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Leverages of floral and nesting resources on foraging ecology and community structure of pollinators throughout disturbed landscapes

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Abstract

Today, our societies are increasingly concerned about the alarming disappearance of insects. Yet they are essential to the proper functioning of our ecosystems, our food and our health. Pollinating insects such as bees, hoverflies and apoid wasps are also affected by this collapse. These insects generate significant ecosystem services for agriculture and the maintenance of natural areas through the pollination of flowering plants and the biological control of crops. A series of causes for these collapses have been widely documented in scientific literature, and it appears that it is mainly landscape degradation that is causing the greatest damage to biodiversity. Agricultural intensification and urbanization are converting complex natural areas into homogeneous, anthropogenic zones, thereby destroying floral and nesting resources, which are key components in the development and maintenance of pollinating insect communities. The homogenization of the landscape also alters the foraging behavior of our pollinating insects. It is in this context that this research thesis will focus, through 4 case studies, on the impact of landscape fragmentation on pollinator communities and foraging ecology in temperate zone of agricultural and urban landscapes.

This thesis is structured around a general introduction (Chapter I), a general methodology (Chapter II) which introduces the technical tools shared between the different case studies, and four chapters constituting the core of this thesis (Chapter III-VI), the first two of which focus on agricultural landscapes, while the last two are more concerned with urban environments. The thesis concludes with a discussion, an outlook on future research and a general conclusion (Chapter VII).

Chapter III looks at the influence of ecological diversification on the biodiversity of pollinators, particularly wild bees and hoverflies, on two farms in the early stages of ecological transition. This study has the merit of providing quality pollinator occurrence data based on standardized sampling techniques established over two years (2018-2019) and reports certain species that may be of conservation concern given their critical conservation status. In view of the specific diversity of wild bees present (101 species) and hoverflies (31 species), the ecological diversification of environments within the two sampled farms (e.g. wetlands, flower strips...) and agroecological practices may constitute new favorable environments for rare, oligolectic or endangered pollinator species.

Building on the methodology and results of the previous chapter, in chapter IV we looked at the impact of a more specific floral resource supply practice: flower strips in intercropping systems with winter wheat. We found that a multifloral composition of flower strips attracted a greater diversity of hoverflies but not wild bees than monofloral oleaginous strips composed of *Dimorphoteca pluvialis* (Asteraceae) and *Camelina sativa* (Brassicacea). Finally, the ecological and economic benefits provided by these oleaginous flower strips are organized on three levels: (i) they provide alternative floral resources to pollinators; (ii) farmers are rewarded by the Agro-Environmental and Climate Measures (MAEC) of the European Union's

Common Agricultural Policy; (iii) and farmers can generate additional income from the resale of harvested oleaginous seeds.

In chapter V, we investigate urban landscapes. Pollen availability is a key factor influencing the population dynamics of pollinating insects in urban disturbed landscapes. In this study, we analyzed the foraged floral resources of honeybee colonies, *Apis mellifera* L., along an urban-rural gradient in a mega-city Tokyo (Japan). After a genomic analysis (i.e., metabarcoding) to identify the floral species foraged on the pollen samples, we showed that the landscape factor explains the differences in composition of the flowers visited and not their specific richness, whereas this richness is significantly dependent on the temporal factor. Honeybees forage more on woody species in spring, and more on herbaceous species in autumn. This study provides us with a better understanding of the urban needs of honeybees in a mega-city.

Urban surfaces are commonly perceived as "ecologically impermeable" and can therefore represent a considerable obstacle to nesting opportunities for ground-nesting insects. In Chapter VI, we looked at the nesting alternative that pavements could offer for a range of ground-nesting Hymenoptera in the city of Brussels-Capital. Our study identified 22 species of wild bees and apoid wasps capable of nesting on Brussels sidewalks. These species mainly nested in old Brussels sidewalks made of sandstone pavers or concrete slabs with unbonded joints averaging one centimeter in width, and where the construction layers beneath the sidewalks were sandy in texture. At the end of this experiment, we were able to design technical recommendations and management practices to encourage these pollinating insects.

The relevance of this research project and the general context are discussed at the end of this thesis in Chapter VII. Taken as a whole, these four studies underline the importance of food and nesting resources for insect pollinator communities and constitute milestones of knowledge providing further arguments in favor of the conservation and restoration of insect pollinators in disturbed environments We concluded this discussion with an open-minded outlook for the continuation scientific work in these topics of agricultural and urban ecology.

Résumé

A l’heure actuelle, nos sociétés s’inquiètent de plus en plus de la disparition alarmante des insectes. Ceux-ci sont pourtant essentielles dans le bon fonctionnement de nos écosystèmes, notre alimentation ou encore notre santé. Les insectes pollinisateurs tels que les abeilles, les syrphes ou encore les guêpes sphéciformes sont également touchés par cet effondrement. Ceux-ci génèrent des services écosystémiques non négligeables pour l’agriculture ou encore le maintien des espaces naturelles par le biais de la pollinisation des plantes à fleurs ou encore le contrôle biologiques des cultures. Une série de causes de ces effondrements a été largement documentées dans la littérature scientifique et il apparait que c’est principalement la dégradation des paysages qui occasionne les plus grands dommages pour la biodiversité. En effet l’intensification agricole et l’urbanisation convertissent des espaces naturels complexes en zones homogènes et anthropiques ce qui détruit principalement les ressources florales et de nidification qui sont les composantes capitales du développement et du maintien des communautés d’insectes pollinisateurs. D’une autre manière, l’homogénéisation du paysage altère également le comportement de butinage de nos insectes pollinisateurs. C’est dans ce contexte que s’inscrit cette thèse de recherche qui va s’intéresser, à travers 4 études de cas, à l’impact de la fragmentation du paysage sur les communautés de pollinisateurs et l’écologie de butinage dans des paysages agricoles et urbains des zones tempérées.

Cette thèse s’articule autour d’une introduction générale (chapitre I), suivie d’une méthodologie générale (chapitre II) qui introduit les outils techniques partagés entre les différentes études de cas et de quatre chapitres constituant le cœur de cette thèse dont les deux premiers se concentrent dans les paysages agricoles alors que les deux derniers s’intéressent plutôt aux milieux urbains. Cette thèse se termine par une discussion générale, une ouverture sur des recherches futures et conclusion générale (Chapitre VII).

Le chapitre III s’intéresse à l’influence de la diversification écologiques de deux fermes en début de transition écologique sur la biodiversité des pollinisateurs présents notamment les abeilles sauvages et les syrphes. Cette étude a le mérite d’apporter des données d’occurrence de pollinisateurs de qualité selon des techniques d’échantillonnage standardisés établies sur deux années et reporte certaines espèces pouvant faire l’objet d’enjeu de conservation étant donné leurs statut critiques de conservation. Au vu de la diversité spécifique d’abeilles sauvages présentes (101 espèces) et de syrphes (31 espèces), la diversification écologiques des milieux au sein des deux fermes échantillonnées (p.ex. zones humides, bandes fleuries…) et les pratiques agroécologiques peuvent constituer des nouveaux milieux favorables pour des espèces de pollinisateurs rares, oligolectiques ou en danger d’extinction.

En se basant sur la méthodologie et les résultats du chapitre précédent, nous nous sommes intéressés dans le chapitre IV à l’impact d’une pratique d’apport de ressources florales plus particulière : les bandes fleuries en interculture avec du blé d’hiver. Nous avons constaté qu’une composition multiflorale de bande fleurie attirait une plus grande diversité de syrphes que dans des bandes monoflorales oléagineuses composées de *Dimorphoteca pluvialis* (Asteraceae) et de *Camelina sativa* (Brassicacea). Enfin, les avantages écologiques et économiques procurés par ces bandes fleuries oléagineuses sont organisés en trois niveaux : (i) elles permettent aux pollinisateurs de disposer de ressources florales alternatives ; (ii) elles sont rétribuées par les Mesures Agro-Environnementales et Climatiques (MAEC) de la Politique Agricole Commune de l'Union Européenne ; (iii) et elles permettent à l'agriculteur dans dégager un revenu complémentaire dans la revente des graines d'oléagineux récoltées.

Dans le chapitre V, nous changeons de décor paysager et passons dans les paysages urbains. La disponibilité en pollen est un facteur primordial qui influence la dynamique des populations d'insectes pollinisateurs dans les paysages perturbés. Dans cette étude, nous avons analysé les habitudes alimentaires de l'abeille mellifère, *Apis mellifera* L., selon un gradient urbain-rural d'une mégalopole de la taille de Tokyo (Japon). Après une analyse génomique (i.e., metabarcoding) pour identifier les essences florales butinées des échantillons de pollen, nous avons montré que le facteur du paysage explique les différences de composition des fleurs visitées et non leur richesse spécifique alors que cette richesse est significativement dépendante du facteur temps. L'abeille mellifère butine plus de fleurs provenant d'essences ligneuses au printemps alors qu'elle profite plus de strate herbacée en automne. Ainsi, cette étude nous permet de mieux comprendre les besoins urbains de l'abeille mellifère pour une ville importante.

Les surfaces urbaines sont communément perçues comme étant « écologiquement imperméables » et peuvent donc constituer un obstacle considérable aux opportunités de nidifications d'insectes terricoles. Au chapitre VI, nous nous sommes intéressés à l'alternative de nidification que pouvait offrir les trottoirs pour tout une série d'Hyménoptères terricoles dans la ville de Bruxelles-Capitale. Notre étude a recensé 22 espèces d'abeilles sauvages et de guêpes apoïdes qui sont capables de nicher dans les trottoirs bruxellois. Ces espèces terricoles ont principalement nichées dans des vieux trottoirs bruxellois constitués de pavés en grés ou de dalles de béton avec des joints non liés d'une largeur moyenne d'un centimètre et dont les couches de construction sous les trottoirs sont de texture sableuse. Au terme de cet expérience, nous avons pu concevoir des recommandations techniques et des pratiques de gestion pour favoriser l'accueil de ces insectes pollinisateurs.

La relevance de ce projet de recherche et le contexte général sont discutés à la fin de cette thèse au chapitre VII. Prises dans leur ensemble, ces quatre études soulignent l'importance des ressources alimentaires et de nidification pour les communautés d'insectes pollinisateurs et constituent des jalons de connaissances apportant des arguments supplémentaires en faveur de la conservation et de la restauration des insectes pollinisateurs dans les milieux perturbés. Nous avons terminé cette discussion en concluant par une ouverture sur des perspectives de continuation de chacune des études.

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Chapter 1

General introduction and objectives of the thesis

Chapter I: General introduction and objectives of the thesis

Until the point 1.3., the beginning of this chapter is an adapted version of the article:

Grégoire Noël, Julien Bebermans, Nicolas Gengler, and Frédéric Francis (2018). "Role of the disease transmission in pollinator decline – a review." *Faunistic Entomology*, 71. doi:10.25518/2030-6318.4096

Abstract - The loss of global biodiversity has become a growing concern for our society. This multifactorial decline also affects pollinator community that ensures plants reproduction in our environment. Pollinators also provide an excellent ecosystem service to humankind, especially for food security and human well-being. The transmission of intra- and interspecific pollinator diseases is considered as factor of pollinator decline which is increasingly studied. The purpose of this review is to provide an update of the main causes of decline, with a particular focus on the impact of disease transmission. In addition to the synergetic effects of landscape degradation, pesticides, climate changes and invasive species, a relationship exists between the introduction of commercial pollinators into new environments and the emergence of new diseases. Through globalization of commercial trades, some pathogens have become substantial threats to pollinators community health. However, there are still significant gaps in knowledge of transmission mechanisms. Scientific and technological advances in this area would enable the authorities to establish more appropriate health regulations and thereby contribute to the protection of pollinators diversity as a whole.

1. General introduction

1.1. The insect collapse in the Anthropocene era

The human civilization has brought so much pressure on natural spaces and planetary dynamics that we have entered a new geological era called the “Anthropocene” (Zalasiewicz et al. 2011). In the last 70 years, human activity exponentially affects the biodiversity of organisms by increasing the natural habitat transformations, the spreading of invasive species, the pollution agents, and the extinction rates. The human activity on Earth re-organized non-linearly the species richness and the species communities, their population size driven by diverse ecological processes such as speciation and colonization (Storch et al. 2022). For example, the installation of the London Underground railway system caused genetic separation between surface and subterranean populations of the mosquito *Culex pipiens* L. 1758 (Diptera: Culicidae) (Byrne and Nichols 1999). Moreover, the biodiversity dynamics have a direct impact on the multifunctionality of ecosystems and consequently on the so-called "ecosystem services" that it provides **(Figure 1-1)**. The global depletion of diversity and abundance of organisms has become a real concern for society (Pimentel et al. 1997; Pereira et al. 2010; Cardinale et al. 2012; Naeem et al. 2012).

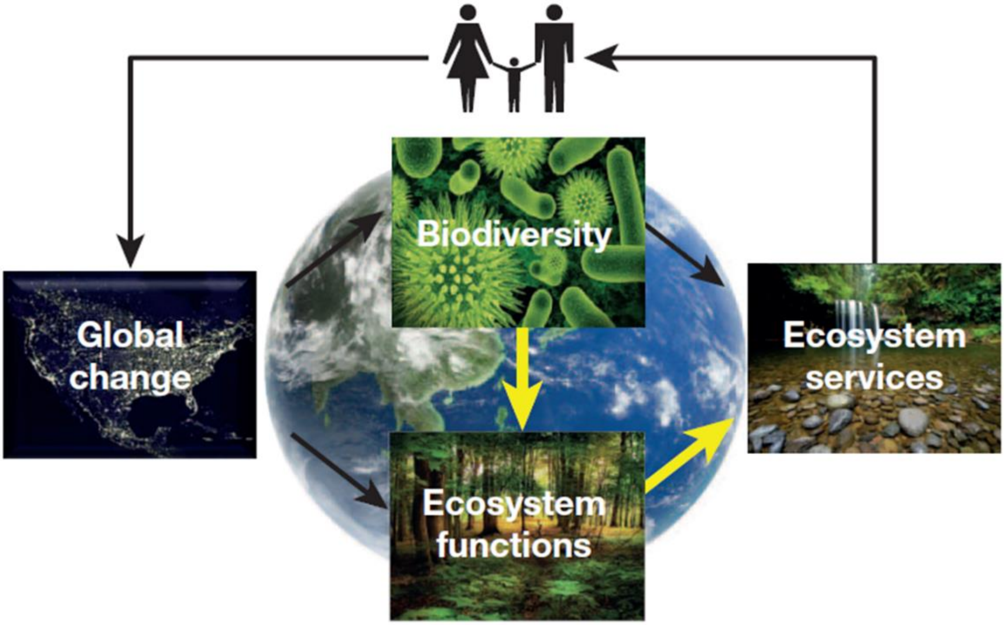


Figure 1-1: Ecosystem main relations from Cardinale et al. (2012)

Following the 6th mass extinction introduced by Barnosky and colleagues (2011), the collapse of insect community is another significant threat to biodiversity and our ecosystem health (Sánchez-Bayo and Wyckhuys 2019). Insects are implicated in many ecosystem functions such as wild plant pollination, organic matter decomposition, water depollution … (Noriega et al. 2018). Their exposition is considerable because the number of described insect species exceeds 1.1 million, and recent estimates suggest that there could be between 5.4 to 7.2 million insect species worldwide (Stork et al. 2015). Recent publication has also shown that flying insect biomass is drastically decreasing on the last 27 years in temperate ecosystems (Hallmann et al. 2017). A recent study, which used data from Hallmann et al. (2017), found a correlation between the decline of insect biomass and species richness decline, specifically in the hoverfly community in German nature reserves between 1989 and 2014. The study revealed that even common hoverfly species are at risk of extinction, highlighting the need for reevaluating conservation measures that currently focus mainly on rare and endangered species (Hallmann et al. 2021). However, obtaining quality data on insect species richness requires a significant investment of time, resources, and expertise, making these studies rather rare. Experienced entomologists, who have spent decades conducting inventories and describing insect species, are in agreement about a significant decline in insect abundance (Aberlenc et al. 2021). This trend is observed even in landscapes with minimal human impact. Moreover, biomass is not always a proxy for estimating the species diversity of insects (see Vereecken et al. 2021).

1.2. The insect pollinator decline

According to the report of the International Platform on Biodiversity and Ecosystem Services (IPBES), pollination and pollinator issues are emerging as one of the most worrying environmental issues of the 21st century (Gilbert 2014). Over 85% of the world's plant species rely on animal pollination for sexual reproduction (Ollerton et al. 2011). At the agricultural level, 75% of the world's major crop diversity depends on flowering plant zoogamy (Klein et al. 2007). The ecosystem service provided by pollinators is estimated to be worth 153 billion euros per year, which represents 9.5% of global agricultural production in 2005 (Gallai et al. 2009). In Belgium, this service for food production was equivalent to 252 million euros in 2010 (Jacquemin et al. 2017).

1.2.1. Generalities on pollinators

Pollinators are mainly insects. Plants that depend on them are said to be 'entomophilous'. This mode of pollination is predominant in the temperate regions (Ollerton et al. 2011). Some vertebrate groups or families such as some bat species and birds (e.g., hummingbirds) are also involved in pollination, especially in tropical regions (Kunz et al. 2011; Wolowski et al. 2013). Insect pollinators belong to four major orders: Hymenoptera, Diptera, Lepidoptera and Coleoptera (Sommaggio 1999; Branquart and Hemptinne 2000; Vaissiere et al. 2005).

The bees

Among Hymenoptera, bees of the superfamily Apoidea are the most efficient group of pollen transporters (Michener 2007). For hundreds of millions of years, bees have co-evolved with flowering plants and played a key role in their diversification (Harder and Johnson 2009; Gómez et al. 2015; Sauquet et al. 2017). Thus, bees have developed biological adaptations over time that make them more efficient. For example, from a morphological point of view, they are covered with gill hairs, specialized morphological features such as scopas (e.g., in *Anthophora* spp. or in *Andrena* spp.) or corbiculas (e.g., in honeybees or social bumblebees) which allow them to maintain and transport large quantities of pollen (Falk 2015). Behaviorally, bees acquired a diet consisting solely of pollen, nectar and sometimes oils produced to make them loyal to floral attractions (Michener, 2007). Scientific evidence is increasingly pointing towards the crucial role of bacterial and fungal microbes from pollen and nectar matrices in the larval development and overall fitness of bees (Dharampal et al. 2019; Rutkowski et al. 2023). Worldwide, more than 20,000 bee species are distributed in 7 families (Michener 2007; Danforth et al. 2013). In Europe, there are 2,051 species (Rasmont et al. 2017) divided into 77 genera, of which more than 403 species have been recorded in Belgium (Drossart et al. 2019). Within this global diversity of bees, about 20 species of social or solitary bees have been domesticated for agricultural production (Stout and Morales 2009). The most common bee species is *Apis mellifera* L., commonly known as the honeybee which is domesticated most of the time while after swarming there is some feral colonies (Pirk et al. 2017).

The hoverflies

Although often overlooked by the scientific community, Diptera (flies) are vital pollinators of flowering plants. More than 150 families of Diptera have been recorded (Evenhuis et al. 2008; Orford et al. 2015), with almost half of these families visiting flowers to collect nectar and pollen for sustenance and development of their sexual organs, respectively (Hickman and Wratten 1996; Kearns 2002). Studies by Rader et al. (2016) have revealed that Diptera pollinators perform a significant number of floral visits, accounting for between a quarter and a half of all floral visits in agricultural systems, compared to bees. Diptera species are also capable of providing pollination services at lower temperatures, higher latitudes, and higher altitudes than bees (Elberling and Olesen 1999). One particular family of Diptera, the Syrphidae or hoverflies, stands out due to their unique ecological features. They contribute ecosystem service in all landscapes by their dual role: pollinator at adult stage and predator of pest at larval stage (Dunn et al. 2020). Also, for other species, the larvae can provide other ecosystem services such as organic matter decomposition or water filtering (Sommaggio 1999). Due to the high range of habitat/diet requirement for the larvae, the hoverfly can be used as bioindicator to assess the human activities on natural ecosystems (Sommaggio 1999, 2014; Ricarte et al. 2011). Therefore, hoverflies are also ideal organisms for studying the effects of agricultural intensification as they are highly mobile compared to many other insect groups (Menz et al. 2019; Wotton et al. 2019). However, the diverse habitat requirements of hoverfly larvae make them vulnerable to the reduction of landscape diversity caused by large-scale monocultures in many parts of Europe, which have destroyed important micro-habitats such as ponds, hedgerows, and copses that are crucial for their survival (Sommaggio, 1999). With more than 6,000 described species and distributed in 284 genera worldwide (excluding Antarctica), hoverflies are a significant insect group. In Europe, over 970 species have been identified, with 357 species present in Belgium (F. Van de Meutter, personal communication). They also improve the quality and quantity of fruiting regardless of the rate of visits made by bees (Garibaldi et al. 2013).

In contrast, the transfer of pollen grains from stamens to recipient stigmas is less efficient than bees (Thorp 2000).

The apoid wasps

Apoid wasps, similar to bees, belong to the superfamily Apoidea. They are considered the sister group of bees and include families such as Heterogynaidae, Ampulicidae, Crabronidae, and Sphecidae (Danforth et al. 2013). However, recent molecular phylogenies have led to the upranking of some subfamilies of apoid wasps to the family level such as Philantidae family (Sann et al. 2018). Apoid wasps exhibit remarkable morphological, behavioral, and ecological diversity. While they generally follow a solitary life cycle, some species nest in aggregations. Most apoid wasps are predatory, hunting arthropod prey to feed their offspring or acting as brood parasites by depositing their eggs in the nests of other carnivorous wasps. In contrast, adult apoid wasps primarily feed on nectar, sap, or honeydew, and some also consume body fluids of prey to sustain their metabolic activities (Bitsch and Leclercq 1993; O’neill 2008).

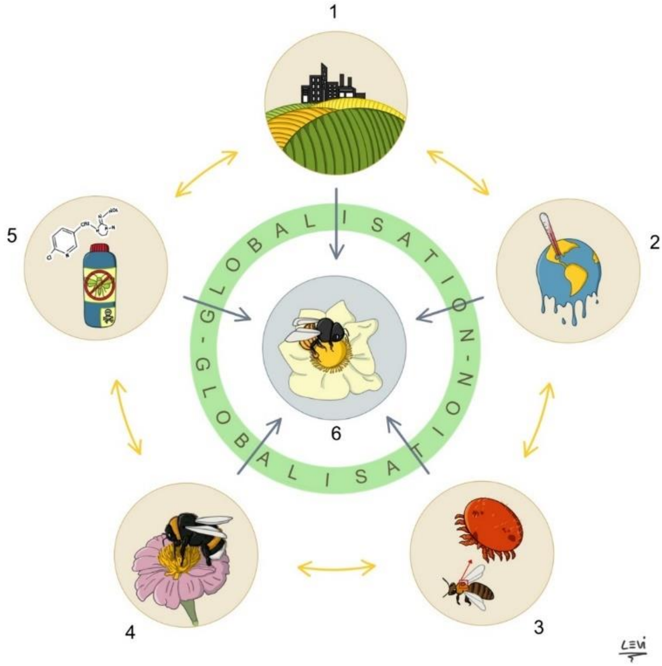
1.2.2. Is there evidence(s) of the insect pollinator decline?

Since the end of World War II, all continents except Antarctica seem to be affected by the decline of pollinator biodiversity. This decline generally affects wild pollinator populations (Allen-Wardell and Others 1998; Kearns et al. 1998; Warren et al. 2001; Donaldson 2002; Biesmeijer et al. 2006; Oldroyd and Wongsiri 2006; Goulson et al. 2008; Freitas et al. 2009; Forister et al. 2010; Keil et al. 2011; Meeus et al. 2011; Cameron et al. 2011; Scheper et al. 2014; Theisen-Jones and Bienefeld 2016). Furthermore, other studies on the evolution of pollinator diversity along gradients of environmental disturbances such as urbanisation (Fortel et al. 2014a; Geslin et al. 2016), agricultural intensification (Kennedy et al. 2013; Le Féon et al. 2013), etc… support the hypothesis of a generalisation of this decline. Concerning the honeybee, certain regions such as the USA or Central Europe are also affected by considerable losses (Haubruge et al. 2006; Potts et al. 2010b; vanEngelsdorp et al. 2011). Over the years and across various countries, the colony losses incurred during winter display a wide range of fluctuations, varying from high to low (Van Der Zee et al. 2012, 2014; Brodschneider et al. 2016). Therefore, colony losses in the overwintering period (Döke et al. 2015) has direct economic consequences for the pollination of early crops and orchards in temperate ecosystems (Degrandi-Hoffman et al. 2019). However, the global number of honeybee colonies has steadily increased by 45% over the last 50 years. This global increase is particularly driven by regions that dominate the world honey market such as China, Turkey, and Argentina, which outweighs the colony losses in other more affected regions (Aizen and Harder 2009).

Despite the studies carried out, it is still difficult to clearly perceive the geographical amplitude and intensity of the decline due to a lack of data as well as a lack of bee taxonomic experts which also in decline as published by the European Red List of insect taxonomist (Hochkirch et al. 2022). Moreover, there are few meta-analyses that jointly address (i) the evolution of biodiversity considering all taxonomic groups of pollinators, either in terms of abundance or species diversity; (ii) the phenology of pollinators (e.g., spring bees, summer bees for temperate regions); (iii) over time spans of several decades (but see Biesmeijer et al. (2006) and Carvalheiro et al. (2013)); (iv) robust and standardised survey methodologies in order to be able to compare data across the diversity of studies (Westphal et al. 2008). Dataset on pollinator diversity and especially abundance remain sparse, except for a few taxonomic groups or geographical regions (Freitas et al. 2009; Vanbergen et al. 2013; Nieto et al. 2014). As an example, a red list has been established for the wild bees of Europe. The authors of this list estimated that more than 50% of the data on wild bees is deficient due to a lack of thorough monitoring (Nieto et al., 2014). Great Britain, the Netherlands, and Belgium have well-established databases that facilitate monitoring of pollinator distribution through online platforms such as BWARS or Atlas Hymenoptera (Rasmont and Haubruge 2002; BWARS 2021). Thanks to remarkable collection efforts, Drossart et al. (2019) and Vereecken et al. (2022) were able to red-list Belgium and Brussels with only 9% and 1% data deficiency, respectively, in terms of bee collection.

1.2.3. A diversity of causes

The scientific community agrees that the global decline of pollinators is multifactorial in origin (Potts et al. 2010a; **Figure 1-2**). Several sources of disturbance interact in a complex spatio-temporal context and could act in synergy with undefined cocktail effects (Vanbergen et al. 2013; Goulson et al. 2015a). Besides the influence of pesticides, climate change, pathogen transfer and the introduction of invasive species, the main cause of pollinator loss seems to be landscape degradation mainly driven by urbanization process and agricultural intensification which convert natural



spaces to anthropogenic areas **destroying essential floral and nesting resources** (see point 1.3.) for insect pollinators and their offspring (Brown and Paxton 2009; Potts et al. 2010a).

Figure 1-2: Main factor of pollinator decline and their interactions (yellow arrow). (1) impact of the landscape degradation (i.e., agriculture intensification and urbanization); (2) impact of the climate change; (3) impact of the disease transmission; (4) impact of the invasive species introduction; (5) impact of the pesticides; (6) impact of the trade exchanges globalization which favor the intensity of the other decline factors.

Landscape degradation:

Landscape heterogeneity shapes biodiversity and associated ecological processes (Wiens 2002). The functional importance of landscape heterogeneity for biodiversity stems from different ecological requirements of organisms in terms of food sources and habitats (Fahrig et al. 2011). The floral sphere offers a range of food resources such as pollen, nectar or certain oils produced by flowers necessary for the life cycle of pollinators and especially bees (Falk 2015). Having co-evolved intimately with bees (Gómez et al. 2015), flowers also have a fascinating array of attraction strategies and biological adaptations. Colour combined with floral architecture (corolla shape, symmetry...), the bouquet of attractive odours are all functional traits responsible for mutualistic plant-pollinator relationships (Fornoff et al. 2017). In Belgian temperate regions, these floral resources must be present in sufficient quantities according to food requirements (for offspring and adults) and specific foraging periods, between the end of winter for the earliest bees such as *Osmia cornuta* (Latreille 1805) or *Bombus terrestris* L., and the beginning of autumn for the latest such as *Colletes hederae* (Schmidt & Westrich 1993).

On the other hand, the presence of nesting sites as well as suitable materials (mud, petals, leaves, etc.) for its building are as important as the available food resources (Potts et al. 2005; Michener 2007). Most bee species nest in the soil. Each ground-nesting bee species requires certain soil characteristics for nesting: vegetation rate, texture, moisture, soil slope, presence of already formed nests, etc… (Cane 1991). The other part of the bee species nests in cavities outside the soil. Depending on the species, they need empty snail shells, dry or fresh stems, holes in wood, cracks in building or road materials... (Fortel et al. 2016; Vereecken 2017). Therefore, a homogenisation of the local landscape inevitably leads to a depletion of both types of resources (floral resources and nesting sites). In rural landscapes, a meta-analysis by Kennedy et al. (2013) showed that, along a gradient of agricultural intensification, the species richness and abundance of wild bees were higher on farms that reduced their use of chemical inputs compared to those using conventional farming practices. The supply of habitats and food resources is favoured by a richer and more complex composition and configuration of semi-natural environments, for example by planting hedges or permanent grasslands (Morandin and Kremen 2013).

Also, the increasing establishment of entomophilous mass flowering crops can lead to spatial and temporal changes in the landscape. The example of monocultures of rapeseed, *Brassica napus* (L. 1753), illustrates this phenomenon of landscape homogenisation (Holzschuh et al. 2011; Kovács-Hostyánszki et al. 2017). Indeed, the flowering time of rapeseed fields offers abundant pollen and nectar resources accessible to pollinators over a period of about one month. This huge temporary peak in available resources favours generalist pollinators and can completely rearrange pollination networks at the landscape level. The flowering period of oilseed rape can also disadvantage the reproduction of neighbouring plants by changing the foraging activity of shared pollinators (Kovács-Hostyánszki et al. 2013; Holzschuh et al. 2016). For example, the study by Holzschuh and colleagues (2011) showed that populations of officinal primrose, *Primula veris* (L. 1753), plants adjacent to oilseed rape crops, showed a 20% reduction in seeds produced due to a dilution of bumblebee populations (main pollinators of officinal primrose). In addition, some populations of wild bees belonging to the family Megachilidae were stimulated by persistent floral and nesting resources offered by semi-natural habitats around the massive oilseed rape blooms. Diekotter and colleagues (2014) concluded that mass flowering crops can maintain solitary bee communities only if semi-natural habitats are present nearby.

Moreover, urbanisation also implies important changes in the landscape through an increase in impervious surfaces and a decrease in green spaces (forests, meadows, wastelands...). Urban areas can be refuges for a significant pollinator community (Hall et al. 2017), but only if these areas have a sufficient cover of natural environments. Studies have shown that the abundance and diversity of wild bees decreases with increasing urbanisation (e.g. in Ahrné et al. 2009; Fortel et al. 2014; see point 1.5.).

Pesticides:

The combination of the degree of exposure and the degree of toxicity of a pesticide constitutes the main risk for pollinators. The latter depends on the ability of the individual or species to detoxify these compounds, its ecological habits (floral preference, foraging frequency), the chemical nature of the active molecules, agricultural practices, and interactions with other stressors such as a decrease in floral resources or the emergence of pathogens (Vanbergen 2013; Goulson et al. 2015a; Collison et al. 2016; Barascou et al. 2021).

Furthermore, the assessment of actual exposure in the field is complicated as it depends on the ecology and foraging behavior of different pollinator species in different landscape contexts. Sub-lethal effects on individually targeted insects may intensify particularly within bee colonies and populations (domestic and wild), especially over the long term (Fairbrother et al. 2014; Woodcock et al. 2016). The synergistic potential and 'cocktail' effects of pesticide mixtures on pollinator communities remain largely unknown. Moreover, most studies on sub-lethal effects of these pesticides have mainly been conducted on few pollinator species and on a small range of chemical compounds (Heimbach et al. 2017). As examples, the cases of neonicotinoids and sulfoximines are discussed below.

In recent years, the direct effects of pesticides on bees, particularly insecticides, have attracted the simultaneous attention of scientists, politicians, and the public. Neonicotinoids are a class of systemic chemical compounds used, among other things, as seed coatings to control insect pests, usually of the biting-sucking type (aphids, thrips, etc.). Neonicotinoids target the central nervous system of targeted insects and can therefore potentially influence pollinator health (Henry et al. 2012; Whitehorn et al. 2013) and impact the ecosystem service of pollination (Stanley et al. 2015). Indeed, the neurotoxic molecules present can concentrate in nectar and pollen (Blacquière et al. 2012) and thus cause irreversible damage to the nervous system of exposed bees, resulting in spatial disorientation during foraging periods (Gill et al. 2012; Fischer et al. 2014; Gill and Raine 2014). Neonicotinoids can also disrupt the reproductive and overwintering phases of affected bee populations (Rundlöf et al. 2015; Fauser et al. 2017).

In addition, the sulfoximines represent a recent class of neurotoxic insecticides acting on nicotinic receptors. One member of this family of insecticides is sulfoxaflor (Sparks et al. 2013), which has been used as a replacement for neonicotinoids in particular against biting crop-sucking pests since cases of resistance to the latter have emerged (Bass et al. 2015). In addition, these chemicals have potentially similar sublethal effects to neonicotinoids (Rundlöf et al. 2015), which may also impact a considerable diversity of insects and agroecosystems (Simon-Delso et al. 2015; Brown et al. 2016). The impact of this family of chemical compounds is likely to be accurately assessed only after several years of use, leaving a high margin of potential negative effects on pollinator biodiversity. They are therefore suspected to be able to contribute to the intensification of pollinator decline (Brown et al., 2016).

Climate change:

Nowadays, the progressive effects of climate change are increasingly quantified and modelled worldwide. As a result, the understanding of their impacts on pollination and pollinators is better interpreted and has evolved significantly (Settele et al. 2016). The increasing frequency, magnitude, and intensity of some extreme weather events (such as heat waves or droughts) may accelerate pollinator declines (Brown et al., 2016). It is becoming clear that such climate events would rapidly lead to extinctions of wild plants and associated pollinators (Rasmont and Iserbyt 2012; Oliver et al. 2015). Thus, it would be interesting to expand our knowledge in this area, especially regarding the relative importance of extreme climatic events to improve the management of habitats dependent on animal pollination. Indeed, pollinator communities are intrinsically linked to the spatial and temporal distribution of floral resources (Potts et al. 2003, 2006; Kremen et al. 2007). Climate change therefore has the potential to restructure or simplify plant-pollinator interaction networks (Schweiger et al. 2010).

Temperature plays a major role in insect and plant growth. Climate change can therefore induce temporal divergences between flowering and the emergence of associated pollinators (Hegland et al. 2009). For example, a meta-analysis by Parmesan (2007) showed that the emergence or arrival of migratory species of butterflies was three times faster than the first flowering of the herbaceous layer. There is therefore a risk that this phenological decoupling could lead to reductions in the availability of floral resources for pollinators and at the same time a reduction in the pollination of associated host plants. However, these considerations must take into account that the structure of plant-pollinator relationships is highly resilient: (i) there is an asymmetry of interaction between specialists and generalists, specialist pollinators would visit generalist plants and vice versa (Bascompte et al. 2003; Fontaine et al. 2011) and (ii) pollinator generalism (polylecticism) is more widespread than specialism (i.e., oligo-, monolecticism) in interactions with flowering plants (Petanidou et al. 2008). Generalist pollinators are therefore more easily able to adapt to changes in floral composition than specialist pollinators, which are more likely to be impacted by climate change (Roberts et al. 2011).

Among other things, climate change has the potential to restrict or expand the ranges of species. Pollinators with a lower migratory capacity will tend to see their range shrink, but for some species that are able to keep pace with climate change, their range may expand (Thomas et al. 2006). Climate predictions are already available for the spatial and temporal distributions of bumblebees in Europe and North America and of European butterflies (Settele et al. 2008; Kerr et al. 2015; Rasmont et al. 2015).

Floral chemical ecology is also involved in plant-pollinator relationships through complex odor clusters associated with nectar and pollen. Variations in temperature and elevated atmospheric carbon dioxide concentration are thought to impact the chemical ecology of plants and the composition of nectar and pollen produced (Griffin et al. 2009; Farré-Armengol et al. 2014; Parachnowitsch and Manson 2015). For example, butterflies feeding on nectar with high concentrations of amino acids lay more eggs and thus increase their fitness than those that took nectar with lower concentrations of amino acids (Mevi-Schutz and Erhardt 2005). Hoover et al. (2012) also concluded that the joint effects of increased atmospheric CO2 and temperature could affect the morphology, phenology, and nectar composition of the flower, thus altering its attractiveness to pollinators. This research topic is still little explored and scientific investigations are underway.

Invasive species introduction:

The ecological effects of invasive species on native pollinators remain complex and still poorly defined. But these can be significant under certain ecological and biogeographical circumstances (Graystock et al. 2016). Invasion or introduction of plant or animal species can act at different trophic scales and thus alters native networks of plant-pollinator interactions. The presence of these new species in an environment also offers new risks of emergence and spread of pathogens from exotic species to native species.

Some entomophilous invasive plants are easily integrated into the plant-pollinator interaction networks of the environment and become additional sources of nectar and pollen for native pollinators (Aizen et al. 2008; Stout and Morales 2009). In general, these exotic plants offer some protection against nectar and pollen shortages under changing climatic conditions (Schweiger et al. 2010). In Europe, a significant proportion of invasive plants are ornamentals (Lambdon et al. 2008) with long flowering times, which can potentially extend the foraging periods of our pollinators (Aizen et al. 2008). The positive effects of invasive plants are limited to generalist pollinators that are more resilient to floral changes (Traveset and Richardson 2006). However, in some cases, the integration of exotic plants into a new ecosystem can dominate plant-pollinator interactions and have a detrimental impact on native floral communities by decreasing their density and diversity (Pyšek et al. 2012; Traveset and Richardson 2014).

The use of pollinators to ensure the pollination of entomophilous crops and increase yields and product quality (almonds, peaches, tomatoes...) has become over time increasingly common (Velthuis and Doorn 2006; Garibaldi et al. 2016). Indeed, many honeybee colonies have also been massively introduced into agricultural and natural ecosystems for the ecosystem service of pollination and honey production (Moritz et al. 2005; Freitas et al. 2009; Graystock et al. 2016). However, some studies show that increasing honeybee hive density can change the floral preference of some wild bumblebees (Forup and Memmott 2005; Walther-Hellwig et al. 2006) or decrease the abundance of wild pollinators present (Lindström et al. 2016). According to a meta-analysis of the literature by Mallinger et al. (2017), the effects of the environmental footprint of honey bees are mainly negative on wild bees, either through competition for floral resources or through disease transmission.

Pathogen and disease transmission:

The dynamics of diseases and their modes of transmission generate impacts at the individual, colony, population, and community levels of pollinators. Parasites and pathogens have their own life cycles. These pathogens can become problematic during overpopulation, domestication, and introduction into new environments (Cameron et al. 2011; Graystock et al. 2016; Geslin et al. 2017).

During the years 2006-2007 and 2007-2008, the USA, for example, experienced considerable and unexplained losses of honeybee colonies. In the absence of known causes, this syndrome was called "Colony Collapse Disorder" (CCD; vanEngelsdorp et al. 2009). While populations of *Varroa destructor* (Anderson and Trueman 2000) and *Nosema ceranae* (Fries et al. 1996) were insufficient to cause a decline in a honey bee colony, it would appear that colonies with CCD showed a higher prevalence and positive relationship with high levels of pathogens (mainly viruses) compared to other weak colonies without CCD and to healthy colonies. A combination of these different pathogens, most of which would be carried by *V. destructor*, would potentially be responsible for this type of syndrome (Cornman et al. 2012).

As another example, Cameron et al. (2016) demonstrated that by examining bumblebee specimens from the collections of various North American museums, the prevalence of *Nosema bombi* (Fantham & Porter 1914), a pathogenic microsporidia specific to the bumblebee group, has increased significantly over the last 20 years. Although there is no certainty regarding the origin of the pathogen strains, the increase in prevalence of the pathogen in declining bumblebee species coincides with the domestication of European bumblebee species in the USA in the mid-1990s such as *Bombus terrestris* complex. This temporal relationship between the arrival of commercial *Bombus* species and the onset of pathogen increase in wild populations would be an important cause of the decline of wild bumblebee populations in North America (Brown 2017).

Trade exchanges globalization:

Changes in human population growth, the consumer system, globalization of trade and less stringent environmental regulation act as indirect factors in the decline of pollinators and thus the impoverishment of the pollination service provided (Watson 2014).

Agriculture is expanding and intensifying to reconcile food and energy supply with population growth (Tilman et al. 2011). In addition, policy decisions play a direct role in land use and agricultural practices. These decisions can significantly influence the composition and configuration of the landscape. The consolidation of agrochemical companies (those producing seeds, fertilizers, and pesticides) is seen as one of the most important issues of the coming years. These industries control access to land and the management of agricultural practices (Howard 2009; Brown et al. 2016). Indeed, these companies favor agricultural systems that generate homogeneous products and cover increasingly large areas. This trend is particularly prevalent in developing countries through new transnational economic agreements. The expansion of maize and soybean over large areas in the USA and Brazil, and palm oil in West Africa and South-East Asia are good examples of this trend (Meade et al. 2016; Pirker et al. 2016).

International trade is also a key player in the shift of land use from natural or semi-natural environments to intensive and industrialized agriculture. It plays a major role in the introduction of new pollinator species, thus offering the possibility for a multitude of pathogens to change host (Goka et al. 2001; Manley et al. 2015; Graystock et al. 2016). Moreover, currently there is no homogeneous regulation of pesticides at the international level. Most countries differ in their regulation of pest management practices. This implies negative impacts on ecological processes (Waterfield and Zilberman 2012). Pesticides banned in developed countries in North America or Europe are sold and exported to developing countries. They are mainly used on export crops (mango, pineapple, sweet potato...) and return to developed countries as residues in imported food products (Galt 2008).

1.2.4. Conclusion

Landscape degradation, pesticides, climate change, invasive species and disease transmission interact in complex ways across different temporal and spatial scales. They affect pollinators throughout their life cycle and no type of environment seems to be spared worldwide. This demonstrates how crucial it is to maintain pollinator diversity and abundance: on the one hand, to ensure the sustainability of our natural ecosystems and agriculture; on the other, to ensure our food security and daily well-being (Potts et al., 2016).

Although research initiatives and knowledge networks (e.g. BELBEES , STEP and BWARS projects) have shed light on some of the issues, many unknowns remain. Thus, increasing knowledge about the links between the different factors would allow to propose more adapted agri-environmental measures (AEM) and thus anticipate in a better way the consequences of pollinators' decline. This short analysis shows that the global decline of pollinators and biodiversity in general is indirectly catalyzed by the financial context of globalization. Capitalism and the principle of free trade have allowed the reduction of international tariff barriers and regulations, which has, among other things, favored the production and trade of pesticides across the world.

Therefore, the economic sphere must be integrated into the understanding of the decline of pollinators.

1.3. Floral and nesting resources

1.3.1. Floral resources

Currently, rapid changes in the structure of plant communities combined with their temporal trends, mainly related to global changes, occurred at diverse spatial scales such as in France (Martin et al. 2019) which can redefine insect pollinator assemblages by their intimate interactions (Bosch et al. 2009). Floral resources, including pollen and nectar, play a vital role in the life cycle of pollinators. Bees, for example, rely on pollen as a source of proteins for the development of their offspring, while nectar serves as their primary fuel for activities such as foraging, reproduction, and winter survival (Alaux et al. 2010; van Rijn and Wäckers 2016). Flowers have evolved to meet the nutritional needs of pollinators, rewarding them in exchange for their assistance in plant reproduction. For instance, in temperate regions, the development of solitary bees, such as *Megachile rotundata* (Fabricius 1787), involves the accumulation of larval provisions. These larval provisions, weighing approximately 90 ± 26 mg per larval cells, consist of approximately 47 ± 11% nectar sugar, 33 ± 5% pollen, and 20 ± 3% nectar water which is the results of the foraging of several thousands of flowers (Klostermeyer et al. 1973; Cane et al. 2011). In contrast, a honeybee colony requires around 120 kg of nectar and 40 kg of pollen per year, which translates to several hundred million rewarding floral visits (Seeley 1995). The availability of floral resources fluctuates in different locations and time periods, and this variability plays a crucial role in shaping the diversity of pollinators. Numerous studies have demonstrated that abundant, diverse, and high-quality floral resources enhance the survival, health, immunity, and detoxification abilities of pollinators. Bees, for example, can adjust their foraging strategies based on the quality of available resources. They can assess the quantity and quality of nectar (Cnaani et al. 2006) and actively search for pollen that contains essential amino acids (Cook et al. 2003).

1.3.2. The foraging ecology of *Apis mellifera* L. as particular case

As eusocial bee, honeybee colony present an interesting behavioral feature: the waggle dance (von Frisch 1965). When a worker bee discovers an interesting patch of pollen and nectar, she can recruit nestmates throughout this recruitment dance. The waggle dance performed by the honeybee scout inform the colony by three capital information: the distance from the hive, the direction regarding to the position of the sun and the odor of the targeted flower. The other workers can decode the distance by the duration of the founder buzzing, the direction by the angle that the founder made from the vertical axis of the comb, and the odor of the flowers at her forage site by her forager wax cuticle and the food that she brings home (Seeley 1995).

1.3.3. Nesting resources – Ecology of ground-nesting bees

According to Danforth et al. (2019), bees can be classified into five groups according to their nesting behavior: soil excavators or ground-nesting bees, wood excavators, squatter bees, epigeal builders and brood parasite bees. According to Ascher and Pickering (2023), there are 20,759 described bee species in the world.

Most solitary bee species build their nests underground, with ground nesters being more prevalent outside of tropical regions (M. Orr, personal communication). For example, all species belonging to the families Andrenidae, Melittidae and Stenotrididae (Australia) as well as the majority of Halicitidae and Colletidae species nest under the ground. Only the long-tongued bees, Apidae and Megachilidae, are content with the full range of nesting modalities. In Belgium, the spring bees (from March to the end of May) are mainly ground bees. These spring communities are generally composed of bees of the genera *Andrena* and *Lasioglossum*. Whereas the summer bee communities (early June to late September) offer a greater variety of nesting behaviors with the emergence of a series of species belonging to the Megachilidae family. Nearly 50% of the 403 species of wild bees present on our territory nest under the ground (Vereecken, 2017; Drossart et al., 2019).

At the beginning of their life cycle, female ground-nesting bees select and prepare their nest by digging underground galleries terminating in chambers or enlarged cells in which they supply larval resources (mixture of pollen and nectar) before laying the egg (Michener 2007; Vereecken 2017). In Belgium, these bees belong to the genera *Andrena*, *Anthophora*, some *Bombus*, *Colletes*, *Dasypoda*, *Dufourea*, *Eucera*, *Halictus*, some *Hoplitis*, *Macropis*, some *Megachile*, *Melitta*, some *Osmia*, *Panurgus*, *Rophites*, *Tetralonia* and *Trachusa* (Drossart et al. 2019). Species belonging to these genera need suitable nesting spaces and places to initiate their cycle. The soils sought by these bees generally vary from sandy to loamy in texture (Cane 1991). Occasionally, some clay soils may also support populations of ground bees (Plateaux-Quénu 1959; Barthell et al. 1988; Radchenko 1988). Although their living behavior is mainly solitary, it is not uncommon to observe an agglomeration of several distinct nests on the ground surface forming a so-called "bourgade" [in French] (**Figure 1-3**). Nest aggregations are likely to be formed by the common attraction of certain environmental factors and not by interactions between the ground-nesting bee individuals (Malyshev 1935; Plateaux-Quénu 1959). The nest aggregation may be composed of a single species or of several different species. Some of the nests of the nest aggregation may be parasitized by cleptoparasitic bees (e.g. of the genus *Nomada*). These colonies can persist for several years or even decades, if the foraging and soil conditions remain constant and appropriate over time. For example, settlements of *Anthophora plumipes* (Pallas 1772) and *A. vaga* have been observed on the same sites for more than 50 years (Ulrich 1956; O'Toole and Raw 1991). *A. vaga* which is the main solitary bees recorded in Brussels (Vereecken et al. 2022) has been present on the Kauwberg sandpit (Uccle) for more than 20 years (A. Pauly, personal communication). In terms of abundance, some settlements have been recorded with several million nests. For example, Blagovestchenskaya's study in 1963 recorded this phenomenon in the presence of *Dasypoda plumipes*, an old synonym for *Dasypoda hirtipes* Fabricius 1793, the pantaloon bee.

Figure 1-3: Main nest aggregation of *Andrena vaga* at Verrewinkel cemetery (Uccle, Belgium). Credit: A. Pauly.

Ground-nesting bees are masters in the art of excavation. Over the course of their evolution, they have developed morphological adaptations and behaviors that facilitate the excavation and construction of their future nests, such as:

- Large enlargements at the base of the hind tibiae to increase their speed of movement in their nest galleries (Radchenko and Pesenko 1996; Michener 2007).

- A reinforced pygidial plate on the sixth tergite in females. This is used as a trowel to move or compact the soil (tamping) during the construction of galleries or during the formation of larval cells at the end of secondary galleries (Radchenko and Pesenko 1996).

- If the soil is compact, females can use their flattened mandibles to break the soil surface (Radchenko and Pesenko 1996). However, they prefer a more granular soil where they can combine the action of their legs with that of their mandibles (Potts and Willmer 1997). If the substrate is too hard, they can wait for a rain event or regurgitate a water or nectar intake which will have the benefit of softening the soil (Butler 1967).

In general, ground-nesting bees reuse the nest of the previous generation or initiate a new nest construction close to their siblings. This nesting choice is a behavioral trait described as philopatric. Indeed, the advantage of this trait is that individuals do not allocate extra energy to locating a new nesting site and building a new underground structure with similar edaphic and landscape characteristics (Yanega 1990; Cane 1997). For example, quantitative data have shown that females of *Halictus rubicundus*, a pre-social species in our regions, overwinter away from their natal nest but return early the following spring to establish their own nest within 50 cm of their natal nest, thus ensuring a certain population structure conducive to social behavior (Yanega 1988, 1990). From population genetic structure point of view, *A. vaga* shows, for example, high inbreeding rates explained by highly philopatric behaviour (Černá et al. 2013). Despite this, the temporal and genetic persistence of these nest aggregations also favors parasitism by a plethora of insects: cuckoo bees of the genus *Nomada* (Apidae) or even *Sphecodes* (Halictidae), Diptera of the genus *Bombylius* or even Strepsiptera of the genus *Stylops* (Polidori et al. 2005; Straka et al. 2015). Some wild bee species may also hunt females that have previously dug their nests to take ownership. For example, the bee *Lasioglossum malachurum* (Kirby 1802) can usurp the ground-nesting populations of *Lasioglossum marginatum* (Brullé 1832) when the latter has completed its nest construction and started its work of feeding the larval cells (Plateaux-Quénu 1960).

The architecture of a nest is highly variable and often species-specific (Michener 2007; Danforth et al. 2019) but a typical ground bee nest generally consists of a main tunnel branched by secondary galleries in which one or more larval cells are erected (Malyshev, 1935, **Figure 1-4 A, B, C & D**). The number of larval cells per nest is dependent on the species and the surrounding abiotic and biotic conditions (Harmon-Threatt 2020). These hypogean nests, whether horizontal or vertical, are preferentially well drained and the entrance areas are generally free of vegetation (Cane 1991). Nests are on average 35 cm deep (Cane and Neff 2011). A certain depth allows for freedom from external temperature fluctuations, especially for the offspring. For example, a nest of L. marginatum at 65 cm depth is exposed to a constant temperature of 11.6°C without varying over the nycthemeral cycle (Plateaux-Quénu 1959).

Generally, the process of building a ground bee nest is as follows: location of the nesting site, construction of a main duct and secondary ducts with damage to the galleries. At the end of these secondary galleries: development of larval cells, supply of nectar and pollen and egg laying, sealing of the cells and sealing of the nest (Plateaux-Quénu 1959; Harmon-Threatt 2020). In most ground bee species, females line their larval cells with a cellophane-like substance to prevent desiccation, flooding or bacteria and fungi (Albans et al. 1980; Almeida 2008). The complexity of the nest may also evolve as it is reused by future generations through philopatric behavior (**Figure 1-4 E**) and population growth (Plateaux-Quénu 1959; Sakagami F. and Hayashida 1960).

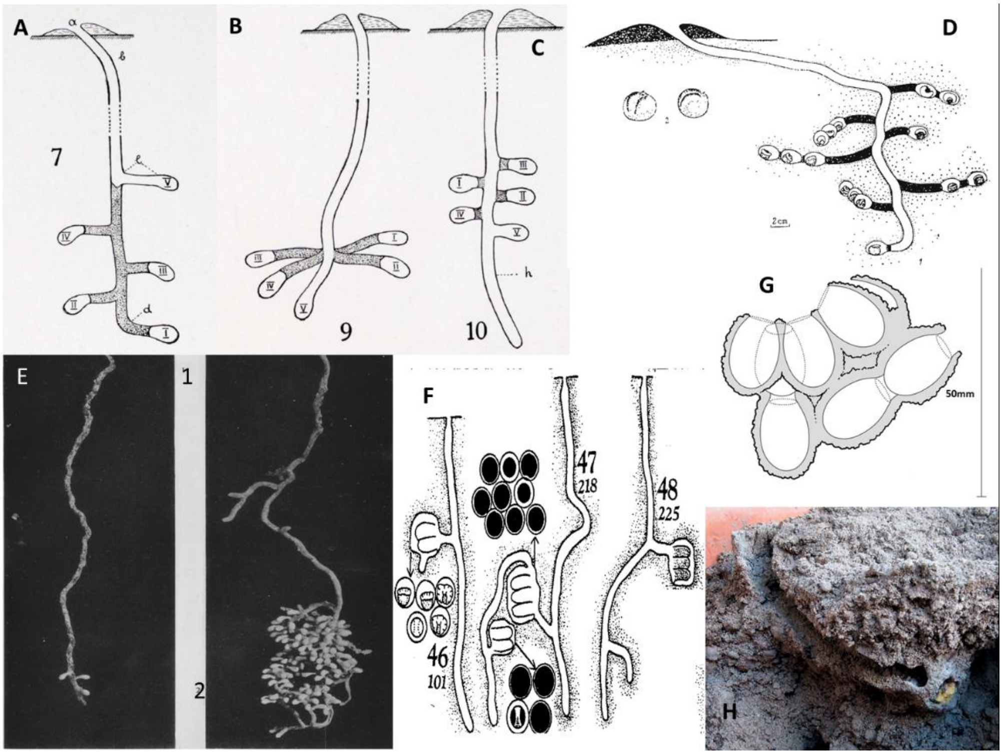


Figure 1-4: Diversity of nest architectures of ground-nesting bees with their larval cells. A: Branched nest of *Colletes cunicularius*, (a,b) entrance to the main duct, (e) lateral ducts, (d) lower section of the nest (Malyshev, 1935). B: Branching nest of *Andrena vaga* (Malyshev, 1935). C: Branched nest of *Halictus sexcinctus* (Malyshev, 1935). D: Nest structure of *Dasypoda braccata* Eversmann 1952 (Radchenko 1988). E: Picture of a clustered nest of *Lasioglossum marginatum* (1) first year and (2) last year of nesting (Plateaux-Quénu, 1959). F: Several nests of *Halictus duplex* Della Torre II (Sakagami F. and Hayashida 1960). G: Structure of the larval cells of *Anthophora plumipes* (Loonstra 2012). H: Main duct of the nest of *Anthophora plumipes* (Loonstra 2012).

The nesting site of ground bees is subject to many abiotic and biotic pressures that can influence the size of the colonies, their survival, or the composition of their community. Soil texture and vegetation cover are the main environmental characteristics reported in the scientific literature (Linsley 1958; Cane 1991). Sandy soil species, also called psammophilous species, represent the main group of the ground-nesting species. For example, the oligolectic species *A. vaga* will nest near willows or willow trees (*Salix* spp.) in sandy-textured, south-facing soils (Vereecken et al. 2006). Other factors such as pH, relative humidity, drainage, or slope of the soils in which the bee nest is more rarely measured (see below).

Soil texture:

Soil texture influences the available oxygen, water content and temperature in the nest, which are essential characteristics to ensure survival and development of the offspring (Fellendorf et al. 2004). Very few studies quantify the texture of the soil of the ground-nesting species, most of the time this notion remains qualitative of the environment, differentiating sandy soils from clay soils, whereas there is a great variety of different textural classes which lie on the classification system use (USDA, …). Taking qualitative measurements of soils would miss out on a detailed understanding of their nesting (Harmon-Threatt, 2020). Furthermore, through their specific ecological niche, it is possible that a ground bee species can tolerate a wide range of different textures to adapt to environmental conditions, as may be the case in urban areas. This issue is relatively unstudied at this level of detail in ground bee populations and communities (but see Cane, 1991; Potts & Willmer, 1997). At the same time, host structures for ground bees, such as soil squares (1m^2) with different grain sizes, have attracted homogeneous communities, demonstrating that these bees can accept a wide range of soil textures from clay to sand (Fortel et al. 2016). In conclusion, although soil texture is important for nesting, this characteristic appears to be variable across species and unknown for most species. Furthermore, the observed preference for sandy soil may be an observation bias as sandy soils generally reduce vegetation which may make nests easier to observe and excavate (Harmon-Threatt, 2020).

Soil cover:

Soils not covered by vegetation are generally more exposed to variations in temperature, moisture and sunlight (Anderson and Harmon-threatt 2016). These abiotic characteristics can be critical in nesting site selection. In addition, if this ground cover is accompanied by visual markers such as a stony carpet, this may help some species to better locate their site (Cane 2015). As with soil texture, it is difficult to determine whether nest development on non-vegetated soils is due to observation bias through the presence of eye-catching ground bee colonies. However, soils with low percentages of vegetation are generally associated with hypogeous nesting (Sardiñas and Kremen 2014).

Other abiotic conditions:

Soil compaction and volumetric weight, slope, pH or even the heterogeneous presence of visible markers can influence nest site selection, development and survival of nest aggregations (Stephen 1960, 1965; Osgood Jr 1972; Potts and Willmer 1997; Wuellner 1999; Sardiñas and Kremen 2014; Cane 2015). Again, we have relatively little knowledge as most studies are not quantitative, target only one species, contain few replicates or focus on one or few localities which makes it difficult to determine potential variation in nesting characteristics at intra- and inter-specific levels (Harmon-Threatt, 2020). In addition, these investigations are generally very time consuming.

In conclusion, the major challenge in understanding the edaphic preferences of ground bees is to be able to adapt management strategies and landscape, urban or agricultural developments by considering the tolerance of the species with respect to the environmental conditions around the nesting site. With the aim of preserving biodiversity, more consistent studies on these fundamental themes must be launched.

Bees nesting under the urban pavement:

The influence of urbanization on wild bee communities is still poorly understood, with some studies showing beneficial effects, others not, and some reporting no significant effects. It is recognized that urbanization leads to a considerable increase in impervious surfaces (pavements, buildings...) which reduces the availability of nesting substrate for ground bees (Wenzel et al. 2020). Furthermore, Cane (2005) stated that nesting opportunities are limited by excessive paving. However, observations already made in the Brussels-Capital Region (BCR) show that some pavements can, on the contrary, become a nesting opportunity.

The ground bee species identified as nesting in pavements in the BCR are *D. hirtipes, Panurgus calcaratus* (Scopoli 1763), *Lasioglossum laticeps* (Schenck 1868), *Andrena barbilabris* (Kirby 1802) and its cleptoparasite *Nomada alboguttata* Herrich-Schaffer 1839 (Pauly 2019a). Although no rigorous study has yet been conducted on the ecology of pavement bees, several observations (Haeseler 1982; Pauly 2019; J. Durieux pers. comm.) suggest that older pavements with sandy soil underneath and uncemented joints are the most hospitable to ground bees. For example, a large population of *D. hirtipes* is present every year in Watermael-Boitsfort (Brussels, Belgium) on the pavements of the Colonial Avenue. The good drainage of the unbound flexible pavements, the recurrent use of sand within the structures and the thermal capacity of the modular elements to capture heat probably contribute to the potential of pavements to host ground bees, providing them with a similar habitat in an urban environment.

Furthermore, the presence of bees on the road sometimes causes concern among residents and can result in the destruction of nests using insecticides or waterproofing re-pavement to make the bees disappear. These are often confused with other Hymenoptera that can also nest in pavements, such as wasps belonging to the families Crabronidae (see point 1.2.1.). In addition, nesting sites can also suffer from inadequate management, mainly due to a lack of knowledge on the part of field workers, such as the use of organic solvents to wash pavements, which prevents ground bees from recognizing the entrance to their nests (Cane 1997). Entire nest aggregation can also disappear overnight following the repair of a pavement deemed obsolete and modernized with bonded jointing materials or following the intervention of firefighters who sometimes struggle to differentiate between the different families of Hymenoptera (Pauly, 2019). Raising the awareness of the various stakeholders is therefore a key point in safeguarding bees in the urban environment. There are still questions regarding ground bees nesting under urban pavements. As no consistent studies on this topic have been carried out, these "pavement bees" may exhibit a wide range of phenotypic plasticity, which is reflected in their nesting behavior. For example, the Californian *Habropoda depressa* Fowler 1899 (Apidae) showed a change in nest structure and larval cell number in urban nesting populations compared to natural nesting populations (Barthell et al. 1988).

1.4. Landscape ecology

1.4.1. Landscape moderating the biodiversity

In recent decades, ecological science has witnessed significant progress in landscape ecology, macroecology, and metapopulation studies. These investigations have shed light on the profound impact of landscape context on biodiversity, ecosystem function, and ecosystem services (With 2019). While the quality of the habitat (provider of survival resources) plays a pivotal role in explaining species distribution, it is essential to consider the influence of the surrounding landscape context on this distribution. Landscape factors, encompassing composition, configuration, and connectivity, exert considerable influence on the structure of pollinator communities (Fahrig et al. 2011; Bukovinszky et al. 2017). Landscape composition refers to the variety and abundance of land-use types, while landscape configuration pertains to the spatial arrangement of different landscape patches (Wiens 2002). Landscape connectivity, on the other hand, signifies the extent to which the landscape facilitates or hinders the movement of organisms through habitats (Taylor et al. 1993).

Therefore, understanding how landscape features influence biodiversity patterns and ecological processes at both local and landscape scales is of utmost importance in addressing the diverse spatial impacts of environmental change. In a pivotal study, Tscharntke and colleagues (2012) put forth eight hypotheses concerning the role of landscape composition and configuration in shaping ecological communities, ecosystem functioning, and services. Their insights, garnered from human-modified landscapes, include the dominance of beta diversity hypothesis, which posits that landscape-mediated dissimilarity among local communities governs biodiversity across the entire landscape, surpassing the negative local effects of habitat fragmentation on biodiversity. Another hypothesis, the landscape-moderated concentration and dilution hypothesis, suggests that spatial and temporal changes in landscape composition can lead to transient concentration or dilution of populations, with functional consequences such as the reduction of temporal stability and resilience of ecosystem functioning. Additionally, the intermediate landscape-complexity hypothesis proposes that the effectiveness of local conservation management is at its peak in structurally simple landscapes, rather than in extremely simplified or highly complex ones. These hypotheses shed light on the intimate interaction between landscape characteristics and ecological dynamics, contributing to our understanding of environmental responses and conservation strategies.

1.4.2. The disturbed landscapes: agricultural and urban ecosystems

Agricultural landscapes:

The current agriculture in temperate landscapes is the result of the 70 years of agricultural intensification since the World War II. This intensification has changed natural and heterogeneous spaces to human and homogenous spaces leading to decline in biodiversity of organisms. Conventional agriculture, which is prevalent in these regions, heavily relies on mechanization, fossil fuels, pesticides, and fertilizers to manage pests and enhance soil fertility (Herzog et al. 2006). These practices have led to increased agricultural productivity and yields (Tilman et al. 2011). Since the end of the century, there has been a resurgence of alternative agricultural practices in Northern countries, including conservation agriculture and agroecology. These approaches are designed to meet society's production requirements while also prioritizing the preservation of soils, human health, the environment, and biodiversity (Dendoncker et al. 2018).

A recent meta-analysis show that re-complexifying agricultural landscapes must lead to improve the biodiversity which also contribute to the agriculture production, ecosystem resilience and human health (Estrada-Carmona et al. 2022). Introducing flower strips in agricultural parcels is a one of the strategies to introduce heterogeneity into landscapes, providing valuable nectar and pollen resources for pollinators and ecosystem services to society. However, a key challenge lies in designing flower strips that can effectively meet the nutritional needs of insect pollinators and optimize their feeding opportunities (Uyttenbroeck et al. 2015, 2017; Filipiak 2018). In EU countries, flower strips belong to the procession of the Agri-Environmental and Climatic Measures (AECM) and farmers who implement them are eligible for financial support (Kleijn and Sutherland 2003).

Some practices of intercropping systems can be associated with flower strips (Hatt et al. 2017b). Intercropping involves cultivating multiple agronomic crops simultaneously on the same agricultural parcels, with the option of harvesting them separately (Lithourgidis et al. 2011). There are different approaches to intercropping: (i) mixed cropping, where two plants are cultivated without spatial distinction, and harvesting is done simultaneously; (ii) Row intercropping, where different crops are arranged in rows and may be harvested at different times; (iii) strip intercropping, which involves placing one or more crops in a strip, possibly within another; (iv) relay cropping, where planting times are staggered, and simultaneous cropping does not occur throughout the entire lifespan of the different plants.

Studies have shown that intercropping systems can yield higher quantities of better-quality crops, provide greater harvest assurance, and mitigate the environmental drawbacks of traditional agriculture (Lopes et al. 2016). These systems also help reduce pest and disease pressure in the fields. Wheat, one of the most widely produced cereals globally, is often associated with intercropping systems, particularly with legumes. The inclusion of legumes in intercropping improves resource utilization, increases yields, and enhances protein levels in wheat (Bedoussac et al. 2015). Similar to conventional flower strips, intercropping systems with flower strips have demonstrated long-term benefits in increasing pollinator populations. Flowering crops, including intercropped systems, can enhance pollinator number (Amy et al.

2018). However, it's important to note that flowering crops can potentially divert pollinators from natural resources during the crop season.

Urban landscapes:

For the first time in human history, more than a half of the world population (54%) lives in urban areas and the urban colonization may reach to 66% at the horizon of 2050 (United Nations 2019). The definition of an urban area (as opposed to rural ones) is not universal, can differ among countries or working domains which establish the used criteria such as the density of human population, the proportion of impervious surfaces,… (Vanderstraeten and Van Hecke 2019). For example, in Belgium, the minimum inhabitants is 75 000 whereas 1 000 and a density of 400 per km² in Canada (Statistics Canada; Vanderstraeten and Van Hecke 2019).

Among the landscape disturbances, urbanization represent a process of landscape transformation from natural or agricultural areas which must be understood as a continuum rather dichotomy evolving with temporal and spatial components. Therefore, the urban-rural gradient should be studied as an ecological or disturbance gradient acting as filter for living species which generates some winners and losers (e.g. in Banaszak-Cibicka and Żmihorski 2012; Fauviau et al. 2022). The urban filter selects the species highly tolerant to disturbance or highly adaptable such as similar group of species. Urbanization gradient is not a linear effect, in some cases, the areas with moderate levels of human developments, such as the suburbs; can harbor higher species richness than in rural or natural areas (Hall et al. 2017). Indeed, the suburbs are shaped by substantial landscape heterogeneity which increase edge effects or the variation in community composition, also called the β diversity (Tscharntke et al. 2012). Also, this anthropic landscape can be viewed as heterogeneous mosaic (Cadenasso et al. 2007) composed of different landscape elements which are subject to their own proportions (e.g. land use) and configurations (i.e. their patch size). In addition, the functional connectivity of the landscape, which is enhanced for example by railway or canals, plays a pivotal role in the distribution of the species (Hardy et al. 2022).

2. Objectives and outline of the thesis

A series of studies have shown that it is the degradation of natural habitats that has the greatest impact on pollinator communities by the disappearance of flower resources (pollen and nectar) as well as their nesting sites (Potts et al. 2010a). To counteract these effects, the conservation and/or restoration of (semi-)natural environments must be carried out head-on to (re)constitute large, connected habitats dedicated to biodiversity. In highly disturbed/fragmented environments, typically agricultural areas and urban areas, other management alternatives can be put in place. In these environments, transforming the conventional and homogenous areas in more diversified landscape patches or setting up buffer zones (flower strips, community gardens, etc.) in a configuration that includes the dispersal capabilities of species (e.g., their foraging radius) is essential to enhance the two key components of pollinators, flower resources and their nesting sites (Drossart and Gérard 2020).

It is within this current context that this thesis project addressed its main question is: “**How floral resources and nesting sites impact the foraging ecology and community structure of pollinators of disturbed landscapes in the temperate zones?**” This thesis aims to encompass a various case studies investigating the leverages of these two key components, the floral resources, and the nesting sites to promote pollinator insects within agricultural and urban landscapes of the temperate zones of the world. Through these case studies, valuable insights will be gained, leading to the development of recommendations for designing effective management additional or new knowledges that conserve these essential resources for insect pollinators. Throughout this thesis, four specific questions pertaining to the study cases conducted in the agricultural zones of Namur province (Belgium), in the city of Brussels (Belgium), and the mega-city of Tokyo (Japan) were thoroughly examined. These questions shaped structure of this thesis, guiding the research in these diverse and disrupted landscapes:

1. Do local farmsteads at the beginning of their ecological transition promote wild bees and hoverfly diversity? – **Chapter III**
2. In local intercropping systems, does customized flower-strips promote wild bee and hoverfly communities? – **Chapter IV**
3. Does the species richness and composition of the foraged flower community by the honeybee vary according to (i) an urban-rural gradient and (ii) to the course of the seasons in Tokyo (Japan) as mega-city? – **Chapter V**
4. Does the urban pavement of Brussels-city promote the opportunity of nesting sites for Apoidea species? – **Chapter VI**

Therefore, the proposed thesis is divided in two part (**Figure 1-5**): review of the literature in relation to the current context of pollinator decline (**Chapter I**) and field-based assessments from agricultural and urban environments (**Chapter III to VI**). Therefore, the core material of this thesis is structured in four different chapters based on field assessments and statistical analysis. The overview and main results are displayed in **Table 1-1**. A general methodology chapter (**Chapter II**) preceded the core material in order introduce and motivate the main experimental designs, techniques and methodologies that are shared by the different study cases. Finally, a general discussion (**Chapter VII**) finalizes this thesis by providing recommendations and new information for the science and society.

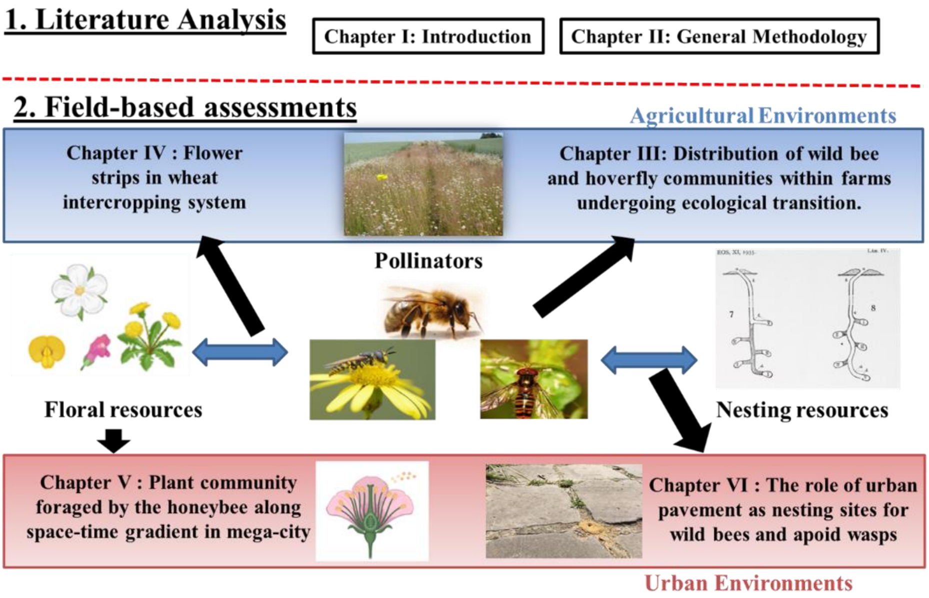


Figure 1-5: Schematic overview of the proposed thesis. Floral resources and pollen are extracted from BioRender. The nesting resources picture originate from Malyshev (1935).

Table 1-1 : Thesis overview and main results.

Chapter's aim	Specific questions	Methods	Main results
Chapter 3: Quantify the distribution of wild bee and hoverfly communities within farms undergoing ecological transition	(i) What are the impacts of farm-scale landscape diversification on bee and hoverfly fauna at early stage of ecological transition?	Use of standardized surveys of pollinator and standardized protocols for their preparation in collections	The on-farm diversification and organic practices may be an important refuge for rare, red-listed and oligolectic pollinator species and is a sustainable option for the generalist ones. **Noël *et al.*, (2021)** published in Biodiversity Data Journal
Chapter 4: Quantify the effects of several flower strip modalities in intercropping system on wild bee and hoverfly communities	(i) How customized flower strips in intercropping systems influenced wild bee and hoverfly communities? (ii) How these two groups of pollinators interact with the different floral species within the flower strips?	Use of standardized collections of pollinator collections and preparation. Compare α diversity metrics by their effective species number (Hill's number) and use β diversity analysis	Differences in hoverfly species richness was showed between the different flower strips. Distinct pollinator communities visited flowers composing the sowed flower strips in wheat crop. **Amy *et al.*, (2018)** published in Insects
Chapter 5: Study the plant community structure foraged by the honeybee along space-time gradient	How does the composition of the foraged flower community (and the foraged plant traits) vary according to (i) an urban-rural gradient and (ii) to the course of the seasons?	Use DNA metabarcoding on corbiculate pollen combined with LMMs and GLMMs to disentangle the influence urban and temporal gradient on foraged plant communities	The landscape class (rural, suburban and urban areas) explains spatial variations in the plant composition foraged by honeybees, but not in taxa richness while pollen diversity and plant composition showed a strong seasonal dependence. **Noël *et al.*, (2023)** published in Urban Forestry and Urban Greening Journal
Chapter 6: Study the role of urban pavement as nesting sites for wild bees and apoid wasps	(i) What is the ground-nesting living in Brussels pavement? (ii) Is there a link between ground-nesting species size and joint? (iii) Does the pavement type have an impact on it? (iv) Is the soil texture having an impact in the selected nesting site?	Use of citizen science pipeline and pedological methods to characterize all the sampling nesting sites	A total of 22 species belonging to 10 Hymenoptera families were identified. Sandstone setts or concrete slabs with an unbound joint size around 1 cm and sandy foundations were found to be best suitable urban pavements for the ground-nesting species. Conservation recommendations were established for new applied solutions for urban planning management **Noël *et al.*, (2023)** submitted to Insect Conservation and Diversity

Chapter 2

General methodology

1. How to collect insect pollinator community?

Once a research question has been identified and the current state-of-the-art has been reviewed, it is important to select an optimal methodology for insect collection. This will allow for the most accurate estimation of biodiversity on the scale of the research question. The methodology for collecting insects is contingent upon several factors, such as the specific insect communities being studied, the chosen sampling design, and available human and financial resources (Montgomery et al. 2021). There is currently no consensus on the optimal method for collecting insect pollinators (Prendergast et al. 2020). Each technique has its own advantages and drawbacks, which are influenced by factors such as the financial costs involved, the experience of the sampler, the level of sampling effort, and the potential bias towards certain taxonomic groups. However, when combining techniques, the estimation of insect pollinator biodiversity can be improved in terms of accuracy and quality (Westphal et al. 2008).

In the study of insect pollinators, we applied two combined techniques that can be used depending on the skill level of the human sampler: **active methods** that require human involvement, and **passive methods** that are independent of human skills (**Figure 2-1**). Active techniques are relatively inexpensive, easily portable, most of the time non-destructive to specimens, and can associate insect pollinators with their visited flowers. These methods also allow for minimal or no degradation of captured specimens, making them easier to identify. Examples of active techniques include hand-made insect vacuums or sweep nets. In contrast, passive techniques require little to no skill or experience from human samplers, can be deployed for days or weeks of sampling, and include colored pan traps, vane traps, baits, malaise traps, or trap-nests. However, both methods have limitations that can introduce bias when estimating biodiversity. Active methods are labor-intensive, limited in duration, and require experienced human samplers to locate tiny insect pollinators (< 0.5mm) such as *Lasioglossum* spp. (Hymenoptera: Halictidae) on flowers (Roulston et al. 2007). Additionally, it is difficult to standardize collection protocols on spatial and temporal scales due to the variability of flower patches and insect pollinator behavior. Passive methods can be taxonomically biased, as larger specimens like *Bombus* spp. are often underestimated due to their ability to avoid traps (Roulston et al. 2007). Furthermore, passive traps cannot provide information on visited flowers, and specimens can degrade over time if traps are left active for several days. Therefore, the most effective approach is to find a balance between active and passive methods to obtain the best possible estimate of insect pollinator biodiversity. Combining both techniques has proven to be the most suitable for sampling a representative fraction of insect pollinators, particularly wild bee communities in temperate regions (Westphal et al. 2008).

The effectiveness of sampling techniques for hoverflies varies depending on the studied ecosystems and geographical location. Typically, methods used to estimate syrphid diversity include malaise traps, colored pan traps (usually yellow), and sweep netting (Campbell & Hanula, 2007; Namaghi & Husseini, 2009; Földesi & Kovács-

Hostyánszki, 2014). The duration of passive techniques influences the amount of collected syrphid specimens. In agricultural areas, malaise traps have been shown to be more effective than yellow cups but less effective when there is abundant flower cover (Namaghi & Husseini, 2009). However, malaise traps are expensive, difficult to transport in large numbers, and affected by wind in open spaces, killing many insects and increasing preparation time (Carboni & Lebuhn, 2000; Evans, 2016). Experienced entomologists using sweep netting can capture hoverflies visiting flowers or oviposition sites, which are not attracted by passive techniques (Namaghi & Husseini, 2009). Combining colored pan traps and sweep netting should improve sampling of these Diptera, which generally have lower population densities in agricultural systems (Campbell & Hanula, 2007; Földesi & Kovács-Hostyánszki, 2014).

In **Chapters III and IV** of this thesis, we used the standardized guidelines of Westphal et al. (2008) to design our experiment for insect pollinator sampling. Our approach involved using colored pan traps (passive technique) and random transects with a sweep net (active technique) to estimate the true biodiversity of wild bee and hoverfly communities in disturbed environment. This selected methodology also allow us to be comparable with other studies such as Uyttenbroeck et al. (2015) or Vereecken et al. (2021).

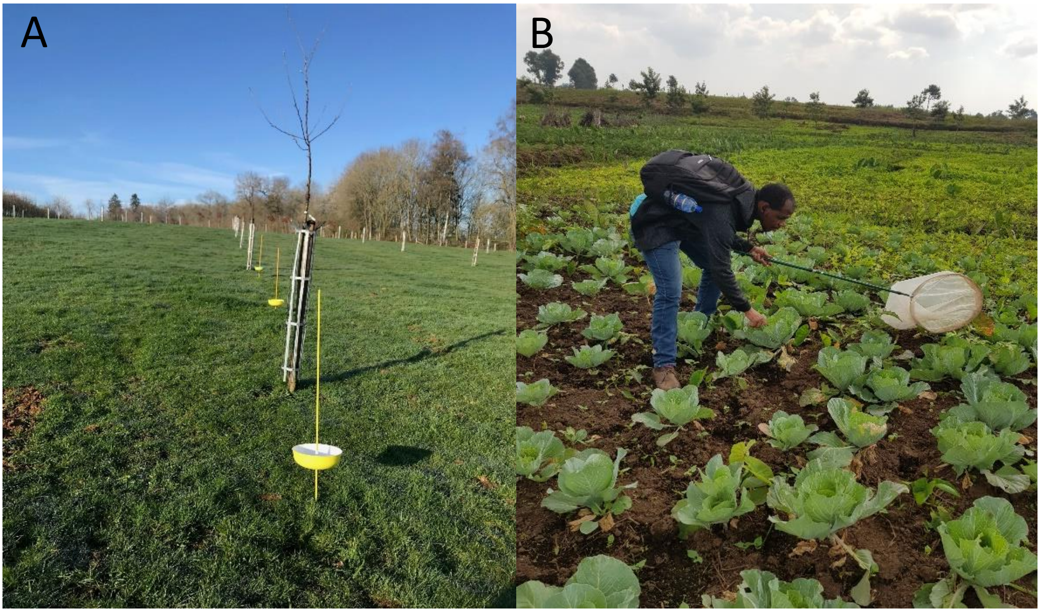


Figure 2-1 : (A) Colored pantraps in Froidefontaine farmstead (Chapter III). (B) Use of the net to capture insects. Credit photos: J. Bonnet & M. Cokola Cuma

2. Biodiversity and community ecology – a matter of space and time

Introduced for the first time in 1988 by E. O. Wilson and F. M. Peter in their book, the term “biodiversity” is a concept originally proposed by ecologists to address the loss of biological diversity, or the variety of organisms on Earth. Biodiversity is a widely used term in both scientific and everyday language and has been extended to other scientific domains such as genetics (e.g. in Parejo et al. 2016) or microbiology (e.g. in Colman et al. 2012). This concept is applied in various ways and aims to define the patterns of species distribution in given system over space and time. In applied situations, we can predict the species distribution based on reliable dataset of species occurrence in space and time over environmental variables such as climate and landscape (Marshall et al. 2015). The knowledge of occurrence of species in specific locations and time periods also provides the generation of species red lists (e.g. in Nieto et al. 2014; Drossart et al. 2019a; Vereecken et al. 2022), the dynamics of an exotic species (e.g. in Lanner et al. 2022), or predictive map of endangered species through climatic scenarios (e.g. in Rasmont et al. 2015). The distribution of species within a specific system is often referred to as a species community, and the interactions between these species are investigated within the framework of community ecology to understand their structure, known as community structure (Gardener 2014).

Biodiversity and community structure are based on an unclear concept, the species concept which classically defined by morphological analysis of the collected specimens also called biological taxonomy. Indeed, species taxonomy is a primer in all ecological studies and one of the most ancient scientific domains (Linné 1758). Last decades, taxonomy science has expanded its range of techniques to identify specimen species, including recognition by genetic markers, chemical cues, more precise biogeographical features (GPS coordinates, datum …), use of deep learning methods on pictures (Spiesman et al. 2021), to become what is called now integrative taxonomy (Orr et al. 2020). After generally tedious taxonomic work to identify species from a sample, it is possible to distinguish the pattern of species occurrence to infer interesting ecological process. But how to measure biodiversity? How can we compare species richness across different environmental or landscape conditions? How is the composition of species communities influenced by spatial and temporal factors? These overarching questions and the motivations behind their exploration in this thesis will be introduced in the upcoming subchapters, where general concepts and selection criteria for this thesis will be discussed.

2.1. The measure of biodiversity

Biodiversity of living organisms encompasses two main components: **the species richness** or the number of species present in a given system and **the species evenness** or the relative abundance of species present in a given system (Magurran 2004).

First mathematically introduced by McIntosh (1967), species richness is founded on two assumptions. The first assumption is that the taxonomy of the organisms being studied is well-established, which unfortunately is not always the case. The second assumption is the application of the species-neutral diversity assumption, where equal weight is given to each species (Marcon 2017), which unfortunately also is not always the case, especially considering functional or phylogenetic components of the biodiversity (i.e., disparity or divergence measures) (Devictor et al. 2010).

On the other hand, species evenness refers to the relative abundance of individuals among different species present in a given system. It takes into account the number of individuals per species, and may be represented by an evenness index. Unlike species richness, evenness index measures the regularity of species distribution and considers the presence of both rare and dominant species in the system.

When studying species diversity, various levels of analysis are used to calculate diversity indices: α and β diversity (Whittaker 1960). α diversity quantifies the number of species within a specific sampling unit, such as a habitat, a season, or a quadrat (Marcon 2017). It represents the smallest level of measurement (**Figure 2-2**). On the other hand, β diversity measures the similarity or dissimilarity in species composition between different sampling units (Tuomisto 2010a) (**Figure 2-2**). The combined total of α and β diversity defines γ diversity, which is typically used to represent landscape or regional biodiversity. Theoretically, the levels of biodiversity studies can be in the form of the **equation 2-1**.

$$\gamma = \sum \alpha + \sum \beta$$

Equation 2-1 : Conceptual equation of the biodiversity levels. The diversity within a single system corresponds to α. The comparison of species composition between systems corresponds to β. The pool diversity of a larger system such as in the landscape or regional scale corresponds to γ

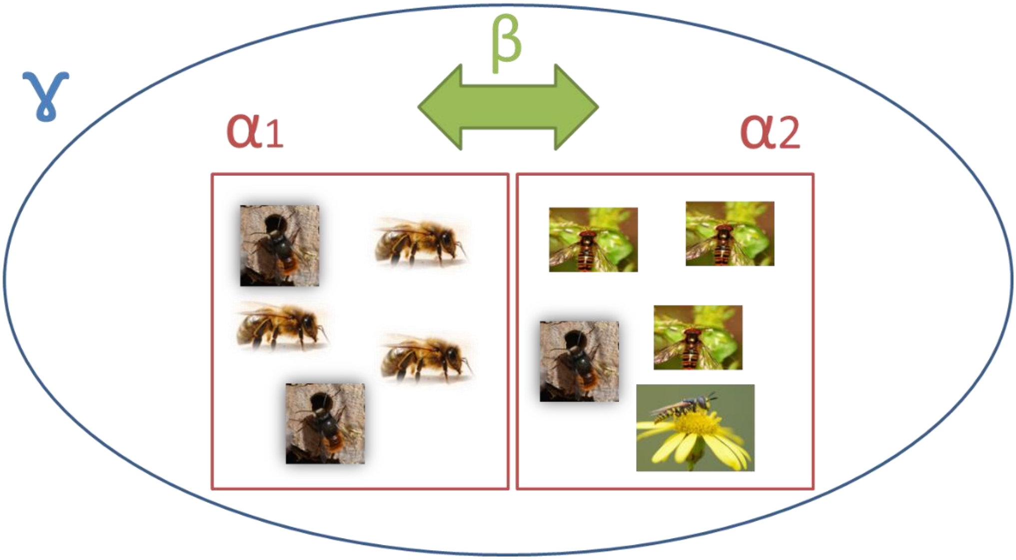


Figure 2-2: Conceptual representation of the different levels of diversity. In red, pollinator local biodiversity of α_1 and α_2; in green, the β biodiversity comparing species composition between α_1 and α_2; and γ biodiversity or total biodiversity of the system over defined space and time, in blue. The images have been credited to G. Noël, J. Bonnet, and J. Mignon.

2.2. *Alpha diversity indexes*

In the majority of studies exploring species diversity and community structure, three traditional α diversity indices are commonly used: the species richness, the Gini-Simpson index (**Equation 2-2**) and the Shannon index (**Equation 2-3**). These indexes are based either on presence-absence or quantitative data. The Gini-Simpson index (also noted the Simpson index in this thesis) calculates the probability that two randomly selected individuals from the sample belong to different species (Simpson 1949; Hurlbert 1971). On the other hand, the Shannon index measures the biodiversity as information quantity. In other words, the probability of each species is not squared but multiplied by the log of the probability (Shannon 1948; Hurlbert 1971). Both indexes are correlated and provide valuable insights into the dominance-rarity pattern within the studied community. However, the Shannon index tends to smooth out the influence of dominant species by considering the overall species composition more broadly (Gardener 2014; Marcon 2017). Due to the distinct aspects they measure, choosing between species richness, Gini-Simpson, and Shannon index becomes challenging as they do not capture the same quantities (Tuomisto 2010b).

$$E = 1 - \sum_{s=1}^{S} p_s^2$$

Equation 2-2 : Gini-Simpson formula (Simpson 1949). The variable p_s corresponds to the probability of two random individuals belong to the same species s.

$$H = -\sum^{S} p_s \ln p_s$$

Equation 2-3 : Shannon formula (Shannon 1948). The variable p_s corresponds to the probability of two random individuals belong to the same species s.

2.3. *Limitations of Gini-Simpson and Shannon indexes – Hill's number framework*

Both the Gini-Simpson and Shannon indexes are affected by sampling effort as they are derived from species sums, resulting in potential bias. Additionally, they do not adhere to the principle of replication in scaling species gain or loss equally (Jost 2006; Tuomisto 2010b). For instance, if a community with uniform species abundance experiences a 20% loss or gain in species, the traditional Gini-Simpson and Shannon indexes would exhibit minimal proportional change.

To address these limitations, Hill (1973) introduced a framework that integrates a consistent terminology based on the concept of the "effective number of species," which represents the number of equally abundant species needed to produce the same value of diversity measure (Jost 2006; Chao et al. 2014a). Hill diversities are a form of averaging, specifically measuring the average rarity of species in the samples, where rarity is defined as the reciprocal of relative abundance (**Equation 2-4**). This framework recognizes a fundamental principle: a community with, on average, rarer species exhibits higher diversity. Both Gini-Simpson and Shannon indexes were mathematically transformed into Hill-Shannon (or exponential Shannon diversity) and Hill-Simpson indexes (reciprocal Gini-Simpson diversity) or (Hill, 1973) to better capture the mean rarity of species. Each form of Hill's diversity numbers scales the mean rarity within each sampling units (Roswell, 2021). The calculation of the mean rarity of species varies for different diversity indices. For species richness, it is calculated as the arithmetic mean, for Hill-Shannon it is the geometric mean, and for Simpson-Hill, it is the harmonic mean (**Table 2-1**).

$${}^{q}D = (\sum_{i=1}^{S} p_i^q)^{1/(1-q)}$$

Equation 2-4 : Hill unification formula (Hill 1973). The variable q corresponds to the Hill's number which can be 0, 1 or 2. The variable p_i corresponds to the proportion of individuals of a species i of the species richness S.

Table 2-1 : Summary of Hill's number (Hill 1973). In the formulas, S represents the number of species, and p_i represents the proportional abundance of the i-th species.

Hill's diversity number	Name	Formula	Sensitivity to rare species	Mean rarity
Hill's diversity of order 0 (q = 0)	Species richness	S	+++	Arithmetic mean
Hill's diversity of order 1 (q = 1)	Exponantial Shannon diversity	$\exp(\sum(p_i \ln(p_i)))$	+	Geometric mean
Hill's diversity of order 2 (q = 2)	Reciprocal Gini-Simpson diversity	$1 / \sum(p_i^2)$	-	Harmonic mean

2.4. The Beta diversity concept

The β diversity represents the change in species composition between defined modality based on space or time: between habitats, spring vs summer species, sites…. In the two last decades, β diversity became an umbrella concept encompassing different kinds of notion such as heterogeneity, complementarity or (dis)similarity (Tuomisto 2010a). As alpha diversity index (see 2.4 subchapter), the estimation of the change in species composition is supported by presence-absence or quantitative data. Two main ways are recognized by Anderson et al. (2011) to conceptualize β diversity: (i) the turnover of species community along an environmental or temporal gradient and (ii) variation of the community composition (sample units) in a given space. In all of this thesis, we selected the second way to show and assess the β diversity by exploring relationships between community structure and landscape factors (e.g. percentage of impervious surface) using unconstrained and constrained ordination techniques (Borcard et al. 2018a). The selected unconstrained ordination of multivariate analysis are explained in the next subchapter (see point 3.1.).

3. Other statistical tools

3.1. Multivariate analysis

The multivariate analysis is preponderant in this thesis because all the generated datasets are constructed on species **x** observations and environmental variables **x** observations. Thus, we applied unconstrained ordinations methods to synthetize the principal of our multidimensional datasets generated by the obtention of the community matrix.

3.1.1. Principal Component Analysis (PCA)

In our multidimensional datasets, PCA will map the objects of the matrix in a space defined by the variables. For each associations of variables (R mode in Borcard et al. 2018b), a new pair of axis are defined from covariances or correlations between the variables in order to explain at best the variance of the scatter plot. To transfer the coordinates to the objects to the reduced dimensional spaces, we divide each correlation by the square root of the proper value in order to result in the proper vectors. These methods aim to preserve the Euclidean distance between objects in the dataset. Typically, we focus on 2 or 3 new axes that capture the highest amount of explained variance. Variables that are closer to axis 1 or 2 contribute the most to the overall variation. However, it is important to note that PCA is sensitive to extreme values or outliers, which can heavily influence the structure of the multidimensional dataset. Additionally, PCA may not be well-suited for datasets with a high number of zeros or low frequencies (Legendre and Legendre 2012a).

3.1.2. Principal Coordinates Analysis (PCoA)

Like PCA, Principal Coordinate Analysis (PCoA) is a method used to ordinate multidimensional objects such as community matrix in a reduced number of dimensions, allowing the visualization of major trends within its dataset (Legendre and Legendre 2012a). It is particularly useful for comparing insect community differences across different sampling spatio-temporal modalities. PCoA is based on a distance matrix, which is not Euclidean (c.f., PCA). In the case of this thesis, since the community matrix contains many zeros, the Bray-Curtis method is applied to generate the distance matrix. One advantage of this method is that it does not consider species that are absent in different spatial or temporal modalities. The total distance is calculated as the sum of distances for each species (Legendre and Legendre 1998). Once the matrix is centered, eigenvalues and eigenvectors are computed. The objects in the matrix are then re-projected in 2-D graphics using these vectors, known as principal coordinates. Each eigenvalue represents the variance explained by the corresponding eigenvector. Typically, the first 2 or 3 dimensions, which explain more than 10% of the observed variance, are used for the PCoA plot. Values below 10% are generally considered less appropriate for interpretation (Bellehumeur and Legendre 1998; Hair et al. 2019).

3.1.3. Nonmetric Multidimensional Scaling (NMDS)

NMDS is a technique that aims to preserve the relative dissimilarities between objects in an ordination plot, rather than accurately representing the exact dissimilarities. Its goal is to effectively capture the ordering relationships among objects using a limited number of axes which are given at the beginning of the calculation process. Like PCoA, NMDS relies on a distance matrix that quantifies the dissimilarities between objects. It is an iterative technique that requires an initial configuration, often obtained from PCoA, to initiate the optimization process and may be computer intensive (Legendre and Legendre 2012a; Borcard et al. 2018b).

3.1.4. PCA, PCoA or NMDS?

The three methods have their own drawbacks and benefits, depends on the nature of the data, and the specific objectives of the research project. In summary, for exploration analysis, the species x observations datasets can be visualized using ordinations scores by linear model for PCA, through the generation of distance matrix for PCoA and NMDS depending on original distances and ranked distances, respectively (Borcard et al. 2018b).

3.2. Modelization

3.2.1. Linear model (LM)

Some of research questions in ecology addresses the influence of one factor to another such as the proportion of impervious surface on the foraged plant species richness (see Chapter V). The Linear Model (LM) or linear regression can be applied by the **equation 2-5**.

$$Y_i = \alpha + \beta \times X_i + \varepsilon_i \text{ where } \varepsilon_i \sim N(0, \sigma^2)$$

Equation 2-5 : Equation of the bivariate linear regression.

Y_i is the variable to explain of i_{th} observation or the response variable while X_i is the explanatory or independent variable of i_{th} observation. The α and β parameters correspond here to the intercept and the slope of the equation, respectively. The deviation from the i_{th} observed value against the i_{th} fitted value is captured by the residuals ε_i which follow a normal distribution with a mean of 0 and a variance of σ^2. The solve of **equation 2-5** results from the use of the least square methods to determine the unknown α and β parameters. After this solving, we can predict values of the response variable based on the values of the explanatory variable with a certain degree of confidence. The extension of LM is the multiple linear regression which involves the use of multiple explanatory variables and slopes to define. LM is based on several statistical assumptions which cannot be violated in theory, but it is a different story in practice. By order of importance, (i) the normality of the observations and the residuals must be tested, (ii) the homoscedasticity (homogeneity of the variance) must be tested, (iii) the independence of the X values must be assessed and (iv) must be deterministic.

3.2.2. Generalized Linear Mixed-Models (GLMM)

GLMMs are an extension of the LM in response of more structured datasets (e.g., nested datasets) containing non-independent or aggregate observations. GLMMs affords an aid to correct the inference of the explanatory variables and a reduction of the probabilities to have false positives or false negatives by structuring the explanatory factors in two classes: fixed effects and random effects (Harrison et al. 2018). Defining random and fixed is dependent on the goals of the analysis (Gelman and Hill 2006). Assuming that different modalities of a variable are all independent of one another and share a similar residual variance corresponds to fixed effect. This could be a set of temperature modalities for the development of an insect. A random sampling encompassing all the value possibilities from a variable is defined as random effect. This could typically be a site position for a sampling.

3.2.3. The combination of modelling and multivariate analysis: constrained ordination – redundancy analysis (RDA)

Redundancy analysis (RDA) is a sophisticated statistical tool combining linear regression and PCA. It works on response matrix (Y) and explanatory variable matrix belonging to X. The distinction from unconstrained ordination (PCA) is based on this approach: in constrained ordination (RDA), the matrix of explanatory variables influences the "weights" (eigenvalues) and orientations of the ordination axes. This means that the axes in RDA genuinely explain or model (in the statistical sense) the variation in the dependent matrix (Legendre and Legendre 2012b; Borcard et al. 2018a). Community matrices generally exhibited a zero-inflated problem owing to the dominance rarity pattern present in the data (Roswell et al. 2021). To address this issue effectively, it was preferable to convert the community matrix into a distance matrix. Consequently, we employed distance-based RDA (dbRDA) in such instances.

4. Application of the shared methodologies and techniques in this thesis

In the next 4 chapters, we applied a diversity of techniques from the citizens sciences to molecular techniques. In function of the experimental design and the datasets generated, we applied appropriate statistical analysis (**Table 2-2**).

Table 2-2 : Summary of the shared methodologies used in this thesis.

	Specific methods	Chapter III	Chapter IV	Chapter V	Chapter VI
Field methods	Colored pantraps and transect netting	X	X		
	Pollen collection			X	
	Transects netting and citizen sciences				X
Identification methods	Morphological species identification	X	X		X
	Molecular species identification			X	
Statistical methods	Parametric tests	X			X
	Non-parametric tests	X	X	X	X
	Multivariate analysis: PCA, PCoA, NMDS, dbRDA	X	X	X	X
	Modelization: LM, GLMM, dbRDA			X	X

Chapter 3

Distribution of wild bee and hoverfly communities within farms undergoing ecological transition

Chapter III: Distribution of wild bee and hoverfly communities within farms undergoing ecological transition

This section is an adapted version of the article:

Grégoire Noël, Julie Bonnet, Sylvain Everaerts, Anouk Danel, Alix Calderan, Alexis de Liedekerke, Clotilde de Montpellier d'Annevoie, Frédéric Francis & Laurent Serteyn (2021). "Distribution of wild bee (Hymenoptera: Anthophila) and hoverfly (Diptera: Syrphidae) communities within farms undergoing ecological transition". Published in *Biodiversity Data Journal* 9: e60665. https://doi.org/10.3897/BDJ.9.e60665

Abstract - In Havelange (Belgium), two farms are experiencing an ecological transition. We aimed to evaluate the impact of their agricultural activities on insect pollinator communities. This article depicts the situation at the very early stage of the farm transition. This study supports the fact that the maintenance of farm-level natural habitats provides environmental benefits, such as the conservation of two important pollinator communities: wild bees and hoverflies. Over two years (2018-2019), by using nets and coloured pan-traps, we collected 6301 bee and hoverfly specimens amongst contrasting habitats within two farmsteads undergoing ecological transition in Havelange (Belgium). We reported 101 bee species and morphospecies from 15 genera within six families and 31 hoverfly species and morphospecies from 18 genera. This list reinforces the national pollinator database by providing new distribution data for extinction-threatened species, such as *Andrena schencki* Morawitz 1866, *Bombus campestris* (Panzer 1801), *Eucera longicornis* (L.) and *Halictus maculatus* Smith 1848 or for data deficient species, such as *A. semilaevis* Pérez 1903, *A. fulvata* (Müller 1766), *A. trimmerana* (Kirby 1802) and *Hylaeus brevicornis* Nylander 1852.

Keywords: organic and regenerative farming, wild bee, hoverfly, ecological transition

1. Introduction

Nowadays, the greatest challenge faced by agriculture is to provide food for everyone, without altering the agro-biodiversity and the related ecosystem services (Duru et al. 2015; Muller et al. 2017; Dendoncker et al. 2018). Indeed, the worldwide intensification of agricultural systems has led to tragic biodiversity losses. During the last decades, many studies showed a strong impoverishment of insect pollinators in intensively farmed landscapes. The depletion of these pollinators - and with them the ecosystem service of pollination - could have severe negative impacts on farmers and consumers welfare (Biesmeijer et al. 2006; Carvalheiro et al. 2013; Potts et al. 2016; Woodcock et al. 2019). The decrease in floral resources and the degradation of nesting sites is one of the main factors of decline (Potts et al. 2010a; Goulson et al. 2015b; Sánchez-Bayo and Wyckhuys 2019). In Belgium, in 2010, the insect-pollination was valuated at around 250 M€ (Jacquemin et al. 2017).

Agroecological farming systems grow crops on small areas, alongside heterogeneous habitats and complex arrangements (e.g. subdivision of plots by hedgerows, fallow areas, flower meadows etc.) that provide shelters and abundant food resources to beneficial insects (Power et al. 2012). Diversified habitats at the plot or at the farm spatial scale help to control pests, weeds and phytopathogens and provide other regulatory ecosystem services, such as pollination and preservation of nutrients and water in soils (Hatt et al. 2018).

The bee community (Hymenoptera: Anthophila) is amongst the most efficient pollinator groups in temperate agriculture landscapes. In Belgium, the latest inventory recorded 403 bee species, which represents almost one quarter of the European bee diversity (Rasmont et al. 2017; Drossart et al. 2019). Their morphological and behavioral traits co-evolved with flowering plants, allowing them to secure pollination (Michener 2007). The richness of bee morphologies, specialization in pollen and nectar diets and sizes greatly supports an increase in yields in small-scale agricultural farms (Garibaldi et al. 2016). Since the end of the 19th Century, Belgium has had great expertise in the monitoring of bees. Since the 70s, this survey has particularly accelerated through mapping, preservation and management of historical collections, taxonomic keys and revision of the Belgian fauna (Drossart et al. 2019).

Besides, the Diptera order represents one of the largest and most diverse groups in the pollinator community (Skevington and Dang 2002). Too often neglected, dipteran pollinators ensure the reproduction of many flowering plants (Ssymank et al. 2008; Rader et al. 2016). By consuming pollen and nectar, adult hoverflies (Syrphidae) play a pivotal role in the pollen transmission of over 70% of wildflowers (Inouye et al. 2015; Doyle et al. 2020). Hoverfly larvae exhibit a wide variety of feeding habits, including phytophagy, zoophagy, aphidophagy, saprophagy and mycophagy (Sommaggio 1999). As they cover a large spectrum of microhabitats (e.g. roots layer, herbs layer, dead wood, ponds...) (Speights 2017), hoverfly larvae can be used as biological indicators to evaluate the conservation status of ecosystems (Sommaggio 1999; Burgio and Sommaggio 2007). The widespread distribution of syrphids in temperate landscapes and the availability of excellent taxonomic keys for European species identification are also characteristics that promote syrphids as bio-indicators. Syrphids are very interesting organisms for studying the effects of agriculture intensification on biodiversity because they are particularly mobile (Gao et al. 2020). Moreover, hoverfly communities are strongly affected by the standardisation in landscape structures and by intensive agricultural practices (Dormann et al. 2007). In Belgium, 357 syrphid species were recorded according to the latest survey (Frank Van de Meutter, personal communication).

The impacts of agroecological transition on pollinator communities remain poorly documented. Such evaluation needs standardized and fine-scaled sampling efforts. Thus, the goal of this study is to provide a local and robust inventory of the bee and hoverfly fauna in two farms undergoing ecological transition in Havelange County (Belgium). The general impacts of farm-scale landscape diversification on bee and hoverfly fauna are discussed. In future research, such inventory will allow an assessment of the impacts of regenerating agricultural landscapes on the pollinator community structure. Moreover, this study feeds in new records and new locations for the national repository of the wild bee and hoverfly communities, owned by the Laboratory of Functional and Evolutionary Entomology (Prof. Frédéric Francis), Gembloux Agro-Bio Tech and the Laboratory of Zoology (Prof. Pierre Rasmont), University of Mons.

2. Material and methods

2.1. Study site and habitats description

The study was conducted in two neighbouring agricultural sites, located in the Municipality of Havelange (**Figure 3-1A**): the Froidefontaine and Emeville farmsteads (**Figure 3-1B**). They are located at 2 km away from each other, in the geological region of Condroz, in Wallonia (Belgium), as defined by Dufrene and Legendre (1991).

Figure 3-1 : Map of both sites. A. Location of Havelange Municipality in Belgium; B. The location of the two farmsteads in Havelange.

2.1.1. The Froidefontaine farmstead

The Froidefontaine farm (50°23'6"N, 5°8'34.799"E) covers an area of 55 hectares, with a mosaic of varied habitats. One of the management objectives is diversifying the land use by conserving natural areas (mesophilic and wet meadows, limestone slopes, ponds...) and hosting different farming projects in a collaborative way on farming areas. Thus, the farm aims at creating a rich and welcoming landscape for diversity, including biodiversity. Within the farm, we defined four adjacent habitats (**Figure 3-2A; Table 3-1**) covering about 10 ha each: a parcel of crops (GC) including a third of the surface with vegetable crops (GC1), a meadow zone (PAT), a young apple orchard (VER) and a wetland (ZH). The parcels were surrounded by hedges principally composed of hornbeam, elderberry, dogwood, hawthorn, maple and European charcoal. One apiary of two black honeybee colonies (*Apis mellifera mellifera*) was on site at 400m of ZH into the farmstead.

2.1.2. The Emeville farmstead

The Emeville farm (50°23'2.4"N 5°10'1.199"E) covers an area of just over 40 ha. In 2016, the farm managers and a committee of various partners converted conventionally managed fields to agroecological farming methods. To allow a complexification of the ecological network and creating an agricultural landscape enriched with biodiversity, the first actions were: laying hedges and grass strips; planting rustic apple trees; breeding Angus cattle (*Bos taurus taurus* L.) in an orchard; alternating temporary and permanent meadows; arranging of flowered grass strips; using no pesticides and amendments. There was no apiary into the farmstead, and we did not know about apiaries around the farmstead.

The sampling zone covered 15 ha and was divided into seven parcels (**Figure 3-2B; Table 3-1**), which included six parcels of crops separated by flower strips and one parcel of orchard. Each flower strip (BF1, BF2 and BF3; **Figure 3-2B**) was composed of three plant mix sequences, including a combination of one "feeder" flower patch (BFV) and one "pollinator" flower patch (BFB), separated by the cover crop patch. The cover crop patch was composed of a grass mix of *Festuca arundinacea* Schreb 1771 and *Dactylis glomerata* L. 1753 sown at 20 kg/ha. The feeder flower patch was composed of a mix of 40% of clover (*Trifolium pratense* L. 1753) and 60% of alfalfa (*Medicago sativa* L. 1753) sown at 25 kg/ha. In order to match Agri-Environmental and Climate Measures (AECM) specifications, the pollinator flower patch was sown at 30 kg/ha and was composed of a mix including 85% of grasses (*Poa pratensis* L., 1753 *Festuca rubra* L. 1753 and *Agrostis capillaris* L. 1753), 2%

of leguminous species (*Lotus corniculatus* L. 1753, *Medicago lupulina* L. 1753 and *T. pratense*), 3% of annual flower (*Papaver rhoeas* L. 1753, *Glebionis segetum* Fourr. 1869 and *Cyanus segetum* Hill 1762) and 10% of other flower species (*Achillea millefolium* L. 1753, *Centaurea jacea* L. 1753, *Daucus carota* L. 1753, *Leucanthemum vulgare* Lam. 1779, *Malva moschata* L. 1753, *Silene latifolia* Poir.

1789, *Melilotus* sp. Mill. 1754, *Knautia arvensis* Coult. 1828 and *Echium vulgare* L. 1753).

Table 3-1 : Habitat description of the sampled parcels and flower strips.

Parcel name	Parcel code	Farmstead	Sampling year	Parcel description
Pavillon	PAV	Emeville	2019	Pastures combined with apple orchard. Flowering fields under young apple trees (many rustic varieties). There are dandelions, shamrocks, meadow cardamine etc. This parcel is mainly surrounded by woods and hedges. A herd of Angus was grazing most of the time, from May.
Frere	FRE	Emeville	2019	Mainly alfalfa, some other fabaceae (red and white clovers). There are dandelions and speedwells at the start of the season. A hedge borders the parcel to the East. A flowery strip runs on the South face (BF 2; **Figure 3-2B**). Harvested during the month of June and after recovery in mid-July.
Epicurien	EPI	Emeville	2019	Divided parcel along the East to the West, composed equally of small and large spelts. Hedgerows border the parcel to the East face.
Dikkekip	DIK	Emeville	2019	The parcel is at the bottom of the slope. Left without plant cover until May, when pea crop was sown. There are some rumex and a lot of chamomile too.
Flower strips	BF	Emeville	2019	Composed of a mix of cover crops and flower crops. See the site description for more details.
Crops	GC	Froidefontaine	2018-2019	Vegetable crops occupy a third of the surface of the cultivated parcel.
Pasture	PAT	Froidefontaine	2018-2019	A hay meadow composed of Poaceae, clovers, dandelions etc. Bordered by hedgerows, except to the South face (sheep fence).
Orchard	VER	Froidefontaine	2018-2019	Flowering fields under young apple trees (many rustic varieties). This parcel is grazed by sheep in April and May. The parcel is bordered by hedges, except to its North face (sheep fence).
Wetland	ZH	Froidefontaine	2018-2019	The vegetation is mainly composed of plants from wetlands: buttercups, nettles, thistles, cradles etc. The meadow is bordered by a brook to the South and a hedge to the North.

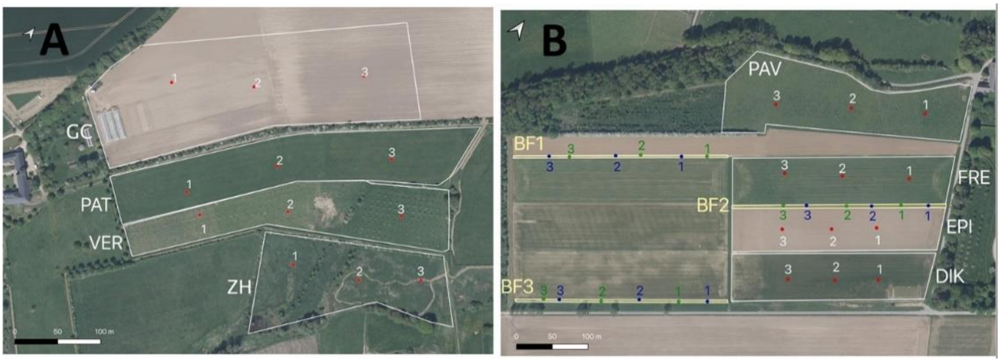


Figure 3-2 : Experimental design on both farmsteads **A.** Froidefontaine farmstead map. GC, PAT, VER and ZH correspond to the sampled parcels, whose details are given in Table 1. Each numbered red dot corresponds to the position of a trio of coloured (white, yellow, blue) pantraps; **B.** Emeville farmstead map. PAV, FRE, EPI and DIK correspond to the sampled parcels, whose details are given in Table 1. Each numbered red dot corresponds to the position of a trio of coloured (white, yellow, blue) pantraps. BF1, BF2 and BF3 correspond to the sampled flower strips. Each blue or green numbered dot corresponds to the position of a trio of coloured (white, yellow, blue) pantraps for the "feeder" flower patch or the "pollinator" flower patch, respectively.

2.1.3. Collection methods

To assess wild bee and hoverfly diversity, we conducted standardised sampling methods by combining coloured pantraps and netting transects (Westphal et al. 2008; Grundel et al. 2011; Földesi and Kovács-Hostyánszki 2014). Sampling was performed in 2018 and 2019, from April to July. At each collection site (**Figure 3-2A & B**), we positioned a triplet of pantraps (FLORA model with a diameter of 26.5 cm, RINGOT, France) coloured with UV reflecting sprays in white, blue and yellow (ROCOL top tracer model, UK). The pantraps were set-up in line and spaced 3 to 5 meters apart, in order to avoid the attraction coverage bias and to reach the same probabilities of insect capture between the pantraps (Droege et al. 2010; Amy et al. 2018). The pantrap triplets were separated by a minimum of 20 metres, in order to cover each parcel as homogeneously as possible (Carboni and Lebuhn 2003; Eeraerts et al. 2017). Each pantrap was filled with odourless and colourless soapy water every two weeks during one day (from 9:00 AM to 5:00 PM). Every two weeks, we also conducted variable transects with an insect net for one hour in the morning and one hour in the afternoon, for each habitat in Froidefontaine and each flower strip in Emeville (**Table 3-1; Figure 3-2**). We selected the sampling dates according to the following climatic conditions: temperature higher than 7°C, calm wind (< 12 km/h) and sunny and cloudless day (Westphal et al. 2008). We stocked insects in 70% ethanol for their conservation.

We followed the protocol of Mouret et al. (2007) to prepare, pin and label our collected specimens. In 2019, we decided to let the yellow pantraps to be continuously activated from mid-May to the end of July with sampling every 10 days to maximise the capture of syrphids and considering that hoverflies have a predilection for the yellow colour (Wäckers and van Rijn 2012; Lunau et al. 2018; An et al. 2018).

2.1.4. Species identification

Bee specimens were identified at the species level following identification keys of Pauly (2019b) for Halictidae, Patiny and Terzo (2010) for Andrenidae and Falk (2015) for the other bee families (Apidae, Colletidae, Megachilidae and Melittidae). All Halicitidae and Andrenidae specimens were confirmed by Alain Pauly (Royal Belgian Institute of Natural Sciences) and Thomas James Wood (University of Mons), respectively. Other bee specimens were confirmed by the reference collections of Gembloux Agro-Bio Tech. Hoverfly specimens were identified at the species level using the identification key of Verlinden (1994). The specimens were then confirmed by Frédéric Francis (University of Liège) and the reference collections of Gembloux Agro-Bio Tech. We applied Belgian Red List of bees for the conservation status of identified species (Drossart et al. 2019).

2.1.5. Historical data of Havelange Municipality

Thanks to Data Fauna-Flora v.5.1 software (Barbier and Rasmont 2015), we queried the database of Belgian wild bees, on 26 June 2020, for the historical diversity of wild bees in the Havelange Municipality. The selected geographical quadrat was encompassed within latitude from 50°21'14.4"N to 50°24'46.8"N and in longitude from 5°7'12"E to 5°19'26.399"E. The syrphid historical data were not available for Havelange Municipality.

2.1.6. Statistical analysis

We conducted one-way ANOVA tests to compare species richness and abundance of bee and hoverfly fauna between sampled parcels of Froidefontaine and Emeville farmsteads, separately. We also validated normal distribution of residuals of each ANOVA test. Subsequently, Tukey’s post-hoc tests were used to compare each parcel pair. We separated the flower strips of Emeville farm from the parcel comparisons because they were not sampled with the same effort as those of the sampled parcels. We compared the species richness and abundance of bee and hoverfly fauna between the feeder flower patch (BFV; **Figure 3-2B**) and the pollinator flower patch (BFB; **Figure 3-2B**) using the Student t-test. All statistical analysis were performed using R 4.0.2 and the resulting graphs were built using *ggplot2* and *ggpubr* packages (Wickham et al. 2016; Kassambara and Kassambara 2020). Last, beta species diversity was analyzed within the dataset of captured insect pollinators according to the both farmsteads and their specific parcels using the Bray–Curtis dissimilarity matrix and Principal Coordinate Analysis (PCoA) in order to show these dissimilarities at each sampling site (Oksanen 2008).

3. Results

Over 2 years (2018-2019) of sampling, we collected 4,303 bees and 1,998 syrphids, representing 92 species and morphospecies from 15 genera and six families for the bees and 31 species and morphospecies from 18 genera for the hoverflies (**Table 3-2**). Polylectic, oligolectic and cuckoo bee species correspond to 61%, 14% and 25% of bee richness, respectively. However, the relative proportion of specialised bee (0.9%) was low, with polylectic and cuckoo bees corresponding to 94% and 5.1% in abundance of the total sampled bees, respectively (**Table 3-2**). All adult hoverfly species were considered as polylectic species (Frank Van de Meutter, pers. comm.), except for *Platycheirus immarginatus* (**Table 3-2**). In the historical database of Belgian wild bees, we retrieved 18 bee species corresponding to 349 specimens between 1918 and 2007. These data are available in Suppl. material 1. With these historical data of the Havelange Municipality, the bee diversity reached 101 different bee species.

Table 3-2 : Abundance of each pollinator species according to the habitat of its collection. The habitat details are given in **Table 3-1**.

	BF	**DIK**	**EPI**	**FRE**	**GC**	**PAT**	**PAV**	**VER**	**ZH**	**Total (%)**
Bee	**285**	**256**	**277**	**244**	**439**	**1145**	**349**	**685**	**623**	**4303 (100)**
Andrena angustior		1	2		13	17	1	10	13	57 (1,32)
Andrena apicata		1								1 (0,02)
Andrena bicolor		1		1	7	1		4	10	24 (0,56)
Andrena carantonica				1		5		4	3	13 (0,3)
Andrena chrysosceles		2	5	5	1	2	6	1	4	26 (0,6)
Andrena cineraria		9	25	42	117	409	42	90	109	843 (19,59)
Andrena dorsata		6	4	7		6	6	12	5	46 (1,07)
Andrena flavipes	4	15	16	10	57	54	20	73	63	312 (7,25)
Andrena fulva		1	1	3	4	7	7	8	1	32 (0,74)
Andrena fulvata		6	10	1	7	3	6	9	6	48 (1,12)
Andrena gravida		2	3	3	8	48	4	23	15	106 (2,46)
Andrena haemorrhoa	1	7	5	19	32	145	24	121	107	461 (10,71)
Andrena humilis			1		2	1	1		2	7 (0,16)
Andrena labialis	2									2 (0,05)

Andrena labiata			1					2		3 (0,07)
Andrena minutula	1	3	2	1	2	1	2	3		15 (0,35)
Andrena mitis		2								2 (0,05)
Andrena nigroaenea		5	3	2	13	9	3	6	13	54 (1,25)
Andrena nitida		4	10	8	11	60	24	40	20	177 (4,11)
Andrena ovatula					3			1		4 (0,09)
Andrena praecox									3	3 (0,07)
Andrena schencki								1		1 (0,02)
Andrena semilaevis							1			1 (0,02)
Andrena subopaca	2		1			1			1	5 (0,12)
Andrena trimmerana					1	1				2 (0,05)
Andrena vaga		2	2	3		4	3	1		15 (0,35)
Andrena wilkella	8							6	1	15 (0,35)
Apis mellifera	114	32	33	35	57	63	128	54	41	557 (12,94)
Bombus campestris									1	1 (0,02)
Bombus hortorum	1		2	2		4			1	10 (0,23)
Bombus hypnorum						3			3	6 (0,14)
Bombus lapidarius	35	1	4	5	4	73	7	29	50	208 (4,83)
Bombus pascuorum	58		1	13	7	26	1	7	20	133 (3,09)
Bombus pratorum		1		2	2	9	1	3	4	22 (0,51)
Bombus terrestris	35	2	2	12	19	17	8	12	18	125 (2,9)
Bombus vestalis								1		1 (0,02)
Chelostoma rapunculi						1				1 (0,02)
Colletes cunicularius				1				1		2 (0,05)
Colletes daviesanus					1					1 (0,02)
Eucera longicornis								1		1 (0,02)
Halictus maculatus			1		1	1		3	3	9 (0,21)
Halictus rubicundus						2	1		2	5 (0,12)
Halictus scabiosae								2	2	4 (0,09)
Hylaeus brevicornis								2		2 (0,05)

Hylaeus communis				1			1			2 (0,05)
Hylaeus hyalinatus								1		1 (0,02)
Hylaeus signatus	1									1 (0,02)
Lasioglossum calceatum		38	43	14	9	29	11	24	16	184 (4,28)
Lasioglossum fulvicorne		2					2			4 (0,09)
Lasioglossum laticeps		1	4	1	4	1	1	2	1	15 (0,35)
Lasioglossum lativentre	5	1	16		2	17	4	28	1	74 (1,72)
Lasioglossum leucopus		3	1		1					5 (0,12)
Lasioglossum leucozonium		4	3	1	1	4	1	5	2	21 (0,49)
Lasioglossum malachurum			1		1		1		1	4 (0,09)
Lasioglossum morio		8	1			3	1	2	2	17 (0,4)
Lasioglossum pauxillum	6	93	62	41	19	24	13	16	20	294 (6,83)
Lasioglossum punctatissimum			1	1			1	1	1	5 (0,12)
Lasioglossum sexstrigatum			1							1 (0,02)
Lasioglossum sp.					1		1	2		4 (0,09)
Lasioglossum villosulum		1	1		4	2	1	3		12 (0,28)
Lasioglossum zonulum		1	1	3	4	4	1	2	4	20 (0,46)
Megachile ericetorum	1								1	2 (0,05)
Megachile willughbiella	1							1		2 (0,05)
Melitta tricincta								1		1 (0,02)
Nomada bifasciata						2	1	2	1	6 (0,14)
Nomada fabriciana						1		1	1	3 (0,07)
Nomada flava			1			2		2	2	7 (0,16)
Nomada flavoguttata						1		1	3	5 (0,12)
Nomada fucata	1		2		4	7	5	11	1	31 (0,72)
Nomada fulvicornis			1	2			1			4 (0,09)
Nomada goodeniana			2		5	24	2	13	7	53 (1,23)
Nomada lathburiana					1	9	1	2	2	15 (0,35)
Nomada leucophthalma						2		1	1	4 (0,09)
Nomada marshamella									2	2 (0,05)

Nomada panzeri						2			3	5 (0,12)
Nomada ruficornis			1		1	21		13	17	53 (1,23)
Nomada signata						2		1	1	4 (0,09)
Nomada succincta						1				1 (0,02)
Nomada zonata						1	1	2	1	5 (0,12)
Osmia bicolor									1	1 (0,02)
Osmia bicornis					3	6	1	2	5	17 (0,4)
Osmia cornuta						1		2		3 (0,07)
Osmia leaiana							1	1		2 (0,05)
Osmia leucomelana	1				1			2		4 (0,09)
Osmia tridentata	1									1 (0,02)
Seladonia tumulorum	7	1		3	4	3		6	2	26 (0,6)
Sphecodes ephippius			1	1	3	3		3	1	12 (0,28)
Sphecodes ferruginatus							1			1 (0,02)
Sphecodes gibbus									1	1 (0,02)
Sphecodes monilicornis								1	1	2 (0,05)
Sphecodes puncticeps					1					1 (0,02)
Sphecodes sp.					1			1		2 (0,05)
Hoverfly	**907**	**228**	**26**	**91**	**266**	**91**	**86**	**72**	**231**	**1998 (100)**
Cheilosia sp.	2				1	15	1	1	55	75 (3,75)
Episyrphus balteatus	124	10	1	36	6	3	5	10	14	209 (10,46)
Eristalis arbustorum	60	10	3	1	5	2	2	5	15	103 (5,16)
Eristalis nemorum	3									3 (0,15)
Eristalis pertinax								1	5	6 (0,3)
Eristalis sepulchralis	1									1 (0,05)
Eristalis similis								1		1 (0,05)
Eristalis tenax	186	13	4	8	37	24	43	4	23	342 (17,12)
Eupeodes luniger	6	9	1	1	3	1	1	1	2	25 (1,25)
Ferdinandea cuprea							2			2 (0,1)

Helophilus trivittatus						1			2	3 (0,15)
Melanostoma mellinum	53	17		13	13	1	3	1	1	102 (5,1)
Metasyrphus corollae	7	15		3	1	1	1	2		30 (1,5)
Metasyrphus latifasciatus						2			4	6 (0,3)
Myathropa florea				1					3	4 (0,2)
Platycheirus albimanus	1	1			1					3 (0,15)
Platycheirus clypeatus									1	1 (0,05)
Platycheirus immarginatus						1		1		2 (0,1)
Platycheirus peltatus						3	1		2	6 (0,3)
Platycheirus scambus					2					2 (0,1)
Rhingia campestris						1				1 (0,05)
Scaeva pyrastri	19	5		3	4	3	1		3	38 (1,9)
Sphaerophoria scripta	401	148	13	23	174	29	17	40	84	929 (46,5)
Syritta pipiens	37		1		13			1	4	56 (2,8)
Syrphus ribesii	3		3	1	3	3	9	1	10	33 (1,65)
Syrphus vitripennis	1				3	1		1	2	8 (0,4)
Volucella bombylans								1		1 (0,05)
Volucella pellucens								1		1 (0,05)
Xanthogramma pedissequum				1					1	2 (0,1)
Xylota segnis	2									2 (0,1)
Xylota sylvarum	1									1 (0,05)
Total of specimens	**1192**	**484**	**303**	**335**	**705**	**1236**	**435**	**757**	**854**	**6301**

For Froidefontaine farmstead, bee richness in VER was significantly higher than in GC (*p*value < 0.05; **Figure 3-3A**) and bee abundance in PAT was significantly higher than in GC, VER and ZH (*p*-values < 0.05; **Figure 3-3B**). Hoverfly diversity in ZH was significantly higher than in VER (*p*-value < 0.05; **Figure 3-3C**), while hoverfly abundance was homogenous amongst the Froidefontaine parcels (**Figure 3-3D**). For Emeville farmstead, bee and hoverfly richness and bee abundance did not vary amongst parcels (**Figure 3-4A, B and C**), while DIK parcel exhibited significantly greater hoverfly abundance than EPI, FRE and PAV parcels (*p*-values < 0.05; **Figure 3-4D**). Only for bee richness and hoverfly abundance, the pollinator flower patch BFB showed significantly higher mean values than the feeder flower patch BFV (*p*-values;).

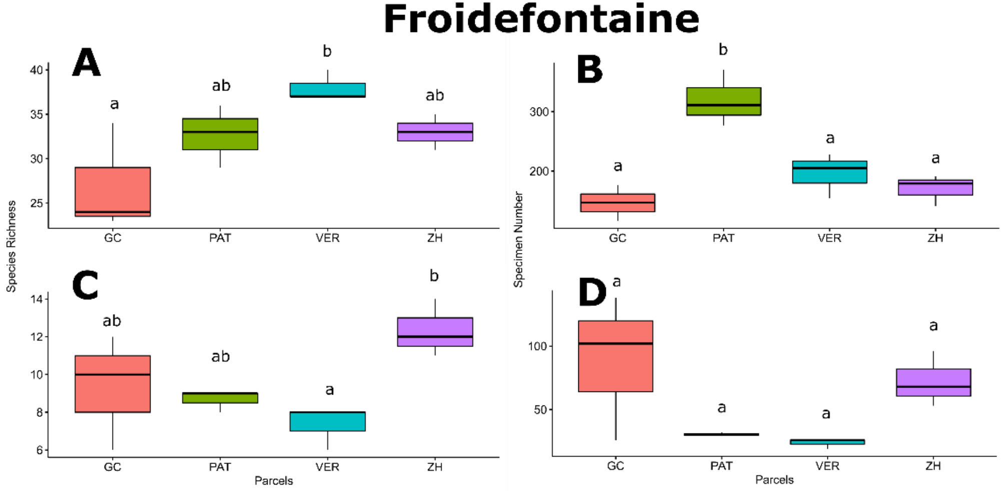


Figure 3-3 : Mean values of species richness and abundance for bee and hoverfly fauna amongst Froidefontaine parcels GC, PAT, VER and ZH (see details given in **Table 3-1**). **A.** Bee richness; **B.** Bee abundance; **C.** Hoverfly richness; **D.** Hoverfly abundance. Letters above the boxplots represent Tukey's post-hoc comparisons.

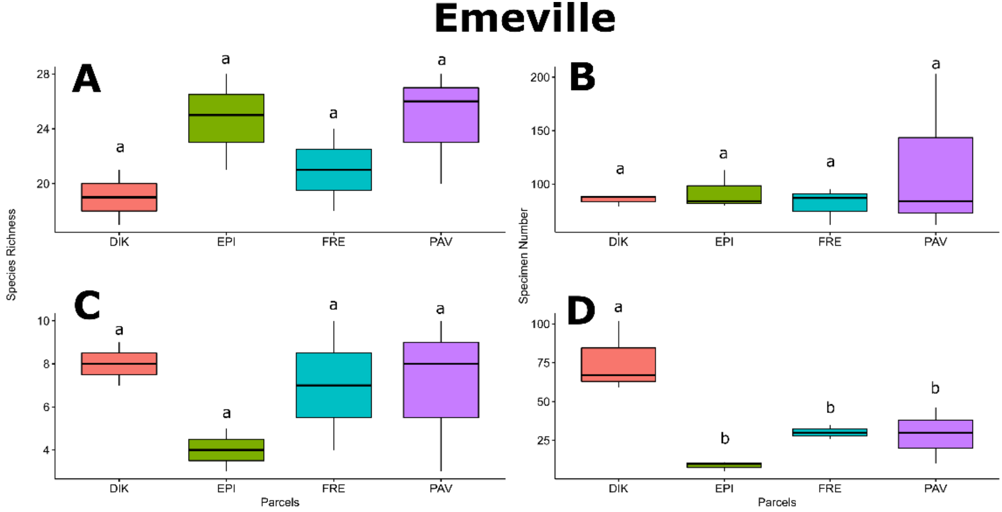


Figure 3-4 : Mean values of species richness and abundance for bee and hoverfly fauna amongst Emeville parcels DIK, EPI, FRE and PAV (see details given in **Table 3-1**). **A.** Bee richness; **B.** Bee abundance; **C.** Hoverfly richness; **D.** Hoverfly abundance. Letters above the boxplots represent Tukey's post-hoc comparisons.

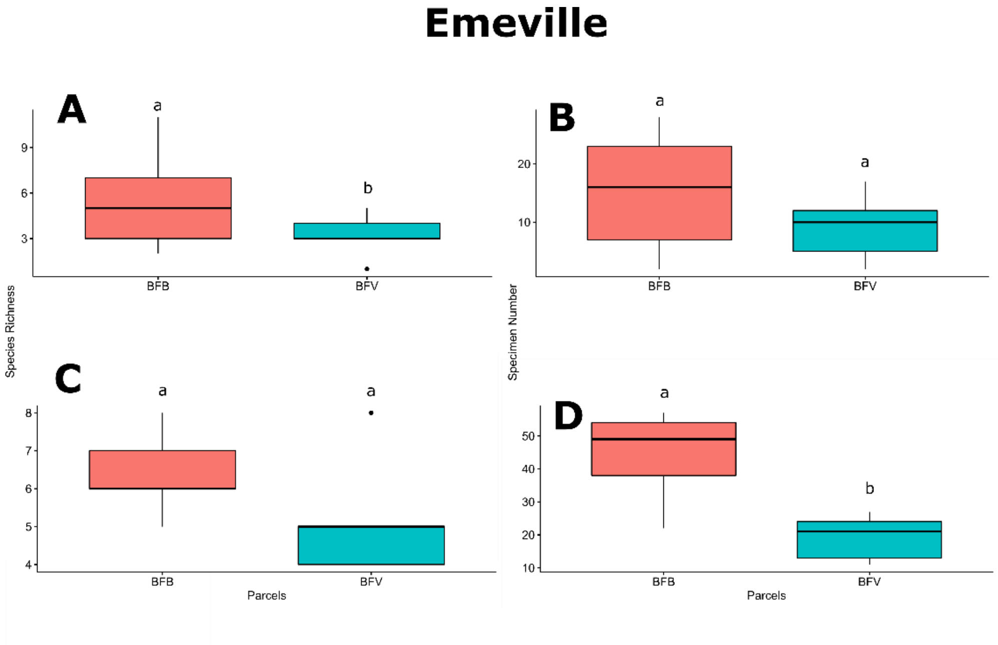


Figure 3-5 : Mean values of species richness and abundance for bee and hoverfly fauna amongst flower strips BFB and BFV (see details given in **Table 3-1**). **A.** Bee richness; **B.** Bee abundance; **C.** Hoverfly richness; **D.** Hoverfly abundance. Letters above the boxplots represent Student t-test comparisons.

Between the two farmsteads, the bee communities were primarily organized along the first axis, indicating the influence of the flower strips **(Figure 3-6A)**. However, this was not observed for the hoverflies, as they exhibited more comparable communities between the two farms **(Figure 3-6B)**. Within the Froidefontaine farmstead, both bee and hoverfly communities displayed distinct compositions across different parcels, except for hoverfly communities between GC and ZH, which showed similar distribution patterns **(Figures 3-7A & 3-8A)**. In contrast, within the Emeville farmstead, bee communities exhibited segregation between parcels and flower strips, while hoverfly communities demonstrated a more gradual pattern **(Figures 3-7B & 3-8B)**.

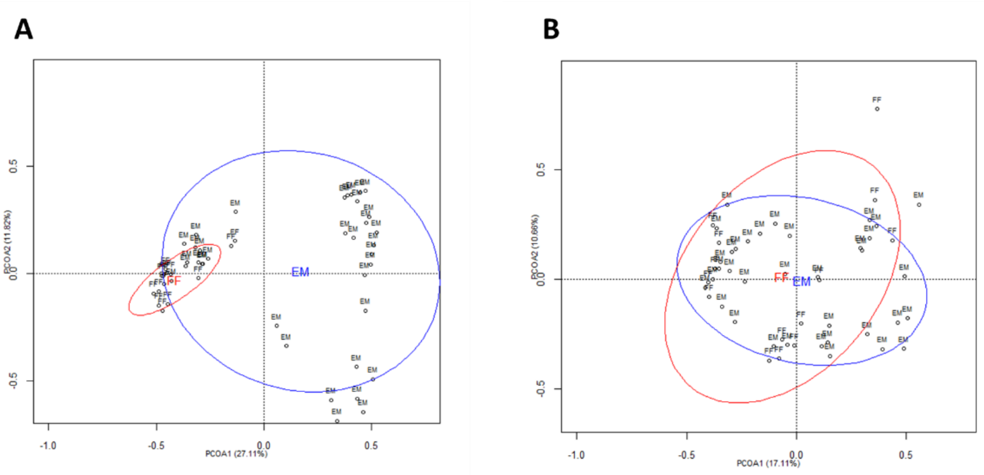


Figure 3-6 : Principal Component Analysis (PCoA) for both farmstead (red for Froidefontaine and blue for Emeville) **A.** Bee beta diversity; **B.** Hoverfly beta diversity. The ellipses are shown with 80% of interval confidence.

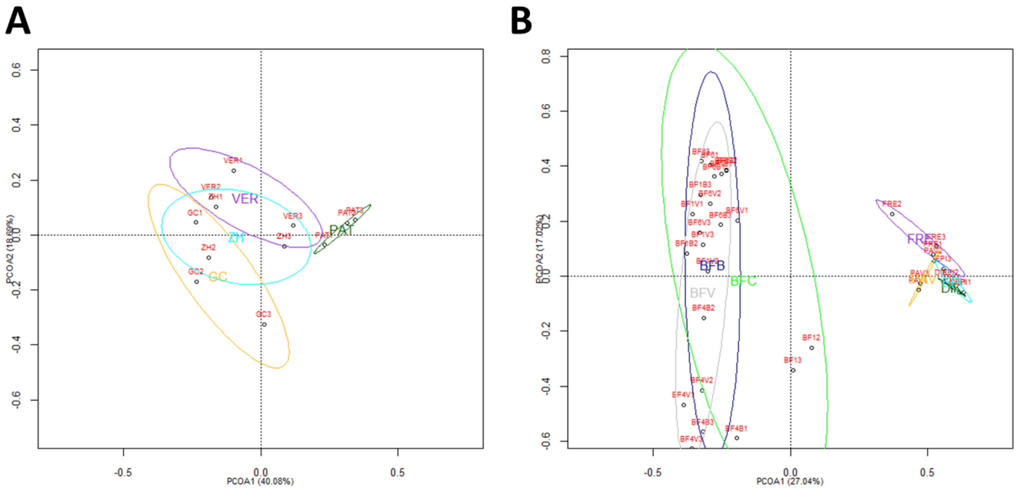


Figure 3-7 : Principal Component Analysis (PCoA) for bee pollinators in Froidefontaine (**A**) and in Emeville (**B**) farmsteads. The colors of each ellipse correspond to the acronyms showed in **Table 3-1**. The ellipses are shown with 80% of interval confidence.

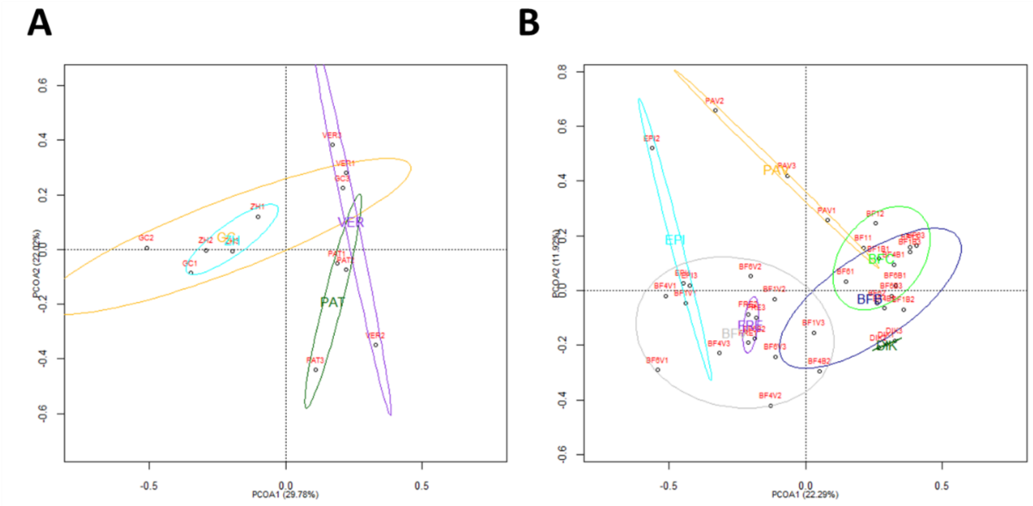


Figure 3-8 : Principal Component Analysis (PCoA) for hoverfly pollinators in Froidefontaine (**A**) and in Emeville (**B**) farmsteads. The colors of each ellipse correspond to the acronyms showed in **Table 3-1**. The ellipses are shown with 80% of interval confidence.

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4. Discussion

4.1. Polylectic bee species

In our study, we identified 101 different bee species, corresponding to almost one quarter of the Belgian bee fauna (Drossart et al. 2019). Depicting 57.32% of the total bee collected material, the top-five bee species in both farms were *Andrena cineraria* (19.59%), *Apis mellifera* (12.94%), *A. haemorrhoa* (10.71%), *A. flavipes* (7.25%) and *Lasioglossum pauxillum* (6.83%).

Both farms presented suitable habitats to these polylectic species, including open wooded spaces, fallow land or lawns. The abundance of *Taraxacum* spp. (Asteraceae), *Salix* spp. (Salicaceae), *Craetegus* spp. (Rosaceae) and fruit trees could explain the dominance of *A. cineraria*, *A. haemorrhoa* and *A. flavipes* populations. Moreover, they usually nest in south exposed sites, in bare soils or in areas with sparse and short vegetation (Falk 2015). The other common polylectic bees were mainly ground-nesting species belonging to *Andrena* and *Lasioglossum* genera, such as *A. nitida*, *A. gravida*, *L. calceatum* or *L. lativentre* (**Table 3-2**).

Uncommon polylectic bee species were also collected. For example, *Andrena trimmerana* and *Halictus maculatus* (**Figure 3-9C**) are rarely observed in the Condroz Region and more largely in Belgium. *H. maculatus* is a little more common in Wallonia and this species is considered as "vulnerable" in Belgium, but "least concern" in Europe (Drossart et al. 2019, Nieto et al. 2014). Moreover, this species forages on *Achillea millefolium* (Asteraceae), *Centaurea* spp. (Asteraceae) or *Daucus* *carota* (Apiaceae) (Pauly 2019b), which were naturally present or cultivated in both farms. In 2019, specimens of *A. trimmerana* were collected only in the Froidefontaine farmstead, where *Rubus* spp. (Rosaceae), orchards, umbellifers or *Cirsium* spp. (Asteraceae) were flowering. Two specimens of *Colletes cunicularius* were sampled from both farms. This species is specialised on *Salix* spp. (Salicaceae) or *Prunus cerasus* L., (Rosaceae) (Falk 2015). While *Lasioglossum leucopus* was observed in both farms - probably because of the presence of several of its preferred host plants, *Ranunculus* spp. (Ranunculaceae), *Taraxacum* spp. (Asteraceae) and *D. carota* - this species is considered as "near threatened" according to the IUCN Red List Criteria in Belgium (Drossart et al. 2019, Pauly 2019b).

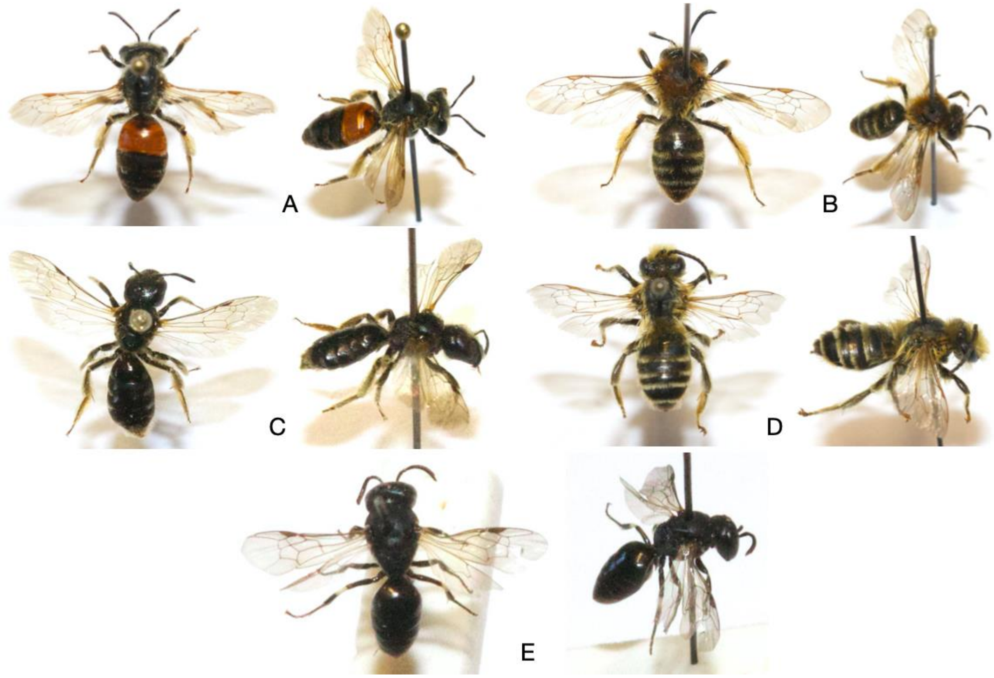

Figure 3-9 : Dorsal and lateral side of some rare bees observed within the farmsteads. **A.** *Andrena schencki* Morawitz 1866; **B.** *Andrena fulvata* (Müller 1766); **C.** *Halictus maculatus* Smith 1848; **D.** *Melitta leporina* (Panzer 1799); **E.** *Hylaeus brevicornis* Nylander 1852.

Rarer species were observed within the farmsteads. Collected in the orchard of Froidefontaine, *Andrena schencki* (**Figure 3-9A**) had not been observed south of the Sambre and Meuse Furrow for more than 30 years (Rasmont and Haubruge 2002). *Andrena semilaevis*, a very rare species since 1990 in Belgium (Rasmont and Haubruge 2002), was captured in the orchard of Emeville. This polylectic species is mostly observed on the umbellifers (Falk 2015). Forty-six specimens (1.12% of total sampling) of *Andrena fulvata* (**Figure 3-9B**) were collected in 2019 in all habitats of both farms, while only one observation was encoded in Atlas Hymenoptera repository for Belgium (Rasmont and Haubruge 2002). That probably means a recent installation of the population on the study sites. However, misidentification due to their morphological resemblance to *A. angustior* could bias its Belgian rarity (T.J. Wood, personal communication). This species nests in calcareous soils and forages principally on Asteraceae flowers, such as *Taraxacum* spp. (Falk 2015).

The high diversity of wild bees in the two farms could be linked to the presence of seminatural habitats around the parcels. Indeed, the implantation of hedgerows, flower strips or shrubby strips between the habitats of both farms provides sufficient floral resources during the foraging activity period of polylectic species (Albrecht et al. 2020).

The massive introduction of honeybee colonies (>3/km²) can have significant negative effects on wild bee fauna due to floral competition and disease transmission (Geslin et al. 2017b), particularly when colonies are concentrated in a single apiary (Henry and Rodet 2018). This can lead to dramatic consequences for the native bee populations (Herbertsson et al. 2016). Our observations revealed a notable presence of honeybees in our dataset (~12% of the be dataset), indicating a potential correlation with the presence of an apiary within the Froidefontaine farmstead and potentially in the vicinity of the Emeville farmstead. In comparison to the literature, our findings show that this is not a substantial value. For instance, a study conducted in European orchards, known to attract a large number of honeybees during flowering, reported a honeybee presence of 40% (with a total of 12,867 bees) (Weekers et al. 2022). In another study focused on more organic farming systems, the honeybee presence was even lower, accounting for only 23% of the entire dataset (Holzschuh et al. 2008).

4.2. Oligolectic bee species

Thirteen bee species were characterised as oligolectic (Drossart et al. 2019), which represented 24 specimens (**Table 3-2**).

Two common species, *A. praecox* and *A. vaga* and two uncommon species, *A. apicata* and *A. mitis*, were collected in different parts of both farms (**Table 3-2**). In Belgium, they are considered as *Salix* spp. specialists. Moreover, these last two species had never been observed in Condroz before and not since 1950 in the south of Wallonia. *A. humilis* is a specialist of Asteraceae plant species, such as *Tragopogon dubius* Scopoli 1772, *Hieracium pilosella* Vaillant 1754 (Scheuchl 2002) or *Cichorium* spp. and *A. labialis* is a specialist of leguminous plants (Fabaceae) (Rasmont and Haubruge 2002).

A single specimen of Melittidae family, *Melitta leporina* (**Figure 3-9D**), was sampled. The female is particurlarly related to the flowers of *M. sativa* and *T. pratense* species (Fabaceae) (Dellicour and Michez 2010), which were abundantly present around the wetland of Froidefontaine Farm. One species of Colletidae family, *Colletes daviesanus*, forages pollen entirely from composite flowers such as tansy, mayweeds or oxeye daisy (Asteraceae) (Falk 2015).

In Froidefontaine habitats, we also sampled a few specimens of *Chelostoma rapunculi*, *Eucera longicornis*, *Hylaeus signatus*, *Megachile ericertorum* and *Osmia* *leaiana*, probably because their preferred flowers were partially present: *Trifolium* sp., *Medicago* sp., *Cirsium* sp., *Rubus* sp., *Centaurea* sp. and *Stachys sylvatica* L. 1753.

4.3. Cuckoo bee species

We only collected two specimens of cuckoo bumble bees (subgenus *Psithyrus* Lepeletier), *Bombus campestris* and *B. vestalis*, in Froidefontaine wetland and in Froidefontaine orchard (**Table 3-2**), respectively. They are considered rare species (Lhomme and Hines 2019) and their presence could be explained by the relative predominance, in the *Bombus* genus, of their associated host species: *B. pascuorum* and *B. terrestris* (**Table 3-2**).

Concerning the nomad bees (*Nomada* spp.), we identified 15 species representing 4.6% of the collected material. They especially parasitise *Andrena* spp. and their relative abundance is dependent on the proportion of their host bee species (Sheffield et al. 2013). Most of their host species were collected throughout the two years of experiment. For example, we found, in a small proportion, *Nomada flavoguttata* and *N. leucophthalma*, which are linked to *Micrandrena* spp. Ashmead 1899 (*Andrena semilaevis*, *A. subopaca*...) and *A. apicata*, respectively. On the contrary, *N. goodeniana* and *N. ruficornis* were largely present due to the strong dominance of *A. cineraria* and *A. haemorrhoa* (Rasmont and Haubruge 2002).

All collected *Sphecodes* spp. are generalist cleptoparasites, except for *S. gibbus* that parasitises the nests of *Halictus* species, such as *H. maculatus* and *H. rubicundus*. Their relative abundance also followed the abundance of their host species: the most collected *S. epphipius* is the cuckoo bee of the most collected halictid bee, *Lasioglossum pauxillum* (Pauly 2019b).

4.4. Hoverfly species

Within both farmsteads, *Sphaerophoria scripta* was, by far, the most abundant hoverfly species, followed by *Eristalis tenax* and *Episyrphus balteatus*, corresponding together to almost three quarters of the total number of collected specimens (**Table 3-2**). These species are the most common syrphids encountered in Central Europe (Nengel and Drescher 1991; Alhmedi et al. 2010; Francuski et al. 2013). Aphidophagous larvae of *S. scripta* and *E. balteatus* are important for pest control in agricultural systems, while *E. tenax* larvae recycle the organic matter in wet manures, muds or ponds (Sommaggio 1999). We also emphasised the presence of *Melanostoma mellinum*, which occured in almost each habitat and particularly in flower strips. Adults *M. mellinum* are specialised in the floral visitation of anemophilous plants (Van der Groot and Grabandt 1970).

Beside these ubiquitous species, rarer species were found in only a few habitats: *Xanthogramma pedissequum*, *Myathropa florea* and *Ferdinandea cuprea* (**Figure 3-10**). Unlike *S. scripta* and *E. tenax*, these species do not migrate. The larvae of *X. pedissequum* feed on aphids reared on the anthills of some *Lasius* sp. Fabricius 1804

(Hymenoptera: Formicidae) (Speights 2017). The species *M. florea* and *F. cuprea* present a microphagous larval stage. In intensified agricultural landscapes, it is conceivable that the environmental requirements of such species are scarcely fulfilled. Notably, microphagous species appear to be particularly sensitive to pesticides (Schweiger et al. 2007). On the contrary, agricultural landscapes of Froidefontaine and Emeville Farms are suitable for these specialist species, because they include semi-natural ecosystems and organic orchards where cattle or sheep are grazing. We also identified two specimens of *Platycheirus immarginatus* that are specialist foragers on *Bolboschoenus maritimus* (L.) (**Table 3-2**) (Speight 2017). We did not find this plant species in Froidefontaine farmstead, meaning that *P. immarginatus* might forage on other plant species. Continuous sampling represented only 4.33% of the total hoverfly specimens. However, it allowed us to reveal two more hoverfly species, in Emeville flower strips: *Xylota sylvarum* and *X. segnis,* whose larvae are saproxylic and live close to roots and dead wood (Speights 2017).

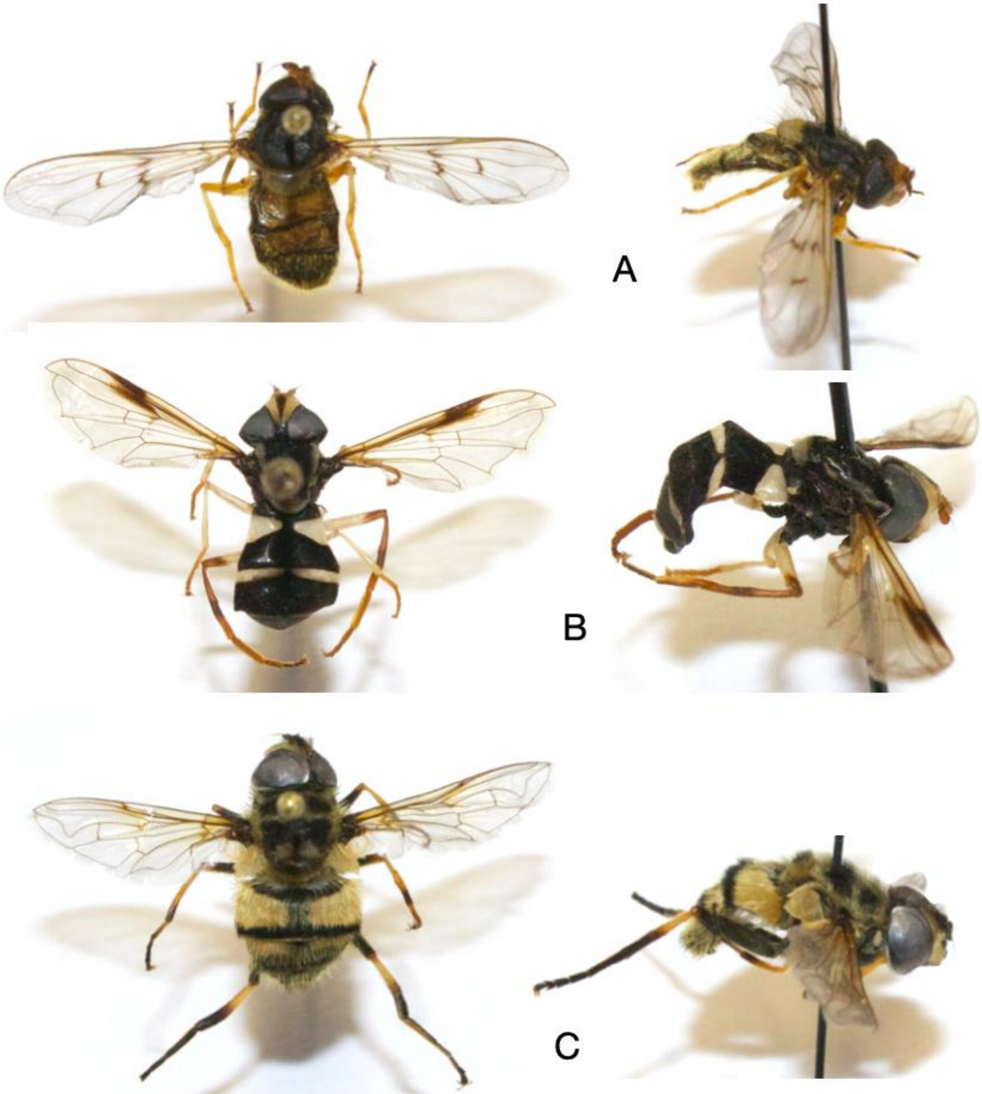

Figure 3-10 : Dorsal and lateral side of some rare hoverfly species observed within the farmsteads. **A.** *Ferdinandea cuprea* (Scopoli 1763); **B.** *Xanthogramma pedissequum* (Harris 1776); **C.** *Myathropa florea* (L.).

4.5. Impact of agroecological practices on wild bees and hoverflies communities at the farm scale

By in-depth sampling, we documented new occurrences of almost 1/4 of Belgian bee fauna in two farms in ecological transition. For the historical region of the Municipality of Havelange, we have almost quintupled the richness of wild bee community despite high quality monitoring of these populations in Belgium (Drossart et al. 2019). There are few studies of this type in a close environment and with comparable methodology. Therefore, comparing our results with other studies seems to be of little relevance. This study leads us to consider that, on small areas undergoing ecological transition, an important richness of pollinators is easily reached. Moreover, it is possible that the conducted survey underestimates the real diversity per plot, even if the pattern of dominance rarity should be maintained. We also lack data at the end of the season, especially for late summer bees, such as *Colletes hederae* (Schmidt and Westrich 1993). For hoverflies, we still lack inventory data on the scale of the Belgian territory (Frank Van de Meutter, pers. comm.).

The practices on and around the studied farms seemed favourable to pollinators (**Figure 3-11**) and especially to the polylectic species. Promoted by agroecology, the diverse parcels within each farmstead of the study have a significant impact on beta diversity, more so than on alpha diversity. As a result, distinct pollinator communities emerge, each exhibiting unique floral and nesting preferences, which enhances the resilience of the environment (Dendoncker et al. 2018). In Froidefontaine Farm, the land tenure showed strong impact on bee richness and abundance by an alternation of floral bee-feeding parcels, like the Froidefontaine pasture (PAT; **Figure 3-3B**) and bee-nesting parcels, like the Froidefontaine orchard (VER; **Figure 3-3A & Figure 3-11B**). On the one hand, late mowing permits the keeping of abundant floral resources throughout the bee activity period (Meyer et al. 2017) and, on the other hand, sheep grazing permits the conservation of some bare soil sites that favour ground-nesting bees (Cane 1991). Landscape micro-habitats, such as ponds, hedgerows or groves, are important to the survival of many pollinator species, especially by providing habitats for hoverfly larvae (Sommaggio 1999). The wetland of Froidefontaine (ZH) (**Figure 3-11A**) harboured higher hoverfly diversity than the other parcels (**Figure 3-3C**), with species like *S. scripta*, *Cheilosia* sp. and *E. tenax*, whose larvae have different diets (i.e. aphidophagous, phytophagous and microphagous, respectively) (Sommaggio 1999, Speight 2017). The cultivated parcel of Froidefontaine (GC) (**Table 3-2**) and the pea crop of Emeville (DIK) (**Figure 3-4D**) showed high abundances of aphidophagous hoverflies, likely caused by the high prevalence of aphids on crops. The flower strips separating the parcels of Emeville Farm consisted of a floral mix especially designed to fill the ecological requirements of bees and hoverflies (**Figure 3-11D**). The floral composition of these flower strips attracted more hoverfly specimens than bees, which were mainly represented by *A. mellifera* (**Table 3-2**). Moreover, they were combined with belatedly-mowed hedges that support floral resources for pollinators throughout their activity season. Similarly, the hedgerows bordering the parcels of Froidefontaine (**Figure 3-11C**), coupled with ecological crop management practices (i.e. no-till, no chemical inputs...), promoted the establishment of wild bee populations (Albrecht et al. 2020). Indeed, hedgerows and other semi-natural habitats usually represent superior floral richness and abundance compared to intensive agricultural land use (Hannon and Sisk 2009).

According to the Belgian Red List of bees (Drossart et al. 2019), we have collected several species indexed in threatened categories from diverse habitats of both farms, especially in the orchard and in the wetland of Froidefontaine. These species were represented by one specimen of *A. schencki*, one specimen of *B. campestris*, one specimen of *E. longicornis* and nine specimens of *H. maculatus*. We also mitigated the data deficiency in Belgium for a few records of bee species, such as *A. semilaevis*, *A. trimmerana* and *Hylaeus brevicornis* (**Figure 3-9E**). Taxonomically recent recognition, split from species complex and morphological similarity with widespread taxa or less studied genera (e.g. *Hylaeus* sp.) reflect current taxonomic impediments for 9.4% of the Belgian bee richness (Drossart et al. 2019).

Pollinator composition of each farmstead harboured both common and rare species, which indicates that on-farm diversification and organic practices may be an important refuge for rare, Red-Listed or oligolectic pollinator species (Guzman et al. 2019b). Restoring or incorporating diverse habitats in agro-ecosystems is therefore a long-term solution for the conservation of pollinating species (St. Clair et al. 2020).

Figure 3-11 : Some field pictures in each farm. **A.** Froidefontaine wetland (ZH); **B.** Froidefontaine orchard (VER); **C.** Double hedgerow between Froidefontaine cultivated parcel (GC) and pasture (PAT); **D.** Emeville flower strip between FRE and EPI parcels (photo credit : I. Van Dorpe); **E.** Emeville orchard (PAV).

Chapter 4

Flower strips in wheat intercropping system

Chapter IV: Flower strips in wheat intercropping system

This section is an adapted version of the article:

Amy, Clara, **Grégoire Noël,** Séverin Hatt, Roel Uyttenbroeck, Frank Van de Meutter, David Genoud, and Frédéric Francis (2018). "Flower Strips in Wheat Intercropping System: Effect on Pollinator Abundance and Diversity in Belgium" Published in *Insects* 9, no. 3: 114. https://doi.org/10.3390/insects9030114

Abstract - The decline of pollinators in agricultural areas has been observed for some decades, this being partly due to landscape simplification in intensive agrosystems. Diversifying agricultural landscapes by sowing flower strips within fields could reduce these adverse effects on biodiversity. In this context, the study presented here aimed at assessing and comparing the abundance and diversity of bees (Hymenoptera: Anthophila) and hoverflies (Diptera: Syrphidae) found and visiting flowers in three types of flower strips in Belgium: (i) a mixture of 11 wild flowers, (ii) a monofloral strip of *Dimorphoteca pluvialis* (Asteraceae) and (iii) a monofloral strip of *Camelina sativa* (Brassicaceae), where the last two are considered to be intercrops since they are valuable on the market, all sown within a field of winter wheat (*Triticum aestivum* L.). Pollinators were captured with pan traps and by netting in standardised transects from May to July 2017. One-thousand one-hundred and eighty-four individuals belonging to 43 bee species and 18 hoverfly species were collected. Significant differences in hoverfly diversity were found between the different flower strips. The multifloral treatment supported a greater diversity of syrphid species. Various pollinator species visited the different flowers composing the mixture and also *D. pluvialis*. The pollinator community proved to be predominantly generalist, with the exception of an oligolectic species in Belgium, *Andrena nitidiuscula*. Moreover, the three tested flower strips were effective in attracting hoverflies, among them natural enemies of insect pests. This study opens new perspectives in the design of intercropping systems with flower strips towards the design of sustainable agro-ecosystems. Improving economic profitability of sowing flower strips could encourage farmers to diversify their agricultural systems and foster conservation biology strategies.

Keywords: sustainable agriculture; ecosystem services; Apoideae; Syrphidae; *Dimorphoteca pluvialis*; *Camelina sativa*; *Coriandrum sativum*; *Fagopyrum esculentum*; *Andrena nitidiuscula*

1. Introduction

With approximately 20,000 species worldwide and more than 2000 species in Europe, bees (Hymenoptera: Anthophila) are among the most speciose pollinator groups in temperate agriculture landscapes (Rasmont et al. 2017). Their long-time coevolution with flowering plants has provided them with both morphological (e.g., scopa, pollen baskets) and behavioural (e.g., lectism, sociality) traits, suitable for plant pollination (Michener 2007; Pfiffner and Müller 2007). Non-bee insects, among other hoverflies (Diptera: Syrphidae), are also important for pollinating plants because they are responsible for 25–50% of the total number of visits to flowers (Fründ et al. 2010; Rader et al. 2016) and contribute significantly to pollination (Jauker et al. 2012; Orford et al. 2015). Pollination is an essential ecosystem service because 70% of the diversity of plants cultivated globally and up to 84% of plants cultivated in Europe depend on it (Breeze et al. 2016). Its economic value has been estimated at 153–285 billion Euros a year (Klein et al. 2007). In Belgium, the contribution of insect pollinators to plant production for human food (i.e., mainly fruits and vegetables) was estimated at about 250 million Euros in 2010 (Jacquemin et al. 2017). For some 50 years now, pollinator diversity and abundance have been declining at a large scale (Cameron et al. 2011; Garibaldi et al. 2013). Important drivers responsible for this decline are the simplification of landscapes and fragmentation of habitats caused by urbanisation processes and agricultural intensification (Potts et al. 2011). Indeed, along with the modernisation of agriculture, parcel size has dramatically increased on 40% of the European landscape (Carrié 2016) due to the suppression of semi-natural habitats (i.e., hedges, groves, fallows). The loss of pollinators from agricultural landscapes threatens the service of pollination (Deguines et al. 2014). In fact, pollinator decline could negatively impact pollinator-dependent crop yields (e.g., orchards, cultivation of vegetables), creating a negative economic impact (Potts et al. 2016). This depletion could have severe implications for producers and consumer welfare (Potts et al. 2016). Current pollinator decline may also lead to deficiency of essential minerals and vitamins for the human diet provided by pollinator-mediated crops (Eilers et al. 2011). Moreover, wild plants could suffer from a dearth of pollination and such effects may cascade further through the food web (Suttle 2003). These threats could have detrimental effects on agro-ecosystems, human food supply and well-being (Potts et al. 2016). In this context, Agri-Environmental and Climate Measures (AECM) have been proposed to farmers in Europe to 'reduce environmental risks associated with modern farming on the one hand and preserve nature and cultivated landscapes on the other hand' (European Commission 2015). Farmers can adopt AECM on a voluntary basis and receive monetary compensation in return for potential losses of income. In Wallonia (Belgium), 11 measures are available to farmers who commit themselves for at least five years (NaTagriWal: Belgium 2015). Some of these measures aim at supporting pollinators, such as wildflower strips. Flowering strips are recognised to support insect populations in general (Haaland et al. 2011) and pollinators particularly (Le Féon 2010; Uyttenbroeck et al. 2017), yet their effect depends strongly on the floral composition of the sown mixtures

(Warzecha et al. 2018). Previous studies explored how pollinator communities are affected by the species diversity of flower mixtures (Potts et al. 2003), by the functional diversity of flower mixtures (Uyttenbroeck et al. 2017) and by specific plant species that are known to be attractive to pollinators (Barbir et al. 2015). Additionally, spatial diversification of agroecosystems is increasingly considered to improve the sustainability of agriculture (Hatt et al. 2018). Within fields, intercropping (i.e., the cultivation of at least two crops simultaneously) can reduce the requirement for fertilisers (Bedoussac et al. 2015) and the risks of infestations by insect pests (Lopes et al. 2016) and diseases (Boudreau 2013). Considering flowering crops in intercropping could moreover benefit pollinators.

The first objective of this study is to estimate the biodiversity of pollinator communities (Hymenoptera: Anthophila and Diptera: Syrphidae) on several flower strips sown in wheat (*Triticum aestivum* (L., 1753)) crops. The second objective is to compare three modalities of flower strips regarding their effect on pollinator abundance and diversity: a multifloral mixture of wildflower species and two oilseed monofloral strips of *Camelina sativa* (Crantz, 1753) (Brassicaceae) and *Dimorphoteca pluvialis* (Moench, 1794) (Asteraceae) are considered intercrops since they are valuable on the market (Campbell 2018; Singer and Weselake 2018). Whereas sowing mixtures of wildflowers can be subsidised through the AECM, the latter two options would offer opportunities of income diversification to farmers (Pinochet 1994; Gugel and Falk 2006). By focusing on bees and hoverflies more particularly, the third objective of the present study is to explore how these two groups of pollinators interact with the different floral species within the flower strips.

2. Materials and methods

2.1. Experimental setup

Three flower strip treatments were established by sowing a multifloral, and two distinct monofloral, strips (i.e., one with *C. sativa* and one with *D. pluvialis*) in a 12-ha field of the “Agriculture Is Life” experimental farm of Gembloux Agro-Bio Tech (University of Liege, Belgium) (50◦30′52.403′′ N; 4◦43′51.153′′ E). The surrounding landscape was mostly composed of large urbanised areas (52%) and agricultural fields (39%) within a 3 km radius from the field (Figure S1). The multifloral treatment contained 11 floral species that were selected for their melliferous potential: *Daucus carota* (L., 1753) (Apiaceae), *Oenothera biennis* (Linnaeus, 1753) (Onagraceae), *Echium vulgare* (L., 1753) (Boraginaceae), *Coriandrum sativum* (L., 1753) (Apiaceae), *Fagopyrum esculentum* (Moench, 1794) (Polygonaceae), *Glebionis segetum* ((L.) Fourr., 1869) (Asteraceae), *Silene latifolia alba* (Poiret, 1789) (Caryophyllaceae), *Malva moschata* (L., 1753) (Malvaceae), *Geranium pyrenaicum* (Burman, 1753) (Geraniaceae), *Trifolium incarnatum* (L., 1753) (Fabaceae), *Trifolium repens* (L., 1753) (Fabaceae). *T. repens* and *T. incarnatum* were especially chosen for their soil cover properties. To ensure high floral diversity and evenness, the seed mixture was assembled using an equal number of seeds for each floral species

(**Table 4-S1**). Eighteen flower strips (4 m × 25 m) were sown in the field on 27 April 2017, each strip being separated by 27 m of winter wheat, finally constituting an intercropping system. Each floral treatment was repeated three times in a Latin square design and each repetition consisted of two similar adjacent strips (**Figure 4-1**). All sampled flower strips were assumed as independent replicates. Winter wheat was sown in November 2016 and no insecticides were used during the experiment.

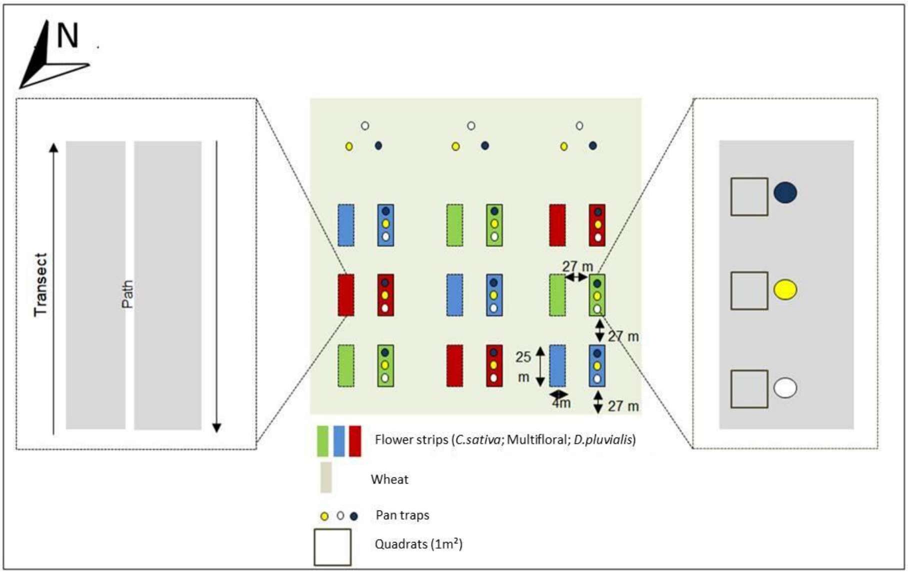

Figure 4-1 : Experimental setup.

2.2. *Pollinator trapping and identification*

All sampling and identification were limited to bees and hoverflies. These families are the ones participating mainly in the pollination process in an effective and substantial way (Rader et al. 2016).

Sampling was conducted during a period of three months, from May (early blooms) to July 2017. A standard protocol for pollinator surveys (Westphal et al. 2008) was used: a combination of white, blue and yellow coloured pan traps (Flora®, 27 cm diameter and 10 cm depth) were installed every 5 m in the centre of the western strip in each block (**Figure 4-1**) every 15 days from 9:00 a.m. to 5:00 p.m. in good weather conditions (i.e., temperature above 15 ◦C, wind speed below 15 km/h and a clear sky) (Fontaine 2008). To be able to offset the effect of flower strips from the background pollinator community, three lots of pan traps were placed in the wheat field, 40 m away from the flower strips. Pan traps were filled with water and some drops of colourless and odourless detergent (wash liquid 'Rainett—Ecologique®') to decrease the surface tension of the water. Insects were collected and kept in 70% ethanol. Additionally, floral visitations were assessed through standardised transects conducted from 11:00 a.m. to 12:00 a.m. and from 2:00 p.m. to 3:00 p.m. (i.e., within the range of the wild bee daily peak of activity) (Geroff et al. 2014; Gezon et al. 2015).

Transects were run in each eastern strip of each block (**Figure 4-1**). Two walks were undertaken for each floral species with a waiting time of two seconds on every floral unit to observe the visits of pollinators (**Figure 4-1**). A floral unit corresponded to one or a set of flowers where the insect can move by walking without needing to fly. When a pollinator landed on a floral unit, it was collected by using a net and kept in a box containing crushed ice. In the laboratory, all collected individuals were preserved in a freezer at −20 ◦C. The transects were repeated twice, spaced a week apart for each flower species during their flowering time. A total of six days of collection with coloured pan trap traps were made and two net traps for each flower species during flowering, for a total of eight transects (on *C. sativa*, *D. pluvalis*, *C. coriander* and *F. esculentum*). The collected data were encoded separately, depending on the type of flower strip and sampling technique. The insects were pinned using a pre-established protocol (Mouret et al. 2007). Insect identification was performed with identification keys (Scheuchl 2000; Mouret et al. 2007; Patiny and Terzo 2010; Falk 2015; Pauly 2019b) and with the help of specialists for species checking and specific taxon groups (Halictidae: Alain Pauly; Syrphidae: Frank Van de Meutter; Andrenidae: David Genoud).

2.3. Vegetation surveys

To survey the vegetation development, three quadrats of 1 m × 1 m were placed in each of the western strip of each block (**Figure 4-1**) (Uyttenbroeck et al. 2015). The number of plants and floral units were counted in each quadrat for every species on 11 July 2017 (i.e., when most of the plants were blooming).

2.4. Statistical analyses

Data analyses were performed with Microsoft Excel 2010 and R software v.3.0.1.

First, the structure of the sampled communities was evaluated with a combination of pan trap and sweep net, and also separately, by considering the abundance of individuals, their species richness and by calculating the following three alpha diversity indexes: Simpson, Shannon and Pielou (‘Vegan’ package (Oksanen et al. 2013; Wickham 2016)). The Simpson index calculates the proportion to which two individuals have accumulated in a community of the same species (Simpson, 1949). It takes into account the abundance of each species in a sample and their proportion in the population. The Shannon index (H) is associated with the Simpson index (Marcon 2017). The proportion of each species is multiplied by its own logarithm. The Shannon index takes better account of important variations of the rarest species (Marcon 2017). The Pielou (R) index, often complementary to the Shannon index, calculates the distribution of individuals between species or the evenness, regardless of species richness (Marcon 2017). Because our sample size (N = 3 per floral treatment) is too small and normality of our data was not met, a non-parametric test (Kruskal-Wallis; *p*-value < 0.05) was used to assess abundance, species richness and the effects of the alpha diversity indices (i.e., Simpson, Shannon, Pielou) between each treatment (i.e., multifloral, *C. sativa*, *D. pluvialis*) on (i) bees + hoverflies trapped and netted, (ii) bees + hoverflies trapped (iii) bees + hoverflies netted, (iv) bees trapped and netted and (v) hoverflies trapped and netted. These non-parametric tests were followed by post hoc comparisons (Dunn’s test) if necessary, to check for pairwise significant differences. Post-hoc comparisons were computed via the ‘dunn.test’ package (Dinno 2017).

Each local community is supposed to be limited in size with defined species number (Magurran 2004). Observed species richness from sampling effort (net + coloured pan traps) is dependent on the sample size. Indeed, new species detection expands with the increase of sample size or sampling effort. To check whether the sampling was conducted in a complete manner, sample coverage curves were plotted for: (i) hoverflies and bees together; (ii) bees alone; and (iii) hoverflies alone (‘iNEXT’ package). Second, the structure of the same sampled communities was evaluated by considering the abundance of individuals and sequence of Hill number (Hill 1973; Chao et al. 2014b) to compare alpha diversity estimations of the floral treatments (‘iNEXT’ and ‘Vegan’ packages) (Oksanen et al. 2013; Hsieh et al. 2016). Indeed, studies proposed a unified framework regarding Hill numbers extended (Chao et al. 2014b) from works based on rarefaction and extrapolation (R/E) sampling curve for species richness and sample completeness (Colwell et al. 2012; Chao and Jost 2012). Each Hill number corresponds to a diversity order q, which defines species diversity measures as a particular feature: species richness (N = 0), the exponential of the Shannon entropy (N = 1) and the inverse Simpson concentration index (N = 2) (Hill 1973). R/E curves were built specifying 100 bootstrap replications on individual-based abundance data to compare the pollinator communities between the floral treatments: (i) hoverflies and bees together, (ii) bees alone and (iii) hoverflies alone.

Third, the structure of the pollinator community in the three treatments was examined through ordination methods using Principal Coordinate Analysis (PCoA) based on Bray-Curtis distance (functions ‘cmdscale,’ ‘ordiplot’ and ‘ordiellipse’ from the ‘Vegan’ package). Data of the pan traps and those from the sweep net were analysed separately. The same analysis was realised for the structure of the pollinator community by floral species using data of the sweep net. The two main components most adequately explaining the variance of the community structures were used to build the PCoA biplots. The community dataframe was standardised using the ‘Hellinger’ method for a one-way analysis of similarities (ANOSIM) also based on Bray-Curtis distance. For every PCoA, ANOSIM was conducted with 9999 permutations to analyse dissimilarity patterns between treatments and flowers.

3. Results

3.1. Pollinator diversity in flower strips

In total, 1184 pollinator individuals belonging to 61 species were collected with pan traps and the net, of which 18 species were hoverflies (583 individuals) and 43 species were bees (601 individuals). The species accumulation curves, reaching a plateau of saturation, show that the sampling effort was sufficient to collect most of the pollinator diversity of the environment (**Figure 4-S2**). *Sphaerophoria scripta* (Linnaeus, 1758)

(Diptera: Syrphidae) was the most abundant species, followed by *Eristalis tenax* (Linnaeus, 1758) (Diptera: Syrphidae), *Lasioglossum pauxillum* (Schenck, 1853) (Hymenoptera: Halictidae), *Lasioglosssum morio* (Fabricius, 1793) (Hymenoptera: Halictidae) and *Andrena flavipes* (Panzer, 1799) (Hymenoptera: Andrenidae) (**Table 4-1**). No rare species were present, except for *Andrena nitidiuscula* (Schenck, 1853) (Hymenoptera: Andrenidae) ranked as minor concern (LC) on the European Red List (Nieto et al. 2014). Concerning hoverflies, the conservation statuses could not be indicated because no red list at the moment exists for this family.

Simpson, Shannon and Pielou indexes describing alpha diversity showed high diversity for each floral treatment (**Table 4-2**) against total species composition of the experimental field. These indexes also exposed that individuals are distributed with several dominant species (**Table 4-1**) which reduced community evenness.

The Kruskal-Wallis tests carried out to compare abundance, species richness, Simpson, Shannon and Pielou indexes in the three flower strip treatments showed no significant evidence of a difference between the mean ranks of at least one pair of groups (**Table 4-2**). However, the abundance of specimens and Pielou's evenness index showed a non-significant trend (p-value ≤ 0.08) to be distinct, suggesting that it would be different pollinator communities among the floral treatments.

The diversity indexes were also analysed with pan trap and sweep net data separately. No significant difference was found. When bees and hoverflies were analysed separately, there was significant evidence of differences for Simpson and Shannon indexes with hoverfly data (**Table 4-2**).

Table 4-1 : Abundance of all the bees and hoverflies species collected with pan traps and during transects in each treatment. The endangered status from the European red list of bees (Nieto et al. 2014) for each bee species is indicated (LC: Minor concern; DD: insufficient data). To our knowledge, no endangered status information is available for hoverflies. Foraging traits are also pointed (P: Polylectic; O: olygolectic with the type of flower; C : cuckoo bees or cleptoparasites).

Species	Status	Foraging	Multifloral	*C. sativa*	*D. pluvialis*	Control	Total (%)
Apoidae							
Andrenidae							
Andrena carantonica	*D.D*	P	0	0	0	1	1 (0.08)
Andrena chrysosceles	*D.D*	P	1	0	0	0	1 (0.08)
Andrena cineraria	*L.C*	P	0	1	1	0	2 (0.17)
Andrena dorsata	*D.D*	P	5	1	3	0	9 (0.76)
Andrena flavipes	*L.C*	P	23	47	14	2	86 (7.29)
Andrena gravida	*D.D*	P	0	2	0	0	2 (0.17)
Andrena haemorrhoa	*L.C*	P	0	0	1	0	1 (0.08)
Andrena humilis	*D.D*	O	0	0	0	1	1 (0.08)
Andrena minutula	*D.D*	P	10	1	0	1	12 (1.02)
Andrena minutuloides	*D.D*	P	4	0	0	0	4 (0.34)

Andrena nigroaenea	*L.C*	P	0	2	1	0	3 (0.25)
Andrena nitida	*L.C*	P	0	1	2	0	3 (0.25)
Andrena nitidiuscula	*L.C*	O	1	0	0	0	1 (0.08)
Apidae							
Apis mellifera	*L.C*	P	6	5	3	5	19 (1.61)
Bombus hypnorum	*L.C*	P	0	0	0	1	1 (0.08)
Bombus lapidarius	*L.C*	P	7	5	14	2	28 (2.37)
Bombus lucorum	*L.C*	P	0	0	4	0	4 (0.34)
Bombus pascuorum	*L.C*	P	0	1	0	0	1 (0.08)
Bombus pratorum	*L.C*	P	0	0	0	1	1 (0.08)
Bombus sylvestris	*L.C*	P	0	0	1	0	1 (0.08)
Bombus terrestris	*L.C*	P	16	9	12	3	40 (3.39)
Bombus vestalis	*L.C*	C	0	1	0	0	1 (0.08)
Nomada fabriciana		C	0	0	0	1	1 (0.08)
Colletidae							
Hylaeus sp.	*L.C*	-	1	1	0	0	2 (0.17)
Crabronidae							
Lindenius sp.	*L.C*	-	2	0	0	0	2 (0.17)
Oxybelus sp.	*L.C*	-	0	0	0	1	1 (0.08)
Halictidae							
Halictus maculatus	*L.C*	P	0	1	0	0	1 (0.08)
Halictus rubicundus	*L.C*	P	0	2	0	0	2 (0.17)
Halictus scabiosae	*L.C*	O	0	0	0	1	1 (0.08)
Lasioglossum calceatum	*L.C*	P	4	7	25	1	37 (3.14)
Lasioglossum fulvicorne	*L.C*	P	2	2	0	3	7 (0.59)
Lasioglossum laticeps	*L.C*	P	6	5	0	3	14 (1.19)
Lasioglossum leucozonium	*L.C*	P	0	0	0	1	1 (0.08)
Lasioglossum malachurum	*L.C*	P	10	12	13	4	39 (3.31)
Lasioglossum minutissimum	*L.C*	P	0	3	0	0	3 (0.25)
Lasioglossum morio	*L.C*	P	19	36	18	41	114 (9.66)
Lasioglossum nitidulum	*L.C*	P	0	0	1	0	1 (0.08)
Lasioglossum nitidiusculum	*L.C*	P	0	1	1	0	2 (0.17)

Lasioglossum pauxillum	*L.C*	P	37	45	32	24	138 (11.69)
Lasioglossum villosulum	*L.C*	P	0	2	1	0	3 (0.25)
Seladonia tumulorum	*L.C*	P	1	2	1	0	4 (0.34)
Sphecodes ephippius	*L.C*	C	1	0	0	0	1 (0.08)
Sphecodes monilicornis	*L.C*	C	2	0	0	0	2 (0.17)
Syrphidae							
Episyrphus balteatus	-	P	16	4	6	0	26 (2.20)
Eristalis arbustorum	-	P	11	0	0	0	11 (0.93)
Eristalis similis	-	P	0	0	0	1	1 (0.08)
Eristalis tenax	-	P	70	12	56	3	141 (11.95)
Eumerus strigatus	-	P	7	4	0	2	13 (1.10)
Eupeodes corolla	-	P	7	3	2	0	12 (1.02)
Eupeodes latifasciatus	-	P	0	0	1	0	1 (0.08)
Eupeodes luniger	-	P	7	0	0	0	7 (0.59)
Melanostoma mellinum	-	P	3	6	7	0	16 (1.36)
Platycheirus clypeatus	-	P	2	1	4	0	7 (0.59)
Scaeva pyrastri	-	P	9	0	3	0	12 (1.02)
Scaeva selenitica	-	P	0	0	1	0	1 (0.08)
Sphaerophoria rueppelli	-	P	1	3	1	0	5 (0.42)
Sphaerophoria scripta	-	P	176	98	7	8	289 (24.49)
Sphaerophoria taeniata	-	P	3	3	0	0	6 (0.51)
Syritta pipiens	-	P	25	3	0	0	28 (2.37)
Syrphus ribesii	-	P	3	0	1	0	4 (0.34)
Syrphus vitripennis	-	P	1	1	1	0	3 (0.25)

Table 4-2 : Mean abundance and species richness of pollinator community, diversity (Simpson, Shannon and Pielou) depending on the type of collection and pollinator family in each treatment (_standard deviation), the degree of freedom (df), Kruskal-Wallis, _2-value and significant differences (*: p-value < 0.05).

Data		***C. sativa***	***D. pluvialis***	**Multifloral**	**df**	**χ2**	***p*-value**
Pan traps *net* hoverflies*bees	Abundance	111±31.43	79.30±23.46	165.67±54.99	2	5.07	0.08
	Species richness	22.67±3.79	19.33±2.08	26.67±1.16	2	4.47	0.12
	Simpson’s Diversity	0.82±0.06	0.88±0.03	0.83±0.04	2	3.29	0.19
	Shannon's Diversity	2.31±0.23	2.47±0.15	2.41±0.16	2	1.16	0.67
	Pielou's evenness	0.74±0.04	0.84±0.04	0.74±0.06	2	5.42	0.07
Pan traps *hoverflies*bees	Abundance	34±24.75	18.3±5.85	17±1	2	0.97	0.61
	Species richness	9.33±2.88	9.66±1.52	8.66±1.52	2	0.85	0.65
	Simpson’s Diversity	0.76±0.08	0.81±0.08	0.81±0.08	2	1.15	0.56
	Shannon's Diversity	1.75±0.35	1.98±0.37	1.94±0.31	2	0.62	0.73
	Pielou's evenness	0.8±0.10	0.87±0.10	0.9±0.07	2	2.22	0.32
Net *hoverflies*bees	Abundance	59±10.58	45.33±27.64	138.33±59.80	2	5.6	0.06
	Species richness	14±2	11.6±3.78	21.66±1.52	2	5.8	0.06
	Simpson’s Diversity	0.71±0.03	0.78±0.02	0.78±0.02	2	5.42	0.06
	Shannon's Diversity	1.81±0.10	1.93±0.11	2.18±0.18	2	5.06	0.07
	Pielou's evenness	0.68±0.02	0.8±0.06	0.7±0.05	2	4.62	0.09
Pan taps*net *bees	Abundance	48.3±33.60	44±7	42±1	2	2.98	0.22
	Species richness	12±3	10±0	13±1	2	3.08	0.21
	Simpson’s Diversity	0.78±0.01	0.83±0.01	0.84±0.01	2	5.95	0.06
	Shannon's Diversity	1.88±0.16	2±0.03	2.18±0.08	2	5.6	0.06
	Pielou's evenness	0.76±0.08	0.86±0.01	0.84±0.02	2	3.2	0.2
Pan traps*net *hoverflies	Abundance	44.26±11.67	29.66±21.36	113.33±6.18	2	5.95	0.05
	Species richness	7±1.73	6.66±2.88	12.33±1.52	2	5.65	0.05
	Simpson’s Diversity	0.44±0.03	0.57±0.05	1.24±0.03	2	7.2	0.02*
	Shannon's Diversity	1.01±0.14	1.24±0.20	1.58±0.04	2	6.48	0.03*
	Pielou's evenness	0.53±0.02	0.68±0.06	0.63±0.03	2	5.95	0.05

Finally, the post-hoc Dunn’s test reveals significant differences between the multifloral treatment and the *C. sativa* treatment for both Simpson (*p*-value = 0.003) and Shannon indexes (*p*-value = 0.005) (**Figure 4-2**).

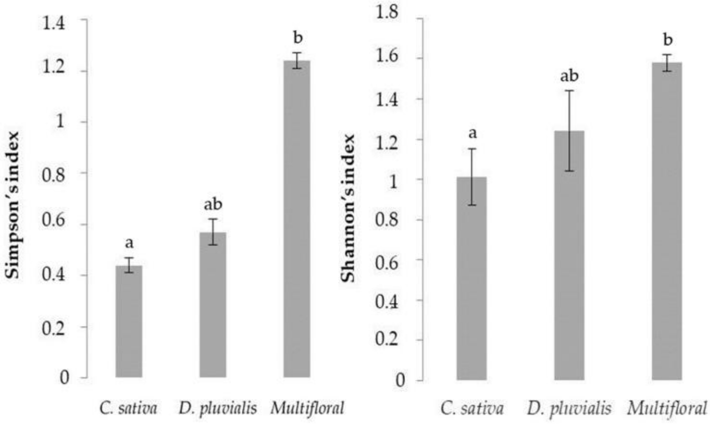

Figure 4-2 : Bar plots of mean values of both Simpson and Shannon indexes for the different treatments. The different letters represent a significant difference calculated from the post-hoc Dunn’s test comparison (*p*-value < 0.05).

Rarefaction/extrapolation curves for Hill numbers show that treatments have similar species richness (N = 0) (**Table 4-3, Figure 4-3**). In contrast, there is a significant difference for N = 2 between *Dimorphoteca* and the other two treatments for hoverflies and bees combined, as suggested by an overlap in the confidence intervals. For both Shannon (N = 1) and Simpson diversities (N = 2), there is one difference between multifloral treatment and the other two treatments (**Figure 4-3**).

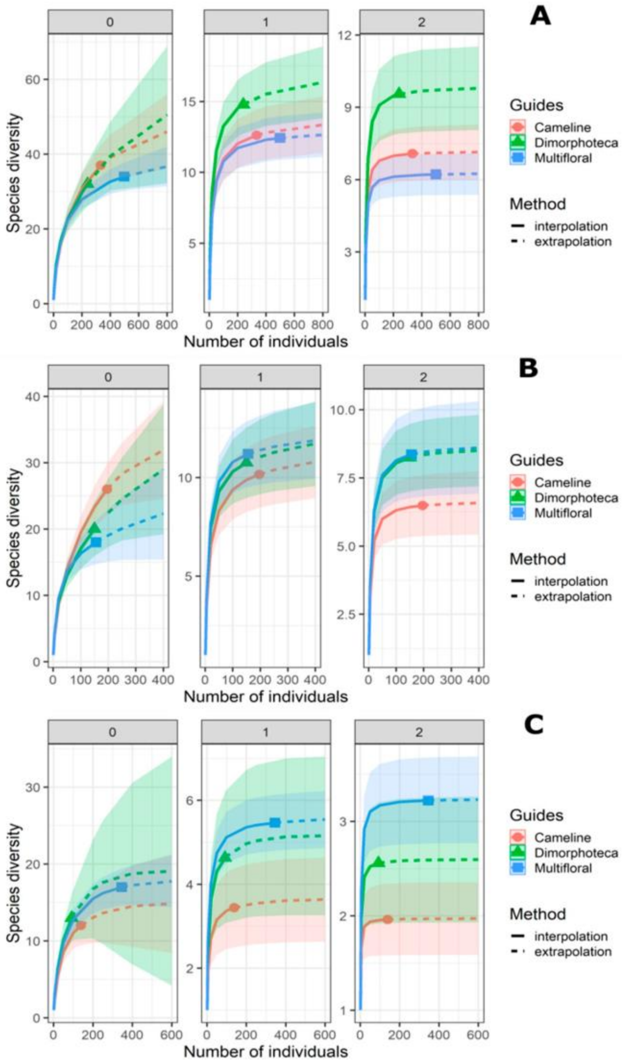

Figure 4-3 : Comparison between pollinator communities from the three floral treatments (denoted by colours and solid dots) by sample-size-based rarefaction (solid lines) and extrapolation (dashed curves) curves based on abundance data of hoverflies and bees together (**A**), bees alone (**B**) and hoverflies alone (**C**). Each panel displays Hill numbers of order N = 0 (left panel), N = 1 (middle panel) and N = 2 (right panel). The 95% confidence intervals (coloured-shaded regions) were obtained by a bootstrap method based on 100 replications.

ANOSIM show no significant dissimilarities in the pollinator communities in the pan traps (global R = −0.037; *p*-value = 0.606) (**Figure 4-4a**). As for the communities captured with the net during transects, the species distribution differed between the three treatments (global R = 0.794; *p*-value = 0.003) (**Figure 4-4b**).

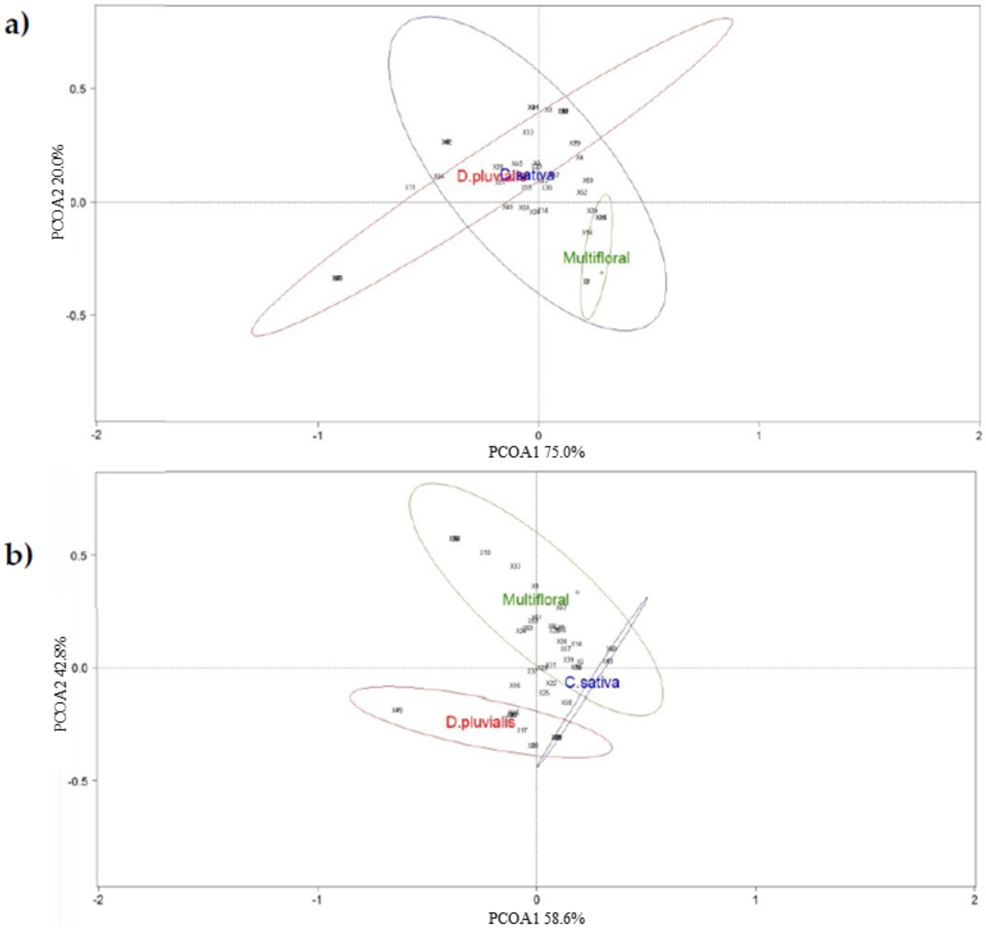

Figure 4-4 : Principal coordinate analysis (PCoA) ordination of the three treatments (red circle: *D. pluvialis*; green circle: Multifloral; blue circle: *C. sativa*) based on the data collected with (**a**) pan traps and (**b**) a net through transects. Ellipses show the 80% confidence interval of the locations grouped by flower strip. Species scores are represented with numbers (**Table 4-S2**).

3.2. The flower identity effect on pollinator visitations

During the transect samplings, coriander *C. sativum* and buckwheat *F. esculentum* were the most abundant species blooming in the multifloral strips (**Figure 4-S3).** Species richness of netted specimens during transects was composed of ten hoverfly species and 16 bee species. ANOSIM showed differences between the pollinator diversity and abundance of flowers studied (global R = 0.713; *p*-value < 0.001) (**Figure 4-5**). Flowers of *D. pluvialis* differed from other flowers. The same is true for *C. sativum* and *F. esculentum* flowers. Only *C. sativa* showed a tendency to attract the same pollinator community as *F. esculentum*.

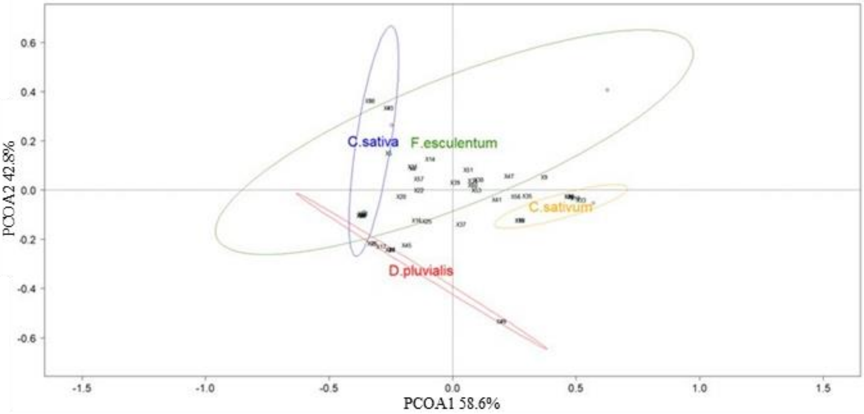

Figure 4-5 : Principal coordinate analysis (PCoA) ordination of the four flower species with data collected with a net (red circle: *D. pluvialis*; yellow circle: *C. sativum*; green circle: *F. esculentum*; blue circle: *C. sativa*). Ellipses show the 80% confidence interval of the locations grouped by flower species. Species scores are represented with numbers (**Table 4-S2**).

4. Discussion

4.1. The biodiversity of pollinators

1184 individuals belonging to 43 bee species and 18 hoverfly species were collected, representing 11.75% and 5.13% of the national richness in Belgium, respectively (Nieto et al. 2014; Speights 2017). These figures are rather low yet considering that land use within a 3 km radius from the field consisted mainly of urbanised areas (52%) and agricultural fields (39%) (Figure S1), such a poor pollinator community is not unexpected (Williams et al. 2010; Potts et al. 2015; Lindgren et al. 2018). Indeed, studies have already shown that pollinator species diversity and abundance generally decrease with landscape simplification, leading to a homogenisation of the insect communities (Andersson et al. 2013; Senapathi et al. 2017). The presence of small shrubs, hedges and fragments of woodland on the remaining 8% of the surface area may have provided the necessary resources of nectar and pollen, nesting sites or larval habitat to support a pollinator community, albeit impoverished to some extent (Taki et al. 2007). This observation may also explain the low presence of oligolectic bees in our study. Indeed, while polylectic bees are less sensitive to agricultural intensification and the increase of urbanised zones, oligolectic bees (which are less flexible in their range of food resources) are more likely to be affected by agricultural and urban stresses, causing a decline in their population (Wood et al. 2016). *Sphaerophoria scripta*, *E. tenax*, *L. pauxillum*, *L. morio* and *A. flavipes* were the most abundant pollinator species. They are all polylectic species common in agricultural landscapes and are recognised as efficient pollinators (Kleijn and van Langevelde 2006; Falk 2015). The composition of the surrounding landscape (i.e., urban areas and agricultural fields) can explain their presence in the field. For example, *L. morio* does not present any particular requirements and nests in anthropogenic areas such as town parks and gardens (Nieto et al. 2014; Falk 2015). With regard to hoverfly species, the high abundance of *S. scripta* is consistent with its ecology: it colonises open landscapes with a short turf and patches of bare ground and often frequents pioneer vegetation which makes it a typical species of agricultural environments (Branquart and Hemptinne 2000). The larvae of *S. scripta* are aphid predators amongst others on cereals (Morales et al. 2007; Chabert and Sarthou 2017). As for the second most abundant hoverfly species, *E. tenax*, its abundance follows from large-scale long-distance migration in summer (Mueller and Dauber 2016).

As for less common species, *A. nitidiuscula* were collected on coriander in the floral mixture. So far, some 15 observations of *A. nitidiuscula* are known from Belgium. The only previous observation in the area of Gembloux dates from 1989 (Waarnemingen.be, BDFGM_GX and BDFGM_Mons database) (**Figure 4-S4**). This species is oligolectic on Apiaceae flowers (Raemakers 2005) and inhabits a variety of open habitats. Flower strips sown in agricultural fields seems to be such a habitat that can support fragile (meta)populations of relatively rare pollinator species.

4.2. Attractiveness of the floral mixture to pollinator

We were able to detect significant differences among floral treatments with pan trap and sweep net data aggregated with Hill number analyses. A difference between the multifloral treatment and Dimorphoteca with the N = 2 index has been observed, indicating that the pollinator diversity was higher in the multifloral than in the Dimorphoteca strips. Parallel to the analysis of Hill indices, the indices of Simpson, Shannon and Pielou indicated that the floral strips have housed a fairly large number of species dominated by particular taxa, suggesting that floral strips were attractive to pollinators. Finally, according to the Pielou index, the treatments brought together communities of species whose dominance is equitable, with hypothetically the dominance of certain species.

Moreover, PCoA and ANOSIM show that the floral strips revealed different pollinator communities with net capture while pan trapping did not. This result suggests that both field collection methods are complementary to conduct exhaustive pollinator sampling (Popic et al. 2013).

Metric analyses using only hoverfly data, however, showed significant differences between the treatments of both Shannon and Simpson indexes and Hill numbers N = 1 and N = 2. These results reveal that multifloral strips make it possible to obtain a greater variety of Syrphidae than when using monofloral strips. Moreover, this result indicates that the pollinator community in monofloral strips tends to be more diverse when dominant pollinator species become more relevant and rare or common species are not favoured. These results can be explained by the various blooms occurring in the flower strips.

The counting of floral units in the quadrats indicates that only two species of the multifloral mixture (i.e., buckwheat and coriander) bloomed in abundance (Figure S3). A first reason for the low germination rate could be the drought wave that occurred in Wallonia in spring 2017 (Anonymous 2017) which dried up the soil preventing the germination of many species. A second explanation could be the density of weeds, particularly the Lamb's quarters *Chenopodium* sp. (Amaranthaceae), which is a nitrophilous species common in conventionally cultivated fields (Figure S3). Nevertheless, some pollinators are attracted by Lamb's quarters, particularly some hoverflies (Warzecha et al. 2018). This phenomenon recalls that weeds in agricultural landscapes can support ecosystem processes and maintaining their diversity is a crucial issue (Rollin et al. 2016). These results therefore highlight that the correct establishment of sown wildflower strips and their expected effects on insect biodiversity and the related ecosystem processes is not systematic and depends on environmental (abiotic and biotic) parameters.

4.3. The role of floral traits

The pollinator communities on the four flower species that bloomed in abundance were different (**Figure 4-3**). Previous studies have demonstrated the importance of floral traits in the attraction of pollinators (Colley and Luna 2000; van Rijn and Wäckers 2016; Fornoff et al. 2017; Bauer et al. 2017), among them the flower colour and the type of the corolla that determines the nectar and pollen accessibility. The present flower species were white or yellow. These colours are effective in attracting hoverflies and some bees of the genus *Bombus* (Bray. 1957) (Colley and Luna 2000). Conversely, the blue flowers, absent in our study, would be more conducive to attracting bees (McCravy and Ruholl 2017). Floral colours could explain the greater presence of hoverflies, especially on buckwheat and coriander. The positive effect of colour on hoverflies may have been supported by the corolla type of these flower species, qualified as 'flower with open nectar' and 'flower with partly hidden nectar' after the classification of Müller (1881) (Darwin 1881) in the BIOLFLOR database (Kolz et al. 2002). Indeed, the corolla type determines the availability of nectar for visitors and species with short corolla depth such as umbel flowers (Apiaceae) (e.g., coriander and some Asteraceae like *D. pluvialis*) or with wide corollas such as buckwheat, are attractive to hoverflies and increase their survivorship (van Rijn and Wäckers 2016). Conversely, nectar in narrow corollas such as that of *C. sativa* is accessible to bees, which could explain the increased abundance of these pollinators in this treatment (Gómez et al. 2008). These observations can explain the significant differences observed with the ANOSIM results for the PCoA representing the pollinator communities for each flower.

5. Conclusion

First, the present study provides an additional list of bees and hoverflies found in a typical agricultural landscape dominated by field crops and urban areas in Wallonia, Belgium. It shows that most of the species collected are generalists in terms of habitats. Moreover, the presence of *A. nitidiuscula* enhances the interest of the flower strips by favouring less frequent pollinator species.

The study also highlights the abundance of aphidophagous hoverflies, which may benefit farmers by naturally controlling aphids (Hemiptera: Aphididae) that are common agricultural pests in the region (Hatt et al. 2017a). This result supports the need for broadening the scope in order that spatial diversification of agro-ecosystems addresses multiple issues simultaneously (Hatt et al. 2018).

Second, the study did not generally reveal significant differences in terms of abundance and diversity of pollinators in the different treatments (i.e., monospecific vs. multifloral strips). Only hoverflies were more diversified (Shannon's and Simpson's diversity) in the multifloral mixture. A reason may be that few species in the multifloral mixture actually bloomed. In addition, this study was conducted on a single experimental site, which makes it impossible to compare the results between different experimental fields that could have shown significant differences in terms of pollinators and species blooms. Further studies are thus required to draw a clearer conclusion on whether multispecies wildflower mixtures or monofloral crops benefit pollinators the best. In particular, flower phenology remains a key element of the effectiveness of flowering strips. Hence, further research should assess the effect of blooming time on pollinator species emerging early in the season as well as on those requiring food resources late in the season. Moreover, it would be useful to evaluate whether an earlier or later sowing of *C. sativa* and *D. pluvialis* would allow their flowering to be spread out over a longer period. Third, the significant difference of pollinator communities observed on each flower species reinforces the interest of identifying the floral traits benefiting visiting insects to improve floral blends. Being conducted in a single year, this work could be completed in the future by exploring the evolution of the obtained results on a longer term. Finally, the economic benefits provided by the cultivation of *C. sativa* and *D. pluvialis* could be compared with the monetary compensation provided to farmers by the AECM for multifloral mixtures. Proving the economic profitability of sowing flower strips could encourage farmers to diversify their agricultural systems as well as their incomes.

Chapter 5

Plant community foraged by the honeybee along space-time gradient in a megacity

Chapter V: Plant community foraged by the honeybee along space-time gradient in a megacity

This section is the original version of the article:

Grégoire Noël, Arnaud Mestrez, Philippe Lejeune, Frédéric Francis, Junko Kawai, Masayuki Miwa, Koichi Uehara, Ayako Nagase (2023). “Pollen meta-barcoding reveals different community structures of foraged plants by honeybees (*Apis mellifera* L.) along space-time gradient in Japan”. Published in *Urban Forestry and Urban Greening Journal*, 79, 127794. https://doi.org/10.1016/j.ufug.2022.127794

Abstract - The availability of pollen in urban-rural landscapes is an essential factor that influences the population dynamics of insect pollinators. The amount and diversity of pollen play a pivotal role in the foraging ecology of pollinators for their growth and health, but investigations on the spatio-temporal patterns of foraged plants remain rare, especially in cities as neo-ecosystems. Here, we explored the temporal foraging habits of a highly polylectic pollinator (*Apis mellifera* L.) in Tokyo, including different landscape classes from rural to urban areas. Mixed-pollen samples in each month and each location (N = 17) were analysed using DNA meta-barcoding to identify plants visited by honeybees. The results showed that the landscape class (rural, suburban and urban areas) explains spatial variations in pollen source-plant composition foraged by honeybees, but not in taxa richness. Furthermore, pollen diversity and pollen source-plant composition showed a strong seasonal dependence. A higher plant richness and foraged woody taxa was found to occur in spring, which was mainly dominated by the genera *Prunus* and *Acer*. In summer and autumn, the genera *Trifolium* and *Plantago* of the herbaceous stratum were the most visited plants. The Fabaceae, Rosaceae, Brassicaceae, Plantaginaceae, and Onagraceae plant families were the most frequently observed in all combined samples. The present study contributes to a deeper understanding of the foraging ecology of *A. mellifera* colonies across urban-rural gradient surrounding mega-cities such as Tokyo.

Keywords: Pollen, metabarcoding, community structure, foraging ecology, *Apis mellifera*, urban ecology

1. Introduction

Ongoing urbanisation is one of the main drivers of landscape degradation and pollinator biodiversity loss (Ahrné et al. 2009; Potts et al. 2010a; Fortel et al. 2014b; Concepción et al. 2015; Sánchez-Bayo and Wyckhuys 2019). Indeed, floral resources are becoming scarcer under the pressure of urban fragmentation, and the increase in impervious surfaces is rendering nesting sites inaccessible to pollinators (Harrison and Winfree 2015; Wenzel et al. 2020). However, recent studies have revealed that cities can also act as a refuge for pollinators (Hall et al. 2017), particularly for bees (Theodorou et al. 2020b): (i) cities are less exposed to pesticides (Fortel et al. 2014b; Muratet and Fontaine 2015), (ii) urban management sustainably permits the maintenance of their floral resources (Pardee and Philpott 2014; Baldock 2020), and (iii) urban areas are configured with a heterogeneity of green spaces, which would be favourable to the foraging preferences of bees (Baldock et al. 2015b). Moreover, flowerbeds in the urban matrix are highly attractive and are a source of pollen and nectar for insect pollinators (Garbuzov and Ratnieks 2014; Garbuzov et al. 2015a). With an increasing popularity in beekeeping activities in cities, honeybees (*Apis mellifera* L.) contribute to urban plant pollination, generate profits of by-products, and provide environmental education (Cho and Lee 2018; Tanaka et al. 2020). However, the massive introduction of urban honeybees has led to growing concerns about detrimental effects on wild pollinators through an increase in floral resource competition and the spillover of shared pathogen agents (Geslin et al. 2017; Ropars et al. 2019).

As a eusocial species, honeybees organise their floral resource collections (i.e. nectar and pollen) through a complex communication system within their colonies. According to plant phenology, honeybee scouts rapidly recruit their siblings to forage on rich new patches of flowers using a characteristic waggle dance (von Frisch 1965). The foraging decision-making system of the colony can vary from day to day or within the same day following real-time nectar and pollen availability in the surroundings. Throughout its active seasons, the colony constantly maintains a balance between its biomass and energy management according to the availability and the diversity of surrounding floral resources. This strategy ensures sufficient food stores and energy reserves for winter (Seeley 1995; Alaux et al. 2017). Pollen diversity provides substantial resources in terms of protein, lipid, vitamin, and mineral supplies (Haydak 1970). Large amounts of pollen (15–30 kg) are collected annually, mainly for brood production during summer (Seeley 1995; Avni et al. 2009, 2014). The quality and diversity of pollen are also essential for better life expectancy and immunity, as well as the parasite or pathogen tolerance of the bees and the colony (Wilde et al. 2003; Alaux et al. 2010; Di Pasquale et al. 2013; Wang et al. 2014).

The preservation of ecosystem functioning relies on the mutualistic networks of pollinators and plants. Several methods are used to assess these interactions: the observation of floral visits, digital tracking systems to capture floral visits, chemical signatures of pollen, pollen genetic sequencing, and pollen light microscopy (Cornman et al. 2015). Identifying a pollen species or genus by light microscopy from mixed pollen samples, also known as melissopalynology (Ohe et al. 2004), is a time-consuming process, even for well-trained experts (De França Alves and De Assis Ribeiro DosSantos 2014) that results in low taxonomic resolution, usually at the family or genus rank at best (Rahl 2008; Kaškoniene and Venskutonis 2010; Richardson et al. 2015a; Bell et al. 2016). With the advent of high-throughput sequencing (HTS) techniques, DNA meta-barcoding approaches have become reliable methods to obtain faster taxonomic profiles with higher resolution of mixed pollen collected from bees or flowers (Sickel et al. 2015; Pornon et al. 2016; Bell et al. 2017). To elucidate the plant taxonomic composition of mixed-pollen samples, the meta-barcoding process can be based on different genetic markers, such as the *rbcL*a, *matK*, *trnH-psbA*, *trnL*, and *ITS* regions (mainly *ITS2*), which require high inter-specific and low intraspecific variability (Kraaijeveld et al. 2014; Galimberti et al. 2014; Sickel et al. 2015; Richardson et al. 2015a; Hawkins et al. 2015). These selected loci, the primer set and the differences in available plant reference sequences for each marker used for amplification drive the range of taxonomic inferences (Bell et al. 2016).

The floral plant composition of agricultural or urban landscapes heavily impacts the foraging ecology of honeybee workers (Danner et al. 2017; Lucek et al. 2019; Richardson et al. 2021). Indeed, the fragmentation of urban matrix usually leads to the creation of small, remote, and intensely maintained green spaces (Bastin and Thomas 1999), which could influence the foraging distance of honeybee workers (Garbuzov et al. 2015b). As the most polylectic bee forager (Butz Huryn 1997), honeybees can adapt to shortages in floral resources by enlarging their foraging area (Steffan-Dewenter and Kuhn 2003; Danner et al. 2016). Moreover, seasonal shifts greatly impact the pollen availability for honeybee colonies, according to the phenology of the floral resources (Danner et al. 2017; Sponsler et al. 2020). In temperate climates, the foraged plant characteristics also vary according to the course of the seasons: spring is dominated by trees and shrubs, summer has more herbaceous species, and autumn is characterized by woody vines (Sponsler et al. 2020). Nonetheless, the space-time effects combined with plant characteristics in the foraged plant community have yet to be studied extensively (Richardson et al. 2021). Understanding the floral range dynamics of honeybee colonies according to urbanization gradient would help to better determine the foraging ecology of the honeybee colonies in cities.

To achieve this aim, the taxonomy of pollen foraged by honeybees was identified over the seasons along an urban-rural gradient from different locations in the Kanto region, Tokyo, and its surroundings in Japan. The research questions addressed were as follows: (i) How does the composition of the foraged flower community (and the foraged plant characteristics) vary along an urban-rural gradient? (ii) How does the composition of foraged flower communities and characteristics of pollen forage plants vary according to the course of the seasons?

2. Material and methods

2.1. Study area and experimental set-up

We selected 17 apiary locations, with homogeneous climatic and altitudinal conditions (**Table 5-S1**), distributed along an urbanisation gradient in the Kanto region of Japan (**Figure 5-1**). One hive per apiary was used for pollen sampling. From March to September 2019, pollen samples were collected using pollen traps at the entrance of the same hive (**Figure 5-S1**). This experimental setup was used to collect pollen balls from the hind legs of honeybee foragers with a standardised honeybee-size mesh and tray (Mahmood et al. 2017). Then, the contents of the pollen traps were discharged into labelled 50-ml conical tubes and stored at –20°C. However, due to meteorological conditions and the personal schedules of the beekeepers, the sampling date, collection frequency, and operational time of the pollen trap varied from site to site for a total of 143 collected pollen samples (**Table 5-S2**). Each corbiculate pollen sample was thoroughly mixed by lightly kneading all collected pollen with a mortar and pestle. Then, all samples were sent to the private company, Bioengineering Lab. Co., Ltd. (https://www.gikenbio.com/, consulted on 20/07/2020) for the processing of the meta-barcoding of the mixed pollen samples.

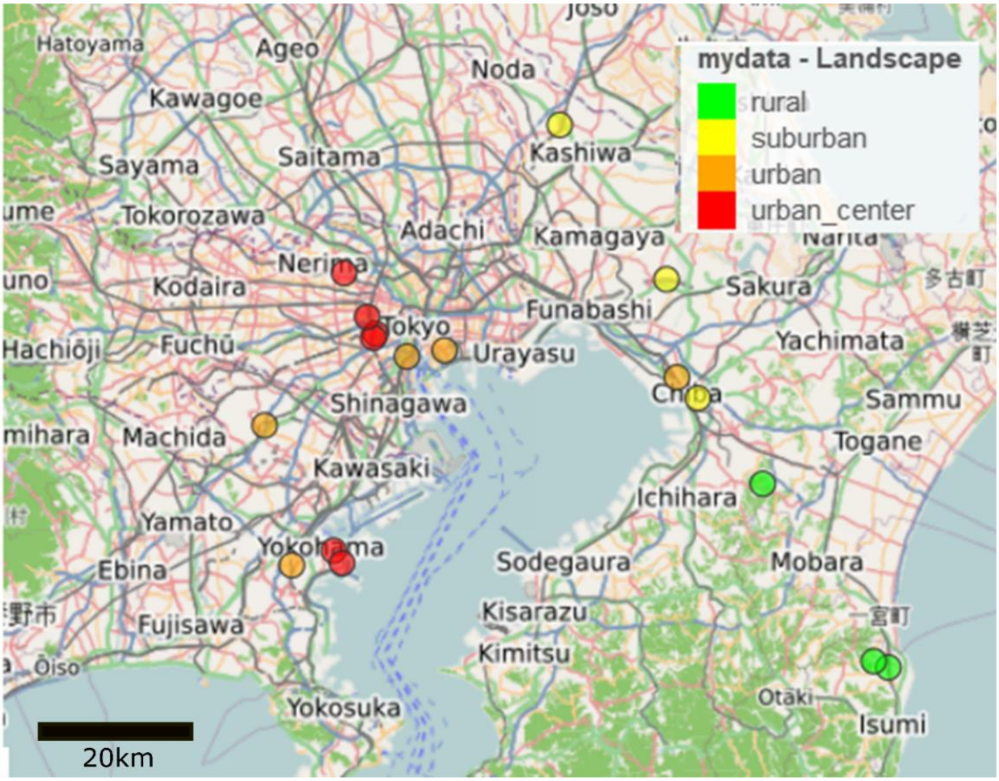

Figure 5-1 : Selected hive locations along Tokyo bay (Japan). Each colour corresponds to the landscape type resulting from the cluster analysis of the study sites based on k-means approach. The map was drawn using *Openstreetmap France* from *mapview* in R (Appelhans et al. 2019).

2.2. Landscape analysis

Using remote sensing techniques, the landscape structure was investigated within a 6-km radius around each hive location (**Table 5-S2**); this distance enclosed 95% of the forage area per colony (Seeley 1995). With the help of Planet Labs Inc. (Planet Core Team 2020), we used multi-spectral images (RGB, NIR) with 3-m pixel resolution. To fully exploit the potential of the data, the cloud cover condition was set to a maximum of 5%. Planet data are relevant for computing and mapping high-resolution terrestrial above-ground vegetation at the landscape scale (Miller et al. 2019). For each planet image, the normalised difference vegetation index (NDVI) was computed using the red and near-infrared bands based on band rationing, which allowed for the delineation of the vegetation cover from other types of land cover (Xue and Su 2017). Classes were created with the function *reclassified* from the *raster* package in R (Hijmans et al. 2020) by defining the NDVI threshold values to distinguish the water (NDVI: from -1 to -0.2), the impervious surface (NDVI: from -0.199 to 0.199) and the vegetation (NDVI: from 0.2 to 1) (Taufik et al. 2016; Hashim et al. 2019). A majority filter, with a 6 × 6 filter kernel size, from the *whitebox* package in R (Lindsay 2016), was applied to smooth the result and aggregate regions of high uncertainty. Landscape classifications at the site level were performed using demographic data (i.e. number of inhabitants per admin units) and landscape metrics from the *lconnect* (Mestre and Silva 2019) and *landscape metrics* (Hesselbarth et al. 2019) packages in R. To classify our sites along an urban-rural gradient (Bastin and Thomas 1999; Hadley and Betts 2012), we retained selected data: number of inhabitants per km^2 (dpop), the integral index of connectivity (IIC) (Saura and Pascual-Hortal 2007), the effective mesh size (MESH) (Spanowicz and Jaeger 2019), Shannon’s evenness index (SHEI) (Shannon 1948), the vegetation cover proportion (veg cover), the vegetation patch density (Threlfall et al. 2015), and the median vegetation class NDVI (NDVI median) (**Table 5-S3**).

We conducted principal component analysis (PCA) of the landscape dataset to visualise the differences among our study sites. The unsupervised k-means clustering method was applied to delineate the landscape category along the urban-rural gradient into k groups. Before initiating the analysis, the data were standardised using the *scale* function in R to make variables comparable. As a result, the clustering algorithm was independent of any variable unit. The number of k groups required to be defined as the first step was determined using the elbow method (Kodinariya and Makwana 2013). The k-means partitioning analysis was performed using the *k-means* function with 25 random sets (Strickland 2014) and the *factoextra* package in R for PCA graphical representations (Kassambara and Fabian 2020).

2.3. *Molecular techniques*

2.3.1. DNA extraction

First, pollen samples (0.5 g) were lyophilized using a lyophilizer freeze dryer VD-250R (TAITEC, Koshigaya, Saitama, Japan). After being ground at 1500 rpm for 2 min using a ShakeMaster NEO homogeniser (bms, Shinjyuku, Tokyo, Japan), DNA was extracted using the protocol of MPure Bacterial DNA Extraction Kit (MP Biomedicals, Irvine, CA, USA). DNA purification of the samples was performed using the MPure-12 Automated Nucleic Acid Purification System (MP Biomedicals, Irvine, CA, USA). Quality control of DNA extracts was conducted using Synergy H1 (BioTek, Winooski, VT, USA) and QuantiFluor dsDNA System (Promega, Madison, WI, USA).

2.3.2. Library preparation and DNA sequencing

One hundred and forty-three libraries were produced using a 2-step tailed polymerase chain reaction (PCR) method. The first PCR amplification was conducted using internal transcribed spacer (ITS1) primers designed by Masamura *et al.*, (2014) (**Table 5-S4**), coupled with MiSeq-specific adapters. This primer pair was selected for its effectiveness (Maeda and Takahashi 2017) to identify more Japanese plant species compared to ITS 1 and ITS 2 region designed by Cheng et al. (2016). The second PCR amplification was conducted using index primers. PCR reactions were carried out in a reaction volume of 10 µL containing 1.0 µL of 10× Ex Buffer, 0.8 µL of nucleoside triphosphate dNTPs (each at 2.5 mM), 0.5 µL for both forward and reverse primer at a concentration of 10 µM, 2.0 µL of DNA template normalized at 0.5 ng/µL, 0.1 µL of DNA polymerase ExTaq at 5 U/µL (TaKaRa, Otsu, Shiga, Japan) and 5.1 µL of double-distilled water. The PCR profile was as follows: 2 min of denaturation at 94°C, followed by 30 cycles with 30 s of denaturation at 94°C, 30 s of annealing at 57°C, 30 s of elongation at 72°C, and a final elongation at 72°C for 5 min. The PCR products were purified using AMPure XP (Beckman Coulter, Brea, CA, USA). The second PCR profile was follows: 2 min of denaturation at 94°C, followed by 10 cycles with 30 s of denaturation at 94°C, 30 s of annealing at 60°C, 30 s of elongation at 72°C, and a final elongation at 72°C for 5 min. Library concentrations were determined using a Synergy H1 microplate reader (BioTek, Winooski, VT, USA) and a QuantiFluor dsDNA System (Promega). Library quality was evaluated using a fragment analyser (Advanced Analytical Technologies, Ankeny, IA, USA) with a dsDNA 915 Reagent Kit (Agilent, Santa Clara, CA, USA). The generated library was sequenced using MiSeq Illumina technology (Illumina, San Diego, CA, USA) through a 2× 300 paired-end run.

2.3.3. Data processing

For subsequent analysis, the sampling dates were discretely pooled by month and analysed in R version 4.0.2 (R Core Team 2020). We used Spearman’s rank-order correlation between the number of taxa per sample and the sampling length in hours to test whether the data could be treated independently of the sampling length. Furthermore, we used “FASTX Barcode Splitter” from Fastx toolkit, a short-reads pre-processing tool, to extract only the target and index sequences from MiSeq reads

(Hannon 2010). Next, the reads were denoised and filtered using Sickle software (Joshi and Fass 2011) with an overlap quality value of 20. Trimmed reads with fewer than 150 bases were discarded. The remaining reads were merged using FLASH (version 1.2.11) paired-end merge script (Magoč and Salzberg 2011) using the following conditions: fragment length after merging of 420 bases, read fragment length of 280 bases, and minimum overlap length of 10 bases. The open-source bioinformatic pipeline Qiime 2.0 (Bolyen et al. 2019) workflow script was used for taxa creation and taxonomic assignment for the filtered reads. The filtered read pairs were clustered with USEARCH 9.0.2132 (Edgar et al. 2011) to remove low quality data with more than 97% sequence identity. Taxon sequences were searched on the 1st February 2020 against the NCBI nucleotide database (Benson et al. 2013) using BLASTN ver. 2.9.0. and the 10 best matches were retained. Only the top 1 taxon was considered if it belongs to Tracheophyta group (i.e. vascular plants), other taxa belonging to other kingdoms were removed. Following the taxonomy classification step, taxa-abundance data and operational taxonomic unit (OTU) data were applied to the R environment (R Core Team 2020). First, the assignment of all OTUs below the identity threshold of 97% was discarded (Danner et al. 2017; Smart et al. 2017). Next, the number of reads was sorted by genus and sample (i.e. site and collection date), and was then expressed as the ratio between the read count and the sum of read number per sample for each genus. Genera accounting for less than 0.05% of the total number of reads for a single sample were excluded to prevent false positives and two samples were removed because it accounted for less than 1000 reads to limit inferences from insufficient sequencing depth (Sponsler et al. 2020).

2.4. Taxonomic analysis

Read-matches to plant taxa were classified to genus, where possible, and treated as incidence (i.e. presence/absence binary arrays) data because ITS1 reference sequences were lacking for species in the region. The richness of the pollen samples (i.e. the number of distinct taxa of foraged plants) was analysed as a function of the month (i.e. converted in integer variable) and landscape variables (i.e. explanatory variables). The non-collinearity among the predictors was assessed using Pearson’s correlation coefficients with a range value from -0.7 to 0.7 as selection criteria (Dormann et al. 2013). Four landscape variables were selected: dpop, SHEI, the proportion of impervious surface (Cov_urb) and the urban patch density (pd_urb) (**Figure 5-S2**). Given that the independence of our measurements could not be controlled because of the pseudoreplication of the temporal data (**Table 5-S2**) and the unbalanced experimental design, general mixed-effects models (GLMMs) were used (Grueber et al. 2011; Harrison et al. 2018). The pollen collection sites were specified as random effects (1| site). Negative binomial distribution was chosen as link function to explain the distribution error of the foraged plant richness (Lindén and Mäntyniemi 2011) using *glmmTMB* R package (Brooks et al. 2017). The selected predictors were specified as fixed effects as a function of the months and landscape variables (i.e. explanatory variables). For the model selection, Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) metrics were assessed. The lowest values of theses metrics optimize the trade-off between the fit and the complexity of the constructed models (Richards 2008). Finally, the residual distributions of all mixed-effects models assumptions (i.e. over- underdispersion, deviance) were inspected and checked with *DHARMa* R package (Hartig 2021).

For multivariate analysis, the pollen source-plant taxonomic composition of the samples was studied across sites, sampling periods and landscape classes using the Jaccard dissimilarity metric from the *vegan* package in R (Oksanen et al. 2019). This asymmetric distance coefficient addresses the problem of double zero, which is essential when studying data on community composition along a gradient. Differences in pollen source-plant composition between sampling periods and landscape classes were investigated by permutation-based multivariate analysis (N = 999) of variance using the *adonis* function (Anderson 2001). If the PERMANOVA results were significant, a post-hoc multilevel pairwise analysis with Bonferroni correction was performed using the *pairwiseAdonis* package in R (Martinez Arbizu 2020). The dissimilarities in the structures of pollinated plant communities were displayed using non-metric multidimensional scaling (NMDS) with 999 permutations. We also tested the selected landscape variables to analyze β diversity using distance-based redundancy analyses (dbRDA) on the Jaccard distance matrix as variable to explain. Afterward, ANOVA with 999 permutations was performed to test the influence of the selected landscape variables on the dissimilarities of the observations. These analyses were performed using the *vegan* R package (Oksanen 2008). All graphics were generated using the *ggplot2* package in R (Wickham 2016).

2.5. Indicator species and trait-based analysis

Similarity percentage (SIMPER) analysis was applied to identify how the taxonomic composition differed from the environmental conditions (landscape type) and changes (season). This step allowed us to identify the sampled taxa that contributed significantly to the dissimilarities among the months or landscapes. Finally, to analyse the characteristics of pollen forage plants, taxa were further classified by their traits including herbaceous (no woody stems above ground) or woody taxon (tree, shrub, liana), and including native, alien, or cultivar taxa. The plant trait database was built using information from Ylist (Yonekura and Kajita 2007) and ©Species2000 (Roskov et al. 2019) for the Japanese plant dataset. To determine if the proportion between the different traits varied with the seasons and landscape types, the G-test of independence for contingency table was performed using the *RVAideMemoire* package in R (Hervé 2020). The G-test is based on the log likelihood ratio and tests whether the relative proportions of one categorical variable (i.e. plant nature or native status) are independent of the second categorical variable (i.e. season or landscape). Next, post-hoc pairwise comparisons were conducted between pairs of proportions using the Bonferroni correction of the p-values (MacDonald and Gardner 2000).

3. Results

3.1. *Landscape classification*

The method of differentiating vegetation from impervious surfaces using NDVI provided convincing results after crosschecking, even in the complex environment of an urban matrix. The two first dimensions of the landscape PCA from landscape variables of our sampling sites described a high percentage of the variance (axis 1 = 81.6% and axis 2 = 11.0%; **Figure 5-2**). According to the elbow method (**Figure 5-S3**) of the k-means partitioning, we classified our sampling sites into four landscape classes according to a rural-urban gradient: rural (N = 3), suburban (N = 3), urban (N = 5), and urban centre (N = 6). The urbanised locations were driven by a much higher demographic density compared to the other landscapes (**Figure 5-S4**). Moreover, following the decrease in the proportion of vegetation along the rural-urban gradient, it can be assumed that the higher patch density in the cities was induced by the presence of many smaller plots, such as private garden patches. In contrast, the rural sites demonstrated a higher connectivity between the patches.

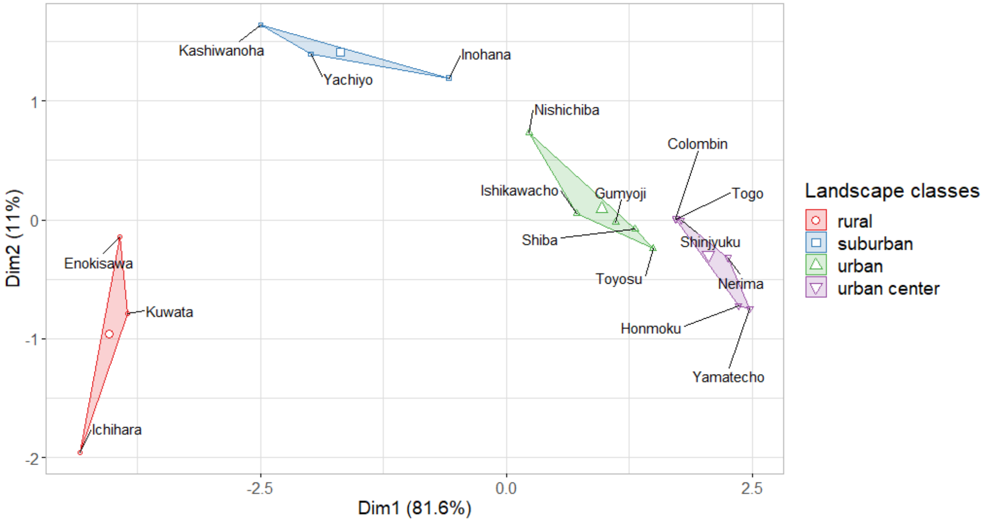

Figure 5-2 : Landscape cluster analysis of selected locations based on k-means approach. The axes represent the first two principal components of the PCA analysis. The dot shapes and colours represent the resulted landscape classes: circle/red = rural landscape; square/blue = suburban landscape; triangle/green = urban landscape; reversed triangle/purple = urban centre landscape.

3.2. *Taxonomic analysis*

Illumina sequencing generated a total of 8,179,602 paired-end raw reads for the 143 pollen samples from the 17 sites throughout the 7 months of pollen sampling. After assembling and filtering, 6,799,314 reads (83.2%) were obtained for analysis, with a mean count of 47,548 ± 27,464 (SD) reads per sample. After taxonomic assignment of the meta-barcoding dataset, we identified 307 plant flower taxa from 74 families and 187 genera. Prior to the analysis, the richness was not correlated with the duration of sampling (**Table 5-S2**), showing a very weak relationship (rs [143] = −0.17, $p < 0.05$), allowing us to consider the statistical independence of all our pollen samples. Plant richness ranged between 3 and 42 pollinated plant taxa per sample, with an average of 12 ($SD = 6.2$). GLMMs were not able to detect a significant interaction between landscape variables and collection time on taxa richness (**Table 5-1**). Only, the pollen richness diminished significantly as the seasons progressed (**Table 5-1**, **Figure 5-3A**) while the impervious surface proportion has no impact on the pollen richness (**Figure 5-3B**).

Table 5-1 : Detailed effects of the season progression and the landscape variables on foraged species richness. Results of the linear mixed-effects models containing the months and the landscape variables as explanatory variables to foraged species richness (N = 143). Model selection was performed according to the AIC and BIC criterions. ‘*’ and ‘***’ correspond to *p-value* < 0.05 and 0.001, respectively.

Model	Predictor	Value	Standard error	P-value	AIC	BIC
1	Intercept	3.060	0.475	***	865.4	889.1
	Month	-0.120	0.020	***		
	log(dpop)	-0.002	0.048	0.954		
	SHEI	-0.118	0.271	0.663		
	Cov_urb	0.308	0.368	0.402		
	pd_urb	0.002	0.002	0.257		
2	Intercept	3.049	0.390	***	863.4	884.1
	Month	-0.126	0.020	***		
	SHEI	-0.120	0.269	0.656		
	Cov_urb	0.296	0.299	0.322		
	pd_urb	0.002	0.002	0.217		
3	Intercept	2.891	0.162	***	861.6	879.3
	Month	-0.124	0.019	***		
	Cov_urb	0.397	0.194	0.04*		
	pd_urb	0.002	0.002	0.112		
4	Intercept	2.991	0.150	***	862.1	876.9
	Month	-0.119	0.019	***		

	Cov_urb	0.265	0.177	0.133		
5	Intercept	3.140	0.114	***	862.3	874.2
	Month	-0.116	0.019	***		

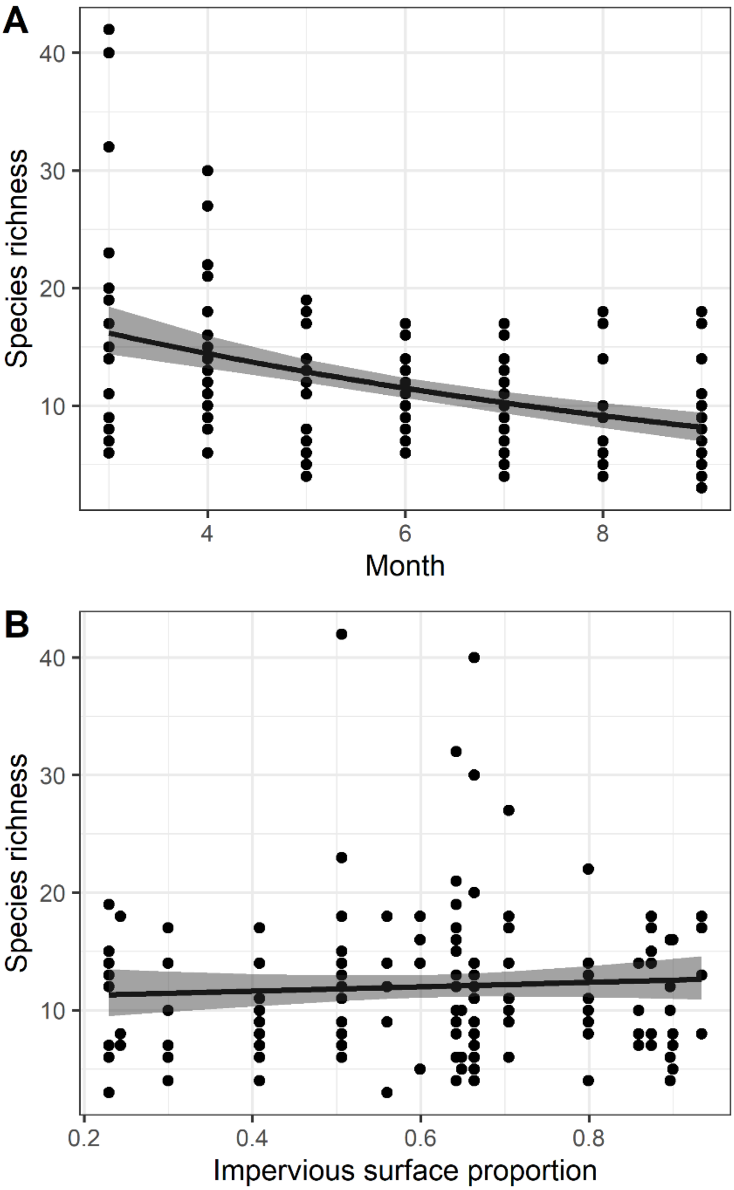

Figure 5-3 : Season progression (A) and impervious surface (B) effect on foraged plant richness. Shaded areas for both graphics correspond to 5% confidence interval superimposed on black lines followed negative binomial family models (N = 143). The months follow the Julian calendar numbers.

NMDS displayed high variability in the composition of foraged pollen across the months and seasons (**Figure 5-4**). The greatest discontinuity separated spring (March, April, and May) from autumn (September). Concerning the floral composition foraged by the honeybees, May and August served as transition months to subsequent seasons. The permutation tests revealed that the month period ($F = 6.87$; $R^2 = 0.23$; $p < 0.001$), site ($F = 1.27$; $R^2 = 0.1$; $p < 0.01$), and landscape class ($F = 2.01$; $R^2 = 0.03$; $p < 0.001$) were significant explanatory variables of pollen source-plant composition in the samples; however, the sampling period was attributed a larger proportion of the variance. From pairwise comparisons (i.e. letters from **Figure 5-4**), the urbanised sites hosted similar plant communities. Moreover, the structure of the plant communities varied significantly over the months until late summer and early autumn (i.e. August and September), when the floral composition harboured similar foraged plant communities.

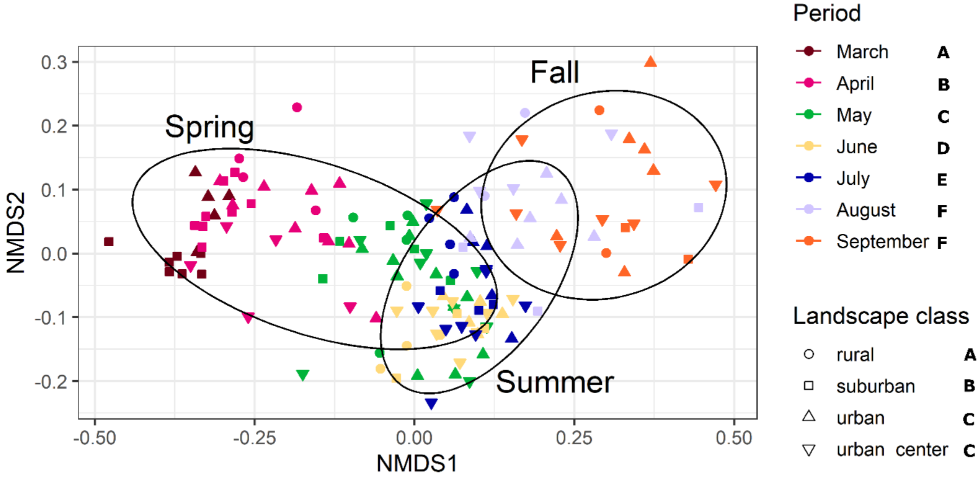

Figure 5-4 : Non-metric multidimensional scaling (NMDS) of plant communities from the pollen incidence data. Dot shapes correspond to the landscape classes of pollen samples. Dot colours correspond to the sampling months, and the seasons are displayed by 80% prediction confidence ellipses. Letters indicate significant differences ($p < 0.05$) according to the pairwise post-hoc comparisons, with Bonferroni correction, of the foraged plant communities among the landscape gradient and the sampling period.

The db-RDA result showed a poor constrained variance explanation (dbRDA1 = 10.95%; dbRDA2 = 7.34%; Figure 6-7) of the pollen distance composition matrix and the landscape variables but a significant linkage (R^2-adjusted = 0.063; p-value =

0.007). The influence of population density (df = 1; F-stat = 1.31; p-value = 0.051) combined with SHEI (df = 1; F-stat = 1.25; p-value = 0.08) on the pollen composition is nearly significant.

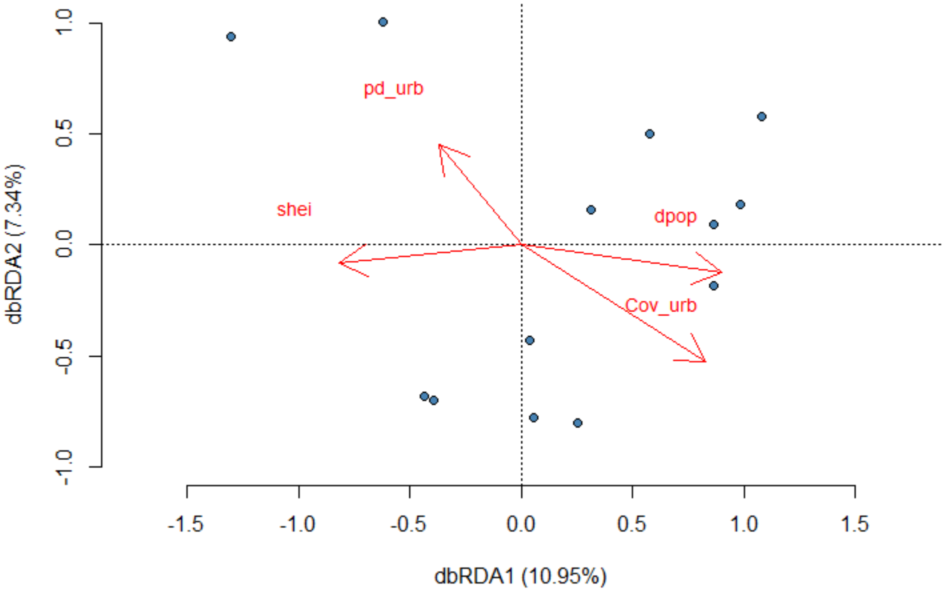

Figure 5-5 : Distance-based Redundancy Analysis (dbRDA) of pollen samples. Dimensions 1 and 2 showed 18.29% of the constrained variance. Blue points (N = 17) correspond to the collection sites. Red arrows correspond to the landscape variables.

3.3. Indicator species and trait-based analysis

The characteristics of pollen forage plants varied significantly according to their stratum (*G* = 99.0, *p* < 0.001) and native status (*G* = 69.1, *p* < 0.001) over the study months (**Figure 5-5B**), while only the plant strata showed significant differences (*G* = 10.7, *p* < 0.05) according to the landscape classes (**Figure 5-5A**).

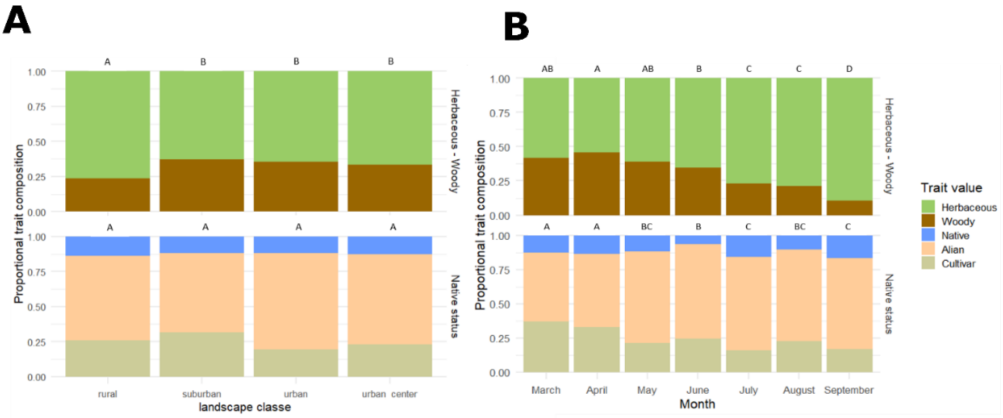

Figure 5-6 : Proportional occurrences of the different plant traits. (A) Proportional occurrences of the different plant traits (plant nature and native status) along the different landscape classes. Letters on top of the bar cluster homogeneous landscapes according to the significant results of post-hoc pairwise comparison with Bonferroni correction ($p < 0.05$). (B) Proportional occurrence of the different plant traits (plant nature and native status) across the sampling period. Letters on top of the bar cluster denote homogeneous sampling periods according to the significant results of post-hoc pairwise comparison with Bonferroni correction ($p < 0.05$).

3.3.1. Effect of landscape

Significantly more herbaceous plant taxa structured the rural landscape (**Figure 5-6A**), even if pollen source-plant composition traits were independent of landscape type. A total of 35 plant taxa were shared among all the landscapes over the sampling months such as some *Trifolium* spp. or *Plantago asiatica* L., while 151 plant taxa were exclusively related to specific landscapes, corresponding to 27%, 22%, 24%, and 26% of the total plant taxa for rural, suburban, urban, and urban centre landscapes, respectively (**Figure 5-S5**). *Sagittaria natans* Pall. and *S. trifolia* L. taxa are tied to rural areas. Plant families, such as Fabaceae, Rosaceae, Brassicaceae, Asteraceae, Plantaginaceae, and Onagraceae, were the most frequent taxonomic families encountered in all samples. However, their proportions varied according to the landscape (**Fig. 5-S6**). In urban and urban centre areas, leguminous plants prevailed more than in rural and suburban landscapes. However, the suburban landscape showed a higher frequency for the Brassicaceae, Ranunculaceae, and Rosaceae plant families. Surprisingly, anemophilous plants of the Poaceae family were more frequently foraged in the urbanised landscape than in the countryside (**Fig. 5-S6**). *Trifolium* genus showed constant occurrence throughout the landscape classes while other main genera foraged by honeybees were more frequent in distinct landscapes such as *Allium*, *Sagittaria* and *Helianthus* taxa for rural landscapes, *Veronica* and *Papaver* taxa for suburban areas, *Plantago* and *Oryza* taxa for urban center landscape (**Figure 5-7A**).

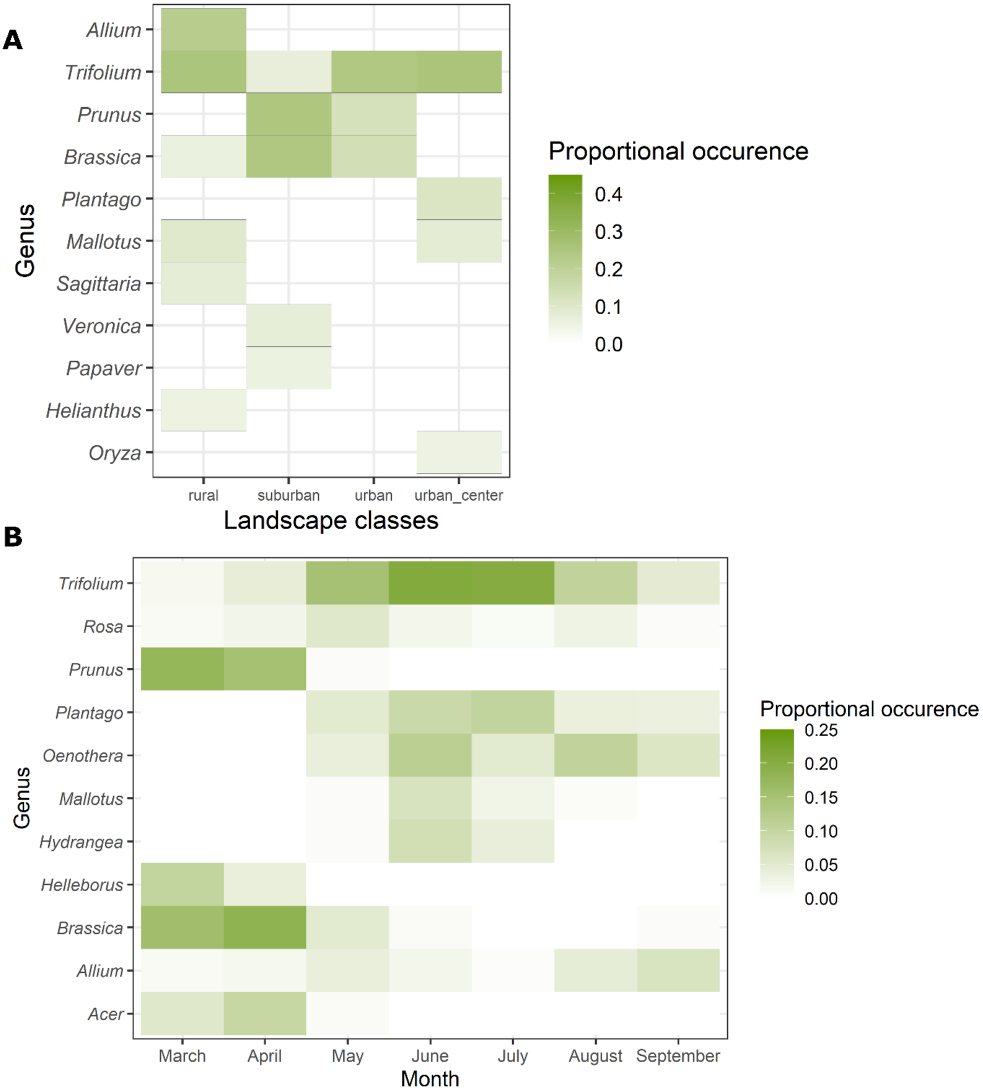

Figure 5-7 : Heatmaps according to the landscape classes and the sampling months. (A) Heatmap representing 11 most frequent genera (N = 46 taxa; 37% of the meta-barcoding dataset) ordered in descending order by their prevalence in all samples according to their landscape proportional occurrence. The proportional occurrence corresponds to the ratio of the number of observations for a genus per month to the number of samples for the specific month. The 11 genera were chosen according to the condition that their observation frequency is superior to 10% of the total occurrence of at least one landscape class. (B) Heatmap representing 11 most frequent genera (N = 62 taxa; 47% of the meta-barcoding dataset). The proportional occurrence corresponds to the ratio of the number of observations for a genus per month to the number of samples for the specific month. The 11 genera were chosen according to the condition that their observation frequency is superior to 10% of the total occurrence of at least one season.

3.3.2. Effect of sampling period

The proportion of foraged woody taxa decreased significantly over the seasons ($G = 87.5$, $p < 0.001$), with a peak of 46% in April and a low of 10% in September. Over the sampling months, honeybees foraged mainly on alien plant taxa (**Figure 5-6B**). Cultivar taxa were more foraged in spring than in the other two seasons ($G = 32.9$, $p < 0.001$). The most visited plant genera in March and April included *Prunus, Helleborus*, *Brassica,* and *Acer* taxa (**Figure 5-7B**). In contrast, four of the 11 most frequent genera emerged in late spring in May. Between April and June, a noticeable phenological turnover in the pollen composition (**Figure 5-7B**) was observed, with May serving as a transition bridge. This has already been highlighted by the discontinuities in the NMDS ordination (**Figure 5-4**). Following this shift, the genus *Trifolium* spp. was highly dominant in the June and July samples. In addition, the herbaceous genera *Plantago* and *Oenothera* spp. were also found in large proportions in combination with the woody genera *Mallotus* and *Hydrangea* spp. In August, the taxa from the genus *Oenothera* were the most represented with *Trifolium,* despite a reduction in its occurrence. A shift in pollen composition trends, with a reduction in highly proportional occurrence genera, was observed in August and September. In other words, plants detected in August and September were more distributed between the genera. Only the genus *Allium* showed an increase from August to September. Finally, *Trifolium* spp., *Rosa* spp., and *Allium* spp. were the only genera that were observed throughout the study period.

4. Discussion

This study revealed interesting patterns of honeybee foraging habits along the urban-rural gradient throughout their active season. In the present study, we used an unconventional approach, namely k-means clustering from landscape metrics, to categorise our sampling sites into four landscape classes. These landscape variables allowed the estimation of the effects of some ecological processes at the landscape level (i.e. foraging and plant dispersal) in assessing the diversity, connectivity, and aggregation of the patches (Baguette et al. 2012; Doherty and Driscoll 2018). Despite the convincing results of the grouping method, several reservations are worth mentioning. First, the selected foraging radius of 6 km accentuated the spatial autocorrelation issues on the landscape variables by increasing the foraging area overlaps among the sites (Plant 2012). This was not tested in the present study because we considered each hive per apiary as independent unit. It can be explained by the high variations in hive foraging behaviour (Visscher and Seeley 1982; Oldroyd et al. 1992), even at the local scale for colonies of the same apiary (De Vere et al. 2017). Indeed, the foraging behaviour of honeybee colonies is mainly driven by: (i) the temporal colony needs; (ii) the high density of pollen and nectar resources available near the colony; (iii) the rapid decision making by colony for the most profitable flower patches; and (iv) the ability to tightly modulate its pollen reserves to protect the colony from seasonal pollen breaks (e.g. long rainy periods, rarefaction of floral resources at the end of summer) (Seeley 1995). Second, the 3-image resolution from satellites may result in some limitations, especially in complex landscapes, such as urban matrices. Therefore, this spatial resolution issue could be counterbalanced with the use of specific cameras, such as red-green-blue (RGB) or multispectral cameras, mounted on unmanned aerial vehicles to characterise floral identification and surfaces (Chen et al. 2019; Librán-Embid et al. 2020). Nonetheless, this research domain is still in its infancy, particularly in the data processing of imagery classification by deep learning (Pritt and Chern 2017). However, despite these spatial limitations, the approach led to satisfactory classification, which paves the way for further investigations. The use of DNA meta-barcoding with one pair of ITS1 primers (Masamura et al. 2014) allowed for the identification of a great diversity of plant resources and highlights foraging patterns, regardless of the landscape variables or classes. This method yields to the identification of 307 taxa, which is higher than previous studies (Danner et al. 2017; Richardson et al. 2021). This could be explained by the important sampling effort of pollen samples (N = 143) over the seasons combined with contrasting landscapes (i.e. urban to rural landscapes). Moreover, the lack of a Tokyo regional plant list and associated ITS1 references to compare our sequences with could have increased the number of detected taxa by introducing false-positive BLAST alignments (Richardson et al. 2015a).

We observed a strong seasonal effect on plant richness (**Figure 5-3**), the foraged plant community, and plant characteristics (**Figure 5-4** and **Figure 5-6B**). We observed higher plant richness and foraged woody taxa in spring than in the other two seasons (**Figure 5-3** and **Figure 5-6B**), as reported previously (Lau et al. 2019; Brodschneider et al. 2019; Sponsler et al. 2020). The genera *Prunus* spp. and *Acer* spp. (**Figure 5-6D**) dominate the foraged woody stratum during this season, as these taxa offer adequate protein:lipid ratios for the development of honeybee early in the foraging season (Requier et al. 2015; Richardson et al. 2015b; Brodschneider et al. 2019; Sponsler et al. 2020; Vaudo et al. 2020). Indeed, bee breads with high proportions of both genera were positively correlated with high protein content (Donkersley et al. 2017; Vaudo et al. 2020). Particularly, the complexity and high range of foraged plants is known to be beneficial to the "nutritional value" of these bee bread stocks, and thus to honeybee immunity (Alaux et al. 2010). After the spring period, the proportion of foraged herbaceous strata gradually substituted woody taxa to reach approximately 90% of herbaceous foraged taxa in September, in agreement with previous studies (Brodschneider et al. 2019; Sponsler et al. 2020). This growing herbaceous stratum is mainly dominated by *Trifolium* spp. and *Plantago* spp. which, because of their long flowering period, might explain the lower richness of taxa foraged in summer and autumn (Donaldson-Matasci and Dornhaus 2012; Liolios et al. 2015; Brodschneider et al. 2019). Also late summer and early autumn are known to be characterized by a depletion of floral resources in temperate regions which could explain specific foraging on the remaining floral communities such as *Trifolium* or *Allium* genera (Requier et al. 2015; Sponsler et al. 2020). Moreover, clover species (i.e. *Trifolium* spp.) are highly ubiquitous in grasslands, such as meadows for rural areas or parks and gardens for urban areas (Critchley et al. 2007; Brodschneider et al. 2019) and may contribute to the concentration effect of amino acid content

(Donkersley et al. 2017). Concerning the temporal dynamics of the biogeographic traits, the observations point to a decrease in the frequency of cultivars in favour of exotic taxa, which contradicts previous studies (Williams et al. 2011; Urbanowicz et al. 2020). The highly anthropized and fragmented environments of the Tokyo region and its surroundings could explain the predominance of non-native floral species. However, this statement should be mitigated and requires further investigation, such as a complete plant inventory of the study site. Finally, we observed a transitional change from August by a collapse of the dominant flower prevalence, which may correspond to the seasonal dearth of floral resources (**Figure 5-4** and **Figure 5-6B**) (Park and Nieh 2017; Brodschneider et al. 2019). The honeybees mitigate this effect by increasing their foraging range, requiring extra effort for sometimes worthless rewards (Park and Nieh 2017). Finally, the study of other co-variables, such as brood monitoring or estimating the pollen collection/reserve of each colony, could be used to compare the conditions of each sampled colony and improve our understanding of the foraging patterns of the colonies (Delaplane et al. 2013).

The landscape variables did not influence forage plant richness, as reported in previous studies (Steffan-Dewenter and Kuhn 2003; Danner et al. 2017). However, considering the spatial variation of taxa composition among the sites of our area of interest (i.e. beta diversity) (Legendre 2014), our results showed a spatial structure of foraged plant communities in countryside, suburban, and urban environments (i.e. by merging the urban and urban centre areas) that could be driven by the population density (**Figure 5-4 and Figure 5-5**). It is likely that honeybee colonies modify their foraging preferences due to the high prevalence of unattractive ornamental flowers in urban landscapes (Garbuzov et al. 2015a). The urban matrix also offers smaller spread patches and less dense floral resources, which contribute to the foraging change of workers (De Vere et al. 2017; Lucek et al. 2019). This shift in the prospected flora is also coupled to fulfil the nutritional demand with a diverse and complementary floral diet (Hendriksma and Shafir 2016; Donkersley et al. 2017). Therefore, it shows the importance of taking beta diversity and not only the local richness into account to understand the community structure of foraged plants throughout space and time scales (Socolar et al. 2016; Richardson et al. 2021). Despite the landscape structure of the foraged plant community, the trait-based analysis revealed no significant pollinated plants traits for honeybee colonies, except for herbaceous plants in rural landscapes (**Figure 5-5A**). However, the forest cover is high in rural areas (Enokisawa and Kuwata=42.2%, Ichihara=35%), mainly composed of artificial forest stands (Enokisawa and Kuwata=53%, Ichihara= 33%) (Chiba 2022). Artificial forest mainly consists of conifers *Cryptomeria japonica* (L. f.) D.Don and *Chamaecyparis obtusa* (Siebold & Zucc.) Endl. which do not provide floral resources for bees, meaning that the attraction of herbaceous plants would be facilitated. Moreover, the inconclusive result of trait-based analysis can be explained by the number and selection of functional traits selected from the foraged plants. The selected characteristics of pollen forage plants are few and not very informative in the context of plant-pollinator relationships. An evaluation of the functional structure of foraged plants with relevant floral characteristics (which honeybee colonies depends on) such as floral symmetry, floral shape, etc. would have helped to refine the understanding of the attractive floral structure (Fornoff et al. 2017; E-Vojtkó et al. 2020).

In all the samples, 35 plant taxa were shared throughout all the landscapes studied, corresponding to 45% of all read counts. The top three plant families were Fabaceae, Rosaceae, and Brassicaceae which are known to be in the foraging preference of honeybee (Sponsler et al. 2020; Richardson et al. 2021). The presence of grasses (Poaceae) may seem surprising among the most frequent families in the samples, given that these plants are considered unsuitable for *A. mellifera* resource needs (Decourtye et al. 2010). In view of their dominance, it seems unlikely that anemophilic pollen is the result of contamination by pollen blown from flowers onto the body of the bees, such as the rice paddy field in Japan (Kimura et al. 2014). Other recent studies have shown that pollinators (i.e. bees and syrphids), particularly *Apis* bees, interact with wind-pollinated plant species for their nutrient or nesting requirements (Saunders 2018). Honeybees foraged floral resources ranging from 28 to 45 taxa, specific to each defined landscape. For example, some parts of rural landscapes in our study correspond to wetlands which are suitable to the population development of *S. natans* and *S. trifolia* (Chen 1989).

5. Conclusion

This study has shown that the foraged plant richness was mainly explained by the season progression. In addition, it reflects large plant communities dominated by the occurrence of alien species independently of the landscape classes. Woody species offered an important source of pollen to the honeybees in the early growth season while herbaceous plants dominated foraged plant structure in summer and autumn. The cultivar species occurrence tends to decrease constantly over the seasons, while native species are sparsely foraged. These findings are consistent with the literature addressing this topic with similar datasets (Requier et al. 2015; Sponsler et al. 2020). Our work revealed that the foraged floral composition is also driven by the landscape context suggesting that bee diet is locally constrained by ecological drivers although 35 foraged plant taxa were exploited by honeybees in all defined landscapes. During periods of floral dearth, honeybees increase their foraging radius and their number of floral sites which require extra effort from the foragers sometimes for worthless rewards (Park and Nieh 2017). Indeed, the nutritional need for the colony is a pivotal factor for the colony health which is mainly affected by the variations in pollen depletion and quality rather than its pollen richness (Di Pasquale et al. 2016). Therefore, in an urban greening context, it would be relevant to put in place measures to alleviate this seasonal dearth by ensuring enough high-quality floral resources close to the apiaries which may also benefit in their tolerance against pathogens or pesticides (Di Pasquale et al. 2013; Barascou et al. 2021).

Chapter 6

The role of urban pavement as nesting site for wild bees and apoid wasps

Chapter VI: The role of urban pavement as nesting site for wild bees and apoid wasps

This section is an adapted version of the article:

Grégoire Noël, Violette Van Keymeulen, Yvan Barbier, Sylvie Smets, Olivier Van Damme, Gilles Colinet, Sophie Lokatis, Julien Ruelle & Frédéric Francis. "Nest aggregations of wild bees and apoid wasps in urban pavements: a "street life" to be promoted in urban planning." In minor revision in *Insect Diversity and Conservation Journal* under the special issue of **urban entomology**.

Abstract - In the last 10 years, the interest in nature-based solutions and ecosystem services like pollination has increased profoundly and with it the need to gather knowledge about wild bees and apoid wasp community dynamics in urban ecology. Research on how the urban environment impacts the conditions of nesting sites is relatively scarce. Recent observations in the Brussels-Capital Region (Belgium) show that urban pavements can be an alternative nesting opportunity for ground-nesting Hymenoptera, such as wild bees and apoid wasps. Here, using a citizen science approach, we investigated the richness of ground-nesting species living under urban pavements, as well as their preferences of sidewalk characteristics. A total of 22 species belonging to 10 families of wild bees, digger wasps and their associated cleptoparasites were identified at 89 sites in the Brussels-Capital Region (Belgium). Sandstone setts or concrete slabs with an unbound joint size around 1 cm were found to be the best suitable urban pavements for the ground-nesting species. The soil texture under the pavement contained mainly sandy particles. We propose management guidelines to support bee and wasp species nesting under urban pavement in highly urbanized areas. Our observations pave the way for further research in the field of urban ecology and highlight the potential of multifunctional pavement designs that promote not only climate adaptation, but also biodiversity.

Keywords: Anthophila, Apoidea, nesting behaviour, sustainable development, urban ecology, urban ecosystem, urban conservation

1. Introduction

Rapid urbanization is one of the most critical drivers of global biodiversity loss (Ives et al. 2016; Driscoll et al. 2018). Recognizing the role that cities play in tackling the global biodiversity crisis, preserving and fostering local biodiversity has become a key objective for municipalities around the world (Pierce et al. 2020). The goal of urban nature strategies, generally, is not only to protect and preserve biodiversity, but also to enhance ecosystem functions, and to grant the urban population access to nature in their day-to-day life. Urban nature however sometimes sprouts in unexpected places, and urban dwellers and ecologists alike, have not payed much attention to the ecosystem that is spreading in the cracks and joints of urban pavements. Urban pavements provide alternative nesting opportunities for certain ground-nesting species (see Dijon et al. 2023), but although the sandy mounds that generally indicate their presence are known to be quite present in several European cities (pers. observations of the authors), only few studies have addressed urban pavements as habitats for aculeate insects (notably Haeseler 1982; Pauly 2019a).

Hymenopteran communities are sensitive to urban landscape conversion (Buczkowski and Richmond 2012; Geslin et al. 2016; Corcos et al. 2019; Theodorou et al. 2020a): flower resources are becoming scarce under the pressure of urban fragmentation, and urban soil is rendered impervious by concrete, asphalt and other pavement, which makes the ground uninhabitable to ground-nesting hymenopterans (Burkman and Gardiner 2014; Harrison and Winfree 2015; New 2015; Geslin et al. 2016; Wenzel et al. 2020; Ayers and Rehan 2021). Research has shown a decline in richness and size of urban bee communities as urbanisation increases (Ahrné et al. 2009; Fortel et al. 2014b; Eggenberger et al. 2019), and the decline of predatory species like wasps (Abrahamczyk et al. 2020). These are additionally affected by habitat fragmentation as a consequence of urbanization, as their ability to locate prey in large landscapes may be restrained (Kareiva 1987), leading to changes in the community structure of apoid wasps in cities (Christie and Hochuli 2009; Burkman and Gardiner 2014). Paradoxically, recent studies have shown that cities can also serve as refuges for wild bee communities (Baldock et al. 2015a; Hall et al. 2017; Theodorou et al. 2020c). Bees are very efficient pollinators and ensure the sexual reproduction of flower plants due to their morphological features and behaviour(e.g. branched hairs or setae, diet composed of pollen and nectar) (Michener 2007). Thus, the bees in urban areas also provide pollination as ecosystem service for urban agriculture as well as wildflowers (Lowenstein et al. 2014) and, in some instances, can be bioindicators of healthy ecosystems (Kevan 1999). In urban areas, several factors can be potentially beneficial to hymenopteran communities, and wild bees in particular: (i) the amount and coverage of biocidal particles in cities tends to be lower than in the surrounding countryside, (ii) the heterogeneity of urban patches offers a wide diversity of habitats with multiple associated ecological niches and (iii) urban parks, gardens and other green spaces can provide sufficient floral resources distributed throughout the year (Fortel et al. 2016; Wenzel et al. 2020; Fenoglio et al.

2021). A surprisingly large number of cavity-nesting species might even benefit from artificial nesting aids (MacIvor 2017).

Of the 20,759 described bee species in the world (Ascher and Pickering 2023), most solitary bees build their nests underground with a higher proportion of ground nesters outside of the tropics (M. Orr, personal communication). In Belgium, more than half of the 403 species of wild bees are nesting in the ground (Drossart et al. 2019). Their nests typically consist of an entrance, often surrounded by a mound of soil (**Figure 6-1a**), which leads into a main gallery that branches into secondary galleries. The larval cells contain food resources (a mixture of pollen and nectar) and one or multiple eggs each (Malyshev 1935; Michener 2007). Ground nesting behaviour is typical for Andrenidae and Melittidae families, as well as the majority of Halictidae and Colletidae families (Danforth et al. 2019). Apoid wasps - including families that have recently been up-ranked (e.g., Philantidae, Psenidae, Bembicidae, Pemphredonidae) by Sann et al. (2018) - also have important ecological roles in urban environments. Adults behave as flower visitors and as predators they capture insect or spider prey to feed their offspring (Bitsch and Leclercq 1993). In Belgium, 199 species of apoid wasps have been documented, again with more than half of them nesting in the ground (i.e., 107 spp., Pauly 1999; Rasmont and Haubruge 2002). Some aculeate species may exhibit a collective nesting strategy in nest aggregation such as *Cerceris* spp. (Willmer 1985; Polidori et al. 2006) or *Andrena* spp. (e.g. in Fellendorf et al. 2004). In ground-nesting wild bees, sub-social and semi-social behavior can be found, particularly among some *Lasioglossum* species such as *Lasioglossum laticeps* (Schenck, 1869) (Packer 1983).

Bee and wasp species that nest in the ground can potentially also inhabit urban pavements, if their requirements are met. On pavements, these species can dig their nests in the joints between pavement tiles, where they find suitable nesting material between and below the tiles. Joint size probably has a direct effect on the nesting ability of bees and wasps: if the joint size is smaller than their thorax size, they will not be able to dig a gallery. The soil texture (Cane 1991), soil cover (Nichols et al. 2020), soil compaction (Wuellner 1999; Sardiñas and Kremen 2014), soil humidity (Wuellner 1999), soil temperature and sun exposition of the soil (Potts and Willmer 1997), as well as soil aesthetics (Cane 2015) can influence the selection of nesting sites. In Brussel-Capital Region (BCR), pavements are built using different jointing material. Only the modular pavements (i.e. slabs that are separated by jointing material) are supposed to provide suitable nesting structures to host Hymenoptera nests. To reach the soil below the pavement plates, digging insects will have to enter through the joint. The composition of the jointing material thus defines the hardness of the substrate and therefore the ability of bees and wasps to tunnel into it, acting as a filter in nest site selection.

As a pioneering study in the urban ecology of ground-nesting Hymenoptera, we addressed the following questions about the community of wild bees and apoid wasps according to their nesting preferences in pavements: (i) What species inhabit the pavements in the Brussels-Capital Region (BCR)? (ii) Is there a relationship between the size of ground nesting species and the diameter of the joints? (iii) Does the pavements type impacts species composition? (iv) Which soil texture under the pavement is favoured by ground-nesting species? To address these questions, we asked volunteers to look for nesting sites throughout the city of Brussels, and share the location, as well as additional information, of their observation. In a second step, the sites that had been preidentified by volunteers were visited and sampled by experts. We characterized the edaphic features of the identified species. Finally, we discuss the implications of our results in view of current challenges of designing urban pavements to encourage ground-nesting insects and human-wildlife cohabitation in cities.

2. Material and methods

2.1. Identification and validation of the potential study sites

As part of a general assessment of wild bee communities in the Brussels-Capital Region (BCR), Pauly (2019a) reported 4 nesting sites in urban pavements. In order to gain more knowledge about this highly understudied habitat, we applied a crowdsourcing method based on citizen science (Newman et al. 2017). We first created and actively disseminated a participatory survey to BCR citizens on social networks in collaboration with the communication department of Brussels Environment and other key actors in BCR conservation (e.g., regional, and local institutions). The online form was launched on March 11, 2020, before the first potential emergence of the expected hymenopteran insects (Pauly 2019a). The participatory survey ended on 17 July 2020. Participants were asked to enter information on (i) the BCR municipality, (ii) street name and building number, (iii) the date of the last observation and, (iv) an image of the nest or the insect (facultative). We removed 74 observations unrelated to taxa and location of our study sampling strategy: ant nests (**Figure 6-1b**), cavity-nesting bees, and locations outside of BCR. A total of 89 locations were found suitable for further field observation and visited within a week after transmittal. On site we examined the pavement cover for 30-45 min and looked for insect activity on the sites. Field work was performed on sunny days with clear sky, little wind (less than 15 km/h) and a daily minimum temperature of 15°C between 09:00 and 17:00 (Ahrné et al. 2009; Fortel et al. 2014b). A site was validated if (i) a bee or wasp showed evidence of entry or exit into a nest between the paving slabs and/or (ii) a cuckoo species (i.e., cuckoo bee or wasp) patrolled near a sandy mound on the pavement.

2.2. Data collection on validated sites

The number of nests was estimated by counting the sandy mounds. On each of the selected sites, each morphologically distinct specimen was captured and killed *in situ* with ethyl acetate ($C_4H_8O_2$) for later species identification. If possible, joint size was measured on 6 nests randomly selected within a site using a millimetre bar next to the nest entrance. If more than one ground-nesting species was present at a site, measurements were taken randomly across the site without distinguishing between species. The joint structure variable was added to the database as a nominal qualitative variable with 2 modalities: degraded rigid joint (**Figure 6-1c**) or unbound joint (**Figure 6-1d**). The type of pavement was assessed from photographs and classified according to 3 modalities: concrete slabs (**Figure 6-1c**); sandstone setts (**Figure 6-1d**) and other types, including ceramic slabs, concrete pavement, limestone (Belgian blue stone) and porphyry setts. Finally, the position of the nest on the pavement was recorded as a qualitative variable according to the following nomenclature: pavement; adjacent to a house front or wall; internal yard; road with car traffic or other (i.e. embankment, junction by pavement and embankment or junction by internal yard and pavement). The sand of the mounds was collected randomly from 3-10 nest entrances (max. 50g), and used to assess the composition of the underlying soil layer (**Figure 6-1a**). Based on preliminary results of the substrate texture under pavements, sandy mounds seem to be a good proxy of soil texture (see supplementary information S1 in **Chapter X**).

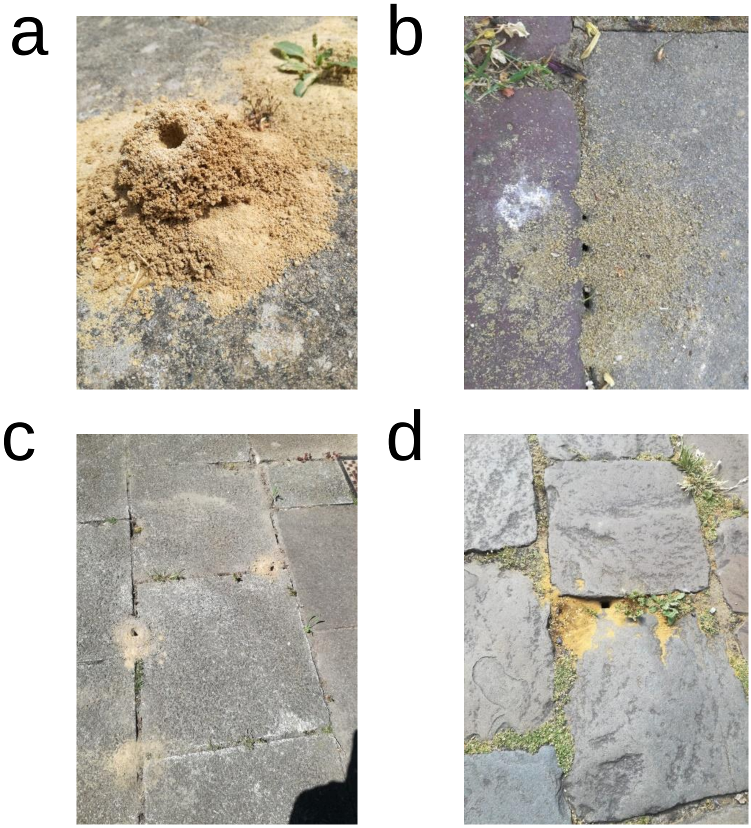

Figure 6-1 : Pictures of nest structures on urban pavements. **(a)** Sandy mound (Auderghem, Brussels) of *Dasypoda hirtipes* (Fabricius, 1793). **(b)** Ant nests are characterized by multiple entrances, widespread substrate and small sticks or blades inserted into entrances. Some nests also show above ground galleries (Anderlecht, Brussels). **(c)** Nest entrances built in degraded rigid joints of concrete slabs (Schaerbeek, Brussels). **(d)** Nest entrance built into the unbound joints of sandstone setts (Schaerbeek, Brussels). Pictures by Grégoire Noël.

2.3. Laboratory data collection

All collected specimens were prepared for identification following Mouret *et al.* (2007). Several identification keys were used to identify bee and wasp species (Bitsch and Leclercq 1993; Bitsch et al. 1997, 2007; Falk 2015; Pauly 2019b). All identified bee specimens were checked against the reference collections of the Functional and Evolutionary Entomology Laboratory (ULiège), the collections of Alain Pauly for captured Halictidae species and the collections of Jean Leclercq for apoid wasp species. We also measured the inter-tegular distance (ITD) – the distance between the two wing insertions – of female individuals only, which serves as proxy for their size (Kendall et al. 2019) using a digital caliper (Electronic Digital Caliper). Sand mound samples were weighed using a precision balance and passed through a sieve shaker

(Haver & Boecker VWR brand) for 10 minutes at an amplitude of 1 mm through five sieves with mesh sizes of 1 mm, 500 µm, 200 µm, 100 µm and 50 µm in order to distinguish sands from clays and silts (50 µm threshold) and to differentiate between very fine, fine, medium and coarse sands. The particles retained by each sieve were then weighed and their value converted to a percentage of the total sample volume. This conversion eliminates weight variations due to moisture and provides a common basis for comparison between samples of different weights. Although the particle rate of silts and clays was not distinguished, we can approximate the average and extreme textures of the collected mounds using the texture triangle by halving the remaining percentages between the silt and clay classes.

2.4. Mapping and statistical analysis

All analyses were performed in the R software environment (version 4.0.2.; R Core Team 2020). Validated sites were mapped using the *mapview* R package (Appelhans et al. 2019). The average mean joint size per site was then assigned to the corresponding species. For the joint size, we used an ANOVA after descriptive statistical analysis of the data to compare joint size measurements between selected ground-nesting species and their respective families. specimens from the families Bembicidae, Crabronidae, Psenidae Due to their parasitic nesting strategy, we excluded cuckoo species in the statistical analysis, as well as and Pemphredonidae because we did not record their respective joint sizes (see Table 1). A *post-hoc* Tukey test with adjustment for multiple comparisons was applied to compare the pair mean of joint size. As ITD represents the proxy of bee size, we applied a Gaussian linear regression to explain the influence of bee size on the joint size selection by ground-nesting bees.

For the particle size analysis, a principal component analysis (PCA) was performed to determine whether similarities in particle size preferences among ground-nesting species could be attributed to specific families (as shown in **Table 6-1**) using *factoextra* (Kassambara and Fabian 2020) and *FactoMineR* (Lê et al. 2008) R packages. Graphs were plotted using the *ggplot2* R package (Wickham 2016).

We used BCR land-use map of the Brussels-Capital Region from Bruxelles Environnement Institute which was produced in 2015 by ULB as part of the LifeWatch project to generate eleven variables (**Table 6S-2**) of land-use classes within a 300-meter radius of each nesting site (n = 52; only bees). Based on this map, we calculated the percentage of impervious surfaces, Shannon's index (SHEI) and vegetation via the *landscape metrics* R package (Hesselbarth et al. 2019). The non-collinearity among these landscape variables was assessed using Pearson's correlation coefficients with a range value from -0.7 to 0.7 as selection criteria (Dormann et al. 2013). Five landscape variables were selected: the proportion of impervious surface, the proportion of arable land, surface of needle leaved trees and proportion of recently (<5 years) disturbed ligneous vegetation. To analyze β diversity, distance-based redundancy analyses (dbRDA) were then performed on the Bray–Curtis dissimilarity matrix with the *dbrda* R function setting up the distance matrix as variable to explain and selected landscape variables as explanatory variables. Afterward, ANOVA with 999 permutations was performed to test the influence of the selected landscape variables on the dissimilarities of the observations. These analyses were performed using the *vegan* R package (Oksanen 2008).

3. Results

3.1. Participatory survey and site validation

Between 5 April and 31 July 2020, 163 sites throughout BCR fulfilled the criteria for our online form and were surveyed subsequently. Of these, a total of 89 sites could be validated (**Figure 6-2**). The municipalities of Ixelles (n=16), Watermael-Boitsfort (n=13) and Uccle (n=13) had the largest number of sites, while only few sites were situated in Berchem-Saint-Agathe (n=1), Forest (n=1) and Auderghem (n=2) (**Figure 6-S1**). There was no nesting activity reported by citizens of Saint-Josse ten-Noode and Koekelberg municipalities.

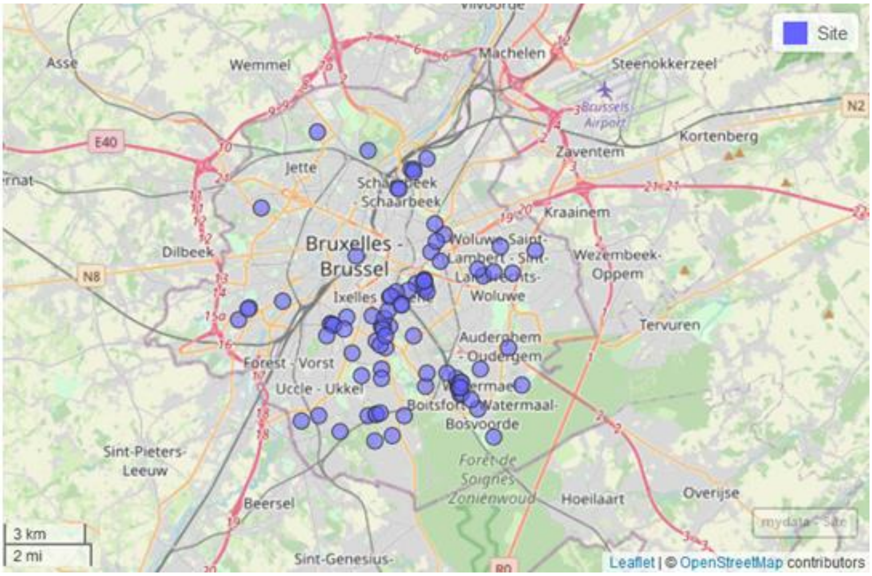

Figure 6-2 : Distribution map of the validated study sites in Brussels Capital Region (N=89).

3.2. Species recorded

We collected 153 specimens belonging to 22 species, including 11 solitary bee species, 9 apoid wasp species and 2 chrysid species (**Table 6-1**). The most abundant species found at the study sites were *A. barbilabris*, *C. arenaria*, *L. laticeps* and *H. nobile*. We also collected the corresponding cleptoparasite and parasitoid species, namely *N. alboguttata*, *Sphecodes* spp., *H. gerstaeckeri*, and *H. nobile* (**Table 6-1**). Most sites had a single ground-nesting species (excluding cleptoparasite and parasitoid species). However, some of the sampled sites showed that co-occurrence of several nesting species was possible between solitary wasps and solitary bees, but also between different bee species and between different wasp species (**Figure 6-S2**).

Table 6-1 : List of apoid and chrysid families (in bold) and species collected at the sampling sites. The specific abundance is given on the right side of each species. Species names in black correspond to ground-nesting, non-parasitoid species, while cuckoo species are highlighted in red. The apoid wasp families were defined according to Sann et al. (2018).

Bees	Abundance	Apoid and chrysid wasps	Abundance
Andrenidae	**33**	**Philantidae**	**37**
Andrena barbilabris (Kirby, 1802)	*30*	*Cerceris arenaria* (L.)	*26*
Andrena vaga (Panzer, 1799)	*3*	*Cerceris quadricincta* (Panzer, 1799)	*4*
Melittidae	***8***	*Cerceris rybyensis* (L., 1791)	*5*
Dasypoda hirtipes (Fabricius, 1793)	*8*	*Philanthus triangulum* (Fabricius, 1775)	*2*
Halictidae	**36**	**Bembicidae**	**1**
Lasioglossum fulvicorne (Kirby, 1802)	*2*	*Gorytes planifrons* (Wesmael, 1852)	*1*
Lasioglossum laticeps (Schenck, 1868)	*20*	**Crabronidae**	**6**
Lasioglossum sexstrigatum (Schenck, 1868)	*7*	*Lindenius pygmaeus armatus* (Rossi, 1794)	*4*
Sphecodes crassus Thomson, 1870	*2*	*Oxybelus bipunctatus* Olivier, 1812	*2*
Sphecodes miniatus Hagens, 1892	*1*	**Psenidae**	**2**
Sphecodes monilicornis (Kirby, 1802)	*1*	*Mimesa lutaria* (Fabricius, 1787)	*2*
Sphecodes pellucidus Smith, 1845	*3*	**Pemphredonidae**	**3**
Apidae	**5**	*Diodontus insidiosus* Spooner, 1938	*3*
Nomada alboguttata (Herrich-Schäffer, 1839)	*5*	**Chrysididae**	**22**
		Hedychrum gerstaeckeri Chévrier, 1869	*4*
		Hedychrum nobile (Scopoli, 1763)	*18*

3.3. *Joint size analysis*

A total of 398 joint measurements at 69 validated sites was correlated with the nest entrance locations of 10 ground-nesting species (**Figure 6-3**). The mean joint size for all species was 1.08 cm ± 0.57 cm with a maximum measured at 3.00 cm and a minimum at 0.20 cm. Details for all studied species are given in **Table 6-S1**. A significant difference in mean joint size was observed between different ground-nesting species (F-stat = 1.97; df = 9; p-value = 0.041). However, after adjusting for multiple comparisons, no pairs of species differing in their joint size were detected (p-value > 0.05). After excluding 9 sites because individuals were too damaged to allow precise measurement of ITD, the linear regression of the mean joint size and ITD size was performed on 80 observations. ITD did not explain the selection of joint size among the species (F-stat = 0.16; df = 78; p-value = 0.69; **Figure 6-4**).

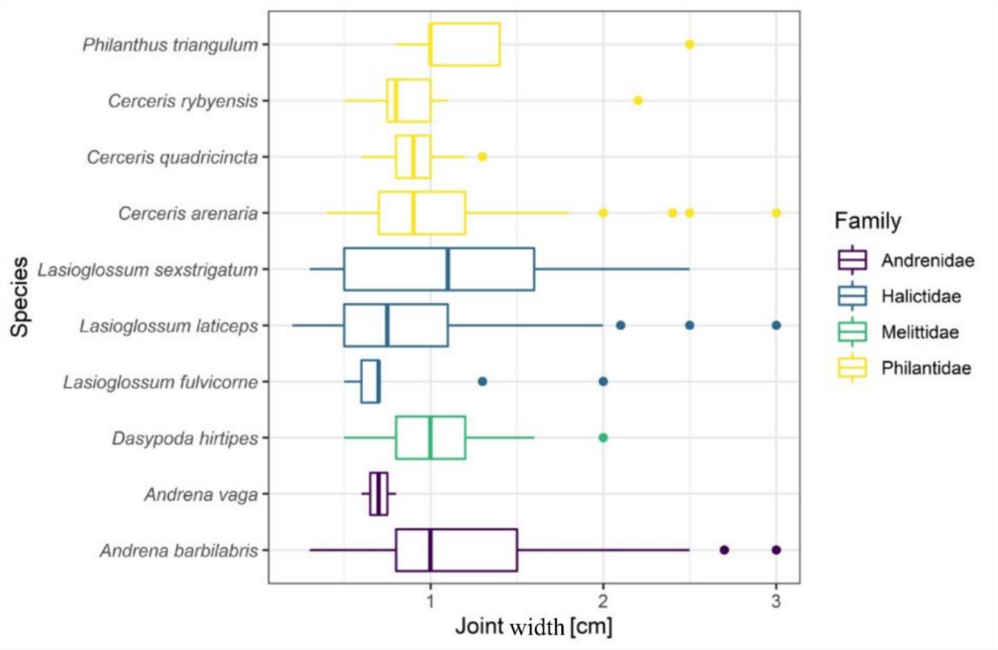

Figure 6-3 : Distribution of joint sizes (in cm) measured next to nest entrances, grouped according to the different ground-nesting species that were present at the sites. For example, nests found on sites where *Andrena barbilabris* (bottom of the figure) was found, were built in joints with a wide variation of size, ranging between less than half a centimeter and three centimeter with a median around one centimeter.

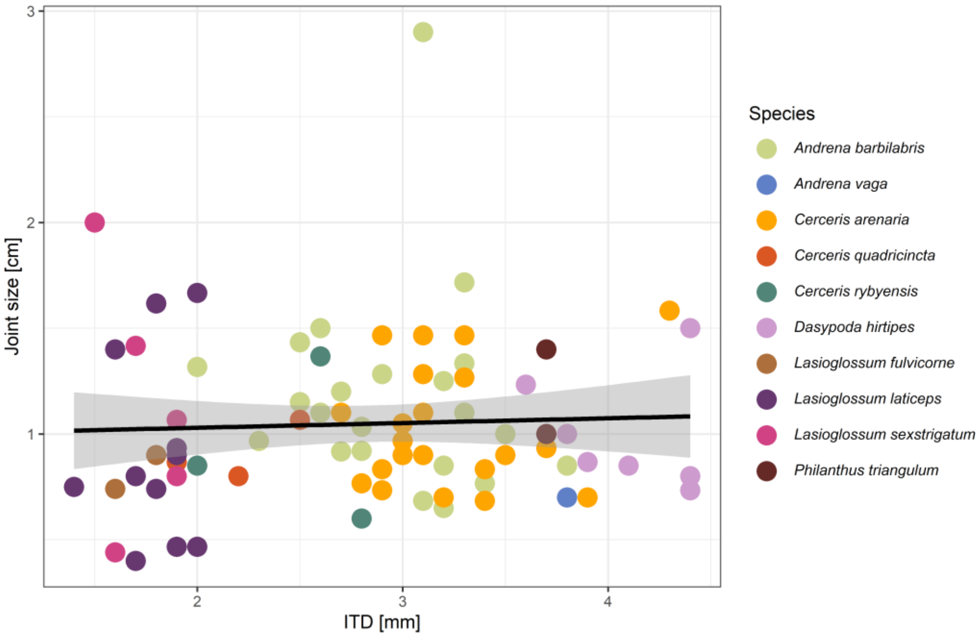

Figure 6-4 : Linear regression of inter-tegular distance average (ITD in mm) and near-entrance joint size average (in cm). Colors corresponded to different ground-nesting species. Grey shade area indicates 95% confidence interval region computed from means.

3.4. Joint structure and pavement type

After excluding 10 sites because they did not meet our classification (i.e. hybrid pavement), joint structure and pavement type of 79 sites were characterized. The joints were unbound at 80% of the sites encountered, while 20% of the joints were characterized as rigid and degraded, leaving openings for ground-nesting species to dig and nest (**Figure 6-5a**). In terms of composition, the pavements were mainly composed of concrete slabs (40 sites) and sandstone setts (29 sites) (**Figure 6-5b**). The remaining sites were composed of sandstone or limestone paving stones (3 sites), concrete paving blocs (4 sites), ceramic paving flags (1 site), porphyry setts (1 site) and blue stone elements (1 site). Regarding the location of nests on the pavements (**Figure 6-5c**), most were located on pavements or sidewalks (53 sites) while some were located on roads (3 sites) and in the internal courtyards of houses (8 sites). Some sites were located at the level of house steps (11 sites) or sometimes spilling over onto sidewalks (4 sites). Only one site was characterized on stair steps and another one was characterized with an overflow of the ground-nesting aggregation from the embankment to the sidewalk.

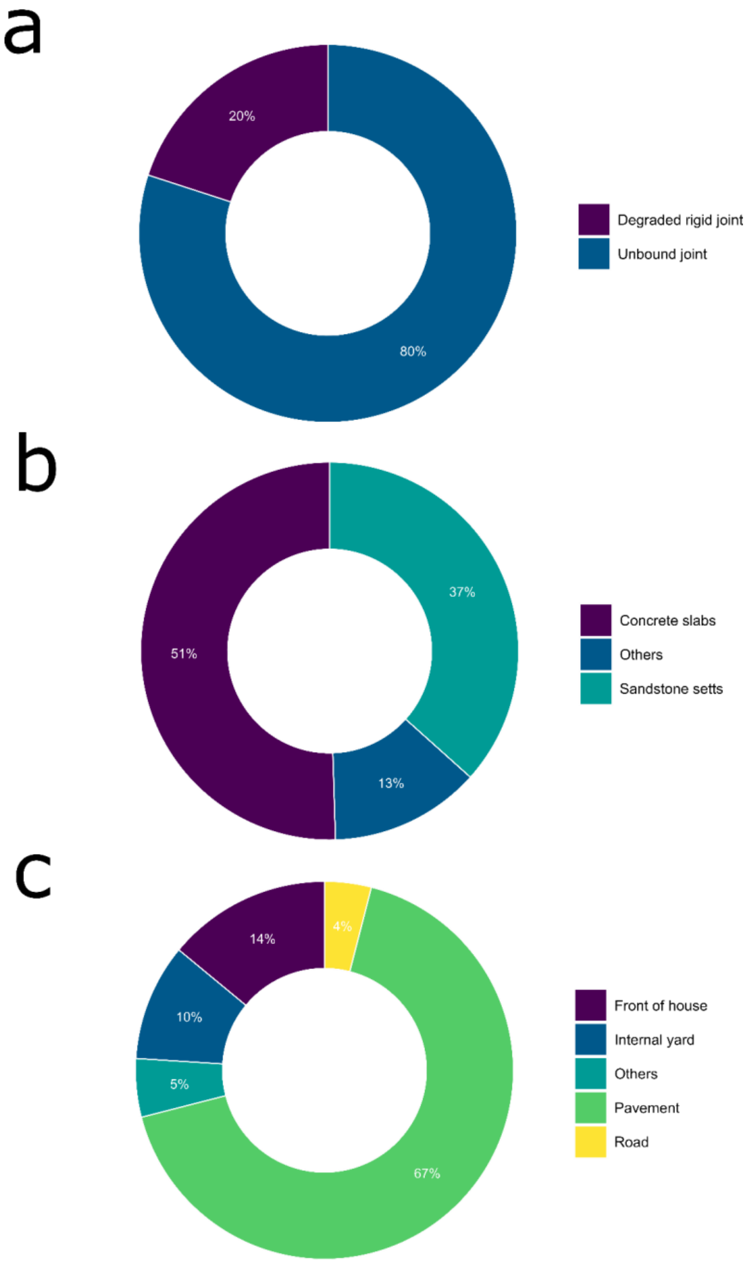

Figure 6-5 : Distribution (%) of joint types from 79 study sites (**a**), types of urban pavement on which nests were located (**b**) and locations of nest entrances on urban pavements (**c**).

3.5. *Soil texture analysis*

A total of 53 sandy mound samples were analysed for grain size. The sandy fraction of the samples was always higher than 85% and the silt and clay fractions were always lower than 10%, classifying all samples as sandy and homogeneous in texture (**Figure 6-S3**). On average, the samples were composed of 2.91% of particles larger than 1mm in diameter (i.e., very coarse sands) and 2.61% smaller than 50 µm (i.e., clays and silts). Particles with a diameter of 500 µm - 200 µm (i.e., medium sands) were the most abundant in the samples with a proportion of 41.71%. The samples contained on average 7.47% of particles in the 1 mm – 500 µm class (i.e., coarse sands), 13.40% of particles with a diameter of 100 µm – 50 µm (i.e., very fine sands) and 31.90% of 200 µm – 100 µm (i.e., fine sands) (**Figure 6-S4**). PCA did not detect separate clusters or discontinuities of the sandy samples depending on the ground-nesting species or their respective families (**Figure 6-6**).

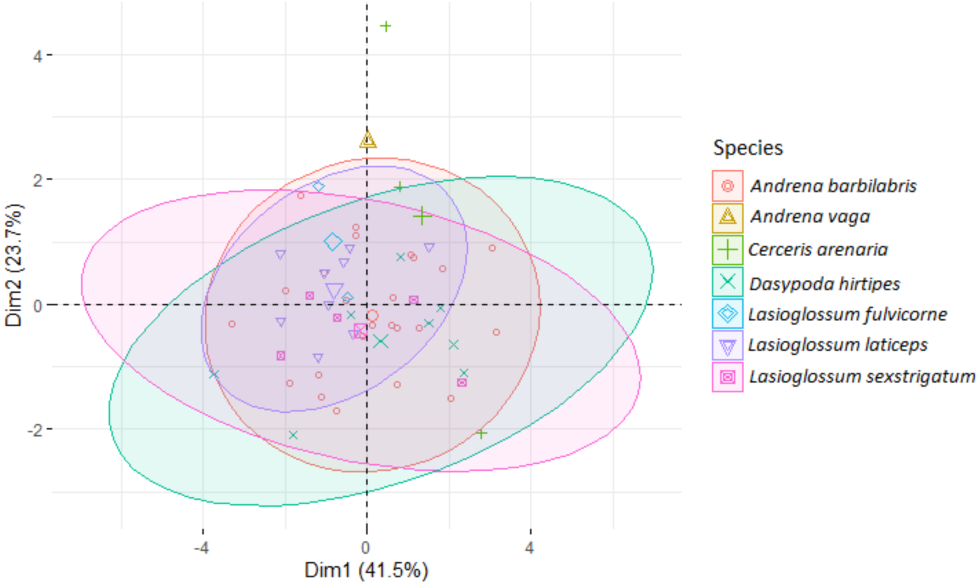

Figure 6-6 : Principal component analysis (PCA) of collected mound samples grouped with 80% confidence ellipses by ground-nesting species. Dimensions 1 and 2 showed 65.2% of the explained variance. Coloured and shaped points (N = 53) corresponded to the ground-nesting species.

3.6. *β diversity analysis*

The dbRDA result showed a poor constrained variance explanation (dbRDA1 = 14.28%; dbRDA2 = 2.41%; **Figure 6-7**) of the distance composition matrix and the landscape variables but a significant linkage (R^2-adjusted = 0.090; p-value = 0.022). The influence of impervious surface (df = 1; F-stat = 2.53; p-value = 0.074) and

SHEI (df = 1; F-stat = 2.16; p-value = 0.088) on the species composition in the pavements is nearly significant.

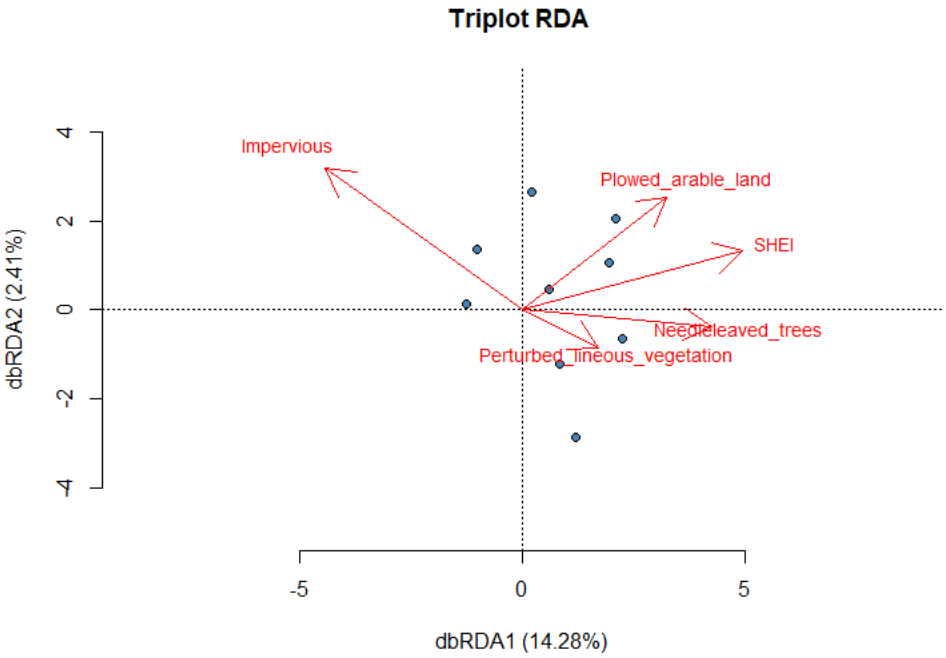

Figure 6-7 : Distance-based Redundancy Analysis (dbRDA) of species samples. Dimensions 1 and 2 showed 16.69% of the constrained variance. Blue points (N = 52) correspond to the collection sites. Red arrows correspond to the landscape variables.

4. Discussion

4.1. Monitored species

At present, knowledge about adaptations of ground-nesting bees to the urban environments is patchy, particularly with regards to their nesting strategies (Wenzel et al. 2020; Antoine and Forrest 2021; Ayers and Rehan 2021). In our study, we were able to confirm the observations of Pauly (2019a) and Haeseler (1982) for *D. hirtipes*, *L. laticeps*, *L. sexstrigatum*, *S. miniatus*, *S. pellucidus* and *A. barbilabris* (the latter also having been mentioned by Falk, 2015), but not for the Halictidae bee species *Lasioglossum calceatum* (Scopoli, 1763), *Halictus rubicundus* (Christ, 1791) and *Sphecodes puncticeps* (Thomson, 1870), and for the Andrenidae bee species *Panurgus calcaratus* (Scopoli, 1763). *P. calcaratus* is an oligolectic species foraging on *Heriacium* spp. (Asteraceae), and we expected it to be present at our sampling sites. We may have missed it due to its summer phenology (Rasmont and Haubruge 2002). However, several species of ground-nesting bees and their associated cleptoparasites were identified for the first time as nesting under urban pavements: *A. vaga*, *L.*

fulvicorne, *S. crassus*, *S. monilicornis* and, *N. alboguttata*. These species of ground-nesting bees and cuckoo bees are rather common, and are listed as having stable populations in Belgium and Europe’s red lists, except for *A. barbilabris* whose Belgian populations are reported to be increasing (Nieto et al. 2014; Drossart et al. 2019). All wild bees were polylectic except for *A. vaga* and *D. hirtipes,* with the former being specialized on *Salix* spp. (Salicaceae) and the latter foraging mainly on Cichorioideae (Asteraceae, Rasmont and Haubruge 2002). Compared to the co-occurrence data with their associated hosts (**Figure 6-S2**), it appears that *L. laticeps* may be a new host for the cuckoo *S. crassus,* although we cannot confirm the association by a direct observation of an entry into the nest or into one of the 4 known hosts of *S. crassus* (Pauly 2019b): *Lasioglossum pauxillum* (Schenck, 1853), *L. punctatissimum* (Schenck, 1853), *L. nitidiusculum* (Kirby , 1802) and *L. parvulum* (Schenck, 1853) were not detected in this study. We also captured a specimen of *S. monilicornis* which is a potential cuckoo bee of *L. laticeps* (Bogusch 2003). We can confirm Vegter's (1993) observations of large numbers of *S. miniatus* parasitizing *L. sexstrigatum* nests. Finally, three specimens of *S. pellucidus* and five of *N. alboguttata* were captured at sites of its known host *A. barbilabris* (Witt 1992; Rasmont and Haubruge 2002).

In the scientific literature, only one study Haeseler (1982) reports the nesting of 9 apoid ground-nesting wasps and 1 spider wasp species (Hymenoptera: Pompilidae) in urban pavements of the city of Oldenburg (Germany). Our document is therefore the second written report of apoid wasps nesting under urban pavements, with 8 new species (only *O. bipunctatus* is present in both studies). As in Haeseler (1982), the species richness of apoid wasps in BCR is greater than that of the identified ground-nesting bees. The three *Cerceris* species are solitary wasps that nest on sandy substrates. They capture and paralyse small Coleoptera for their offspring, especially Curculionidae or Chrysomelidae specimens for *C. arenaria* and *C. quadricincta,* while *C. rybyiensis* specialises in capturing small species of halictid bees (Bitsch et al. 1997). *H. gerstaekeri* and *H. nobile* are known as their associated parasitoid wasps. *P. triangulum*, commonly known as the "beewolf", is a predatory apoid species that is widespread in Europe. It is a specialist predator of *Apis mellifera* L., but can as an exception substitute with other wild bee genera (e.g. *Andrena* spp., *Dasypoda* spp.) (Bitsch et al. 1997).

Surprisingly, other species of apoid wasps belonging to other smaller and less frequently captured genera, were also found among the pavement inhabitants. These were *G. planifrons*, *D. insidiosus*, *M. lutaria*, *O. bipunctatus* and *L. pygmaeus armatus*. Their nests often consist of a single gallery leading to the larval cell(s) and are built in sandy soils. Like *Cerceris* spp., the adults are generalist predators. Their prey consists mainly of specimens belonging to the families Cicadellidae, Fulgoridae, Cercopidae, Membracidae, micro-Diptera or micro-Hymenoptera (Bitsch and Leclercq 1993; Bitsch et al. 1997, 2007). *Cerceris* spp. are small and common apoid wasps (Bitsch et al. 1997) that have no conservation status in Belgium, although *G.*

planifrons is considered to be very rare (Schmid-Egger et al. 2010). *L. pygmaeus armatus* is a rare psammophilous species in Belgium, with only two observations since 1950 despite its wide European range (Bitsch and Leclercq 1993; Rasmont and Haubruge 2002). This species was observed on 4 different sites in BRC, suggesting that it nests frequently on BRC pavements and could therefore be the subject of a potential conservation project. Another interesting finding of our study was that in several instances, sites were occupied by multiple species, such as *C. arenaria* and *D. hirtipes* (**Figure 6-S2**). From our sampling protocol, it was difficult to observe nest differentiation or sharing of nest entrances between ground-nesting species. However, intraspecific individuals of *Cerceris* species can co-occupy the same nest (Willmer 1985; Polidori et al. 2006), which calls into question their strictly solitary behaviour. Therefore, it would be interesting to study the subterranean nesting structures in more depth and observe whether individuals of presumably solitary species, and even individuals from different species occupy the same nest structure.

Our sampling was limited to a single sampling period of 30 - 45 minutes. It is likely that the entire diversity of hymenopterans that inhabited a site was not sampled. It should be noted that nest-aggregations are dynamic systems, and our assessment only reflects a moment in time in the life of ground-nesting insect populations and communities. From a spatial perspective, this study was limited to the Belgian capital with its own urbanization and climatic conditions. Therefore, an extension of this study to other large cities (e.g., Paris or Berlin) could highlight other species of ground-nesting bees and wasps in urban pavements, and potentially different conservation issues. Also, larger species such as *Andrena* spp., *D. hirtipes*, *P. triangulum* or even *Cerceris* spp. may have been more likely to detect. On the other hand, small species nesting within pavements such as *Lasioglossum* spp. or other small-sized apoid species (**Table 6-1**) may not have been detected because they were located in less frequented streets and characterized by small nest clusters with poorly visible sand mounds. We can therefore assume that our sampling effort may have overestimated the number of large species, while underestimating small species.

4.2. Joint size and pavement structure

Joint sizes in BCR vary from 1 mm to 150 mm depending on the shape of the pavement element and the maximum diameter of the joints (Bruxelles-Mobilité 2016). However, during our observations, we found that the size of the joints where the entrances to the galleries were located fluctuated around a wider average, with no real preference in joint size among species or families of apoids. An increase in distance between two tiles or slabs is likely to accelerate the degradation of the pavement. Otherwise, we did not observe any preference of different taxa or differently sized species for larger or smaller joint diameters. The measured ITD was generally small enough to allow a wide range of species to pass through the narrowest joint measured.

All sites were characterized by pavement tiles that were separated by joints that allowed the ground-nesting species to dig their galleries. Nest entrances could be found in joints where (1) jointing material was absent, (2) jointing material was unbound or (3) bound jointing material was degraded. The presence of nests in cracks of bonded joints highlighted in the results was rather surprising. These structures were designed to be completely closed and originally did not allow insects to nest. However, we observed deterioration of the pavement and fragmentation of the jointing material. This could be explained by the poor quality of the rigid joint or its age, which affected the durability and cohesion of the material, making it more prone to disintegration during any disturbance such as shrinkage cracking or freeze-thaw episodes. It is possible that ground-nesting species of bees and wasps could be involved in the degradation of the modular structures and in particular their joints, as bees are able to dig into hard-packed soils (Barthell et al. 1988; Cane 1991).

A BCR pavement with unbound jointing materials is, in theory, always placed on underlying permeable and draining layers (i.e., sand, gravel, stone) in order to avoid water stagnation in the structure and its deterioration (CRR 2009, 2018). This combination seems to meet the criteria of sandy texture and drainage of the soil material generally required by ground-nesting bees and wasps in their natural environment. Indeed, soil that is too waterlogged, flooded or too dry can impede the survival capacity of individuals in immature stages (Wuellner 1999). Most of the pavements studied here consisted of sandstone blocks and concrete slabs. These results raised many questions about the architecture of the galleries within the substrate. Although we were able to determine the depth at which we found individuals of *A. barbilabris* (see supplementary information 1), the architecture of the sub-pavement nests remains unknown: do the galleries penetrate deep into the different layers that make up the paving, or do they only extend to the first few centimetres below the paving?

On several occasions, we reported nesting sites at the level of house steps and stairways. These observations were accompanied by a lack of jointing material along the terraced houses, which allowed the ground-nesting insects to access the sandy stratum under the pavements. It was mainly *Lasioglossum* spp. and non-Philantidae wasp species that preferred to nest in this type of location, which also should allow them to benefit from higher temperatures due to indirect solar radiation (Cane 2015) that first reaches the facades of the terraced houses.

4.3. Soil texture analysis

The particle size analysis revealed that the mound samples consisted of material with a sandy texture. This is consistent with observations made by Cane (1991) on 32 species of ground-nesting bees in the USA, by Vereecken *et al.*, (2006) for *A. vaga*, Malyshev (1935) and Michez (2008) for *D. hirtipes*, and Falk (2015) for the 6 remaining bee species, which report these ground-nesting bees to build their nests in sandy-textured soils. However, while these authors noted a variety of other used textures, such as silt loam and clay loam for Cane (1991), sandy-clay soils also for Vereecken *et al.*, (2006) or clay soils also for Michez (2008), our study highlighted only one type of texture used by the bees to dig their galleries. This means that from the perspective of soil-nesting insects, the foundation layer can be homogeneous in all BRC pavements if it is of sandy origin. However, promoting a sandy and homogeneous texture through urban redevelopment may disproportionately favour species associated with this ecological niche.

4.4. Implications for urban pavement design and management

A suitable pavement for ground-nesting species consists of sandstone pavers or concrete slabs with an unbound jointing size around 1cm on an unbound foundation. These characteristics are derived from the typical BCR pavements assessed in our study (**Figure 6-8**). Although it may be difficult to modify the requirements of the standard specifications established by the BCR specifications, as these are the basic requirements of pavements to guarantee the durability of pavements in BCR (Bruxelles-Mobilité 2016). While natural stone pavements with wide and unbound joint are favourable as nesting sites for the studied apoid species, these generally offer a lower level of pedestrian comfort (Bertrand et al. 2019) and are not fully compatible with the durability requirements of the BCR specifications, which recommend modular structures of the bonded type and a joint width less than or equal to 1 cm (Bruxelles-Mobilité 2016).

Our findings highlight a mismatch between the societal demands of BCR and the environmental opportunities. We thus propose that future studies should assess the possibility of designing multifunctional pavements that simultaneously meet the challenges of comfort, durability, entomobiodiversity and rainwater filtration. Such synergies in pavement design could for example be reached through advances in paving block design, or by differentiating pavements with a central area dedicated to pedestrians (no or thin joints) and a peripheral area dedicated to ground-nesting species and water infiltration (wide joint openings, draining material, vegetated or not), or by creating and/or maintaining a vegetated strip at the periphery of pavements, as may exist in BCR allotments (Fig. S5). In addition, it would be interesting to foster actions to initiate and reinforce a paradigm shift in the city's aesthetic criteria: "untidy" and "unfunctional" pavements in the human-centred city could become "multifunctional" pavements in the bio-centred city (Aronson et al. 2017; Rivkin et al. 2019). It would be very interesting to link this multifunctionality not only to biodiversity (i.e., offering nesting opportunities for ground-dwelling wild bees and other insects), but also with a potential for water leaching and an urgently needed reduction of soil sealing in urban areas (Fini et al. 2017).

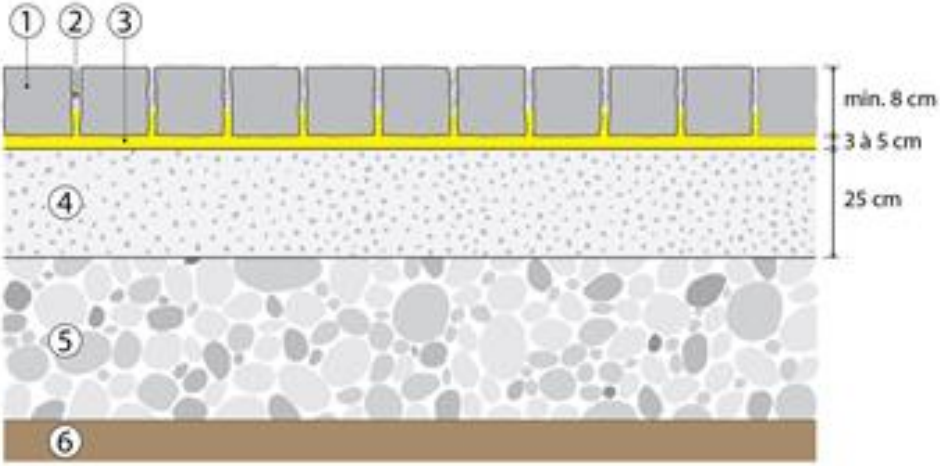

Figure 6-8 : The pavement structure : (**1**) Paving elements ; (**2**) Joints with an opening size of 0.8 to 1.0cm, filled with sand 0/6.3 or 0/8 (fine content less or equal than 10%); (**3**) Laying course: gravel 2/6,3 or 2/8 (fine content less or equal than 2%); (**4**) Road base: unbound aggregate 0/20 or 0/40; (**5**) Sub-base with a defined thickness according to the construction plan; (**6**) Subgrade

5. Conclusion

Our results show that sealed surfaces in urban areas do not always lead to the total loss of nesting opportunities as suggested by Cane et al. (2006) and Fortel et al. (2016). On the contrary, our study highlighted the opportunity of pavements to provide nesting opportunities in the city. Our observations tend to confirm the hypothesis put forward by Pauly (2019a), who stated that among all the pavement types in BRC, the old pavements in BCR - where the soil under the paving stones was sandy and where the joints were not cemented - were the most hospitable for ground-nesting bees and consequently for ground-nesting wasps. Unfortunately, these older BCR pavements are more prone to be redeveloped into pavement that is less suitable as nesting sites by the city of Brussels. This study suggests that pavements, previously thought to be unsuitable for biodiversity, may provide a refuge for some insect populations. Therefore, by turning to older pavement designs or including the properties of pavements that provide suitable nesting opportunities to aculeate insects in alternative construction models, we could make the city a more welcoming place for biodiversity.

Chapter 7

General discussion and conclusion

1. Reminder of the research questions and the β diversity importance in the overall biodiversity

1.1. Thesis reminder

We explored an interdisciplinary scientific framework from morphological taxonomy for species identification to genomics, landscape and community ecology, or social sciences to fill some scientific gaps or to provide additional scientific knowledge in pollination ecology and community structure of insect pollinator in disturbed temperate landscapes. In this thesis, we investigated four specific study cases to provide additional insights and new elements for future biodiversity and ecosystem service management. To do so, we raised four specific questions related to our four study cases:

1. *Do local farmsteads at the beginning of their ecological transition promote wild bees and hoverfly diversity? In our case it seems so.*

In **Chapter III**, we investigated the impact of habitat diversification at the plot or farm spatial scale on insect pollinators in agricultural landscapes. Our findings revealed that such diversification supports a significant biodiversity (in α and β levels) of insect pollinators, which play a crucial role in providing insect pollination and biological control of pests. We observed a higher representation of oligolectic species in terms of species richness rather than specimen abundance, suggesting that their specific floral targets are spatially close but may require further implantation to support oligolectic pollinator populations. Through our deep sampling efforts, we were able to discover rare species and gather new data on the population dynamics of lesser-known insect pollinator species in Belgium, such as *Andrena fulvata* Stoeckhert 1930 and *Halictus maculatus* Smith 1848 for wild bees, as well as *Xanthogramma pedissequum* (Harris 2006) and *Xylota sylvarum* (Linnaeus 1758) for hoverfly species.

2. *In local intercropping systems, does customized flower-strips promote wild bee and hoverfly communities? Not in our case, except for hoverfly community in multifloral flowerstrip.*

In the **Chapter IV**, our focus shifted towards a specific technique for landscape diversification: the implementation of flower strips to support pollinator species in the context of AECM (see **Chapter I**, point 1.4.1). Through a short-term experiment conducted on a single site, we demonstrated that our flower strip modalities, consisting of one multifloral strip and two monofloral strips, resulted in a limited biodiversity comprised primarily of generalist and highly mobile insect pollinators. We hypothesized that this poor diversity of sampled pollinators was influenced by the highly anthropized landscape of the experimental site. This study also underscores the importance of carefully designing the floral composition of multifloral strips and considering the inclusion of oilseed floral species in monofloral strips, which could potentially benefit farmers' income.

3. Does the species richness and composition of the foraged flower community by the honeybee vary according to (i) an urban-rural gradient and (ii) to the course of the seasons in Tokyo (Japan) as mega-city? Yes, but only for the floral composition according to the urban gradient and the diminution of floral richness according to the season course.

In our exploration of urban environments (from **Chapter V**), we delved into the foraging behavior of a super-generalist pollinator, *Apis mellifera* L., along an urbanization and temporal gradient in the mega-city of Tokyo, Japan. Our findings revealed that the diversity of visited flower structures, measured by β diversity, was influenced by the urban-rural gradient (especially the population density), while taxonomic richness remained unaffected. Furthermore, we observed a significant decline in pollen diversity as the season progressed. Honeybees exhibited a preference for woody species during the spring and shifted towards herbaceous species in the autumn. Considering the asymmetry of plant-pollinator interactions, focusing on specific plant species to ensure a sufficient pollen supply for honeybee colonies, particularly at the end of the activity season when the resources were disappearing, may also contribute to the dietary needs of other insect pollinators, including oligolectic or rare species.

4. Does the urban pavement of Brussels-city promote the opportunity of nesting sites for Apoidea species? Yes, for certain species in particular Brussels pavements.

In our final case study presented in **Chapter VI**, we examined the impact of urban pavements, acting as an urban filter, on Apoidea pollinators. Through an extensive sampling effort conducted in the Brussels Region of Belgium, we made some exciting discoveries - a rich diversity of Apoidea species nesting beneath the urban pavements combined with specific urban requirements. These species showed a preference for nesting in old Brussels pavements constructed with sandstone pavers or concrete slabs featuring unbonded joints. Additionally, the presence of sandy construction layers beneath these pavements seemed to be conducive to their nesting habits. Finally, we were able to develop technical recommendations and management practices aimed at promoting the well-being of these important pollinating insects.

1.2. The β diversity dominance

In this thesis, the remaining habitats within and around the study sites in both disturbed landscapes considered (i.e., agricultural and urban landscapes) have not been identified as a significant factor in the homogenization of community structure. This suggests that the habitats offered enough floral and nesting resources to maintain diverse communities of insect pollinators. Moreover, β diversity (rather than α diversity) was a significant pattern regarding to the distinct communities in function of the habitats (**Chapter III and IV**) or the landscape (**Chapter V**). It was more difficult to reveal any underlying trends in **Chapter VI** due to the structure of the data and the experimental design. Therefore, this thesis suggests that the landscape matrix of three study cases on four can mitigate the loss of landscape-wide biodiversity by harboring more dissimilar insect pollinator communities which reinforces the dominance of β diversity hypothesis as hypothesized by Tscharntke et al. (2012). Indeed, with his colleagues, Pr. Tscharntke (University of Göttingen, Germany) proposes three distinct explanations why landscape fragmentation *per se* has been overestimated to generate biodiversity losses and underestimated the impact of β diversity in the mitigation of these biodiversity decline.

(1) Within a specific spatial area, community dissimilarity amplifies in combination with the increasing of spatial heterogeneity, and this phenomenon intensifies over distance. Consequently, augmenting habitat diversity through subdivision to a limit size (i.e., smallest insect pollinator scale) in the designated spatial expanse increases β diversity and so γ diversity, which potentially leads to a reduction in α diversity within these contrasted patches. This pattern affects the generalist pollinators species as well as the endangered species (Tscharntke et al. 2002). In the context of our study cases, conducted at the local or landscape scale, the dominance of β diversity's influence on overall diversity in each case can be attributed to factors like habitat fragmentation, crop/land-use diversification (**Chapter III and IV**), and landscape heterogeneity (**Chapter V – Figure 5-2**). The potential dominance of β diversity in these studies may result in more resilient (agro-/urban-)ecosystems.

(2) If the surrounding landscape matrix of our fragmented elements contains usable resources such as pollen and nectar, it also positively impacts β diversity of pollinator communities (e.g., in Medeiros et al. 2019). But this pattern may vary according to the spatial scale considered, the ecosystems or biogeographic regions. At the local/landscape scale or our three study cases, the surrounding landscape seems favorable to insect pollinator by offering diverse habitats and edge effects suggesting again a reinforcement of a dominance of β diversity hypothesis (Tscharntke et al. 2012; Ren et al. 2023).

(3) Many studies struggle to accurately differentiate between habitat fragmentation and habitat loss (Fahrig 2003). Habitat fragmentation typically encompasses two elements: habitat loss and the partitioning of habitats, the habitat fragmentation *per se*. When it is well defined, the direct effects of habitat fragmentation *per se* on biodiversity are generally less pronounced (and could be either positive or negative) compared to the impact of habitat loss. In this thesis, the habitat fragmentation was clearly not defined and tested as in Fahrig's review (2003) even if habitat fragmentation influences the pollen collection of generalist pollinators (Steffan-Dewenter and Kuhn 2003) as seen in **Chapter V** by dissimilar floral communities visited by honeybee colonies.

Therefore, Tscharntke and his colleagues (2012) demonstrated that β diversity may be a better contributor to the γ diversity of a given area. It is important to note, however, that these trends of β diversity dominance may hold true in landscapes of various structural complexities, ranging from intermediate to very complex. This pattern might not apply in extremely simplified areas, like vast monocultures of Poaceae, where only highly pollinator communities, typically generalist/polylectic ones, could survive (Tylianakis et al. 2005). While not explicitly tested, I feel by the field experience that our study systems (across all four chapters) were situated within landscapes of moderate complexity which means that our landscape were not so disturbed by the human footprint. This could potentially explain the robust patterns of β diversity observed in **Chapters III, IV, and V**. Moreover, we also showed that ecological processes such as environmental filters of landscape lead to changes in species assemblages and influencing regional, landscape, and local biodiversity in our four chapters (see **Figure 7-1**). Particularly, **Chapter III** and **IV** showed more polylectic pollinators in abundance than oligolectic ones suggesting that edge effects in intermediate disturbed landscapes may drive the insect pollinator communities (Medeiros et al. 2019). Taking all the study in consideration, we organized the rest of our discussion around three key themes: the importance of obtaining quality data, the multifaceted of biodiversity, and the specific case of *A. mellifera*. We finished this discussion part by outlining future perspectives related to agroecological transition, urban pavement, and the floral competition posed by other honeybee species.

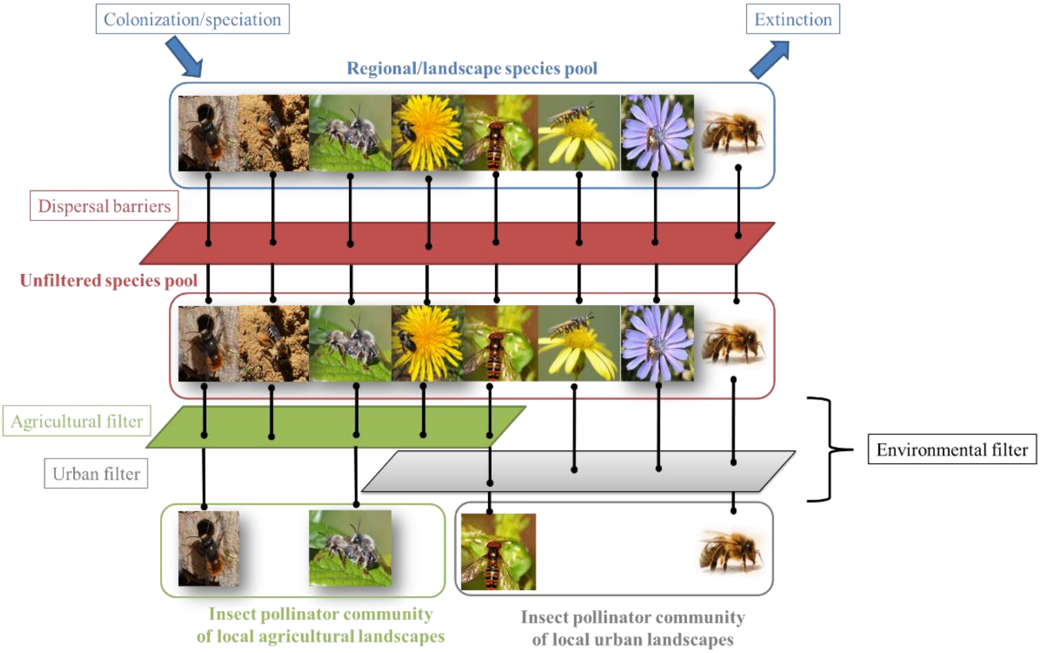

Figure 7-1 : Representation of ecological processes structuring biodiversity from regional species pool to local communities. This figure is inspired from Cornell and Harrison (2014) and adapted in this thesis framework on insect pollinators community. Ecological processes correspond to enclosed text boxes.

2. Obtaining and conserving insect pollinator specimens

In the context of pollinator decline in the Anthropocene era, three study cases on four reported quality data of pollinator occurrences (i.e., wild bees, hoverflies and apoid wasps) in different landscapes in Belgium through standardized methodologies.

As usual, we included occurrence data, but also related co-variable with each specimen such as the trap catch method, the visited flower, the type of pavement… Actually, it is a tedious task to record species, it requires cutting-edge expertise and relatively high finance to pay the salary of the technician/engineer, the displacements or train students. Moreover, traditional entomologists identifying insect specimens are on the decline (Lee 2000; Hochkirch et al. 2022) and still less academia positions for that kind of research while most of the insect biodiversity must be described or reported (Orr et al. 2020). For example, according to research by Forbes et al. (2018), the order Hymenoptera, which includes bees, wasps, and ants, has the potential to surpass the richness of the Coleoptera order (beetles) by a factor of two. However, it is important to note that the current understanding of Hymenoptera's species diversity is limited due to the presence of many undescribed species, particularly within the Parasitica subgroup of Hymenoptera. Currently, the number of described species in the Hymenoptera order (~153 000 sp.) is approximately 2.2 times less than that of the Coleoptera order (387 000 sp.) (Aberlenc et al. 2021). A study in Sweden, renowned for its insect taxonomy, found that 26% of the country's "true diversity" of insects remains unknown, while 15% are yet to be described as new species (Ronquist et al. 2020). Therefore, the insect taxonomy currently needs of huge investments in money and skilled human means (i.e., in French "jus de cerveau" as J.M. Jancovici says for the climate change mitigations). Indeed, the description or identification of insect species are losing momentum because the reliance of "good research" measures by the Science Citation Index is incompatible with basic taxonomic work as taxonomic paper are not highly cited except for some exceptional taxa (Valdecasas et al. 2000), for example the so-damaging mite for honeybee colonies, *Varroa destructor* (Anderson and Trueman 2000). But, the advent of new identification techniques, which define the modern or integrative taxonomy (Orr 2020), than classical morphological identification emerged and are complementary of classical taxonomy since the last two decades following the common use of molecular technique and the rise of the computer processing speed such as the DNA (meta)barcoding of the specimens (e.g. in Schmidt et al. 2015) or the deep/machine learning methods (e.g. in Spiesman et al. 2021). But, these new techniques can be expensive, unreliable and/or incomplete in certain cases (Sonet et al. 2018).

In order to predict the dynamics of insect pollinators, it is crucial to consider the spatial and temporal fluctuations in their abundance and community composition. Various studies have highlighted the importance of understanding these variations (e.g. in Tylianakis et al. 2005; Petanidou et al. 2008). However, the complex and highly variable nature of ecological data associated poses challenges in assessing their population status. As a result, it becomes difficult to implement proactive management strategies to prevent potential declines, as declines are often recognized only after they have been observed (Murray et al. 2009), which is called the baseline effect by Didham et al. (2020). In Belgium, we have the chance to get access to diverse very useful tools for the distribution and ecological of our wild bees to understand the first layer of their biological conservation through national historical dataset (Rasmont and Haubruge 2002) and red-list at national (Drossart et al. 2019) and regional level

(Vereecken et al. 2022). There are more limitations to get access for Belgian hoverflies and apoid wasps because not such updated dataset exists for their Belgian distribution or the collection data are mainly in their not accessible analogic form (i.e., physical collection). Regarding to this last point, a lot of efforts are done to digitize insect collections and insect sheets in Brussels or in Wallonia. Currently, the insect collection of Gembloux Agro-Bio Tech including more than 3 million of specimen especially Belgian is digitizing using numeric databases at two levels: insect box and insect specimens (Noël et al. 2023).

Preserving insect collections is equally vital as capturing and identifying them (Mouret et al. 2007; Fagot et al. 2022). These collections serve as invaluable traces from the past, providing crucial information for future scientific investigations (e.g., in Fürst et al. 2022 and Zimmermann et al. 2023). Properly stored insect specimens are rich repositories of biodiversity data and historical records, essential for taxonomic research, conservation assessments, and studying climate change impacts (Brown and Paxton 2009). However, ensuring the preservation of these collections requires adequate infrastructures (storage spaces, known climatization, informatic means…) and human resources for their maintenance. Proper curation, cataloging, and conservation efforts are necessary to safeguard these most valuable informations for the benefit of scientific knowledge and ecological understanding.

3. Biodiversity measurement, an evolving field

Even if the first diversity indexes were introduced at the end of the 40's, the field of biodiversity estimates evolved so fast in the last 20 years with the advent of the omics and big data (Kondratyeva et al. 2019). Also, the mathematical concepts were gradually extended and tested as biodiversity became a hot scientific topic with the development of numerical ecology and the power of computer calculations (Legendre and Legendre 2012b). In this thesis, the alpha and beta diversity richness are the lines linking the four field assessments chapters. But species richness estimations were only based on the taxonomic facet of biodiversity while we could have gone deeper in the community structure (especially for **Chapter III & V**) by taking account of the functional and phylogenetic facets (Webb et al. 2002; Devictor et al. 2010; Buchholz and Egerer 2020). The investigation of functional and phylogenetic structure could refine the interaction between the species assemblages and thus provide better recommendations in terms of biological conservation or restoration management (e.g. in Freilich and Connolly 2015; Normandin et al. 2017; Vereecken et al. 2021). In **Chapter IV** and **Chapter V**, we emphasize the importance of considering the functional traits of flower plants to effectively target the needs of insect pollinators (Fornoff et al. 2017). By utilizing *A. mellifera* as a powerful bio-sampler, we can evaluate the local/regional functional community of floral plant (**Figure 7-1**) through the prism of multidimensional spaces for selected functional trait (Villéger et al. 2008; Mouillot et al. 2013). However, it should be noted that we currently lack a comprehensive functional trait database for temperate wild flora, although initiatives like FLORITUDE are emerging to compile functional trait for the entire French flora that are foraged by insect pollinators (Martin et al. 2021). Several of these traits, such as floral shape, floral depth, floral symmetry, and floral color, are particularly relevant to plant-pollinator networks.

Moreover, to meaningfully compare diversity of our modalities (e.g., see **Chapter IV**), we standardized our samples by equalizing the sampling effort. Here, the effort was measured as the time spent with the net to collect samples (pollinator) and the number of pantraps. But species richness is dependent on the number of individuals that contains the sample: if the sample contains more individuals, the number of species also tends to increase, resulting in greater sample diversity (Preston 1948). Thus, the samples containing less individuals were underestimated in terms of true biodiversity (Gotelli and Colwell 2001). Another way to standardize the samples is to compare all the samples or modalities by the smallest number of individuals from a sample having removed the individuals in surplus in the other samples. In this case, we standardized the samples by the sample size, we rarefied all the other samples (rarefaction method). But the rarefaction methods do not account for the relative abundance of each specimen in the more abundant samples (Willis 2019) which provide bias in the richness estimations (McMurdie and Holmes 2014). Rarefaction underestimates diverse communities (Chao and Jost 2012). An elegant method called coverage has been developed to address the limitations of the previous two methods. It aims to estimate the number of species that have not been observed yet. Coverage specifically calculates the proportion of individuals in the entire community that belong to species already detected in the sample. As this proportion increases, it indicates a decrease in the proportion of individuals in the community that belong to species that have not been detected yet (Chao et al. 2014b; Hsieh and Chao 2017; Roswell et al. 2021). Once the coverage approach has been used to standardize the sample richness, Hill's framework (Hill 1973) can be applied to compare the biodiversity. In its work, Segers and colleagues (2023) estimated species richness, Hill–Shannon and Hill–Simpson indexes by standardizing their coverage (i.e., at the lowest coverage sample) to assess the efficiency of different pheromones traps, to kill the weevil pest of field bean crop *Bruchus rufimanus* Boheman 1833, on wild bees and hoverflies as non-target organisms.

We applied some β diversity analysis in all the thesis chapters as complementary approach to evaluate the biodiversity. However, the analysis of community composition variation and turnover is currently booming (Mori et al. 2018) as β diversity provide useful information on the intensity of landscape degradation on overall biodiversity (Tuomisto 2010a; Tscharntke et al. 2012). For instance, in a study by Tylianakis et al. (2005) comparing bee and wasp communities across a land-use gradient, it was observed that diversity was highest in intensively used agroecosystems. On the other hand, this study also showed that plot-level β diversity was significantly greater in less intensively used systems, attributed to higher habitat heterogeneity, and resulting in greater community dissimilarity. It is crucial to exercise caution when examining extremely simplified landscapes, such as large-scale agricultural monocultures or highly urbanized city center such as in **Chapter V** with Tokyo, as they may only support a limited number of surviving populations and exhibit nested, spatially homogenized communities (Tscharntke et al. 2012). In this thesis, other aspect of β diversity could have been analyzed such as the species turnover between communities using Jaccard, Sørensen or Bray-Curtis coefficients to isolate actual gradient(s) that influence the species turnover or our datasets. Other methods such as multivariate dispersion measures or diversity partitioning could have been considered to better assess the species composition structure (Anderson et al. 2011).

4. The particular case of honeybee *Apis mellifera*

The depletion of floral resources through the homogenization of the landscape is one of the most important factors in the weakening of pollinator populations (Potts et al. 2010a; Scheper et al. 2014, 2015). In order to stem the decline of pollinators, many actions have been implemented. Among others, these concern the establishment and multiplication of honeybee colonies in different types of environments (Geldmann and González-Varo 2018). However, numerous publications have shown that high honey bee colony densities can negatively affect wild bee communities via two indirectly interacting ecological mechanisms: competition for food resources and disease transmission (i.e., apparent competition) (Graystock et al. 2016; Herbertsson et al. 2016; Ropars et al. 2019). Studies related to food niche overlaps have been widely used to explain the distribution and use of floral resources by different pollinator species that comprise the community. As a result, food niche overlap is common, particularly for functionally related species (e.g., similar size, similar tongue length) (Goulson and Darvill 2004; Barônio and Torezan-Silingardi 2017). The majority of bee species are generalists and thus can adapt their foraging behavior based on available floral resources to fulfill their primary objective of securing nutrient resources for their offspring. In addition, shared flowers act as a hub for the spread of disease among visiting pollinators (Durrer and Schmid-Hempel 1994; Graystock et al. 2015). Infected bees are likely to deposit parasites and/or pathogens on floral surfaces, including via their contaminated feces or simply by contact (Manley et al. 2015). By visiting flowers, healthy pollinators can contract these diseases via contaminated pollen and/or nectar (Singh et al. 2010). Flower-pollinator interactions thus present themselves as an important route for horizontal disease transmission (McMahon et al. 2018). However, the epidemiology and dynamics of transmission are still poorly characterized and the question of the direction of transmission remains (but see Figueroa et al. 2019). Following the globalization of trade, some of these pathogens belonging to domestic pollinators, notably RNA-strand-positive viruses and microsporidia (of the genus *Nosema*), have seen their prevalence increase, wreaking havoc on wild bee populations, which sometimes have no adapted defense strategies (Ravoet et al. 2014; Cameron et al. 2016).

The foraging behavior of honeybees has been extensively studied in rural areas (Danner et al. 2017; Bänsch et al. 2020), but there is relatively less understanding of their foraging ecology in urban environments (but see in Nagase et al. 2022). Here, the application of pollen metabarcoding provides a rapid and efficient method to generate a large amount of data. It offers valuable insights into the availability of flower plant communities within large cities (Sponsler et al. 2020; Richardson et al.

2021). However, it is important to approach the results with caution due to the semi-quantitative nature of this technique (Kraaijeveld et al. 2014).

The availability of floral resources needs to be sufficient to host both domesticated honeybees and local wild pollinators. The percentage of impervious surfaces plays a major role in the pollination of biodiversity (Geslin et al. 2016). Therefore, populations of managed honeybees must be regulated to ensure wild pollinator populations are not adversely affected (Geslin et al. 2017; Mallinger et al. 2017; Ropars et al. 2019). In contrast, green areas must be managed and well distributed to meet the demands of the pollinator community (Blackmore and Goulson 2014). In summary, well-managed cities could play an active role in the preservation of insect pollinators, and thus provide hotspots for pollination services (Baldock et al. 2019). However, to do so, decision-makers will need to focus on regulating the introduction of honeybees (selection of native bee species, colony density, and control of pathogens and parasites) and on the availability of resources: diminution of the proportion of impermeable surface area, melliferous plant species, green spaces connectivity and landscape diversity (Hennig and Ghazoul 2011; Wang et al. 2021).

The recommendation of this thesis is not to promote the massive and non-oriented implantation of urban honeybee hives especially throughout the prism of plant pollination or urban agriculture but rather to accompany apicultural projects with impact and environmental studies on other insect pollinators.

5. Perspectives

5.1. The future of ecological transition in farmstead

Agroecology and organic farming are recognized to be relevant alternatives to conventional agriculture, which heavily relies on chemical, mechanical, and fossil-based inputs (Wezel et al. 2020). These sustainable farming areas are defined by the use of diverse, heterogeneous landscapes that have a positive impact on the ecological and functional roles of biodiversity within agroecosystems (Altieri 1999). By incorporating on-farm diversification, these agroecosystems provide crucial habitats and resources for pollinators, pest predators, and parasitoid species that rely on floral and nesting resources (Holzschuh et al. 2008; Hatt et al. 2017a, 2018; Guzman et al. 2019a). While these effects are more pronounced at local scales compared to regional scales, both richness and abundance of arthropods show overall increases at both levels in landscape-diversified farms (Lichtenberg et al. 2017). In Wallonia, a significant number of farms have initiated their ecological transition, adopting more sustainable practices to produce food and resources in a more environmentally friendly manner.

Currently, both farmsteads examined in this thesis will continue their ecological transition. For example, since 2021 Froidefontaine farmstead has initiated the conversion of its conventional parcel (referred to as the GC plot in **Chapter III**) into an agroforestry system, which is known to provide benefits in floral and nesting resources for insect pollinators (Staton et al. 2019; Kay et al. 2020). In addition to these on-farm changes, efforts have been made to raise awareness in society. As part of a socio-cultural project, a pollinator trail called "sentier des pollinisateurs" has been established at Froidefontaine farm. This trail features informative posters placed strategically throughout the farm, providing easy-to-understand information about the diversity and biology of recorded pollinators found in the different areas of the farm. This approach aims to communicate and transfer knowledge to the public, fostering a greater understanding and appreciation for pollinators (e.g., Burns et al. 2021). In future, Froidefontaine farmstead might become an observational station of the pollinator community fluctuations after several year of agroecological impact with the same deep experimental protocol.

5.2. Hymenoptera nesting in pavements – What's next?

The technical and scientific outcomes of the STREETBEES project (Chapter VI) opened more questions than it found answers.

5.2.1. Questions related to pollinator species

Among the morphological characteristics that may be influenced by urbanization, the body size has received particular attention. It is an important predictor of foraging and dispersal abilities in wild bees (Greenleaf et al. 2007; López-Uribe et al. 2019). Therefore, fragmentation of the urban matrix may cause an increase in body size as floral resources are sparser, and flight duration and distance must be increased to ensure minimal food resource supply (Merckx et al. 2018; Theodorou et al. 2020a). Nevertheless, as cities are more impervious to pavement (macadam, building...), the ambient temperature compared to the surrounding rural areas may increase through the heat island effect (Manoli et al. 2019). In addition, it has been observed that larger bees are more susceptible to higher ambient air temperatures compared to smaller individuals, primarily due to their lower surface-to-volume ratio (Goulson 2010). Therefore, if temperature is the main factor influencing bee performance, it would be expected that cities, as described by Eggenberger et al. (2019) for *Bombus* spp., would exhibit smaller bee body sizes.

As the STREETBEES project (**Chapter VII**) is the first deep description of their edaphic preferences, it is possible that "pavement" pollinators show a phenotypic plasticity translated by a nesting behavioral adaptation. Concerning the selected soil texture, to our knowledge, no literature reports observations on the similarity or differentiation of the textures used by ground-nesting pollinators to build their nest according to the surrounding matrix (urban versus rural; see **Figure 7-1**). However, Cane (1991) stated that this parameter may be the most important element in the selection of the nesting site for ground-nesting species. Moreover, understanding the genetic basis of local adaptation is fundamental to predicting the evolutionary responses of species to urban change (Rivkin et al. 2019). However, the recent development of "next generation" DNA sequencing technologies has allowed the genome-wide identification of candidate loci involved in the population genetic structure of some wild bee species (Kahnt et al. 2018; Theodorou et al. 2018). To date, there is no consensus on the influence of the city on population genetic traits, although recent studies have shown an association of urbanization with genetic drift or gene flow, highlighting the strong impact of urbanization on the non-adaptive evolution of urban populations (Theodorou et al. 2018; Miles et al. 2019). Therefore, including analyses from distinct populations of ground-nesting pollinator will add new elements to the understanding of their urban adaptations. Lastly, many multi-scalar landscape characteristics such as landscape heterogeneity, habitat type, floral resource richness and abundance influence pollinator community structure (Wenzel et al. 2020).

Therefore, it could be interesting to study the influence of urban environments on the eco-evolutive dynamics of targeted ground-nesting pollinators such as bee species *Dasypoda hirtipes* (Fabricius 1793) or *Andrena barbilabris* (Kirby 1802). Why and how they change their strategy behavior nesting into the soil of natural spaces to highly urbanized pavement? A new research project would investigate the differences between populations nesting in pavements and populations nesting in natural soil regarding: (i) individual size, (ii) nesting behavior, and (iii) genetic traits of the populations. Additionally, the study would explore (iv) the potential relationship between these traits and the surrounding matrix by comparing urban populations nesting between pavements and rural populations at regional scale.

5.2.2. Questions related to the urban pavements

While our study case focuses on a single year of sampling in one city, STREETBEES project was the first study deeply dealing with this issue at hand and can serve as a valuable starting point for future research in the rapidly evolving field of urban ecology (Rega-Brodsky et al. 2022; Lokatis et al. 2023). The methodology employed in our study can be readily extended to other Belgian cities like Antwerpen or Liège, as well as European cities such as Paris or Berlin. Building upon the recommendations presented in **Figure 6-7**, it would be beneficial to conduct field tests on prototype pavement structures in Brussels. However, this endeavor would require the support of environmental and politic institutions and the full cooperation of municipalities and other stakeholders to successfully design innovative pavement solutions during renovation projects. Indeed, Deparis and colleagues (2023) showed that all hierarchical levels of municipal operative system must be engaged to favor spontaneous vegetation in French medium-sized city. Moreover, applying sensibilization to the city dwellers related to the insect pollinators inhabiting the pavement would enhance the integration of people-nature relationship in city like Brussels.

Nevertheless, it is possible that Brussels Region redesign ancient pavement based on asphalting hard surfaces as this is cheaper for private construction managers (J. Ruelle, personal communication). Thus, we need to claim at the policy level against these practices as they are incompatible with the living biodiversity, flora and fauna, up and under the streets (Uchida et al. 2014; Pescott 2016; Bonthoux et al. 2019b; Dijon et al. 2023). As the surface of Brussels pavement is 1,400 000m² (E. Flamée, personal communication), the impact of pavement (re)conversion to host biodiversity mitigate negative effects of urbanization process could be considerable. In future research, it is crucial to explore the integration of fauna and flora in the design of new pavements, especially considering the potential benefits of permeable pavement in enhancing environmental conditions (Ferguson 2012). With the emergence of numerous Nature-based Solutions projects (e.g. in Cortinovis et al. 2022; Epelde et al. 2022) and the positive perception of vegetated streets by the public (Bonthoux et al. 2019a), it becomes imperative to investigate how incorporating fauna and flora can contribute to sustainable pavement design.

5.3. The other honeybee species

The Western honeybee, *A. mellifera,* is not the unique species of the genus *Apis*. The subgenera of all these species are differentiated by the size: *Megapis*, *Apis* and *Micrapis*. They are all polylectic and eusocial pollinator (Hepburn and Radloff 2011a, b). There are six other species than *A. mellifera* which are recognized by Pauly (2015): *Apis (Apis) cerana* Fabricius 1793, *Apis (Apis) koschevnikovi* Enderlein 1906, *Apis (Apis) nigrocincta* Smith 1861, *Apis (Megapis) dorsata* Cockerell 1906, *Apis (Micrapis) florea* Fabricius 1787 and *Apis (Micrapis) andreniformis* Smith 1858. As *A. mellifera,* other medium and large size *Apis* species focus on large available floral resources in the environment (Roubik 1989). But the foraging ecology and visited plant species are poorly documented for the other *Apis* species especially in disturbed landscapes (Corlett 2011). In the context of cityscape, it could be interesting to use metabarcoding of floral matrix such pollen or honey to understand the floral overlapping of massively introduced Western honeybee in Japan against the natively Japanese honeybee *A. cerana* (Fabricius 1793) and evaluate the impact of surrounding landscape on the generated overlapping coefficients (Namin et al. 2022).

6. Final conclusion

This thesis is in line with the times, as it addresses the pressing need for high-quality data on organism biodiversity, which its decline is a significant threat to humanity. The heterogeneity of the landscapes, where the spatial connectivity of the different land use is ideally configured, reaches the most optimal outcome in maintaining the biodiversity of insect pollinators in urban or agricultural matrices. This thesis primarily focused on three taxonomic groups of insect pollinators: bees, hoverflies, and digger wasps. However, there are many other groups, including some other Diptera families (Tachinidae, Calliphoridae…), some Coleoptera families (Cantharidae, Scarabaeidae…), and Lepidoptera (some Heterocera and Rhopalocera), that also play significant roles as pollinators. They need to be considered in more extensive studies. Even in Wallonia or Belgium, there is a lot of work to map, date, and record regional species in numeric databases throughout new sampling campaign or the conversion of historical collections in numeric data. Establishing national and regional insect databases or local inventories will help us to articulate more detailed scenario to protect and maintain insect populations as well as mobilizing the policy makers and other societal stakeholders.

However, it is important to note that the relationship between landscape complexity and common diversity components, such as species richness, is not always linear. To gain a more comprehensive understanding of biodiversity patterns, it is valuable to invest time in conducting in-depth analyses that consider additional measures, such as β diversity. This will refine our understanding of biodiversity patterns driven by the underlying ecological processes at play in the environment (e.g., environmental filter). However, as the analysis becomes more complex, it poses challenges in effectively communicating new knowledge to the political world and society at large. Therefore, there is an urgent need to engage various resources in popularizing and disseminating knowledge to the public. These efforts are crucial in ensuring the successful implementation of ecological transition in cities and agricultural areas. By actively engaging and informing the public, we can equip them with the necessary tools to contribute to this transition and foster a sustainable future.

Chapter 8

Scientific communications

1. Scientific communications related to the thesis

1.1. Scientific publications

Amy, C., **Noël, G.**, Hatt, S., Uyttenbroeck, R., Van de Meutter, F., Genoud, D., & Francis, F. (04 September 2018). Flower strips in wheat Intercropping system: effect on pollinator abundance and diversity in Belgium. *Insects, 9 (3), 114*. doi:10.3390/insects9030114 https://hdl.handle.net/2268/228310

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Under revision:

Noël, G., Van Keymeulen, V., Barbier, Y., Smets, S., Van Damme, O., Colinet, G., Lokatis, S., Ruelle, J., & Fancis, F.. Nest aggregations of wild bees and apoid wasps in urban pavements: a "street life" to be promoted in urban planning. In minor revision in *Insect Diversity and Conservation Journal* under the special issue of **urban entomology**.

1.2. International/national conference

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Noël, G., Serteyn, L., Calderan, A., de Liedekerke, A., Dufrêne, M., & Francis, F. (2018). *Assessment of pollination ecosystem service throughout wild bees biodiversity and associated phytometer experiment – a case study at farmstead Froidefontaine in ecological transition (Havelange, Belgium).* In ZOOLOGY 2018 - Zoology in the Anthropocene 13-15. https://hdl.handle.net/2268/231648

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Nagase, A., Pouilloux, L., Francis, F., & **Noël, G.** (15 December 2021). *Investigation of Apis mellifera L. foraging behaviour to promote pollinator-friendly urban cities in Japan.* Paper presented at VIII International Conference on Landscape and Urban Horticulture, Catania, Italy. https://hdl.handle.net/2268/263575

1.3. Technical reports

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2. Scientific communications not related to the thesis

2.1. Scientific publications

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Chapter 9

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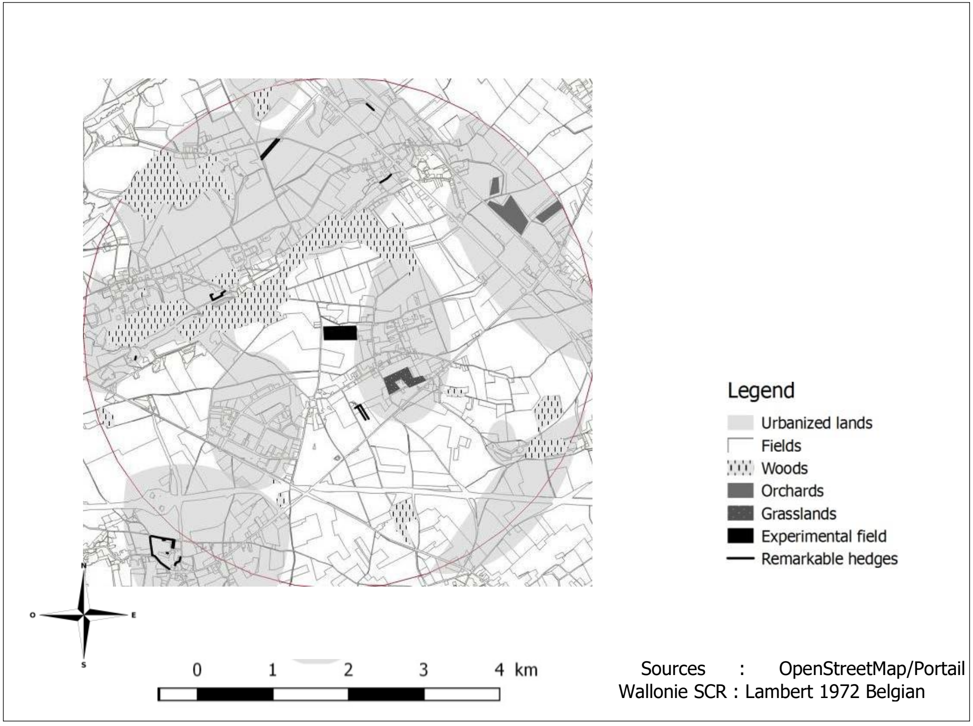
Chapter 10

Appendixes

1. Chapter IV

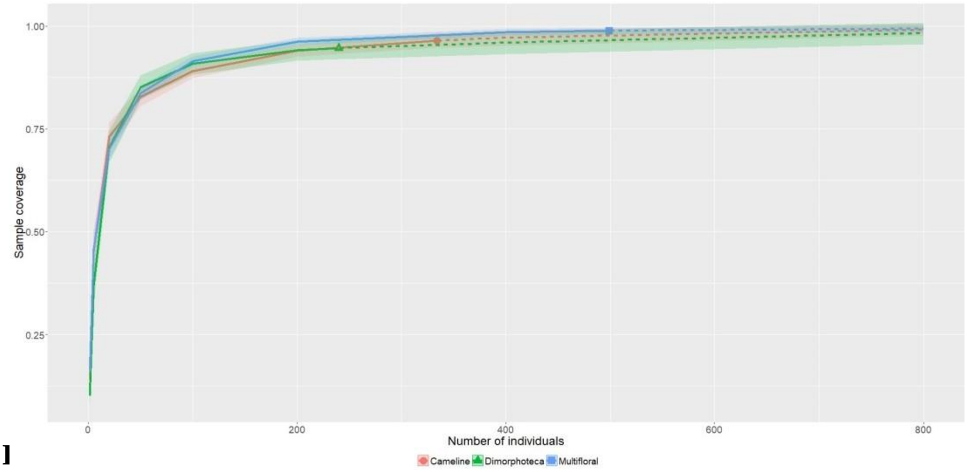
Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Floral mixtures of the three treatments, Table S2: Species scores of the PCOA, Figure S1: Mapping of the landscape around the experimental field on a radius of 3 km, Figure S2: Species accumulation curves based on abundance data of hoverflies and bees together (A), bees alone (B) and hoverflies alone (C), Figure S3: Number of floral units per floral species in the quadrats of multifloral flower strips, Figure S4: Mapping of the Andrena nitidiuscula distribution in Belgium since 1929 (Source: Rasmont (2017); Atlas Hymenoptera).



Sources : OpenStreetMap/Portail Wallonie SCR : Lambert 1972 Belgian

Figure 4-S1 Mapping of the landscape around the experimental field on a radius of 3 km

A


1.00
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Sample coverage
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Number of individuals
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Dimorphoteca
Multifloral

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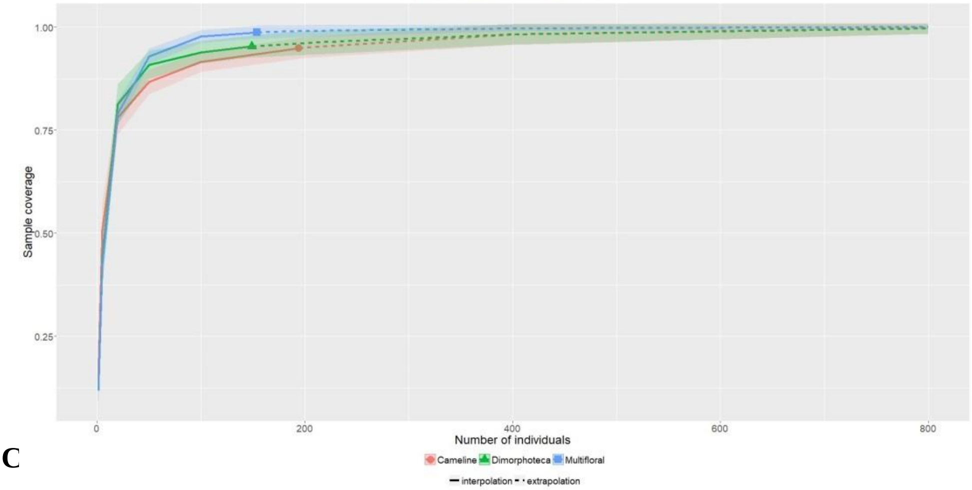
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Number of individuals
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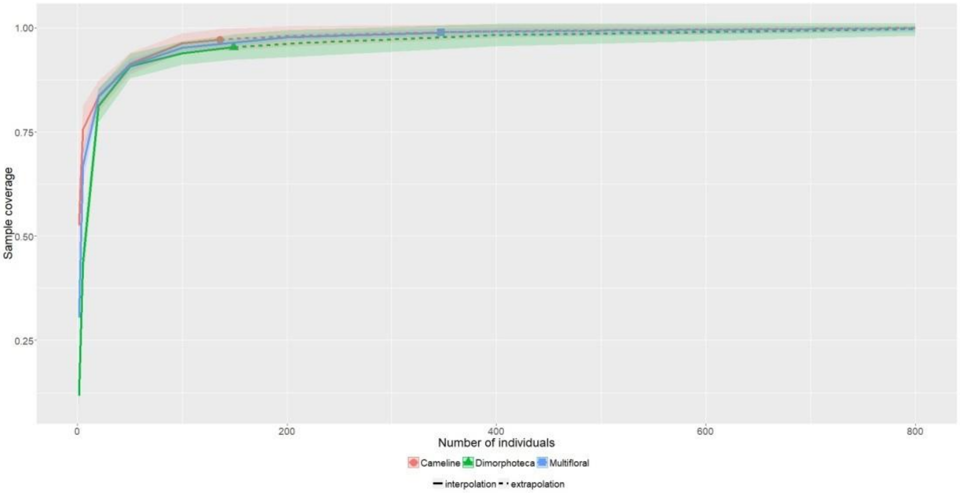
Dimorphoteca
Multifloral
interpolation
extrapolation

C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Number of individuals
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Multifloral
interpolation
extrapolation

Figure 4-S2 Species accumulation curves based on abundance data of hoverflies and bees together (A), bees alone (B) and hoverflies alone (C)

Table 4-S1 Floral mixtures of the three treatments

Treatment 1	**Kind Species**	**Phenology**	**Weight (g)/ha**	**g/16m²**
Floral mixture				
Wild carrot	*Daucus carota*	Biennial	175	0,28
Evening primrose	*Oenothera biennis*	Biennial	70	0,112
Viperine	*Echium vulgare*	Biennial	507,5	0,812
Coriander	*Coriandrum sativum*	Annual	1400	2,24
Buckwheat	*Fagopyrum esculentum*	Annual	1750	2,8
Chrysanthemum of the harvest	*Glebionis segetum*	Annual	157,5	0,252
Whit mate	*Silene latifolia alba*	Sustainable	140	0,224
Mallow	*Malva moschata*	Sustainable	350	0,56
Pyrenees Geranium	*Geranium pyrenaicum*	Sustainable	350	0,56
		Total	**4900**	**7,84**
Background cover				
Crimson clover	*Trifolium incarnatum*		100	0,16
White clover	*Trifolium repens*		100	0,16
		Total	**5000**	**8**
Treatment 2				
Dimorphoteca	*Dimorphoteca pluvialis*		5000	8
Treatment 3				
Camelina	*Camelina sativa*		5000	8

Table 4-S2 Species scores of the PCOA

1	*Andrena carantonica*	32	*Sphecodes ephippius*
2	*Andrena chrysosceles*	33	*Sphecodes monilicornis*
3	*Andrena cineraria*	34	*Episyrphus balteatus*
4	*Andrena dorsata*	35	*Eristalis arbustorum*
5	*Andrena flavipes*	36	*Eristalis similis*
6	*Andrena gravida*	37	*Eristalis tenax*
7	*Andrena haemorrhoa*	38	*Eumerus strigatus*
8	*Andrena humilis*	39	*Eupeodes corolla*
9	*Andrena minutula*	40	*Eupeodes latifasciatus*
10	*Andrena minutuloides*	41	*Eupeodes luniger*
11	*Andrena nigroaenea*	42	*Halictus maculatus*
12	*Andrena nigroena*	43	*Halictus rubicundus*
13	*Andrena nitida*	44	*Halictus scabiosae*
14	*Apis mellifera*	45	*Lasioglossum calceatum*
15	*Bombus hypnorum*	46	*Lasioglossum fulvicorne*
16	*Bombus lapidarius*	47	*Lasioglossum laticeps*
17	*Bombus lucorum*	48	*Lasioglossum leucozonium*
18	*Bombus pascuorum*	49	*Lasioglossum malachurum*
19	*Bombus pratorum*	50	*Lasioglossum minutissimum*
20	*Bombus terrestris*	51	*Lasioglossum morio*
21	*Bombus vestalis*	52	*Lasioglossum nitidiusculum*
22	*Melanostoma mellinum*	53	*Lasioglossum pauxillum*
23	*Nomada fabriciana*	54	*Lasioglossum villosulum*
24	*Oxybelus sp*	55	*Lindenius sp*
25	*Platycheirus clypeatus*	56	*Syritta pipiens*
26	*Bombus sylvestris*	57	*Syrphus ribesii*
27	*Scaeva selenitica*	58	*Syrphus vitripennis*
28	*Seladonia tumulorum*	59	*Lasioglossum nitidulum*
29	*Sphaerophoria rueppelli*	60	*Scaeva pyrastri*
30	*Sphaerophoria scripta*	61	*Hyaleus sp*

31	*Sphaerophoria taeniata*	

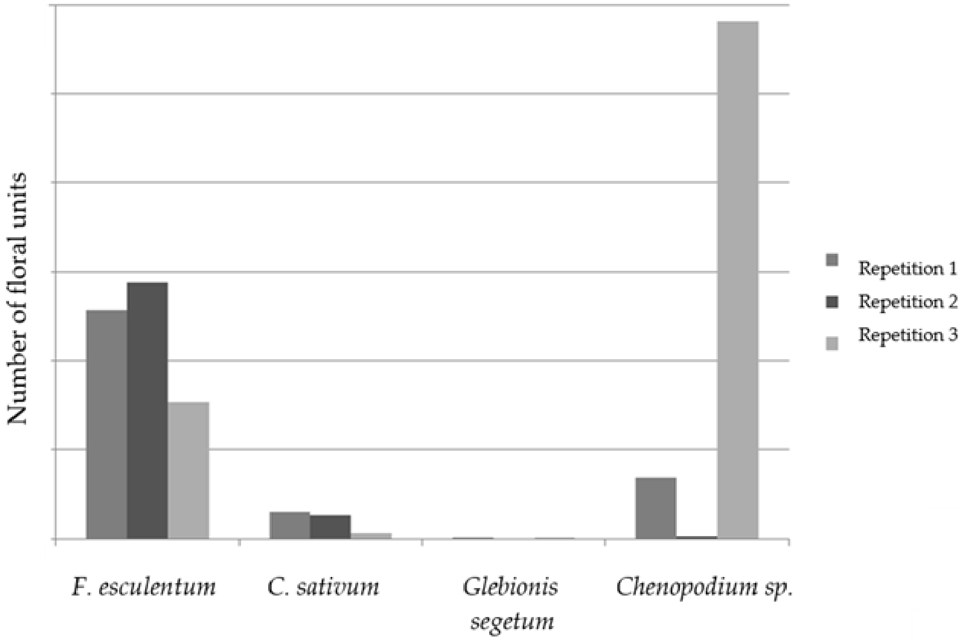
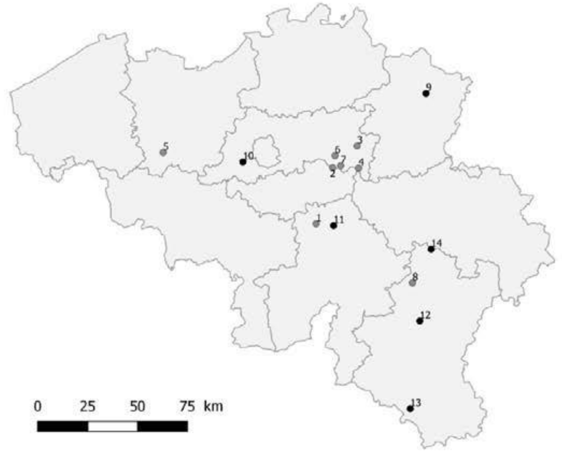


Figure 4-S3 Number of floral units per floral species in the quadrats of multifloral flower strips



N°	Year	Determiner	Collector	Data source
1	2017	Genoud D.	Amy C.	Faculty of Gembloux Agro-Bio-Tech
2	2016	D'Haeseleer J.	-	Waarneming
3	2016	De Grave D.	-	Waarneming
4	2016	D'Haeseleer J.	-	Waarneming
5	2015	Janssen K.	-	Waarneming
6	2014	Vanormelingen P.	-	Waarneming
7	2012	Vanormelingen P.	-	Waarneming
8	2007	Patiny S.	UMH	BDFGM_MONS
9	1989	Warncke K	-	BDFGM_Mons
10	1989	Warncke K	-	BDFGM_Mons
11	1989	Warncke K	-	BDFGM_Mons
12	1989	Warncke K	-	BDFGM_Mons
13	1989	Warncke K	-	BDFGM_Mons
14	1929	Leclercq J	LIEGE	BDFGM_GX

Legend

1929< ● <2000< ● <2018

Figure 4-S4 Mapping of the *Andrena nitidiuscula* repartition in Belgium since 1929 (Source: Rasmont (2017); Atlas hymenoptera)

2. Chapter V

Table 5-S1. Climatic data of the study sites

Location	Prefecture	Elevation [m]	Mean Temperature [°C]	Precipitation [mm]
Enokisawa	Chiba	14	15.6	1428
Kuwata	Chiba	23	15.6	1428
Yachiyo	Chiba	31	14.9	1394
Ichihara	Chiba	36	15.5	1550
Nerima	Tokyo	38	15.1	1448
Shiba	Tokyo	6	15.4	1442
Togo	Tokyo	29	15.4	1442
Colombin	Tokyo	24	15.4	1442
Shinjyuku	Tokyo	32	15.4	1442
Toyosu	Tokyo	6	15.4	1442
Yamatecho	Kanagawa	23	15.6	1554
Ishikawacho	Kanagawa	29	14.7	1488
Gumyoji	Kanagawa	13	15.6	1554
Honmoku	Kanagawa	12	15.6	1554
Nishichiba	Chiba	17	15.3	1435
Kashiwanoha	Chiba	19	14.7	1358
Inohana	Chiba	17	15.3	1435

Table 5-S2. Details of the pollen sample collection

Sample name	Site	Season	Duration of sampling in day (hours)	Date	Number of reads
A01	Enokisawa	Spring	10	23 April 2019	27162
A02	Enokisawa	Spring	9	15 May 2019	17449
A03	Enokisawa	Summer	7	14 June 2019	36532
A04	Enokisawa	Summer	9	25 July 2019	38620
A05	Enokisawa	Summer	9,5	21 August 2019	28147
A06	Enokisawa	Autumn	8	19 September 2019	37096
A07	Enokisawa	Spring	8,5	23 April 2019	44486
A08	Enokisawa	Spring	9	15 May 2019	34536
A09	Enokisawa	Summer	9	14 June 2019	38232
A10	Enokisawa	Summer	9	25 July 2019	39107
A11	Kuwata	Spring	8,5	23 April 2019	3031
A12	Kuwata	Spring	9,25	15 May 2019	38122
A13	Kuwata	Summer	7,6	14 June 2019	44687

A14	Yachiyo	Spring	9	16 April 2019	24841
A15	Yachiyo	Spring	9	22 May 2019	29450
A16	Yachiyo	Summer	9	25 June 2019	36628
A17	Yachiyo	Summer	9	31 July 2019	30570
A18	Yachiyo	Summer	9	31 August 2019	30528
A19	Ichihara	Spring	9	23 April 2019	14947
A20	Ichihara	Spring	8,17	30 May 2019	38302
A21	Ichihara	Summer	4	18 June 2019	33007
A22	Ichihara	Summer	8	26 July 2019	33024
A23	Ichihara	Summer	3,5	30 July 2019	29326
A24	Ichihara	Summer	9	26 August 2019	49940
A25	Ichihara	Autumn	6,5	20 September 2019	37844
A26	Nerima	Spring	9	17 April 2019	28230
A27	Nerima	Spring	2,25	13 May 2019	25196
A28	Nerima	Summer	2,5	8 June 2019	44719
A29	Nerima	Summer	2,7	8 July 2019	36120
A30	Nerima	Summer	7,42	11 August 2019	38379
A31	Nerima	Autumn	5,25	15 September 2019	52069
A32	Shiba	Spring	24	22 April 2019	41195
A33	Shiba	Spring	46	7 May 2019	52261
A34	Shiba	Spring	23,25	15 May 2019	36055
A35	Shiba	Spring	24	25 May 2019	61431
A36	Shiba	Summer	22	5 June 2019	51687
A37	Shiba	Summer	24	19 June 2019	60453
A38	Shiba	Summer	25	26 June 2019	56414
A39	Shiba	Summer	23	10 July 2019	42158
A40	Shiba	Summer	21	24 July 2019	30333
A41	Shiba	Summer	22	31 July 2019	30568
A42	Shiba	Summer	22	7 August 2019	34332
A43	Shiba	Summer	21	28 August 2019	39619
A44	Shiba	Autumn	21	4 September 2019	36774

A45	Togo	Spring	13	29 April 2019	15269
A46	Togo	Spring	11	26 May 2019	3787
A47	Togo	Summer	8	16 June 2019	39939
A48	Togo	Summer	9	25 July 2019	50185
A49	Togo	Summer	10	25 August 2019	35302
A50	Togo	Autumn	10	20 September 2019	52669
A51	Colombin	Spring	9	16 April 2019	40372
A52	Colombin	Spring	9	17 May 2019	19450
A53	Colombin	Summer	9	18 June 2019	48985
A54	Colombin	Summer	9,5	17 July 2019	44909
A55	Colombin	Summer	9	20 August 2019	21298
A56	Colombin	Autumn	9	24 September 2019	36168
A57	Shinjyuku	Spring	9,17	18 April 2019	17229
A58	Shinjyuku	Spring	9	15 May 2019	10698
A59	Shinjyuku	Summer	9,08	19 June 2019	53316
A60	Shinjyuku	Summer	8,92	17 July 2019	47707
A61	Shinjyuku	Autumn	9,08	17 September 2019	41739
A62	Toyosu	Spring	8	22 April 2019	46494
A63	Toyosu	Spring	6,5	27 May 2019	43642
A64	Toyosu	Summer	29	18 July 2019	12996
A65	Yamatecho	Spring	8,75	15 April 2019	38172
A66	Yamatecho	Spring	9	17 May 2019	35457
A67	Yamatecho	Summer	11	26 June 2019	42468
A68	Yamatecho	Summer	8	26 July 2019	41181
A69	Yamatecho	Summer	7,5	9 August 2019	32438
A70	Yamatecho	Autumn	8	11 September 2019	45092
A71	Ishikawacho	Spring	70,17	20 April 2019	36329
A72	Ishikawacho	Spring	24	31 May 2019	23883
A73	Ishikawacho	Summer	48	17 June 2019	42006
A74	Ishikawacho	Summer	24	9 August 2019	50688
A75	Ishikawacho	Summer	25	23 August 2019	32507

A76	Ishikawacho	Autumn	53	5 September 2019	34608
A77	Ishikawacho	Autumn	55	20 September 2019	44411
A78	Gumyoji	Spring	42	19 April 2019	42933
A79	Gumyoji	Spring	47	27 April 2019	20266
A80	Gumyoji	Spring	27	10 May 2019	29566
A81	Gumyoji	Spring	26	17 May 2019	27689
A82	Gumyoji	Spring	26	24 May 2019	31583
A83	Gumyoji	Spring	26	31 May 2019	30013
A84	Gumyoji	Summer	26	30 August 2019	29781
A85	Gumyoji	Autumn	26	16 September 2019	3083
A86	Gumyoji	Autumn	55	28 September 2019	36072
A87	Honmoku	Spring	9	2 May 2019	6329
A88	Honmoku	Spring	9	8 May 2019	44052
A89	Honmoku	Spring	9	15 May 2019	25479
A90	Honmoku	Spring	9	24 May 2019	24244
A91	Honmoku	Summer	9	5 June 2019	46150
A92	Honmoku	Summer	8	13 June 2019	47213
A93	Honmoku	Summer	9	26 June 2019	33434
A94	Honmoku	Summer	9	3 July 2019	32991
A95	Honmoku	Summer	9	10 July 2019	21744
A96	Honmoku	Summer	9	30 July 2019	17047
A97	Honmoku	Autumn	9	7 September 2019	41125
A98	Honmoku	Autumn	9	25 September 2019	35486
A99	Nishichiba	Spring	#N/A	March 2019	63254
A100	Nishichiba	Spring	#N/A	March 2019	91542
A101	Nishichiba	Spring	#N/A	March 2019	60283
A102	Nishichiba	Spring	#N/A	March 2019	77721
A103	Nishichiba	Spring	#N/A	March 2019	120804
A104	Nishichiba	Spring	#N/A	April 2019	84532
A105	Nishichiba	Spring	#N/A	April 2019	50209
A106	Nishichiba	Spring	#N/A	April 2019	73426

A107	Nishichiba	Spring	#N/A	April 2019	105278
A108	Nishichiba	Spring	#N/A	May 2019	93263
A109	Nishichiba	Spring	#N/A	May 2019	38892
A110	Nishichiba	Summer	14	12 June 2019	38889
A111	Nishichiba	Summer	17	13 July 2019	39870
A112	Nishichiba	Summer	13	17 August 2019	31579
A113	Nishichiba	Autumn	25	18 September 2019	39989
A114	Nishichiba	Spring	#N/A	15 March 2019	105717
A115	Kashiwanoha	Spring	#N/A	15 March 2019	118884
A116	Kashiwanoha	Spring	#N/A	15 March 2019	97738
A117	Kashiwanoha	Spring	#N/A	15 March 2019	88100
A118	Kashiwanoha	Spring	#N/A	15 March 2019	116558
A119	Kashiwanoha	Spring	#N/A	15 March 2019	90033
A120	Kashiwanoha	Spring	#N/A	20 April 2019	107139
A121	Kashiwanoha	Spring	#N/A	20 April 2019	103150
A122	Kashiwanoha	Spring	#N/A	20 April 2019	119990
A123	Kashiwanoha	Spring	#N/A	20 April 2019	67123
A124	Kashiwanoha	Spring	#N/A	May 2019	68620
A125	Kashiwanoha	Spring	#N/A	May 2019	67740
A126	Kashiwanoha	Summer	12	June 2019	33353
A127	Kashiwanoha	Summer	13	July 2019	35435
A128	Kashiwanoha	Summer	16	August 2019	43975
A129	Kashiwanoha	Autumn	3	September 2019	39363
A130	Kashiwanoha	Spring	#N/A	March 2019	108066
A131	Inohana	Spring	#N/A	March 2019	99031
A132	Inohana	Spring	#N/A	March 2019	83932
A133	Inohana	Spring	#N/A	April 2019	84692
A134	Inohana	Spring	#N/A	April 2019	85468
A135	Inohana	Spring	#N/A	April 2019	56590
A136	Inohana	Spring	#N/A	April 2019	60466
A137	Inohana	Spring	#N/A	May 2019	98072

A138	Inohana	Spring	#N/A	May 2019	118489
A139	Inohana	Summer	11	June 2019	45067
A140	Inohana	Summer	17	July 2019	46397
A141	Inohana	Summer	14	August 2019	34237
A142	Inohana	Autumn	9	September 2019	7036
A143	Inohana	Spring	#N/A	March 2019	138045

Table 5-S3. Mean and standard deviation of variables among the landscape classes. The units of landscape variables are given in square brackets. The standard deviation is provided after the mean.

Cluster	**dpop [in./km^2]**	**IIC [-]**	**MESH [ha]**	**NDVI median [-]**	**SHEI [-]**	**veg cover [%]**	**Pd [nbr/km^2]**
rural	398 (291)	0.3533 (0.0366)	0.49 (0.05)	2414.8 (904.9)	0.93 (0.05)	0.67 (0.05)	0.000044 (0.00004)
suburban	6475 (4839)	0.0572 (0.0342)	0.48 (0.05)	186.3 (122.9)	0.93 (0.1)	0.56 (0.08)	0.000117 (0.00003)
urban	10741 (2104)	0.0023 (0.0021)	0.32 (0.04)	2.7 (1.3)	0.51 (0.12)	0.27 (0.08)	0.000276 (0.00003)
urban centre	16532 (1499)	0.0009 (0.0006)	0.32 (0.03)	1.4 (1.1)	0.38 (0.14)	0.19 (0.07)	0.000437 (0.00010)

Table 5-S4 Details of primer used in this study. Underline indicates the Illumina sequencing primer region. Ns are for improving the Illumina sequencing quality. Bold indicates the plant specific primer sequence for ITS1 (Masamura et al., 2014). The 2ndF and 2ndR consist of the Illumina P5 or P7 flow cell binding sequence, index sequence, and sequencing primer sequence, respectively.

1st PCR	
1st-18S-MiseqF	5’-ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNN**GACGTCGCGAGAAGTCCAYTG**-3’
1st-5.8S-MiseqR	5’-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNN**TCGCATTTCGCTACGTTCTTCATCG**-3’
2nd PCR	
2ndF	5’-AATGATACGGCGACCACCGAGATCTACAC-Index2-ACACTCTTTCCCTACACGACGC-3’
2ndR	5’-CAAGCAGAAGACGGCATACGAGAT-Index1-GTGACTGGAGTTCAGACGTGTG-3’

Figure 5-S1. Pollen traps. (A) Pollen trap set-up at the entrance of the selected hive in Nishi-Chiba campus of the Chiba University, 25 March 2020. (B) Pollen trap close-up with pollen balls collected in the trail in Nishi-Chiba campus of the Chiba University, 17 June 2020.

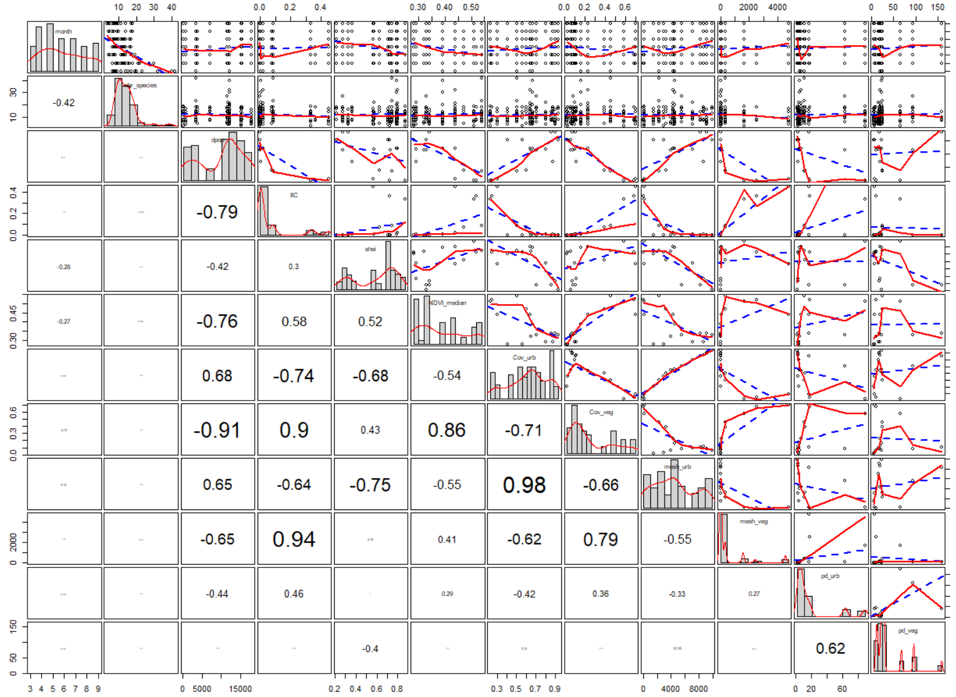


Figure 5-S2. Collinearity graphic. The landscape variables are represented by month, number of species (nbr_species), number of inhabitants per km² (dpop), the integral index of connectivity (IIC), the effective mesh size for vegetation (mesh_veg) and urban areas (mesh_urb), Shannon's evenness index (SHEI), the vegetation (Cov_veg) and urban (Cov_urb) cover proportion, the vegetation (pd_veg) and urban (pd_urb) patch density, and the median vegetation class NDVI (NDVI median)

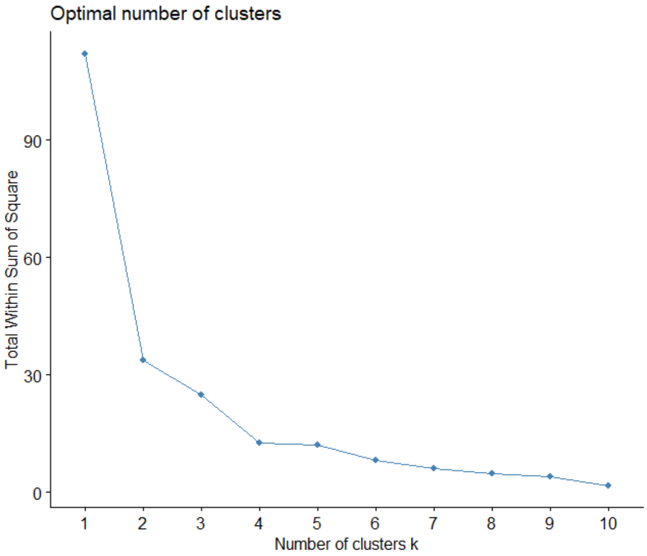


Figure 5-S3. Elbow clustering plot. Elbow method plotting the total within sum of square explained in function of the number of k clusters. The elbow of the curve suggests the number of groups to retain for k-means clustering analysis.

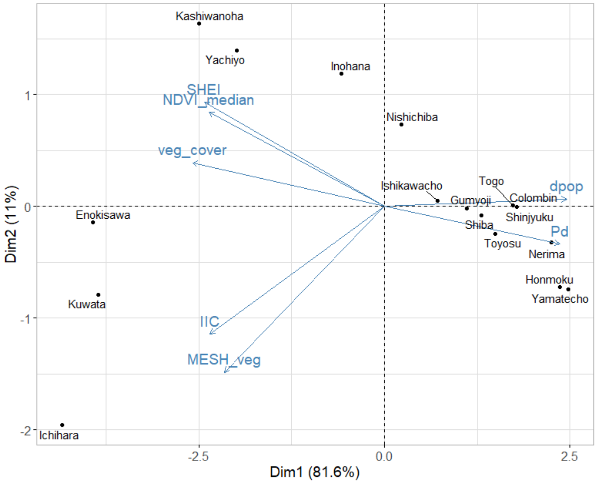


Figure 5-S4. Principal component analysis (PCA) biplot of individual sites and landscape variables. The landscape variables (in blue) are Pd: Patch density [patches/km²], dpop: demographic density (number of inhabitant/km²), MESH_veg: effective mesh size of vegetation [-], IIC: Integral index of connectivity [-], veg_cover: vegetation cover (%), NDVI_median: median of the NDVI of the cells superior to 0.2 [-] and SHEI: Shannons's evenness index [-]

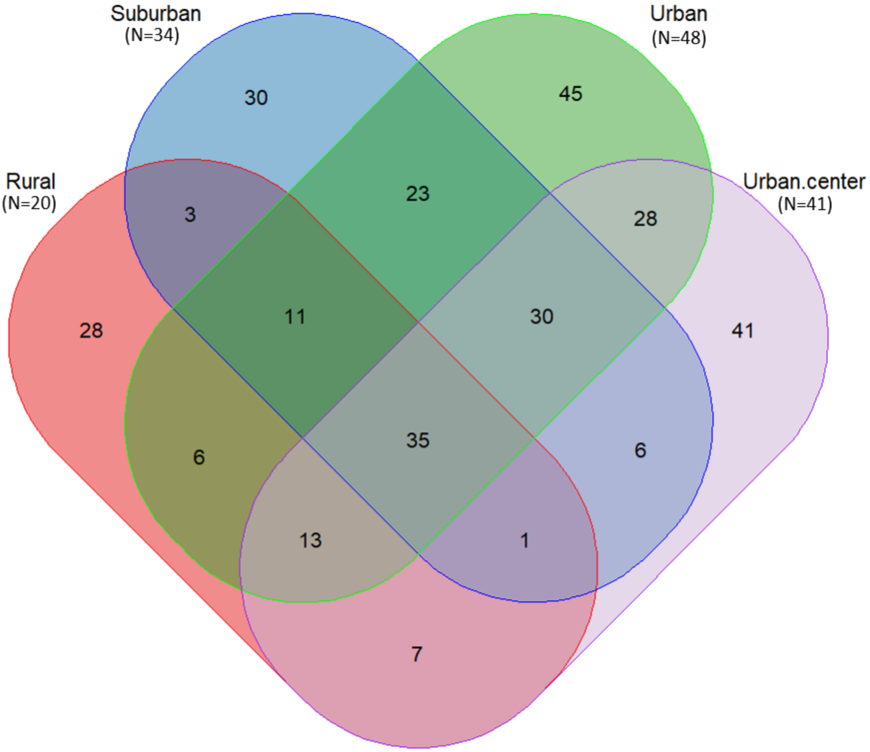


Figure 5-S5. Venn diagram Venn diagram indicating the overlap of plant taxa according to the different landscape classes. "*N*" corresponds to the number of samples per landscape.

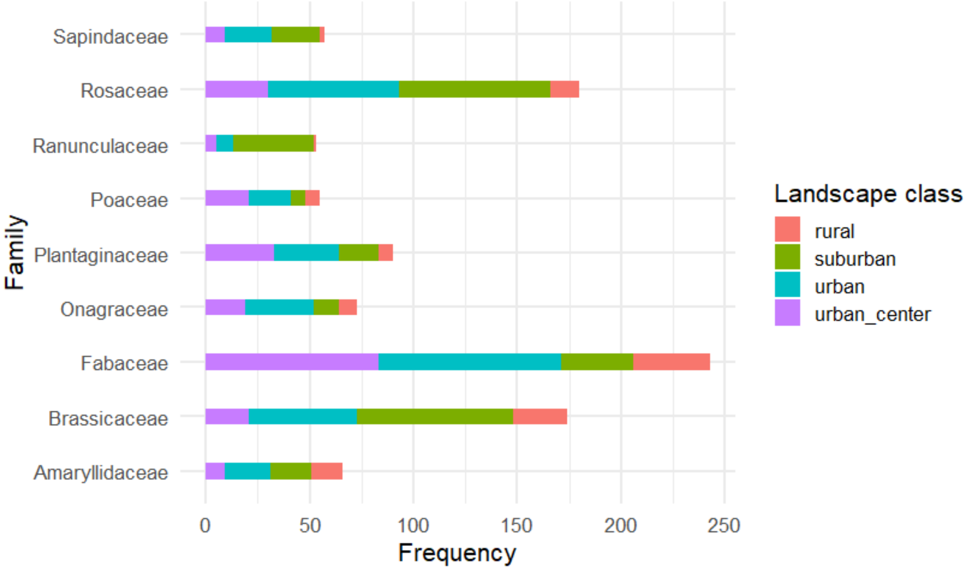


Figure 5-S6. Bar plot showing the 9 most frequent family observed in the samples in contrast with the landscape classes. Color proportions correspond to the landscape classes.

3. Chapter VI

Supplementary information 1:

Supplementary soil texture material & method

We dismantled several modular elements from pavement structure, assessed the nature of the laying course and, where possible, excavated its foundation to determine its nature and thickness, and searched for nest structures. A 250-300g sample of sand from two sites was taken for grain size determination.

The two sand samples taken on site were sieved in BRRC's laboratories according to the requirements of NBN EN 933-1 and 2, applied in road engineering. A dry sieving was carried out. The opening of the sieves was chosen to be as close as possible to the sieves applied in pedology, which are slightly different from those applied in road engineering. Thus the 100µm sieve was replaced by the 90µm sieve and the 50µm sieve by the 63µm sieve.

Results of the excavation

1) Description of the first excavation (concrete slabs in Watermael-Boitsfort, Bruxelles):

The layer course consisted of a thick layer of mortar, contrary to what was expected in the presence of sandy joints (sometimes mixed with mortar). The removal of the pavement was limited to one slab in order not to destabilize the structure. As a result, it was not possible to excavate the foundation to determine its thickness. The concrete slabs (30 x 30cm) probably laid in a full mortar bath, show joints filled with mortar (or damaged) or sand.

The joint width was very irregular and ranged between 1 and 15mm (average 8mm). The thickness of the mortar layer was irregular at 3-4cm. We observed a layer of yellow sand (foundation or laying course).

First excavation (Watermael-Boitsfort, Bruxelles) - Left: Removal of the concrete slab. Right: Concrete slab and profile mortar thickness. Images by Sylvie Smets

2) Description of the second excavation (sandstone setts in Etterbeek, Bruxelles):

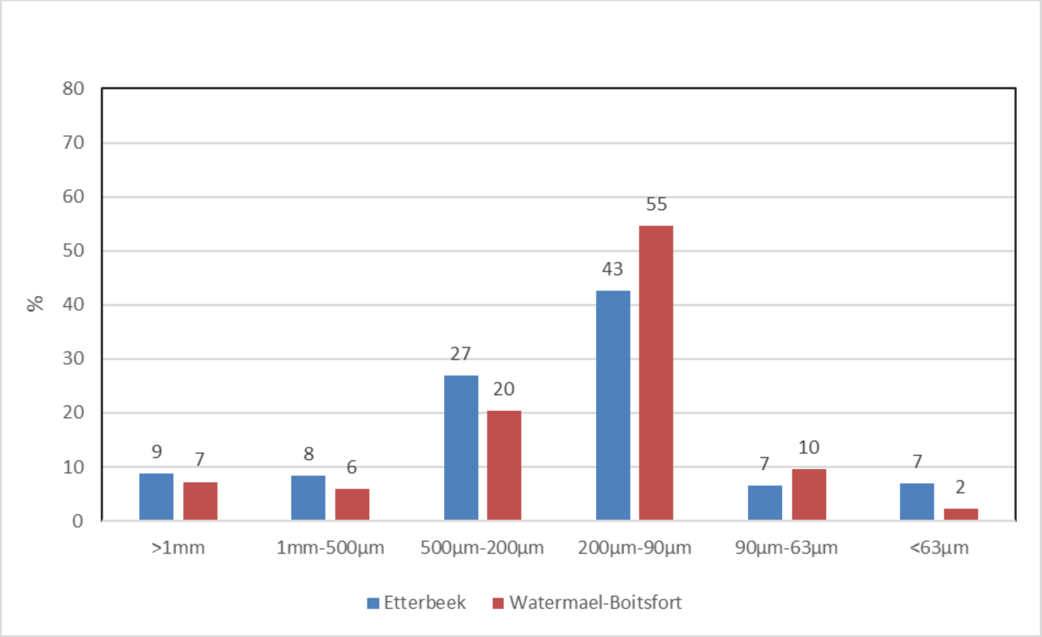
The sandstone setts (12cm * 12cm) have a thickness of 9cm and are jointed with sand. The width of the jointing was on average 5mm. The laying course with a thickness of few centimeters is composed of yellow sand. The limit between the laying course and the basis was unclear. The foundation is composed of aggregates with some brick debris, with 35cm thick, including the laying course. The sub-base or soil was composed of indurated material and could not be identified.

We also looked for nesting structures below the pavement tiles. We found two living individuals of *Andrena barbilabris* at 20 and 27cm depth, and one dead individual of *Lasioglossum laticeps* at 7cm depth measured from the bottom of the tile and living ants at different depths. We also discovered some chambers containing weevil corpses at depths of 10, 20 and 30cm and eggs, indicating nesting structures belonging to *Cerceris* spp. (probably *Cerceris arenaria*).

Second excavation (Etterbeek, Brussels) - 1: Removal of paving stones; 2: Profile of the first few cm; 3: Excavation of the yellow sand basis; 4: Gallery in the basis layer; 5: Weevil corpse; 6: Andrena barbilabris in the basis (20-27 cm deep). Images by Sylvie Smets (CRR) and Violette Van Keymeulen (ULiège).

Measurement of the grain size of the samples taken on site

The particle size analysis of the two sand samples taken on these sites was comparable to those carried out on the mound sand, except for slight differences in the limits of the classes (90µm instead of 100µm and 63µm instead of 50µm). We noted that the most represented category of base layer sands is the 200 to 90µm class, whereas for the mound sands, it is the 500 to 200 µm class that is most represented. It should also be noted that the measurements on mound sand are much more numerous. We also noted that the percentage of material below 63µm is 4.5% on average. There is therefore less than 4.5% of the fraction (silt + clay, limits 2 and 50µm) and we are therefore in a predominantly sandy texture, as for the materials recovered from the sandy mounds at the surface of the pavements.



Size distribution of sand taken from under the mortar layer (Watermael-Boitsfort) and laying course (Etterbeek) taken on site.

Supplementary Tables & Figures

Participatory survey

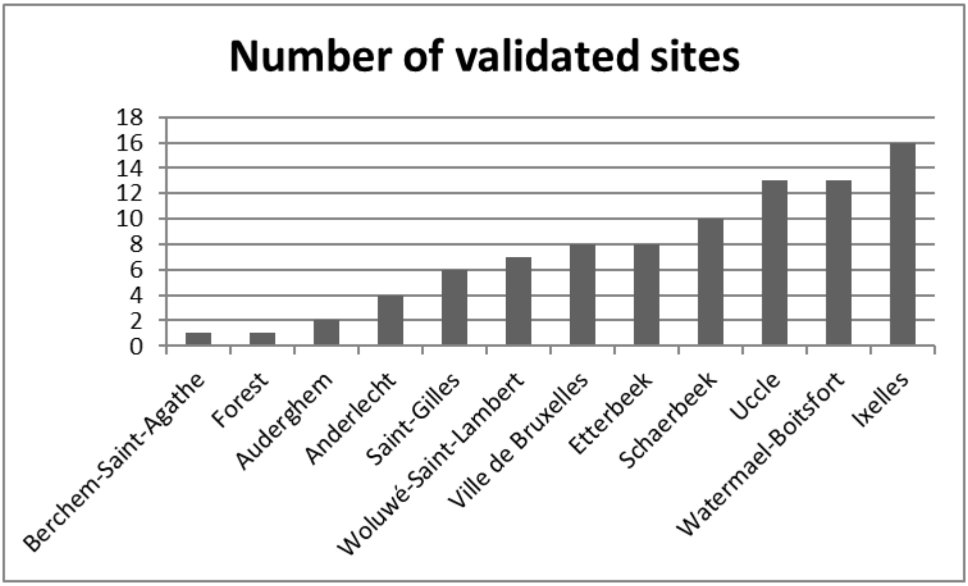


Figure 6-S1 Number of validated sampling sites by municipality

Co-occurrence of species

All species occurred at sites with at least one other species present, except for *A. vaga*, which may however be due to its earlier activity in the spring. Thus, co-occurrence of species is very pronounced. The most pronounced co-occurrences for ground-nesting species are between *C. arenaria* and *L. pygmaeus*, between *C. rybiensis* and *L. laticeps*, between *A. barbilabris* and *L. laticeps*, and between *D. hirtipes* and *C. arenaria*. The co-occurrence between cuckoo species and their associated hosts was also observed: between *H. nobile* and *C. arenaria*, between *H. gerstaeckeri* and *M. lutaria*, between *H. gerstaeckeri* and *C. arenaria*, and between *S. crassus* and *L. laticeps*.

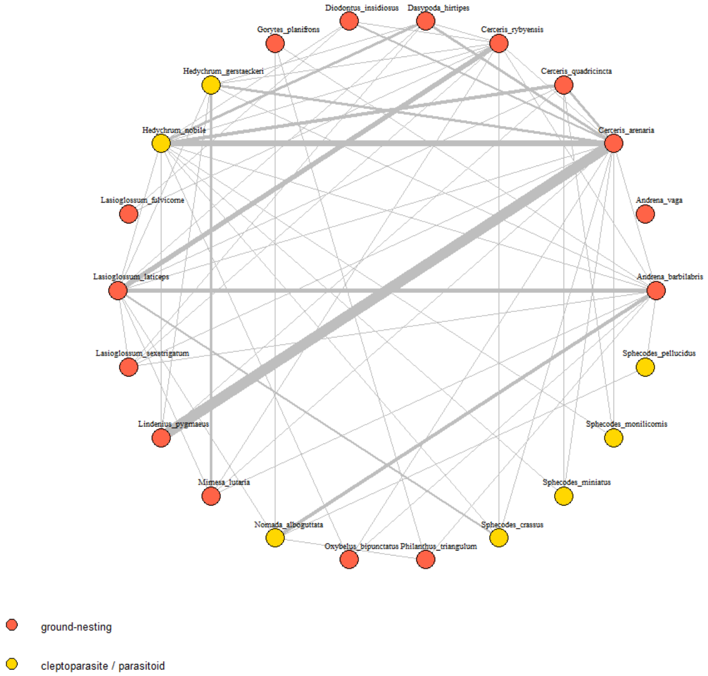


Figure 6-S2 Co-occurrence network for identified bee and wasp species. The red and yellow colours represent a species' nesting strategy: ground-nesting species are colored in red, and cleptoparasitic/parasitoid species in yellow. A link (grey line) between two species indicates that they were present at the same site at least once. The size of the linkage increases with increasing the number of times these species co-occurred at the same sites, with *C. arenaria* and *L. pygmaeus* being simultaneously present at 4 sites.

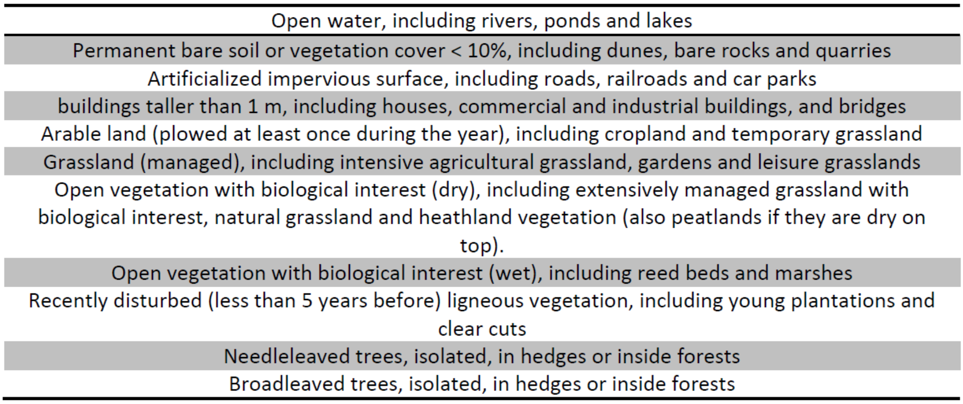
The number of nests present on the sidewalk shows great variability and ranges from 2 to 500 nests with an average of 107 nests per site and a median of 50 nests per site. This range between the median and the mean indicates an asymmetric distribution with a small amount of data with high values. As for the nest density, it presents a mean of 12 nests/m² and oscillates between 0.167 and 100 nests/m². It shows an asymmetric distribution rather similar to the number of nests with a median at 5 nests/m².

Joint Size Analysis

Table 6-S1 Measurements of the joint sizes (width) near the nest entrance of ground-nesting species in urban pavements.

Species	**Family**	**Measure number**	**Mean size ± standard deviation [cm]**	**Size min/max [cm]**
Andrena barbilabris	Andrenidae	145	1,20 ± 0,62	0,3/3,0
Andrena vaga	Andrenidae	3	0,7 ± 0,1	0,6/0,8
Cerceris arenaria	Crabronidae	110	1,04 ± 0,51	0,4/3,0
Cerceris quadricincta	Crabronidae	9	0,91 ± 0,23	0,6/1,3
Cerceris rybyensis	Crabronidae	7	1 ± 0,56	0,5/2,2
Dasypoda hirtipes	Melittidae	29	1 ± 0,34	0,4/3,0
Lasioglossum fulvicorne	Halictidae	11	0,82 ± 0,44	0,5/2,0
Lasioglossum laticeps	Halictidae	56	0,93 ± 0,63	0,2/3,0
Lasioglossum sexstrigatum	Halictidae	19	1,19 ± 0,75	0,3/2,5
Philanthus triangulum	Crabronidae	9	1,27 ± 0,51	0,8/2,5

Table 6-S2 Land use classes defined by ULB as part of the Lifewatch project (2015) and used by Bruxelles Environnement to characterize the landscape of the regional territory.



Open water, including rivers, ponds and lakes
Permanent bare soil or vegetation cover < 10%, including dunes, bare rocks and quarries
Artificialized impervious surface, including roads, railroads and car parks
buildings taller than 1 m, including houses, commercial and industrial buildings, and bridges
Arable land (plowed at least once during the year), including cropland and temporary grassland
Grassland (managed), including intensive agricultural grassland, gardens and leisure grasslands
Open vegetation with biological interest (dry), including extensively managed grassland with biological interest, natural grassland and heathland vegetation (also peatlands if they are dry on top).
Open vegetation with biological interest (wet), including reed beds and marshes
Recently disturbed (less than 5 years before) ligneous vegetation, including young plantations and clear cuts
Needleleaved trees, isolated, in hedges or inside forests
Broadleaved trees, isolated, in hedges or inside forests

Granulometry analysis

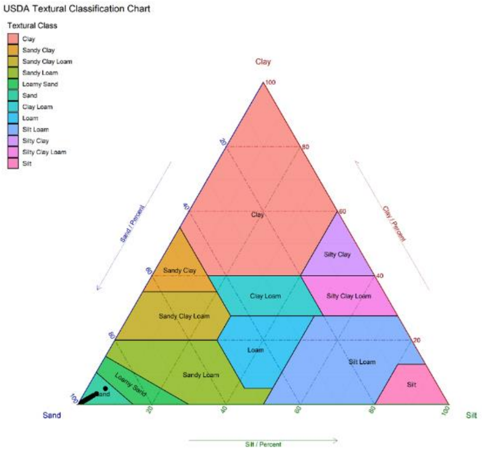


Figure 6-S3 USDA textural triangle and the location of the collected tumuli samples in black dot points (N = 53)

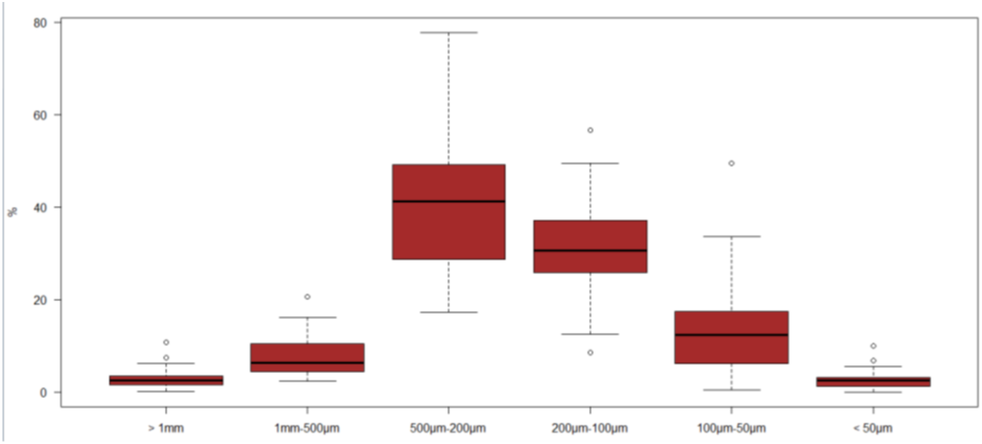


Figure 6-S4 Boxplot of the quantitative variables related to the rates of particles size fractions of the sandy mounds of bees and wasps nesting in the pavements of the Brussels-Capital Region

Figure 6-S5 Pavements in the municipality of Uccle that have grassy or non-vegetated beds suitable to accommodate species of ground-nesting Hymenoptera.