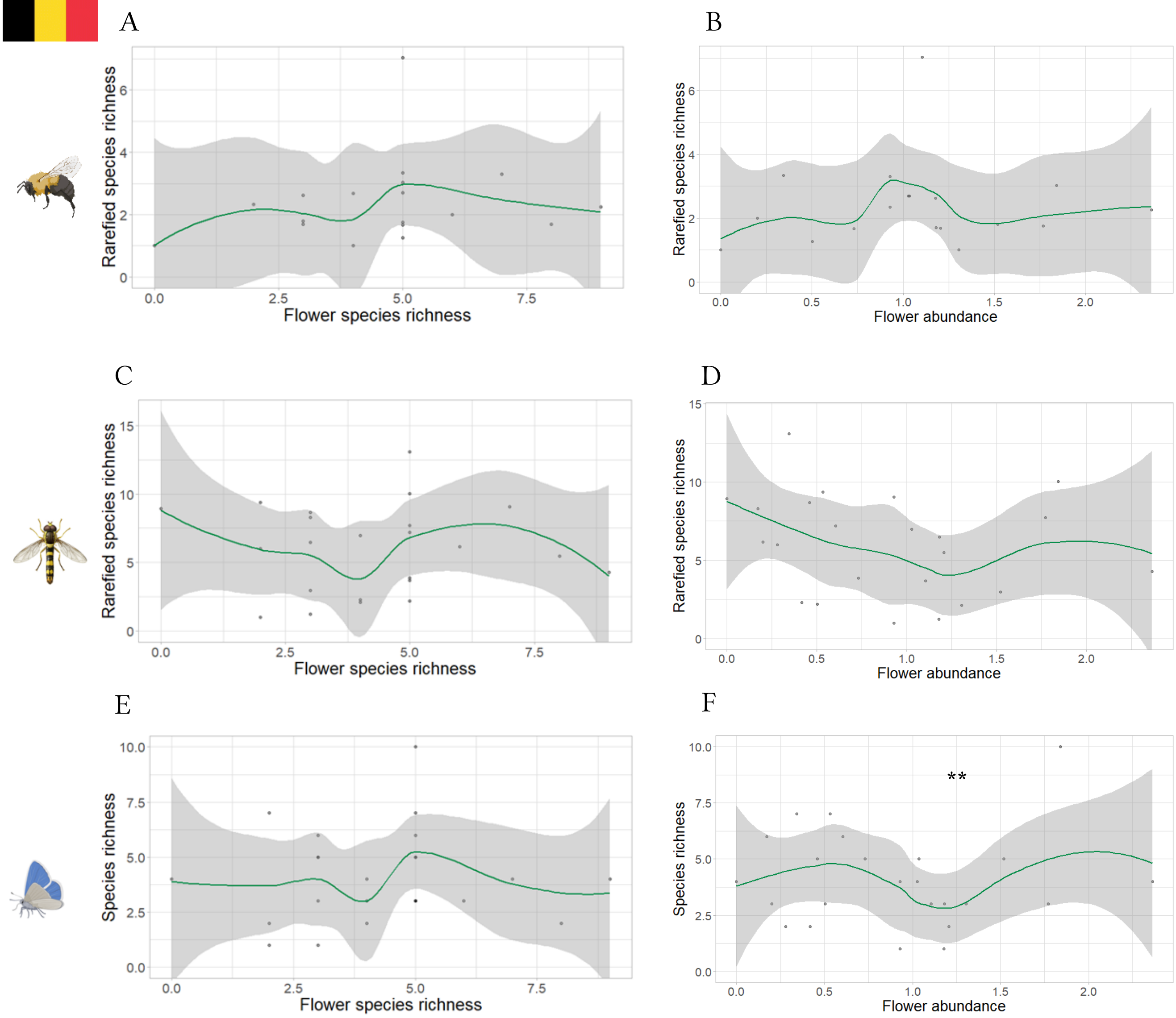

*Figure 15: Impact of flower species richness on the **A:** rarefied species richness of bee (P.value=0.3839), **C:** rarefied species richness of hoverfly (P.value=0.381855), **E:** species richness of butterfly (P.value=0.81503) assemblages and impact of flower abundance on the **B:** rarefied species richness of bee (P.value=0.6493), **D:** rarefied species richness of hoverfly (P.value=0.404408), **F:** species richness of butterfly (P.value=0.00422) assemblages along Belgian roadsides.*

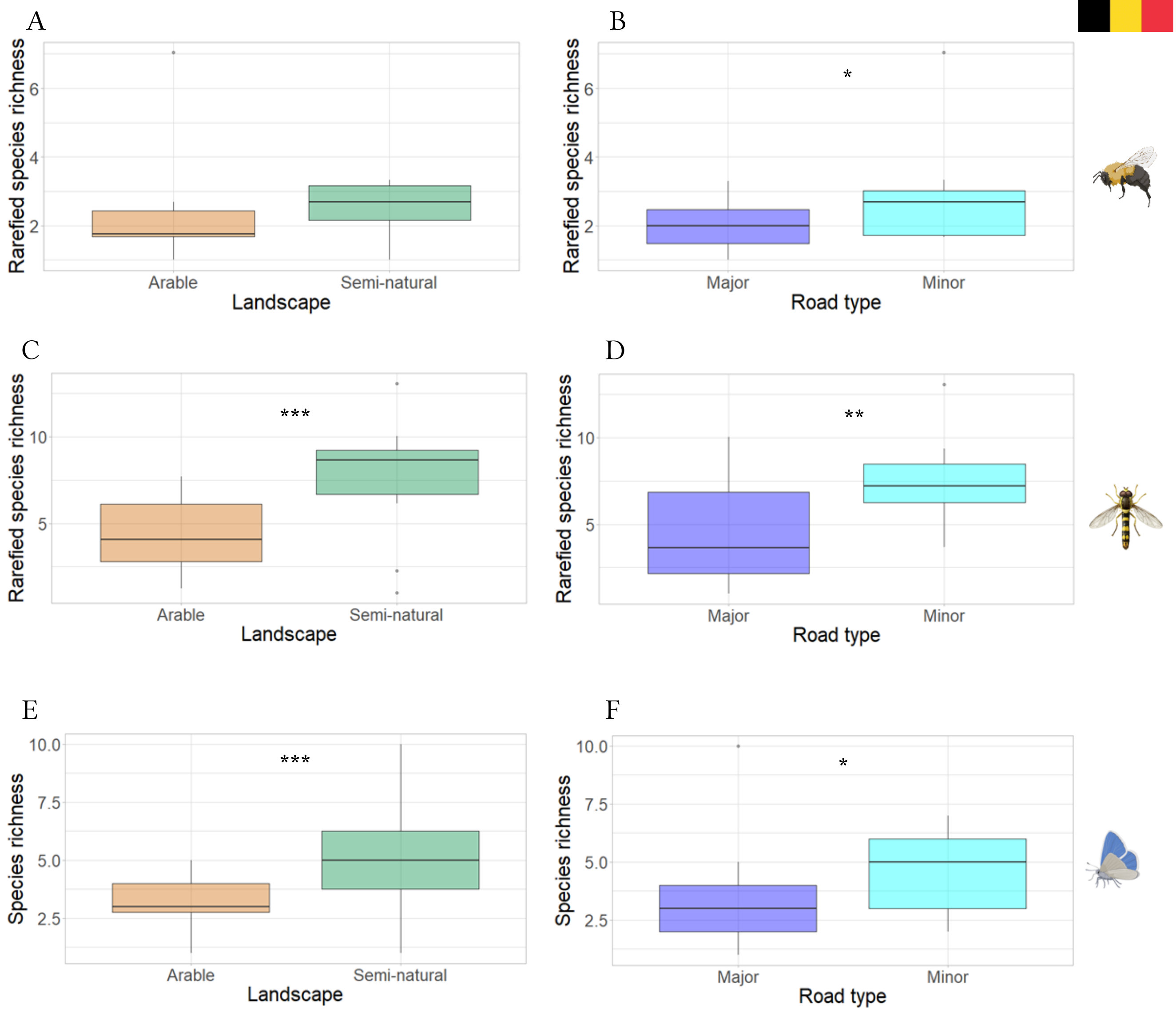

*Figure 16: Impact of the landscape on the species richness of **A:** bee (P.value=0.5343), **C:** hoverfly (P.value=0.000242), **E:** butterfly (P.value=7.93e-06) assemblages and impact of the road type on the species richness of **B:** bee (P.value=0.0241), **D:** hoverfly (P.value=0.001194), **F:** butterfly (P.value=0.01556) assemblages along Belgian roadsides.*

*Table 4: Summary of the generalized Poisson model of rarefied species richness in bees and hoverflies, and species richness in butterflies in Belgium. It presents the model results predicting the species richness of Belgian bees, hoverflies and butterflies. The predictor variables included in the model are **Flower_N0** : Floral species richness, **SNH** : Type of semi-natural habitat, **Minor** : Type of minor road, and **Flower_ab** : Floral abundance.*

	Estimate	Std. Error	Z value	Pr(>|z|)
Bees				
(Intercept)	0.11159	0.48283	0.231	0.8172
Flower_N0	0.06267	0.07196	0.871	0.3839
SNH	0.17529	0.28209	0.621	0.5343
Minor	0.58283	0.25841	2.256	0.0241
Flower_ab	0.12149	0.26717	0.455	0.6493
Hoverflies				
(Intercept)	0.78180	0.32830	2.381	0.017249
Flower_N0	0.04213	0.04818	0.874	0.381855
SNH	0.71156	0.19384	3.671	0.000242
Minor	0.56653	0.17484	3.240	0.001194
Flower_ab	0.15336	0.18394	0.834	0.404408
Butterflies				
(Intercept)	0.45498	0.27761	1.639	0.10124
Flower_N0	-0.01046	0.04472	-0.234	0.81503
SNH	0.70620	0.15809	4.467	7.93e-06
Minor	0.37848	0.15645	2.419	0.01556
Flower_ab	0.44101	0.15415	2.861	0.00422

6.1.2. Serbia

Figure 17 illustrates the impact of floral species richness and abundance on pollinator species richness in Serbia. Figure 18 highlights the impact of landscape and road type on pollinator species richness in Serbia. Table 5 provides a summary of the generalized Poisson model in Serbia.

Statistical analysis (p.value = 0.005858) indicates that flower species richness significantly impacts the number of hoverfly species. For a floral species richness value of 28.3, the average number of hoverfly species is 6.85 (SE = 0.416, IC 95% = [6.08, 7.72]). An increase in 5 flower species results in a 10% increase in the number of hoverfly species.

Statistical analysis (p.value = 2.4e-06) indicates that flower species richness significantly impacts the number of butterfly species. For a floral species richness value of 28.3, the average number of butterfly species is 8.03 (SE = 0.645, IC 95% = [6.86, 9.4]). An increase in 5 flower species results in a 23% increase in the number of butterfly species.

We did not detect a significant effect of flower species richness on the species richness of bees (p.value = 0.7420). The model could not identify an effect where adding five flower species to the floral community would result in a 17% increase in bee richness (Estimate = 0.005324, SE = 0.016175)

We did not detect a significant effect of flower abundance on the species richness of wild bees (p.value = 0.5411), hoverflies (p.value = 0.512012) and butterflies (p.value = 0.1740). However, concerning bees, the large standard error of the estimated effect indicates limited power to detect substantial effects (Estimate = -0.236521, SE=0.386996). For instance, our model would be unable to identify an effect where increasing flower coverage by 1% results in an 113% increase in bee richness. Concerning hoverflies, the model cannot identify an effect where adding 1% flower cover would result in a 35% increase in hoverfly species richness (Estimate = 0.100845, SE=0.153796). Concerning butterflies, the model cannot identify an effect where adding 1% flower cover would result in a 50% increase in butterfly species (Estimate = 0.279735, SE = 0.205770).

We did not detect a significant effect of the road type on the species richness of wild bees (p.value = 0.9206), hoverflies (p.value = 0.082584), and butterflies (p.value = 0.4754). However, concerning bees, the standard error of the estimated effect is large (Estimate = 0.029848, SE = 0.299285), indicating that our study may not have sufficient power to detect substantial effects. Indeed, the smallest increase detected by the model is 80%. Concerning hoverflies, the smallest increase detected by the model is 30% (Estimate = 0.230371, SE = 0.132711). Concerning butterflies, the smallest increase detected by the model is 38% (Estimate = -0.116678, SE=0.163475).

Statistical analysis (p.value = 0.043196) confirms that the landscape significantly impacts hoverfly species richness along the roadside in Serbia. The species richness is significantly higher in SNH, with an average of 7.73 (SE = 0.619, IC 95% = [6.61, 9.04]) compared to 6.07 (SE = 0.546, IC 95% = [5.09, 7.24]) in the agricultural landscape. This means there are around 27% more hoverfly species in SNH than in agricultural habitats.

We did not detect a significant effect of landscape on the species richness of wild bee assemblages (p.value = 0.7959) and butterfly assemblages (p.value = 0.8550). However, concerning bees, the standard error of the estimated effect is large, indicating that our study may not have sufficient power to detect substantial effects (Estimate = 0.076386, SE = 0.295304). Indeed, the smallest increase detected by the model is 78%. Concerning butterflies, the smallest increase detected by the model is 35% (Estimate = -0.027845, SE = 0.152363).

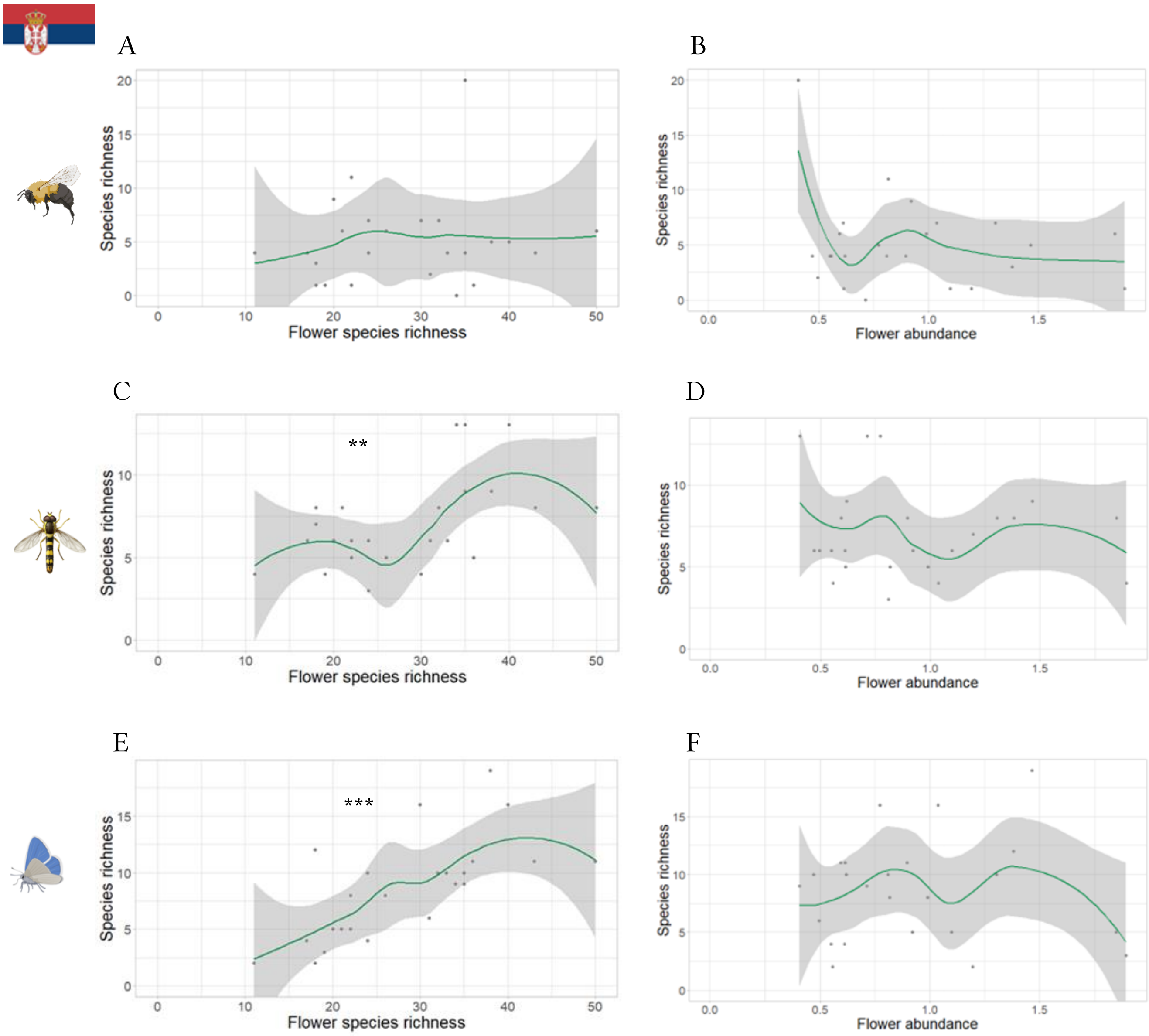

Figure 17: Impact of flower species richness on the species richness of ***A:*** *bee (P.value=0.7420),* ***C:*** *hoverfly (P.value=0.005858),* ***E:*** *butterfly (P.value=2.4e-06) assemblages and impact of flower abundance on the species richness of* ***B****: bee (P.value=0.5411),* ***D:*** *hoverfly (P.value=0.512012),* ***F:*** *butterfly (P.value=0.1740) assemblages along Serbian roadsides.*

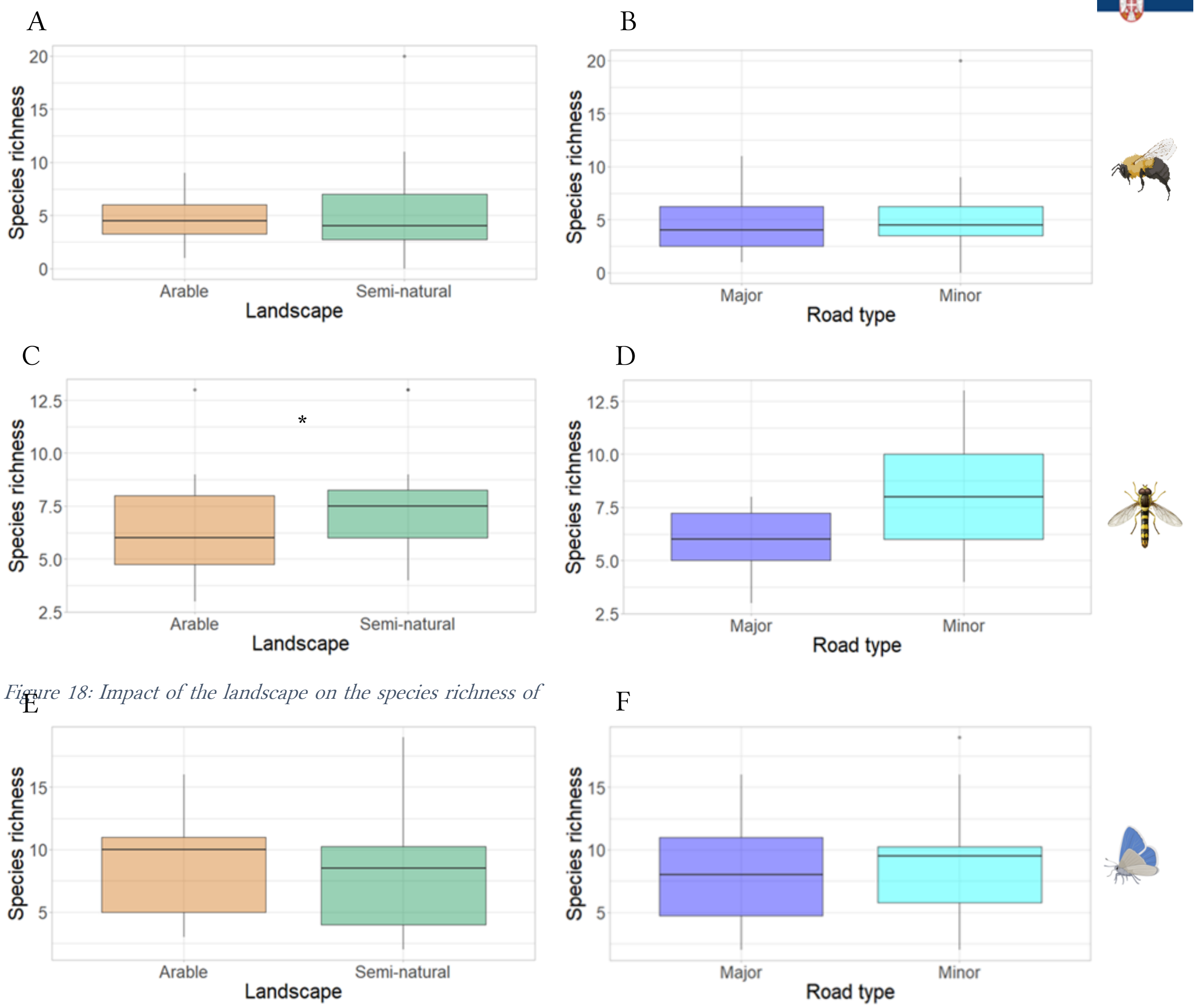

Figure 18: Impact of the landscape on the species richness of ***A:*** *bee (P.value=0.7959),* ***C:*** *hoverfly (P.value=0.043196),* ***E:*** *butterfly (P.value=0.8550) assemblages and impact of the road type on the species richness of* ***B:*** *bee (P.value=0.9206),* ***D:*** *hoverfly (P.value=0.082584),* ***F:*** *butterfly (P.value=0.4754) assemblages along Serbian roadsides.*

*Table 5: Summary of the generalized Poisson model of species richness in bees, hoverflies and butterflies in Serbia. It presents the model results predicting the species richness of Serbian bees, hoverflies and butterflies. The predictor variables included in the model are **Flower_N0** : Floral species richness, **SNH** : Type of semi-natural habitat, **Minor** : Type of minor road, and **Flower_ab** : Floral abundance.*

	Estimate	Std. Error	Z value	Pr(>|z|)
Bees				
(Intercept)	1.631246	0.703856	2.318	0.0205
Flower_N0	0.005324	0.016175	0.329	0.7420
SNH	0.076386	0.295304	0.259	0.7959
Minor	0.029848	0.299285	0.100	0.9206
Flower_ab	-0.236521	0.386996	-0.611	0.5411
Hoverflies				
(Intercept)	1.038460	0.303660	3.420	0.000627
Flower_N0	0.019705	0.007151	2.756	0.005858
SNH	0.241374	0.119385	2.022	0.043196
Minor	0.230371	0.132711	1.736	0.082584
Flower_ab	0.100845	0.153796	0.656	0.512012
Butterflies				
(Intercept)	0.715435	0.397914	1.798	0.0722
Flower_N0	0.041805	0.008863	4.717	2.4e-06
SNH	-0.027845	0.152363	-0.183	0.8550
Minor	-0.116678	0.163475	-0.714	0.4754
Flower_ab	0.279735	0.205770	1.359	0.1740

6.2. Attractiveness of flowers for pollinators along roadsides

6.2.1. Belgium

Figure 19 illustrates the top 10 flowers that attract the most bee specimens and species, hoverfly specimens and species, and the top 5 flowers that attract the most butterfly species between June and September in Belgium.

Cirsium arvense attracted the highest number of bees, with 59 individuals observed on the plant, representing 8 different bee species. *Rubus fruticosus* attracted 23 individuals and 9 bee species. *Eupatorium cannabinum* drew 13 specimens but only 3 species. *Convolvulus arvensis* attracted 9 individuals across 5 bee species. *Heracleum sphondylium* attracted 6 specimens from 4 bee species. Additionally, we found 3 bee species each on *Lythrum salicaria*, *Filipendula ulmaria*, *Eupatorium cannabinum*, *Epilobium angustifolium*, *Convolvulus sepium*, and *Centaurea nigra*.

Heracleum sphondylium attracted the most hoverflies, with 150 individuals and 14 species observed. This is followed by *Convolvulus arvensis*, which hosted 118 individuals and 9 species. *Cirsium arvense* supported 102 hoverfly specimens across 13 species. *Anthriscus sylvestris* attracted 41 hoverfly specimens and 9 species, while *Jacobaea vulgaris* attracted 39 specimens. Both *Eupatorium cannabinum* and *Achillea millefolium* attracted 27 specimens each. *Sonchus arvensis* attracted 19 specimens from 6 species, and *Crepis biennis* attracted 17 individuals across 5 species.

The most butterfly-attracting plants are, in order: *Cirsium arvense*, *Eupatorium cannabinum*, *Cirsium vulgare*, *Lythrum salicaria*, and *Brassica napus*.

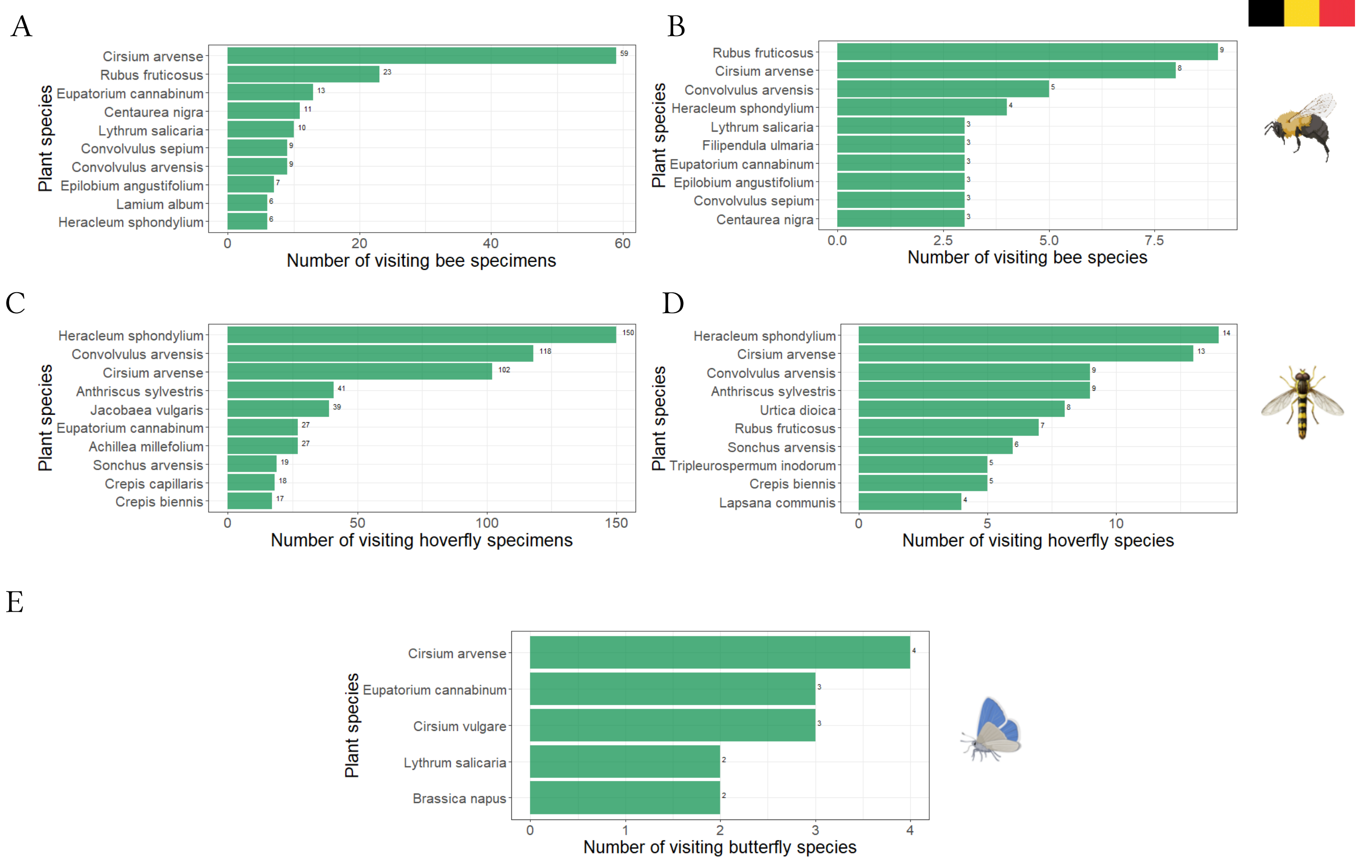

*Figure 19: Top 10 flowers attracting **A:** wild bee specimens, **B:** wild bee species, **C:** hoverfly specimens, **D:** hoverfly species, **E:** butterfly species in Belgian roadsides.*

6.2.2. Serbia

Figure 20 illustrates the top 10 flowers that attract the most bee species, hoverfly species and the top 5 flowers that attract the most butterfly species between May to July in Serbia.

Regarding bees, the flower that attracts the most species is *Carduus acanthoides*, followed by *Convolvulus arvensis*. Then, *Trifolium pratense*, *Salvia nemorosa*, and *Astragalus onobrychis* each attracted 5 bee species. *Symphytum officinale*, *Securigera varia*, *Ranunculus repens*, *Lotus corniculatus*, and *Cichorium intybus* each attracted 4 bee species.

The flowers attracting the most species of hoverflies were *Galium mollugo* and *Achillea millefolium*, with 9 species. *Ranunculus acris* attracted 8 species, while *Sambucus ebulus* and *Rosa canina* each attracted 7. *Tordylium maximum*, *Orlaya grandiflora*, *Cornus sanguinea*, and *Conium maculatum* each attracted 6 species. *Ranunculus repens* attracted 5 species of hoverflies.

Serbia's top 5 plants attracting butterflies are *Salvia nemorosa*, *Carduus acanthoides*, *Astragalus onobrychis*, *Vicia sativa*, and *Centaurea stoebe*.

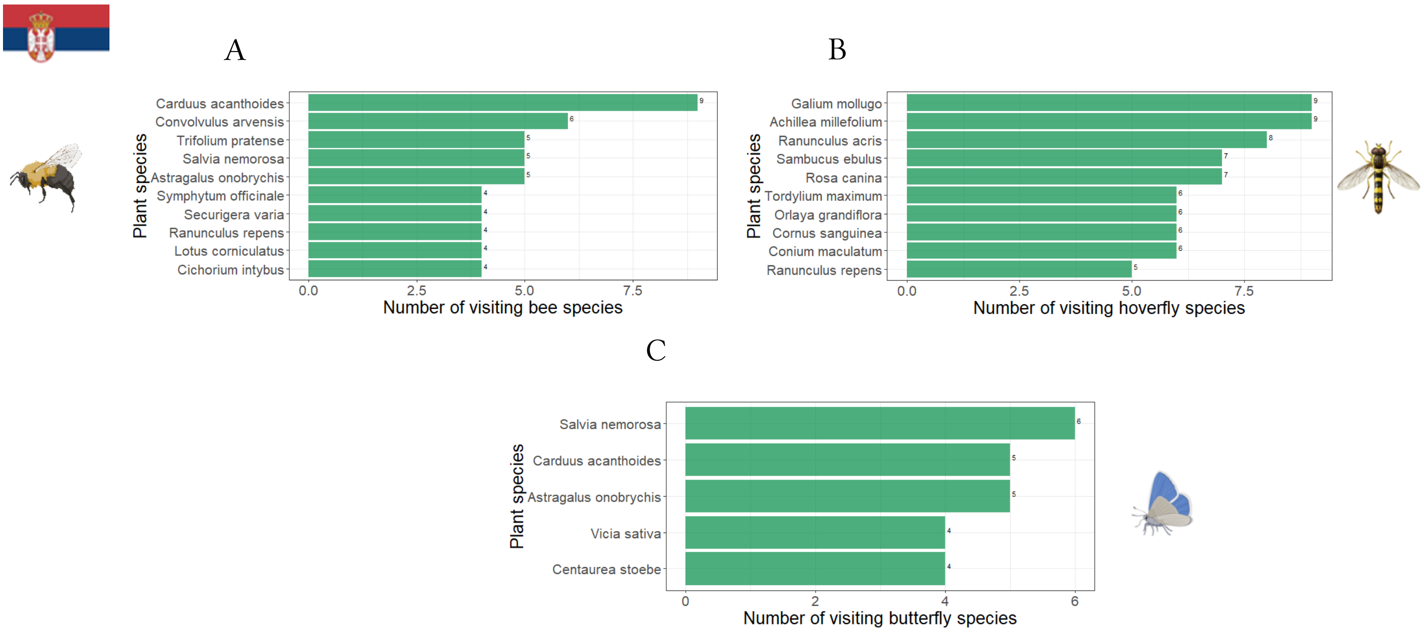

*Figure 20: Top 10 flowers attracting **A:** wild bee species, **B:** hoverfly species, **C:** butterfly species on Serbian roadsides.*

6.3. Relationship between flower-rich roadsides and pollinator mortality by vehicle collisions

6.3.1. Belgium

Figure 21 and Table 6 illustrate the impact of flower species richness, flower abundance, landscape and road type on invertebrate collision numbers in Belgium.

We did not detect a significant effect of flower species richness on roadkill number (p.value = 0.566). The model cannot identify an effect where adding five flower species to the floral community would result in a 73% increase in roadkill number (Estimate = 0.03226, SE = 0.05617).

We did not detect a significant effect of flower abundance on roadkill number (p.value = 0.447). The model cannot identify an effect where adding 1% flower cover would result in a 54% increase in collision number (Estimate = -0.16713, SE = 0.21967).

We did not detect a significant effect of road type on road collisions (p.value = 0.102). The smallest increase detected by the model is 49% (Estimate = 0.33247, SE = 0.20341).

Statistical analysis (p.value = 7.87e-07) confirms that the landscape significantly impacts the roadkill number. It is significantly higher in the agricultural landscape, with an average of 44.2 (SE = 5.65, IC 95% = [34.43, 56.8]) compared to 13.4 (SE = 2.72, IC 95% = [8.98, 19.9]) in the SNH. This means there is around 70% less roadkill in SNH than in the agricultural landscape.

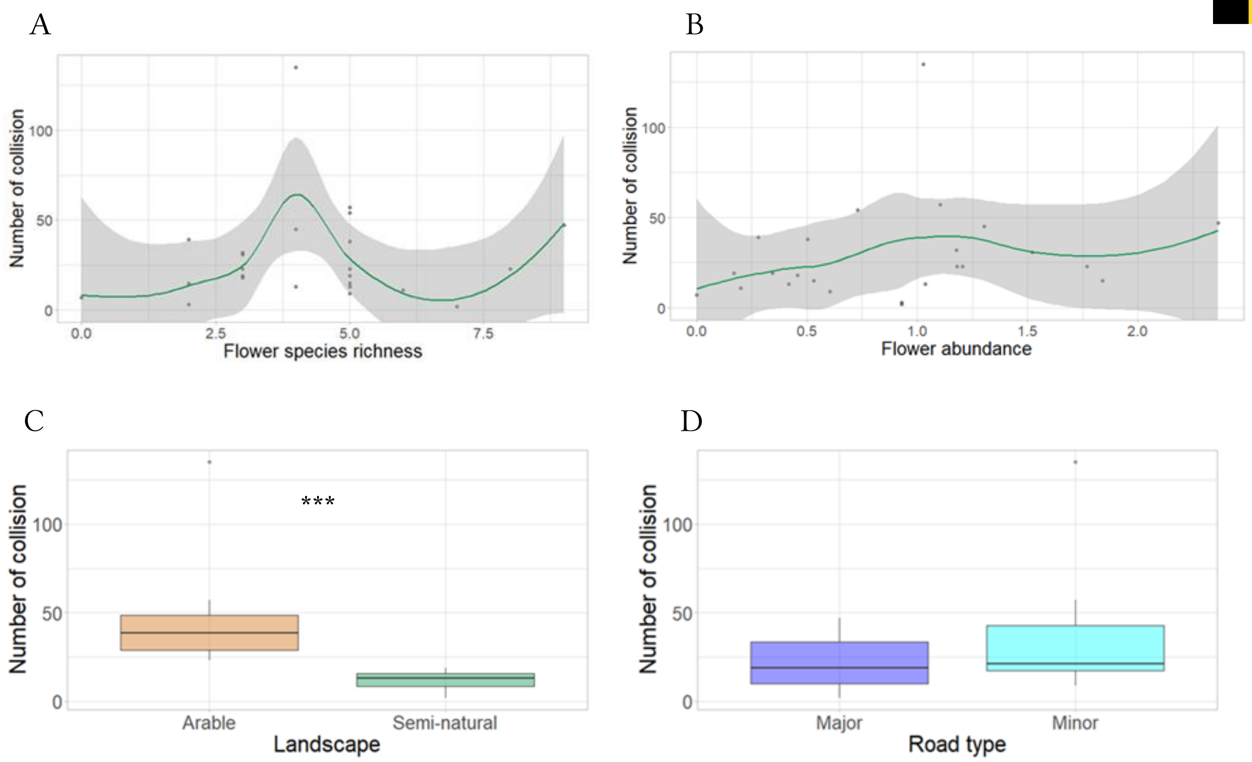

*Figure 21: Impact of **A:** flower species richness (p.value = 0.566), **B:** flower abundance (p.value = 0.447), **C:** landscape (p.value = 7.87e-07), **D:** road type (p.value = 0.102) on invertebrate collision number in Belgium.*

*Table 6 : Summary of the generalized Poisson model of roadkill number in Belgium. It presents the results of the model predicting the roadkill number in Belgium. The predictor variables included in the model are **Flower_N0** : Floral species richness, **SNH** : Type of semi-natural habitat, **Minor** : Type of minor road, and **Flower_ab** : Floral abundance.*

	Estimate	Std. Error	Z value	Pr(>|z|)
(Intercept)	3.63511	0.34657	10.489	< 2e-16
Flower_N0	0.03226	0.05617	0.574	0.566
SNH	-1.19559	0.24209	-4.939	7.87e-07
Minor	0.33247	0.20341	1.634	0.102
Flower_ab	-0.16713	0.21967	-0.761	0.447

6.3.2. Serbia

Figure 22 and Table 7 illustrate the impact of flower species richness, flower abundance, landscape and road type on invertebrate collision numbers in Serbia.

We did not detect a significant effect of flower species richness on roadkill number (p.value = 0.6697). The model is unable to identify an effect where adding five flower species to the floral community would result in a 10% increase in roadkill numbers (Estimate = 0.004115, SE = 0.009649)

We did not detect a significant effect of flower abundance on roadkill number (p.value = 0.9721). The model cannot identify an effect where adding 1% flower cover would result in a 57% increase in collision number (Estimate = -0.008109, SE=0.231501).

We did not detect a significant effect of road type on road collisions (p.value = 0.9509). The smallest increase detected by the model is 45% (Estimate = 0.011727, SE = 0.190341).

Statistical analysis (p.value = 0.0432) confirms that the landscape significantly impacts the roadkill number. It is significantly higher in the agricultural landscape, with an average of 23.3 (SE = 2.62, IC 95% = [18.7, 29.1]) compared to 16.3 (SE = 2.17, IC 95% = [12.5, 21.1]) in the SNH. This means there is around 30% less roadkill in SNH than in the agricultural landscape.

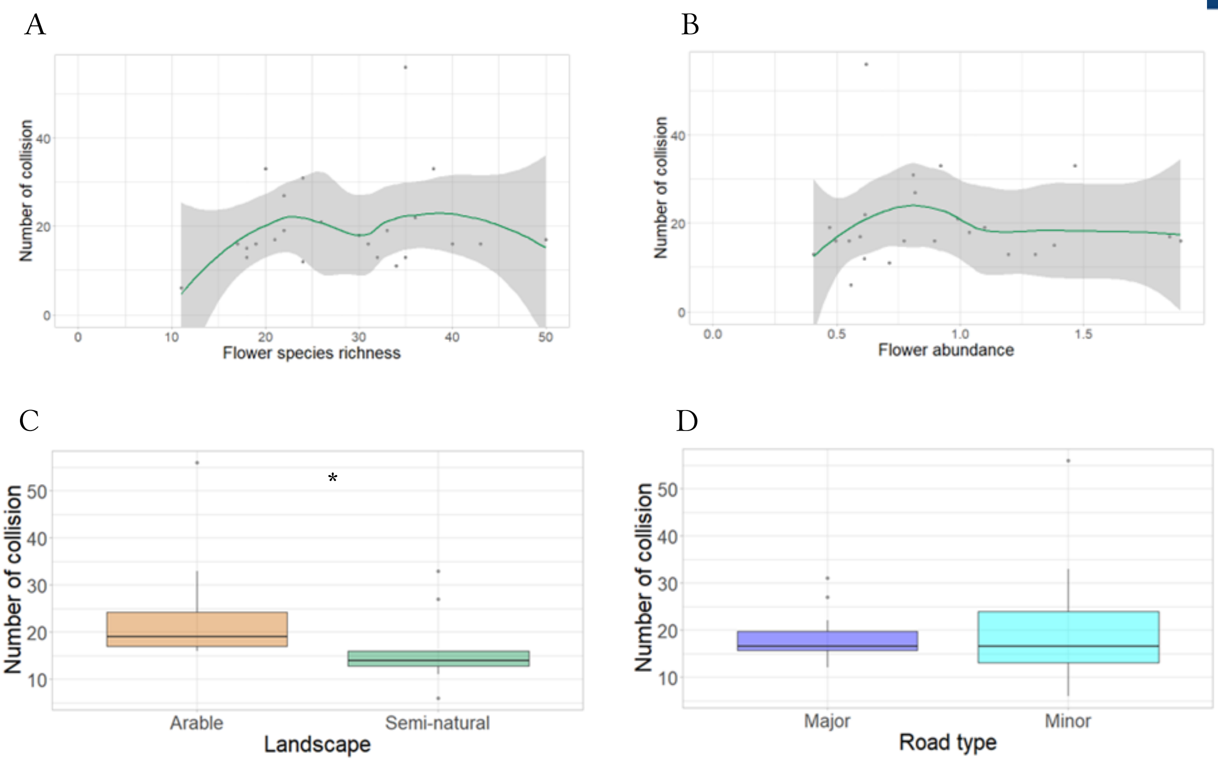

*Figure 22 : Impact of **A:** flower species richness (p.value = 0.6697), **B:** flower abundance (p.value = 0.9721), **C:** landscape (p.value = 0.0432), **D:** road type (p.value = 0.9509) on invertebrate collision number in Serbia*

*Table 7: Summary of the generalized Poisson model of roadkill number in Serbia. It presents the results of the model predicting the roadkill number in Serbia. The predictor variables included in the model are **Flower_N0** : Floral species richness, **SNH** : Type of semi-natural habitat, **Minor** : Type of minor road, and **Flower_ab** : Floral abundance.*

	Estimate	Std. Error	Z value	Pr(>|z|)
(Intercept)	3.034077	0.452153	6.710	1.94e-11
Flower_N0	0.004115	0.009649	0.427	0.6697
SNH	-0.358793	0.177507	-2.021	0.0432
Minor	0.011727	0.190341	0.062	0.9509
Flower_ab	-0.008109	0.231501	-0.035	0.9721

6.4. Impact of vehicle collisions on pollinator communities

As shown in Figure 23, we collected 691 invertebrates on the sticky trap in Belgium, including 387 Thysanoptera, 127 Diptera, 89 Hymenoptera, 29 Hemiptera and 18 Coleoptera. 22 specimens could not be identified. In Serbia, we collected 476 invertebrates, including 136 Hymenoptera, 96 Diptera, 86 Thysanoptera, 58 Hemiptera and 27 Heteroptera. 7 specimens could not be identified.

Table 8 provides data on bees, hoverflies, and butterflies, including their lengths, collected on sticky traps in both Belgium and Serbia. In Belgium, we captured four hoverfly species: *Sphaerosphoria scripta*, *Syritta pipiens*, *Eumerus sp.*, and *Platycheirus albimanus*. In Serbia, the collections included six bee specimens (two *Andrena sp.*, one *Lasioglossum sp.*, and three *Apis mellifera*), four hoverfly specimens (*Euperodes corollae*), and two butterfly specimens (*Polyommatus icarus* and *Maniola jurtina*).

Figure 24 and Table 9 show the distribution of the length of invertebrates killed on the road in Belgium and Serbia. In Belgium, the distribution ranges from a minimum of 1 mm to a maximum of 37.35 mm, with several extreme high values. In Serbia, the distribution ranges from 1 mm to 20 mm, also with many extreme high values. The average length observed was 2.025 mm in Belgium and 2.374 mm in Serbia.

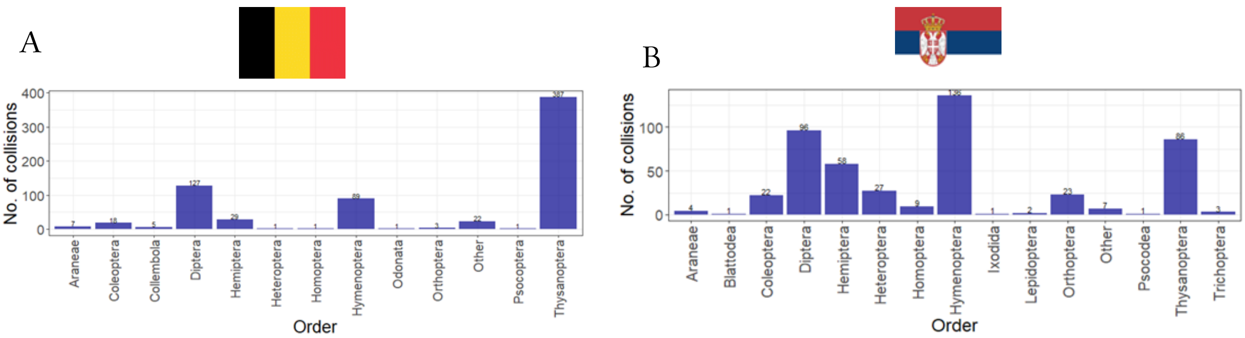

Figure 23 : Number of invertebrates collected on the sticky trap by order in Belgium and Serbia

Table 8: Summary of pollinators collected on the sticky trap in Belgium and Serbia

ID	Country	Species	Length (mm)
1	Belgium	*Sphaerophoria scripta*	10,32
2	Belgium	*Syritta pipiens*	5.99
3	Belgium	*Eumerus sp.*	3.81
4	Belgium	*Platycheirus albimanus*	6.74
5	Serbia	*Andrena sp.*	8
6	Serbia	*Andrena sp.*	10
7	Serbia	*Lasioglossum sp.*	3
8	Serbia	*Apis mellifera*	12
9	Serbia	*Apis mellifera*	13
10	Serbia	*Apis mellifera*	12
11	Serbia	*Eupeodes corollae*	10
12	Serbia	*Eupeodes corollae*	8
13	Serbia	*Eupeodes corollae*	8
14	Serbia	*Eupeodes corollae*	8
15	Serbia	*Polyommatus icarus*	11
16	Serbia	*Maniola jurtina*	15

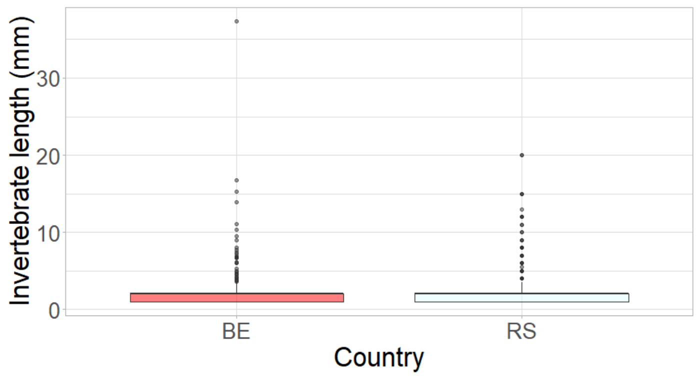

Figure 24: Length of invertebrates collected on sticky trap in Belgium (BE) and Serbia (RS)

Country	Mean	Min	Median	Max	N
BE	2.025	1	2	37.35	691
RS	2.374	1	2	20.00	476

Table 9: Summary of the length of invertebrates collected on the sticky trap by country

7. Discussion

7.1. Relationship of plant diversity and pollinator diversity along roadsides

7.1.1. Bees

Firstly, our study revealed a low diversity of bee species along roadsides in Belgium and Serbia. Indeed, compared to local studies, only 11% of the region's species were present along Belgian roadsides (Fiordaliso et al., 2022). On a national scale, this percentage is even lower, with 5% of all the Belgian bee species and 9% of all the Serbian bee species (Drossart et al., 2019; Mudri-Stojnić et al., 2021). Our results remain poor even if completeness is considered *(cf. Annex 9.7)*. Abundance, which has only been assessed in Belgium, underscores the dominance of specific species such as *Bombus pascuorum*, *Bombus sensus stricto*, and *Bombus lapidarius*, representing 76% of all specimens collected. Interestingly, this pattern aligns with findings from the 2022 Fiordaliso study, where these three species were also identified as the most abundant. This abundance is likely due to these species' eusocial and opportunistic nature, enabling them to adapt to various resources and habitats (Folschweiller et al., 2020). However, it's important to note that net collection over-represents slow and large species (Leclercq et al., 2022; Prendergast et al., 2020).

Considering the limited diversity observed, questions regarding conservation interest in these environments can be raised. Our study highlights an interesting discovery along Serbian roadsides. Indeed, we collected one threatened species with a vulnerable status (Mudri-Stojnić et al., 2021), *Systropha planidens*, on major and minor roads in cultivated fields and semi-natural areas. *Systropha planidens*, a univoltine species with a flight period from June to late July, exhibits oligolectic behavior, specializing in *Convolvulus*, where mating also occurs (Westrich, 2019). No endangered bees have been found in Belgium, although 24 have already been found in the region (Fiordaliso et al., 2022). While the contribution to species conservation from our study is minimal, the example from Serbia is encouraging, suggesting that it is possible to find endangered species along roadsides. Moreover, in Belgium, rare species have already been found along highway verges, such as *Lasioglossum xanthopus* (Pauly & Vautier, 2019).

Let's examine floral diversity and its impact on bee diversity. At first, flower abundance and species richness don't improve bee species richness in Belgium and Serbia. However, these results should be interpreted with caution due to the insufficient power of the model, which is a consequence of the limited number of bee specimens collected. Moreover, the low floral coverage in both countries, which ranged on average from 0% to 2.4% in Belgium and from 0.4% to 1.8% in Serbia, may probably not have a wide enough range to measure the impact of floral abundance on bee species richness. Regarding the literature, the study by Hopwood et al. (2008) found that increasing the species richness and abundance of native flowers increases bee species richness. They focused on the diversity of wild bees along restored roadsides, which have been reseeded with native herbaceous plants and mown every 2 to 4 years, compared to weedy roadsides dominated by non-native grasses and with over 50% non-native herbaceous plant cover. These findings align with Potts (2003), who reported a positive correlation between floral diversity and bee species richness. Most bees in our samples were polylectic, capable of exploiting a wide range of flowers, suggesting they can thrive even with lower floral diversity. However, in Serbia, some species exhibit more specialized ecologies.

For example, *Andrena taraxaci* feeds on Asteraceae, with known pollen sources including dandelion (*Taraxacum officinale*) and coltsfoot (*Tussilago farfara*). *Andrena viridescens* is strictly oligolectic, specializing on *Veronica* species. Additionally, *Eucera nigrescens* specializes in Fabaceae, while *Rophites quinquespinosus* specializes in Lamiaceae. An interesting ecological behavior is observed in *Osmia bicolor*, also collected in Serbia, which nests in snail shells.

One element reinforcing the low specific richness of bees in Belgium is the devastating effect of the type of road. This impact is so significant that it remains observable despite the model's low power. Few studies have examined the impact of traffic volume on pollinators along roadside verges. In their review, Phillips et al. (2020) highlight that this question remains unresolved. However, passing cars generate turbulence along the roadsides. Higher traffic densities create more turbulence, making it more difficult for pollinators to feed (Fitch & Vaidya, 2021; Phillips et al., 2019). This could explain our results of a significantly negative impact of major roads on the species richness of bees in Belgium. The results for Serbia contrast with the lack of observed effects from traffic volume on the number of bee species. This difference may be linked to the density of the road network and road use. In Belgium, the busiest road in the study had an average of 179.9 vehicles per 15 minutes, whereas in Serbia, it had only 22.4 vehicles. The major roads in Serbia likely cause less turbulence and do not disturb the feeding behavior of bees as much as Belgian roads. In addition, lower road density means natural and semi-natural habitats are less fragmented. This allows bees to benefit from larger, more continuous areas for feeding and reproduction, reducing the potential impact of roads on their behavior and diversity. However, these hypotheses should be taken cautiously because the Serbian model lacks power (the smallest increase detected by the model is 80%).

7.1.2. Hoverflies

Our first striking finding concerning hoverflies is that diversity is low along roadsides. Our Belgian result represents 10% of all the Flemish hoverfly species, and our Serbian result is 3% of all the Balkan Peninsula hoverfly species (Van de Meutter et al., 2021). Although a few species are still to be discovered, the species richness estimates were reasonable, suggesting that most species have been found *(cf. Annex 9.7.1)*. The three most dominant species in Belgium are *Episyrphus balteatus*, *Sphaerosphoria scripta*, and *Eristalis pertinax (cf. Annex 9.7.1)*. The first two were mainly found along roadsides in agricultural areas. *Episyrphus balteatus* is an opportunistic species, typically abundant during the summer months, corresponding to most of our sampling dates. During this period, the species exhibits its 2nd generation flight, as documented by Van Veen (2010), and resident populations supplement the migrating populations(Ball & Morris, 2015). Like *Sphaerosphoria scripta*, its larvae feed on aphids, which probably explains the presence of these two species in agricultural areas. *Eristalis pertinax* flies from March to November (Schulten, 2019). The larvae are aquatic and tolerant to eutrophic waters (Van Veen, 2010). Thus, they are generally found in humid environments (Van Veen, 2010). This aligns with our findings, where most *Eristalis pertinax* were located along roadsides in semi-natural environments. Unfortunately, like bees, we cannot study the abundance of hoverflies in Serbia because the sampling was non-lethal.

From a conservation point of view, we found three threatened species along Belgian roads: *Meliscaeva cinctella*, *Platycheirus peltatus* and *Cheilosia illustrata*, all of which have vulnerable status on the red list of hoverfly species in Flanders (Van de Meutter et al., 2021). *Meliscaeva cinctella* is a forest species (Ball & Morris, 2015; Schulten, 2019) and was found along forest roads, including areas of great biological interest. *Cheilosia illustrata* is often found at forest edges associated with hogweed, where the larvae exploit the roots and stems (Ball & Morris, 2015). We suppose that roads help to create this edge effect, bringing light into the forest (Hanula et al., 2016 in Phillips et al., 2020). The presence of *Platycheirus peltatus* along agricultural roads is consistent with its larvae's aphidophagous diet (Ball & Morris, 2015). Unfortunately, Serbia does not have an IUCN status, and we cannot discuss it.

To answer our biological question, let's look at the impact of floral diversity on the species richness of hoverflies. In Serbia, flower species richness significantly impacts the hoverfly species richness. This impact translates to a 10% increase in hoverfly species for every five additional flower species. Unfortunately, we haven't found any articles concerning the influence of floral diversity on the species richness of hoverflies along roadsides. Nonetheless, our results align with Phillips' (2020) review, which studied pollinators along road verges. It indicates that roadsides with a high flower species richness increase the species richness of pollinators along roads. In Belgium, flower species richness doesn't seem to improve hoverfly species richness. However, this result must be taken cautiously because the model cannot identify an effect where adding five flower species to the floral community would result in a 60% increase hoverfly richness. Furthermore, the floral species richness in Belgium exhibits a narrower range, from 0 to 9 species, compared to Serbia's broader range of 11 to 50 species. It suggests that the floral species richness in our Belgian sites might not cover a wide enough range to measure their effect on hoverfly species richness. Concerning flower abundance, our results suggest that flower cover does not significantly impact the species richness of Belgian and Serbian hoverflies. However, this result needs to be taken with caution because the model cannot identify an effect where adding 1% flower cover would result in a 43% and 35% increase in hoverfly richness in Belgium and Serbia, respectively, and because of the low flower cover mentioned above *(cf. Section 7.1.1)*. Moreover, these findings contradict existing literature, notably Phillips (2020), which underscores the positive correlation between roadside flower abundance and pollinator diversity.

Another exciting result of our study was the impact of road type on Belgian hoverflies, with species numbers increasing by 76% along minor roads. As described above with bees, these results can be explained by the fact that feeding behavior is disturbed by passing cars, which generate turbulences, which are more pronounced along major roads *(cf. Section 7.1.1)*. The results for Serbia contrast with the lack of observed effects from traffic volume on the number of hoverfly species. We also put forward the same theory as bees, linked to the different road traffic density and use in Belgium and Serbia *(cf. Section 7.1.1)*.

Our results indicate that landscape significantly impacts the species richness of Belgian and Serbian hoverflies. In Belgium, the effect is high, with the number of hoverfly species doubling in semi-natural areas. In Serbia, the effect is smaller, with a 27% increase in species in semi-natural habitats. Forest landscapes are favorable places for many species of hoverflies to overwinter and develop their larvae (Branquart et al., 2000 in Meyer et al., 2009). Many hoverfly species find numerous microhabitats in the forest, such as decaying wood, small ponds between branches, sap runs as well as aphids in deciduous and coniferous trees (Speight et al., 2006 in Meyer et al., 2009).

7.1.3. Butterflies

Along the Belgian roadsides, we found 24% of all the Wallonia butterfly species and along the Serbian roadsides, we found 20% of all the Serbian butterfly species (Fichefet, 2008; Popović et al., 2017). Among them, we collected two threatened species in Belgium and one threatened species in Serbia. *Speyeria aglaja*, which has an endangered status, and *Iphiclides podalirius*, which has a vulnerable status were collected in Belgium (Fichefet, 2008). *Speyeria aglaja* was found at one site in Fagne Famenne region. This species is associated with forest edges and is associated with violets, where the female lays her eggs (Fichefet et al., 2019b). The individual noted was indeed found in a forest environment. *Iphiclides podalirius* was collected along a road in an agricultural area. As for it, the female lays her eggs on *Prunus* (Fichefet et al., 2019a). In Serbia, we collected a species with vulnerable status, *Neozephyrus quercus*, which, as its name indicates, is associated with oak (*Quercus*). Indeed, adults seem to feed strictly on aphid honeydew collected on oak leaves, neglecting plant nectar, carrion, excrement, and moist soil (Tolman & Lewington, 2015).

Regarding floral diversity and its impact on butterfly diversity, our results show that the species richness of flowers in Serbia impacts the specific richness of Serbian butterflies, with an effect of 23% more species when the number of flower species increases by 5. This aligns with Ries' (2001) and Skórka's (2018) studies, which indicate that roadsides with a high flower species richness increase the species richness of butterflies along roads. In Belgium, flower species richness doesn't seem to improve butterfly species richness. However, this result must be taken cautiously because the model cannot identify an effect where adding five flower species to the floral community would result in a 55% increase in butterfly species richness. Moreover, and as mentioned before, the low number of flower species in Belgium may not have a wide enough range to measure their effect on butterfly species richness. Concerning flower abundance, despite its low cover, our Belgian results show a positive impact of floral cover on the number of butterfly species, with an increase of 55% of butterfly species with a rise of 1% in flower cover. This finding is consistent with the literature, as Saarinen et al. (2005) suggest, that a high abundance of nectar strongly correlates with butterflies' species richness along roadsides. The Serbian result indicates that flower cover does not significantly impact the butterfly species' richness. However, this result needs to be taken with caution because the model cannot identify an effect where adding 1% flower cover would result in a 50% increase in butterfly species richness and because of the low flower cover mentioned above *(cf. Section 7.1.1)*.

The impact of roads on species richness is again evident in Belgium, with a 46% increase in butterfly species along minor roads compared to major roads. Here again, we return to the theory expressed earlier with bees and hoverflies about feeding behavior disturbed by passing cars, which create turbulence, all the greater on major roads *(cf. Section 7.1.1)*. The results for Serbia contrast with no observed effects from traffic volume on the number of butterfly species. We also proposed the same theory as bees and hoverflies, linked to the different road traffic densities and use in Belgium and Serbia *(cf. Section 7.1.1)*.

In addition, our results indicate that landscape significantly impacts the species richness of Belgian butterflies, with the number of butterfly species doubling in semi-natural areas. Saarinen et al. (2005) studied the diversity of butterflies along roads in Finland and found that the landscape surrounding the roadside affected butterflies' abundance and species richness. In agricultural areas, butterfly abundance was lower, whereas in forest landscapes, species richness increased (Saarinen et al., 2005). Similarly, Berg et al. (2011) found greater butterfly density and species richness in forested roadside areas. Although we do not have abundance data for butterflies and cannot discuss it, our Belgian results align with the literature, showing butterfly species richness increases in a landscape with a strong forest component. The results for Serbia contrast with the absence of observed effects from the landscape on the number of butterfly species. It could potentially be attributed to the forestry component of the semi-natural sites in Serbia. Unlike Belgium, where the forest component of semi-natural sites was dominant, this was not the case in Serbia.

7.2. Attractiveness of flowers for pollinators along roadsides

Our results indicate that bees, hoverflies, and butterflies show distinct preferences for specific roadside flowers, with variations among the three groups.

In Belgium, bees predominantly visited *Cirsium arvense*, a finding consistent with Pywell (2005), who also recorded a high proportion of bumblebee visits to this species (Pywell et al., 2005). Thistles (*Cirsium spp.* and *Carduus spp.*), ranking highest for both Belgian and Serbian bees, are crucial food plants for wild bees (Westrich, 2019). These plants attract long-tongued bees for nectar and short-tongued bees for pollen. For example, bumblebees, particularly males, are regular visitors to thistles, which provide essential nectar resources in late summer when males require significant energy for courtship behavior (Vray et al., 2017). *Cirsium arvense* supports both generalist bees, such as *Lasioglossum pauxillum* and *Halictus rubicundus*, and oligolectic bees, including *Osmia leaiana*, *Osmia niveata*, and *Osmia spinulosa*, present in Belgium territory (Drossart et al., 2019; Westrich, 2019). *Carduus acanthoides* also supports both generalist bees, such as *Lasioglossum pauxillum* and *Osmia aurulenta*, and oligolectic bees, including *Eucera dentata* present in Serbia (Mudri-Stojnić et al., 2021). Despite their ecological importance, thistles are often considered « weeds » by the general public and within agricultural landscapes. In several European countries, legislative rules mandate their eradication. We, therefore, support Vray et al.'s (2017) call for the abolition of these laws and advocate for finding alternatives that balance agricultural requirements with biodiversity conservation. The second most visited plant by bees in Belgium is *Rubus fruticosus*, commonly known as blackberry; it also ranks first in attracting most bee species. In his book, Westrich (2019) observed numerous generalist bee species collecting pollen from *Rubus fruticosus*, including *Andrena bicolor*, *Andrena minutula*, *Halictus rubicundus*, and *Lasioglossum calceatum*, species also noted in our study in Belgium. Moreover, *Rubus fruticosus* can serve as nesting sites for cavity-nesting species that excavate their nests within the stem, such as *Megachile versicolor*, *Megachile centuncularis*, and *Heriades truncorum*, species present in the Belgian territory (Drossart et al., 2019; Westrich, 2019). The third most visited plant in Belgium in terms of abundance is *Eupatorium canabinum*, which is surprising and not referenced in Westrich's book. However, when we examine the species of bees visiting this flower, we find *Bombus sensus stricto* and *Bombus pascuorum*, notably polylectic groups (Folschweiller et al., 2020). *Centaurea* is considered as crucial as thistles for wild bees and ranks fourth in Belgium for attracting bee specimens in Belgium (Westrich, 2019). They serve as valuable nectar and pollen sources for many bee species (Vray et al., 2017; Westrich, 2019). In addition, in England, Wood's (2017) study examined the foraging behavior of bees and the pollen contribution of sown flowers from an agri-environmental seed mix. The study indicates that *Centaurea nigra* is among the most important sown flowers for solitary bees (Wood et al., 2017). Common in fields and observed in our study, *Convolvulus* is among the top 3 in both countries for attracting the highest number of bee species. Wild bees often use these flowers for sleeping, but they are also a valuable source of nectar and pollen (Westrich, 2019). Besides bumblebees, species of *Halictus* and *Lasioglossum* collect pollen from *Convolvulus*. Indeed, we found two species of Halictidae in these flowers in Belgium: *Lasioglossum calceatum* and *Lasioglossum pauxillum*. *Convolvulus* can also attract oligolectic species. In Serbia, we collected *Systropha planidens* on *Convolvulus arvensis*, a strictly oligolectic species of the *Convolvulus* genus. Given that this species is classified as vulnerable on the Serbian red list (Mudri-Stojnić et al., 2021). *Trifolium pratense* and *Lotus corniculatus* are among the most visited sown flowers by bumblebees on farmland, according to Wood's (2015) study. These Serbia's two plants are in the top 10 for attracting the most bee species. *Trifolium pratense* exclusively attracted bumblebees, including *Bombus pascuorum*, *Bombus sensus stricto*, *Bombus haematurus*, *Bombus ruderatus*, and *Bombus lapidarius*. While *Heracleum sphondylium* may rank lower among the top 10 visited plants in Belgium and isn't featured in Serbia, its significance is underscored by Wood et al. (2015). It is classified as one of the preferred wild plants for most bee species in England's farmland (Wood et al., 2015).

In their book, Stuart Ball and Roger Morris (2015) indicate that most hoverflies do not have specialized mouthparts and prefer easy access to nectar and pollen via umbellifers such as hogweeds and flowers of the family of Asteraceae, *Cirsium spp.* and *Carduus spp.*, and *Centaurea spp.*. This aligns with our results in Belgium and Serbia. Moreover, hoverflies tend to visit flowers of white and yellow colors more frequently (Ball & Morris, 2015; Haslett, 1989). Interestingly, most of our study's flowers also manifest these color traits. Recent research focusing on the subfamily-level preferences within Syrphidae indicates that the affinity for white flowers is particularly pronounced among Eristalinae, whereas Syrphinae displays lower selectivity regarding flower color (Klecka et al., 2018). This could explain the presence of *Cirsium arvense* in the top 3 in the list, attracting a lot of hoverfly specimens and species. Furthermore, thistles serve as a crucial food source for the larvae of certain phytophagous hoverflies such as *Cheilosia grossa* and *Cheilosia albipila*, which are present in Belgium territory (Ball & Morris, 2015; Van de Meutter et al., 2021). These hoverfly adults lay their eggs on thistles, and the larvae subsequently develop within the stems. Moreover, Ball et al. (2015) recommend a selection of plants to attract hoverflies, including *Achillea spp.* and *Cornus spp.*. Interestingly, our study in Serbia identified these two plants as the most ten attractive to syrphid species, with 9 and 6 species respectively. *Achillea millefolium* also emerged as a prominent feeding resource for syrphids in Belgium. The presence of *Rubus fruticosus* and *Eupatorium cannabinum* in the top 10 in Belgium resonates with Ball et al.'s (2015) suggestion to plant *Rubus spp.* and *Eupatorium spp.*. In addition to thistles, which serve as food for the larvae of certain *Cheilosia* species, bulbous plants, particularly daffodils, provide sustenance for *Merodon* and *Eumerus* species (Ball & Morris, 2015).

Adult butterflies do not feed exclusively on flowers and expand their diet with wet sand, fruit, mud, dung, or carrion by sucking up the liquid (Bonebrake et al., 2010; Hardy et al., 2007). Lack of floral diversity along roadsides and this behavioral plasticity could explain their low presence in flowers in our study, prompting us to present the top 5 rather than the top 10 preferred floral species. Stefanescu et al. (2009) published the results of a long-term study of the flowers most visited by adult butterflies in Spain, highlighting the generalist foraging behavior exhibited by most butterfly species. However, they noted that some families, Lycaenidae and Nymphalidae, have more specialists. For instance, the adult *Celastrina argiolus* is listed as a specialist, showing a preference for *Lytrhum salicaria*. Interestingly, our Belgian study aligns with these findings, as we observed *Celastrina argiolus* feeding on *Lytrhum salicaria*. Furthermore, our research identified *Cirsium arvense* as the plant attracting the highest number of butterfly species in Belgium. Notably, *Cirsium arvense* also plays a crucial role for specialist species like adult *Thymelicus acteon*, an endangered species listed on the Walloon Red List of endangered butterflies (Fichefet, 2008; Stefanescu & Traveset, 2009).

Although we collected a few pollinator species along the roads, and their feeding preferences may not be representative of the entire community of bees, hoverflies, and butterflies, we feel that the thistle's position at number one is legitimate, as it is discussed in the literature (Vray et al., 2017; Westrich, 2019). Moreover, special attention should be given to the red-listed species identified in our study. In Belgium, these include *Speyeria aglaja* and *Iphiclides podalirius*, whose larvae depend respectively on host plants such as violets and *Prunus*, as well as *Cheilosia illustrata*, which the larvae exploit the roots and stems of hogweed. In Serbia, *Systropha planidens* and *Neozephyrus quercus* in the red-listed species require *Convolvulus arvensi*s and *Quercus*, respectively. In their book, Terzo et al. (2014) emphasize plant species of significant food value for pollinators in meadows, collectively spanning the entire flight period. We also recommend incorporating these species along roadsides: *Bellis perennis*, *Trifolium spp.*, *Lotus corniculatus*, *Geranium spp.*, *Malva sylvestris*, Ranunculus *spp.*, *Daucus carota*, and *Achillea millefolium* (Terzo & Vereecken, 2014).

In their review, Philips and colleagues (2020) provide indications for maintaining road verges for insect pollinator density and richness. Firstly, they report to create high-quality habitats along the roadside. This involves having high plant species richness, high flower species richness and abundance. We suggest including the plant species listed above. Philips and colleagues highlight that the creation of high-quality habitat also requires the management of invasive plant species. Secondly, they recommend limiting the mowing to 0 to 2 cuts per year, employing the mosaic mowing technique. For instance, implementing two cuts per year near the road while refraining from mowing in adjacent habitats. Additionally, they advise to avoid mowing during the period between spring and late summer when pollinators are most active (Phillips et al., 2020; Skórka et al., 2013). Another article proposes the removal of mowing residues as a strategy to mitigate the dominance of Poaceae over dicotyledons, which represent a more important food source for pollinators (François & Le Féon, 2017). Grass management could also be achieved by *Rhinanthus*, a parasitic plant that feeds pollinators and reduces the dominance of Poaceae (Bullock & Pywell, 2005). As mentioned in the introduction *(cf. Section 4.5.1)*, some Walloon roadsides are mown late using the mosaic principle. This practice is implemented in 226 municipalities, covering 3,600 hectares, or 20% of the road network (BdM, 2023). We strongly encourage the authorities to extend the late mowing network to support pollinator populations further and enhance roadside habitat

7.3. Relationship between flower-rich roadsides and pollinator mortality by vehicle collisions

Our results regarding our second biological question suggest that rich-floral road verges do not act as honeytraps for insects because flower abundance and species richness don't improve insect collisions in Belgium and Serbia. However, these results should be interpreted with caution due to model precision. As described above, the low flower species richness (Belgium) and the low floral abundance may be limiting factors *(cf. Section 7.1.1)*. Furthermore, our results diverge from existing literature suggesting that high-quality roadside habitats reduce the number of pollinators killed (Dániel-Ferreira et al., 2022; Polic et al., 2014; Ries et al., 2001; Skórka et al., 2013). For instance, the study by Skorka et al. (2013) indicates that increasing the flower species richness on roadsides reduces the number of butterflies killed. Similarly, Ries et al. (2001) demonstrate that roadsides abundant in flowers have lower butterfly mortality rates compared to rich-grassy roadsides. The hypothesis proposed is that pollinators remain on the flower-rich side of the road when sufficient floral resources are available.

Surprisingly, our statistical models suggest that the type of road does not impact the number of insects killed in either Belgium or Serbia. However, these results should be interpreted with caution due to the model's lack of power. Moreover, our findings contradict most articles reviewed, which found a strong positive correlation between traffic volume and insect mortality (Prasad Rao & Saptha Girish, 2007; Skórka et al., 2013; Soluk et al., 2011).

In addition, our results indicate that the landscape significantly impacts the number of roadkill in Belgium and Serbia, with fewer insects killed in semi-natural areas. In Belgium, there is 70% less roadkill in SNH than in the agricultural landscape. The effect is lower in Serbia, with 30% less roadkill in semi-natural landscapes than in arable areas. Keilsonhn et al. (2017) investigated the impact of roadside habitat on insect mortality in Pennsylvania, comparing forest, grassland, and meadow roads. Their method involved collecting dead insects from the road. They found that the mortality rate of insects killed by vehicles is significantly lower when roads are surrounded by forests than when meadows and lawns surround them (Keilsohn et al., 2018). Although our study focused on agricultural environments rather than meadows and lawns, their hypothesis is interesting. They suggest fewer insects are killed in forests because fewer diurnal insects are active in these areas. This hypothesis could be relevant to our findings in Belgium and Serbia in landscapes with a strong forest component. Additionally, even though we highlighted the adverse effects of intensive monoculture agriculture in the introduction; Croxton et al. (2022) show that this environment can be hospitable to pollinators (Croxton et al., 2002). Finally, our results may also be influenced by the large number of Thysanoptera collected along field roads, as their larvae are phytophagous on most cultivated plants.

7.4. Impact of vehicle collisions on pollinator communities

Let's first discuss the invertebrates collected. One of the first striking results of our study is the large number of Thysanoptera collected, mainly in Belgium, accounting for more than half of the specimens collected, mainly in agricultural areas in July. Thysanoptera are known for mass flights at certain times of the year. These flights occur on days with above-average pressure, sunshine and temperature, and below-average rainfall (Lewis, 1964). Since most Thysanoptera were collected in July, we believe our vehicle hit mass thrips flights several times this month. The following two largest groups are the orders Diptera (19%) and Hymenoptera (13%). The top three orders in Serbia are the same but appear in a different ranking, with 20% of Diptera, 29% of Hymenoptera, and 18% of Thysanoptera. Diptera and Hymenoptera are among the top 3 insects killed on the road in the study by Martin et al. (2018) which investigated the number of insects killed by vehicles in Canada using a methodology similar to ours, employing sticky traps on cars. However, Diptera were numerous, accounting for 77% of their sample. In addition, Hymenoptera and Diptera are also well-represented in other studies collecting dead insects on the roadside (Baxter-Gilbert et al., 2015; Keilsohn et al., 2018).

To answer our last biological question, it seems that pollinator communities are little affected by vehicle collisions. Indeed, few bees, hoverflies and butterflies have collided with vehicles, perhaps reflecting the poor diversity along the roadside. In Belgium, they represent 0.006% of specimens collected, and in Serbia, 0.025%. We didn't collect any bees in Belgium, while we collected six bees in Serbia: three honeybees, *Apis mellifera*, *one Lasioglossum sp.*, and two *Andrena sp*. Unsurprisingly, no bumblebees were collected in either country. This aligns with the findings of Bhattacharya et al. (2003), who marked and captured bumblebees along roads and observed that they never cross roads unless displaced and forced to feed at alternative sites. We were surprised by the absence of butterfly specimens collected in Belgium, and only two in Serbia. In the literature, butterflies are a group that is studied along roadsides. The typical collection method involves picking up dead specimens directly from the roads (Ries et al., 2001; Skórka et al., 2013). In contrast to our results, butterflies ranked in the top three orders in studies by Keilsohn et al. (2018) and Baxter-Gilbert et al. (2015), which used the method of collecting dead insects on the roadside. However, using their collection methodology, they also collected moths that were not part of our study. The question of nocturnal insects will be addressed in the outlook *(cf. 7.5 Conclusion and outlooks)*. Regarding hoverflies, we collected four species in Belgium: *Platycheirus albimanus*, *Sphaerophoria scripta*, *Syritta pipiens*, and *Eumerus* sp. In Serbia, they collected four hoverflies, but all belonged to the species *Eupeodes corollae*. Interestingly, two species, *Sphaerophoria scripta* and *Eupeodes corollae*, are migratory (Ball & Morris, 2015). We might think that nomadic species, which are more mobile and travel longer distances, tend to be more killed on roads than sedentary, less mobile species. This hypothesis was verified in a study on butterflies (De la Puente et al. 2008 in Muñoz et al., 2015) but not in any other (Skórka et al., 2013).

The size of the specimens also affects the collision risk. The size of the bees, hoverflies, and butterflies is larger than the majority of the insects collected which may explain their low number. Our results for both Belgium and Serbia align with existing literature, indicating that vehicle collisions kill mostly smaller insects. For instance, Martin et al. (2018) studied insect mortality due to vehicles in Canada using a methodology similar to ours, employing sticky traps on cars. Their findings showed that 96.8% of the insects in their sample were smaller than 5 mm. Our findings are comparable, with 96.8% of the insects killed in Belgium and 92.3% in Serbia being smaller than 5 mm. Additionally, a study conducted in Poland on butterflies collected dead on roads also showed an over-representation of the smallest species (Skórka et al., 2013). While we did not capture any butterflies in Belgium, we collected *Polyommatus icarus* and *Maniola jurtina* in Serbia, with lengths of 11 and 15 mm, respectively. The higher mortality rate of smaller insects can be attributed to their flight height. Smaller insects fly close to the asphalt at lower altitudes, increasing their likelihood of colliding with vehicles. In contrast, larger species typically cross roads at higher altitudes, often above the height of cars (Fitch & Vaidya, 2021; Phillips et al., 2020; Skórka et al., 2013).

More generally, one question remains: Would the insects collected during the car experiment really have hit and died without the sticky trap? We frequently observed still-alive insects when removing the sticky trap from the car. This observation leads us to consider that collisions with vehicles may not be lethal but possibly sublethal, likely depending on the vehicle's speed. In the outlook section, we will discuss alternative sampling methods to address this issue *(cf. 7.5 Conclusion and outlooks)*.

7.5. Conclusion and outlooks

In conclusion, our study highlights the low diversity of pollinators along roadsides. Despite this, we demonstrate that the abundance of flowers along roadsides increases species richness among Belgian butterflies, while flower specific richness positively impacts both hoverfly and butterfly species richness in Serbia. These flowers, however, do not attract the three main groups of pollinators equally. *Cirsium arvense* and *Carduus acanthoides* are particularly beneficial, supporting the greatest diversity of bees in Belgium and Serbia and being the favorite plants of Belgian hoverflies and butterflies. An interesting finding is that road type positively impacts the bee, hoverfly and butterfly species richness in Belgium, a pattern not observed in Serbia, likely due to the lower density and usage of the Serbian road network.

Regarding vehicle collisions, the insect orders most frequently killed by vehicles in Belgium and Serbia are Thysanoptera, Hymenoptera, and Diptera. Within the primary pollinator groups, bees, hoverflies, and butterflies were few killed by vehicle collisions, whereas smaller insects were more dominant. Finally, we have not identified floral roadsides as honey traps for pollinators in the broad sense.

Looking forward, to addressing the sampling bias toward slow and large species and collecting the entire bee community, many studies suggest combining passive and active collection methods (European Commission, Joint Research Centre, 2021; Krahner et al., 2021; Montgomery et al., 2021; Schindler et al., 2013; Templ et al., 2019; Westphal et al., 2008 in Klaus et al., 2024). We recommend conducting a new inventory along roadsides using a combination of pan traps, passive method, and net sampling, active method. Instead of linear transects, the net method should focus on micro-habitats likely to attract bees, such as potential nesting sites and flower strips.

Our research focused on the direct mortality of insects caused by the road. However, an important question that should be addressed is its barrier effect. Roads fragment habitats, preventing the movement of animals that avoid crossing them or are killed crossing them. To study this barrier effect, we could imagine capturing and marking insects along roads to study their behavior and analyze road crossings. Although this methodology has been utilized in other countries, its application remains unexplored in Belgium and Serbia along roads. In addition, we could conduct a population genetics study by sampling insects from various habitat fragments separated by roads.

To extend our research, we propose including nocturnal insects and investigating their diversity along roadsides, utilizing methods such as setting up window traps overnight on roadside verges. Additionally, conducting the car experiment during nighttime hours would allow us to assess the insects most susceptible to collision and we could compare the results with our study. Moreover, considering the allure of street lighting along roadsides to nocturnal insects, studying its impact could offer valuable insights. Further, we suggest analyzing the behavior of these nocturnal insects by capturing and tagging them to observe their road-crossing frequency, facilitating a comparative analysis with daytime insect behavior.

In our study, we found that many small insects were hit by cars. It would be interesting to investigate whether these small insects are more vulnerable to collision risk. To determine their vulnerability, we propose studying the entire insect community on roads and measuring their traits using sampling methods such as malaise traps. By comparing these results with our existing data, we could assess whether small insects are indeed more susceptible to collision risks.

Finally, to answer the question of the lethal effect of collisions on insects, we suggest conducting laboratory studies. Through these experiments, we will subject insects of diverse sizes to objects moving at varying speeds, aiming to determine the point of lethality. Furthermore, we aim to evaluate any sublethal effects ensuing from collisions that do not immediately result in lethality.

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9. Annex

9.1. Poem « Le vieux canal » by André Lescot

Je le revois si bien dans ma mémoire
Ce beau canal près de qui je suis né ;
C'est Napoléon - quel titre de gloire ! -
Qui lui fit relier Mons à Condé.

Son profil est net, sans un seul méandre.
Sur les rives, des platanes géants
Lui ont conféré cet aspect si tendre
Des plus beaux tableaux des peintres d'antan.

Lentement, glissant sur l'eau profonde
Qu'ils enflent aux abords des portails
De longs bateaux passent en faisant des ondes
Et de gros remous près du gouvernail.

D'autres, légers, avec les flancs vides,
Portant le front haut arrivent, hagards,
Et surprennent un peu tant ils sont rapides,
Ils ont des allures de malabars.

Ils se nomment La Seine ou l'Indomptable,
La Méduse, Quimper, Guadalcanal,
Ils s'affairent comme de beaux diables
Tout près des ponts et des chantiers navals.

Les jets d'eau ont des bruits de lessive
Près de l'écluse aux pertuis ruisselants ;
Et tout contre les portes massives
L'amont conspire en longs bouillonnements.

Les bateliers manoeuvrent, alertes,
A petits pas pressés sur les plats-bords,
Jettent leurs cordes par-dessus l'eau verte
Et le chaland tresaille sous l'effort.

De quel pays, de quels lointains rivages
Viennent-ils donc ? Ces vaillants voyageurs,
Dont les embruns ont tanné le visage,
Ont des tatouages en forme de cœur.

Fiers messagers, coursiers infatigables,
Comme ils paraissent emporter avec eux
D'autres coins de terre, des bancs de sable
De l'air marin et des morceaux de cieux !

On ne le verra plus, bientôt une route
Passera en ces lieux. Ô sort fatal !
Tout cela prendra fin, mais je doute
Que je puisse oublier mon beau canal !

9.2. European transport networks

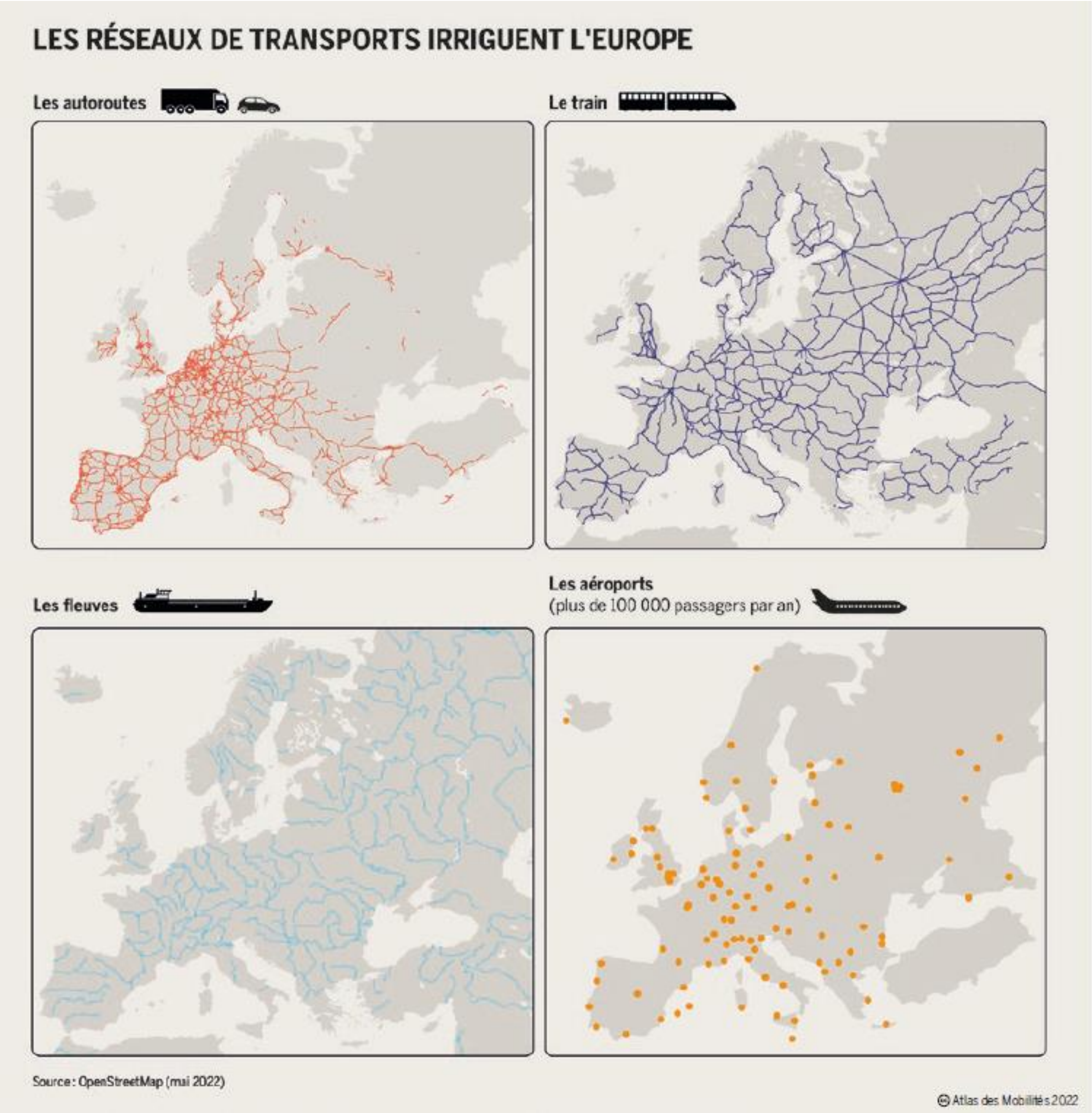

Figure 25: European transport networks 2022 represented by highways (les autoroutes), high-speed trains (le train), rivers (les fleuves), airports (les aéroports) (Bigo et al., 2022)

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9.3. Field data sheet : butterflies

Surveyor name	Study site	Page of
Date	Survey round	Comments
Starting time		
End time		
Wind (Beaufort)	Management type	
Temperature (°C)	Last treatment *	
Cloudiness (%)		
Sun shine (% time)		

Butterfly/ Burnet Species

Five 3-minute sub-transects of 50m each (total 250m). Transect width 1m to either side (2m total width).
Identify to species/ morphospecies or include as much taxonomic description as possible.
Record plant species when butterfly was visiting a flower.

		0-3min	4-6min	7-9min	10-12min	13-15min	Flower visit	Outside transect

*last treatment - include any management practice e.g. verge mowed/ hedge cut one week ago

9.4. Field data sheet : bees and hoverflies

Surveyor name	Study site	Page of
Date	Survey round	Comments
Starting time		
End time		
Wind (Beaufort)	Management type	
Temperature (°C)	Last treatment*	
Cloudiness (%)		
Sun shine (% time)		

Bees and hoverflies

Five 3-minute sub-transects of 50m each (total 250m). Transect width 1m to either side (2m total width).
Identify to species or collect specimen. If neither is possible, include as much taxonomic description as possible.
Record plant species when insect was visiting a flower.

sub-transect (1-5)	pollinator	abundance	ID if caught (nc = not caught)	visited plant	comment

*last treatment - include any management practice e.g. verge mowed/ hedge cut one week ago

9.5. Field data sheet : Quadrats

Flower Cover Quadrats

Record all flowering plant species and % cover for each

Surveyor name: Study site: Date: Survey round:

SubTr 1: Sward Height: Total veg cover:

Species	**% cover**	**Species**	**% cover**

SubTr 2: Sward Height: Total veg cover:

Species	**% cover**	**Species**	**% cover**

SubTr 3: Sward Height: Total veg cover:

Species	**% cover**	**Species**	**% cover**

SubTr 4: Sward Height: Total veg cover:

Species	**% cover**	**Species**	**% cover**

SubTr 5: Sward Height: Total veg cover:

Species	**% cover**	**Species**	**% cover**

9.6. Field data sheet : Traffic survey

Traffic Survey

Record all vehicles passing in each direction in a 15 minute period

Surveyor name	Study site
Date	Survey round
Time	

Vehicle Type	**Tally**
Motorcycle/ Scooter	
Car	
Van/ Light goods vehicle	
Lorry/ Heavy Goods vehicle	
Other:	

9.7. Description of walking experiment data

9.7.1. Belgium

Table 10: Inventory of bees collected: the table shows the proportion of individuals collected according to the landscape and type of route, as well as the total number of specimens collected in Belgium. The IUCN status is from Drossart (2019).

Taxonomy	IUCN Red List Category (Belgium)	Arable		Semi-natural		Total
		Minor	Major	Minor	Major	
		Andrenidae				
Andrena bicolor	LC	0	0	0	1	1
Andrena dorsata	LC	1	0	1	2	4
Andrena minutula	LC	0	0	1	0	1
		Apidae				
Bombus hortorum	NT	1	0	1	1	3
Bombus hypnorum	LC	0	0	1	0	1
Bombus lapidarius	LC	14	17	2	1	34
Bombus pascuorum	LC	13	19	26	20	78
Bombus pratorum	LC	0	0	1	2	3
Bombus sensus stricto	/	10	9	6	13	38
Bombus sylvestris	LC	0	0	2	0	2
Bombus vestalis	NT	1	0	5	0	6
		Colletidae				
Colletes daviesanus	LC	0	1	0	0	1
Hylaeus communis	LC	0	0	1	0	1
		Halictidae				
Halictus rubicundus	LC	0	0	1	0	1
Lasioglossum calceatum	LC	3	0	8	5	16
Lasioglossum fulvicorne	LC	0	0	1	0	1
Lasioglossum lativentre	LC	0	0	0	1	1
Lasioglossum parvulum	LC	0	0	2	0	2
Lasioglossum pauxillum	LC	1	0	1	0	2
Seladonia tumulorum	LC	1	0	0	0	1
TOTAL specimens		**45**	**46**	**60**	**46**	**197**
TOTAL species		**9**	**4**	**16**	**9**	**20**
TOTAL threatened species (VU+EN+CR+RE)	**0**	**0**	**0**	**0**	**0**	**0**

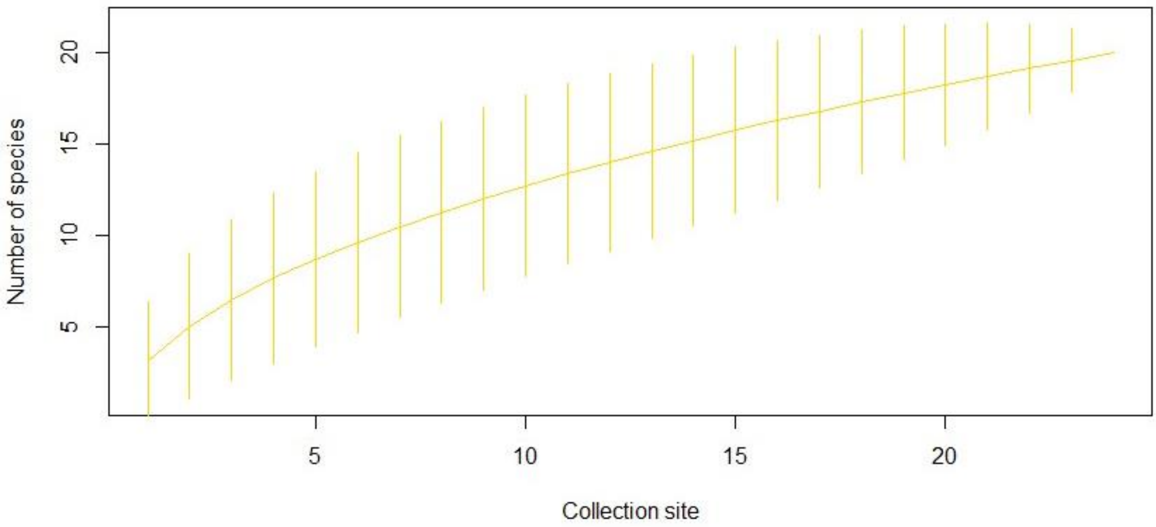

Figure 26: Belgian bee species accumulation curve by site

Table 11: Estimators of the total specific richness of bees in Belgium

Species	Chao ± se	Jack1 ± se	Jack2	Bootstrap ± se
20	36 ± 14	29 ± 5	36	24 ± 2

Using species richness estimators, we determined that between 55% and 83% of the bee species present at the studied sites were captured.

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Table 12: Inventory of hoverflies collected: the table shows the proportion of individuals collected according to the landscape and type of route, as well as the total number of specimens collected in Belgium. The IUCN status is from Van de Meutter (2021).

Taxonomy	IUCN Red List Category (Flanders)	Arable		Semi-natural		Total
		Minor	Major	Minor	Major	
Syrphinae						
Baccha elongata	LC	0	0	2	0	2
Episyrphus balteatus	LC	55	124	18	37	234
Eupeodes corollae	LC	14	2	1	0	17
Eupeodes luniger	LC	6	1	0	0	7
Melanostoma mellinum	LC	15	1	1	3	20
Melanostoma scalare	LC	0	0	8	5	13
Meliscaeva cinctella	VU	0	0	5	0	5
Paragus haemorrhous	LC	0	0	0	3	3
Paragus pecchiolii	LC	0	0	0	1	1
Platycheirus albimanus	LC	1	0	1	0	2
Platycheirus peltatus	VU	1	0	0	0	1
Platycheirus scutatus	LC	0	0	4	0	4
Scaeva pyrastri	LC	0	2	0	0	2
Sphaerophoria scripta	LC	82	127	9	11	229
Syrphus ribesii	LC	0	1	1	0	2
Syrphus vitripennis	LC	4	4	1	0	9
Xanthandrus comtus	LC	0	0	0	1	1
Eristalinae						
Cheilosia illustrata	VU	0	0	5	0	5
Cheilosia pagana	LC	0	0	3	0	3
Cheilosia proxima	LC	1	0	0	2	3
Chrysogaster solstitialis	LC	0	0	7	0	7
Eristalis arbustorum	LC	6	2	0	1	9
Eristalis nemorum	LC	2	1	5	4	12
Eristalis pertinax	LC	0	1	73	44	118
Eristalis tenax	LC	5	8	14	6	33
Ferdinandea cuprea	LC	0	0	0	2	2
Pipizella CF virens	LC	1	0	0	0	1
Rhingia campestris	LC	5	1	2	0	8
Syritta pipiens	LC	3	2	0	0	5
Volucella pellucens	LC	0	0	0	1	1
TOTAL specimens		**201**	**277**	**160**	**121**	**759**
TOTAL species		**15**	**14**	**18**	**14**	**30**
TOTAL threatened species (VU+EN+CR+RE)	**3**	**1**	**0**	**2**	**0**	**3**

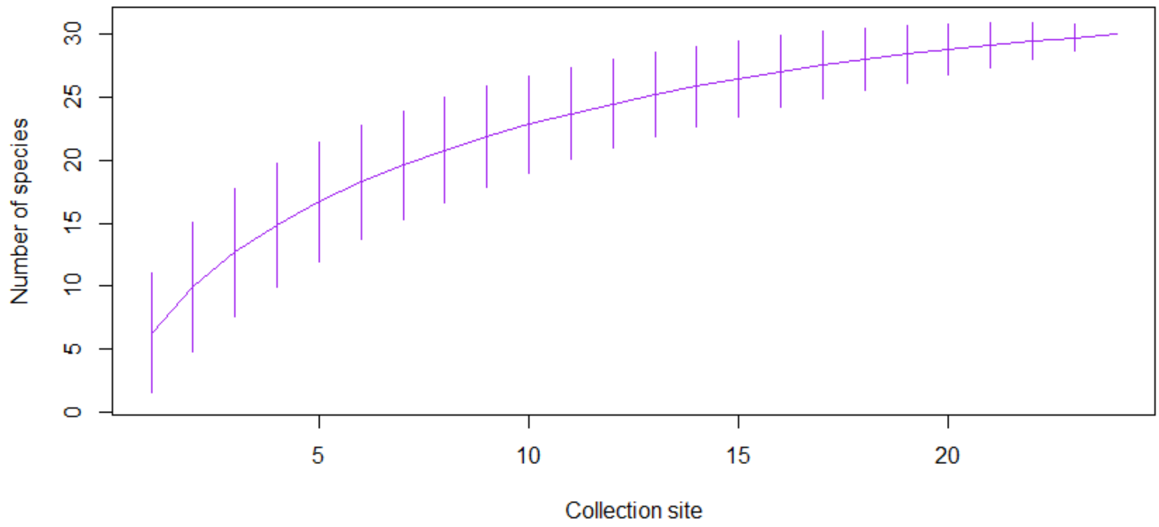

Figure 27: Belgian hoverfly species accumulation curve by site

Table 13: Estimators of the total specific richness of hoverflies in Belgium

Species	Chao ± se	Jack1 ± se	Jack2	Bootstrap ± se
30	32 ± 2	36 ± 3	34	33 ± 2

The species richness estimates show that between 83% and 93% of the hoverfly species present at the studied sites were captured.

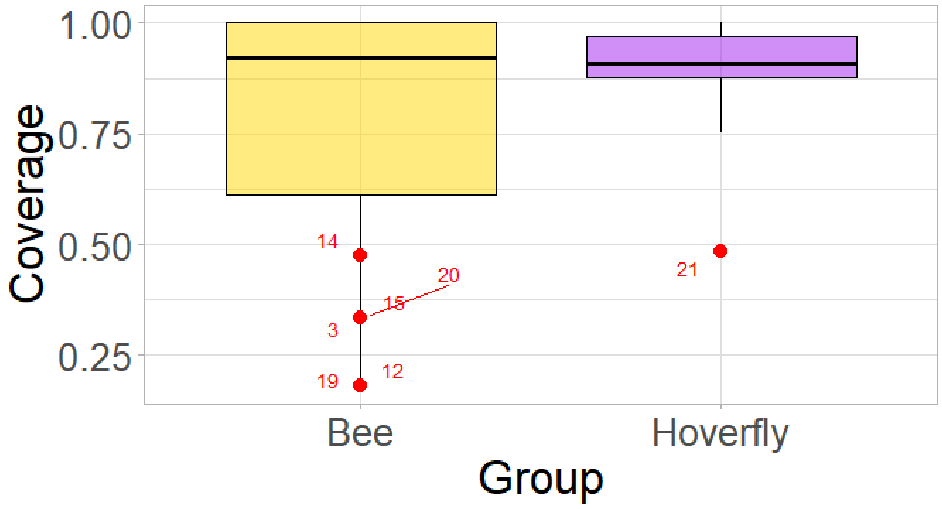

Figure 28:Boxplot of coverage distribution of Belgian bee and hoverfly samples in Belgium

Table 14: Inventory of butterflies: the table provides data on the presence and absence of butterflies based on the landscape and type of route in Belgium. The IUCN status is from Fichefet (2008)

Taxonomy	IUCN Red List Category (Wallonia)	Arable		Semi-natural	
		Minor	Major	Minor	Major
Hesperiidae					
Thymelicus lineola	NT	✗	✓	✗	✗
Lycaenidae					
Celastrina argiolus	LC	✗	✗	✓	✓
Nymphalidae					
Aglais io	LC	✓	✗	✓	✓
Aphantopus hyperantus	LC	✗	✗	✓	✗
Araschnia levana	LC	✗	✗	✓	✓
Brenthis ino	LC	✗	✗	✗	✓
Coenonympha pamphilus	LC	✓	✗	✗	✗
Pararge aegeria	LC	✓	✗	✓	✗
Polygonia c-album	LC	✗	✗	✓	✓
Pyronia tithonus	LC	✓	✓	✓	✓
Speyeria aglaja	EN	✗	✗	✓	✗
Vanessa atalanta	NE	✓	✓	✓	✓
Papilionidae					
Iphiclides podalirius	VU	✗	✓	✗	✗
Pieridae					
Gonepteryx rhamni	LC	✗	✓	✓	✓
Pieris brassicae	LC	✓	✓	✓	✓
Pieris napi	LC	✗	✗	✓	✓
Pieris rapae	LC	✓	✓	✓	✓
TOTAL species		**7**	**7**	**13**	**11**
TOTAL Threatened species (VU+EN+CR+RE)	**2**	**0**	**1**	**1**	**0**

Table 15: Inventory of flowers: the table provides data on the presence and absence based on the landscape and type of route in Belgium

Taxonomy	Arable		Semi-natural	
	Minor	Major	Minor	Major
Asteraceae				
Achillea millefolium	✓	✓	X	✓
Arctium minus	X	X	✓	X
Bellis perennis	X	X	✓	X
Carduus acanthoides	X	✓	X	X
Centaurea nigra	X	✓	X	X
Cirsium arvense	✓	✓	✓	✓
Cirsium vulgare	X	✓	X	X
Crepis capillaris	X	✓	X	✓
Eupatorium cannabinum	X	X	✓	✓
Galinsoga parviflora	X	X	X	✓
Jacobaea vulgaris	X	✓	X	✓
Lapsana communis	X	X	X	✓
Leontodon autumnalis	X	X	X	✓
Leucanthemum vulgare	X	✓	X	X
Matricaria chamomilla	X	✓	X	X
Sonchus arvensis	✓	X	X	X
Tanacetum vulgare	X	✓	X	✓
Apiaceae				
Anthriscus sylvestris	✓	✓	X	X
Conium maculatum	✓	X	X	X
Heracleum sphondylium	✓	✓	✓	✓
Brassicaceae				
Brassica napus	X	✓	X	X
Sinapis arvensis	✓	X	X	X
Betulaceae				
Corylus avellana	X	X	✓	X
Clusiaceae				
Hypericum perforatum	X	✓	✓	X
Convolvulaceae				
Convolvulus arvensis	✓	✓	X	X
Convolvulus sepium	✓	X	✓	✓
Fabaceae				
Lotus pedunculatus	X	✓	X	✓
Medicago falcata	X	✓	X	X
Medicago lupulina	X	✓	X	X
Trifolium sp.	✓	X	X	X
Vicia cracca	✓	X	X	X
Vicia sp.	X	X	X	✓
Geraniaceae				
Geranium robertianum	X	✓	✓	X
Geranium sp.	X	X	✓	X
Lamiaceae				
Lamium album	X	X	X	✓
Mentha arvensis	X	X	X	✓
Prunella vulgaris	X	X	X	✓
Lythraceae				
Lythrum salicaria	X	X	✓	X
Onagraceae				
Circaea lutetiana	X	X	✓	✓
Epilobium hirsutum	X	X	✓	✓
Plantaginaceae				
Linaria vulgaris	✓	X	X	X
Ranunculaceae				
Ranunculus repens	X	X	✓	X
Rosaceae				
Filipendula ulmaria	X	X	✓	✓
Rubus fruticosus	X	X	X	✓
Rubiaceae				
Galium mollugo	X	X	X	✓
Valerianaceae				
Valeriana dioica	X	X	✓	X

9.7.2. Serbia

Table 16: Inventory of bees: the table provides data on the presence and absence of bees based on the landscape and type of route in Serbia. The IUCN status is from Mudri-Stojnić et al. (2021).

Taxonomy	IUCN Red List Category (Serbia)	Arable		Semi-natural	
		Minor	Major	Minor	Major
Andrenidae					
Andrena cordialis	DD	X	X	X	✓
Andrena curvana	DD	X	X	X	✓
Andrena dorsalis	DD	X	X	✓	X
Andrena flavipes	LC	✓	✓	✓	✓
Andrena gelriae	DD	✓	X	X	X
Andrena hattorfiana	NT	X	X	X	✓
Andrena lagopus	LC	X	X	✓	X
Andrena nitida	LC	X	X	✓	X
Andrena polita	LC	X	X	✓	X
Andrena simontornyella	LC	X	X	✓	✓
Andrena symphyti	DD	X	X	✓	X
Andrena taraxaci	DD	X	X	✓	✓
Andrena viridescens	DD	X	X	✓	✓
Apidae					
Anthophora plumipes	LC	X	X	X	✓
Anthophora salviae	DD	✓	X	✓	X
Biastes brevicornis	LC	✓	X	X	X
Bombus haematurus	LC	✓	X	✓	✓
Bombus lapidarius	LC	X	X	✓	X
Bombus pascuorum	LC	✓	X	✓	✓
Bombus pratorum	LC	X	X	X	✓
Bombus ruderatus	LC	X	X	✓	X
Bombus sensus stricto	/	✓	✓	✓	✓
Ceratina cyanea	LC	✓	✓	✓	X
Ceratina nigrolabiata	LC	✓	X	X	✓
Eucera chrysopyga	LC	X	✓	X	X
Eucera nigrescens	LC	✓	X	✓	✓
Eucera tricincta	LC	✓	✓	X	X
Xylocopa violacea	LC	X	✓	✓	✓
Colletidae					
Hylaeus brevicornis	LC	✓	X	X	X
Halictidae					
Halictus maculatus	LC	X	✓	✓	X
Halictus patellatus	LC	✓	X	X	X
Halictus quadricinctus	NT	✓	✓	X	X
Halictus scabiosae	LC	X	X	✓	X
Halictus sexcinctus	LC	✓	X	X	X
Halictus simplex	LC	✓	✓	✓	X
Halictus tetrazonius	DD	X	X	X	✓
Lasioglossum bischoffi	DD	X	X	X	✓
Lasioglossum calceatum	LC	X	X	✓	X
Lasioglossum corvinum	LC	X	X	✓	X
Lasioglossum discum	LC	✓	✓	X	X
Lasioglossum glabriusculum	LC	X	X	X	✓
Lasioglossum interruptum	LC	X	✓	X	✓
Lasioglossum laterale	/	X	X	✓	✓
Lasioglossum leucozonium	LC	X	✓	X	X
Lasioglossum malachurum	LC	X	X	X	✓
Lasioglossum marginatum	LC	X	X	✓	✓
Lasioglossum pauxillum	LC	✓	X	X	X
Lasioglossum truncaticolle	DD	✓	X	✓	X
Lasioglossum villosulum	LC	X	✓	X	✓
Nomiapis diversipes	LC	✓	✓	X	X
Rophites quinquespinosus	NT	X	X	X	✓
Seladonia kessleri	LC	✓	X	X	X
Seladonia subaurata	LC	✓	X	✓	X
Systropha curvicornis	NT	✓	X	X	X
Systropha planidens	VU	✓	✓	✓	X
Megachilidae					
Chelostoma florisomne	LC	X	X	✓	X
Heriades truncorum	LC	X	✓	X	X
Megachile centuncularis	LC	✓	X	X	X
Megachile melanopyga	LC	✓	X	X	X
Megachile pilidens	LC	✓	X	X	X
Osmia aurulenta	LC	X	✓	X	X
Osmia bicolor	LC	X	✓	X	X
Osmia bicornis	LC	X	X	✓	X
Osmia rufohirta	LC	✓	X	X	X
TOTAL species		**28**	**18**	**30**	**24**
TOTAL threatened species (VU+EN+CR+RE)	**1**	**1**	**1**	**1**	**0**

Table 17: Inventory of hoverflies: the table provides data on the presence and absence of hoverflies based on the landscape and type of route in Serbia. The presence status is from (Vujić et al., 2001)

Taxonomy	Presenting species category (Serbia)	Arable		Semi-natural	
		Minor	Major	Minor	Major
Eristalinae					
Cheilosia albitarsis	P	X	X	✓	X
Eristalinus aeneus	P	X	X	✓	X
Eristalis arbustorum	P	✓	✓	✓	✓
Eristalis tenax	P	✓	✓	✓	✓
Helophilus trivittatus	P	✓	✓	✓	✓
Merodon analis	P	X	X	✓	X
Merodon ruficornis	P	X	X	✓	X
Myathropa florea	P	✓	X	✓	X
Syritta pipiens	P	✓	✓	✓	✓
Volucella zonaria	P	X	X	✓	X
Syrphinae					
Chrysotoxum cautum	P	X	X	✓	✓
Chrysotoxum festivum	P	X	X	✓	X
Chrysotoxum vernale	P	✓	X	X	X
Episyrphus balteatus	P	✓	✓	✓	✓
Eupeodes corollae	P	✓	✓	✓	✓
Eupeodes luniger	P	X	X	✓	✓
Melanostoma mellinum	P	X	✓	✓	✓
Meliscaeva auricollis	P	✓	X	X	X
Paragus bicolor	P	X	X	✓	X
Paragus haemorrhous	P	X	✓	✓	✓
Paragus quadrifasciatus	P	✓	X	X	X
Paragus testaceus	P	✓	X	X	X
Platycheirus albimanus	P	X	X	X	✓
Scaeva dignota	P	✓	X	✓	✓
Scaeva pyrastri	P	✓	X	✓	✓
Scaeva selenitica	P	✓	X	X	X
Sphaerophoria scripta	P	✓	✓	✓	✓
Syrphus ribesii	P	✓	✓	✓	X
Syrphus vitripennis	P	✓	X	✓	✓
Xanthogramma dives	P	X	X	X	✓
TOTAL species		**17**	**10**	**23**	**16**

Table 18: Inventory of butterflies: the table provides data on the presence and absence of butterflies based on the landscape and type of route in Serbia. The IUCN status is from Popović et al. (2017)

Taxonomy	IUCN Red List Category (Bosnia-Herzegovina)	Arable		Semi-natural	
		Minor	Major	Minor	Major
Hesperiidae					
Ochlodes sylvanus	LC	X	X	✓	X
Pyrgus malvae	LC	✓	✓	✓	✓
Thymelicus lineola	LC	✓	✓	✓	✓
Lycaenidae					
Aricia aegestis	LC	✓	X	X	X
Celastrina argiolus	LC	✓	✓	✓	✓
Cupido argiades	LC	X	X	✓	X
Favonius quercus	VU	✓	X	X	X
Glaycopsyche alexis	LC	X	✓	✓	X
Lycaena dispar	NT	X	✓	✓	✓
Plebejus argus	LC	✓	✓	✓	✓
Plebejus argyrognomon	NT	✓	✓	✓	X
Polyommatus bellargus	LC	✓	✓	✓	✓
Polyommatus icarus	LC	✓	✓	✓	✓
Satyrium acaciae	NT	X	X	X	✓
Nymphalidae					
Aglais io	LC	X	✓	X	X
Aphantopus hyperanthus	LC	X	X	✓	X
Boloria dia	LC	✓	X	X	✓
Brenthis daphne	LC	X	✓	X	X
Coenonympha pamphilus	LC	✓	✓	✓	✓
Isoria lathonia	LC	✓	✓	X	✓
Lasiommata megera	LC	✓	✓	✓	X
Maniola jurtina	LC	✓	✓	✓	✓
Melanargia galathea	LC	✓	✓	✓	✓
Melitaea didyma	LC	✓	X	✓	X
Minois dryas	LC	X	X	✓	X
Pararge aegeria	LC	X	X	X	✓
Vanessa atalanta	LC	✓	✓	✓	✓
Vanessa cardui	LC	✓	✓	✓	✓
Papilionidae					
Iphiclides podalirius	LC	✓	X	✓	✓
Papilio machaon	NT	✓	✓	X	X
Pieridae					
Anthocharis cardamines	LC	✓	X	✓	X
Aporia crataegi	LC	X	X	X	✓
Colias crocea	LC	✓	✓	✓	✓
Colias hyale	LC	✓	X	X	X
Gonepteryx rhamni	LC	✓	✓	X	X
Pieris brassicae	NT	✓	✓	✓	✓
Pieris napi	LC	✓	✓	X	✓
Pieris rapae	LC	✓	✓	✓	✓
Pontia edusa	LC	✓	✓	X	X
TOTAL species		**28**	**25**	**25**	**22**
TOTAL Threatened species (VU+EN+CR+RE)	**1**	**1**	**0**	**0**	**0**

Table 19: Inventory of flowers: the table provides data on the presence and absence based on the landscape and type of route in Serbia.

Taxonomy	Arable		Semi-natural	
	Minor	Major	Minor	Major
Adoxaceae				
Sambucus ebulus	X	✓	✓	X
Sambucus nigra	✓	X	✓	✓
Apiaceae				
Aegopodium podagraria	X	X	X	✓
Anthriscus sylvestris	X	X	✓	✓
Conium maculatum	X	✓	✓	✓
Daucus carota	✓	✓	✓	✓
Falcaria vulgaris	✓	✓	X	X
Orlaya grandiflora	✓	✓	✓	✓
Tordylium maximum	✓	X	X	X
Torilis arvensis	✓	X	✓	X
Asteraceae				
Achillea millefolium	✓	✓	✓	✓
Anthemis arvensis	✓	✓	✓	X
Bellis perennis	✓	X	✓	✓
Carduus acanthoides	✓	✓	✓	✓
Carthamus lanatus	✓	X	X	X
Centaurea jacea	✓	✓	✓	X
Centaurea scabiosa	✓	X	X	X
Centaurea stoebe	✓	X	X	X
Cichorium intybus	✓	✓	✓	✓
Cirsium arvense	✓	✓	X	X
Cota tinctoria	✓	X	X	X
Crepis biennis	✓	✓	✓	✓
Crepis foetida	X	X	✓	✓
Erigeron annuus	✓	✓	✓	✓
Lactuca serriola	X	X	✓	X
Lapsana communis	X	X	X	✓
Matricaria chamomilla	✓	✓	✓	✓
Senecio inaequidens	X	X	X	✓
Senecio vulgaris	X	X	✓	X
Sonchus arvensis	✓	X	X	✓
Sonchus asper	X	X	X	✓
Taraxacum officinale	X	X	✓	✓
Tragopogon dubius	X	X	X	✓
Xeranthemum annuum	✓	✓	X	X
Xeranthemum inapertum	X	X	✓	X
Boraginaceae				
Anchusa ochroleuca	✓	X	X	X
Anchusa officinalis	✓	✓	✓	✓
Myosotis arvensis	✓	✓	✓	✓
Symphytum officinale	X	X	✓	X
Brassicaceae				
Alliaria petiolata	X	X	X	✓
Arabidopsis thaliana	X	X	X	✓
Berteroa incana	✓	X	✓	X
Brassica napus	✓	✓	✓	✓
Calepina irregularis	✓	X	✓	X
Capsella bursa-pastoris	✓	✓	✓	✓
Descurainia sophia	X	X	X	✓
Diplotaxis tenuifolia	X	✓	X	X
Lepidium draba	✓	✓	✓	✓
Sinapis arvensis	X	X	✓	✓
Sisymbrium officinale	✓	X	X	X
Campanulaceae				
Knautia arvensis	✓	✓	✓	X
Caprifoliaceae				
		1		0
Caryophyllaceae				
Arenaria serpyllifolia	✓	X	✓	X
Dianthus pontederae	✓	X	X	X
Petrorhagia saxifraga	✓	✓	X	X
Silene latifolia	X	✓	X	X
Silene nutans	✓	X	X	X
Silene vulgaris	X	X	X	✓
Stellaria graminea	X	X	✓	X
Stellaria holostea	X	X	X	✓
Stellaria media	X	X	X	✓
Celastraceae				
Euonymus europaeus	X	X	✓	✓
Clusiaceae				
Hypericum perforatum	✓	✓	✓	✓
Convolvulaceae				
Convolvulus arvensis	✓	✓	✓	✓
Cornaceae				
Cornus sanguinea	✓	✓	✓	✓
Dipsacaceae				
Scabiosa ochroleuca	✓	✓	✓	✓
Euphorbiaceae				
Euphorbia cyparissias	✓	✓	✓	✓
Euphorbia esula	✓	✓	✓	✓
Euphorbia glareosa	✓	X	X	✓
Euphorbia helioscopia	✓	X	X	X
Euphorbia seguieriana	✓	X	X	X
Fabaceae				
Astragalus onobrychis	✓	✓	✓	✓
Cytisus austriacus	✓	✓	X	X
Dorycnium herbaceum	✓	X	X	X
Genista tinctoria	✓	X	✓	X
Lathyrus aphaca	X	✓	X	X
Lathyrus hirsutus	X	✓	X	X
Lathyrus sphaericus	X	X	✓	X
Lathyrus sylvestris	✓	X	X	X
Lathyrus tuberosus	✓	✓	✓	✓
Lembotropis nigricans	✓	X	X	X
Lotus corniculatus	✓	✓	✓	X
Medicago arabica	✓	X	X	X
Medicago falcata	✓	✓	✓	✓
Medicago lupulina	✓	X	✓	✓
Medicago minima	✓	✓	X	✓
Medicago sativa	✓	✓	✓	✓
Melilotus albus	✓	X	✓	X
Melilotus officinalis	✓	✓	X	X
Ononis spinosa	✓	X	X	X
Robinia pseudoacacia	X	X	X	✓
Securigera varia	✓	✓	✓	✓
Trifolium campestre	✓	✓	✓	X
Trifolium pratense	✓	✓	✓	✓
Trifolium repens	✓	✓	✓	✓
Vicia hirsuta	X	✓	✓	X
Vicia pannonica	✓	✓	✓	X
Vicia sativa	✓	✓	✓	✓
Vicia tenuifolia	✓	✓	✓	✓
Geraniaceae				
Erodium cicutarium	X	✓	✓	X
Geranium columbinum	X	✓	✓	X
Geranium dissectum	✓	✓	✓	✓
Geranium molle	X	X	X	✓
Geranium phaeum	X	X	X	✓
Geranium pusillum	✓	✓	✓	✓
Geranium robertianum	X	X	✓	✓
Lamiaceae				
Ajuga reptans	X	X	✓	✓
Ballota nigra	✓	✓	✓	✓
Clinopodium vulgare	X	✓	✓	✓
Glechoma hederacea	X	X	✓	✓
Lamium galeobdolon	X	X	X	✓
Lamium maculatum	X	X	X	✓
Lamium purpureum	✓	X	✓	✓
Mentha longifolia	✓	X	X	X
Prunella vulgaris	X	X	X	✓
Salvia nemorosa	✓	✓	✓	✓
Salvia pratensis	X	X	✓	✓
Stachys recta	✓	X	X	X
Teucrium chamaedrys	X	✓	✓	X
Thymus serpyllum	✓	X	✓	✓
Liliaceae				
Allium ursinum	X	X	X	✓
Allium vineale	✓	X	X	X
Linaceae				
Linum perenne	✓	✓	✓	X
Malvaceae				
Malva sylvestris	✓	✓	X	✓
Oleaceae				
Ligustrum vulgare	X	X	✓	X
Orobanchaceae				
Rhinanthus alectorolophus	✓	X	X	X
Papaveraceae				
Chelidonium majus	X	X	✓	X
Papaver rhoeas	✓	✓	✓	✓
Plantaginaceae				
Linaria genistifolia	✓	✓	X	✓
Plantago lanceolata	✓	X	X	✓
Veronica austriaca	✓	X	✓	✓
Veronica persica	X	X	✓	✓
Primulaceae				
Anagallis arvensis	X	X	✓	✓
Ranunculaceae				
Clematis vitalba	X	✓	✓	✓
Consolida regalis	✓	✓	✓	X
Ranunculus acris	✓	✓	✓	✓
Ranunculus arvensis	X	X	✓	X
Ranunculus repens	X	X	✓	✓
Thalictrum minus	X	X	✓	✓
Resedaceae				
Reseda lutea	X	X	✓	X
Rosaceae				
Agrimonia eupatoria	✓	✓	✓	✓
Crataegus monogyna	X	X	X	✓
Geum urbanum	X	X	X	✓
Potentilla argentea	✓	✓	X	X
Potentilla recta	✓	X	X	X
Potentilla reptans	X	✓	X	✓
Rosa canina	✓	✓	✓	✓
Rubus caesius	✓	✓	✓	✓
Rubiaceae				
Asperula cyanchica	X	X	✓	X
Cruciata laevipes	X	✓	✓	✓
Galium aparine	✓	X	✓	✓
Galium mollugo	✓	✓	✓	✓
Galium verum	✓	✓	✓	X
Sherardia arvensis	✓	X	✓	X
Scrophulariaceae				
Verbascum lychnitis	X	X	✓	X
Verbascum nigrum	✓	✓	X	✓
Solanaceae				
Lycium barbarum	✓	X	X	X
Solanum dulcamara	X	✓	X	X
Valerianaceae				
Valerianella carinata	✓	X	X	X
Valerianella dentata	X	X	X	✓
Verbenaceae				
Verbena officinalis	✓	✓	X	X
Violaceae				
Viola arvensis	✓	✓	✓	X

Z

9.8. Belgian sites and associated variables

		Bee			Hoverfly		Butterfly
Site index	N0	N0r	Abundance	N0	N0r	Abundance	N0
1	2	1,69	14	9	5,48	63	2
2	3	2,34	11	1	1	2	1
3	3	/	3	3	2,28	29	2
4	3	2,62	17	2	1,24	38	1
5	2	2,25	2	4	4,28	14	4
6	1	1	1	7	8,95	16	4
7	2	2	3	6	6,15	25	3
8	1	1	3	3	2,11	19	3
9	2	1,8	6	5	2,98	28	5
10	2	1,26	4	6	2,2	115	3
11	4	2,69	15	8	6,97	38	4
12	4	/	4	7	6	32	2
13	4	3,34	12	9	13,07	29	7
14	9	/	13	8	8,31	24	6
15	3	/	3	9	8,67	28	5
16	5	7,04	8	5	3,69	31	3
17	5	3,03	23	8	10,04	15	10
18	3	3,3	5	9	9,05	34	4
19	4	/	4	9	7,21	48	6
20	3	/	3	7	9,37	24	7
21	4	2,7	25	5	/	7	5
22	2	1,67	6	5	3,86	29	5
23	2	1,7	5	7	6,49	27	3
24	2	1,75	7	9	7,71	44	3

9.9. Serbian sites and associated variables

Site index	Bee N0	Hoverfly N0	Butterfly N0
1	20	13	9
2	11	5	8
3	4	6	4
4	4	4	2
5	2	6	6
6	7	6	4
7	0	13	9
8	5	13	16
9	4	9	10
10	1	5	11
11	1	6	5
12	1	7	2
13	4	8	11
14	7	8	10
15	6	8	11
16	6	5	8
17	1	4	3
18	7	4	16
19	6	8	5
20	9	6	5
21	4	3	10
22	4	6	10
23	3	8	12
24	5	9	19

9.10. Description of car transect data

9.10.1. Belgium

Table 20: Inventory of invertebrates collected in Belgium: the table shows the total number of specimens collected by family

Family	Sum
Araneae	
NA	7
Coleoptera	
Chrysomelidae	2
Coccinellidae	1
Curculionidae	3
Latridiidae	2
Melyridae	1
Staphylinidae	9
Collembola	
Sminthuridae	1
NA	4
Diptera	
Acalyptratae	1
Cecidomyiidae	24
Chironomidae	20
Chloropidae	12
Dolichopodidae	2
Drosophilidae	1
Empididae	2
Fanniidae	1
Heleomyzidae	2
Hybotidae	2
Muscidae	5
Mycetophilidae	1
Phoridae	5
Rhagionidae	1
Sarcophagidae	1
Scatopsidae	8
Sciaridae	16
Sphaeroceridae	1
Syrphidae	4
NA	18
Hemiptera	
Aphididae	18
Cicadellidae	6
Lygaeidae	1
Miridae	2
Tettigometridae	1
NA	1
Heteroptera	
Miridae	1
Homoptera	
Cicadellidae	1
Hymenoptera	
Cynipidae	8
Ichneumonidae	7
Pompilidae	1
NA	73
Odonata	
Libellulidae	1
Orthoptera	
Acrididae	3
Other	
NA	22
Psocoptera	
NA	1
Thysanoptera	
Aeolothripidae	3
Thripidae	383
NA	1

BB

9.10.2. Serbia

Table 21: Inventory of invertebrates collected in Serbia: the table shows the total number of specimens collected by family

Family	Sum
Araneae	
NA	4
Blattodea	
NA	1
Coleoptera	
NA	22
Diptera	
Syrphidae	4
NA	92
Hemiptera	
Aphididae	48
NA	10
Heteroptera	
NA	27
Homoptera	
NA	9
Hymenoptera	
Andrenidae	2
Apidae	3
Formicidae	4
Halictidae	1
Mymaridae	1
NA	125
Ixodida	
NA	1
Lepidoptera	
Lycaenidae	1
Nymphalidae	1
Orthoptera	
NA	23
Other	
NA	7
Psocodea	
NA	1
Thysanoptera	
NA	86
Trichoptera	
NA	3

10. Illustration table

Figure 1 : Canal Mons-Condé buried under asphalt in Saint-Ghislain 1

Figure 2 : Representation of the six European bee families 5

Figure 3 : Representation of the three European hoverfly subfamilies 7

Figure 4: Representation of the six European butterfly families 9

Figure 5: Modal distribution of domestic passenger transport, 2013 (in % of total domestic transport, in passenger-kilometers) 12

Figure 6: Map of the Belgian and Serbian road network (Credit : OpenStreetMap, 2024; Lescot, 2024) 13

Figure 7: Amphibian (Credit : Lescot, 2024) and butterfly (Credit : Oren Ravid, 2023) hit by a vehicle 14

Figure 8: Illustration of biological questions; according to (Phillips et al., 2020) (Credit : Lescot, 2024). **Q1a** : Does the diversity of floral species increase the diversity of pollinator assemblages ? **Q1b** : Which flowers are most attractive to pollinators in the plant communities along roadsides? **Q2** : Do rich-floral road verges act as honeytraps for pollinators? **Q3** : Are pollinator communities impacted by vehicle collisions? 18

Figure 9: The Belgium study area (Credit : Lescot, 2023) 19

Figure 10: The Serbia study area (Credit : Lescot, 2023) 19

Figure 11: Schematic showing the location of the walking and car experiment, with verge, boundary feature and adjacent land use (Credit : Safeguard Task 2.6 (ii) Traffic study) 22

Figure 12: Capture imaginary box (Credit : Lescot, 2023) 23

Figure 13: Illustration of the various stages of the materials and methods (Credit : Lescot, 2023): **1** : Paint mark on the road ; **2** : Butterfly identification ; **3** : 1m² quadrat ; **4** : Plastic plate fixed on the car ; **5** : One sticky trap glued on the plastic plate on the car ; **6** : Small plastic beads on a section of the sticky trap ; **7** : Specimen pinned and labeled 26

Figure 14: Choice of response variables depending on the sampling method 28

Figure 15: Impact of flower species richness on the **A:** rarefied species richness of bee (P.value=0.3839), **C:** rarefied species richness of hoverfly (P.value=0.381855), **E:** species richness of butterfly (P.value=0.81503) assemblages and impact of flower abundance on the **B**: rarefied species richness of bee (P.value=0.6493), **D:** rarefied species richness of hoverfly (P.value=0.404408), **F:** species richness of butterfly (P.value=0.00422) assemblages along Belgian roadsides. 32

Figure 16: Impact of the landscape on the species richness of **A:** bee (P.value=0.5343), **C:** hoverfly (P.value=0.000242), **E:** butterfly (P.value=7.93e-06) assemblages and impact of the road type on the species richness of **B:** bee (P.value=0.0241), **D**: hoverfly (P.value=0.001194), **F**: butterfly (P.value=0.01556) assemblages along Belgian roadsides. 33

DD

Figure 17: Impact of flower species richness on the species richness of **A:** bee (P.value=0.7420), **C:** hoverfly (P.value=0.005858), **E:** butterfly (P.value=2.4e-06) assemblages and impact of flower abundance on the species richness of **B**: bee (P.value=0.5411), **D:** hoverfly (P.value=0.512012), **F:** butterfly (P.value=0.1740) assemblages along Serbian roadsides. 36

Figure 18: Impact of the landscape on the species richness of **A:** bee (P.value=0.7959), **C:** hoverfly (P.value=0.043196), **E:** butterfly (P.value=0.8550) assemblages and impact of the road type on the species richness of **B:** bee (P.value=0.9206), **D**: hoverfly (P.value=0.082584), **F**: butterfly (P.value=0.4754) assemblages along Serbian roadsides. 37

Figure 19: Top 10 flowers attracting **A:** wild bee specimens, **B:** wild bee species, **C:** hoverfly specimens, **D:** hoverfly species, **E:** butterfly species in Belgian roadsides. 39

Figure 20: Top 10 flowers attracting **A:** wild bee species, **B:** hoverfly species, **C:** butterfly species on Serbian roadsides. 40

Figure 21: Impact of **A:** flower species richness (p.value = 0.566), **B:** flower abundance (p.value = 0.447), **C:** landscape (p.value = 7.87e-07), **D:** road type (p.value = 0.102) on invertebrate collision number in Belgium. 41

Figure 22 : Impact of **A:** flower species richness (p.value = 0.6697), **B:** flower abundance (p.value = 0.9721), **C:** landscape (p.value = 0.0432), **D:** road type (p.value = 0.9509) on invertebrate collision number in Serbia 43

Figure 23 : Number of invertebrates collected on the sticky trap by order in Belgium and Serbia44

Figure 24: Length of invertebrates collected on sticky trap in Belgium (BE) and Serbia (RS) 45

Figure 25: European transport networks 2022 represented by highways (les autoroutes), high-speed trains (le train), rivers (les fleuves), airports (les aéroports) (Bigo et al., 2022) .... N

Figure 26: Belgian bee species accumulation curve by site .... S

Figure 27: Belgian hoverfly species accumulation curve by site .... U

Figure 28:Boxplot of coverage distribution of Belgian bee and hoverfly samples in Belgium V

Table 1 : Bee family diversity in Europe, adapted from (Ghisbain et al., 2023). 4

Table 2: Butterfly family diversity in the European continent, adapted from (Van Swaay et al., 2010)........ 8

Table 3: Sites list including the country, the latitude and longitude of walking transect start (cf. Section 5.3) in CRS WG 84 Decimal degree and Safeguard Task 2.6 Traffic protocol road characterization 21

Table 4: Summary of the generalized Poisson model of rarefied species richness in bees and hoverflies, and species richness in butterflies in Belgium. It presents the model results predicting the species richness of Belgian bees, hoverflies and butterflies. The predictor variables included in the

model are **Flower_N0** : Floral species richness, **SNH** : Type of semi-natural habitat, **Minor** : Type of minor road, and **Flower_ab** : Floral abundance. 34

Table 5: Summary of the generalized Poisson model of species richness in bees, hoverflies and butterflies in Serbia. It presents the model results predicting the species richness of Serbian bees, hoverflies and butterflies. The predictor variables included in the model are **Flower_N0** : Floral species richness, **SNH** : Type of semi-natural habitat, **Minor** : Type of minor road, and **Flower_ab** : Floral abundance. 38

Table 6 : Summary of the generalized Poisson model of roadkill number in Belgium. It presents the results of the model predicting the roadkill number in Belgium. The predictor variables included in the model are **Flower_N0** : Floral species richness, **SNH** : Type of semi-natural habitat, **Minor** : Type of minor road, and **Flower_ab** : Floral abundance. 42

Table 7: Summary of the generalized Poisson model of roadkill number in Serbia. It presents the results of the model predicting the roadkill number in Serbia. The predictor variables included in the model are **Flower_N0** : Floral species richness, **SNH** : Type of semi-natural habitat, **Minor** : Type of minor road, and **Flower_ab** : Floral abundance. 43

Table 8: Summary of pollinators collected on the sticky trap in Belgium and Serbia 45

Table 9: Summary of the length of invertebrates collected on the sticky trap by country 45

Table 10: Inventory of bees collected: the table shows the proportion of individuals collected according to the landscape and type of route, as well as the total number of specimens collected in Belgium. The IUCN status is from Drossart (2019). S

Table 11: Estimators of the total specific richness of bees in Belgium S

Table 12: Inventory of hoverflies collected: the table shows the proportion of individuals collected according to the landscape and type of route, as well as the total number of specimens collected in Belgium. The IUCN status is from Van de Meutter (2021). U

Table 13: Estimators of the total specific richness of hoverflies in Belgium U

Table 14: Inventory of butterflies: the table provides data on the presence and absence of butterflies based on the landscape and type of route in Belgium. The IUCN status is from Fichefet (2008) V

Table 15: Inventory of flowers: the table provides data on the presence and absence based on the landscape and type of route in Belgium W

Table 16: Inventory of bees: the table provides data on the presence and absence of bees based on the landscape and type of route in Serbia. The IUCN status is from Mudri-Stojnić et al. (2021) X

Table 17: Inventory of hoverflies: the table provides data on the presence and absence of hoverflies based on the landscape and type of route in Serbia. The presence status is from (Vujić et al., 2001) Y

Table 18: Inventory of butterflies: the table provides data on the presence and absence of butterflies based on the landscape and type of route in Serbia. The IUCN status is from Popović et al. (2017) Y

Table 19: Inventory of flowers: the table provides data on the presence and absence based on the landscape and type of route in Serbia. .... Z
Table 20: Inventory of invertebrates collected in Belgium: the table shows the total number of specimens collected by family .... BB
Table 21: Inventory of invertebrates collected in Serbia: the table shows the total number of specimens collected by family CC