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Pollinator conservation in the Anthropocene

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Padua, 29 September 2023

Andree Cappellari

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Summary

Pollinators play a key role in ecosystems, ensuring the reproduction of most wild and cultivated flowering plant species. However, pollinator communities are rapidly changing due to multiple anthropogenic drivers, with potential effects also on the fundamental ecosystem service they provide. Through the chapters of my thesis, I analysed how the factors that shape the abundance, diversity and distribution of pollinators in landscapes and thus determine their thriving or decline, *i.e.*, land-use changes, the use of pesticides, urbanization, and the introduction of managed species, affect both managed and wild pollinators.

After a brief introduction on pollinators and the key determinants of their abundance, diversity and distribution (*Chapter 1*), I investigated the effects of landscape composition and seasonality on the properties of pollen collected by managed honey bees, in particular on pollen diversity (*Chapter 2*) and pollen contamination by pesticides (*Chapter 3*), both of which strongly impact bee health. For both works, we collected pollen samples monthly, from April to September, for two consecutive years at 13 locations in Northern Italy. We highlighted that landscape composition affected both pollen features, and in particular that a high amount of semi-natural habitats in landscapes helped both to increase the heterogeneity of pollen collected by honey bees and to minimise contamination by pesticides.

I then focused on the effects of urbanization on wild pollinators (*Chapter 4*). In particular, I explored how increasing temperatures and the amount of green areas affected wild bee communities and their functional traits in the city of Rome, in central Italy. We sampled wild bees 7 times during spring and summer in 36 sites. We found that higher temperatures were generally associated with a higher abundance and species richness of wild bees, but they also led to a homogenisation of wild bee community traits, favouring a few traits such as small body size and polylectic diet. On the other hand, the amount of green areas did not affect wild bee communities.

In the following chapter, I examined the potential impacts of massively-introduced managed species on wild pollinator communities (*Chapter 5*). Through the study of 51 plant-pollinator networks sampled in northern Italy, I examined how potential competition between managed honey bees and wild pollinators was influenced by the functional traits of both pollinators and the plants they forage on. We highlighted that plant communities characterised by high functional richness could help mitigate potential competition between managed and wild pollinators by providing alternative resources on which wild pollinators can forage, and that

pollinators characterised by functional traits similar to those of the honey bee were more prone to potential competition.

Last, I analysed how two pollinator-friendly measures, *i.e.*, habitat restoration and habitat enhancement for pollinators, affected pollinator diversity and ecosystem multi-functionality (*Chapter 6*). We selected 96 sites in northern Italy belonging to three habitat types (crop field margins, semi-natural patches, and urban green areas) with a gradient of flower coverage. We sampled wild pollinators and a large number of ecosystem services, mostly biodiversity-based, using which we calculated two indices of ecosystem multi-functionality. We found that while habitat restoration from intensive to semi-natural habitats benefited both pollinators and multiple ecosystem services, habitat enhancement for pollinators promoted pollinator diversity, but did not affect ecosystem multi-functionality.

In conclusion, the results obtained from my thesis could help develop targeted strategies for the conservation of both wild and managed pollinators. I highlighted that semi-natural areas play a key role in supporting pollinators, that honey bees may pose a threat to specific categories of pollinators, and that rising temperatures will lead to drastic changes in pollinator communities. I also showed that functional traits of both plants and pollinators have a strong influence on pollinator responses to the factors that threaten their survival. Finally, I pointed out how conservation measures for pollinators may – or may not – also impact other fundamental ecosystem services. It is therefore clear that multiple factors must be considered in order to get a clear picture of how pollinator communities are changing and what we can do to slow, stop or reverse their decline. Species conservation is a complex science, and further studies are needed to investigate the potential effects of interactions between drivers threatening managed and wild pollinators in the Anthropocene.

Riassunto

Gli insetti impollinatori svolgono un ruolo fondamentale negli ecosistemi, consentendo la riproduzione della maggioranza delle specie di angiosperme sia selvatiche che coltivate. Tuttavia, le comunità di impollinatori stanno rapidamente cambiando a causa di molteplici fattori di origine antropica, con potenziali effetti anche sul fondamentale servizio ecosistemico che questi insetti forniscono. Attraverso i capitoli della mia tesi ho analizzato nel dettaglio come i fattori che determinano l'abbondanza, la diversità e la distribuzione degli impollinatori nei paesaggi, in particolare i cambiamenti nell'uso del suolo, l'uso di pesticidi, l'urbanizzazione e l'introduzione di specie esotiche e gestite, influenzino sia gli impollinatori gestiti che quelli selvatici.

Dopo una breve introduzione sugli impollinatori e sui fattori che determinano la loro abbondanza, diversità e distribuzione (*Capitolo 1*), ho indagato gli effetti della composizione del paesaggio e della stagionalità sulle caratteristiche del polline raccolto dalle api mellifere gestite, in particolare sulla diversità del polline (*Capitolo 2*) e sulla sua contaminazione da prodotti fitosanitari utilizzati in agricoltura (*Capitolo 3*), due fattori che possono impattare fortemente la salute delle api. Per entrambi i lavori, abbiamo raccolto campioni di polline mensilmente, da aprile a settembre, per due anni consecutivi in 13 località in Nord Italia. Abbiamo evidenziato come la composizione del paesaggio abbia un effetto molto forte su entrambe le caratteristiche del polline, e in particolare come una elevata percentuale di habitat semi-naturali nel paesaggio possa contribuire sia ad aumentare l'eterogeneità del polline raccolto dalle api mellifere, sia a minimizzare la contaminazione da prodotti fitosanitari.

Mi sono poi concentrata sugli effetti dell'urbanizzazione sugli impollinatori selvatici (*Capitolo 4*). In particolare, abbiamo esplorato l'effetto dell'aumento delle temperature e della quantità di aree verdi sulle comunità di api selvatiche e i loro tratti funzionali nella città di Roma, in Italia centrale. Abbiamo campionato le api selvatiche per 7 volte durante la primavera e l'estate in 36 siti. Abbiamo riscontrato che temperature elevate erano associate a una maggiore abbondanza e ricchezza di specie di api selvatiche, ma anche a un'omogeneizzazione dei tratti della comunità di api, favorendo specifici tratti come dimensioni del corpo limitate e dieta generalista. Non abbiamo invece evidenziato alcun effetto della quantità di aree verdi sulle comunità di api selvatiche.

Nel capitolo successivo ho esaminato i potenziali impatti degli impollinatori gestiti sulle comunità di impollinatori selvatici (*Capitolo 5*). Attraverso lo studio di 51 network di interazione tra piante e impollinatori

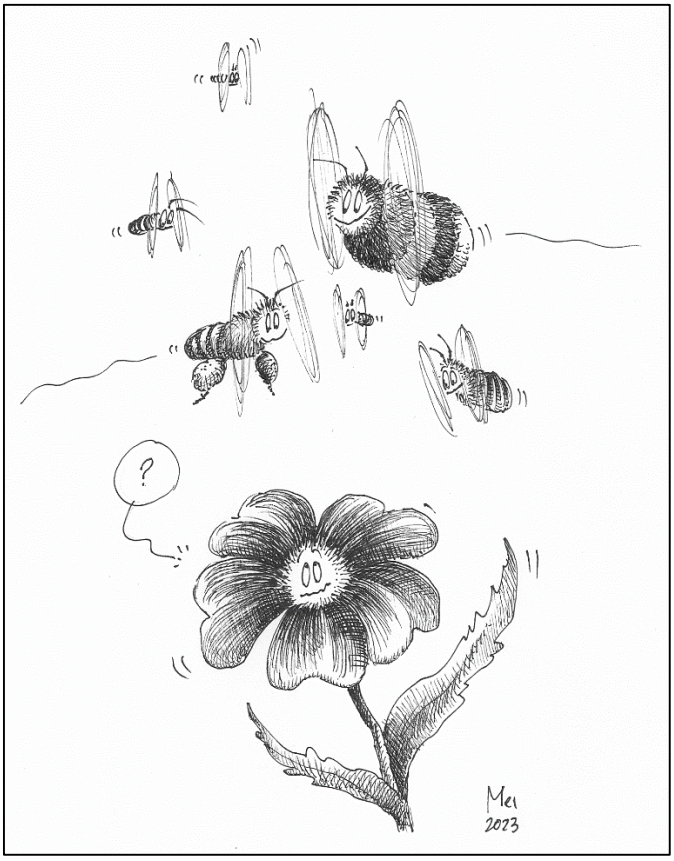
in Nord Italia, abbiamo indagato come la potenziale competizione tra api mellifere gestite e impollinatori selvatici fosse influenzata dai tratti funzionali degli impollinatori e delle piante fiorite su cui foraggiano. Abbiamo sottolineato come comunità di piante caratterizzate da alta ricchezza funzionale possano aiutare a mitigare la potenziale competizione tra impollinatori gestiti e selvatici, e come gli impollinatori caratterizzati da tratti funzionali simili a quelli dell'ape mellifera siano più inclini alla potenziale competizione.

Infine, ho analizzato come due misure *pollinator-friendly*, cioè il ripristino degli habitat semi-naturali e il miglioramento degli habitat per gli impollinatori, influissero sulla diversità di impollinatori e sulla multi-funzionalità degli ecosistemi (*Capitolo 6*). Abbiamo selezionato 96 siti in Nord Italia in tre habitat diversi (margini di campo, aree semi-naturali, e aree verdi urbane) con copertura di piante fiorite variabile. Abbiamo campionato gli impollinatori selvatici e un elevato numero di servizi ecosistemici, per la maggior parte legati alla biodiversità, con i quali abbiamo calcolato due indici di multi-funzionalità ecosistemica. Abbiamo rilevato che mentre il ripristino degli habitat da intensivi a semi-naturali può favorire sia gli impollinatori che molteplici servizi ecosistemici, il miglioramento degli habitat può beneficiare gli impollinatori, ma non ha alcun effetto sulla multi-funzionalità ecosistemica.

I risultati ottenuti dalla mia tesi si configurano come uno strumento utile per l'elaborazione di precise strategie per la conservazione degli impollinatori sia selvatici che gestiti. Ho sottolineato che le aree semi-naturali svolgono un ruolo chiave per il benessere degli impollinatori, che le api mellifere possono rappresentare una minaccia per specifiche categorie di impollinatori selvatici, e che l'aumento delle temperature può portare a cambiamenti drastici nelle comunità di impollinatori. Ho anche mostrato che i tratti funzionali, sia delle piante che degli impollinatori, possono avere una forte influenza sulle risposte degli impollinatori ai fattori che ne minacciano la sopravvivenza. Infine, ho evidenziato come misure di conservazione per gli impollinatori possano – o meno – aver un impatto anche su altri fondamentali servizi ecosistemici. È quindi evidente come sia necessario considerare numerosi fattori per avere un quadro chiaro di come le comunità di impollinatori stiano cambiando e cosa possiamo fare per rallentare, arrestare o invertirne il declino. La conservazione delle specie è una scienza complessa, e ulteriori studi sono necessari per indagare gli effetti delle possibili interazioni tra i fattori che minacciano gli impollinatori gestiti e quelli selvatici nell'Antropocene.

CHAPTER 1

Introduction



1.1. Wild and managed pollinators

Pollination is a vital ecosystem service. Animal pollination, in particular, is fundamental for the reproduction of more than 85% of angiosperms (Ollerton et al. 2011), including 75% of global food crop types (Potts et al. 2016). In temperate regions, insects are the most important group of pollinators, with a monetary value of their contribution to crop production of about €153 billion (Gallai et al. 2009). Among them, bees (Hymenoptera: Anthophila) and hoverflies (Diptera: Syrphidae) stand out for their efficiency. Bees include more than 20,000 species worldwide, classified into 7 families (Michener 2007). Since both adults and larvae of most species feed on nectar and pollen, bees are strongly specialized for collecting these resources. Most bee species are characterized by specific pollen-carrying structures on their legs or abdomen, called *scopa* or *corbicula*, which make them particularly efficient in transporting pollen from one flower to another (Michener 2007). The western honey bee (*Apis mellifera* Linnaeus) is the most widespread bee species, as it has been managed for millennia (Roffet-Salque et al. 2015), mostly for the production of honey. The honey bee is a super-generalist pollinator and it is fundamental for the pollination of a large proportion of wild and cultivated plant species (Garibaldi et al. 2013; Hung et al. 2018), despite being less efficient than wild pollinators in some contexts (Rollin and Garibaldi 2019; Eeraerts et al. 2020). Hoverflies are a relatively large family of flies, including about 6,000 species worldwide (Rotheray and Gilbert 2011). Hoverflies have different lifestyles and habitat preferences in different stages of their life cycle: while larvae could be saprotrophs, insectivores, phytophagous or coprophagous and therefore linked to a high variety of habitat types, almost all adults feed on nectar and pollen and often prefer open habitats (Vujić et al. 2022). Many hoverfly species migrate and, as opposed to bees, they do not build nests for their larvae. Therefore, hoverflies usually have large home ranges and are able to transport pollen over considerable distances – even up to 100 km (Wotton et al. 2019; Doyle et al. 2020; Fisler and Marcacci 2023).

In the past 50 years, the demand for insect pollination in crops has tripled (Goulson et al. 2015). Nevertheless, several works showed that pollinators are declining due to multiple anthropogenic causes (Potts et al. 2010). A global study highlighted that the number of collected and observed bee species has been declining since 1990, with 25% fewer species found in recent years (Zattara and Aizen 2021). Also, regional studies showed that pollinators strongly declined in the Netherlands and Great Britain in the last 40 years (Biesmeijer et al. 2006; Carvalheiro et al. 2013; Powney et al. 2019; Van Strien et al. 2019), and bumblebee

community composition changed in red clover fields in Sweden in the last 70 years (Bommarco et al. 2012). Some bumblebee species have declined up to 90% and have contracted their surveyed geographic ranges by 23-87% in North America in the last decades (Cameron et al. 2011; Bartomeus et al. 2013; Jacobson et al. 2018; Richardson et al. 2019), while declines in abundance and richness of non-bumblebee wild pollinators in the United States seem to be modest (Bartomeus et al. 2013). Besides this decline, pollinator communities in general are undergoing major alterations: Bartomeus et al. (2013) showed that 56% of wild pollinator species significantly changed their relative abundance in the United States over the last century, while Mathiasson and Rehan (2020) highlighted profound changes also in plant-pollinator interactions.

However, not all pollinator species are impacted by environmental changes in the same way. Changes may negatively affect some species, while others, more adaptable to the new conditions, may be favoured and become dominant (Bartomeus et al. 2013). These differences in species responses are mostly related to their functional traits. For example, species characterized by oligolecty (high resource specialization), univoltinism (one generation per year), small phenological breadth, and large body size may be more prone to decline (Biesmeijer et al. 2006; Bartomeus et al. 2013). On the other hand, short-tongued bumblebees became prevalent in bumblebee communities of Northern Europe (Biesmeijer et al. 2006; Bommarco et al. 2012; Jacobson et al. 2018; Richardson et al. 2019), and dominant crop pollinators increased their abundance by 10% in Great Britain in the last 30 years, potentially following agri-environment scheme management for pollinators (Powney et al. 2019).

1.2. Pollinators in dynamic landscapes

Managed and wild pollinators live in dynamic, rapidly evolving landscapes. Depending on their features, landscapes will host specific pollinator communities which will change over time, being affected by several anthropogenic drivers that shape species abundance and diversity. These drivers are the same potentially determining pollinator decline and include, among the most relevant ones, land-use changes and habitat loss, the use of pesticides, urbanization, and the introduction of managed species (Potts et al. 2010; Goulson et al. 2015).

Land-use changes related to human activities include modifications in land cover, configuration and management that may result in the loss of suitable habitats for pollinators (Potts et al. 2016). Semi-natural habitats are of crucial importance for pollinators, being especially rich in floral resources and areas suitable

for nesting or where larvae can develop. Therefore, the conversion of semi-natural habitats to intensive agriculture is known to strongly negatively affect pollinator communities (Carvalheiro et al. 2013; Koh et al. 2016; Habel et al. 2019), and increasing distance from semi-natural areas reduces wild pollinator abundance and richness (Ricketts et al. 2008; Moquet et al. 2018). Moreover, habitat loss and fragmentation can influence the foraging activity of pollinators, for example by increasing search and travel times to gather resources, with potential negative effects on pollinator health (LeBuhn and Vargas Luna 2021). Oligolectic and ground-nesting bees appear to be more vulnerable to habitat loss than polylectic and cavity-nesting bees (LeBuhn and Vargas Luna 2021), while hoverflies and dipterans in general are more resilient because of their polylecty and high mobility (Millard et al. 2021). Clarify how pollinators and their foraging activity are affected by land-use changes and habitat loss is a central theme in conservation ecology, as evidenced by the large body of literature on the topic (Winfree et al. 2009; Tonietto and Larkin 2018; Millard et al. 2021; Raven et al. 2021; Liang et al. 2023). Nevertheless, significant knowledge gaps remain to be addressed even for the most well-studied species, such as the honey bee (Härtel and Steffan-Dewenter 2014).

The conversion of habitats from semi-natural to agricultural also involves changes in management practices. Due to the sharp increase in cultivated areas in the last 50 years, nowadays pesticides are applied more than ever (Bernhardt et al. 2017). Pesticides are commonly used for crop protection and include, among the most important categories, insecticides, fungicides, and herbicides (Zioga et al. 2020). The toxicity of these compounds is highly variable, based on the target species, pollinator species sensitivity, pesticide formulation, and landscape context. In general, insecticides include compounds that pose major threats to pollinators, since they are specifically formulated to negatively affect insect health. Neonicotinoids, in particular, are systemic insecticides that have been largely shown to have important negative effects on non-target insects (Tooker and Pearsons 2021) and have therefore been banned in most EU countries. Nevertheless, newly formulated insecticides have also been shown to negatively impact pollinators (Siviter and Muth 2020). Fungicides are usually characterized by lower toxicity compared to other groups of pesticides, but they can interact synergistically among themselves and with other pesticides to exacerbate negative effects on pollinators (Siviter et al. 2021). Herbicides, on the other hand, could affect pollinator health both directly and indirectly, *i.e.*, by reducing the abundance and diversity of flowering plants (Potts et al. 2016). However, pollinators foraging in landscapes are exposed to multiple pesticides, which can synergistically interact to exacerbate the

negative effects on pollinator health (Zhao et al. 2020). While it is crucial to investigate the toxicity of single compounds for pollinators, it is also essential to understand how the mixes of compounds to which insects are exposed are modulated by landscape structure and composition.

In addition to the conversion of semi-natural habitats to agricultural habitats, the development and expansion of urban areas are among the major land-use changes that characterized the 20th century, and nowadays more than 50% of the world population lives in cities (OECD 2015). Urbanization, however, has strong impacts on biodiversity and pollinators for multiple causes. The increase in built-up areas is the most obvious and direct effect of urbanization, as it leads to the loss or reduction of green areas suitable for pollinators (Herrmann et al. 2023). Moreover, increased impervious surfaces can result in the so-called "urban heat island effect", making cities warmer than surrounding rural areas (Polidori et al. 2023). Warmer and drier climates associated with high urbanization can lead to homogenization of pollinator communities (Ganuza et al. 2022), and most pollinator groups, such as hoverflies and butterflies, struggle to adapt to urban environments (Burdine and McCluney 2019; Theodorou et al. 2020; Piano et al. 2020; Fenoglio et al. 2020). However, some species, characterized for example by high thermal limits, could adapt more easily and seem to thrive in cities (Papanikolaou et al. 2017; Hamblin et al. 2017). Since urban sprawl is predicted to further increase in the next decades (OECD 2015), additional studies are needed to understand how pollinators respond and adapt to urban environments.

Recently, another potential threat to wild pollinators has emerged. The presence in landscapes of the so-called “Massively Introduced Managed Species” (MIMS) (Geslin et al. 2017), which include the honey bee and a few other species, can cause major changes in pollinator communities and plant-pollinator interactions. The density of managed honey bee colonies increased exponentially in the last 50 years in the Mediterranean areas (Herrera 2020), and each honey bee colony can host more than 50,000 bees. As a result, honey bees are often dominant in pollinator communities (Hung et al. 2019; Herrera 2020). Despite being essential for crop pollination, extremely high abundances of honey bees found in areas where beekeeping is widely practised could potentially threaten wild pollinators (Mallinger et al. 2017; Geldmann and González-Varo 2018; Wojcik et al. 2018; Ropars et al. 2019; Angelella et al. 2021). These negative effects could be direct, *i.e.*, via competition for floral and nesting resources or spread of diseases and pathogens, or indirect, *i.e.*, via changes in plant communities to which wild pollinators are not able to adapt (Traveset and Richardson 2006; Mallinger

et al. 2017). However, these potential negative effects are often context-dependent, and it is still unclear which pollinator groups might be most affected by a high abundance of managed honey bees, and how local conditions might mitigate this potential competition.

1.3. Pollinator conservation

Changes in pollinator communities related to land-use changes, the use of pesticides, urbanization and the introduction of managed pollinators potentially impact both human well-being and biodiversity (Potts et al. 2016). Pollination deficit resulting from drastic changes in pollinator communities and pollinator decline may endanger wild plant reproduction and diversity (Clough et al. 2014). Moreover, in agricultural landscapes, it could cause yield loss and a reduction in the quality of produced food, but also impair the long-term resilience of food production systems (Olhnuud et al. 2021). Equally important, however, are the potential negative effects on the so-called *bio-cultural diversity* (IPBES 2016; Dicks et al. 2021), a concept which recognises that culture and biodiversity are linked and may be mutually constituted (Hill et al. 2019). It is therefore clear how crucial it is to develop specific conservation plans to protect these key organisms.

Improving land management with pollinator-friendly measures is a key action to safeguard pollinator populations, for example through habitat restoration and habitat enhancement for pollinators (Kennedy et al. 2013; Van Strien et al. 2019; Warren et al. 2021). Habitat restoration involves the transformation from intensively managed habitats, such as croplands, to semi-natural habitats (Ricketts et al. 2008), and the presence in the landscape of different types of semi-natural habitats in particular has been shown to boost wild pollinator richness (Pindar and Raine 2023). Habitat enhancement involves the creation of flower strips, flower-rich margins, and hedgerows, usually in agricultural or urban-dominated landscapes (Wratten et al. 2012). Both flower strips and hedgerows showed important conservation benefits for pollinators (Ouvrard et al. 2018; Buhk et al. 2018; Ponisio et al. 2019) and for biodiversity in general (Albrecht et al. 2020). However, it is also essential to ensure that semi-natural habitat patches, flower strips or hedgerows are not isolated within intensive agricultural landscapes but are adequately connected, in order to allow pollinator movement in the landscape (Potts et al. 2016).

Conservation actions aimed at protecting one group of pollinators do not necessarily benefit another, since different pollinators have different necessities. For example, the presence of green roofs in urban environments has been shown to increase wild bee populations, but not hoverfly populations (Jacobs et al.

2023). In fact, different pollinator groups often require specific resources. For central-place foragers, the availability of nesting resources is also crucial. Interventions such as leaving patches of bare ground – since nearly 75% of wild bees nest on the ground (Antoine and Forrest 2021) – or providing suitable nesting cavities have been shown to boost pollinator populations (Potts et al. 2005). Also, for some species, other types of resources such as resin and floral oils can be limiting (Requier and Leonhardt 2020). Similarly, conservation measures for pollinators can impact other key ecosystem services, such as biological control and water quality regulation, thus underscoring how critical it is to carefully evaluate the most appropriate interventions to implement in order to maximize the benefits not only for different pollinator groups but also for multiple ecosystem services.

1.4. Research objectives and thesis structure

The overall aim of my PhD thesis was to analyse how land-use changes, the use of pesticides, urbanization, the introduction of managed species, and conservation measures for pollinators shaped the relative abundances and species diversity of pollinator communities, focusing on both managed and wild pollinators.

In *Chapter 2*, we explored how the provision of pollen by managed honey bees was influenced by landscape composition and seasonality. For two consecutive years, we collected pollen samples monthly, from April to September, from apiaries placed in 13 locations in a mountainous area of Northern Italy, and identified pollen grains to the lowest possible taxonomic level. We determined landscape composition at two different spatial scales using regional land cover maps. Then, we tested how landscape composition and seasonality shaped the diversity of pollen collected by honey bees.

In *Chapter 3*, we analyzed the effect of landscape composition and seasonality on pesticide contamination of pollen collected by honey bees. Similarly to *Chapter 2*, we collected pollen samples monthly from apiaries placed in 13 locations in a mountainous area of Northern Italy for two consecutive years. Using a multi-residual analysis, we searched for almost 400 compounds in pollen, including insecticides, herbicides, and fungicides. For each pollen sample, we calculated the Pollen Hazard Quotient, a measure of potential pollen toxicity, and then tested how it changed depending on landscape composition, seasonality, and pesticide category.

In *Chapter 4*, we investigated the effects of urbanization on wild bee communities and their functional diversity. We sampled bees using pan-traps in 36 sites in the city of Rome (central Italy) characterized by

independent gradients of temperature and amount of open habitat cover. We considered four functional traits of bees, *i.e.*, body size, nesting strategy, diet breadth, and social behaviour. We then tested how wild bee communities changed in relation to temperature and open habitat cover in terms of abundance of individuals, species richness, and functional diversity.

In *Chapter 5*, we used a combination of ecological network analysis and functional traits analysis to disentangle the relationships between managed honey bees and wild pollinators in semi-natural habitats. We sampled plant-pollinator networks in 51 grasslands in Northern Italy and calculated the resource overlap between managed honey bees and wild pollinators. We analyzed both functional traits of plants, *i.e.*, corolla length, flower shape and flower colour, and of pollinators, *i.e.*, tongue length, body size, type of foraging range, and taxonomic family. Then, we tested how the resource overlap was influenced by managed honey bee abundance, functional composition of plant communities, and pollinator traits.

In *Chapter 6*, we explored how two pollinator-friendly measures, *i.e.*, habitat restoration and habitat enhancement, affected pollinators and multiple ecosystem services. We sampled 96 sites belonging to three habitat types, *i.e.*, semi-natural patches, urban green areas, and crop field margins. We sampled pollinators using pan-traps and measured seven ecosystem services: honey bee-related ecosystem services, ground-dwelling arthropod-related ecosystem services, pest control, seed predation, disease control, soil nutrient cycling, and flood control. We calculated ecosystem multi-functionality using two approaches and then tested how ecosystem multi-functionality and pollinator diversity were shaped by habitat type (habitat restoration) and increasing flower cover (habitat enhancement for pollinators).

Finally, in *chapter 7*, I summarized the results of my PhD thesis.

CHAPTER 2

Seasonality and landscape composition drive the diversity of pollen collected by managed honey bees

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*These authors have contributed equally to this work and share first authorship

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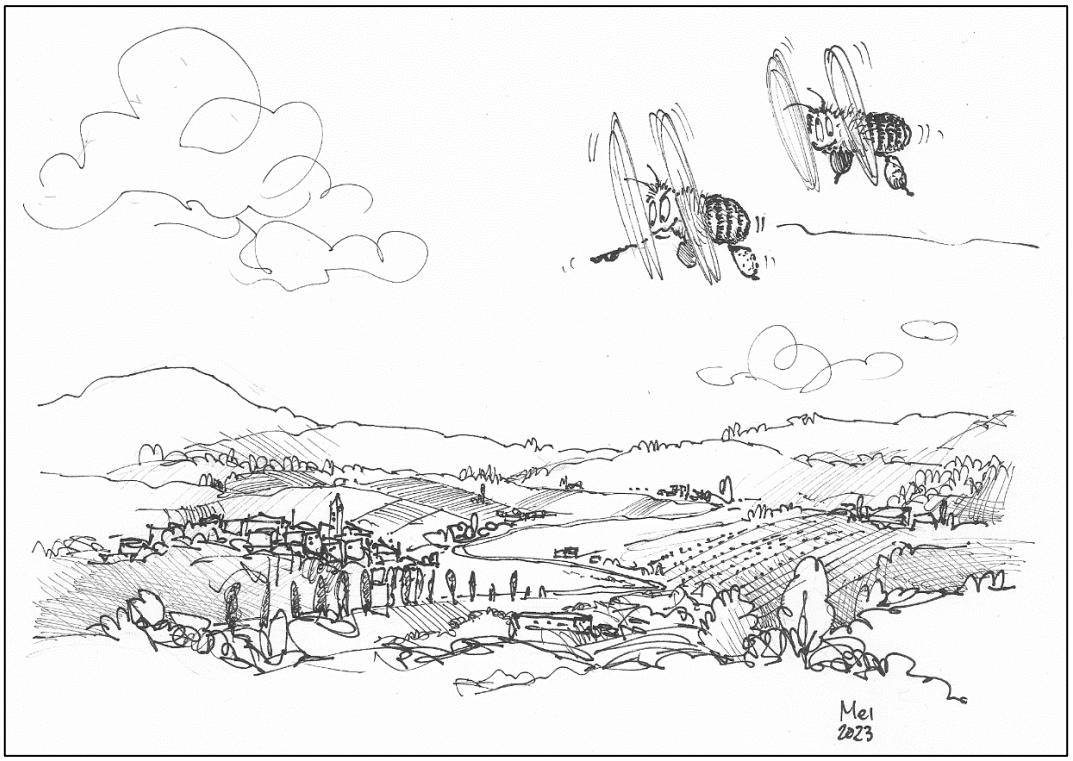

2.1. Abstract

The western honey bee, *Apis mellifera*, is the most important and widespread managed pollinator species. Honey bee diet is based on nectar and pollen, and pollen diversity and composition, in particular, affect colony health and fitness. As landscape composition is strongly linked to floral resource heterogeneity, it could influence the resource intake of honey bees. This work aimed to explore how the composition of pollen collected by honey bees was modulated by seasonality and landscape composition heterogeneity in a mountainous cultivated area of Northern Italy. We selected 13 locations, and at each location, we placed two honey bee colonies from which we collected pollen samples every month during the whole flowering season for two consecutive years. We then analyzed pollen samples in the laboratory and determined the Shannon diversity index of each pollen sample and the temporal pollen taxon replacement. We extracted the cover of the main habitat types at three spatial scales and tested the effect of landscape diversity and composition using Principal Component Analysis. Honey bees foraged on a high number of floral resources, however, they mostly collected pollen from a small number of taxa, with pollen type composition changing throughout the flowering season. In early spring and late summer, most pollen grains were collected from a few plant species, while from May to August the number of collected pollen types was significantly higher. Landscape composition affected pollen diversity only at the end of the flowering season. While honey bees were able to collect highly diverse pollen throughout spring and summer regardless of landscape composition, in late summer, when pollen collected is fundamental for the overwintering of the colony and its development in the following season, semi-natural areas became crucial for honey bee foraging activities, with pollen diversity increasing with increasing percentages of semi-natural areas. Our research highlighted the importance for honey bees of certain seasonal resources and of semi-natural habitats at the end of the flowering season, which ensure the subsistence of their colonies throughout the year.

2.2. Introduction

In recent years, pollinator abundance and diversity faced a strong decline due to multiple anthropogenic pressures (Potts et al. 2010). One of the main causes of this decline is the loss and fragmentation of natural areas, which led to a decrease in plant diversity, potentially determining insufficient nutrition for pollinators (Goulson et al. 2008). In particular, the honey bee, *Apis mellifera* Linnaeus, is the most widespread managed pollinator species and its presence is crucial not only for ensuring the reproduction of plant species in natural habitats but also for crop production, which is positively impacted by honey bee pollination both in terms of quantity and quality (Hung et al. 2018; Rollin et al. 2019).

The honey bee is a eusocial species whose colonies can host more than 50,000 individuals (Von Frisch 1954; Fontana 2019). Its diet is based on nectar and pollen. Nectar is a source of energy and, after being transformed into honey, it constitutes the food stock through which the colony survives during winter. Pollen is a source of protein and lipids, and in addition to direct feeding of larvae and adult workers, it is necessary for the secretion of two substances essential for the life of the colony, *i.e.*, the royal jelly, which is the food for all the larvae in the first 3 days and for queens during their whole life (Winston 1991), and the wax, of which honeycombs are made (Hepburn 1986; Tautz 2008). Pollen availability not only influences the development and reproduction of the colony in the short term, but as for honey, it is also fundamental for the overwintering of the colony and its development in the following season (Alaux et al. 2017).

The quality of pollen in terms of nutrient content varies from one plant species to another (Roulston and Cane 2000), and for this reason, honey bees must have access to diverse pollen sources in order to assure colony health. Only landscapes with a certain degree of floristic diversity can therefore guarantee adequate resources for honey bees (Di Pasquale et al. 2016). Recent studies showed that the proportion of semi-natural habitat within the landscape is positively related to honey bee pollen diversity and protein content (Donkersley et al. 2014; Cannizzaro et al. 2022) and to the probability of winter survival of the colonies (Rutschmann et al. 2022), while habitat fragmentation negatively affects the abundance of pollen collected by honey bees (Ochungo et al. 2021). Moreover, heterogeneous landscapes have been shown to support honey bees also by reducing their foraging distances, therefore allowing them to consume fewer resources to obtain food (Danner et al. 2017).

In this work, we aimed to understand the effect of landscape composition and seasonality on the diversity of pollen collected by honey bees in a mountainous cultivated area in Northern Italy. We selected 13 locations, from which we collected pollen samples monthly from two honey bee colonies during the spring and summer of 2019 and 2020. After analyzing pollen samples in the laboratory, we determined the Shannon diversity index for each pollen sample and the temporal β-diversity of pollen at each location. To assess landscape heterogeneity, we calculated the cover of the main habitat types at 1, 3, and 5 km radius buffers around the sampling locations, and analyzed landscape composition through Principal Component Analysis and Shannon diversity index. We hypothesize that pollen composition would change throughout the flowering season, following plant phenology at least in early spring and late summer when floral resources are relatively scarce. Moreover, we expect that landscape composition would strongly affect pollen composition, with high-diverse landscapes supporting honey bee colonies by offering a wider range of pollen types in comparison to homogeneous landscapes.

2.3. Materials and methods

2.3.1. Study area

The study was carried out in the Trentino province, an area in Northern Italy covering about 6,214 km^2. The area is generally mountainous, but it is characterized by a considerable landscape heterogeneity, with about 80% semi-natural areas, 15% agricultural areas, and 5% urban areas. As a result, the climate is highly variable. The mean annual temperature is about 9 °C, and the mean annual precipitation at 200 m a.s.l. is 1,200 mm.

We selected 13 sampling locations, which were characterized by great variability in landscape composition (Table S2.1; Figure 2.1). Three sites were close to apple orchards, three were close to vineyards, three were in an urban setting, and three were far from agricultural areas. The average elevation of the sampling locations was 533 m a.s.l. (min = 93 m a.s.l., max = 1,481 m a.s.l.). The mean air temperature at the sampling sites during the sampling periods, *i.e.*, from April to September of 2019 and 2020, was 17 °C.

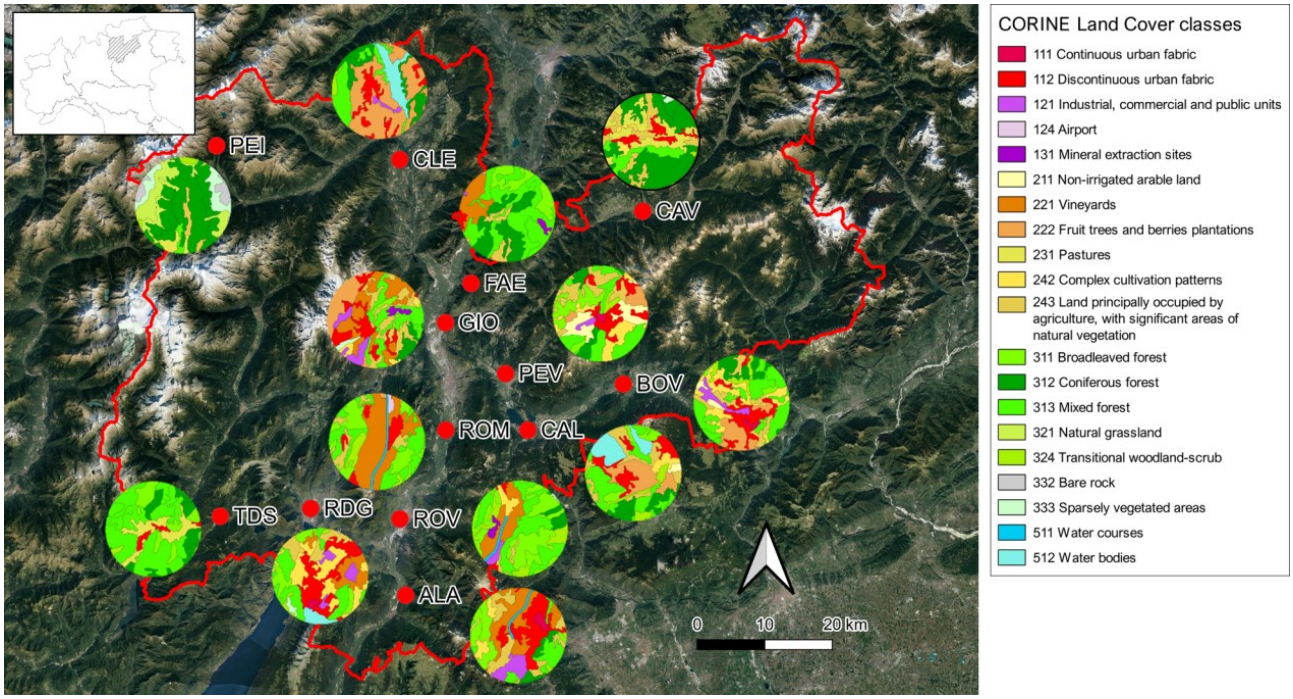

Figure 2.1: Map of the 13 sampling locations, also showing the landscape composition at 3 km radius buffers around the sampling locations using CORINE Land Cover classes. Abbreviations for locations are ALA (Ala), BOV (Borgo Valsugana), CAL (Caldonazzo), CAV (Cavalese), CLE (Cles), FAE (Faedo), GIO (Giovo), PEI (Peio), PEV (Pergine Valsugana), RDG (Riva del Garda), ROM (Romagnano), ROV (Rovereto), and TDS (Tiarno di Sopra). See Table S2.1 for additional information on sampling locations.

2.3.2. Experimental design

In 2019 and 2020, we placed one small apiary consisting of two honey bee colonies at each sampling location. All colonies originated from the livestock managed by the Edmund Mach Foundation and had sister queens of *A. m. carnica* x *A. m. ligustica*. The colonies were managed directly by Edmund Mach Foundation personnel according to the local beekeeping practice. From April to September, we carried out pollen samplings every month, for a total of six pollen samples collected per colony per year. At some locations, however, the number of pollen samples was lower due to adverse climatic conditions. In particular, in 2019, at one location only three samples could be collected, and only five at two others. In 2020, only five samples were collected at three locations. Pollen samples were collected by activating pollen traps at the hive entrance for 48 h, Pollen samples were then stored at -20 °C.

2.3.3. Landscape composition

For each sampling location, we extracted the cover of the main habitat types using the CORINE Land Cover (CLC) database (© European Union, Copernicus Land Monitoring Service 2018, European Environment Agency) at three spatial scales, *i.e.*, the local foraging scale of honey bees (1 km radius buffer around the sampling locations) and two landscape foraging scales of honey bees (3 and 5 km radius buffers around the

sampling locations). Following the CLC classification, we considered a total of 24 land-use classes (Table S2.2). Landscape composition was heterogeneous across sites. In 3 km radius buffers around the sampling locations, an average of 33% of the land was covered by agricultural areas (min = 7%, max = 52%), 51% by semi-natural areas (min = 26%, max = 92%), 13% by urban areas and other artificial surfaces (min = 0, max = 30%), and 2% by other areas (min = 0, max = 13%) (Table S2.1).

As most of these classes were highly correlated, we performed a Principal Component Analysis (PCA) to extract the landscape composition at each of the three spatial scales. We extracted the first two eigenvalues, PC1 and PC2, which explained 38, 48, and 45% of the total landscape variability at 1, 3, and 5 km radius buffers around the sampling locations (Table S2.3). PC1 was positively related to semi-natural areas, in particular coniferous forests, natural grasslands, and areas with sparse vegetation, and negatively related to intensive areas, in particular urban areas and vineyards (Table S2.3). Therefore, high values of PC1 can be interpreted as a high proportion of semi-natural areas within the landscapes.

Moreover, we calculated the Shannon diversity index for landscape composition using the 24 land-use classes at each of the three spatial scales. Shannon diversity index quantifies the heterogeneity of landscapes, taking into account both richness and evenness of land-use classes, with low values of the index indicating a low landscape heterogeneity. Shannon diversity index was calculated using the *R* package *vegan* (Oksanen et al. 2019). All statistical analyses were performed using the *R* software version 3.6.1 (R Development Core Team 2019).

2.3.4. Pollen analysis

From each pollen sample, we extracted two grams of pollen pellets, which were dissolved in distilled water and mixed using an advanced vortex mixer (VELP Scientifica, ZX3). We took 20 µl of the obtained suspension and placed it on a microscopic slide. Once the suspension of water and pollen was dry, we placed a drop of glycerin jelly on top of the sediment and covered it with a slide. Pollen was then observed under the optical microscope (Optika, B500PPH). For each sample, we counted about 500 pollen grains by applying the "transect" method (Tamic et al. 2011). Pollen grains were identified at the lowest possible taxonomic level according to available literature (Ricciardelli d'Albore 1998; Bucher 2004; El-Labban 2020) and palynological databases (PalDat 2000; PollenAtlas 2021). The identified pollens were classified following the "pollen types" nomenclature proposed by Persano Oddo and Ricciardelli d'Albore (1989).

For each pollen sample, we calculated the Shannon diversity index. As for landscape composition, the Shannon index for pollen reflects both the richness and evenness of pollen samples, with lower values indicating a lower diversity in pollen sample composition. Moreover, to understand how pollen composition changed throughout the flowering season, we calculated the mean β-richness and replacement at each location over the six sampling months, based on presence/absence data. All pollen indices were calculated using the *R* package *vegan* (Oksanen et al. 2019).

2.3.5. Statistical analysis

First, to determine the effect of landscape composition and seasonality on pollen diversity, we built two linear mixed-effect models for each spatial scale using the R package *nlme* (Pinheiro et al. 2019). In all models, the response variable was pollen Shannon index. Selected explanatory variables were collection month, year, landscape Shannon index, and the interaction between month and landscape Shannon index for the first model, and collection month, year, landscape PC1, landscape PC2, and the interactions between month and landscape PC1 and between month and PC2 for the second model. We also included the sampling location as random factor in all models. Starting from each full model, we used a backward deletion procedure, removing one-by-one the interactions with p value > 0.05, and re-ran the model to correctly interpret model main effects.

Second, to explore the effect of landscape composition on temporal β-diversity of pollen, we built four linear models for each spatial scale. We selected β-richness and replacement of pollen as response variables, PC1 and PC2 as explanatory variables for the first model, and landscape Shannon index as explanatory variable for the second model.

2.4. Results

We analyzed a total of 116,979 pollen grains in 224 samples collected during 2 years. We identified 122 plant taxa, most of them (n = 93) at the genus level (Table S2.4). We observed 48 pollen types in April, 80 in May, 77 in June, 67 in July, 69 in August, and 50 in September. The most abundant types were *Hedera* spp. (n = 16,896 pollen grains), Plantaginaceae (n = 10,303 pollen grains), and *Malus*/*Pyrus* spp. (n = 7,826 pollen grains). On the other hand, the most prevalent taxa were Compositae T-form, which includes the genera *Taraxacum* and *Cichorium* (found in 149 pollen samples), Compositae H-form, which includes the genera *Helianthus*, *Petasites*, and *Senecio* (found in 117 pollen samples), and *Trifolium repens* group (found in 110 pollen samples).

The monthly pollen samples were dominated by a handful of taxa, and there was a strong temporal turnover in the composition of pollen samples (Figure 2.2). In spring, honey bees mostly collected pollen on *Malus*/*Pyrus* spp. (21% of total pollen grains), *Salix* spp. (18%), and Compositae T-form (12%), with only three species making up half of the collected pollen grains (Figure 2.2 *a*, *b*). In June, *Castanea sativa* became the prevalent pollen type (29%), together with *T. repens* group (8%), *Filipendula* spp. (7%), and *Vitis* spp. (6%) (Figure 2.2 *c*). In July, the most visited taxa were Plantaginaceae (39%), *T. repens* group (9%), *Clematis* spp. (7%), and *Parthenocissus* spp. (7%) (Figure 2.2 *d*). Plantaginaceae were also found in August (16%), but pollen was mostly collected on *Artemisia* spp. (22%) and, to a lesser extent, on Compositae H-form (9%) and *Thalictrum* spp. (8%) (Figure 2.2 *e*). In September, almost all pollen was collected on *Hedera* spp. (79%) (Figure 2.2 *f*).

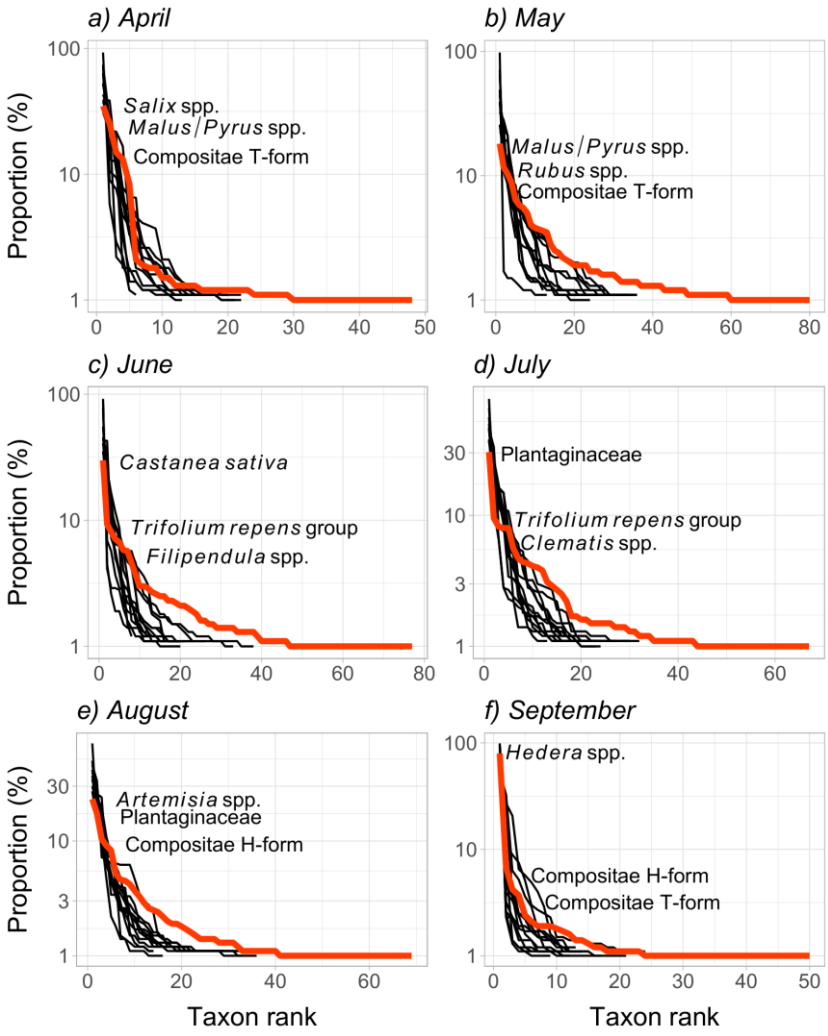

Figure 2.2: Rank abundance plots showing the relative proportion of the taxa found in pollen samples collected in *a)* April, *b)* May, *c)* June, *d)* July, *e)* August, and *f)* September of 2019 and 2020. Black lines represent single sampling locations and red lines represent the average of all locations for each sampling month. Species are ranked on the X-axis from left to right from the most to least abundant. The Y-axis was log-transformed to improve clarity.

Pollen Shannon index was strongly influenced by the collection month (Table 2.1). Pollen diversity was higher in May, July, and August, while pollen samples of April and particularly September were more homogeneous (Figure 2.3 *a*). Moreover, pollen Shannon index responded to the interaction between month and landscape PC1 at 3 and 5 km. Landscape composition had no effect on pollen diversity from April to August, however, in September the diversity of collected pollen increased with increasing landscape PC1, suggesting a positive effect of semi-natural habitat on pollen collection in late summer (Figure 2.3 *b*, *c*).

Table 2.1: Results of the linear mixed-effect models testing the response of pollen Shannon index to month, year, landscape Shannon index, and the interaction between month and landscape Shannon index; and month, year, landscape PC1 and PC2, and the interactions between month and landscape PC1 and month and landscape PC2. Landscape Shannon index, PC1 and PC2 were calculated at the three spatial scales, *i.e.*, *a)* 1 km radius buffer, *b)* 3 km radius buffer, and *c)* 5 km radius buffer around the sampling locations. Values in bold indicate significant effects (p value < 0.05). Only significant results after a backward stepwise model selection procedure are reported.

Spatial scale	Explanatory variable	χ^2	df	*p* value
a) 1 km	Month	105.494	5	< 0.001
	Year	0.013	1	0.911
	Landscape Shannon index	1.156	1	0.282
	Month	104.570	5	< 0.001
	Year	0.019	1	0.891
	Landscape PC1	2.467	1	0.116
	Landscape PC2	0.283	1	0.595
b) 3 km	Month	105.054	5	< 0.001
	Year	0.000	1	0.992
	Landscape Shannon index	0.018	1	0.894
	Month	103.378	5	< 0.001
	Year	0.001	1	0.980
	Landscape PC1	1.357	1	0.244
	Landscape PC2	0.030	1	0.862
	Month x Landscape PC1	16.795	5	**0.005**
c) 5 km	Month	105.493	5	<0.001
	Year	0.000	1	0.990
	Landscape Shannon index	1.292	1	0.256
	Month	98.978	5	< 0.001
	Year	0.002	1	0.961
	Landscape PC1	1.938	1	0.164
	Landscape PC2	0.050	1	0.823
	Month x Landscape PC1	17.397	5	**0.004**

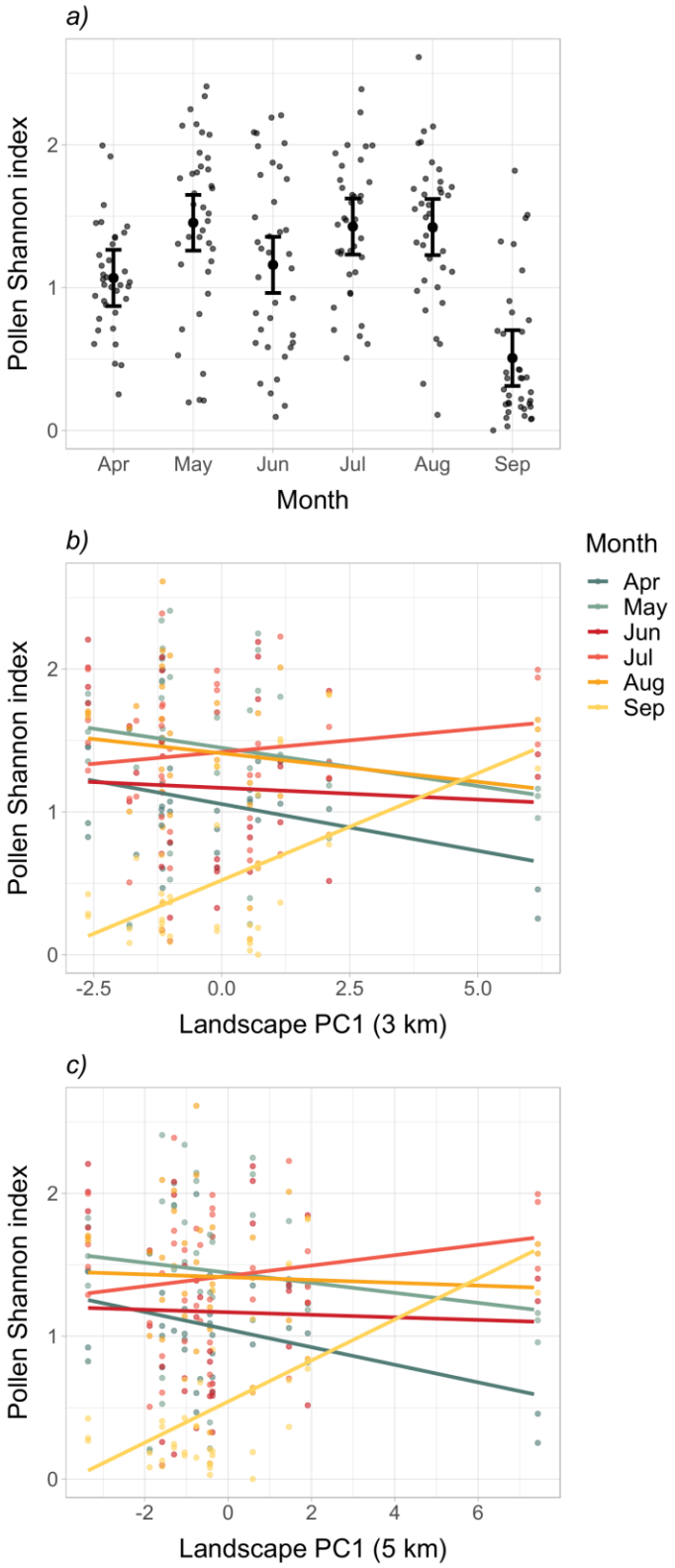

Figure 2.3: Plots showing the effect on pollen Shannon index of *a)* collection month, *b)* the interaction between month and landscape PC1 calculated at 3 km radius buffer around the sampling locations, and *c)* the interaction between month and landscape PC1 calculated at 5 km radius buffer around the sampling locations. Small points represent raw data points, large black points and coloured lines represent model estimates and black bars represent the 95% confidence intervals.

Temporal β-richness and replacement did not change in response to landscape composition at any of the selected spatial scales (Table 2.2 *a*, *b*). In general, β-richness values were high at all locations (min = 0.783, max = 0.880), while β-replacement values were particularly low (min = 0.235, max = 0.342).

Table 2.2: Results of the linear models testing the response of *a)* mean β-richness and *b)* mean β-replacement of pollen samples to landscape PC1 and PC2, and landscape Shannon index, at the three spatial scales, *i.e.*, 1 km, 3 km, and 5 km radius buffers around the sampling locations.

Response variable	Spatial scale	Explanatory variable	Estimate	SE	*t* value	*p* value
a) Mean β-richness	1 km	Landscape PC1	0.002	0.007	0.302	0.769
		Landscape PC2	0.001	0.008	0.104	0.919
		Landscape Shannon index	0.035	0.023	1.558	0.147
	3 km	Landscape PC1	-0.010	0.005	-2.060	0.066
		Landscape PC2	-0.004	0.006	-0.643	0.535
		Landscape Shannon index	0.032	0.031	1.057	0.313
	5 km	Landscape PC1	-0.009	0.005	-2.062	0.066
		Landscape PC2	-0.001	0.006	-0.119	0.908
		Landscape Shannon index	0.017	0.036	0.482	0.639
b) Mean β-replacement	1 km	Landscape PC1	0.005	0.006	0.966	0.357
		Landscape PC2	-0.005	0.007	-0.670	0.518
		Landscape Shannon index	0.025	0.022	1.164	0.269
	3 km	Landscape PC1	-0.007	0.005	-1.403	0.191
		Landscape PC2	0.004	0.006	0.585	0.571
		Landscape Shannon index	0.035	0.028	1.285	0.225
	5 km	Landscape PC1	-0.006	0.005	-1.300	0.223
		Landscape PC2	-0.003	0.006	-0.516	0.617
		Landscape Shannon index	0.023	0.033	0.720	0.487

2.5. Discussion

The survival, prosperity, and reproduction of honey bee colonies depend on the ability of honey bees to collect and store honey and pollen (Brodschneider and Crailsheim 2010). In this study, by observing almost 117,000 pollen grains, we were able to collect information on honey bee foraging behaviour from early spring to late summer. Moreover, we highlighted how pollen diversity was strongly shaped by seasonality, while landscape composition affected pollen diversity only at the end of the flowering season.

2.5.1. Effect of seasonality on honey bee foraging preferences and pollen diversity

Honey bees, despite being extraordinarily polylectic, usually select a limited number of flowering plant species to forage on (Lau et al. 2019). Here, we observed a strong temporal turnover in the composition of pollen collected by honey bees, which partly reflects honey bee foraging preferences, and partly reflects plant phenology and pollen availability in the study area, at least in the early and late flowering season. In particular, trees were revealed to be a key resource for honey bees in spring. *Salix* spp. in April, *Malus*/*Pyrus* spp. in May, and *Castanea sativa* in June were the main pollen taxa collected by honey bees. The importance of trees for honey bees is well-known (Donkersley 2019) as they are often among the early-flowering species. We showed that Compositae such as *Taraxacum* spp., *Helianthus* spp., and *Senecio* spp., and the legume *Trifolium repens* strongly supported honey bees throughout spring and summer. In August, *Artemisia* spp. pollen was highly represented, as this taxon is common in fallows and urban areas. In September, almost all pollen was collected on *Hedera* spp., which was the most abundant flowering plant species in late summer in the study area (Prosser et al. 2019).

Pollen diversity was also shaped by seasonality. We observed that the start and end of the flowering season, *i.e.*, April and September, were characterized by a dearth of floral resources, while we observed a peak of pollen diversity in May. Interestingly, despite the high percentage of agricultural areas in certain landscapes, we did not highlight a strong effect of mass flowering crops such as apple, which flowers in April and May, which can potentially reduce the diversity of pollen collected by honey bees, as bees tend to focus on these resources. The diversity of pollen collected in August was surprisingly high, given that resources are usually relatively scarce at the end of summer (Garbuzov et al. 2015; Requier et al. 2015; Danner et al. 2017; Sponsler et al. 2020). The high diversity found in August could be explained by the mid-elevation of sampling sites, which causes a shift in pollen decline from August to September.

Since many of the pollen taxa that we collected were grouped at the family level, and almost all the remaining ones were identified at the genus level, pollen diversity could have been even higher in some seasons, if we had been able to achieve species-level identifications of pollen.

2.5.2. Interactive effect of seasonality and landscape composition on pollen diversity

Our results highlighted that the diversity of pollen collected by honey bees was influenced by the interaction between collection month and landscape composition, *i.e.*, the proportion of semi-natural areas.

Pollen diversity was independent of the proportion of semi-natural areas from April to August. Several studies found that pollen composition was not affected by landscape composition (Danner et al. 2017; Guzman et al. 2019; Simanonok et al. 2020; Jones et al. 2021). This can be explained by both the structure of selected landscapes and the foraging behaviour of the honey bee. Even if some landscapes were strongly modified by anthropogenic activities, they always included a certain proportion of semi-natural habitats, ranging from 25 to 83%, which comprised both open habitats and forests. Moreover, many agricultural areas, which ranged from 7 to 52%, were intermixed with semi-natural habitats. In all landscapes, honey bees were therefore able to collect pollen in areas that offered a high amount of resources, at least until mid-summer. Moreover, honey bees can travel more than 10 km from their hive, although they usually forage < 1 km away from the hive (Von Frisch 1967; Visscher and Seeley 1982; Tautz 2008; Seeley 2019). Several studies highlight that landscape composition affects the distance to which honey bees forage, and in particular that their foraging distance increases in simplified landscapes (Steffan-Dewenter and Kuhn 2003; Abou-Shaara 2014; Danner et al. 2017). A study from the UK reports that in landscapes dominated by the common heather (*Calluna vulgare*), the average distance of foraging honey bees strongly changed during the season: while in May it was about 1 km, in August, during the flowering period of the common heather, the average foraging distance increased up to 5.5 km (Beekman and Ratnieks 2000). In our study, however, we did not collect data on how far honey bees travelled to collect pollen. In some areas, honey bees may need to travel much further to obtain food resources, with potentially negative consequences for colony fitness.

On the other hand, in September, pollen diversity increased with increasing proportion of semi-natural habitats at 3 and 5 km radius buffers. While honey bees are able to collect heterogeneous pollen independently of landscape composition from spring to mid-summer, the scarcity of floral resources in late summer may turn semi-natural areas into key habitats. In this part of the season, when nectar sources are decreasing (Tew et al. 2022), honey bees search for the most diverse pollen sources in order to breed winter individuals that must develop adequate fat bodies (Frias et al. 2016). Semi-natural areas in the landscape can promote late-season pollen protein and winter survival of honey bee colonies (Kuchling et al. 2018; Simanonok et al. 2020; Rutschmann et al. 2022). Therefore, the higher number of resources offered by these habitats can be crucial for honey bees at such a critical stage of the colony cycle.

2.6. Conclusions

Our work highlighted that the diversity of pollen was shaped by seasonality, as we observed a strong temporal turnover in the diversity of pollen collected by honey bees. Landscape composition only affected pollen diversity at the end of the flowering season. In spring and summer, honey bees were able to efficiently forage in all landscapes, probably even due to the presence of a few key plant species such as *Trifolium repens* that could strongly support colonies (Filipiak et al. 2017). In late summer, when resources were generally scarce, semi-natural areas became fundamental for honey bees, as they offered a wider range of floral resources. However, more research on this topic is needed, as landscape composition could also affect other aspects of honey bee ecology. For example, complementing this study with observations on foraging flight distances and colony fitness could help elucidate the potential effect of landscape simplification on honey bees. Moreover, another aspect that should be taken into account is the potential contamination of food sources for bees, as the presence of intensively cultivated areas can affect pollen quality due to pesticide presence (Zioga et al. 2020).

2.7. Acknowledgments

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CHAPTER 3

Impact of landscape composition on honey bee pollen contamination by pesticides: A multi-residue analysis

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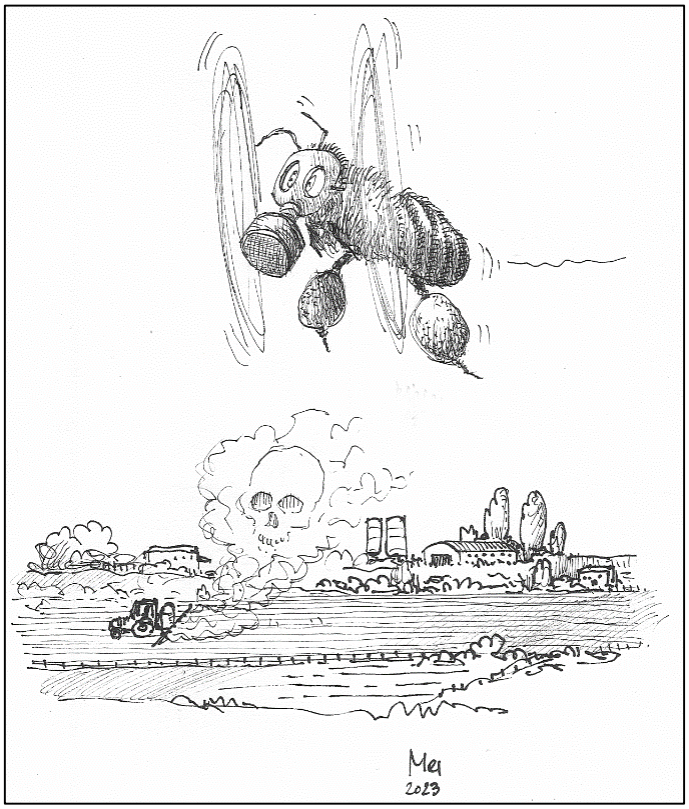

3.1. Abstract

The honey bee is the most common and important managed pollinator of crops. In recent years, honey bee colonies faced high mortality for multiple causes, including the use of plant protection products (hereafter pesticides). This work aimed to explore how contamination by pesticides of pollen collected by honey bees was modulated by landscape composition and seasonality. We placed two honey bee colonies in 13 locations in Northern Italy in contrasting landscapes, from which we collected pollen samples monthly during the whole flowering season in 2019 and 2020. We searched for almost 400 compounds, including fungicides, herbicides, and insecticides and acaricides. We then calculated for each pollen sample the Pollen Hazard Quotient (PHQ), an index that provides a measure of multi-residue toxicity of contaminated pollen. Almost all pollen samples were contaminated by at least one pesticide. We detected 95 compounds, mainly fungicides, but insecticides and acaricides showed the highest toxicity. Fifteen % of the pollen samples had medium-high or high levels of PHQ, which could pose serious threats to honey bees. Fungicides showed a nearly constant PHQ throughout the season, while herbicides and insecticides and acaricides showed higher PHQ values in spring and early summer. Also, PHQ increased with increasing cover of agricultural and urban areas from April to July, while it was low and independent of landscape composition at the end of the season. The cover of perennial crops, *i.e.*, fruit trees and vineyards, but not of annual crops, increased PHQ of pollen samples. Our work highlighted that the potential toxicity of pollen collected by honey bees was modulated by complex interactions among pesticide categories, seasonality, and landscape composition. Due to the large number of compounds detected, our study should be complemented with additional experimental research on the potential interactive effects of multiple compounds on honey bee health.

3.2. Introduction

The honey bee, *Apis mellifera* Linnaeus, is the most important managed pollinator species, with an estimated economic value to crop yield of about $6.4 billion in the USA alone (Reilly et al. 2020). Despite a global increase of 85% in the number of managed honey bee colonies since the 1960s, in recent years honey bees have been experiencing high mortality, especially in North America and Europe (Osterman et al. 2021). This syndrome is often referred to as Colony Collapse Disorder (CCD) (vanEngelsdorp et al. 2009), and it is related to several causes (Goulson et al. 2015). Among these causes, the most relevant ones seem to be the spread of parasites and pathogens, such as the parasitic mite *Varroa destructor* Anderson & Trueman and the fungus *Nosema ceranae* (Rosenkranz et al. 2010; Le Conte et al. 2010; Geffre et al. 2020), and nutritional stress related to a restricted diet due to limited availability of floral resources, often caused by semi-natural habitat loss (Naug 2009; Branchiccela et al. 2019). Moreover, the use of plant protection products (hereafter, pesticides) that could contaminate pollen and nectar can also play a key role in the decline of these pollinators (Henry et al. 2012; Sánchez-Bayo et al. 2016; Tsvetkov et al. 2017; Woodcock et al. 2017).

Agriculture has increased by c. 40% globally in the last 50 years (Aizen et al. 2019), and consequently the use of pesticides and their potential impact on bees (DiBartolomeis et al. 2019). Fungicides, herbicides and insecticides are commonly used for crop protection (Zioga et al. 2020). Insecticides include the compounds that pose major threats to arthropods, since they are designed to directly affect them (Fairbrother et al. 2014; Lundin et al. 2015; Tsvetkov et al. 2017; Woodcock et al. 2017; Wood and Goulson 2017; Holder et al. 2018). Few studies have investigated the effect of fungicides and herbicides on honey bees and pollinators in general, despite being the most widely used compounds in terms of applied tonnes (Tamburini et al. 2021, EUROSTAT, 2023). However, effects such as the reduction of bee foraging efficiency, longevity and survival rate, and changes in gut microbiota have been reported, with a large variability among compounds (Cullen et al. 2019; Rondeau and Raine 2022).

Since most pesticides are applied in crop fields, bees foraging in landscapes dominated by intensive farming should be more exposed to these compounds (David et al. 2016; Böhme et al. 2018). A high cover of semi-natural habitats could help dilute pollen contamination since honey bees could collect pollen from uncontaminated floral resources. The amount of crops in the landscape is known to potentially boost insecticide concentration in pollen, especially for some highly toxic neonicotinoids, such as thiamethoxam and

imidacloprid, and organophosphates, such as chlorpyrifos (Calatayud-Vernich et al. 2018; Wood et al. 2019). Also, the cover of specific crop categories in the landscape, such as apple and cherry orchards and blueberry plantations, could predict pesticide residue concentration in pollen (McArt et al. 2017; Graham et al. 2021, 2022). However, pesticide drift from crops could lead to high contamination also in surrounding areas, resulting for example in a high number of pesticides detected in pollen collected by bees in semi-natural habitats (Lambert et al. 2013; Calatayud-Vernich et al. 2018).

The use of pesticides is not continuous throughout the year. Therefore, seasonality can play a strong role in increasing or reducing the level of contamination by pesticides in pollen collected by honey bees, even because mechanisms of exposure to pesticides of honey bees might change throughout the season (Krupke et al. 2012). The highest concentration of pesticide residues in pollen collected by honey bees is usually observed in April and May since a large part of pesticide applications is made in spring (Lambert et al. 2013; Tong et al. 2018; Liu et al. 2022). However, some studies reported contamination peaks in mid-season or even later, *e.g.*, between July and September (Long and Krupke 2016; Tosi et al. 2018). Moreover, previous works reported a reduction in pesticide concentration after the blooming of focal crop species, which might be related to different foraging preferences, but also to the biodegradation of pesticides with increasing temperatures (David et al. 2016). Most of these studies, however, focused on specific pesticide categories such as insecticides, or a limited range of compounds, while multi-residue analyses on temporal and spatial variability of pollen contamination are largely still missing. This approach can provide a comprehensive picture of the importance of single crops and associated pesticides across heterogeneous agricultural landscapes.

In this work, we explored how pesticide residues in pollen collected by honey bees were affected by seasonality, landscape composition, and compound category. We selected 13 sampling locations in Northern Italy from which we collected pollen samples monthly for two consecutive years. For each pollen sample, we used liquid chromatography-tandem mass spectrometry and gas chromatography-tandem mass spectrometry to search for 375 compounds, including insecticides, acaricides, fungicides and herbicides. Then, for each compound and each pollen sample, we calculated the Pollen Hazard Quotient (PHQ), a measure of potential pollen toxicity for honey bees. We expected that insecticide would have a major impact on the potential toxicity of pollen, especially for some categories, such as neonicotinoids. We also expected higher pollen

contamination at the beginning of the season, especially in areas with a high cover of crops and fruit orchards in particular.

3.3. Materials and methods

3.3.1. Study area and site selection

The study was carried out in Northern Italy, in the Trentino-Alto Adige and Veneto regions (NE Italy), where we selected 13 sampling locations characterized by contrasting landscapes. In 3 km radius buffers around the sampling locations, the cover of semi-natural areas ranged from 1 to 92% (mean = 50%), the cover of agricultural areas ranged from 8 to 87% (mean = 38%), and the cover of urban areas ranged from 0 to 30% (mean = 12%) (Table S3.1, Figure S3.1). Site elevation ranged between 91 and 1,481 m a.s.l. (mean = 535 m a.s.l.). As a result, the climate in the sampling areas was highly variable: the mean annual temperature ranged between 6.8 °C (1500 m a.s.l.) and 13.5 °C (90 m a.s.l.) (mean = 10.8 °C), while the total precipitation ranged between c. 1,100 and 1,700 mm/year (mean = 1,260 mm/year).

In 2019 and 2020, we placed two honey bee colonies at each location. Activating pollen traps at the hive entrance for 48 hours, we collected pollen samples monthly from April to September, for a total of six samples per year per location. Due to adverse climatic conditions, we were not able to collect pollen samples each month at a few locations: in 2019 we collected only five samples at two locations and three at one, while in 2020 we collected only five samples at three locations. Pollen samples were then stored at -20 °C.

3.3.3. Landscape composition

We extracted the cover of the main habitat types at each sampling location using the regional land-use map (© European Union, Copernicus Land Monitoring Service 2018, European Environment Agency) at two scales considering the foraging distance of honey bees, *i.e.*, 3 km and 5 km radius buffers around the sampling locations (Table S3.2). Since most of the 15 land-use classes were correlated with each other, we performed a Principal Component Analysis (PCA) to extract the landscape composition at each sampling location. We extracted the first three eigenvalues, PC1, PC2, and PC3, which respectively explained 25.9%, 18.9%, and 14.21% of the total landscape variability at 3 km radius buffers (Figure S3.2 *a*), and 31.2%, 20.6%, and 12.75% at 5 km radius buffers (Figure S3.2 *b*). All statistical analyses were performed using the *R* software version 3.6.1 (R Core Team 2019).

3.3.4. Pesticide analysis

We searched for 375 active ingredients in pollen samples, including insecticides and acaricides (N = 169), fungicides (N = 117), and herbicides (N = 89) (Table S3.3, Figure S3.3 *a*). For the chemical analyses, pollen was grounded using a mill in liquid nitrogen. From each sample, we extracted two grams of pollen according to the QuEChERS method (EN 15662:2018) (European Standard EN 15662:2018 2018). The extracts were then analysed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and gas chromatography-tandem mass spectrometry (GC-MS/MS) (Tables S3.4, S3.5).

LC-MS/MS analyses were performed using an Acquity UPLC coupled with a XEVO TQ mass spectrometer equipped with an electrospray ion source (Waters Corporation, Milford, USA) and operating in MRM mode recording two specific transitions for each pesticide. The column used was an Acquity UPLC BEH C18 (1.7 µm particle size, 100 × 2.1 mm), and the mobile phases were A (water with 0.1% formic acid) and B (methanol with 0.1% formic acid). The gradient conditions were as follows, based on times (t): t_1 = 0-0.25 min, hold 95% A, 5% B; t_2 = 0.25-6 min, ramp linearly to 70% B; t_3 = 6-7.5 min, hold 70% B; t_4 =7.5-9.5 min, ramp linearly to 100% B; t_5 = 9.5-12 min, hold 100% B.

GC-MS/MS analyses were performed by Agilent 8890 gas chromatograph coupled to a TQ 7010B mass spectrometer (Agilent Technologies Inc., USA) equipped with an electron impact ion source (ionization energy = 70 Ev EI). GC analysis was conducted on a Restek Rxi-5Sil MS capillary column (20 m × 0.18 mm internal diameter × 0.18 µm) (Restek, USA) and the following conditions were used: He constant flow 1 mL/min, inlet temperature 260 °C, injection volume 1 µL (split, 1:10), MS transfer line temperature 280 °C, temperature program: 60 °C for 1 minute, then 60 °C/min ramped to 170 °C, followed by 20 °C/min ramped to 320 °C (held for 1 minute). The acquisition, as well as for the LC/MS system, was carried out in MRM mode.

Glyphosate was quantified following the QuPPe-PO-Method (M1.9, Version 12) (Anastassiades et al. 2020) which involves the use of an LC-MS/MS (Acquity UPLC coupled with a XEVO TQ mass spectrometer) system equipped with a Raptor Polar X column.

3.3.5. Validation method

Analytical parameters of the pollen multi-residue method such as matrix effect, limits of quantification (LOQs), limits of detection (LODs), linearity, precision and trueness were evaluated according to SANTE guidelines (SANTE/12682/2019; European Commission, 2020) (Tables S3.6, S3.7). All pesticide parameters

were quantified using five-point matrix-matched calibration curves ($R^2 > 0.98$) and triphenyl phosphate as internal standard. Matrix effects were evaluated by comparing the slope of the calibration curve done in solvent and the slope of that prepared in the extract of the pollen matrix. To verify the recovery (Rec%) and the repeatability (RSD%) of the method, a blank pollen matrix (no pesticide contamination) was used. Pesticides were added to the matrix at three concentration levels: 10, 50, and 200 µg/kg, and each added concentration level was analysed sixfold. Average values of Rec% and RSD% over three concentration levels complied with the SANTE guidelines (Rec % 70-120% and RSD% < 20%) (European Commission, 2020). The sensitivity of the method was estimated by establishing the LOQs according to SANTE guidelines, and LODs were estimated as one-third of the quantification limit. According to the SANTE guidelines, all obtained pesticide data were not corrected by the recovery since it was found to be between 80% and 120%.

3.3.6. Pesticide risk assessment

After determining the concentration in ppm, we calculated the Pollen Hazard Quotient (PHQ) (Stoner and Eitzer 2013) for each compound in each pollen sample. PHQ is a measure of hazard from pesticide residues in pollen in relation to acute toxicity to honey bees, and it is calculated as the ratio between the compound concentration in ppb (µg/kg) and the oral or contact LD_{50} for honey bees. We retrieved oral LD_{50} from the University of Hertfordshire Pesticide Properties DataBase (Lewis et al. 2016). However, we used contact LD_{50} for five compounds (bromophos ethyl, emamectin benzoate, fluazifop-p-butyl, piperonyl butoxide, and tetradifon), for which we could not obtain oral LD_{50}. Then, we determined the total PHQ of each pesticide category (fungicides, herbicides, and insecticides/acaricides) in each pollen sample by summing PHQ values of each category in each sample, and the total PHQ for each pollen sample by summing PHQ values of all compounds in each sample. We assumed additive toxic effects of multiple pesticides due to the lack of information on possible synergistic or antagonistic effects.

In addition, we calculated the acute risk quotient (RQ) for honey bees for each compound in each pollen sample using the US Environmental Protection Agency BeeREX model. While PHQ is ideal for evaluating the effect of specific drivers on multi-residue contamination of pollen, it does not take into account the amount of pollen consumed by honey bees, as opposed to the BeeREX model. First, we calculated the total dose of each compound consumed by each bee as the product between the concentration of the compound in µg/mg and the dose of pollen consumed by the honey bee in mg/day. Since we were not interested in testing

how pesticide toxicity varied for different bee castes, we considered the highest consumed dose, which is 9.6 mg/day for nurse workers. Second, we calculated the acute RQ as the ratio between the total dose of pollen consumed by each bee and the oral LD_{50} for the compound. An acute RQ > 0.4 exceeds the concern threshold and indicates high toxicity of the compound for honey bees.

3.3.7. Statistical analyses

In order to determine the effect of seasonality, landscape composition, and pesticide category on PHQ of each pollen sample, we used linear mixed-effects models. We included the total PHQ of each pesticide category (fungicide, herbicide, and insecticide/acaricide) as response variable (ln-transformed), while selected explanatory variables were the year and the interaction between the sampling month and pesticide category, between the sampling month and landscape PC1, between the sampling month and landscape PC2, and between the sampling month and landscape PC3. Landscape PC1, PC2 and PC3 were calculated at both spatial scales, *i.e.*, 3 km and 5 km radius buffers around the sampling locations. To account for the repeated measures, we included the sample ID nested within the location ID as random factor. Then, starting from the full model, we used a backward deletion procedure, removing one-by-one interactions with *p*-value > 0.05, and re-ran the model to correctly interpret the main effects. We tested whether model residuals were spatially auto-correlated using Moran's I in the *R* package *ape* (Paradis and Schliep 2019) and we detected no spatial autocorrelation (global test, *p*-value = 0.859).

Then, we focused on the effect of specific crop categories on PHQ of pollen samples. We built two linear mixed-effects models using the total PHQ of pollen samples as response variable, and the cover of annual crops (including non-irrigated arable land, complex cultivation patterns, and agriculture with significant areas of natural vegetation) and perennial crops (including fruit trees, berry plantations, and vineyards) in the landscape at 3 km and 5 km radius buffers around the sampling locations as explanatory variables. We also included the location ID as random factor. Since the results of the models at the two spatial scales were similar, we presented in the main text only the results of the models at the 3-km radius scale.

3.4. Results

Out of the total 147 samples, only 4% were free of pesticide residues. We detected a total of 97 compounds in pollen samples, mostly fungicides (N = 48), followed by insecticides (N = 32) and only a few herbicides (N =

17) (Figure S3.3 *a*). The proportion of the detected compounds was similar throughout the season (Figure S3.3 *b*). On average, we detected 11 compounds in each pollen sample.

The concentration of detected pesticides was significantly higher for fungicides than for insecticides/acaricides and herbicides (Figure 3.1 *a*). The most abundant compounds were all fungicides, *i.e.*, captan, found in 30% of samples with a total concentration (summed across all pollen samples) of 320.135 ppm (max = 142 ppm, mean = 2.178 ppm); folpet, found in 10% of samples with a total concentration of 28.409 ppm (max = 15.6 ppm, mean = 0.193 ppm); and zoxamide, which was the most common detected compound, found in 80% of samples, with a total concentration of 16.955 ppm (max = 3.99 ppm, mean = 0.115 ppm) (Table S3.3). The fungicides spiroxamine and penconazole were also commonly detected in our samples, respectively found in 62% and 50% of samples (Table S3.3).

However, the overall toxicity of fungicides was low, as these compounds are mostly characterized by high LD_{50} values. The highest contribution to the total PHQ of pollen samples was made by insecticides/acaricides, in particular neonicotinoids and organophosphates (Figure 3.1 *b*). The compounds with the highest total PHQ (summed across all pollen samples) were all insecticides, *i.e.*, dimethoate, imidacloprid, and indoxacarb (Table S3.3). Dimethoate showed a total PHQ of 31,870 and, despite its toxicity, it was very common, being found in 23% of samples, with a total concentration of 3.187 ppm (max = 1.370 ppm, mean = 0.022 ppm). Three pollen samples showed particularly high dimethoate concentrations, which led to PHQ values for the compound of 13,700, 7,470, and 4,840, corresponding to 137%, 75%, and 48% of the oral LD_{50}, respectively. Dimethoate in these three pollen samples exceeded the concern threshold for acute RQ, with acute RQ values of 0.132, 0.072, and 0.046, respectively. Imidacloprid showed a total PHQ of 25,405, and it was also common, being found in 20% of samples, with a total concentration of 0.094 ppm (max = 0.038 ppm, mean = 0.001 ppm). One pollen sample showed a peak of imidacloprid concentration, which led to a PHQ value of 10,270, corresponding to 102% of the oral LD_{50}, and an acute RQ value of 0.099, beyond the concern threshold. Indoxacarb showed a total PHQ of 4,821 and was less common than dimethoate and imidacloprid, being found in 7% of samples with a total concentration of 1.119 ppm (max = 0.812 ppm, mean = 0.008 ppm). One sample showed a peak of indoxacarb concentration, which led to a PHQ value of 3,500, corresponding to 35% of the oral LD_{50}, which however did not exceed the concern threshold for acute RQ.

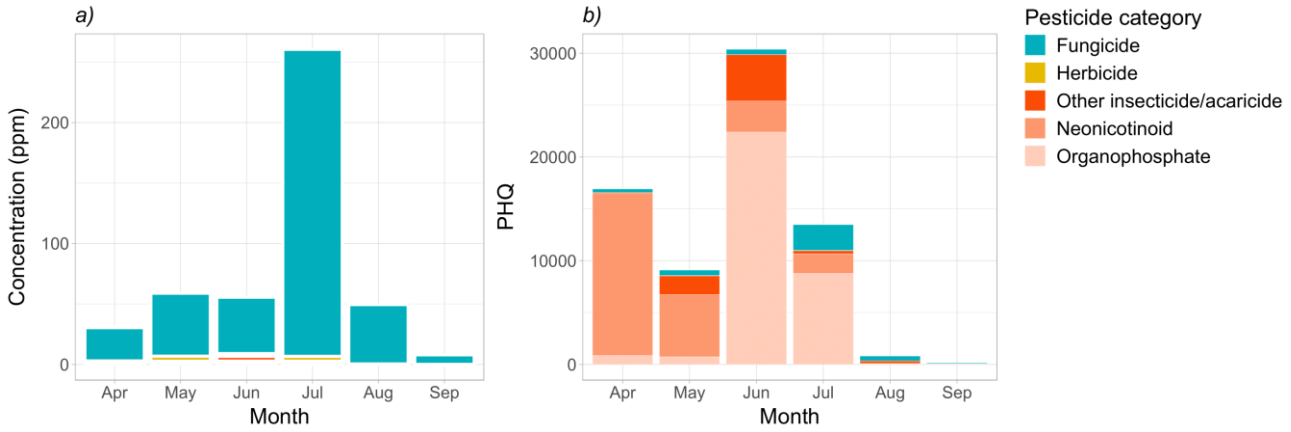

Figure 3.1: Bar plots showing *a)* total concentrations in ppm of pesticide categories for each sampling month and *b)* total PHQ of pesticide categories for each sampling month.

Although the toxicity of single compounds in terms of acute RQ was relatively low, total PHQ values of pollen samples were high (total PHQ > 1000) in 8% of samples, medium-high (500 < total PHQ < 1000) in 7% of samples, medium (50 < total PHQ < 500) in 22% of samples, and low (total PHQ < 50) in 58% of samples. Total PHQ was influenced by the interactions among seasonality, landscape composition, and pesticide category at both 3 km and 5 km radius buffers around the sampling locations (Tables 3.1, S3.8). PHQ of pollen samples changed throughout the season based on the pesticide category. The peak of PHQ of fungicides was in July, but PHQ was approximately constant in all months. On the other hand, PHQ of herbicides and insecticides and acaricides was higher from April to June, and it decreased, especially for insecticides and acaricides, at the end of the season (Figure 3.2).

Table 3.1: Results of the linear mixed-effects model testing the effect of the interaction between the sampling month and pesticide category, the interaction between the sampling month and landscape PC1, the interaction between the sampling month and landscape PC2, and the sampling year on PHQ of pollen samples (ln-transformed). Landscape PC1, PC2 and PC3 were calculated using the regional land-use map categories in 3 km radius buffers around the sampling locations. Values in bold indicate significant effects (*p* value < 0.05). Only significant results after a backward stepwise model selection procedure are reported.

Explanatory variable	χ^2	**df**	***p* value**
Intercept	0.001	1	0.980
Month	14.108	5	**0.015**
Pesticide category	44.770	2	**< 0.001**
Landscape PC1 (3 km)	15.363	1	**< 0.001**
Landscape PC2 (3 km)	0.137	1	0.711
Landscape PC3 (3 km)	0.103	1	0.749
Year	0.787	1	0.375
Month x Pesticide category	34.736	10	**< 0.001**
Month x Landscape PC1 (3 km)	23.513	5	**< 0.001**

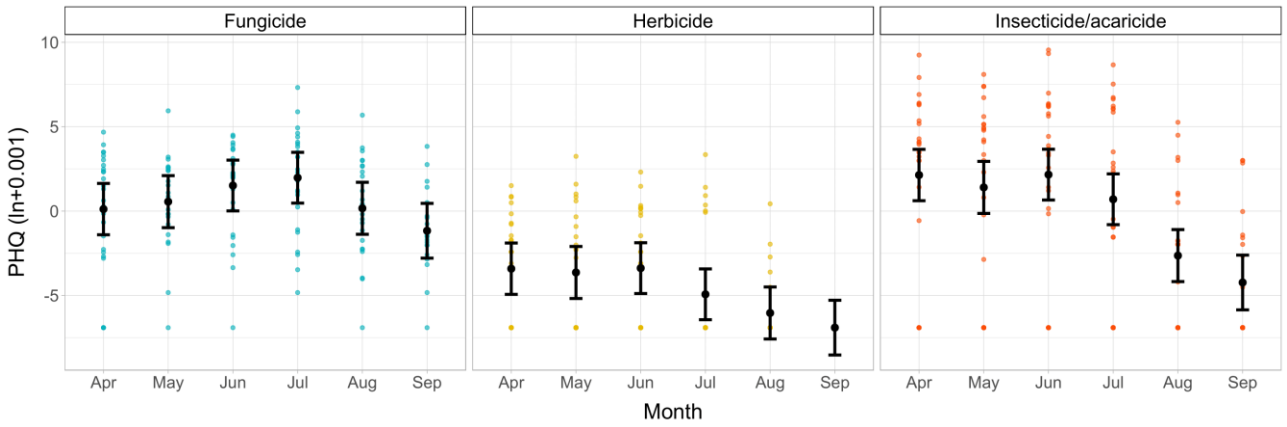

Figure 3.2: Plots showing the effect of the interaction between the sampling period and the pesticide category on PHQ of pollen samples (ln-transformed). Small coloured points represent raw data points, large black points represent model estimates, and bars represent the 95% confidence intervals.

PHQ was also influenced by the interaction between the sampling month and landscape PC1 calculated at both 3 km and 5 km radius buffers around the sampling locations. PHQ increased with increasing landscape PC1 from April to July, while in August and September, landscape composition did not affect PHQ of pollen samples (Figures 3.3, S3.4). Low values of landscape PC1 were related to semi-natural areas, in particular coniferous forests, natural grasslands, and sparsely vegetated areas, while high values of landscape PC1 were related to both agricultural and urban areas (Figure S3.2 *a*, *b*).

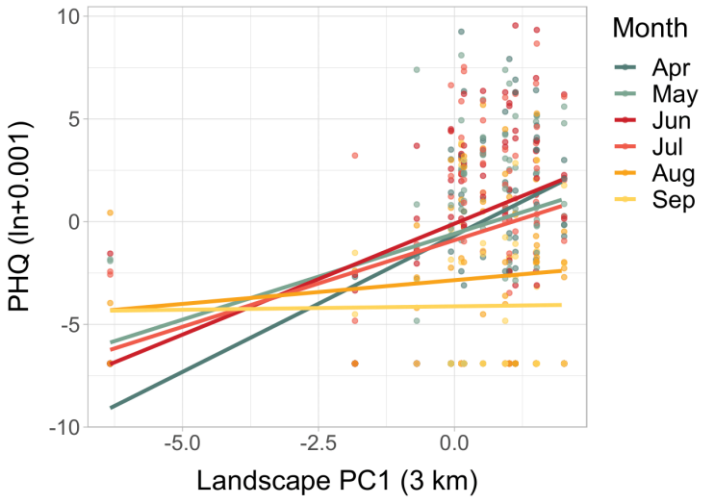

Figure 3.3: Plot showing the effect of the interaction between the sampling period and landscape PC1 on PHQ of pollen samples (ln-transformed). Landscape PC1 was calculated using the regional land-use map categories in 3 km radius buffers around the sampling locations. Points represent raw data points and lines represent model estimates.

Total PHQ of pollen samples increased with increasing cover of perennial crops, *i.e.*, fruit trees and berries plantations and vineyards, at both 3 km and 5 km radius buffers around the sampling locations (Tables 3.2, S3.9, Figures 3.4, S3.5). However, total PHQ was only marginally affected by the cover of annual crops

in 3 km radius buffers around the sampling locations (Table 3.2), and the effect was not significant at a larger scale (Table S3.9).

Table 3.2: Results of the linear mixed-effects model testing the effect of the percentage of annual and perennial crops in 3 km buffers around the sampling locations on PHQ of pollen samples (ln-transformed). Values in bold indicate significant effects (*p* value < 0.05).

Explanatory variable	Estimate	SE	df	*t* value	*p* value
Intercept	0.264	1.012	64	0.261	0.795
Annual crop % (3 km)	5.865	2.892	10	2.028	0.070
Perennial crop % (3 km)	13.108	3.646	10	3.595	**0.005**

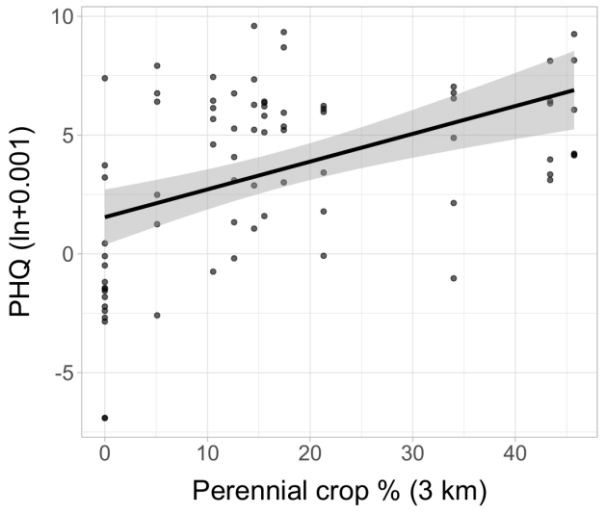

Figure 3.4: Plot showing the effect of the percentage of perennial crops (fruit trees and vineyards) in 3 km radius buffers around the sampling locations on PHQ of pollen samples (ln-transformed). Points represent raw data points, the line represents model estimates, and the shaded area represents the 95% confidence interval.

3.5. Discussion

Contamination by pesticides of pollen collected by honey bees can seriously threaten the health of honey bees and their colonies. Here, we performed a multi-residue analysis, testing for almost 400 compounds, and explored how the potential toxicity of pollen changed based on the pesticide category and how it could be modulated by landscape context and seasonality. For the first time, we demonstrated the interactive effects of these three variables on the potential toxicity of pollen collected by honey bees. In particular, we found that the peak of potential toxicity of pollen for honey bees changed for fungicides, herbicides, and insecticides and acaricides. Moreover, the effect of landscape composition, in particular of agricultural and urban areas, was modulated by the sampling month. Also, a high cover of perennial crops in the landscape, but not of annual crops, was associated with a higher potential toxicity.

We detected at least one compound in 96% of analysed pollen samples. The few pesticide-free pollen samples were all collected in the areas with the highest cover of semi-natural areas. Similar works carried out in Italy found a lower rate of pollen sample contamination, with 50-62% of samples contaminated by pesticides (Tosi et al. 2018; Martinello et al. 2019), however, a study conducted in our same study region found no samples free of pesticides (Favaro et al. 2019). Out of the 375 compounds searched in pollen samples, we identified 97 compounds (26%), a percentage similar to the one reported by Böhme et al. (2018) but much higher than Favaro et al. (2019), which only detected 13% of the searched compounds. Our surveys were done halfway through each month, but we had no information on whether pesticide treatments were applied in the surrounding areas before our surveys due to the high number of farmers and fields. Although the investigated areas were mainly mountainous and characterized by a relatively high cover of semi-natural areas, the agricultural and urban areas around the hives boosted the presence of pesticides in pollen.

Herbicides were rare in our pollen samples and comprised the least toxic compounds, despite their toxicity being considered moderate for honey bees (Iwasaki and Hogendoorn 2021). By far, the most common and abundant pesticides detected in pollen samples were fungicides, as also found in other works (Mullin et al. 2010; Friedle et al. 2021). The most commonly detected were zoxamide, which affects cytoskeleton and motor proteins, and spiroxamine and penconazone, which affect sterol biosynthesis in membranes. These fungicides are used for the control of fungal pathogens in a variety of crops, in particular vineyards, fruit orchards and cereal fields. The toxicity of fungicides for honey bees is generally low since they do not directly impact insects, and therefore their potential negative effects on honey bees are still debated (Iwasaki and Hogendoorn 2021). For example, Tamburini et al. (2021) highlighted no effect of azoxystrobin, a systemic broad-spectrum fungicide, on honey bee colonies at field-realistic exposure, while Al Naggar et al. (2022) showed that the same compound could have detrimental effects on the gut microbiota of bees. However, the major threat of fungicides is related to their interaction with other compounds (Iwasaki and Hogendoorn 2021; Ward et al. 2022): for example, the acute toxicity of some insecticides dramatically increases in the presence of fungicides (Tsvetkov et al. 2017). Moreover, even low doses of pesticide mixtures, considered not harmful for honey bees, may reduce the efficiency of insects exposed in early development stages (Prado et al. 2019). While laboratory experiments are fundamental to test the effect of single pesticides, it is crucial to also investigate the effect of multiple pesticides to which bees could be exposed in nature.

In general, only a few single compounds exceeded the concern threshold for the acute RQ. However, multi-residue analysis showed that 15% of analysed pollen samples had medium or high levels of PHQ, which could pose serious threats to honey bees. Insecticides and acaricides were less common than fungicides, however, they contributed most to the total PHQ of pollen (Friedle et al. 2021; Knapp et al. 2023). This result was expected since insecticides and acaricides are specifically formulated to negatively affect arthropods, therefore including non-target species. The most toxic insecticide categories were neonicotinoids, especially abundant in April and May, and organophosphates, which boosted PHQ of pollen in June and July. Neonicotinoids are highly efficient in controlling target species, but consequently also highly toxic to bees. These insecticides are known to strongly negatively impact bee survival, also during overwintering, and bee general health, especially the immune and reproductive systems (Tsvetkov et al. 2017; Woodcock et al. 2017). Since the use of neonicotinoids has led to higher risks to bees in the last decades (Goulson et al. 2018), strict regulations have been imposed. In the analysed pollen samples, imidacloprid was the neonicotinoid with the highest PHQ, as also found by Tosi et al. (2018). Imidacloprid is an insecticide with immunosuppressive activity, that also showed detrimental effects on bee memory (Williamson and Wright 2013; Di Prisco et al. 2013; Delkash-Roudsari et al. 2022, but see Dai et al. 2019). Despite a shift towards neonicotinoids in the last few years (DiBartolomeis et al. 2019), organophosphates are still commonly used in Italy (Porrini et al. 2016), as demonstrated by the widespread use of dimethoate.

3.5.1. Banned pesticides

In our pollen samples, we detected a few compounds that were banned for use in the EU because of health and environmental concerns, as already reported in Italy (Perugini et al. 2018). We identified the fungicide carbendazim, which was banned in the EU since 2014, at 6 locations and in 14 samples, 7 from 2019 and 7 from 2020, at higher concentrations in 2020 (mean = 0.002 ppm) than in 2019 (mean = 0.0003 ppm). The use of carbendazim is still widespread in the EU (Pesticide Action Network Europe, 2020), and it was also detected in food produced in Italy (EFSA 2022). However, carbendazim is also a metabolite of thiophanate-methyl, a fungicide which was still legal to use until 2021 and was also found in our samples. Therefore, the contamination of some of our pollen samples by carbendazim could be related to thiophanate-methyl conversion - although carbendazim and thiophanate-methyl were not found in association in two pollen samples, pointing out the need for stricter controls on pesticide use in Europe. The fungicide quinoxifen and

the insecticide chlorpyrifos were banned in Italy in March and April 2020 respectively, and after these dates, we detected them in 2 samples from 2 locations and 4 samples from 4 locations respectively. Some highly toxic compounds were banned in Italy after our surveys, *e.g.*, the neonicotinoid insecticides thiamethoxam and imidacloprid, and the oxadiazine insecticide indoxacarb, which was one of the compounds with the highest total PHQ. Lastly, in June 2020, the use of dimethoate was banned in Italy following the EU Regulation 2019/1090, however, its use was allowed in olive orchards until October 2020, after our samplings.

3.5.2. Interactive effect of pesticide category and seasonality on pollen toxicity

Seasonality differently affected PHQ of the three pesticide categories. For herbicides and insecticides and acaricides, PHQ was higher in spring and early summer and started to decrease in July. While herbicide contamination was generally low, the result for insecticides is probably largely related to applications in apple orchards, which in Italy are mainly made in spring and early summer (Garthwaite et al. 2015) and may have boosted pollen contamination. Similarly, other works found that pollen contamination by insecticides decreased at the end of summer (Tong et al. 2018; Friedle et al. 2021; Murcia-Morales et al. 2021). Some studies highlighted a peak of acaricide PHQ at the end of the season caused by treatments against *Varroa* mites (Murcia Morales et al. 2020), which we did not observe since our honey bee colonies were only treated with oxalic acid.

On the other hand, PHQ of fungicides slightly decreased in August and September, but it was more uniform throughout the season. The presence of fungicides in pollen samples was probably related to both apple orchards and vineyards in the landscapes. In apple orchards, fungicides are mainly applied to control diseases such as powdery mildews, apple scab, and cankers, while in vineyards they are used to prevent downy mildew, powdery mildew, and grey mould. These treatments are usually applied throughout the season, from the beginning of the vegetative growth to post-flowering of crops, and therefore caused little seasonal variation in fungicide PHQ.

3.5.3. Interactive effect of landscape composition and seasonality on pollen toxicity

The effect of landscape composition on PHQ of pollen collected by honey bees was modulated by the sampling month. PHQ increased with increasing cover of agricultural and urban areas from April to July, while it was lower in August and September and unrelated to landscape composition. It is well known that seasonality can have a strong effect on the detection of pollen contaminations (Koech et al. 2023), with fewer pesticides found

at the end of the season according to plant protection practices (Murcia-Morales et al. 2021). On the other hand, the effect of landscape context on honey bee pollen contamination is still debated. For example, some works highlighted that pesticide contamination was independent of landscape composition (Raimets et al. 2020; Koech et al. 2023; Knapp et al. 2023), while others showed that samples collected from hives placed in agricultural areas exhibited a higher concentration of pesticides (David et al. 2016; Meikle et al. 2020; Zaller et al. 2022). Our honey bee hives were not placed in intensive agricultural landscapes, since all selected landscapes were characterized by a certain cover of semi-natural areas (mean cover = 50%). Nevertheless, pollen contamination was high in areas with a higher cover of urban and agricultural areas, emphasizing that even a small amount of these areas may seriously threaten bees (Main et al. 2020).

An additional factor that should be considered when exploring the effect of landscape composition on pollen contamination is pesticide drift, which could increase risks to honey bees in agricultural-dominated landscapes. Pesticide residues could drift from focal crops to surrounding areas, contaminating pollen and nectar of wildflowers at field margins (Ward et al. 2022). Since a high diversity of floral resources could be related to a higher contamination risk of pollen collected by bees (Bednarska et al. 2022), the lower diversity of floral resources typical of the end of the season could have also minimized pollen contamination by pesticides.

Unexpectedly, urban areas emerged as key pathways of pollen contamination by pesticides. Contamination is usually lower in urban areas compared to rural areas (David et al. 2016, Siviter et al. 2023). However, recent studies underlined that urban habitats could be associated with high pollen contamination by pesticides, which may exceed that of agricultural habitats in some sampling periods (Benner et al. 2023), in particular for specific compounds such as neonicotinoid insecticides (Botías et al. 2017, Kavanagh et al. 2021). In addition, urban areas pose additional risks to honey bee health due to air pollution, which can negatively affect odour learning and memory (Leonard et al. 2019). The impacts of pesticide applications and pollution in general on pollinators in urban areas are still largely unexplored, despite the widespread use of pesticides in public and private gardens and the growing interest in urban beekeeping in most cities (Matsuzawa and Kohsaka, 2021), underscoring the need for further studies on this topic.

3.5.4. Effect of annual and perennial crops on pollen toxicity

The cover of annual crops, which in the study area mostly included maize, did not affect pollen contamination, as also found by Wintermantel et al. (2020). On the other hand, pollen collected in landscapes with a high cover of perennial crops, *i.e.*, fruit orchards and vineyards, was characterized by a higher PHQ. Böhme et al. (2018) analysed pesticide residues in pollen collected by honey bees in different habitats, highlighting the lowest pollen contamination in semi-natural habitats, intermediate contamination in grain fields, and the highest contamination in fruit orchards, similar to what we observed. Most of the perennial crops in our landscapes were apple orchards, which are crucial for the economy of our study area, and covered a large portion of our landscapes, ranging between 0 and 45% (mean = 9%). Apple is one of the most sprayed crops, with an average of 25 pesticide applications per year (Garthwaite et al. 2015), and therefore pollen collected in apple orchards often shows high levels of pesticide contamination (Knapp et al. 2023). However, it is also important to emphasize that most pesticide applications, especially for insecticides, are not permitted during blooming to safeguard pollinators. The high PHQ observed during apple blooming could be therefore related to pre-blooming pesticide applications, since most treatments in apple orchards in the study region are made between mid-March and the end of May (Garthwaite et al. 2015), but also to contamination at non-focal crops, as also highlighted by McArt et al. (2017).

3.5.5. Study limitations

Like most of the works performed under field conditions, we were not able to account for the potential interactive effects among pesticides, thus considering only additive effects when estimating multi-residue pollen toxicity, and potentially underestimating the negative effects on bee health. In fact, laboratory trials showed that the toxicity of pesticide mixtures can increase synergistically and also lead to an amplification of the sub-lethal effects of the least-toxic compounds, resulting in detrimental effects on bee health and colony longevity (Di Prisco et al. 2013). Therefore, future studies should also consider possible synergies among pesticides, in order to have an accurate and realistic assessment of the impacts of pesticides on honey bees.

3.6. Conclusions

Honey bees are key pollinators and are seriously threatened by pesticide applications. Here, we showed that the potential toxicity of pollen was related to the interaction of multiple factors, *i.e.*, the pesticide category, seasonality, and landscape composition. We highlighted that contamination was generally higher in spring and early summer, and that semi-natural areas can contribute to decreasing pollen contamination. We also found

that pesticide applications in urban and agricultural areas, especially in perennial crops, were probably responsible for a high contamination of pollen. To ensure the well-being not only of pollinators but also of humans, without overlooking crop protection, specific actions can be implemented. For example, farmers should decrease their dependency on pesticides, moving towards more sustainable management practices such as the use of pheromones and biopesticides (Pretty 2018, Baker et al. 2020). Agrochemical companies should formulate compounds that are more selective and less toxic to non-target organisms, focusing on new technologies such as controlled release systems (Singh et al. 2020). Lastly, beekeepers should always carefully assess where to place honey bee hives, preferring whenever possible areas surrounded by semi-natural habitats, in order to provide potentially uncontaminated resources for bees.

3.7. Acknowledgements

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CHAPTER 4

Temperature and not landscape composition shapes wild bee communities in an urban environment

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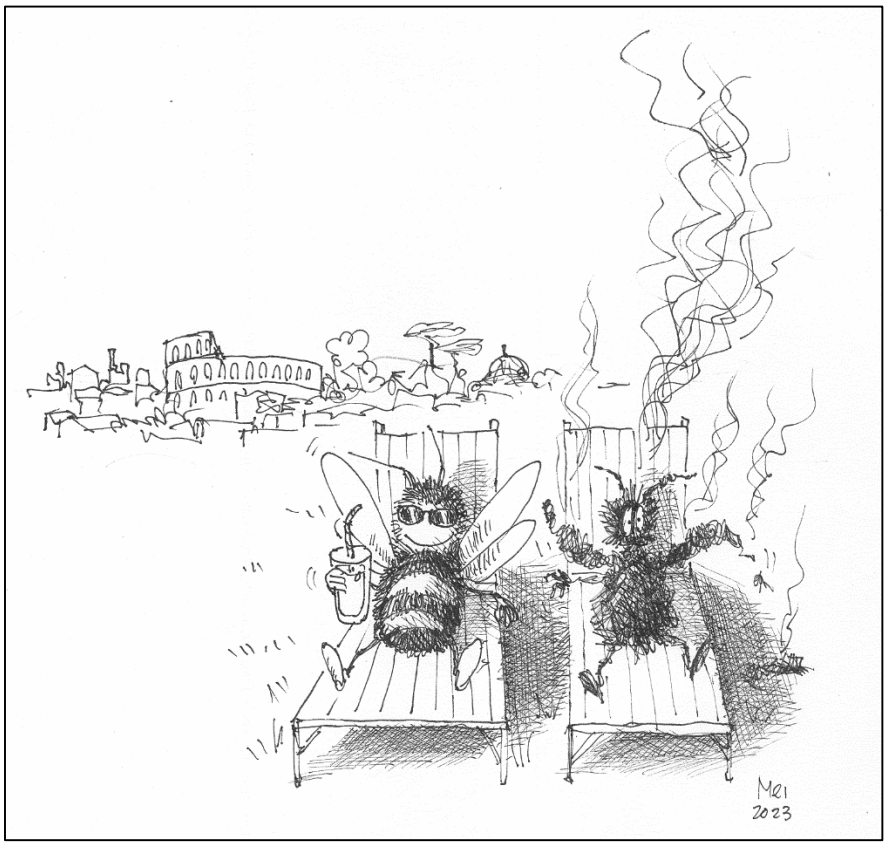

4.1. Abstract

More than half of the world's population lives in urban areas, a proportion that is expected to increase. Even if urbanisation is widely regarded as a major threat to global biodiversity, recent research highlighted the potential ecological importance of cities for pollinators. Key determinants of cities' ability to sustain pollinators are the presence of green areas and the connectivity between them. However, also temperature is expected to be of primary importance for pollinator activities. Here, we aimed to disentangle the effects of temperature, open habitat cover, and distance from the city centre on wild bee communities in the city of Rome (Italy). We selected 36 sites along two statistically independent gradients of temperature and open habitat cover, and we sampled wild bee communities using pan-traps for 4 months. Then, we measured functional traits of wild bee species, that is, body size, social behaviour, nesting strategy, and diet breadth. Temperature emerged as the main driver of wild bee communities, with communities richer in species and individuals at warmer temperatures. We found little species replacement between cold and warm sites. In addition, with increasing temperatures, bee communities were dominated by polylectic and small-bodied species. Here, we showed that in a highly urbanised environment, temperature shapes pollinator communities irrespective of other landscape metrics. Even if warming seemed beneficial for urban pollinator abundance and richness, it might strongly homogenise bee communities by selecting those traits that make species more easily adaptable.

4.2. Introduction

Globally, urban areas are expanding, while natural habitats shrink and become more remote (Grimm et al. 2008). Today, more than half of the world's population lives in urban landscapes, a proportion that is expected to increase to 85% by 2100 (OECD 2015). Urbanisation is widely regarded as a major threat to global biodiversity (Sala et al. 2000; Grimm et al. 2008); however, high levels of biodiversity may also thrive inside cities (Beninde et al., 2015). In particular, recent research highlighted the ecological importance of cities for pollinators (Hall et al. 2017; Theodorou et al. 2020; Wenzel et al. 2020). Urbanisation generally enhanced pollinator diversity compared to more intensified agricultural landscapes (Wenzel et al. 2020). Moreover, urbanisation appeared to shift the functional diversity of bee assemblages (Fournier et al. 2020). Over the last few years, it has been well established that insect pollinators are declining worldwide, mostly due to habitat fragmentation, loss and land-use intensification (Potts et al. 2010; Kennedy et al. 2013). In this context, understanding the potential role of cities as pollinator refuge becomes fundamental.

Key determinants of cities' ability to sustain pollinators are often related to the amount of green areas that are rich in nesting and food resources and to the connectivity between green fragments (Beninde et al. 2015; Wenzel et al. 2020; Biella et al. 2022). Moreover, besides the well-known positive effects of flower availability and high landscape connectivity, temperature is expected to be of primary importance for pollinator activities (Kühsel and Blüthgen 2015). As for ectotherms in general, temperature is one of the main drivers of insect pollinators' activities (Bale et al. 2002; Kühsel and Blüthgen 2015). Warmer environments are expected to be associated with higher growth rates, reduced development time, and increased probability of survival (Zuo et al. 2012). However, excessive climate warming can also lead to negative effects such as increased desiccation impairing insect growth, reproduction, and survival (Hamblin et al. 2018; Dale and Frank 2018). For pollinators, changes in climate are also expected to cause spatial and temporal mismatches with their food plants (Papanikolaou et al. 2017). The urban heat island effect makes cities warmer than surrounding natural areas (Oke 1973), providing an ideal system to study warming effects.

Considering the high diversity of bee life-history strategies, different species might respond to environmental changes in different ways (Bale et al. 2002). Because certain traits can be favoured in different environmental conditions, pollinator communities are likely to exhibit shifts in functional group composition in response to urbanisation and warming. Usually, under warming temperatures, organisms show a smaller

body size because warmer temperature increases metabolic rates and the associated costs for a given body size (Brown et al. 2004; Eggenberger et al. 2019). However, responses to increasing temperatures can be different from taxon to taxon, for example, bumblebees and Halictidae bees showed dissimilar thermal limits and desiccation tolerances (Burdine and McCluney 2019). In contrast, the relationship between wild bees' traits and urbanisation is more variable. However, most studies highlighted that urban areas act as a strong environmental filter on wild bees and that some functional traits are particularly beneficial to thrive in urban areas (Buchholz and Egerer 2020; Gathof et al. 2022). For example, cavity-nesting and polylectic species seemed to profit more from urbanisation than ground-nesting and oligolectic species (Wenzel et al. 2020; Sexton et al. 2021).

In this study, we aimed to disentangle the effects of temperature, open habitat cover, and distance from the city centre on wild bee communities in the metropolitan city of Rome (Italy). Mediterranean ecosystems are among the most vulnerable to climate change and belong to the world biodiversity hotspots for wild bees (Orr et al. 2021). In particular, Italy hosts an incredible diversity of bee species: more than half of the species listed for the entire Europe (Quaranta et al. 2018). However, few studies focus on Mediterranean bees and even less on bees in Mediterranean urban environments. Here, we selected 36 sites along two statistically independent gradients of temperature and open habitat cover and we sampled bee communities using pan-traps for 4 months. We then measured several functional traits of pollinator species. We hypothesised that wild bee diversity and abundance would increase with warmer temperatures and with a higher cover of open habitat at the landscape scale. In addition, we expected that communities would be dominated by species adapted to warm conditions at higher temperatures. In particular, we hypothesised traits to be filtered by the environment, with small bees being favoured at warmer temperatures near the city centre and below-ground nesters and oligolectic bees in areas with a higher cover of open habitat.

4.3. Materials and methods

4.3.1. Study area

The study area was the metropolitan city of Rome (Italy, 41.889956 N, 12.492286 E) (Figure 4.1 *a*), defined as the territory circumscribed by the great motorway ring (c. 360 km^2). Rome is the third most populated city in the European Union, with a population estimated at 3.8 million, and a density of 2,232 people/km^2 in 2016 (World Population Review 2016). The climate is temperate, with mild wet winters and warm summers. From

1970 to 2000, maximum mean annual temperature was 21.4 °C, minimum mean annual temperature was 9.1 °C, and mean precipitation was 140.9 mm. Over the last 40 years in Italy, summer temperatures increased on average by 0.52 °C every 10 years (Fioravanti et al. 2020). Approximately 54% of the study area is represented by urban areas (residential, industrial, and commercial areas), 16% by urban green areas (non-agricultural green areas, both artificial and semi-natural, including historical and archaeological sites, public parks and gardens, grasslands, shrublands, and forests), and the remaining 30% is covered in agricultural lands, pastures and water.

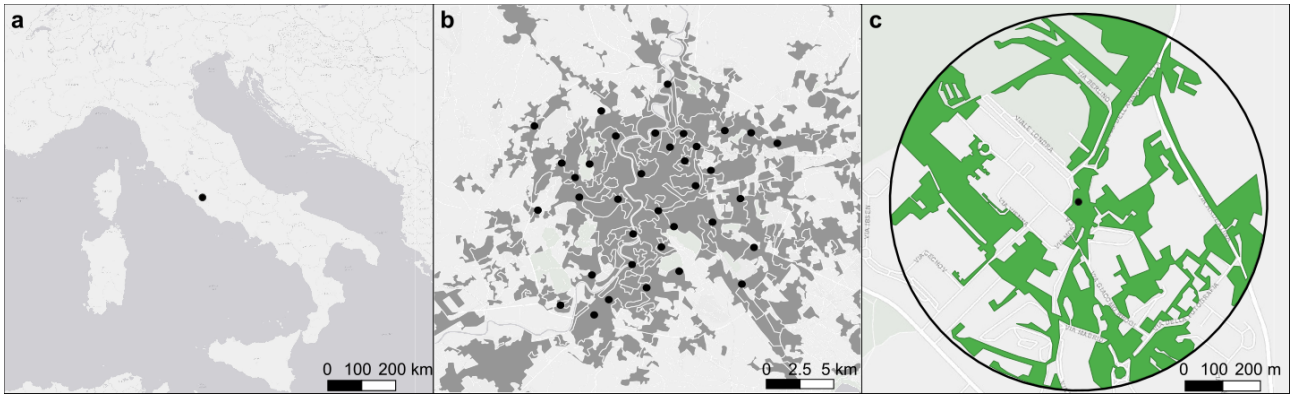

Figure 4.1: *a)* Study area in the city of Rome, Italy; *b)* spatial distribution of the 36 selected sampling sites (black points) along a gradient of urbanisation (shaded); *c)* example of open green habitat cover (in green) in a 500 m buffer. The centroid of the buffer is the point where pan-traps were placed. Maps were obtained from OpenLayers Plugin, QGIS.

4.3.2. Sampling design

We selected 36 sampling sites with open grassland vegetation with 2 km minimum and 26 km maximum distance from each other (Table S4.1, Figure 4.1 *b*). Sampling sites were chosen along two independent gradients: a gradient of median surface temperatures from 34 to 43 °C, and a gradient of open habitat cover in a buffer of 500 m radius spanning from 4% to 53%. We selected a 500 m radius because it emerged from several studies as the most appropriate landscape scale for wild bees (Gathmann and Tscharntke 2002; Steffan-Dewenter et al. 2002).

To obtain surface temperatures, we extracted the radiative skin temperature of the land surface, using Landsat 8 images with 30 m resolution. For each pixel, we calculated the median of the temperatures recorded over the sampling period, from June to September 2016, using Google Earth Engine (Ermida et al. 2020). This temperature metric is considered very relevant for insects and it has been used as a source of temperature data in several insect population models (Chuang et al. 2012; Blum et al. 2015).

To quantify the cover of the main habitat categories in a radius of 500 m around each sampling site, we identified urban, woody, and open habitat areas (*i.e.*, covered in herbaceous vegetation) and digitised polygons in Google Earth Pro manually (Google Earth 7.1.5.1557, 2015). Then, with a field survey, we validated the habitat classification obtained digitised polygons (Figure 4.1 *c*).

Moreover, we calculated the distance of each site from the city centre, that is, the Colosseum (41.890149 N, 12.492298 E). For Rome, this variable is a good proxy of decreasing disturbance along an urban-rural gradient (Fattorini 2014), as suburban areas are richer in semi-natural habitats than the central areas (Figure 4.1 *b*). Lastly, we assessed collinearity between all landscape variables, that is, land surface temperature, open habitat cover, urban cover, woody habitat cover, and distance from the city centre (Figure S4.1 *a*).

4.3.3. Wild bee sampling

At each sampling site, we collected wild bees (Apoidea: Anthophila) using a set of six yellow pan-traps, composed of plastic cups (750 ml, Ø 12.5 cm, h 4.5 cm) filled with a solution of water and 2% biodegradable dish detergent. As the vegetation in the sampling sites was below 50 cm, we placed pan-traps on the ground approximately 10 m apart, in two parallel lines of three pan-traps each. Due to the well-documented relationship between pollinator diversity and flower cover, we chose our sampling sites to reflect a similar amount of flower availability, allowing us to focus on the broader landscape context, that is, open habitat cover and temperature. We placed the pan-traps in small patches of open grassland vegetation characterised by similar plant composition and similar vegetation height (between 20 and 50 cm). Fieldwork was carried out every 2 weeks from mid-June to mid-September 2016, for a total of seven sampling rounds. For each sampling round, pan-traps were set out for 48 h. We excluded honey bees from this study because in our sampling area most honey bees are managed; therefore, their abundance strongly depends on beehive presence. The material was sorted by D.C. and identified by M.M. using identification keys (Additional references in Supplementary Information) and the reference collection of the Museum of Zoology of Sapienza, University of Rome. Species names follow Discover Life (Perlmutter 2010). Specimens are preserved at the Museum of Zoology of Sapienza, University of Rome.

Pan-trap sampling is a well-established method of collecting Hymenoptera and it usually captures a greater diversity of bee species compared to netting (Boyer et al. 2020). Even if the potential bias was constant

across all sites, by using pan traps to sample wild bees we may have under-sampled certain taxa (Prendergast et al. 2020), in particular larger bees (Roulston et al. 2007). In addition, several studies assessed colour preference in Hymenoptera, showing that trap colour affects the diversity of sampled bees and that, in most cases, yellow pan traps collected the largest numbers of bees (Buffington et al. 2021; Krahner et al. 2021). To evaluate the completeness of our sampling effort, we estimated the rarefaction curves using a coverage-based method (Chao et al. 2020) (Figure S4.2 *a*, *b*). With a few exceptions, the curves presented similar slopes and did not cross indicating that our species richness estimates were comparable across sites. However, the quick saturation showed by most curves stressed again that some groups of bees might have been under-sampled (Prendergast et al. 2020).

4.3.4. Wild bee functional traits

To investigate how life history and ecological characteristics mediate bee response to temperature, open habitat cover, and distance from the city centre, we sorted all recorded species based on functional traits. For each bee species, we collected 1) body size, 2) social behaviour (solitary or social), 3) nesting strategy (above-ground or below-ground), and 4) diet breadth (oligolectic or polylectic) (Table S4.2, Additional references in Supplementary Information). We selected the most informative functional traits in predicting bee responses to environmental change according to current literature and our knowledge (Williams et al. 2010). For body size, we measured body length of pinned specimens from head to metasoma end using graph paper. We measured one to five individuals, proportionally to how many specimens we collected in the field. For each species, whenever possible, we measured at least one female and one male. We then calculated the mean body size value for each species. We considered semi-social, social, and eusocial bees as social. Concerning nesting strategies, nesting categories were collapsed to below-ground and above-ground nesting to increase sample size and provide greater generality (Williams et al. 2010). Above-ground nesting bees included those species which build their nests in stems or pre-existing cavities. For diet breadth, we classified as oligolectic those bee species that are specialised to forage on one specific plant taxon, for example, one single plant family (Cane 2021). Finally, we assessed collinearity between all functional traits of wild bees (Figure S4.1 *b*).

4.3.5. Statistical analyses

First, we estimated the effects of surface temperature, open habitat cover, and distance from the city centre on wild bee abundance, species richness, and community evenness. We calculated wild bee community evenness

using the *R* package *codyn* (Hallett et al. 2016) with the default settings that calculate evenness as *Evar* (Smith and Wilson 1996). Then, we fitted three linear regressions using surface temperature, open habitat cover, distance from the city centre, and their two-way interactions as fixed factors and wild bee abundance, species richness, and community evenness as response variables. We used a natural logarithmic transformation of wild bee abundance and species richness to meet the assumption of normally distributed residuals. Pan-traps were placed in herbaceous open habitats that are considered to be the most influential habitat types for wild bees (Michener 2007; Winfree et al. 2011). However, some oligolectic species, in particular the ones nesting in wood, might be associated with trees. Therefore, we tested also the effect of woody cover on wild bees and wood-nesting bees, separately. As woody cover was negatively correlated with surface temperature ($r = -0.49$, p value = 0.002), we could not test for the effect of both variables in the same models. Woody cover did not affect the abundance, species richness, and community evenness of either wild bees or wood-nesting bees (Table S4.3). Therefore, we decided to present in the main text only models testing for the effects of open habitat, surface temperature and distance from the city centre on all wild bees.

Second, we measured changes in the community composition. Based on presence/absence community data, we calculated richness and replacement, the two components of pairwise Jaccard dissimilarity, using the function *betadiver* in the *R* package *vegan* (Oksanen et al. 2019). Then, we generated a temperature distance matrix, a habitat cover distance matrix, and a distance from the city centre distance matrix using the *vegdist* function with Euclidean distance, and a geographical distance matrix using the *R* package *geosphere* (Hijmans 2019). To test the effects of temperature, open habitat cover and geographic distance on wild bee community dissimilarity, we performed multiple regressions on the obtained distances using the *MRM* function in the *R* package *ecodist* with 1000 permutations (Goslee and Urban 2007). We used richness and replacement dissimilarities as response variables.

Third, to measure functional diversity, we used functional dispersion (FDis) and functional evenness (FEve). Functional dispersion represents the dispersion of bee species in a multi-dimensional trait space, that is, the distance of species to the centroid of all species in the community, weighted by their abundance (Laliberté and Legendre 2010). Functional evenness describes the regularity of species distribution in the trait space weighted by their abundance. First, we created a distance matrix using Gower distance for traits. Then, we calculated both indices based on abundance data and Gower distances for traits using the *R* package *FD*

(Laliberté et al. 2014). Finally, we fitted two linear models using functional dispersion and functional evenness as response variables and surface temperature, open habitat cover, distance from the city centre, and their two-way interactions as fixed factors.

Fourth, to assess shifts in trait values within communities due to environmental selection, we used community-weighted means (CWMs), which allow extracting community-level trait values weighed by species abundances. CWMs are particularly useful as the distribution of traits is one of the best methods to describe the community functional composition (Moretti et al. 2009). We calculated CWM for all wild bee functional traits, expanding nominal traits, that is, social behaviour, nesting strategy, and diet breadth, into binary traits (Podani 2005). Then, we fitted four linear regressions using surface temperature, open habitat cover, distance from the city centre, and their two-way interactions as fixed factors and CWMs for each of the four traits as response variables. We excluded kleptoparasite species from all models of functional traits, as they lack pollen-collecting structures and do not build their nests, and morphospecies from social behaviour and diet breadth models, as we lack these data. Moreover, when analysing nesting strategy, we excluded one site because it contained extreme values of above-ground nesting bees compared to all other sites, distorting our analysis (Grubbs test for outlier p value < 0.001), and violating assumption of residual normality.

Starting from each of the full linear models, we used a backward deletion procedure, removing one-by-one the interactions with p value > 0.05, and re-ran the model with all main effects to avoid overfitting and to correctly interpret the main effects. Moreover, in all models, we estimated variance inflation factors (VIFs) to assess possible collinearity issues between fixed effects. All VIF values were close to 1, indicating very little collinearity among predictors (Akinwande et al. 2015). All statistical analyses were performed using the *R* software version 3.5.1 (R Development Core Team 2019).

4.3.6. Multi-model inference

To evaluate the uncertainty of model selection, we also performed a multi-model inference analysis and compared the fit of all possible candidate models nested within each of the full models presented above. Within each set, models were ordered based on their second-order Akaike information criterion (AICc), with the best-fitting model showing the lowest AICc. For each model, we calculated the difference between the model AICc and the lowest AICc of the entire set of models ($\Delta AICc_i = AICc_i - AICc_{min}$). A model in a set can be considered plausible if its ΔAICc is below 2. Multi-model inference analyses were performed with the *MuMIn* package

(Burnham et al. 2011; Barton 2020). The final models selected according to the backward stepwise deletion were consistent with the ranking of the plausible models based on AICc (Tables S4.4, S4.5). Hence, we presented the results of the reduced models from the backward deletion procedure in the main text and reported the multi-model inference analyses only in the Supplementary Information.

4.4. Results

Overall, we collected 3,280 individuals of 96 species and morphospecies of wild bees (Table S4.2, Figure S4.3). The most abundant species was *Lasioglossum malachurum* (Kirby, 1802) (n = 897 individuals), followed by *Lasioglossum glabriusculum* (Morawitz, 1853) (n = 456 individuals) and *Seladonia gemmea* Dours, 1872 (n = 275 individuals). Among the collected species, 77% were polylectic bees, 22% showed a social lifestyle and 38% nested above ground.

Surface temperature was the only factor affecting wild bee abundance and richness (Table 4.1). Both abundance and species richness increased with increasing temperatures (Figure 4.2 *a*, *b*), while community evenness did not respond. Open habitat cover and distance from the city centre did not affect wild bee abundance, species richness, and community evenness (Table 4.1).

Table 4.1: Results of the linear models testing the effect of temperature, open habitat cover, and distance from the city centre on *a)* wild bee abundance (ln-transformed), *b)* species richness (ln-transformed), and *c)* community evenness. Values in bold indicate significant effects (p value < 0.05). No significant interactions were found.

Response variable	Explanatory variable	Estimate	SE	*t* value	*p* value
a) Wild bee abundance (ln)	Intercept	0.278	1.775	0.157	0.877
	Temperature	0.107	0.045	2.389	0.023
	Open habitat cover	-0.011	0.009	-1.164	0.253
	Distance from the city centre	0.015	0.041	0.362	0.720
b) Wild bee richness (ln)	Intercept	1.154	0.765	1.509	0.141
	Temperature	0.048	0.020	2.418	**0.016**
	Open habitat cover	-0.007	0.004	-1.814	0.081
	Distance from the city centre	< 0.001	0.018	-0.015	0.988
c) Community evenness	Intercept	1.070	0.366	2.919	0.006
	Temperature	-0.015	0.009	-1.567	0.127
	Open habitat cover	-0.004	0.008	-0.512	0.612
	Distance from the city centre	0.001	0.002	0.715	0.480

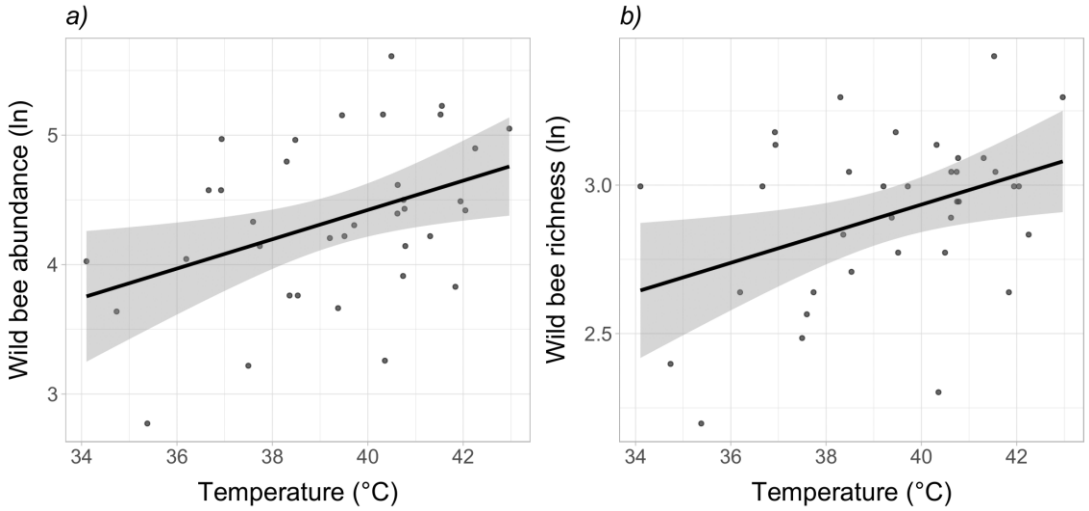

Figure 4.2: Plot showing the effect of surface temperature on *a)* abundance (ln-transformed) and *b)* species richness (ln-transformed) of wild bees. Points represent raw data points, lines represent model estimates, and the shaded areas represent the 95% confidence intervals.

Multiple regressions on distance matrices showed that temperature distance affected only community dissimilarity related to species richness difference (Table 4.2). Species richness difference increased with increasing temperature distance, that is, sites with similar temperatures shared a subset of the occurring species and showed more similar bee communities (Figure 4.3). In contrast, the species replacement component was not affected by temperature. In addition, open habitat distance, distance from the city centre and geographic distance did not have any effect on both richness and replacement components (Table 4.2).

Table 4.2: Results of multiple regression models on distance matrices testing the effects of temperature distance, open habitat cover distance, and geographic distance on wild bee composition dissimilarity components, *i.e.*, *a)* richness dissimilarity and *b)* replacement dissimilarity. Values in bold indicate significant effects (p value < 0.05).

Response variable	**Explanatory variable**	**Estimate**	***p* value**
a) Richness dissimilarity	Intercept	< 0.001	0.823
	Temperature distance	0.015	**0.027**
	Open habitat distance	< 0.001	0.764
	Distance from the city centre	-0.004	0.524
	Geographic distance	< 0.001	0.701
b) Replacement dissimilarity	Intercept	< 0.001	0.682
	Temperature distance	-0.001	0.910
	Open habitat distance	< 0.001	0.923
	Distance from the city centre	0.004	0.571
	Geographic distance	< 0.001	0.451

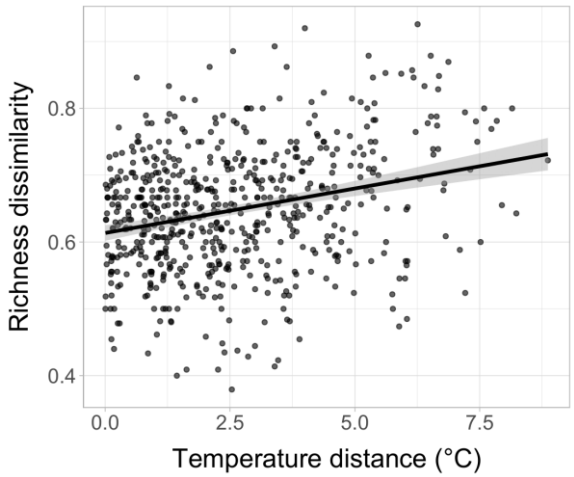

Figure 4.3: Plot showing the effect of temperature distance on richness dissimilarity of wild bee communities among sites. Composition dissimilarity was calculated using the richness component of Jaccard index (Legendre 2014). The line represents the estimate of a multiple regression model on distance matrices.

Functional diversity analyses showed that functional evenness decreased at higher temperatures (Table 4.3 *a*, Figure 4.4) while it was not affected by open habitat cover or distance from the city centre (Table 4.3 *a*). Functional dispersion did not respond to temperature, open habitat cover or distance from the city centre (Table 4.3 *b*).

Table 4.3: Results of the linear models testing the effect of temperature, open habitat cover, and distance from the city centre on *a)* functional evenness and *b)* functional dispersion of wild bee communities. Values in bold indicate significant effects (p value < 0.05). No significant interactions were found.

Response variable	**Explanatory variable**	**Estimate**	**SE**	***t* value**	***p* value**
a) Functional evenness	Intercept	1.190	0.223	5.338	< 0.001
	Temperature	-0.014	0.006	-2.488	**0.018**
	Open habitat cover	0.001	0.001	0.737	0.467
	Distance from the city centre	0.001	0.005	0.289	0.774
b) Functional dispersion	Intercept	0.359	0.119	3.004	0.005
	Temperature	-0.003	0.003	-0.848	0.403
	Open habitat cover	-0.001	0.001	-1.183	0.245
	Distance from the city centre	-0.003	0.003	-1.024	0.314

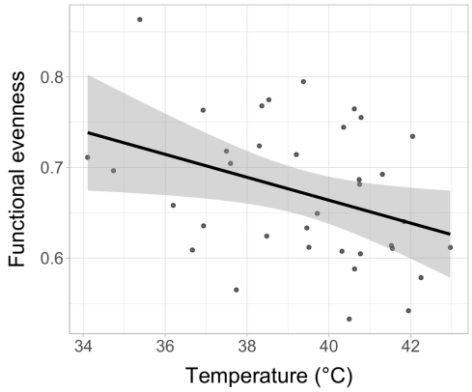

Figure 4.4: Plot showing the effect of surface temperature on functional evenness of wild bee communities. Points represent raw data points, the line represents model estimate, and the shaded area represents the 95% confidence interval.

By analysing CWMs for body size, social behaviour, nesting strategy, and diet breadth, we found that communities were functionally diverse depending on temperature and distance from the city centre (Table 4.4). Communities were characterised by smaller individuals when they were close to the city centre or when temperatures were warmer (Figure 4.5 *a*, *b*). Moreover, bee communities showed a higher proportion of individuals of polylectic species with warmer temperatures (Figure 4.5 *c*). In contrast, CWMs for nesting strategy and social behaviour did not respond to surface temperature, open habitat cover or distance from the city centre. However, we found a positive trend between sociality and open habitat cover (Figure S4.4).

Table 4.4: Results of the linear models testing the effect of temperature, open habitat and distance from the city centre on CWMs for *a)* body size, *b)* nesting strategy (above-ground), *c)* diet breadth (polylecty), and *d)* social behaviour (sociality) of wild bee communities. Values in bold indicate significant effects (p value < 0.05). No significant interactions were found.

Response variable	**Explanatory variable**	**Estimate**	**SE**	***t* value**	***p* value**
a) CWM body size	Intercept	10.892	1.826	5.965	< 0.001
	Temperature	-0.117	0.046	-2.524	0.017
	Open habitat cover	< 0.001	0.009	0.050	0.941
	Distance from the city centre	0.096	0.042	2.284	**0.031**
b) CWM nesting strategy (above-ground)	Intercept	< 0.001	0.003	0.036	0.570
	Temperature	< 0.001	0.003	0.036	0.972
	Open habitat cover	-0.001	0.000	-1.302	0.203
	Distance from the city centre	< 0.001	0.002	-0.186	0.854
c) CWM diet breadth (polylecty)	Intercept	0.533	0.280	1.903	0.066
	Temperature	0.007	0.002	3.710	**0.001**
	Open habitat cover	0.001	0.000	1.578	0.128
	Distance from the city centre	-0.001	0.002	-0.283	0.602
d) CWM social behaviour (sociality)	Intercept	-0.041	0.436	0.095	0.925
	Temperature	0.015	0.011	1.360	0.174
	Open habitat cover	0.004	0.002	1.830	0.077
	Distance from the city centre	0.007	0.010	0.717	0.496

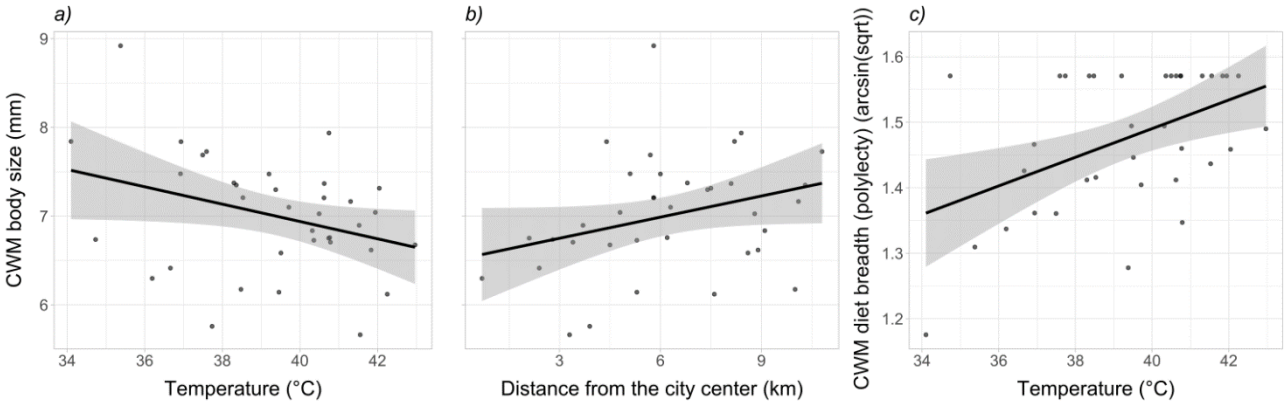

Figure 4.5: Plots showing the effects of *a)* temperature, *b)* distance from the city centre on CWM body size, and the effect of *c)* temperature on CWM diet breadth (polylecty) (arcsin(sqrt)-transformed). Points represent raw data points, lines represent model estimates, and the shaded areas represent the 95% confidence intervals.

4.5. Discussion

Here, we showed that in a highly urbanised environment, temperature was the key driver of wild bee diversity, abundance, composition and functional diversity, shaping pollinator communities irrespective of the cover of open habitat and the distance from the city centre. Warmer sites showed communities richer in individuals and species but dominated by similar traits. In response to warming and distance from the city centre, bee assemblages exhibited clear shifts in functional composition.

4.5.1. Temperature as the main driver of wild bee communities

Both wild bee abundance and species richness were driven by temperature, with a positive effect of warmer temperatures. In addition, temperature was the only factor filtering community composition and, even with a very high variability, it led to communities that differed because of the number of species, and not because of species turnover. Warm temperatures are often beneficial to insects, as they might increase growth rate and survival, and reduce development time (Zuo et al. 2012). Most studies investigating the relationship between temperatures and pollinators found that warm temperatures increased insect activities, abundance, diversity or biomass (Kühsel and Blüthgen 2015; Schürch et al. 2016; Burdine and McCluney 2019; Welti et al. 2022, p. 20; but see Papanikolaou et al. 2017; Hamblin et al. 2018; Casanelles-Abella et al. 2022). However, the reported positive effect of warming should be taken with caution. Large deviations from long-term temperature averages were found to negatively affect flying insects, as rapid temperature rises may exceed locally established tolerance (Welti et al. 2022). To assess more precisely temperature warming effects on bees, we should gain knowledge on mid- and long-term effects of temperature and on species thermal optima. However, little is still known about bee thermal and humidity limits, besides that they could strongly differ from species

to species and even from one population to another (Burdine and McCluney 2019; Sánchez-Echeverría et al. 2019; Martinet et al. 2021).

Cities usually experience much warmer temperatures than nearby rural or semi-natural areas because of heat-absorbing and impervious building materials (Oke 1973). In contrast, increasing vegetation cover decreases temperatures (De Frenne et al. 2013). Also in this study, we found a negative correlation between temperature and tree cover, that is, warmer sites were embedded in highly urbanised landscapes, while colder sites showed a lower percentage of urbanisation. Besides increasing local temperatures with a potentially positive effect on bee growth and survival, high urbanisation might provide locally a large amount of floral resources, for example, in parks, gardens and roadsides, therefore sustaining a high number of species and individuals (Hall et al. 2017; Baldock et al. 2019; Wilson and Jamieson 2019). However, in our study, wild bee abundance and diversity did not respond to open green habitat cover and distance from the city centre. Therefore, it is likely that different sites offered a similar amount of floral resources irrespective of the amount of open habitat in the landscape. Another hypothesis is that all sampled species had been already selected for intensive anthropogenic habitat types (Corcos et al. 2019). In cities, wild bee communities should be the result of centuries of human disturbance and therefore, they might be composed mostly of species adapted to an urban environment. Many studies revealed that only a subset of species, consisting of the most tolerant to anthropogenic activities, is able to survive in highly disturbed environments (Banaszak-Cibicka and Żmihorski 2012; Gámez-Virués et al. 2015; Fournier et al. 2020). In particular, it has been found that insect diversity increases with the age of an urban settlement (Sattler et al. 2010), as its insect fauna has probably been selected for high tolerance to fragmentation and colonisation potential.

4.5.2. Warm urban communities are dominated by specific functional traits

Our results show that in warmer sites, functional evenness decreased. This means that at high temperatures, the most abundant species shared similar traits different from the rest of the community. Probably, few species characterised by specific traits can cope better with warm conditions. As a consequence, these few dominant species may be better adapted to future climate change scenarios, while others, characterised by different traits, may disappear. By analysing community mean traits, we were able to identify which traits seemed beneficial with increasing temperatures. Community mean trait values shifted depending on temperature and distance from the city centre. As expected, we found that mean body size decreased with increasing temperatures, that

is, communities adapted to warm conditions showed on average smaller individuals. Similar results were reported for spiders, beetles, and aquatic taxa in urban environments (Merckx et al. 2018). It is well known that usually smaller animals dissipate heat better (Burdine and McCluney 2019). Larger wild bee species might be therefore negatively affected by increasing temperatures in cities (Wilson and Jamieson 2019). In addition, mean body size increased further away from the city centre, irrespective of the cover of open habitat. Similar results were reported in other studies, where mean body size of several invertebrate species increased with increasing distance from the city centre regardless of local site characteristics (Tóth and Hornung 2019; Braschler et al. 2021). For ground-dwelling arthropods, the decrease in body size has been related to a combination of reduced soil moisture and increased soil contamination (Braschler et al. 2021). However, for mobile flying organisms such as wild bees, this result is probably linked to foraging distances. Larger bee individuals forage further away, while smaller individuals travel closer to their nest (Greenleaf et al. 2007). Cities seem to favour smaller-bodied species because small bees may be more likely to use local and isolated floral spots in the city centre (Braschler et al. 2021; Prendergast et al. 2022). An additional possible explanation is that smaller species require a much limited amount of resources compared to larger species (Winfree et al. 2011; Eggenberger et al. 2019).

Besides filtering for smaller body sizes, warm temperatures increased the number of individuals of polylectic species in the community. In our study, all wild bee communities showed a high level of generalisation, with most species having a polylectic diet. This is typical of highly urbanised environments, where oligolectic species are usually uncommon (Lizée et al. 2011; Casanelles-Abella et al. 2022). Polylectic wild bee species are better able to exploit resources in urban areas as they can access and forage on a great variety of flowers. In this study, the few oligolectic species occurring at colder temperatures disappeared at warmer temperatures. A possible explanation for this might be that specialised species strongly depend on a particular range of conditions and are, consequently, more vulnerable to habitat disturbance in general and warming in particular (Winfree et al. 2011; Hopfenmueller et al. 2014; Martinet et al. 2021). It might also be that the abundance of the favoured host plants of some oligolectic species decreased at warmer temperatures but, unfortunately, we lack the data to confirm this hypothesis.

Finally, we did not find any effect of temperature, open habitat cover, and distance from the city centre on nesting strategy and social behaviour, except for a positive trend between sociality and open habitat cover.

Williams et al. (2010) also found that social species responded strongly to the amount of natural habitat. In our study, most social species nested below ground and, therefore, the availability of bare ground in open areas might have been a key resource for them.

4.6. Conclusions

In a highly urbanised environment, such as the metropolitan city of Rome, wild bee abundance and diversity did not change in response to open habitat cover or distance from the city centre. In contrast, temperature was the main driver shaping wild bee communities. Under future global warming, we expect that heat-tolerant wild bee species will benefit from increasing temperatures in urban settlements and that warm-temperature communities will be dominated by polylectic and small-bodied bees. Further research is needed to understand the potential role of cities as pollinator refuge under global change, focusing not only on wild bees, but even on other fundamental pollinator taxa such as Coleoptera, Diptera, and Lepidoptera.

4.7. Acknowledgements

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CHAPTER 5

Functional traits of plants and pollinators explain resource overlap between honey bees and wild pollinators

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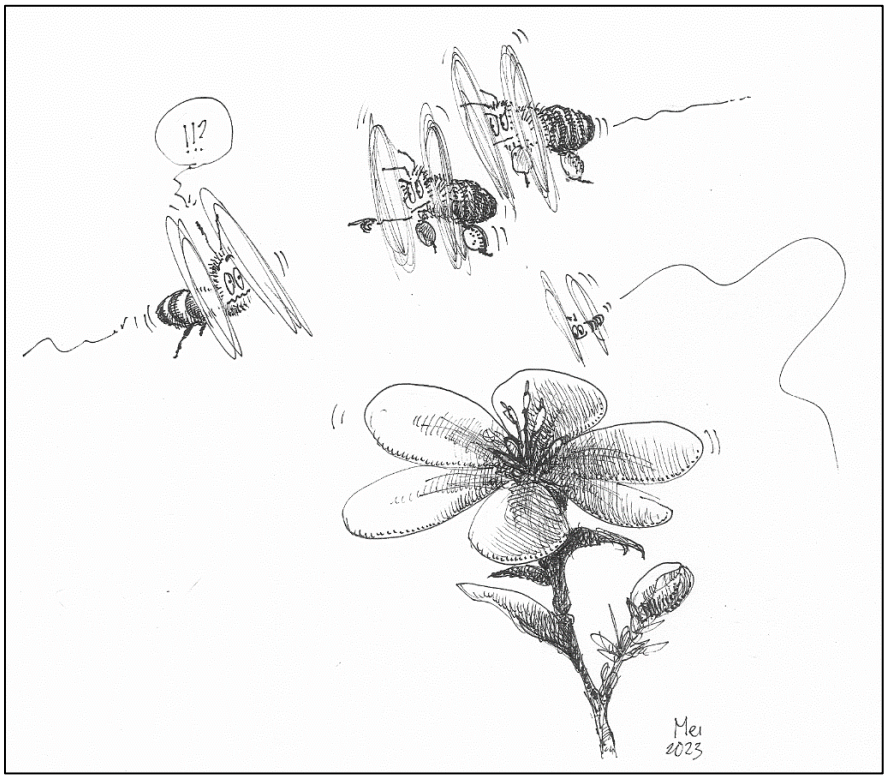

5.1. Abstract

Managed and wild pollinators often cohabit in both managed and natural ecosystems. The western honey bee, *Apis mellifera*, is the most widespread managed pollinator species. Due to its density and behaviour, it can potentially influence the foraging activity of wild pollinators, but the strength and direction of this effect are often context-dependent. Here, we observed plant-pollinator interactions in 51 grasslands, and we measured functional traits of both plants and pollinators. Using a multi-model inference approach, we explored the effects of honey bee abundance, temperature, plant functional diversity, and trait similarity between wild pollinators and the honey bee on the resource overlap between wild pollinators and the honey bee. Resource overlap decreased with increasing honey bee abundance only in plant communities with high functional diversity, suggesting a potential diet shift of wild pollinators in areas with a high variability of flower morphologies. Moreover, resource overlap increased with increasing trait similarity between wild pollinators and the honey bee. In particular, central-place foragers of family Apidae with proboscis length similar to the honey bee exhibited the highest resource overlap. Our results underline the importance of promoting functional diversity of plant communities to support wild pollinators in areas with a high density of honey bee hives. Moreover, greater attention should be paid to areas where pollinators possess functional traits similar to the honey bee, as they are expected to be more prone to potential competition with this species.

5.2. Introduction

As a managed and super-generalist pollinator, the western honey bee, *Apis mellifera* Linnaeus, plays a fundamental role in the pollination of both crops (Garibaldi et al. 2013) and wild plants (Hung et al. 2018). However, managed honey bees might adversely impact wild pollinator communities, as they are often extremely abundant, have a prolonged flight season, and tend to forage on the most abundant and rewarding floral resources (Goulson 2003). Nevertheless, observed effects are often idiosyncratic and seem to depend on local conditions, the composition of wild pollinator communities, and the different methodological approaches adopted (Goulson 2003; Cane and Tepedino 2017; Mallinger et al. 2017).

Against this background, functional traits of both plants and pollinators can help to identify the likelihood, strength and direction of the interactions between managed and wild pollinators (Violle et al. 2007; Eklöf et al. 2013; Schleuning et al. 2015; Bergamo et al. 2020). Floral morphological traits are fundamental in shaping plant-pollinator interactions (Junker et al. 2013). Plant species with greater flower size and longer flowering periods are usually more generalist, being attractive to many pollinator species, while flowers with deep corolla are usually accessible only to a few specialized pollinator species (Lázaro et al. 2020). Although the effect of functional diversity of plant communities on pollinators is still debated (Fornoff et al. 2017; Uyttenbroeck et al. 2017; Goulnik et al. 2020), one expectation is that increased functional diversity should reduce the plant resource overlap between wild pollinators and a dominant species such as the honey bee by providing a larger number of alternative nectar and pollen resources (Figure 5.1).

Similarly, pollinator traits can affect both how pollinators interact with plant species and how they interact with each other (Albrecht et al. 2012; Garibaldi et al. 2015; Woodcock et al. 2019). In particular, the competition of wild pollinators with honey bees in areas with a high abundance of managed pollinators could be stronger for central-place foragers, which are forced to collect pollen and nectar near their nest (Walther-Hellwig et al. 2006), and for oligolectic pollinators, which have a limited ability to shift to alternative resources (Cane and Tepedino 2017). On the contrary, large-sized pollinators with longer proboscis usually have a larger diet breadth, as they are able to exploit a wider range of resources compared to smaller ones (Greenleaf et al. 2007; Lara-Romero et al. 2019). Hence, we expect that a high trait similarity between wild pollinators and the honey bee should increase their resource overlap (Figure 5.1).

Environmental variables can also have a strong effect on species phenology and behaviour. Air temperature and weather, in particular, modulate the activity of pollinators (Trøjelsgaard and Olesen 2013; Giannini et al. 2015). For example, bumblebees are often active at low temperatures and under unfavourable weather conditions (Goulson 2010), while butterflies are strongly negatively affected by low air temperatures (Abrahamczyk et al. 2011). Honey bees are more sensitive to low temperatures than many wild pollinators (Jaffé et al. 2010), so potential competition between wild pollinators and honey bees should be more severe at high temperatures (Figure 5.1).

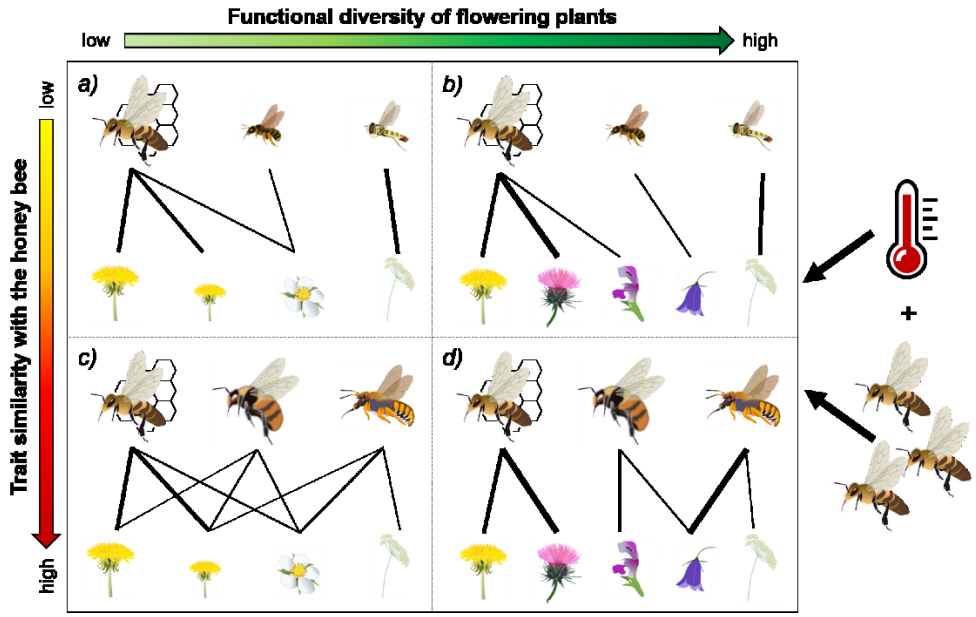

Figure 5.1: Expected effects of functional diversity of plant community and trait similarity between wild pollinator community and the honey bee on plant-pollinator interactions. We hypothesise that: *a)* in sites with a low functional diversity of plant community and a low trait similarity between wild pollinator community and the honey bee, the resource overlap between wild pollinators and the honey bee would be generally low, as pollinator species with functional traits different from those of the honey bee would exploit different resources; *b)* in sites with a high functional diversity of plant community and a low trait similarity between wild pollinator community and the honey bee, the resource overlap would be even lower, as pollinator species would spread on different floral resources; *c)* in sites with a low functional diversity of plant community and a high trait similarity between wild pollinator community and the honey bee, pollinator species would share an important portion of plants with the honey bee, therefore, resulting in a high resource overlap; *d)* in sites with a high functional diversity of plant community and a high trait similarity between wild pollinator community and the honey bee, the resource overlap would decrease, as pollinator species would have much more resources to forage on. Increasing honey bee abundance and higher temperatures would intensify the observed effects.

A promising approach to elucidate potential mechanisms shaping the interactions between plants and pollinators is the use of network tools integrated with functional trait analysis. Here, we investigated how functional traits of both plants and pollinators, together with the abundance of honey bees and temperature, affected the foraging behaviour of wild pollinators. In particular, this study aimed to explore how functional richness and dispersion of plant communities influenced the resource overlap between wild pollinators and the honey bee, also testing the effect of trait similarity between wild pollinators and the honey bee. We observed

plant-pollinator networks in 51 grasslands in Northern Italy and computed the resource overlap between each wild pollinator species and the honey bee. We calculated functional richness and functional dispersion of plant communities using flower corolla length, flower colour, and flower shape, while trait similarity between wild pollinators and the honey bee was calculated using proboscis length, body size, type of foraging range, and taxonomic family.

5.3. Materials and methods

5.3.1. Sampling design

Fieldwork was carried out in 51 grasslands in Northern Italy (Alps and Prealps), approximately 50 × 30 m in size. Grasslands were selected across a steep elevational gradient ranging from 150 to 2100 m a.s.l., and had a wide range of honey bee abundance (Table S5.1, Figure S5.1). The selection of the sites was adjusted during the sampling season to have statistical independence between temperature and honey bee abundance (Pearson's correlation = 0.11, p value = 0.41). Each sampling site was at least 0.53 km from the nearest one (mean = 4.60 km). We were not able to determine the exact number of beehives near the sampling sites, but the mean density in the study area was c. 5 beehives per km^2 (data provided by the National Data Bank of the Zootechnical Registry established by the Ministry of Health at the National Service Centre of the "G. Caporale" Institute of Teramo).

5.3.2. Sampling of ecological interactions

Between May and September 2019, we observed plant-pollinator interactions in the selected sites. Sampling occurred between 9:00 and 17:00 only with air temperature > 15 °C, low wind, no rain, and cloud coverage < 70%. Each site was visited only once. At each site, we identified all flowering plant species and assessed their relative abundance. All the individuals of each plant species were then observed for 15 min in total, during which all hymenopterans and dipterans touching the reproductive parts of flowers were counted and collected. Both plant and pollinator species were identified in the field when possible, otherwise, plants were collected and prepared in a herbarium, while pollinators were placed in vials filled with 70% ethanol. Later identification was entrusted to experts (Filippo Prosser and LM for plants, and AC, MM, DP, and PC for pollinators). During the sampling, we also measured the air temperature using a Tinytag Plus 2 TGP-4017 data logger.

5.3.3. Resource overlap between wild pollinators and the honey bee

Starting from the observed interactions, we built 51 bipartite plant-pollinator networks, one for each sampling site. For each network, we calculated the resource overlap between each wild pollinator species (*i.e.*, excluding the honey bee) and the honey bee using Morisita's index (Morisita 1959) in the *R* package *spaa* (Zhang 2016). The index ranges from 0 to 1, with increasing values indicating an increase in the plant resource overlap between the two pollinator species. In each network, we then calculated the community weighted mean (hereafter, CWM) resource overlap between wild pollinators and the honey bee as the mean resource overlap value of all wild pollinator species weighted by their abundance. We used CWM resource overlap instead of resource overlap values of single species as no model using species as replicates met statistical assumptions, even after changing the distribution or transforming the variables. All analyses were performed using *R* version 3.6.1 (R Development Core Team 2019).

5.3.4. Functional traits of plant species

For each flowering plant species, we measured flower corolla length with a calliper, and recorded flower type after Kugler (1970) and flower colour (Table S5.2). These are among the most important morphological traits for the definition of pollinator feeding niches: flower colour affects the attractiveness and selectivity of flowers, while flower type and corolla length determine the accessibility of flowers to pollinators (Junker et al. 2013). We then calculated two indices of functional diversity of plant communities for each network, *i.e.*, the standardized functional richness and the functional dispersion, which provide complementary information (Villéger et al. 2008; Laliberté and Legendre 2010). First, for each network, we built an Euclidean distance matrix by projecting flowering plant species into a three-dimensional trait space with each axis corresponding to a functional trait. The distance matrix was analysed through Principal Coordinate Analysis (PCoA), and the PCoA axes were then used as new combined traits to compute the functional diversity indices. Categorical variables were transformed into dummy variables (*i.e.*, binary). Functional richness measures the functional space filled by the plant community, *i.e.*, the volume of the convex hull. For each network, we standardized the index value by the "global" functional richness, including all plant species in all networks (Laliberté and Legendre 2010). Its value ranges from 0 to 1, with increasing values of the index indicating an increase in community functional richness. Functional dispersion additionally takes into account the relative abundance of plant species. The index represents the dispersion of plant species in the trait space, *i.e.*, the distance of species to the centroid of all species in the community, weighted by their relative abundance. Its value ranges

from 0 to infinity, with increasing values indicating an increase in functional dispersion, *i.e.*, a strong difference in traits between dominant plant species and low abundant ones. Both indices were calculated using the *R* package *FD* (Laliberté et al. 2014).

5.3.5. Functional traits of pollinator species

For each pollinator species, we selected one to four individuals, depending on the availability, and extracted the proboscis which was measured along with total body length (body size). We derived from the literature two additional traits: type of foraging range (two classes: central-place forager, for species which build a nest, and non-central-place forager), and taxonomic family (Table S5.3, Additional References in Supplementary Information). As for corolla shape and length, proboscis length and body size affect the way a pollinator species can exploit a floral resource. The type of foraging range does not directly influence resource selection, but it determines how far pollinators can travel to collect pollen and nectar. Finally, the taxonomic family is often linked to floral preferences or particular mouthpart morphology. Using these traits, we estimated the trait similarity between each wild pollinator species and the honey bee using Gower's similarity coefficient (Gower 1971) as described by Podani (1999), calculated using the *R* package *FD* (Laliberté et al. 2014). For each site, we then determined the CWM trait similarity between the community of wild pollinators and the honey bee by calculating the mean trait similarity value of all wild pollinator species (*i.e.*, excluding the honey bee) weighted by their abundance.

5.3.6. Potential collinearity between predictors

Before performing the statistical analyses described below, we analysed potential collinearity in our data by computing the variance inflation factors (VIFs) using the *R* package *car* (John and Weisberg 2019). Plant species richness and standardized functional richness of plant community were strongly correlated (Pearson's correlation = 0.876, p value < 0.001), as well as temperature and elevation (Pearson's correlation = 0.751, p value < 0.001). We, therefore, chose to build our models using plant standardized functional richness and temperature as explanatory variables. Functional traits of pollinators were also correlated with each other (Table S5.4, Figure S5.2), so their effect on resource overlap was analysed separately. The explanatory variables of the six global models described in the next paragraph fitted without the interactions had VIFs < 1.5, indicating low collinearity.

5.3.7. Statistical analyses

For the statistical analyses, we followed an information-theoretic approach (Burnham and Anderson 2002), which allows comparing the fit of a set of models rather than selecting one single best model based on *p* values. The first global model (Model 1) included resource overlap between wild pollinator community and the honey bee as response variable, and the main effects of honey bee abundance, temperature, standardized functional richness of plant community, and trait similarity between wild pollinator community and the honey bee as explanatory variables. The model also included all the interactions that could strongly affect the resource overlap, *i.e.*, the two-way interactions between honey bee abundance and plant standardized functional richness, between honey bee abundance and trait similarity between wild pollinator community and the honey bee, between plant standardized functional richness and trait similarity between wild pollinator community and the honey bee, and the three-way interaction between honey bee abundance, plant standardized functional richness and trait similarity between wild pollinator community and the honey bee. The structure of the second model (Model 2) was similar, but standardized functional richness of plant community was replaced by functional dispersion of plant community.

Second, we explored the effect of single pollinator traits on resource overlap. We, therefore, built four linear mixed-effect models, one for proboscis length (Model 3), one for body size (Model 4), one for type of foraging range (Model 5), and one for taxonomic family (Model 6). Proboscis length and body size of wild pollinators were categorized according to trait values of the honey bee, which possesses a proboscis of c. 5 mm and body size of about 12 mm. Proboscis length categories for wild pollinators were: proboscis shorter than the honey bee < 3.9 mm, proboscis similar to the honey bee = 4-6.9 mm, and proboscis longer than the honey bee > 7 mm. Body size categories for wild pollinators were: smaller than the honey bee < 7.9 mm, similar to the honey bee = 8-14.9 mm, and larger than the honey bee > 15 mm. We categorized continuous trait variables due to the poor outcome of model residual diagnostics using traits as continuous variables. For taxonomic family, we aggregated families with less than ten collected individuals, *i.e.*, Cimbicidae, Megalodontesidae, Melittidae, and Scoliidae. For each network, we calculated the CWM resource overlap between wild pollinators and the honey bee for each trait category, *e.g.*, for body size, we had one value of CWM resource overlap for wild pollinators smaller than the honey bee, one for wild pollinators similar in size, and one for wild pollinators larger than the honey bee. Each global model included honey bee abundance, temperature, trait category, and the interaction between honey bee abundance and trait category as explanatory

variables, and network identity as random factor. In all models described above, the continuous explanatory variables were scaled to mean 0 and standard deviation 1 to make slopes comparable (Gelman 2008). For a summary of the six global models, see Table S5.5.

Within each set, models were ordered based on their second-order Akaike information criterion corrected for small sample size (AICc), with lower values indicating models that better fit the data. For each model, we calculated the ΔAICc, *i.e.*, the difference between the model AICc and the lowest AICc of the entire set of models (with the best model having ΔAICc = 0), and the Akaike model weight, which indicates the probability that the model is the best one. As a measure of goodness-of-fit, we estimated the R^2. Lastly, we calculated the model-averaged partial coefficient for each explanatory variable using all models within each set and estimated the 95% confidence intervals around model-averaged partial coefficients. We presented in the tables all models with ΔAICc < 6 (Harrison et al. 2018). All multi-model analyses were conducted using the *R* package *MuMIn* (Barton 2020).

Lastly, we tested for potential spatial autocorrelation of residuals of all models using Moran's I in the *R* package *ape* (Paradis and Schliep 2019). The analyses highlighted no spatial autocorrelation in any of the model (Model 1 *p* value = 0.692; Model 2 *p* value = 0.478; Model 3 *p* value = 0.336; Model 4 *p* value = 0.842; Model 5 *p* value = 0.539; Model 6 *p* value = 0.075).

5.3.8. Methodological considerations

In this study, we opted to sample many sites with a single visit, as we wanted to include a wide range of plant and pollinator functional traits and temperatures. In network ecology, it is common practice to aggregate data collected in multiple sampling events within a single plant-pollinator network (*e.g.*, Montero-Castaño and Vilà 2017; Norfolk et al. 2018; Valido et al. 2019). However, this operation can potentially create artificial species assemblages, *i.e.*, cumulative communities composed of species observed on different days, weeks or seasons, often with non-overlapping phenology (CaraDonna et al. 2020; Schwarz et al. 2020). Using single-visit networks, we aimed at exploring the realized interactions between co-occurring individuals of honey bees and wild pollinators, rather than achieving high sampling completeness of pollinator species or interactions. Our interactions can, therefore, be interpreted as short-term, behavioural responses.

5.4. Results

5.4.1. General results

Across the 51 networks combined, we observed 262 plant species (Table S5.2) and 325 pollinator species or morphospecies (Table S5.3), for a total of 10,841 pollinator visits to flowers. During the 255 h of observation, we recorded 1497 unique plant-pollinator interactions. We identified to the species level 99% of collected wild pollinators (Table S5.3). We observed an average of 81 wild pollinator individuals (min = 16, max = 332), and 24 pollinator species (min = 9, max = 49) per site (Table S5.1). The honey bee was found in all sites and was the most abundant pollinator with 6718 collected individuals (min = 2, max = 768, mean = 132), and the most generalist one, visiting 111 flowering plant species. Other common, abundant and generalist species were *Eristalis tenax* (Linnaeus), a hoverfly species found at 39 sites with 597 individuals that visited 76 flowering plant species, *Bombus pascuorum* (Scopoli), a bumblebee species found at 35 sites with 411 individuals that visited 45 flowering plant species, and *Sphaerophoria scripta* (Linnaeus), a hoverfly species found at 37 sites with 366 individuals that visited 77 flowering plant species. Pollinator proboscis length ranged from 0.4 mm for *Entomognathus brevis* (Vander Linden) to 16 mm for *Bombus gerstaeckeri* Morawitz, while body length ranged from 4 mm for *Hylaeus taeniolatus* Förster and *H. imparilis* Förster to 22.5 mm for *Xylocopa violacea* Linnaeus (Table S5.3).

We observed an average of 20 flowering plant species (min = 8, max = 35) per site (Table S5.1). The most frequently visited species were *Rubus* sp. L. (931 total visits, 97% by the honey bee), *Centaurea nigrescens* Willd. (823 total visits, 84% by the honey bee), and *Epilobium angustifolium* L. (560 total visits, 93% by the honey bee), while the species most frequently visited only by wild pollinators were *Galeopsis pubescens* Besser (278 visits), *Leucanthemum vulgare* Lam. (191 visits), and *Erigeron annuus* (L.) Pers. (153 visits). Few plant species (N = 9) were exclusively visited by honey bees, while many species were exclusively visited by wild pollinators (N = 102), among which there were many umbellifers such as *Daucus carota* L., *Anthriscus sylvestris* (L.) Hoffm., and Heracleum sphondylium L. The most generalist plant species were *Ranunculus acris* L. (attracting 40 pollinator species), *Trifolium pratense* L. (attracting 39 pollinator species), and *E. annuus* (attracting 37 pollinator species). Flower corolla length ranged from 0.05 mm of open disc flowers to 33 mm of *Calystegia sepium* (L.) R. Br. (Table S5.2).

5.4.2. Overall functional traits of plants and pollinators

For Model 1, fifteen models showed a ΔAICc < 6 (Table S5.6). Model averaging indicated that both plant and pollinator functional traits affected the resource overlap between wild pollinator community and the honey bee

(Figure 5.2). The impact of plant functional traits on resource overlap varied with honey bee abundance: resource overlap decreased as honey bee abundance increased in sites with high plant functional richness, while there was no change in resource overlap with increasing honey bee abundance in sites with low plant functional richness (Figure 5.3 *a*). Moreover, resource overlap increased as trait similarity between wild pollinator community and the honey bee increased (Figure 5.3 *b*). Temperature and other interactions did not affect the resource overlap (Table S5.6, Figure 5.2).

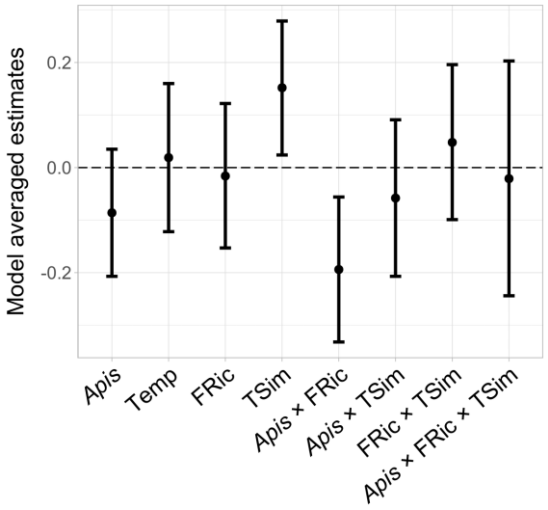

Figure 5.2: Model estimates from the model-averaging procedure based on the set of models with all functional traits of both plants and pollinators (Model 1). Explanatory variables of the global model are honey bee abundance (*Apis*, ln-transformed), temperature (Temp), standardized functional richness of plant community (FRic), trait similarity between wild pollinator community and the honey bee (TSim), and the interactions *Apis* × FRic, *Apis* × TSim, FRic × TSim, and *Apis* × FRic × TSim. All explanatory variables were scaled to mean 0 and standard deviation 1. Points represent model estimates and bars represent the 95% confidence intervals. The variable effect is supported when the confidence interval does not include zero.

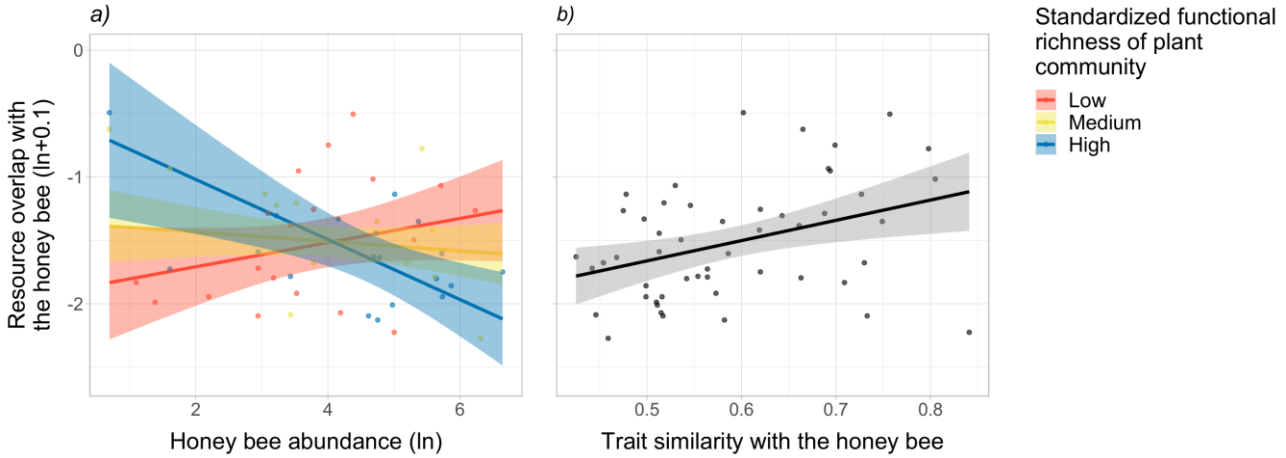

Figure 5.3: Plots showing the effect of *a)* the interaction between honey bee abundance (ln-transformed) and standardized functional richness of plant community, with the three standardized functional richness levels representing the 10th, 50th, and 90th percentiles, and *b)* trait similarity between wild pollinator community and the honey bee on resource overlap between wild pollinator community and the honey bee (ln-transformed) (Model 1). Points represent raw data points, lines represent model estimates, and the shaded areas represent the 95% confidence intervals.

For Model 2, twenty-eight models showed a $\Delta AICc < 6$ (Table S5.7). The resource overlap was affected only by the trait similarity between wild pollinator community and the honey bee (Figure S5.3).

5.4.3. Single functional traits of pollinators

For Model 3, the multi-model inference analysis selected five models with a $\Delta AICc < 6$ (Table S5.8 *a*). Proboscis length was the only variable affecting the resource overlap between wild pollinator community and the honey bee (Figure 5.4 *a*), *i.e.*, pollinators with proboscis length similar to the honey bee showed the highest overlap (Figure 5.5 *a*).

For Model 4, five models had a $\Delta AICc < 6$ (Table S5.8 *b*). Body size was the only variable affecting resource overlap between wild pollinator community and the honey bee (Figure 5.4 *b*), *i.e.*, resource overlap increased with increasing body size (Figure 5.5 *b*). Models for body size showed the highest values of R^2 compared to other functional traits (Table S5.8).

For Model 5, six models had a $\Delta AICc < 6$ (Table S5.8 *c*). Again, only the trait category strongly affected resource overlap between wild pollinator community and the honey bee (Figure 5.4 *c*), *i.e.*, central-place foragers showed a higher overlap with honey bees compared to non-central-place foragers (Figure 5.5 *c*).

For Model 6, four models showed a $\Delta AICc < 6$ (Table S5.8 *d*). The taxonomic family strongly affected resource overlap between wild pollinator community and the honey bee (Figure 5.4 *d*). Bees of family Apidae showed a higher resource overlap than the other families (Figure 5.5 *d*), but the resource overlap was also relatively high for other families such as Conopidae, Halictidae, Megachilidae, and Syrphidae (Figure 5.5 *d*). We did not find an interactive effect of honey bee abundance and trait category in any of the models (Figure 5.4), meaning that the difference in resource overlap between trait categories was independent of honey bee abundance.

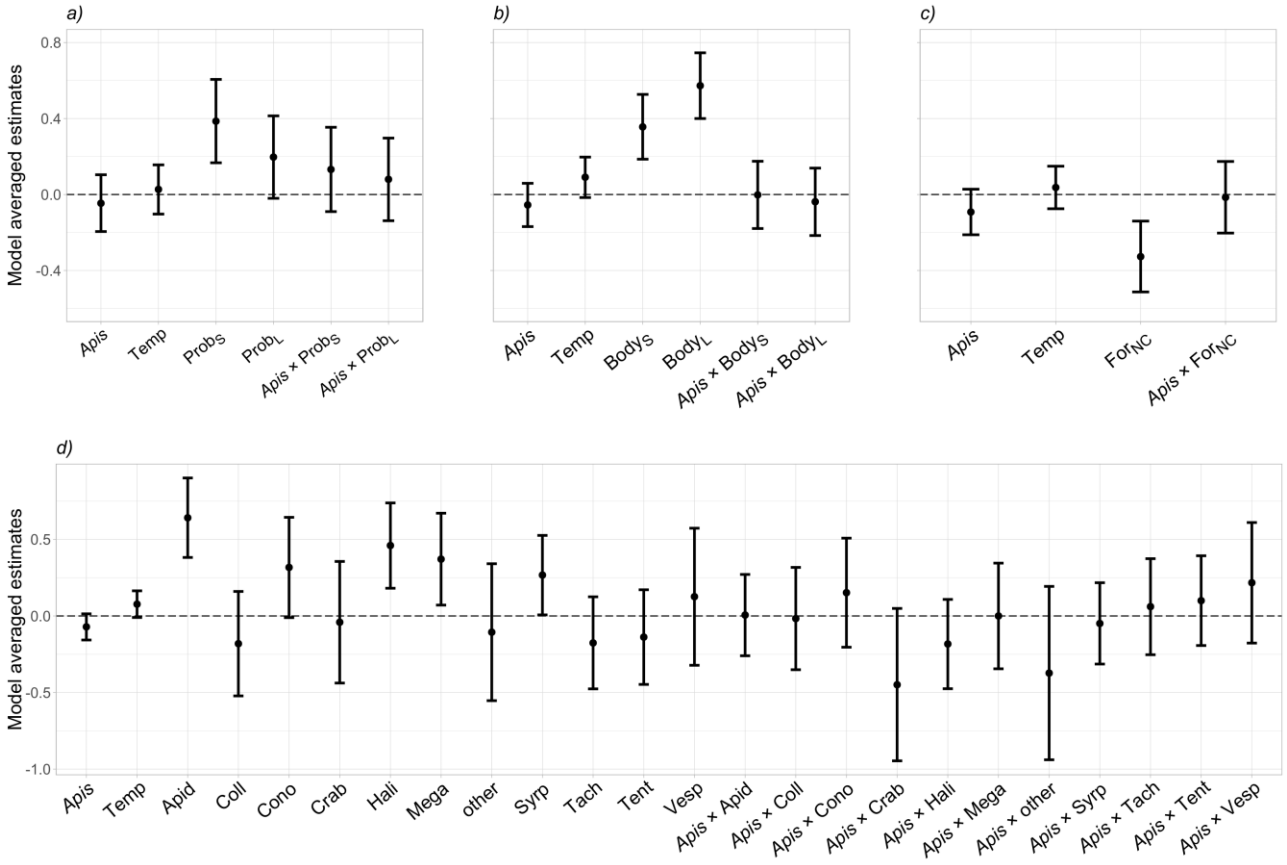

Figure 5.4: Model estimates from the model-averaging procedure based on the four sets of models considering single traits of pollinators, *i.e.*, *a)* proboscis length (Model 3), *b)* body size (Model 4), *c)* type of foraging range (Model 5), and *d)* taxonomic family (Model 6). Explanatory variables of the four global models are honey bee abundance (*Apis*, ln-transformed), temperature (Temp), the levels of the four trait categories (Prob$_S$ = proboscis similar to the honey bee, Prob$_L$ = proboscis longer than the honey bee, Body$_S$ = body size similar to the honey bee, Body$_L$ = body size larger than the honey bee, For$_{NC}$ = non-central forager, Apid = Apidae, Coll = Colletidae, Cono = Conopidae, Crab = Crabronidae, Hali = Halictidae, Mega = Megachilidae, other = other families, *i.e.*, Cimbicidae, Megalodontesidae, Melittidae, and Scoliidae, Syrp = Syrphidae, Tach = Tachinidae, Tent = Tenthredinidae, Vesp = Vespidae) and the interactions between honey bee abundance and each levels of the traits. All continuous explanatory variables were scaled to mean 0 and standard deviation 1. Points represent model estimates and bars represent the 95% confidence intervals. The variable effect is supported when the confidence interval does not include zero.

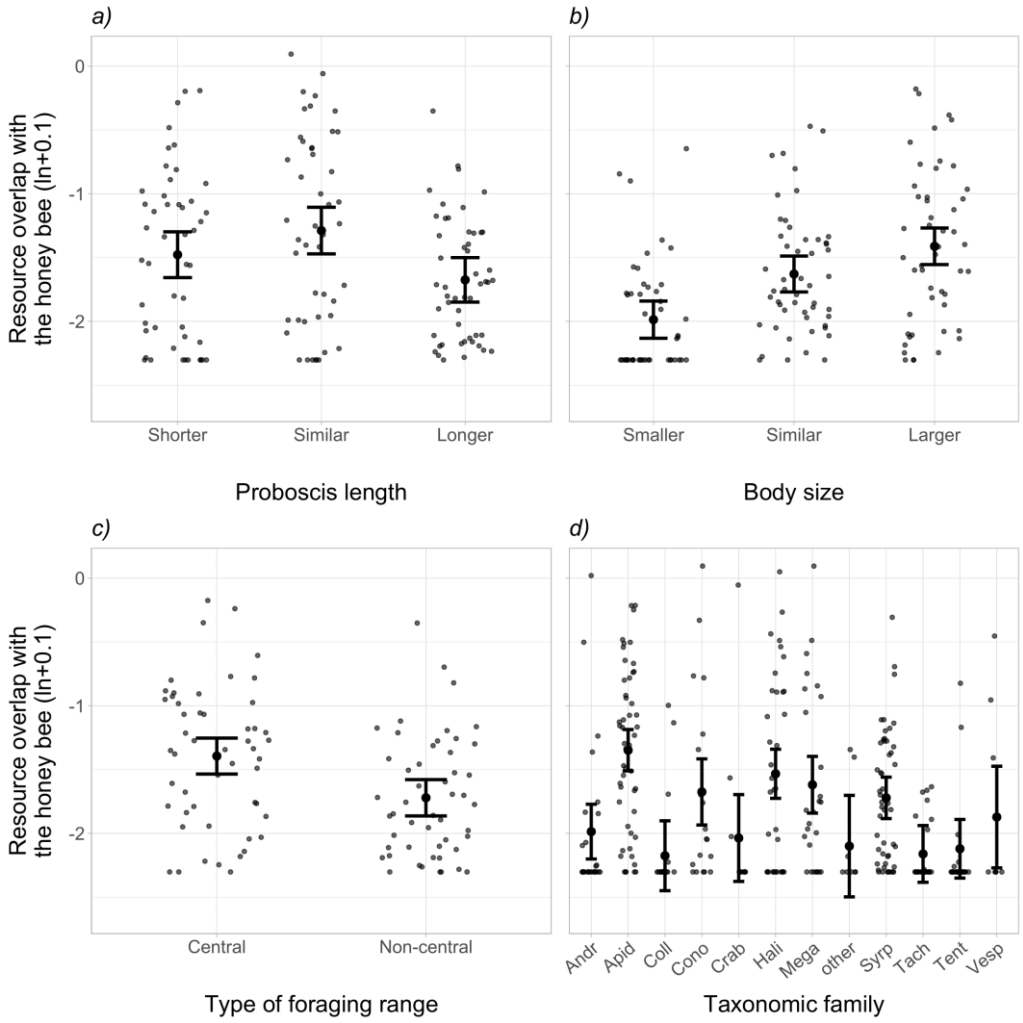

Figure 5.5: Plots showing the effect of *a)* proboscis length (Model 3), *b)* body size (Model 4), *c)* type of foraging range (Model 5), and *d)* taxonomic family (Model 6) on resource overlap between wild pollinator community and the honey bee (ln-transformed). Small black points represent raw data points, large black points represent model estimates, and black bars represent the 95% confidence intervals.

5.5. Discussion

Incorporating functional traits into ecological network analyses helped to elucidate the degree of resource overlap between wild pollinators and the honey bee. In particular, a low functional diversity of plant community combined with a high trait similarity between wild pollinators and the honey bee appeared to increase the risk of potential negative impacts of a high honey bee abundance on wild pollinator communities.

In areas with a high abundance of managed pollinators, resource overlap between wild pollinators and the honey bee could be mitigated by a high functional richness of plant community, in which pollinators could shift to alternative food resources, as opposed to areas with a low functional richness. To our knowledge, this is the first time that plant functional diversity was used to explore the changes in the resource overlap between wild pollinators and the honey bee. Previous works highlighted a similar effect of plant diversity and honey

bee abundance on pollinator communities, with a reduction of potential competition in sites rich in plant species despite an increase in honey bee abundance (Rodríguez et al. 2021). Similarly, heterogeneous landscapes have been shown to support wild pollinators by reducing competition with honey bees (Herbertsson et al. 2016), while a lower availability of differentiated floral resources might increase competition among pollinator species (Thomson 2016; Wignall et al. 2020a, b). However, in contrast with previous research, we found that the resource overlap between wild pollinators and the honey bee never increased with increasing honey bee abundance (Lindström et al. 2016 but see Hudewenz and Klein 2015), even in sites with low plant functional diversity. This might be related to the honey bee foraging behaviour, as it often focuses on the most abundant and rewarding resources, especially in areas with low diversity of plants (Magrach et al. 2017). On the other hand, the lower resource overlap observed in sites with high functional diversity of plant community and high honey bee abundance could be related to the foraging behaviour of wild pollinators that could be forced to forage on plants that are not visited by honey bees. However, while we found an effect of functional richness of plant community, we observed no effect of functional dispersion. This could be partly explained by the fact that many sites were characterized by the same dominant plant species (*e.g.*, *E. annuus* and *Melilotus albus* Medikus) and many different species with lower abundances, so functional dispersion values were similar across sites.

As expected, the resource overlap increased with increasing trait similarity between wild pollinators and the honey bee. Species with similar functional traits usually exploit similar floral resources (Fontaine et al. 2006; Albrecht et al. 2012), so potential competition is expected to be higher for wild pollinators which share traits with the honey bee. First, proboscis length is one of the main constraints of resource selection, affecting whether a pollinator species can obtain nectar from specific flowers. Pollinators are usually more efficient when foraging on plants with flower corolla length matching their mouthpart length (Inouye 1980; Madjidian et al. 2008; Klumpers et al. 2019). For example, hoverflies with a short proboscis tend to prefer flowers that are flat or have a shallow corolla (Fontaine et al. 2006), while long-tongued bumblebees tend to forage on flowers with deep corolla (Balfour et al. 2013). While pollinator species with proboscis shorter or longer than the honey bee mostly foraged on plant species that were not visited by honey bees, pollinators with a similar proboscis visited the same plant species, therefore, increasing their potential competition. Second, body size determines how far pollinators are able to forage, with large pollinators usually having a longer

foraging range compared to small species (Gathmann and Tscharntke 2002; Greenleaf et al. 2007). Here, we found that body size was a key functional trait, driving the resource overlap between wild pollinators and the honey bee. The latter increased with increasing body size, even if we expected a higher overlap for species similar in size to the honey bee. Potential competition with honey bees was, therefore, higher for large species, such as bumblebees. Third, we also observed an increase in resource overlap for central-place foragers. These species are obliged to forage relatively near the nest, based on their foraging range, and are, therefore, unable to expand their foraging area, even when the local density of honey bees is high (Walther-Hellwig et al. 2006). Fourth, many Hymenoptera families such as Apidae, Halictidae, and Megachilidae showed a high level of resource overlap with honey bees. Surprisingly, both thick-headed flies (Diptera: Conopidae) and hoverflies (Diptera: Syrphidae), which we expected to mostly visit open disc flowers, also showed a relatively high resource overlap. While the potential negative effects of honey bees on wild pollinators have often focused on wild bees (*e.g.*, Mallinger et al. 2017), other groups of insects might also be affected.

As the honey bee is not particularly active at low temperatures (Jaffé et al. 2010), we expected that its effect on wild pollinators would be stronger in sites with relatively high temperatures. However, similarly to what was observed in other works (*e.g.*, Corcos et al. 2020; Seoane et al. 2021), we did not find any effect of temperature on resource overlap between wild pollinators and the honey bee, even if the observed temperature range was large (min = 18 °C, max = 38 °C).

5.6. Conclusions

Honey bees have been introduced worldwide, and, therefore, often cohabit with wild pollinators. As their hives can host more than 50,000 individuals, their abundance in natural and managed habitats can be extremely high. Here, we showed that the potential interactions between wild pollinators and honey bees depended on functional traits of both plants and pollinators. In particular, our results highlight the potential role of plant functional diversity in supporting wild pollinators in areas with high honey bee density by decreasing the resource overlap between wild pollinators and the honey bee. Moreover, as pollinator species with traits similar to those of the honey bee tended to visit the same plant species, they could be more vulnerable to potential competition. From a conservation point of view, particular attention should be paid to the potential effects of beekeeping in sites where pollinator species of conservation concern possess functional traits similar to those

of the honey bee. More research is needed to quantify potential short- and long-term effects of high honey bee abundance on fitness, health, and population dynamics of wild pollinators.

5.7. Acknowledgements

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CHAPTER 6

Does pollinator conservation promote environmental co-benefits?

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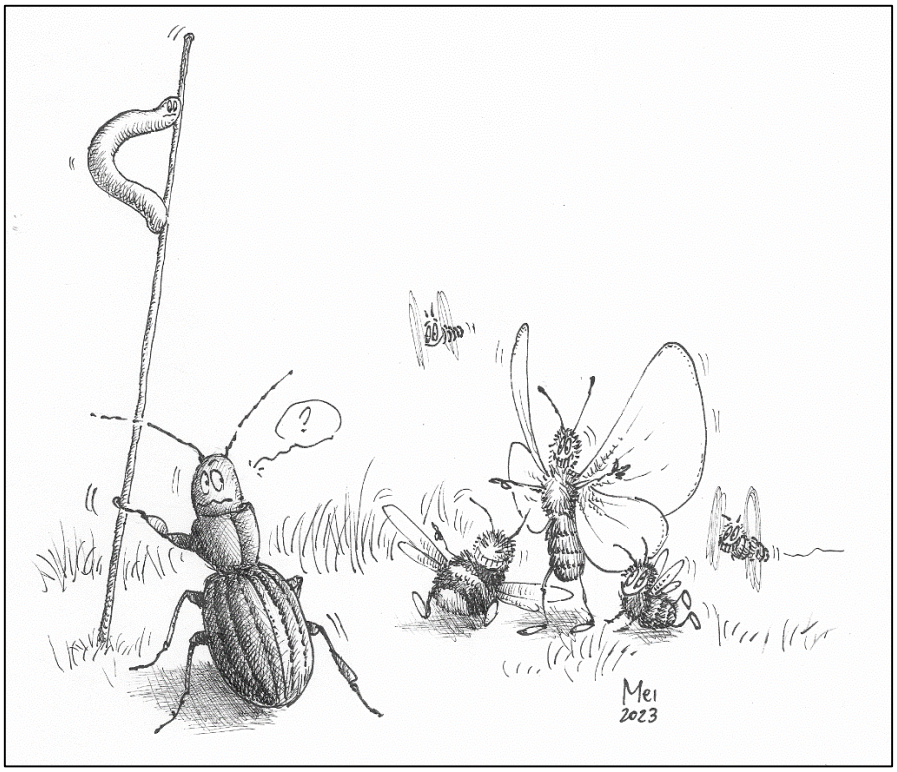

6.1. Abstract

The decline of pollinators is an urgent issue that has gained global attention and many initiatives have been implemented to promote conservation actions. However, interventions aimed at safeguarding pollinators can have ripple effects on multiple ecosystem services that are equally important for human well-being. In this work, we investigated whether environmental conditions favouring pollinators are positively associated with the provision of multiple ecosystem services across three different habitats. We selected 96 sites belonging to three habitat types with different roles in supporting pollinators, *i.e.*, crop field margins, semi-natural patches, and urban green areas. We sampled wild pollinators and seven ecosystem services, which included provisioning, cultural, and regulatory services, using which we calculated two ecosystem multi-functionality metrics. Semi-natural patches and crop field margins exhibited both the highest diversity of pollinators and ecosystem multi-functionality, *i.e.*, habitats that supported pollinators also delivered a higher number of environmental co-benefits. However, increasing habitat quality for pollinators did not result in increased multi-functionality, indicating that single ESs exhibited non-linear responses. Therefore, improving local conditions for wild pollinators did not enhance ecosystem multi-functionality, while specific habitat types have been shown to have the potential to improve pollinator diversity while generating multiple environmental co-benefits.

6.2. Introduction

Pollination is one of the most valuable ecosystem services (ESs), with an estimated overall monetary value of about US$195 billion (Bauer and Sue Wing 2016). Animal pollination, in particular, is essential for ensuring wild plant reproduction (Aguilar et al. 2006; Ollerton et al. 2011) and maintaining crop productivity (Klein et al. 2007; Garibaldi et al. 2013). Since the decline of pollinators could strongly impact pollination (Reilly et al. 2020), maintaining or increasing pollinator diversity and abundance has become a central target in biodiversity conservation (Brittain et al. 2013; Hallmann et al. 2017; Lemanski et al. 2022). Common interventions to support pollinators include management actions at the local scale, *e.g.*, improving habitat quality by increasing flower cover and diversity (Gill et al. 2016; Sutter et al. 2017; Klaus et al. 2021), but also the enhancement of landscapes, *e.g.*, by restoring natural and semi-natural habitats (Scheper et al. 2013; Tonietto and Larkin 2018). However, any intervention designed for pollinators should be carefully assessed, as it could affect multiple ESs both positively and negatively (Galler et al. 2015).

In the best-case scenario, habitat or landscape manipulations to boost pollinator diversity also increase multiple ESs, leading to enhanced ecosystem multi-functionality (EMF). EMF is the capacity of a landscape, habitat, or ecosystem to provide multiple functions at the same time, implying social, economic, and ecological benefits (Byrnes et al. 2014). Until now, most studies on EMF have focused on its association with biodiversity, highlighting positive relationships between EMF and above- and below-ground diversity (Maestre et al. 2012; Lefcheck et al. 2015; Mensah et al. 2020; Delgado-Baquerizo et al. 2020; Fan et al. 2023; but see Gamfeldt and Roger 2017). A key research gap concerns the response of EMF to conservation actions in different habitat types. In particular, it is not known yet the extent to which management actions designed to conserve pollinators in different environments will lead to positive effects on other ESs, potentially generating environmental co-benefits.

ES provision worldwide strongly depends on land use change (Millennium Ecosystem Assessment 2005; Haddad et al. 2015; Gomes et al. 2020). In general, habitats with a favourable conservation status enhance both regulating and cultural ESs (Maes et al. 2012), and a high amount of semi-natural areas enhances biodiversity-based ESs compared to urban and agricultural areas (Baral et al. 2014). For example, pest control is strongly related to the presence of semi-natural habitats in the landscape (Rusch et al. 2016; Holland et al.

2016; Rega et al. 2018), and pollinator diversity declines with increasing distance from semi-natural areas (Ricketts et al. 2008). However, even urban areas, especially those characterized by a moderate level of urbanization and rich in green areas, seem to better support pollinators and the ESs they provide than agricultural areas (Theodorou et al. 2020; Wenzel et al. 2020, but see Baldock et al. 2015). Usually, agricultural areas are fundamental for crop production but are poor in delivering other ESs, particularly regulating ones (Maes et al. 2012; Laura et al. 2017; Tóth et al. 2018). Nonetheless, most of these studies analysed how single ES provisioning changed in different habitat types, without taking into account the possible interactions among ESs.

In this work, we measured wild pollinator diversity, flower cover and diversity and seven ESs, comprising provisioning, regulating and cultural ESs, through eight ES indicators. We selected 96 sampling sites in north-eastern Italy belonging to three habitat types, *i.e.*, crop field margins, semi-natural patches, and urban green areas. Selected habitats represent common land-use categories, each potentially suitable to support pollinators but characterised by a distinct degree of relevance to pollinators. Moreover, sites belonging to the same habitat were selected along a gradient of habitat quality for pollinators, estimated through flower cover and diversity. Our specific aims were 1) to understand how wild pollinator diversity and EMF varied among different habitat types, and 2) to test whether improving local conditions for pollinators would also boost EMF. We expect that both pollinator diversity and EMF would be higher in semi-natural patches and that EMF would increase with increasing flower cover and diversity, suggesting that both restoring semi-natural habitats and improving existing habitat quality for pollinators should produce multiple environmental co-benefits.

6.3. Materials and methods

6.3.1. Study area and sampling design

We selected four regions in north-eastern Italy (Table S6.1, Figure S6.1). Within each region, we selected 24 sampling sites representing three habitat types: crop field margins, which included simple herbaceous margins and complex margins; semi-natural patches, which included grasslands and open abandoned areas; and urban green areas, which included both private and public gardens. Within each region, we selected 8 sites for each habitat type. Within each habitat, sites were chosen a priori along a gradient of quality for pollinators, taking into account both the cover and diversity of floral resources. Sites belonging to the same habitat type were at least 500 m away from each other. Climatic conditions of sites were similar since elevation ranged between

10 and 550 m above sea level. Minimum annual temperatures ranged from 0 °C in January and 18 °C in July, maximum annual temperatures ranged from 6 °C in January and over 30 °C in July, and total annual precipitation ranged from 800 to 1100 mm.

6.3.2. Wild pollinator and plant sampling

We sampled wild pollinators, *i.e.*, wild bees (Hymenoptera: Apoidea: Anthophila) and hoverflies (Diptera: Syrphidae), using pan traps. At each site, we placed three pan traps (yellow, blue, and white; 750 ml capacity, 12.5 cm diameter, 4.5 cm height), 1 m apart from each other, filled with water and a drop of biodegradable dish soap with no fragrance. We did not perform standard transect observations since the sampling was performed by people with different skills, and due to COVID-19 restrictions, it was not possible to work in teams. Pan traps were placed on the ground, in areas with short grass, so that they were clearly visible to pollinators. Pan traps were exposed for 48 h during sunny days, with low wind and temperatures > 20 °C. Wild pollinators were morphologically identified to the species or morphospecies level by DP (hoverflies), and AC and MM (wild bees). Wild pollinator samplings were repeated three times, once per month, between May and July 2021. Since pan traps are considered an unreliable method for estimating pollinator abundance (Westphal et al. 2008; Portman et al. 2020), we focused on pollinator diversity. We calculated α-diversity, *i.e.*, the number of wild pollinator species at each site, and γ-diversity, *i.e.*, the total number of wild pollinator species for each habitat type.

The cover and diversity of flowering plant species are strong indicators of habitat quality for pollinators, and can therefore be used as proxies for habitat enhancement for pollinators (Wratten et al. 2012; Zamorano et al. 2020; von Königslöw et al. 2022). At each site, we identified all flowering plant species in a 10-m radius buffer around the pan traps and assessed their relative abundance. The sampling was repeated three times, once per month, between May and July 2021. At each site, we then calculated flowering plant species α-diversity and mean flower cover.

6.3.3. Assessment of multiple ESs

Between April and September 2021, we measured eight indicators of seven ESs at each site. ESs were chosen based on the Common International Classification of Ecosystem Services (CICES) 5.1 categories and included provisioning, regulating and cultural ESs, mostly related to biodiversity (Table 6.1) (Haines-Young and Potschin 2018). We chose a high number of ESs that are fundamental in both agricultural and natural areas

(Garland et al. 2021), but are rarely assessed in urban environments (Pereira et al. 2023). Moreover, all selected ESs could be quickly and easily measured in all habitat types.

Table 6.1: List of the assessed ESs, with information on the corresponding Common International Classification of Ecosystem Services (CICES) 5.1 category and code (Haines-Young and Potschin 2018) and the measured ES indicators.

ES	CICES 5.1 category	CICES 5.1 code	ES indicator(s)
1) Honey bee-related ESs	Provisioning, regulating, cultural	1.1.3.1, 2.2.2.1, 3.1.1.2, 3.1.1.3	Managed honey bee abundance
2) Ground-dwelling arthropod-related ESs	Regulating	2.2.2.2, 2.2.3.1	Ground-dwelling arthropod abundance
3) Pest control	Regulating	2.2.3.1	Dummy caterpillar predation rate
4) Seed predation	Regulating	2.2.2.2	Seed predation rate
5) Disease control	Regulating	2.2.3.2	Asian tiger mosquito egg abundance
6) Soil nutrient cycling	Regulating	2.2.4.2	Soil stabilisation factor S and decomposition rate k
7) Flood control	Regulating	2.2.1.3	Water infiltration rate in soil

6.3.3.1. Honey bee-related ESs

The honey bee (*Apis mellifera* Linnaeus) is the most important managed pollinator species (Hung et al., 2018). ESs provided by honey bees include several regulating, provisioning, and cultural services. Since large-sized pollinators are often under-sampled using pan traps (Roulston et al. 2007), we opted for direct observations of honey bees on flowering plants to assess their abundance. At each site, we counted honey bees on flowers for 10 min. Honey bee samplings were repeated three times, once per month, between May and July 2021. At each site, we then calculated the total honey bee abundance.

6.3.3.2. Ground-dwelling arthropod-related ESs

Ground-dwelling arthropods include key groups of pest and seed predators (Bohan et al. 2011; Nyffeler and Birkhofer 2017). We assessed ground-dwelling arthropod abundance using pitfall traps. At each site, we placed two pitfall traps, consisting of a buried plastic cup (500 ml capacity, 11 cm diameter, 15 cm height) protected by a plastic cover (Spence and Niemelä 1994). Traps were activated with 70% ethylene glycol for four weeks from June to August 2021, for a total of three sampling rounds. Collected arthropods were stored in 75% ethanol and sorted in the laboratory. At each site, we then determined the total abundance of target ground-dwelling arthropods, *i.e.*, ground beetles (Coleoptera: Carabidae), rove beetles (Coleoptera: Staphylinidae), and spiders (Araneae).

6.3.3.3. Pest control

Pest control by natural enemies is a major regulating ES, especially in agroecosystems (Holland et al. 2016; Rega et al. 2018). Dummy caterpillars are commonly used to assess the intensity of pest predation by actively hunting sight predators (Howe et al. 2009). We moulded 30 mm × 3 mm dummy caterpillars using green plasticine and glued the caterpillars on wood skewers. We placed eight dummy caterpillars at each site, which were exposed for 72 hours. The sampling was repeated two times, in June and July 2021. We then checked all predation marks on caterpillars and determined the mean predation rate of dummy caterpillars at each site.

6.3.3.4. Seed predation

We used seed cards to assess the intensity of predation of weed seeds by seed predators. Seed cards were made of small rectangles (8 × 3 cm) of P80 grit sandpaper, on which seeds were glued using a repositionable glue (3 M Spray Mount) (Westerman et al. 2003). On each seed card, we glued forty seeds of *Taraxacum officinale* (Weber) ex Wiggers, a native plant species, and forty seeds of *Oenothera biennis* L., an invasive exotic species. At each site, we placed three seed cards that were fixed to the ground using nails and were exposed for 72 hours, during sunny days with low wind. Then, we collected the seed cards and counted the remaining seeds of each species. The sampling was repeated twice, in June and July 2021. At each site, we then estimated the mean seed predation rate.

A limitation of this study is that we assumed that weed seeds removed from the cards were predated, thus indicating a service, although we cannot ensure that the organisms that removed the seeds actually destroyed them. However, seed cards have been used for decades to specifically assess seed predation rather than dispersal (Brust and House 1988; Westerman et al. 2003). Moreover, the most common predators of both *T. officinale* and *O. biennis* are insects (Honek et al. 2005; Anstett et al. 2014), such as ground beetles, which are key seed predators (Kulkarni et al. 2015; Carbonne et al. 2020). Seeds of both species are relatively small in size (weight of 1000 seeds for both species: 0.45 gr) and birds and rodents probably predated them to a minimal extent (Hulme 1998).

6.3.3.5. Disease control

We used ovitraps to estimate the abundance of Asian tiger mosquitoes, *Aedes albopictus* (Skuse), a species of medical importance (Benedict et al. 2007). Ovitraps consisted of a small dark container (400 ml capacity, 8 cm diameter, 10 cm height) filled with water and containing a masonite stick where mosquitoes laid their eggs. At each site, we placed one ovitrap on the ground and exposed it for two weeks at the end of July 2021, during

the peak season of egg laying (Petrić et al. 2021). Ovitraps were collected, and the number of eggs was counted using a stereoscope.

6.3.3.6. Soil nutrient cycling

We estimated the decomposition rate of organic matter in soil using the Tea Bag Index (TBI) methodology (Keuskamp et al. 2013). We weighed the green tea and rooibos before placing the bags in the field. At the end of April 2021, we buried two pairs of bags in two 8-cm-deep holes at each site. For each pair, we used one green tea bag and one rooibos bag. After three months, at the end of July 2021, bags were collected, oven-dried at 65 °C for 48 h, and their contents were weighed. At each site, following the TBI protocol, we calculated the stabilisation factor *S* and the decomposition rate *k* (Keuskamp et al. 2013).

Since we were not able to collect all green tea and rooibos bags after three months, we had a few missing values for both the stabilisation factor and the decomposition rate, which we replaced with the respective averaged values to have the same number of measured ESs in all sites. However, to ensure that the use of averaged values would not affect the results of our models, we also performed all the statistical analyses excluding sites with missing bags, *i.e.*, those for which it was not possible to calculate soil stabilisation factor and/or soil decomposition rate. This sensitivity analysis indicated that all models did not show significant differences, therefore, we only present the results of models including averaged values.

6.3.3.7. Flood control

We assessed flood control by measuring the rate of water infiltration in soil (United States Department of Agriculture 2014). The measurements were taken after the soil had been saturated by rain, in September 2021. At each site, we selected a spot with short grass, where we hammered a plastic tube (20 cm diameter) in the ground for about 10 cm. Then, we poured 1 L of water into the plastic tube and assessed the water depth at the beginning of the experiment and after 6 min to obtain the water infiltration rate. We repeated the process three times per site. At each site, we then calculated the mean water infiltration rate as the average value of the three trials.

6.3.4. Assessment of EMF

We assessed EMF at each site including measures for honey bee-related ESs, ground-dwelling arthropod-related ESs, pest control, seed predation, disease control, soil nutrient cycling, and flood control. We used two

approaches: 1) the averaging approach (Mouillot et al. 2011), and 2) the multiple threshold approach (Byrnes et al. 2014). All statistical analyses were performed using *R* version 3.6.1 (R Core Team, 2019).

Using the averaging approach, we calculated a simple EMF index based on normalized values for each ES indicator. First, we normalized each ES indicator value by its maximum, using the formula $X_{norm} = (X_{raw} - X_{min}) / (X_{max} - X_{min})$, where X_{norm} is the normalized ES indicator value, X_{raw} is the raw ES indicator value, X_{min} is the minimum ES indicator value and X_{max} is the maximum ES indicator value. We considered as X_{min} and X_{max} the minimum and maximum ES indicator values observed over the whole dataset, including all three habitat types. For the abundance of Asian tiger mosquito eggs, the only indicator for which low values indicate higher levels of the ES, raw indicator values were reflected before normalization as $X_{ref} = X_{max} - X_{raw}$. Second, we calculated the averaged EMF index for each site as the mean value of all normalized indicator values. Averaged EMF was calculated using the *R* package *caret* (Kuhn 2008).

The multiple threshold approach allows for evaluating whether multiple functions are simultaneously performing at high levels. We considered the full range of thresholds, from 1% to 99% of the maximum value of each ES indicator, and then counted the number of ES indicators that surpassed each threshold at each site. To compute the multiple threshold EMF, we used the *R* package *multifunc* (Byrnes et al. 2014).

6.3.5. Statistical analyses

First, we visually assessed the differences among the three habitat types for wild pollinator α-diversity, flower cover, flowering plant α-diversity and ES indicators. To do so, we compared normalized variable values among the three habitat types using a radar plot.

Second, we analysed how wild pollinator α-diversity and EMF changed in the three habitat types (indicator of habitat restoration) and in relation to flower cover (indicator of habitat enhancement). As flower cover and flowering plant α-diversity were strongly correlated (Pearson's correlation coefficient = 0.606, p value < 0.001), we could not include both in the same models. Therefore, all models were run twice, first using flower cover as explanatory variable, and then using flowering plant α-diversity as explanatory variable. All models including flower cover showed a lower AIC, therefore, we chose flower cover as an indicator of habitat enhancement for pollinators. We built two linear mixed-effect models using wild pollinator α-diversity and averaged EMF as response variables, and habitat type and flower cover as explanatory variables. We also included the region ID as random factor. To run these models, we used the *R* package *nlme* (Pinheiro et al.

2019). Moreover, using the multiple threshold approach, we analysed the effect of habitat type and flower cover on the number of ESs beyond a certain level of performance. To visually assess the significance of each threshold, we calculated the slope of these relationships and plotted them against the corresponding threshold value. Figures were plotted using the *R* package *ggplot2* (Wickham 2016).

Third, to quantify the relationships between wild pollinator α-diversity, flower cover, flowering plant α-diversity and ES indicators and test how these relationships changed among the three habitat types, we calculated the Pearson's correlation coefficients between pairs of variables within each habitat type. Correlations were plotted using the *R* package *corrplot* (Wei et al. 2017).

Landscape context, in particular the amount of semi-natural areas, could affect both wild pollinators and EMF. However, our study was not designed to explore the effect of landscape context, since due to COVID-19 restrictions during fieldwork, we could only sample sites relatively close to the area where the authors who did the fieldwork resided. Therefore, we decided to reduce as much as possible the variation in landscape composition during site selection. To evaluate any potential effect of landscape variables, we fitted three models for each response variable, *i.e.*, wild pollinator α-diversity and averaged EMF, using maximized log-likelihood and compared them using ΔAICc. Model 1 included as explanatory variables habitat type and flower cover. Model 2 included as explanatory variable only the percentage of semi-natural habitats in a 250 m radius buffer around the sampling sites. Model 3 included as explanatory variables habitat type, flower cover, and the percentage of semi-natural habitats in a 250 m radius buffer around the sampling sites. For all response variables, the ΔAICc between Model 1 and Model 3 was below 2, indicating little improvement with the addition of landscape variables, while the difference between Model 1 and Model 2 was always above 2, indicating a better predictive power of local variables (Table S6.2).

6.4. Results

6.4.1. Wild pollinators

We collected 1516 wild pollinator individuals belonging to 144 species or morphospecies (Table S6.3). The most represented wild pollinator family was Halictidae, with 1,080 individuals and 45 species collected, which included the three most abundant wild pollinator species, *i.e.*, *Lasioglossum glabriusculum* (Morawitz) (295 individuals), *L. malachurum* (Kirby) (125 individuals), and *L. minutissimum* (Kirby) (118 individuals). While wild bees were relatively common, we only collected 96 hoverfly individuals belonging to 30 species.

6.4.2. Effect of habitat type on wild pollinators and EMF

Semi-natural patches were characterized by a higher provision of most ESs compared to crop field margins and urban green areas, and results were similar for pollinators and flowering plants (Table S6.4, Figure 6.1). However, the abundance of ground-dwelling arthropods was higher in crop field margins. Urban green areas generally showed the lowest variable values, except for honey bee abundance, flower cover and flowering plant α-diversity. Soil-related ESs were comparable among the three habitat types.

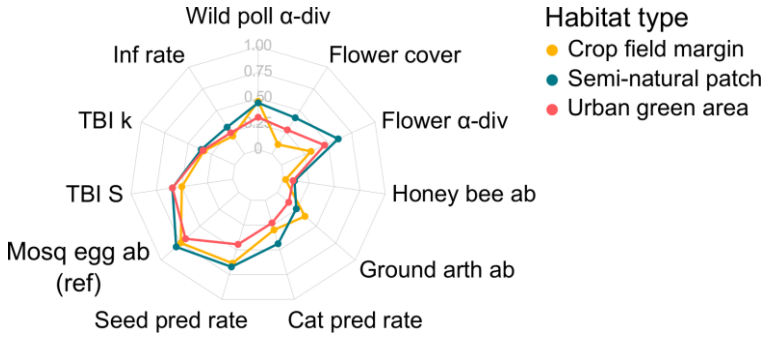

Figure 6.1: Radar plot showing the mean normalized value of each variable at each habitat. Abbreviations are: "Wild poll α-div" for wild pollinator α-diversity, "Flower cover" for flowering plant cover, "Flower α-div" for flowering plant α-diversity, "Honey bee ab" for managed honey bee abundance (honey bee-related ESs), "Ground arth ab" for ground-dwelling arthropod abundance (ground-dwelling arthropod-related ESs), "Cat pred rate" for dummy caterpillar predation rate (pest control), "Seed pred rate" for seed predation rate (seed predation), "Mosq egg ab (ref)" for Asian tiger mosquito egg abundance (reflected) (disease control), "TBI S" for soil stabilisation factor S and "TBI k" for soil decomposition rate k (soil nutrient cycling), and "Inf rate" for water infiltration rate in soil (flood control).

Habitat type affected both wild pollinator diversity and EMF. Wild pollinator α-diversity was comparable in semi-natural patches and crop field margins, and it was lower in urban green areas (Table 6.2 *a*, Figures 6.2 *a*, S6.2 *a*). However, wild pollinator γ-diversity was higher in semi-natural patches than in other habitats. We observed 111 wild pollinator species in semi-natural patches, 77 species in crop field margins, and only 59 species in urban green areas.

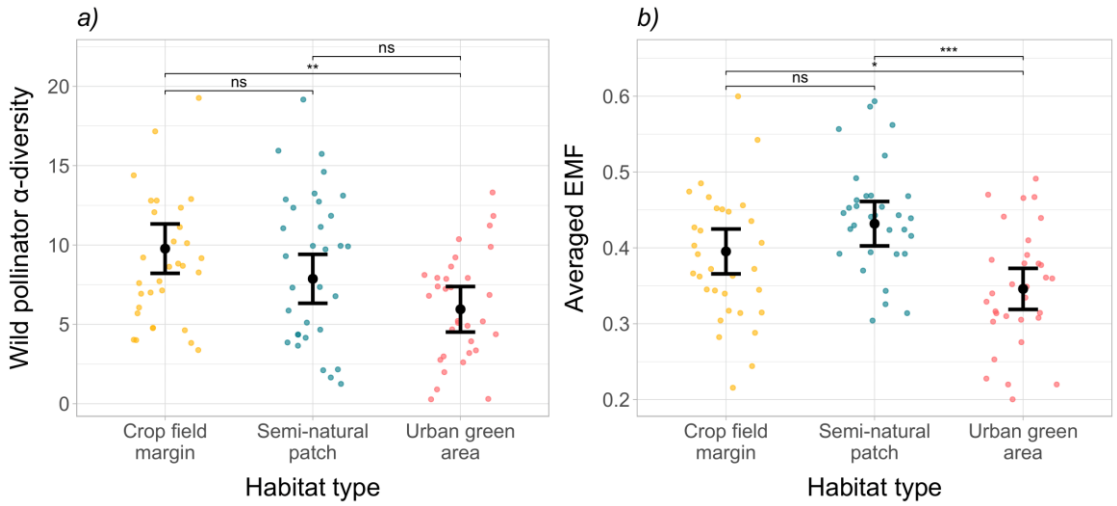

Figure 6.2: Plots showing the effect of habitat type on *a)* wild pollinator α-diversity and *b)* averaged EMF. Small coloured points represent raw data points, large black points represent model estimates, and bars represent the 95% confidence intervals.

Averaged EMF was also higher in semi-natural patches and crop field margins (Table 6.2 *b*, Figures 6.2 *b*, S6.2 *b*). EMF calculated using the multiple threshold approach showed a similar response to habitat type. EMF in semi-natural patches and crop field margins was generally comparable at low thresholds, but their differences increased at higher thresholds, with semi-natural patches providing higher levels of multiple ESs (Table S6.5 *a*, Figure 6.3 *a*, *d*). We observed no differences in multiple threshold EMF between crop field margins and urban green areas (Table S6.5 *b*, Figure 6.3 *b*, *e*), while the comparison between semi-natural patches and urban green areas revealed higher values of EMF in semi-natural patches (Table S6.5 *c*, Figure 6.3 *c*, *f*).

Table 6.2: Results of the linear mixed-effect models testing the effect of habitat type and flower cover on *a)* wild pollinator α-diversity and *b)* averaged EMF. Values in bold indicate significant effects (*p* value < 0.05).

Response variable	Explanatory variable	Estimate	SE	df	*t* value	*p* value
a) Wild pollinator α-diversity	Intercept (Crop field margin)	8.113	0.782	89	10.380	< 0.001
	Semi-natural patch	-1.902	1.136	89	-1.674	0.098
	Urban green area	-3.822	1.023	89	-3.735	**< 0.001**
	Flower cover	0.128	0.047	89	2.751	**0.007**
b) Averaged EMF	Intercept (Crop field margin)	0.377	0.015	89	25.371	< 0.001
	Semi-natural patch	0.037	0.022	89	1.626	0.108
	Urban green area	-0.049	0.020	89	-2.434	**0.017**
	Flower cover	0.001	0.001	89	1.558	0.123

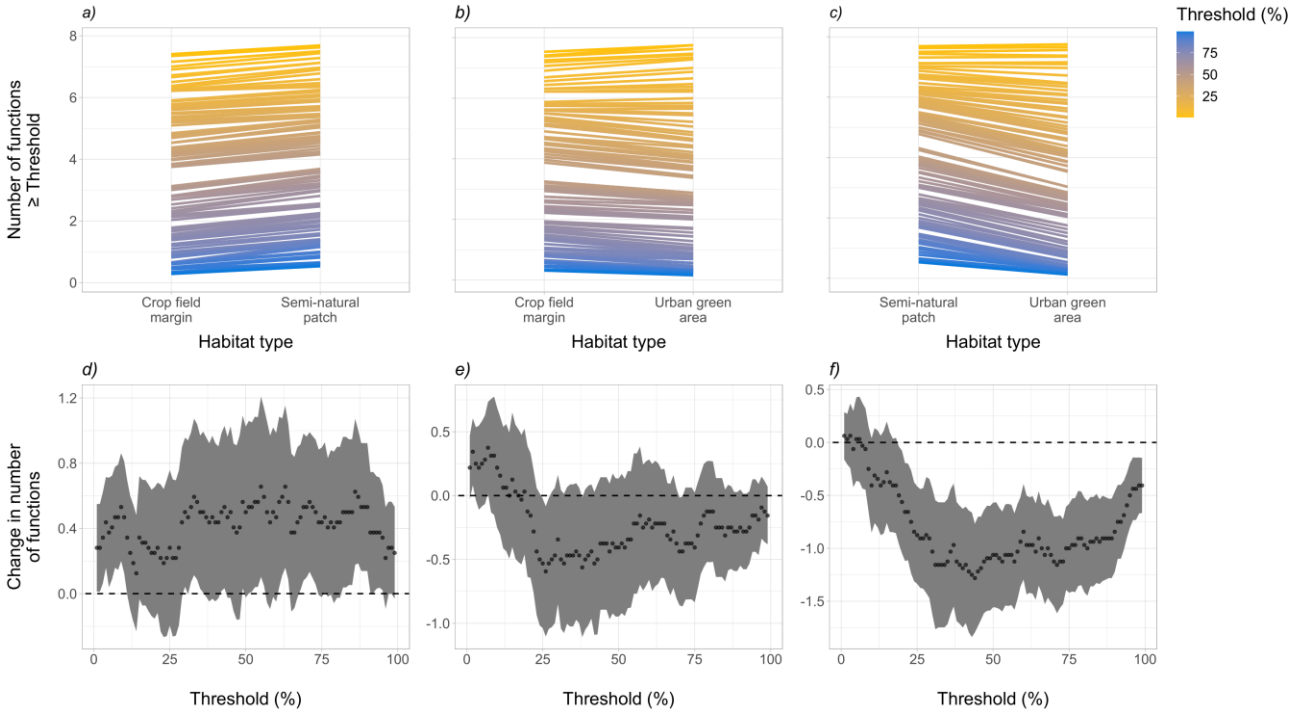

Figure 6.3: Plots showing the effect of habitat type on multiple threshold EMF, *i.e.*, the number of ESs maximized at a certain threshold level. Panels *a)* and *d)* compare crop field margins and semi-natural patches, panels *b)* and *e)* compare crop field margins and urban green areas, and panels *c)* and *f)* compare semi-natural patches and urban green areas. Panels *a)*, *b)*, and *c)* show the relationship between pairs of habitats and the number of functions that performed higher than a certain threshold. We considered the full range of thresholds, from 1% to 99% of the maximum value of each ES indicator, and each line represents a given threshold. Panels *d)*, *e)*, and *f)* show the corresponding relationship between the threshold value and the slope of the relationship between habitat type and the number of functions reaching a certain threshold. Black points represent fitted values and the shaded areas represent the 95% confidence intervals. For each threshold, the relationship with habitat type is significant if the confidence interval does not overlap zero.

6.4.3. Effect of flower cover on wild pollinators and EMF

Wild pollinator α-diversity strongly increased with increasing flower cover (Table 6.2 *a*, Figure 6.4). On the other hand, flower cover did not affect averaged and multiple threshold EMF (Tables 6.2 *b*, S6.6, Figure S6.3). To explain this result, we analysed the correlations between wild pollinator α-diversity, flower cover, flowering plant α-diversity, and ES indicators (Figure S6.4). We highlighted several co-benefits (positive correlations) and only a few trade-offs (negative correlations) among variables in all habitat types. However, correlations changed depending on habitat type. In crop field margins, wild pollinator α-diversity showed a trade-off with infiltration rate, but we also observed synergies between honey bee abundance and flower cover, flower cover and soil decomposition indices, and abundance of ground arthropods and predation rate of dummy caterpillars (Figure S6.4 *a*). Semi-natural patches showed the lowest number of significant correlations among variables, of which only one was a trade-off, and we observed no significant relationships between wild pollinator α-diversity and other variables (Figure S6.4 *b*). In urban green areas, wild pollinator α-diversity was positively

correlated to flowering plant α-diversity and ground-dwelling arthropod abundance, and negatively correlated to the abundance of Asian tiger mosquito eggs (Figure S6.4 *c*).

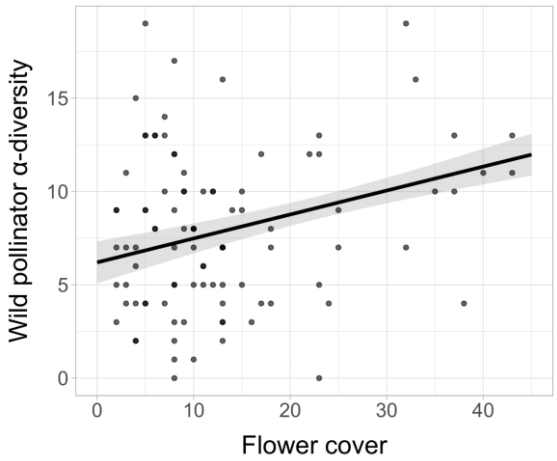

Figure 6.4: Plot showing the effect of flower cover on wild pollinator α-diversity. Points represent raw data points, the line represents model estimate, and the shaded area represents the 95% confidence interval.

6.5. Discussion

To our knowledge, this is the first study investigating how conservation actions for pollinators, *i.e.*, habitat restoration and enhancement, affected EMF calculated using a considerable number of ESs. We found that habitat types supporting a higher diversity of wild pollinators were also associated with higher EMF. On the other hand, we did not find any relationship between flower cover and EMF in the three habitat types, meaning that improving local conditions for pollinators did not lead to higher EMF and indicating non-linear responses of multiple ESs.

6.5.1. Effect of habitat type on wild pollinators and EMF

Contrary to our expectations, we found that semi-natural patches and crop field margins hosted a comparable number of wild pollinator species. However, even if the number of species at each site was similar, the total species diversity of crop field margins was considerably lower, with 34 fewer pollinator species than in semi-natural patches, *i.e.*, species assemblages of field margins were more homogeneous and characterized by a low spatial turnover. Wild pollinators are usually negatively affected by agricultural intensification (Le Féon et al. 2010; Williams et al. 2010) since floral resources are often insufficient and the use of pesticides can pose a serious threat (Goulson et al. 2015). However, unmanaged field margins can be a crucial resource for pollinators in agricultural areas (Arnold et al. 2021; Slupik et al. 2022). In our study, we sampled both simple herbaceous field margins and complex field margins characterized by hedgerows and trees that might have

boosted pollinator diversity (Aviron et al. 2023). Also, we found that urban green areas hosted the lowest number of pollinator species. This result is quite unexpected since recent studies highlighted the potential importance of urban areas for pollinators (Hall et al. 2017; Wenzel et al. 2020). However, these positive effects have been mostly reported for wild bees (but see Herrmann et al. 2023), while other pollinator groups such as hoverflies are known to be negatively affected by urbanization (Lagucki et al. 2017; Theodorou et al. 2020; Herrmann et al. 2023).

EMF also changed among the three habitat types. Averaged EMF showed comparable values in semi-natural patches and crop field margins and lower values in urban green areas. Semi-natural areas and, in general, habitats with a low management intensity have been shown to exhibit higher EMF (Lavorel et al. 2022; Moi et al. 2022; Olimpi et al. 2022). In particular, our crop field margins showed a high abundance of ground-dwelling arthropods and a high predation rate of seeds, as they often provide shelter and alternative prey (Allan et al. 2015; Samnegård et al. 2019). However, the multiple threshold approach revealed that at higher thresholds the difference between semi-natural patches and crop field margins was consistent, meaning that semi-natural patches, unlike crop field margins, were able to simultaneously provide high levels of multiple ESs. On the other hand, lower EMF values in urban green areas were expected, since regulating services have been shown to strongly decrease with increasing urbanization (Wang et al. 2019). Therefore, both wild pollinator diversity and EMF were maximized in semi-natural patches and crop field margins, also highlighting the potential role of field margins for sustaining pollinators while generating multiple environmental co-benefits (Mkenda et al. 2019). Habitat conversion from intensively managed to pollinator-friendly habitats might not be the only way to increase pollinator diversity and EMF.

6.5.2. Effect of flower cover on wild pollinators and EMF

As expected, we found a positive relationship between flower cover and wild pollinator α-diversity. Habitat enhancement for pollinators, *i.e.*, the increase in diversity and cover of flowering plant species, is an effective measure specifically designed to boost pollinator abundance and diversity in different habitat types (Morandin and Kremen 2013; Woodcock et al. 2014; Andrieu et al. 2018; Zamorano et al. 2020; Dietzel et al. 2023; Hussain et al. 2023) since floral resources are one of the central factors in shaping pollinator populations.

Contrary to our expectations, we did not find any relationship between flower cover and EMF. This is in contrast with other studies since habitat enhancement seems to benefit not only pollinators but also other

ESs, especially those related to biodiversity (Wratten et al. 2012). Moreover, there is a large body of literature that showed positive relationships between biodiversity and EMF across different land use types, and most studies on the effect of above-ground biodiversity on EMF focused on plant species (Maestre et al. 2012; Jing et al. 2015; Lefcheck et al. 2015; Soliveres et al. 2016; Schittko et al. 2022; Zhou et al. 2022). However, here we did not sample the complete plant community, since we were only interested in understanding how flower cover and diversity, as indicators of habitat enhancement for pollinators, could affect EMF. Flower cover did not emerge as a good predictor of EMF, highlighting that improving the quality of existing habitats for pollinators does not positively affect EMF. Within the same habitat type, the analysis of the correlation among ESs indicated that probably the underlying drivers that promoted wild pollinator diversity were distinct from those promoting EMF. Moreover, the lack of consistent relationships among services within the three habitats suggested that specific drivers may lead to non-linear responses depending on the habitat type. For instance, pollinator-targeted interventions are often beneficial not only to pollinators but also to predators of pests (Albrecht et al. 2020; Savage et al. 2021). However, sown flower strips do not always benefit pollinator populations as their effects may vary depending on the chosen flower mixture (Wood et al. 2015), and they might also increase the abundance of herbivores, resulting in a trade-off between pollination and pest control (Wäckers et al. 2007). Therefore, the net effects of pollinator-targeted interventions are not straightforward, and it is crucial to investigate which drivers determine high levels of different ESs among habitats (Bullock et al. 2021)

6.6. Conclusions

Maximising the delivery of multiple ESs across different habitat types is a complex task, but it is of central importance for the well-being of humans and ecosystems across human-impacted landscapes. Here, we showed that both semi-natural patches and crop field margins were associated with higher pollinator diversity and EMF, highlighting not only the key role of undisturbed habitats but also the potential importance of field margins. Nevertheless, it is fundamental to emphasise that the total diversity of pollinator species collected in crop field margins was much lower than in semi-natural patches, which are therefore able to support more heterogeneous pollinator communities. Moreover, we found no association between flower cover and EMF in any of the three investigated habitats, meaning that improving habitat quality for pollinators proved to be insufficient to enhance EMF. Our study indicated that promoting pollinators does not always increase the

number of co-benefits that could be delivered to society. Future investigations are needed to understand how pollinator interventions could affect ESs and EMF in different habitat types, and how landscape composition and structure could modulate these relationships.

6.7. Funding information

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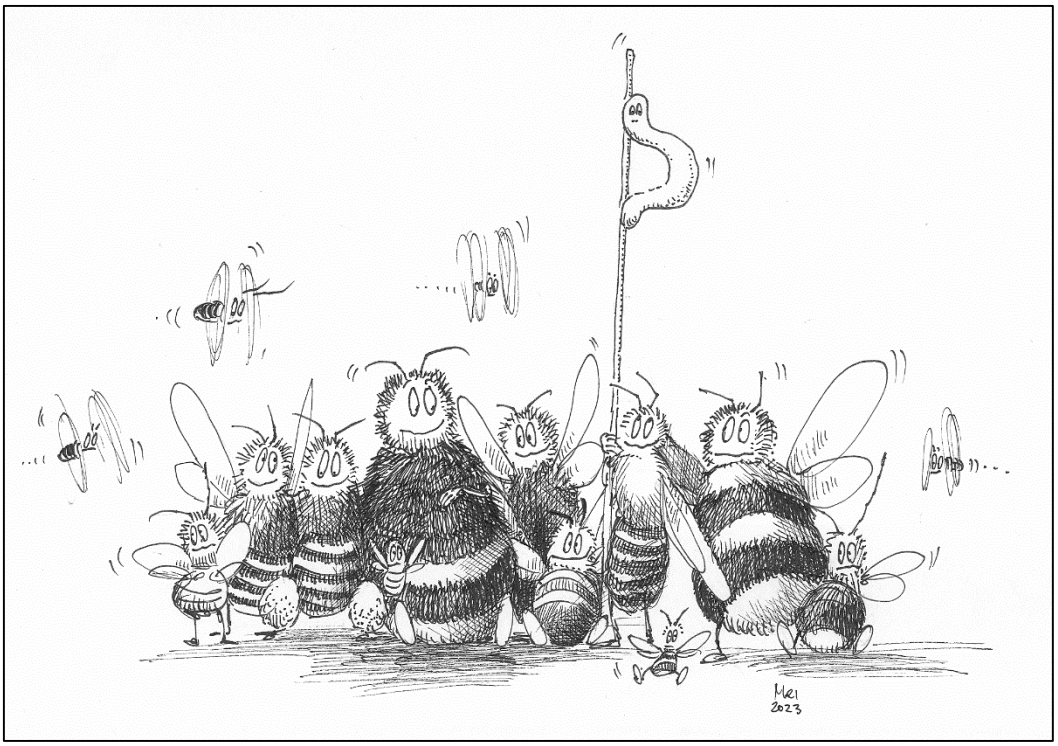
CHAPTER 7

General conclusions



Through the chapters of my PhD thesis, I was able to analyse in detail how managed and wild pollinators are affected by the drivers that determine their abundance and diversity in dynamic landscapes, *i.e.*, land-use changes and habitat loss, urbanization, and the introduction of managed species. Landscape alterations are now unavoidable and usually occur rapidly, leaving pollinator communities little time to adapt. While some species, the more resilient ones, may continue to thrive, other species will not be able to cope with the new conditions and will therefore decrease in abundance or, in the worst case, disappear. Understanding how pollinators respond to these factors will allow us to counteract their negative effects through tailor-made conservation interventions, summarized in Table 7.1.

Table 7.1: Summary of conservation interventions for pollinators that emerged in the chapters of my thesis, including information on the pollinator groups that can benefit from them and the chapters of the thesis in which the topic was addressed.

Conservation intervention	**Affected pollinator group**	**Tested in**
Preserve or restore semi-natural habitats	All pollinators	*Chapter 2* *Chapter 3* *Chapter 6*
Promote richness, cover and functional richness of flowering plants	All pollinators	*Chapter 5* *Chapter 6*
Limit the density of managed honey bees when floral resources are scarce	Wild pollinators	*Chapter 5*
Develop tailored conservation strategies for pollinators based on their functional traits	Wild pollinators	*Chapter 4* *Chapter 5*
Create climate change refugia in urban environments	Wild bees	*Chapter 4*
Provide early-season floral resources, *e.g.*, flowering trees such as willows	Honey bees	*Chapter 2*
Consider landscape structure when placing honey bee hives, *e.g.*, away from perennial crops	Honey bees	*Chapter 2* *Chapter 3*

7.1. The importance of protecting diverse semi-natural habitats and the resources they provide

Semi-natural habitats in landscapes are crucial for sustaining pollinator communities, as seen in *Chapter 2* and *Chapter 3*. However, semi-natural habitats are heterogeneous and not all of them support pollinators to the same degree. Maurer et al. (2022) showed that extensively and conventionally managed meadows, flower strips, hedgerows and forest edges hosted unique sets of pollinator species, and that the importance of these habitats changed throughout the flowering season. Bartual et al. (2019), on the other hand, showed that although forests and woodlands may be optimal for nest building and larvae development, they harboured the lowest abundance of bees compared to open semi-natural habitats. Different types of semi-natural habitats also

offer distinct nesting resources for wild bees, therefore, different bee taxa may benefit more from the presence of certain types of semi-natural habitats than others (Eeraerts and Isaacs 2023). These differences are mainly related to semi-natural habitat structure and vegetation composition, and thus to the amount of resources they can provide, particularly floral resources. For example, in *Chapter 2* we observed how plant phenology and bee preferences affected the composition of pollen collected by honey bees. Even if the abundance of pollinators in forests is generally low, we found that trees played a key role in supporting managed honey bees at the beginning of the flowering season, being replaced later in the season by herbaceous flowering plants, further confirming how different habitat types are needed for the well-being of pollinator populations.

The restoration of different types of semi-natural habitats, therefore, could be a key action to safeguard pollinator populations. However, whenever pollinator-friendly measures are implemented, the potential impact on other organisms and ecosystem services fundamental to our well-being should also be assessed, in order to maximise the positive effects of these conservation actions, as we observed in *Chapter 6*. In Europe, agri-environment schemes in agricultural landscapes are widely sponsored not only for the protection of pollinators but also for biodiversity and their associated ecosystem services. However, these programmes are not sufficient even to protect pollinators: to effectively protect insects, we would need three times the amount indicated by the current policy guidelines of a diverse range of habitats (Pindar and Raine 2023). Targeted policies for pollinators and other key organisms are essential (Cole et al. 2020), however, to date no government worldwide has delivered specific legislation to address biodiversity decline (Hall and Steiner 2019; Van Der Sluijs 2020).

7.2. Conservation of pollinator functional traits

The analysis of pollinator functional traits can be crucial for accurately assessing the response of pollinators to the drivers that determine their abundance and diversity. In *Chapter 4*, we found that wild bees characterized by specific functional traits were threatened by high temperatures in cities, and in *Chapter 5* we highlighted that potential competition with managed honey bees changed based on wild pollinator functional traits. Indeed, the impacts of these drivers on pollinator species are not related to their taxonomic identity, but rather to their functional identity. We observed that wild pollinator responses were influenced by their morphological features such as body size and proboscis length, ecological features such as foraging and nesting preferences, and evolutionary history such as taxonomic family. For example, although bees are generally considered a thermophilic group, negative impacts of increasing temperatures have been highlighted for bumblebees

(Janousek et al. 2023; Sepúlveda and Goulson 2023) and for above-ground nesting bees (Ulyshen and Horn 2023), stressing how climate effects strongly depend on pollinator traits (Dorian et al. 2023). This also emphasises that, despite being a common practice, the clustering of large groups of pollinators, *e.g.*, considering wild bees or hoverflies as single groups, should be evaluated carefully, as it can lead to results that do not give an accurate picture of the actual changes in communities.

Exploring the functional composition of pollinator communities and how they change is also crucial because these modifications can strongly impact the ecosystem service pollinators provide (Gagic et al. 2015) and, therefore, ecosystem resilience (Mouillot et al. 2013). Functional traits determine species role in ecosystems (Coux et al. 2016) and, for pollinators, traits such as body size and proboscis length are strongly related to pollination efficacy (Chase et al. 2023). Pollinator communities characterized by a high functional diversity are generally more efficient, potentially leading to higher crop yield (Hoehn et al. 2008; Woodcock et al. 2019), and changes in the functional composition of pollinator communities can also impact plant-pollinator networks and lead to major changes in plant communities (Simpson et al. 2022). Moreover, the loss of specific functional traits, for example large body size, could impact the reproduction of specific plant species (Zaragoza-Trello et al. 2023). It is therefore clear that not only the conservation of pollinator species but also the preservation of their functional traits should be a priority.

7.3. Managed and wild pollinators: Matching conservation strategies?

The honey bee is a key pollinator species, and the pollination and production of many crops depend on its activity. Therefore, it is crucial to ensure that honey bee colonies maintain optimal health, which could be achieved, for example, by placing their hives in landscapes with a high amount of semi-natural areas, as highlighted in *Chapter 2* and *Chapter 3*. However, honey bees are considerably different from other pollinators and bee species, being eusocial, competitive, super-generalist and, most importantly, managed by humans. These characteristics make managed honey bees potentially harmful to wild pollinators, as seen in *Chapter 5* – including feral honey bee populations, which in Europe are endangered due to the lack of nesting sites and transfer of pathogens and parasites from managed hives (Requier et al. 2019). In Europe, the vast majority of honey bees are managed and feral honey bee colonies are rare and scattered, with densities usually lower than 0.2 colonies km^{-2} and a high rate of winter mortality (Rutschmann et al. 2022; Kohl et al. 2022). The density of managed hives is much higher in Europe, with more than 5 hives km^{-2} in Italy (data provided by the National

Data Bank of the Zootechnical Registry established by the Ministry of Health at the National Service Centre of the "G. Caporale" Institute of Teramo). These unnatural abundances of managed honey bees could result, for example, in increasing competition for floral resources with wild pollinators: during the flowering season, a single honey bee colony could collect the pollen needed for the development of 100,000 wild solitary bees (Cane and Tepedino 2017).

Several strategies have been recommended to limit these potential negative effects on wild pollinators. For example, it has been proposed to limit the placement of bee hives in natural areas, or establish a threshold of hive density in landscapes (Geslin et al. 2017), however, some of these actions could be detrimental to managed honey bee health. Therefore, it is clear that we need an inclusive approach to find a balance between managed pollinator health and wild pollinator conservation (Kleijn et al. 2018). Specific legislation should be adopted that would lead to more sustainable and conscious beekeeping, without impacting bee health, crop pollination and honey production.

7.4. Knowledge of pollinator species

A key, but often neglected, aspect of pollinator conservation is that it cannot be accomplished without proper knowledge of pollinator species, their ecology, and distributions. Although pollinators are crucial for ecosystems and our well-being, our knowledge of some pollinator groups is extremely limited. Hoverflies are a relatively well-known group and for only 5% of European species, out of the total 890 species, the International Union for the Conservation of Nature (IUCN) could not evaluate the risk of extinction due to insufficient information (Vujić et al. 2022). For bees, on the other hand, the scenario is quite different: for more than half of the European species (55.6%), out of the total 1,965 species, we have too little information to define their overall population trend (Nieto et al. 2014). For instance, the Italian Red List of Bees only assessed the conservation status of less than 15% of the species reported for the country, and yet 55% were classified as "Data Deficient" (Quaranta et al. 2018). For the studies presented in *Chapter 4, Chapter 5* and *Chapter 6* we sampled managed and wild pollinators and gathered a large amount of data on species distribution and their floral and habitat preferences. By sampling for only three years and mostly in areas characterized by moderate or high anthropogenic pressure, we collected more than 400 pollinator species and morphospecies, including more than 100 hoverfly species and almost 250 bee species. These data are of critical

importance for our knowledge of pollinators and will contribute to future assessments of pollinator conservation status.

7.5. Future steps and final remarks

The factors that determine pollinator abundance and diversity are not independent of each other. For example, climate could interact with pesticides, either improving or worsening their effect on pollinator health (Kenna et al. 2023), but it can also interact with land-use changes, with effects changing from one pollinator guild to another (Ganuza et al. 2022). In my PhD thesis, I analyzed how pollinators were affected by individual factors, but future studies need to take into account the potential interactive effects among drivers in order to have a realistic and accurate overview of pollinator status that will allow us to effectively protect them. In addition, we know that even the more resilient pollinator species are experiencing change, *e.g.*, pollinator individuals of common species found in warmer urban areas are becoming smaller than those of the same species in cooler areas (Eggenberger et al. 2019; Tommasi et al. 2022). In my PhD thesis, I focused on community-level responses, which are the most easily observed in the short-term, but future studies should explore long-term adaptations at the intra-specific level.

Pollinator conservation is a multifaceted science and an ongoing challenge. The factors that determine the abundance and diversity of pollinators are multiple, complex, often context-dependent, and interconnected. It is our responsibility to work to slow, stop or reverse this process of decline, not only for their value for biodiversity but also because our well-being strongly depends on pollinators. To protect pollinators, the key ecosystem service they provide, and the fundamental contribution they make to biodiversity, tailored conservation actions are needed: to limit land consumption and restore natural and semi-natural habitats that are adequately connected – even in cities; to more strictly regulate beekeeping and become less dependent on honey bee pollination in favour of wild pollinators; to decrease the use of highly toxic pesticides, moving towards an integrated pest management approach, which could benefit not only pollinators and other organisms but also crops; and last, to pursue pollinator monitoring programs that allow determining how pollinator populations are changing over time.

Supplementary information

CHAPTER 2

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Table S2.1: List of the 13 sampling locations with information on coordinates (WGS84, decimal degrees), elevation (m a.s.l.), and percentage of agricultural (Agric), semi-natural (Semi-nat), man-made (Man-made), and other (Other) areas in 3 km radius buffers around the sampling locations.

Location	Lat (N)	Lon (E)	Elev	Agric	Semi-nat	Man-made	Other
Ala	45.782728	11.029251	291	20	73	5	1
Borgo Valsugana	46.055169	11.455965	406	44	41	15	0
Caldonazzo	45.997705	11.270409	462	33	42	12	13
Cavalese	46.283778	11.50333	894	37	55	8	0
Cles	46.361671	11.039318	650	46	31	12	11
Faedo	46.194805	11.169871	700	18	79	3	0
Giovo	46.143398	11.118596	339	52	25	21	0
Peio	46.38582	10.687238	1481	8	76	0	0
Pergine Valsugana	46.073691	11.231101	503	38	43	19	0
Riva del Garda	45.901052	10.852101	93	40	28	27	4
Romagnano	46.000702	11.113726	192	44	45	8	3
Rovereto	45.884123	11.021881	175	41	28	29	2
Tiarno di Sopra	45.893395	10.679264	747	13	83	3	0

Table S2.2: CORINE Land Cover (CLC) classes and merged categories.

CLC code	CLC class	Category
111	Continuous urban fabric	Urban
112	Discontinuous urban fabric	
121	Industrial, commercial and public units	
122	Road and rail networks and associated land	
124	Airport	
131	Mineral extraction sites	
132	Dump sites	
211	Non-irrigated arable land	Crop
221	Vineyards	
222	Fruit trees and berries plantations	
231	Pastures	
242	Complex cultivation patterns	
243	Crop with significant amount of natural vegetation	
311	Broadleaved forest	Semi-natural
312	Coniferous forest	
313	Mixed forest	
321	Natural grassland	
322	Moors and heathland	
324	Transitional woodland-scrub	
332	Bare rock	
333	Sparsely vegetated areas	
335	Glaciers and perpetual snow	
511	Water courses	Other
512	Water bodies	

Table S2.3: Results of the Principal Component Analysis (PCA) for 1, 3, and 5 km radius buffers around the sampling locations, showing variable loadings and their eigenvector values.

	1 km		**3 km**		**5 km**	
	PC1	**PC2**	**PC1**	**PC2**	**PC1**	**PC2**
Explained variability	22.4%	15.8%	26.1%	19.9%	28.8%	16.3%
CLC class						
111 - Continuous urban fabric	-0.382	-0.277	0.231	0.272	-0.203	-0.087
112 - Discontinuous urban fabric	-0.331	0.284	0.281	0.347	-0.266	-0.193
121 - Industrial, commercial and public units	-0.308	0.178	0.255	0.227	-0.233	0.098
122 - Road and rail networks and associated land	-	-	-	-	-0.146	0.056
124 - Airport	-	-	0.096	-0.288	-0.029	0.343
131 - Mineral extraction sites	0.137	-0.094	0.070	-0.211	-0.092	0.087
132 - Dump sites	-	-	-	-	-0.069	0.037
211 - Non-irrigated arable land	-0.382	-0.277	0.078	0.157	-0.065	-0.189
221 - Vineyards	-0.019	-0.180	0.225	-0.260	-0.178	0.360
222 - Fruit trees and berries plantations	-0.064	0.393	0.000	0.145	-0.017	-0.149
231 - Pastures	0.119	-0.098	-0.204	0.002	0.213	-0.107
241 - Crop with significant amount of natural vegetation	0.318	-0.019	0.161	0.259	-0.246	-0.099
242 - Complex cultivation patterns	-0.176	0.202	0.195	0.282	-0.199	-0.204
311 - Broadleaved forest	-0.043	-0.411	0.194	-0.363	-0.164	0.377
312 - Coniferous forest	0.280	-0.028	-0.401	0.050	0.215	-0.247
313 - Mixed forest	0.204	-0.327	0.085	-0.220	-0.173	0.050
321 - Natural grassland	0.213	0.016	-0.356	0.020	0.335	0.071
322 - Moors and heathland	-	-	-	-	0.267	0.262
324 - Transitional woodland-scrub	-0.394	-0.281	0.109	-0.240	-0.052	0.197
332 - Bare rock	-	-	-0.356	0.020	0.325	0.072
333 - Sparsely vegetated areas	-	-	-0.347	0.044	0.321	0.064
335 - Glaciers and perpetual snow	-	-	-	-	0.324	0.073
511 - Water courses	-0.051	-0.081	0.187	-0.328	-0.111	0.433
512 - Water bodies	-0.102	0.354	0.001	0.131	-0.051	-0.210

Table S2.4: Pollen types identified in pollen samples and their average proportion in the six sampling months and in total. The identified pollens were classified following the pollen types nomenclature proposed by Persano Oddo and Ricciardelli d'Arbore (1989).

Pollen type	April	May	June	July	August	September	Total
Acer spp.	0.062	0.030	0.004	-	-	-	0.015
Acidanthera spp.	-	-	-	-	0.004	-	0.001
Actinidia spp.	-	0.011	0.008	-	-	-	0.004
Aesculus spp.	0.009	0.013	-	-	-	-	0.004
Agrimonia spp.	-	-	-	-	0.002	-	-
Ailanthus spp.	-	-	0.004	-	-	-	0.001
Alnus spp.	-	-	0.002	-	-	-	-
Ambrosia spp.	-	-	-	-	0.006	-	0.001
Apiaceae	-	0.009	0.040	0.031	0.010	0.011	0.018
Artemisia spp.	-	-	-	0.004	0.052	0.022	0.013
Aruncus spp.	-	0.004	0.013	0.012	0.017	0.015	0.010
Asparagus spp.	0.009	0.004	0.006	0.004	-	0.004	0.004
Begonia spp.	-	-	0.002	-	0.006	0.015	0.003
Betulaceae	0.047	0.015	-	-	-	-	0.009
Boraginaceae	-	0.008	0.002	0.004	0.004	-	0.003
Buddleja spp.	-	0.006	0.006	0.012	0.017	-	0.008
Buxus spp.	0.009	0.002	-	-	-	-	0.002
Camerops spp.	0.006	0.017	0.002	-	0.002	-	0.005
Campanula spp.	-	0.004	0.002	0.008	0.002	-	0.003
Cannabaceae	-	0.002	0.002	0.008	0.014	-	0.005
Caprifoliaceae	0.021	0.021	0.002	-	0.004	-	0.008
Carex spp.	0.018	0.021	0.004	-	-	-	0.007
Carpinus spp.	-	0.002	-	-	-	-	-
Caryophyllaceae	0.018	0.011	0.002	0.012	0.023	0.004	0.012
Castanea sativa	-	-	0.045	0.014	-	-	0.011
Centaurea spp.	-	-	-	0.004	-	0.004	0.001
Chelidonium spp.	-	0.006	-	-	-	-	0.001
Chenopodiaceae	-	0.002	-	0.006	0.035	0.041	0.013
Clematis spp.	-	0.023	0.025	0.035	0.023	0.022	0.023
Compositae A-form	-	0.006	0.006	0.043	0.023	0.026	0.018
Compositae H-form	0.018	0.013	0.032	0.057	0.062	0.112	0.045
Compositae J-form	0.003	0.004	0.025	0.023	0.021	0.019	0.016
Compositae S-form	-	0.004	0.002	0.023	0.027	0.011	0.012
Compositae T-form	0.103	0.057	0.034	0.062	0.047	0.064	0.058
Convolvulus spp.	-	0.004	0.017	0.023	0.029	0.004	0.014
Cornus spp.	-	0.032	0.002	-	0.002	-	0.007
Corylaceae	0.018	-	-	-	-	-	0.002
Cotinus spp.	-	-	-	-	0.002	-	-
Crocus spp.	0.015	0.004	-	-	-	-	0.003
Cruciferae	0.018	0.015	0.030	0.043	0.023	0.041	0.028
Datura spp.	-	-	-	0.002	0.004	0.015	0.003
Echium spp.	-	-	0.002	0.006	0.002	-	0.002

Eleagnus spp.	-	-	-	0.004	0.006	0.007	0.003
Epilobium spp.	-	-	-	-	-	0.004	-
Ericaceae	0.009	-	0.008	0.008	0.002	-	0.005
Eucalyptus spp.	-	-	0.002	0.002	-	-	0.001
Fagopyrum spp.	-	-	0.004	0.004	0.002	0.004	0.002
Fagus spp.	0.026	0.002	-	-	-	-	0.004
Filipendula spp.	-	0.008	0.013	0.008	0.006	0.007	0.007
Fragaria/*Potentilla* group	0.003	0.017	0.028	0.010	0.010	0.007	0.013
Frangula spp.	-	0.004	-	-	-	-	0.001
Fraxinus spp.	0.050	0.043	0.015	-	-	-	0.018
Genista spp.	0.003	0.004	0.006	0.002	-	-	0.003
Geraniaceae	0.009	0.015	0.013	0.010	0.008	0.007	0.011
Gleditzia spp.	-	0.002	0.002	0.008	-	0.004	0.003
Hedera spp.	-	-	-	0.006	0.033	0.139	0.022
Hedisarum spp.	-	-	0.002	-	-	-	-
Helianthemum spp.	-	0.011	0.019	0.021	0.021	0.034	0.017
Humulus spp.	-	-	-	0.004	0.006	0.004	0.002
Hypericum spp.	-	-	0.008	0.029	0.008	0.007	0.009
Ilex spp.	-	0.021	-	-	-	-	0.004
Impatiens spp.	-	-	-	-	0.025	0.019	0.007
Juglans spp.	0.003	0.002	-	-	-	-	0.001
Knautia spp.	-	-	0.004	0.004	0.006	0.004	0.003
Labiatae	0.029	0.017	0.006	0.008	0.010	0.007	0.013
Lagestroenia spp.	-	-	-	0.018	0.045	0.041	0.016
Lamium spp.	-	0.002	0.008	-	-	-	0.002
Laurus spp.	-	0.002	-	-	-	-	-
Ligustrum spp.	-	-	0.019	0.010	0.004	-	0.006
Liliaceae	0.024	0.038	0.023	0.002	0.004	0.019	0.018
Liriodendron spp.	0.003	0.009	0.004	0.002	0.002	-	0.004
Lonicera spp.	0.006	0.006	0.002	-	-	-	0.002
Lotus spp.	-	0.004	0.002	0.004	0.010	-	0.004
Luzula spp.	-	-	0.017	0.006	-	-	0.004
Lytrhum spp.	-	-	-	-	0.002	-	-
Magnolia spp.	0.009	-	0.011	-	0.004	-	0.004
Malus/*Pyrus* spp.	0.085	0.049	0.006	-	-	0.004	0.023
Malva spp.	-	-	-	0.002	0.012	0.004	0.003
Melilotus spp.	-	0.004	0.023	0.021	0.025	0.015	0.015
Ocinum spp.	-	-	-	0.002	0.016	0.007	0.004
Oenothera spp.	-	-	-	0.002	-	0.004	0.001
Oleaceae	0.026	0.013	0.013	0.014	0.010	-	0.013
Onobrychis spp.	-	0.006	0.002	-	-	-	0.002
Ostrya spp.	0.003	0.002	-	-	-	-	0.001
Papaveraceae	0.026	0.025	0.015	0.006	0.006	-	0.013
Parthenocissus spp.	-	0.004	0.045	0.051	0.016	0.007	0.022
Passiflora spp.	-	-	-	-	0.002	-	-
Phacelia spp.	-	0.002	0.004	-	-	-	0.001
Picea spp.	0.006	0.008	-	-	-	-	0.002
Pinus spp.	0.006	0.028	-	-	-	-	0.007

Plantaginaceae	0.009	0.019	0.038	0.064	0.068	0.045	0.042
Platanus spp.	0.003	-	-	-	-	-	-
Poaceae	0.006	0.023	0.032	0.025	0.025	0.026	0.023
Poterium spp.	0.003	0.002	-	0.002	-	-	0.001
Prunus spp.	0.076	0.019	-	-	-	-	0.014
Quercus spp.	0.038	0.023	0.006	0.002	-	-	0.011
Ranunculaceae	0.035	0.038	0.002	0.002	-	-	0.013
Rhamnaceae	0.003	0.002	-	-	0.002	-	0.001
Robinia spp.	0.003	0.023	-	-	-	-	0.005
Rosa spp.	-	-	0.008	0.002	0.002	-	0.002
Rosaceae	0.009	0.019	0.008	0.006	0.008	-	0.009
Rubiaceae	-	-	-	0.002	-	0.004	0.001
Rubus spp.	0.009	0.032	0.051	0.031	0.019	0.019	0.028
Rumex spp.	0.006	0.006	0.008	0.008	-	0.004	0.005
Salix spp.	0.094	0.025	0.004	-	-	-	0.018
Sambucus spp.	0.006	0.015	-	-	-	-	0.004
Sambucus ebulus	0.003	0.006	-	-	0.002	-	0.002
Trifolium incarnatum group	-	0.002	0.002	-	-	-	0.001
Trifolium pratense group	-	0.009	0.004	0.006	-	-	0.004
Trifolium repens group	-	0.026	0.057	0.062	0.050	0.052	0.043
Thalictrum spp.	-	0.008	0.028	0.031	0.025	0.034	0.021
Tilia spp.	-	-	0.038	0.004	-	-	0.008
Typha spp.	-	-	0.002	-	0.002	0.004	0.001
Umbellifarae	-	0.002	-	-	-	-	-
Urticaceae	-	0.002	-	-	-	-	-
Verbascum spp.	-	0.002	0.015	0.021	0.019	0.011	0.012
Viburnum spp.	-	-	-	-	0.002	-	-
Vicia spp.	-	-	0.006	-	-	-	0.001
Viola spp.	-	-	0.002	-	0.002	0.007	0.002
Vitis spp.	-	0.002	0.030	0.004	-	-	0.007
Xantium spp.	-	-	0.002	-	0.002	-	0.001
Zea mays	-	0.004	0.002	0.021	0.010	0.007	0.008

CHAPTER 3

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Table S3.1: List of the 13 sampling locations with information on coordinates (WGS84, decimal degrees), elevation (m a.s.l.), and percentage of semi-natural (Semi-nat), agricultural (Agric), and man-made (Man-made) areas calculated using the regional land-use map categories in 3 km radius buffers around the sampling locations.

Location	Lat (N)	Lon (E)	Elev	Semi-nat	Agric	Man-made
Ala	45.78273	11.02925	291	74	20	5
Borgo Valsugana	46.05517	11.45597	406	41	44	15
Caldonazzo	45.99771	11.27041	462	49	38	13
Cavalese	46.28378	11.50333	894	56	37	8
Cles	46.36167	11.03932	650	35	52	14
Cogolo di Peio	46.38582	10.68724	1481	92	8	0
Faedo	46.19481	11.16987	700	79	18	3
Giovo	46.1434	11.1186	339	26	52	22
Pergine Valsugana	46.07369	11.2311	503	43	38	19
Rovereto	45.88412	11.02188	175	29	41	30
Salorno	46.24121	11.20504	216	47	45	7
Tiarno di Sopra	45.8934	10.67926	747	84	13	3
Valeggio sul Mincio	45.4085	10.72586	91	1	87	11

Table S3.2: Regional land-use map classes and corresponding categories used in the Principal Component Analysis.

CLC code	CLC class	Category	Type
111	Continuous urban fabric	Urban	Man-made
112	Discontinuous urban fabric	Urban	Man-made
121	Industrial commercial and public units	Industrial	Man-made
122	Road and rail networks and associated land	Urban	Man-made
131	Mineral extraction sites	Mineral extraction sites	Man-made
142	Sport and leisure facilities	Urban	Man-made
211	Non-irrigated arable land	Non-irrigated crop	Agric
221	Vineyard	Vineyard	Agric
222	Fruit trees and berries plantations	Fruit trees	Agric
231	Pastures	Pastures	Agric
242	Complex cultivation patterns	Complex crop	Agric
243	Agriculture with significant areas of natural vegetation	Crop-natural	Agric
311	Broadleaved forest	Broadleaved forest	Semi-nat
312	Coniferous forest	Coniferous forest	Semi-nat
313	Mixed forest	Mixed forest	Semi-nat
321	Natural grassland	Natural grassland	Semi-nat
324	Transitional woodland-scrub	Transitional woodland-scrub	Semi-nat
332	Bare rock	Other	Semi-nat
333	Sparsely vegetated areas	Other	Semi-nat
335	Perpetual snow	Other	Semi-nat
511	Water courses	Other	Semi-nat
512	Water bodies	Other	Semi-nat

Table S3.3: List of compounds searched in pollen samples, including information on the pesticide category (Cat; Fung = fungicide, Herb = herbicide, Ins = insecticide and/or acaricide), chemical group (Chem group), frequency at locations (Freq loc), frequency in samples (Freq sam), maximum and mean concentration (Conc max, Conc mean), maximum and mean PHQ (PHQ max, PHQ mean), and maximum risk quotient (RQ max).

Compound	Cat	Chem group	Freq loc	Freq sam	Conc max	Conc mean	PHQ max	PHQ mean	RQ max
2, 4-DDD	Ins	NA	0	0	0	0	0	0	0
2, 4-DDE	Ins	NA	0	0	0	0	0	0	0
2, 4-DDT	Ins	NA	0	0	0	0	0	0	0
3, 5-dichloroaniline	Fung	NA	0	0	0	0	0	0	0
3-hydroxycarbofuran	Ins	NA	0	0	0	0	0	0	0
3-ketocarbofuran	Ins	Carbamates	0	0	0	0	0	0	0
4, 4-DDE	Ins	NA	0	0	0	0	0	0	0
4, 4-DDT	Ins	NA	0	0	0	0	0	0	0
6-benzylaminopurine	Herb	NA	8	20	0.041	0.001619	0.698	0.027558	0.000007
Abamectin	Ins	Avermectins milbemycins	0	0	0	0	0	0	0
Acephate	Ins	Organophosphates	0	0	0	0	0	0	0
Acetamiprid	Ins	Neonicotinoid	10	57	0.267	0.009844	18.376	0.677476	0.000176
Acetochlor	Herb	α-chloroacetamides	0	0	0	0	0	0	0
Acibenzolar-S-methyl	Fung	Benzo thiadiazole	0	0	0	0	0	0	0
Acrinathrin	Ins	Pyrethroids and pyrethrins	0	0	0	0	0	0	0
Alachlor	Herb	α-chloroacetamides	0	0	0	0	0	0	0
Aldicarb (sum)	Ins	Carbamates	0	0	0	0	0	0	0
Aldicarb sulfone	Ins	Carbamates	0	0	0	0	0	0	0
Aldicarb sulfoxide	Ins	Carbamates	0	0	0	0	0	0	0
Aldrin	Ins	Cyclodiene organochlorines	0	0	0	0	0	0	0
Allethrin	Ins	Pyrethroids and pyrethrins	0	0	0	0	0	0	0

Ametoctradin	Fung	QoSI fungicide	12	59	1.4	0.043844	12.556	0.393231	0.000121
Ametryn	Herb	Triazines	0	0	0	0	0	0	0
Amidosulfuron	Herb	Sulfonylureas	0	0	0	0	0	0	0
Amisulbrom	Fung	QiI fungicides	0	0	0	0	0	0	0
Amitraz	Ins	Amitraz	0	0	0	0	0	0	0
Atrazine	Herb	Triazines	1	2	0.002	0.000020	0.020	0.000204	<0.000001
Azaconazole	Fung	DMI fungicides	0	0	0	0	0	0	0
Azinphos-ethyl	Ins	Organophosphates	0	0	0	0	0	0	0
Azinphos-methyl	Ins	Organophosphates	0	0	0	0	0	0	0
Azoxystrobin	Fung	QoI fungicides	5	9	0.239	0.002224	9.560	0.088980	0.000092
Beflubutamid	Herb	Phenyl ethers	0	0	0	0	0	0	0
Benalaxyl	Fung	PA fungicides	1	7	0.084	0.001204	3.717	0.053272	0.000036
Bendiocarb	Ins	Carbamates	0	0	0	0	0	0	0
Benfluralin	Herb	Dinitroanilines	0	0	0	0	0	0	0
Benfuracarb	Ins	Carbamates	0	0	0	0	0	0	0
Benomyl	Fung	MBC fungicides	0	0	0	0	0	0	0
Bensulfuron-methyl	Herb	Sulfonylureas	0	0	0	0	0	0	0
Benthiavalicarb isopropyl	Fung	CAA fungicides	0	0	0	0	0	0	0
Benzoximate	Ins	Benzoximate	0	0	0	0	0	0	0
Benzoylprop-ethyl	Herb	NA	0	0	0	0	0	0	0
Bifenazate	Ins	Bifenazate	1	2	0.015	0.000116	0.150	0.001156	0.000001
Bifenox	Herb	Diphenyl ethers	0	0	0	0	0	0	0
Bifenthrin	Ins	Pyrethroids and pyrethrins	0	0	0	0	0	0	0
Bitertanol	Fung	DMI fungicides	0	0	0	0	0	0	0
Boscalid	Fung	SDHI	12	35	0.182	0.003075	1.096	0.018490	0.000011
Bromacil	Herb	Uracils	0	0	0	0	0	0	0
Bromophos-ethyl	Ins	Organophosphates	1	1	0.001	0.000007	2.273	0.015463	0.000022
Bromophos-methyl	Ins	Organophosphates	0	0	0	0	0	0	0
Bromopropylate	Ins	NA	0	0	0	0	0	0	0
Bromoxynil	Herb	Nitriles	0	0	0	0	0	0	0
Bromuconazole	Fung	DMI fungicides	0	0	0	0	0	0	0

Bupirimate	Fung	Hydroxypyrimidines	10	34	0.303	0.005245	1.515	0.026224	0.000015
Buprofezin	Ins	Buprofezin	2	3	0.004	0.000048	0.024	0.000286	<0.000001
Cadusafos	Ins	Organophosphates	0	0	0	0	0	0	0
Captan	Fung	Phthalimides	10	44	142.000	2.177789	1420.000	21.777891	0.013632
Carbaryl	Ins	Carbamates	2	3	0.004	0.000054	19.048	0.259156	0.000183
Carbendazim	Fung	MBC fungicides	6	14	0.176	0.001551	1.760	0.015510	0.000017
Carbofuran	Ins	Carbamates	0	0	0	0	0	0	0
Carbosulfan	Ins	Carbamates	0	0	0	0	0	0	0
Carboxin	Fung	SDHI	0	0	0	0	0	0	0
Carfentrazone-ethyl	Herb	N-phenyl triazolinones	1	1	0.001	0.000007	0.012	0.000082	<0.000001
Chinomethionat	Fung	Quinoxalines	0	0	0	0	0	0	0
Chlorantraniliprole	Ins	Diamides	8	24	1.020	0.011599	9.798	0.111415	0.000094
Chlorfenapyr	Ins	Pyrroles, dinitrophenols, sulfluramid	0	0	0	0	0	0	0
Chlorfenson	Fung	Chloronitriles	0	0	0	0	0	0	0
Chlorfenvinphos	Ins	Organophosphates	0	0	0	0	0	0	0
Chlormephos	Ins	Organophosphates	0	0	0	0	0	0	0
Chlorpropham	Herb	Carbamates	0	0	0	0	0	0	0
Chlorpyrifos	Ins	Organophosphates	9	29	0.052	0.001626	208.000	6.503401	0.001997
Chlorpyrifos-methyl	Ins	Organophosphates	0	0	0	0	0	0	0
Chlozolinate	Fung	Dicarboximides	0	0	0	0	0	0	0
Chromafenozide	Ins	Diacylhydrazines	0	0	0	0	0	0	0
Clethodim (sum)	Herb	Cyclohexanediones	0	0	0	0	0	0	0
Clofentezine	Ins	Clofentezine, diflovidazin, hexythiazox	0	0	0	0	0	0	0
Cloquintocet	Herb	NA	0	0	0	0	0	0	0
Cloquintocet-mexyl	Herb	NA	0	0	0	0	0	0	0
Clothianidin	Ins	Neonicotinoid	0	0	0	0	0	0	0
Coumaphos	Ins	Organophosphates	0	0	0	0	0	0	0
Cyanazine	Herb	Triazines	0	0	0	0	0	0	0
Cyantraniliprole	Ins	Diamides	0	0	0	0	0	0	0
Cyazofamid	Fung	QiI fungicides	4	4	0.007	0.000095	0.046	0.000626	<0.000001

Cycloxydim	Herb	Cyclohexanediones	1	1	0.029	0.000197	0.285	0.001939	0.000003
Cyflufenamid	Fung	Phenyl acetamide	10	48	0.348	0.006361	3.480	0.063605	0.000033
Cyflumetofen	Ins	β-ketonitrile derivates	0	0	0	0	0	0	0
Cyfluthrin (sum)	Ins	Pyrethroids and pyrethrins	0	0	0	0	0	0	0
Cyhalofop-butyl	Herb	Aryloxyphenoxy propionates	0	0	0	0	0	0	0
Cymoxanil	Fung	Cyanoacetamide oxime	1	1	0.010	0.000068	0.117	0.000796	0.000001
Cypermethrin (sum)	Ins	Pyrethroids and pyrethrins	0	0	0	0	0	0	0
Cyproconazole	Fung	DMI fungicides	0	0	0	0	0	0	0
Cyprodinil	Fung	AP fungicides	11	66	1.040	0.023320	9.244	0.207313	0.000089
Deltamethrin	Ins	Pyrethroids and pyrethrins	0	0	0	0	0	0	0
Demeton-S-methyl	Ins	Organophosphates	0	0	0	0	0	0	0
Demeton-S-methylsulfone	Ins	Organophosphates	0	0	0	0	0	0	0
Desethyl-atrazine	Herb	Triazines	0	0	0	0	0	0	0
Desisopropyl-atrazine	Herb	Triazines	0	0	0	0	0	0	0
Desmedipham	Herb	Phenlcarbamates	0	0	0	0	0	0	0
Diazinon	Ins	Organophosphates	0	0	0	0	0	0	0
Dicamba	Herb	Benzoates	0	0	0	0	0	0	0
Dichlobenil	Herb	Nitriles	0	0	0	0	0	0	0
Dichlofenthion	Ins	Organophosphates	0	0	0	0	0	0	0
Dichlofluanid	Fung	Sulfamides	0	0	0	0	0	0	0
Dichlorvos	Ins	Organophosphates	1	1	0.006	0.000041	20.690	0.140748	0.000199
Dicloran	Fung	AH fungicides	0	0	0	0	0	0	0
Dicofol (sum)	Ins	NA	0	0	0	0	0	0	0
Dicrotophos	Ins	Organophosphates	0	0	0	0	0	0	0
Dieldrin	Ins	Cyclodiene organochlorines	0	0	0	0	0	0	0
Diethofencarb	Fung	N-phenyl carbamates	0	0	0	0	0	0	0
Difenoconazole	Fung	DMI fungicides	11	31	0.266	0.007027	1.503	0.039694	0.000014

Diflubenzuron	Ins	Benzoylureas	0	0	0	0	0	0	0
Diflufenican	Herb	Phenyl ethers	0	0	0	0	0	0	0
Dimethoate	Ins	Organophosphates	11	34	1.370	0.021680	13700.000	216.802721	0.131520
Dimethomorph	Fung	CAA fungicides	10	38	1.360	0.043075	41.975	1.329469	0.000403
Dimoxystrobin	Fung	QoI fungicides	0	0	0	0	0	0	0
Diniconazole	Fung	DMI fungicides	0	0	0	0	0	0	0
Dinotefuran	Ins	Neonicotinoid	0	0	0	0	0	0	0
Dioxathion	Ins	Organophosphates	0	0	0	0	0	0	0
Diphenamid	Herb	Acetamides	0	0	0	0	0	0	0
Diphenylamine	Fung	NA	0	0	0	0	0	0	0
Disulfoton	Ins	Organophosphates	0	0	0	0	0	0	0
Ditalimfos	Fung	NA	0	0	0	0	0	0	0
Diuron	Herb	Ureas	0	0	0	0	0	0	0
Dodemorph	Fung	Amines	0	0	0	0	0	0	0
Dodine	Fung	Guanidines	10	30	3.800	0.059170	19.000	0.295850	0.000182
Emamectin benzoate	Ins	Avermectins milbemycins	3	5	0.010	0.000170	277.778	4.724116	0.002667
Endosulfan alpha	Ins	Cyclodiene organochlorines	0	0	0	0	0	0	0
Endosulfan beta	Ins	Cyclodiene organochlorines	0	0	0	0	0	0	0
Endosulfan sulfate	Ins	Cyclodiene organochlorines	0	0	0	0	0	0	0
EPN	Ins	Organophosphates	0	0	0	0	0	0	0
Epoxiconazole	Fung	DMI fungicides	0	0	0	0	0	0	0
Etaconazole	Fung	DMI fungicides	0	0	0	0	0	0	0
Ethalfluralin	Herb	Dinitroanilines	0	0	0	0	0	0	0
Ethion	Ins	Organophosphates	0	0	0	0	0	0	0
Ethirimol	Fung	Hydroxypyrimidines	6	10	0.032	0.000401	20.000	0.250850	0.000192
Ethofumesate	Herb	Benzofurans	2	2	0.007	0.000075	0.140	0.001497	0.000001
Ethoprophos	Ins	Organophosphates	0	0	0	0	0	0	0
Ethoxyquin	Fung	NA	0	0	0	0	0	0	0

Etofenprox	Ins	Pyrethroids and pyrethrins	2	3	0.017	0.000163	46.448	0.446075	0.000446
Etoxazole	Ins	Etoxazole	0	0	0	0	0	0	0
Etridiazole	Fung	AH fungicides	0	0	0	0	0	0	0
Etrimfos	Ins	Organophosphates	0	0	0	0	0	0	0
Famoxadone	Fung	QoI fungicides	0	0	0	0	0	0	0
Fenamidone	Fung	QoI fungicides	0	0	0	0	0	0	0
Fenamiphos	Ins	Organophosphates	0	0	0	0	0	0	0
Fenarimol	Fung	DMI fungicides	0	0	0	0	0	0	0
Fenazaquin	Ins	Meti-acaricides and insecticides	0	0	0	0	0	0	0
Fenbuconazole	Fung	DMI fungicides	1	2	0.006	0.000068	1.154	0.013082	0.000011
Fenbutatin-oxide	Ins	Organotin miticides	0	0	0	0	0	0	0
Fenchlorphos	Ins	Organophosphates	0	0	0	0	0	0	0
Fenhexamid	Fung	KRI fungicides	6	16	0.158	0.003395	1.548	0.033265	0.000015
Fenitrothion	Ins	Organophosphates	0	0	0	0	0	0	0
Fenothiocarb	Ins	NA	0	0	0	0	0	0	0
Fenoxaprop	Herb	Aryloxyphenoxy propionates	0	0	0	0	0	0	0
Fenoxycarb	Ins	Fenoxycarb	0	0	0	0	0	0	0
Fenpropathrin	Ins	Pyrethroids and pyrethrins	0	0	0	0	0	0	0
Fenpropidin	Fung	Amines	0	0	0	0	0	0	0
Fenpropimorph	Fung	Amines	0	0	0	0	0	0	0
Fenpyrazamine	Fung	KRI fungicides	2	5	0.009	0.000150	0.090	0.001497	0.000001
Fenpyroximate	Ins	Meti-acaricides and insecticides	0	0	0	0	0	0	0
Fenson	Ins	Cyclodiene organochlorines	0	0	0	0	0	0	0
Fenthion	Ins	Organophosphates	0	0	0	0	0	0	0
Fenthion-sulfone	Ins	Organophosphates	0	0	0	0	0	0	0
Fenthion-sulfoxide	Ins	Organophosphates	0	0	0	0	0	0	0

Fenvalerate (sum)	Ins	Pyrethroids and pyrethrins	0	0	0	0	0	0	0
Fipronil	Ins	Phenylpyrazoles	0	0	0	0	0	0	0
Fipronil-sulfone	Ins	Phenylpyrazoles	0	0	0	0	0	0	0
Flazasulfuron	Herb	Sulfonylureas	0	0	0	0	0	0	0
Flonicamid	Ins	Flonicamid	1	2	0.095	0.001272	0.950	0.012721	0.000009
Florasulam	Herb	Triazolopyrimidine type1	0	0	0	0	0	0	0
Fluazifop	Herb	Aryloxyphenoxy propionates	0	0	0	0	0	0	0
Fluazifop-P-butyl	Herb	Aryloxyphenoxy propionates	1	1	0.009	0.000061	0.143	0.000973	0.000001
Fluazinam	Fung	NA	13	72	1.580	0.059626	15.800	0.596259	0.000152
Flubendiamide	Ins	Diamides	0	0	0	0	0	0	0
Flucythrinate (sum)	Ins	Pyrethroids and pyrethrins	0	0	0	0	0	0	0
Fludioxonil	Fung	PP fungicides	10	30	7.750	0.085204	77.500	0.852041	0.000744
Flufenacet	Herb	α-chloroacetamides	5	5	0.004	0.000061	0.040	0.000612	<0.000001
Flufenoxuron	Ins	Benzoylureas	0	0	0	0	0	0	0
Fluopicolide	Fung	Benzamides	1	1	0.014	0.000095	0.058	0.000395	0.000001
Fluopyram	Fung	SDHI	7	11	0.013	0.000245	0.127	0.002401	0.000001
Flupyradifurone	Ins	Butenolides	5	8	0.166	0.001633	138.333	1.360544	0.001328
Fluquinconazole	Fung	DMI fungicides	0	0	0	0	0	0	0
Fluroxypyr	Herb	Pyridyloxy carboxylates	1	1	0.012	0.000082	0.323	0.002197	0.000003
Fluroxypyr-1-methylheptyl ester	Herb	Phenoxy carboxylates	0	0	0	0	0	0	0
Flusilazole	Fung	DMI fungicides	0	0	0	0	0	0	0
Flutriafol	Fung	DMI fungicides	0	0	0	0	0	0	0
Fluvalinate Tau	Ins	Pyrethroids and pyrethrins	9	14	0.172	0.002449	13.651	0.194361	0.000131
Fluxapyroxad	Fung	SDHI	11	53	0.486	0.007803	4.382	0.070327	0.000042
Folpet	Fung	Phthalimides	8	14	15.600	0.193259	66.102	0.818898	0.000635
Fonofos	Ins	Organophosphates	0	0	0	0	0	0	0
Fosthiazate	Ins	Organophosphates	0	0	0	0	0	0	0

Fuberidazole	Fung	MBC fungicides	0	0	0	0	0	0	0
Furalaxyl	Fung	PA fungicides	0	0	0	0	0	0	0
Furathiocarb	Ins	Carbamates	0	0	0	0	0	0	0
Glyphosate	Herb	Glycine	9	22	2.950	0.072687	28.365	0.698905	0.000272
Heptachlor	Ins	Cyclodiene organochlorines	0	0	0	0	0	0	0
Heptenophos	Ins	Organophosphates	0	0	0	0	0	0	0
Hexachlorobenzene	Fung	NA	0	0	0	0	0	0	0
Hexaconazole	Fung	DMI fungicides	0	0	0	0	0	0	0
Hexaflumuron	Ins	Benzoylureas	0	0	0	0	0	0	0
Hexythiazox	Ins	Clofentezine, diflovidazin, hexythiazox	1	2	0.012	0.000116	0.107	0.001034	0.000001
Imazalil	Fung	DMI fungicides	0	0	0	0	0	0	0
Imazaquin	Herb	Imidazolinones	0	0	0	0	0	0	0
Imazosulfuron	Herb	Sulfonylureas	0	0	0	0	0	0	0
Imidacloprid	Ins	Neonicotinoid	10	20	0.038	0.000639	10270.270	172.825905	0.098595
Indoxacarb	Ins	Oxadiazines	5	10	0.812	0.007612	3500.000	32.811395	0.033600
Ipconazole	Fung	DMI fungicides	0	0	0	0	0	0	0
Iprodione	Fung	Dicarboximides	0	0	0	0	0	0	0
Iprovalicarb	Fung	CAA fungicides	2	2	0.002	0.000027	0.010	0.000136	<0.000001
Isofenphos	Ins	Organophosphates	0	0	0	0	0	0	0
Isofetamid	Fung	SDHI	0	0	0	0	0	0	0
Isopropalin	Herb	Dinitroanilines	0	0	0	0	0	0	0
Isoproturon	Herb	Ureas	0	0	0	0	0	0	0
Isopyrazam	Fung	SDHI	0	0	0	0	0	0	0
Kresoxim-methyl	Fung	QoI fungicides	0	0	0	0	0	0	0
Lambda-cyhalothrin	Ins	Pyrethroids and pyrethrins	0	0	0	0	0	0	0
Lenacil	Herb	Uracils	1	1	0.001	0.000007	0.005	0.000034	<0.000001
Linuron	Herb	Ureas	0	0	0	0	0	0	0
Lufenuron	Ins	Benzoylureas	0	0	0	0	0	0	0
Malaoxon	Ins	Organophosphates	0	0	0	0	0	0	0

Malathion	Ins	Organophosphates	0	0	0	0	0	0	0
Mandipropamid	Fung	CAA fungicides	7	21	0.190	0.003374	0.950	0.016871	0.000009
MCPA	Herb	Phenoxy carboxylates	2	2	0.478	0.003714	2.390	0.018571	0.000023
Mecarbam	Ins	Organophosphates	0	0	0	0	0	0	0
Mecoprob	Herb	Phenoxy carboxylates	0	0	0	0	0	0	0
Mepanipyrim	Fung	AP fungicides	1	1	0.005	0.000034	0.100	0.000680	0.000001
Mepronil	Fung	SDHI	0	0	0	0	0	0	0
Meptyldinocap	Fung	Dinitrophenyl crotonates	0	0	0	0	0	0	0
Metalaxyl	Fung	PA fungicides	6	19	0.050	0.002599	0.186	0.009667	0.000002
Metamitron	Herb	Triazinones	5	7	0.064	0.001095	0.658	0.011265	0.000006
Metazachlor	Herb	α-chloroacetamides	0	0	0	0	0	0	0
Metconazole (sum)	Fung	DMI fungicides	0	0	0	0	0	0	0
Methamidophos	Ins	Organophosphates	0	0	0	0	0	0	0
Methidathion	Ins	Organophosphates	0	0	0	0	0	0	0
Methiocarb (sum)	Ins	Carbamates	0	0	0	0	0	0	0
Methiocarb-sulfone	Ins	Carbamates	0	0	0	0	0	0	0
Methiocarb-sulfoxide	Ins	Carbamates	0	0	0	0	0	0	0
Methomyl	Ins	Carbamates	0	0	0	0	0	0	0
Methoxychlor (sum)	Ins	DDT methoxychlor	0	0	0	0	0	0	0
Methoxyfenozide	Ins	Diacylhydrazines	7	14	0.917	0.011265	0.459	0.005667	0.000004
Metolachlor	Herb	α-chloroacetamides	5	11	0.009	0.000252	0.082	0.002279	0.000001
Metrafenone	Fung	Aryl phenyl ketones	12	54	0.795	0.023558	6.974	0.206667	0.000067
Metribuzin	Herb	Triazinones	5	5	0.118	0.000871	1.538	0.011347	0.000015
Mevinphos	Ins	Organophosphates	0	0	0	0	0	0	0
Monocrotophos	Ins	Organophosphates	0	0	0	0	0	0	0
Monolinuron	Herb	Ureas	0	0	0	0	0	0	0
Monuron	Herb	Ureas	0	0	0	0	0	0	0
Myclobutanil	Fung	DMI fungicides	2	6	0.029	0.000367	0.855	0.010823	0.000008
Napropamide	Herb	Acetamides	0	0	0	0	0	0	0
Nicosulfuron	Herb	Sulfonylureas	0	0	0	0	0	0	0
Nitenpyram	Ins	Neonicotinoid	0	0	0	0	0	0	0
Nuarimol	Fung	DMI fungicides	0	0	0	0	0	0	0

Omethoate	Ins	Organophosphates	0	0	0	0	0	0	0
Ortophenylphenol	Fung	NA	0	0	0	0	0	0	0
Oryzalin	Herb	Dinitroanilines	0	0	0	0	0	0	0
Oxadiazon	Herb	N-phenyl oxadiazolones	0	0	0	0	0	0	0
Oxadixyl	Fung	PA fungicides	0	0	0	0	0	0	0
Oxamyl	Ins	Carbamates	0	0	0	0	0	0	0
Oxathiapiprolin	Fung	OSBPI oxysterol binding protein homologue inhibition	5	8	0.188	0.002993	4.670	0.074354	0.000045
Oxycarboxin	Fung	SDHI	0	0	0	0	0	0	0
Paclobutrazol	Herb	NA	0	0	0	0	0	0	0
Paraoxon	Ins	Organophosphates	0	0	0	0	0	0	0
Paraoxon-methyl	Ins	Organophosphates	0	0	0	0	0	0	0
Parathion-ethyl	Ins	Organophosphates	0	0	0	0	0	0	0
Parathion-methyl	Ins	Organophosphates	0	0	0	0	0	0	0
Penconazole	Fung	DMI fungicides	12	73	0.328	0.010952	29.286	0.977925	0.000281
Pencycuron	Fung	Phenylureas	0	0	0	0	0	0	0
Pendimethalin	Herb	Dinitroanilines	3	10	0.013	0.000524	0.128	0.005177	0.000001
Penoxsulam	Herb	Triazolopyrimidine type2	0	0	0	0	0	0	0
Penthiopyrad	Fung	SDHI	5	9	0.859	0.012381	1.718	0.024762	0.000017
Permethrin	Ins	Pyrethroids and pyrethrins	0	0	0	0	0	0	0
Pethoxamid	Herb	α-chloroacetamides	0	0	0	0	0	0	0
Phosalone	Ins	Organophosphates	0	0	0	0	0	0	0
Phosmet	Ins	Organophosphates	0	0	0	0	0	0	0
Phoxim	Ins	Organophosphates	0	0	0	0	0	0	0
Picoxystrobin	Fung	QoI fungicides	0	0	0	0	0	0	0
Piperonyl butoxide	Ins	NA	3	6	0.072	0.000592	0.245	0.002014	0.000002
Piridafention	Ins	Organophosphates	0	0	0	0	0	0	0
Pirimicarb	Ins	Carbamates	7	18	0.319	0.002864	79.750	0.715986	0.000766
Pirimicarb-desmethyl	Ins	Carbamates	5	6	0.073	0.000585	18.250	0.146259	0.000175
Pirimiphos-methyl	Ins	Organophosphates	0	0	0	0	0	0	0

Prochloraz	Fung	DMI fungicides	0	0	0	0	0	0	0
Procymidone	Fung	Dicarboximides	0	0	0	0	0	0	0
Profenofos	Ins	Organophosphates	0	0	0	0	0	0	0
Profluralin	Herb	Dinitroanilines	0	0	0	0	0	0	0
Promecarb	Ins	Carbamates	0	0	0	0	0	0	0
Prometon	Herb	Triazines	0	0	0	0	0	0	0
Prometryn	Herb	Triazines	0	0	0	0	0	0	0
Propamocarb	Fung	Carbamates	1	1	0.037	0.000252	0.440	0.002993	0.000004
Propanil	Herb	Amides	0	0	0	0	0	0	0
Propaquizafop	Herb	NA	0	0	0	0	0	0	0
Propargite	Ins	Propargite	0	0	0	0	0	0	0
Propham	Herb	Carbamates	0	0	0	0	0	0	0
Propiconazole	Fung	DMI fungicides	0	0	0	0	0	0	0
Propoxur	Ins	Carbamates	0	0	0	0	0	0	0
Propoxycarbazone	Herb	Triazolinones	0	0	0	0	0	0	0
Propyzamide	Herb	Benzamides	0	0	0	0	0	0	0
Proquinazid	Fung	Aza-naphthalenes	1	1	0.001	0.000007	0.008	0.000054	<0.000001
Prosulfocarb	Herb	Thiocarbamates	0	0	0	0	0	0	0
Prosulfuron	Herb	Sulfonylureas	0	0	0	0	0	0	0
Prothioconazole	Fung	DMI fungicides	1	1	0.026	0.000177	0.366	0.002490	0.000004
Prothioconazole-desthio	Fung	DMI fungicides	0	0	0	0	0	0	0
Prothiofos	Ins	Organophosphates	0	0	0	0	0	0	0
Pyraclostrobin	Fung	QoI fungicides	6	11	0.201	0.001653	1.827	0.015014	0.000018
Pyraflufen	Herb	Phenylpyrazoles	0	0	0	0	0	0	0
Pyraflufen-ethyl	Herb	Phenylpyrazoles	0	0	0	0	0	0	0
Pyrazophos	Fung	Dithiolanes	0	0	0	0	0	0	0
Pyrethrins (sum)	Ins	Pyrethroids and pyrethrins	0	0	0	0	0	0	0
Pyridaben	Ins	Meti-acaricides and insecticides	0	0	0	0	0	0	0
Pyrifenox	Fung	DMI fungicides	1	1	0.003	0.000020	0.051	0.000347	<0.000001
Pyrimethanil	Fung	AP fungicides	10	39	0.828	0.025796	8.280	0.257959	0.000080

Pyriofenone	Fung	Aryl phenyl ketones	1	1	0.001	0.000007	0.010	0.000068	<0.000001
Pyriproxyfen	Ins	Pyriproxyfen	1	2	0.004	0.000048	0.040	0.000476	<0.000001
Quinalphos	Ins	Organophosphates	0	0	0	0	0	0	0
Quinoxyfen	Fung	Aza-naphthalenes	3	7	0.189	0.001878	1.890	0.018776	0.000018
Quintozene	Fung	AH fungicides	0	0	0	0	0	0	0
Quizalofop-P-ethyl	Herb	Aryloxyphenoxy propionates	1	1	0.004	0.000027	0.040	0.000272	<0.000001
Sedaxane	Fung	SDHI	0	0	0	0	0	0	0
Sethoxydim	Herb	Cyclohexanediones	0	0	0	0	0	0	0
Simazine	Herb	Triazines	0	0	0	0	0	0	0
Spinetoram	Ins	Spinosyns	1	1	0.001	0.000007	7.143	0.048592	0.000069
Spinosad (sum)	Ins	Spinosyns	1	2	0.015	0.000136	267.857	2.429544	0.002571
Spirodiclofen	Ins	Tetronic and tetramic acid derivatives	0	0	0	0	0	0	0
Spirotetramat	Ins	Tetronic and tetramic acid derivatives	4	6	0.892	0.008707	8.313	0.081143	0.000080
Spirotetramat-enol	Ins	Tetronic and tetramic acid derivatives	0	0	0	0	0	0	0
Spirotetramat-enol-glucoside	Ins	Tetronic and tetramic acid derivatives	0	0	0	0	0	0	0
Spirotetramat-ketohydroxy	Ins	Tetronic and tetramic acid derivatives	0	0	0	0	0	0	0
Spiroxamine	Fung	Amines	13	92	1.940	0.029435	19.400	0.294354	0.000186
Sulfotep	Ins	Organophosphates	0	0	0	0	0	0	0
Sulfoxaflor	Ins	Sulfoximines	1	1	0.079	0.000537	541.096	3.680925	0.005195
Tebuconazole	Fung	DMI fungicides	7	14	0.222	0.002932	2.673	0.035299	0.000026
Tebufenozide	Ins	Diacylhydrazines	1	1	0.051	0.000347	0.614	0.004177	0.000006
Tebufenpyrad	Ins	Meti-acaricides and insecticides	2	3	0.004	0.000068	0.066	0.001129	0.000001
Tebupirimifos	Ins	Organophosphates	0	0	0	0	0	0	0
Tecnazene	Fung	AH fungicides	0	0	0	0	0	0	0
Teflubenzuron	Ins	Benzoylureas	0	0	0	0	0	0	0

Tefluthrin	Ins	Pyrethroids and pyrethrins	0	0	0	0	0	0	0
Tembotrione	Herb	Triketones	0	0	0	0	0	0	0
Tepraloxydim	Herb	Cyclohexanediones	0	0	0	0	0	0	0
Terbufos	Ins	Organophosphates	0	0	0	0	0	0	0
Terbumeton	Herb	Triazines	0	0	0	0	0	0	0
Terbuthylazine	Herb	Triazines	11	25	0.014	0.000558	0.619	0.024653	0.000006
Terbutryn	Herb	Triazines	0	0	0	0	0	0	0
Tetrachlorvinphos	Ins	Organophosphates	0	0	0	0	0	0	0
Tetraconazole	Fung	DMI fungicides	9	40	0.051	0.002816	0.392	0.021660	0.000004
Tetradifon	Ins	Tetradifon	1	1	0.005	0.000034	0.455	0.003095	0.000004
Tetramethrin	Ins	Pyrethroids and pyrethrins	0	0	0	0	0	0	0
Thiabendazole	Fung	MBC fungicides	0	0	0	0	0	0	0
Thiacloprid	Ins	Neonicotinoid	9	19	0.328	0.004136	18.938	0.238803	0.000182
Thiamethoxam	Ins	Neonicotinoid	1	1	0.005	0.000034	1000.000	6.802721	0.009600
Thifensulfuron-methyl	Herb	Sulfonylureas	0	0	0	0	0	0	0
Thiobencarb	Herb	Thiocarbamates	0	0	0	0	0	0	0
Thiodicarb	Ins	Carbamates	0	0	0	0	0	0	0
Thiometon	Ins	Organophosphates	0	0	0	0	0	0	0
Thiophanate-methyl	Fung	MBC fungicides	8	24	0.994	0.007755	8.666	0.067585	0.000083
Tolclofos-methyl	Fung	AH fungicides	0	0	0	0	0	0	0
Tolylfluanid	Fung	Sulfamides	0	0	0	0	0	0	0
Triadimefon	Fung	DMI fungicides	0	0	0	0	0	0	0
Triadimenol	Fung	DMI fungicides	0	0	0	0	0	0	0
Tri-allate	Herb	Thiocarbamates	0	0	0	0	0	0	0
Triasulfuron	Herb	Sulfonylureas	0	0	0	0	0	0	0
Triazamate	Ins	Carbamates	0	0	0	0	0	0	0
Triazophos	Ins	Organophosphates	0	0	0	0	0	0	0
Trichlorfon	Ins	Organophosphates	0	0	0	0	0	0	0
Triclopyr	Herb	Pyridyloxy carboxylates	0	0	0	0	0	0	0
Tricyclazole	Fung	MBI-R	0	0	0	0	0	0	0

Trifloxystrobin	Fung	QoI fungicides	9	21	0.461	0.004837	2.305	0.024184	0.000022
Triflumizole	Fung	DMI fungicides	0	0	0	0	0	0	0
Triflumuron	Ins	Benzoylureas	5	12	0.594	0.004912	2.628	0.021735	0.000025
Trifluralin	Herb	Dinitroanilines	0	0	0	0	0	0	0
Triticonazole	Fung	DMI fungicides	0	0	0	0	0	0	0
Tritosulfuron	Herb	Sulfonylureas	0	0	0	0	0	0	0
Valifenalate	Fung	CAA fungicides	1	1	0.008	0.000054	0.075	0.000510	0.000001
Vamidothion	Ins	Organophosphates	0	0	0	0	0	0	0
Vinclozolin	Fung	Dicarboximides	0	0	0	0	0	0	0
Zoxamide	Fung	Benzamides	13	117	3.990	0.115340	27.143	0.784578	0.000261

Table S3.4: Instrument acquisition data for pesticides analysed by LC-MS/MS. The table reports the ionization mode and its polarity (Ion mode), cone voltage (CV, expressed as V), quantification trace (Quant trace), collision energy (CE, expressed as eV), and qualification trace (Qual trace).

Compound	Ion mode	CV	Quant trace	CE	Qual trace	CE
3-hydroxycarbofuran	ES+	30	238 > 163	16	238 > 181	10
3-ketocarbofuran	ES+	22	236 > 161	24	236 > 179	15
6-benzylaminopurine	ES+	30	226.1 > 91	26	226.1 > 65	36
Abamectin	ES+	74	895.52 > 751.48	44	895.52 > 183.1	52
Acephate	ES+	8	184.1 > 143	8	184.1 > 125.1	18
Acetamiprid	ES+	32	223 > 126	22	223 > 56	14
Acibenzolar-S-methyl	ES+	40	211 > 135.93	28	211 > 91.06	20
Aldicarb (sum)	ES+	26	208.1 > 89	15	NA	NA
Aldicarb sulfone	ES+	15	240.1 > 86.2	20	240.1 > 148.2	13
Aldicarb sulfoxide	ES+	12	207.1 > 132.1	10	207.1 > 89.1	14
Allethrin	ES+	15	303.2 > 90.9	40	303.2 > 107	20
Ametoctradin	ES+	68	276.223 > 149.0999	36	276.223 > 176.213	36
Ametryn	ES+	28	228.1 > 186.1	18	228.1 > 96	36
Amidosulfuron	ES+	20	370.1 > 69	50	370.1 > 261.1	15
Amisulbrom	ES+	15	466 > 227	20	468 > 229	20
Amitraz	ES+	20	294 > 163	15	294 > 122	28
Atrazine	ES+	29	216.1 > 174	18	NA	23
Azaconazole	ES+	30	300 > 159	28	300 > 231	18
Azinphos-ethyl	ES+	10	346 > 132	16	346 > 77.1	36
Azinphos-methyl	ES+	12	318 > 261	8	318 > 160	8
Azoxystrobin	ES+	17	404 > 372	15	404 > 329	30
Beflubutamid	ES+	25	356.1 > 91.1	35	356.1 > 65.1	40
Benalaxyl	ES+	17	326.1 > 148	20	326.1 > 91	34
Bendiocarb	ES+	15	224.1 > 109	20	224.1 > 167.1	10
Benfuracarb	ES+	22	411.2 > 195	23	411.2 > 190	13
Benomyl	ES+	26	291 > 160	20	291 > 192	20
Bensulfuron-methyl	ES+	28	411 > 149	22	411 > 182	20
Benthiavalicarb isopropyl	ES+	20	382 > 180	30	382 > 116	20
Benzoximate	ES+	9	364 > 199.1	8	364 > 105	26
Benzoylprop-ethyl	ES+	16	366 > 105	14	366 > 77	45
Bifenazate	ES+	16	301.159 > 198.112	10	301.159 > 170.089	20
Bifenthrin	ES+	16	440.25 > 181.125	14	440.25 > 165.96	44
Bitertanol	ES+	12	338.1 > 99.1	16	338.1 > 70.1	8
Boscalid	ES+	32	342.9 > 307	20	342.9 > 139.9	20
Bromacil	ES+	20	261 > 205	14	261 > 188	28
Bromophos-methyl	ES+	30	365 > 229	24	365 > 291	30
Bromoxynil	ES-	38	275.8 > 78.9	30	275.8 > 80.8	30
Bromuconazole	ES+	32	376 > 158.9	35	378 > 159	25

Bupirimate	ES+	31	317 > 108	28	317 > 166	28
Buprofezin	ES+	22	306.1 > 201	12	306.1 > 57.4	20
Cadusafos	ES+	17	271 > 159	15	271 > 97	32
Carbaryl	ES+	19	202 > 145	22	202 > 117	28
Carbendazim	ES+	26	192.1 > 160.07	16	192.1 > 132.1	28
Carbofuran	ES+	20	222.1 > 165.1	12	222.1 > 123	22
Carbosulfan	ES+	25	381 > 118	22	381 > 76	34
Carboxin	ES+	22	236.1 > 143	16	236.1 > 87	22
Carfentrazone-ethyl	ES+	36	412.096 > 345.999	24	NA	14
Chlorantraniliprole	ES+	20	482.07 > 450.99	18	482.07 > 283.95	12
Chlorfenvinphos	ES+	18	358.9 > 155	12	358.9 > 99	30
Chlorpyrifos	ES+	27	349.9 > 97	32	349.9 > 198	20
Chlozolinate	ES+	34	331 > 268	24	331 > 81	28
Chromafenozide	ES+	15	395 > 175	18	395 > 339	10
Clethodim Isomer A	ES+	25	360.1 > 164	20	360.1 > 268.1	12
Clethodim Isomer B	ES+	25	360.1 > 164	20	360.1 > 268.1	12
Clofentezine	ES+	19	303 > 138	22	303 > 102	35
Cloquintocet	ES+	30	238 > 179	22	238 > 192	20
Cloquintocet-mexyl	ES+	25	336 > 192	35	336 > 238	18
Clothianidin	ES+	20	250.03 > 168.99	12	250.03 > 131.98	14
Coumaphos	ES+	30	363 > 227	25	363 > 307	16
Cyanazine	ES+	27	241 > 214	17	NA	20
Cyantraniliprole	ES+	24	474.99 > 285.89	18	474.99 > 111.97	62
Cyazofamid	ES+	17	325 > 107.9	20	325 > 261	10
Cycloxydim	ES+	24	326.223 > 280.197	10	NA	22
Cyflufenamid	ES+	24	413.09 > 295.05	14	413.09 > 241.01	22
Cyflumetofen	ES+	21	465 > 173	22	465 > 249	12
Cymoxanil	ES+	14	199 > 128	8	199 > 111	18
Cyproconazole	ES+	27	292.2 > 70.2	18	292.2 > 125.1	24
Cyprodinil	ES+	47	226 > 93	33	226 > 108	25
Demeton-S-methyl	ES+	28	231 > 89	10	231 > 61	30
Demeton-S-methylsulfone	ES+	30	263 > 169	17	263 > 121	17
Desethyl-atrazine	ES+	30	188 > 146	16	188 > 79	26
Desisopropyl-atrazine	ES+	34	174 > 96	18	174 > 78.9	18
Desmedipham	ES+	18	318 > 182	10	318 > 136	22
Diazinon	ES+	20	305.1 > 169	22	305.1 > 96.9	35
Dicamba	ES-	12	219 > 175	10	221 > 177	10
Dichlorvos	ES+	23	221 > 109	22	221 > 79	34
Dicrotophos	ES+	17	238 > 112	10	238 > 193	10
Diethofencarb	ES+	16	268.1596 > 124.0039	30	268.1596 > 152.0901	22
Difenoconazole	ES+	37	406 > 251.1	25	406 > 111.1	60
Diflubenzuron	ES-	20	309 > 289	9	309 > 156	11
Dimethoate	ES+	12	230.1 > 125	20	230.1 > 199	10
Dimethomorph	ES+	30	388.1 > 300.9	20	388.1 > 165	30
Dimoxystrobin	ES+	15	327.1 > 116	21	327.1 > 205	10

Diniconazole	ES+	37	326.1 > 70.2	25	328 > 70	25
Dinotefuran	ES+	15	203.1 > 157.2	8	203.1 > 129.2	12
Diphenamid	ES+	30	240.1 > 134.1	20	240.1 > 167.1	25
Ditalimfos	ES+	15	300.1 > 130.1	34	300.1 > 148.1	20
Diuron	ES+	22	233 > 72.1	18	233 > 46.1	15
Dodemorph	ES+	30	282 > 116	21	282 > 98	28
Dodine	ES+	46	228.223 > 57.046	22	228.223 > 60.038	22
Emamectin benzoate	ES+	52	886.59 > 158.11	34	886.59 > 82.01	50
Epoxiconazole	ES+	25	330 > 121	25	330 > 101	25
Etaconazole	ES+	16	328.1 > 159	25	328.1 > 205	13
Ethion	ES+	16	385 > 199	20	402 > 199	20
Ethirimol	ES+	30	210 > 140	23	210 > 98	25
Ethofumesate	ES+	25	287.1 > 121.1	15	287.1 > 259.1	10
Ethoprophos	ES+	18	243.2 > 131	20	243.2 > 97	31
Ethoxyquin	ES+	30	218.1 > 148	22	218.1 > 160.1	32
Etofenprox	ES+	17	394.3 > 177	15	394.3 > 106.9	43
Etoxazole	ES+	34	360.2744 > 141.0321	30	360.2744 > 113.0764	58
Etrimfos	ES+	30	293 > 125	25	293 > 265	18
Fenamidone	ES+	22	312.1 > 92	25	312.1 > 236.1	14
Fenamiphos	ES+	27	304.1 > 217.1	24	304.1 > 202.1	36
Fenarimol	ES+	37	331 > 268	22	331 > 81	34
Fenazaquin	ES+	27	307.2 > 57.2	25	307.2 > 161	19
Fenbuconazole	ES+	29	337 > 125	36	337 > 70.1	20
Fenbutatin-oxide	ES+	52	519.15 > 90.99	44	519.15 > 196.89	52
Fenhexamid	ES+	32	302.1 > 97.2	22	302.1 > 55.3	38
Fenothiocarb	ES+	15	270 > 95	25	270 > 242	12
Fenoxaprop	ES+	26	334.1 > 288	20	334.1 > 70	20
Fenoxycarb	ES+	22	302.22 > 88	20	302.22 > 116.04	10
Fenpropidin	ES+	50	274 > 147	30	274 > 86	30
Fenpropimorph	ES+	45	304 > 147	28	304 > 117	28
Fenpyrazamine	ES+	26	332.16 > 230.412	18	332.16 > 216.117	28
Fenpyroximate	ES+	23	422.2 > 366.1	15	422.2 > 138.1	32
Fenthion	ES+	25	279.1 > 247.1	13	279.1 > 169.1	16
Fenthion-sulfoxide	ES+	30	295 > 109	32	295 > 280	18
Fipronil	ES-	22	435 > 330	15	435 > 250	25
Fipronil-sulfone	ES-	25	451 > 415	15	451 > 282	26
Flazasulfuron	ES+	23	408.1 > 181.9	20	NA	44
Flonicamid	ES+	36	230.03 > 203.01	18	230.03 > 147.96	28
Florasulam	ES+	30	360 > 129	22	360 > 192	25
Fluazifop	ES+	30	328.1 > 282.1	20	328.1 > 255.1	23
Fluazifop-P-butyl	ES+	32	384.16 > 282.107	22	NA	16
Fluazinam	ES-	34	462.9 > 415.98	22	462.9 > 397.97	14
Flubendiamide	ES+	20	683.2 > 274.1	28	683.2 > 408	20
Fludioxonil	ES-	42	247 > 180	28	247 > 126	35

Flufenacet	ES+	15	364.1 > 152.1	23	364.1 > 194.1	11
Flufenoxuron	ES+	31	489.12 > 158.02	22	489.12 > 141	46
Fluopicolide	ES+	30	383.05 > 173	22	383.05 > 144.9	54
Fluopyram	ES+	32	397.096 > 173.008	32	397.096 > 145.051	52
Flupyradifurone	ES+	25	289 > 126	25	289 > 99	40
Fluquinconazole	ES+	32	376.0319 > 349.079	20	376.0319 > 107.999	48
Fluroxypyr	ES+	74	254.102 > 91.04	28	NA	34
Fluroxypyr-1-methylheptyl ester	ES+	15	367 > 255	11	367 > 181	32
Flusilazole	ES+	27	316 > 247	18	316 > 165	28
Flutriafol	ES+	28	302.16 > 69.99	16	302.16 > 123.06	28
Fluvalinate Tau	ES+	15	503 > 181.1	30	503 > 208.1	12
Fluxapyroxad	ES+	20	382.1 > 362.1	15	382.1 > 342.1	22
Fonofos	ES+	15	247.1 > 109	20	247.1 > 137	10
Fosthiazate	ES+	15	284 > 104	22	284 > 228	10
Fuberidazole	ES+	35	185 > 157	22	185 > 65	22
Furalaxyl	ES+	20	302.1 > 95	29	302.1 > 242.1	16
Furathiocarb	ES+	20	383.2 > 195.1	18	383.2 > 252.1	13
Heptenophos	ES+	15	251 > 127	14	251 > 125	14
Hexaconazole	ES+	31	314 > 70.1	22	316 > 70	25
Hexythiazox	ES+	21	353 > 228.1	14	353 > 168.1	26
Imazalil	ES+	30	297 > 159	23	297 > 69	22
Imazaquin	ES+	35	312.1 > 128	45	312.2 > 267.2	20
Imazosulfuron	ES+	22	413 > 155.9	22	413 > 152.8	12
Imidacloprid	ES+	23	256.1 > 175.1	20	256.1 > 209.1	15
Indoxacarb	ES+	25	528 > 203	40	528 > 150	22
Ipconazole	ES+	28	334.2 > 70	20	334.2 > 125	35
Iprovalicarb	ES+	19	321.1 > 119.06	16	321.1 > 203.1	10
Isofetamid	ES+	15	360.1 > 125	30	360.1 > 210	10
Isopropalin	ES+	30	310.2 > 226.2	20	310.2 > 268.2	15
Isoproturon	ES+	26	207.2 > 72.1	22	NA	NA
Isopyrazam	ES+	25	360.2 > 244.1	22	360.2 > 340.2	19
Lenacil	ES+	20	235.1 > 153.1	16	235.1 > 136.1	32
Linuron	ES+	26	249.1 > 160	20	249.1 > 182.1	15
Lufenuron	ES+	26	511.2 > 141	44	511.2 > 158	20
Malaoxon	ES+	15	315 > 98.9	24	315 > 127	12
Mandipropamid	ES+	20	412.16 > 328.16	16	412.16 > 125.01	34
MCPA	ES-	18	199 > 140.9	17	201 > 143	17
Mecarbam	ES+	12	330 > 227.1	8	330 > 97	35
Mecoprob	ES-	16	213 > 141	18	NA	18
Mepanipyrim	ES+	37	224.1 > 106	25	224.1 > 77	40
Mepronil	ES+	30	270.1 > 119.1	28	270.1 > 91	44
Meptyldinocap	ES-	46	295.16 > 193.24	30	295.16 > 134.06	52
Metalaxyl	ES+	15	280.1 > 220.1	13	280.1 > 192.1	17
Metamitron	ES+	30	203 > 175	15	NA	20
Metazachlor	ES+	15	278 > 134.1	22	NA	10
Metconazole (sum)	ES+	30	320.1 > 70	22	320.1 > 125	36

Methamidophos	ES+	17	142 > 93.9	13	142 > 124.9	13
Methidathion	ES+	10	303 > 145	10	303 > 85.1	20
Methiocarb (sum)	ES+	22	226.11 > 169.06	10	226.11 > 121.12	18
Methiocarb-sulfone	NA	NA	258.1 > 122.1	NA	258.1 > 107.1	NA
Methiocarb-sulfoxide	ES+	22	242.1 > 185	14	242.1 > 122	28
Methomyl	ES+	10	163 > 106	10	163 > 88	10
Methoxyfenozide	ES+	25	369.1 > 149.1	18	369.1 > 313.2	8
Metolachlor	ES+	18	284.1 > 252.1	15	NA	25
Metrafenone	ES+	19	409 > 209.1	14	409 > 226.9	16
Metribuzin	ES+	33	215 > 187	20	NA	20
Mevinphos	ES+	13	225.1 > 127.1	15	225.1 > 193.1	8
Monocrotophos	ES+	25	224 > 127	15	224 > 98	10
Monolinuron	ES+	22	215.1 > 126.1	20	215.1 > 99	30
Monuron	ES+	20	199 > 72	16	199 > 126	25
Myclobutanil	ES+	25	289.1 > 70.2	18	289.1 > 125.1	32
Napropamide	ES+	25	272.15 > 129	16	272.15 > 171.1	18
Nicosulfuron	ES+	26	411.1 > 106	32	411.1 > 182	22
Nitenpyram	ES+	22	271.1 > 224.9	12	271.1 > 125.9	25
Nuarimol	ES+	37	315 > 252	22	315 > 81.1	28
Omethoate	ES+	16	214.1 > 183.1	11	214.1 > 125.1	22
Oryzalin	ES+	28	345.15 > 281.1	19	345.15 > 78	35
Oxadiazon	ES+	32	345.15 > 220	18	345.15 > 177	27
Oxadixyl	ES+	31	279 > 219	10	279 > 132	34
Oxamyl	ES+	15	237 > 72	12	237 > 90	10
Oxathiapiprolin	ES+	35	540.4 > 480.4	25	540.4 > 350.3	28
Oxycarboxin	ES+	22	268.1 > 174.8	16	268.1 > 146.9	25
Paclobutrazol	Es+	27	294.1 > 125.1	38	294.1 > 70.2	20
Paraoxon	ES+	20	276 > 220	20	276 > 94	20
Penconazole	ES+	25	284 > 70.1	16	284 > 159	34
Pencycuron	ES+	40	329.1 > 124.98	20	329.1 > 218	15
Pendimethalin	ES+	12	282.2 > 212.2	10	NA	17
Penoxsulam	NA	NA	484 > 195	NA	484 > 164	NA
Penthiopyrad	ES+	24	360.2 > 276.2	15	360.2 > 256.1	22
Permethrin	ES+	16	408.175 > 183	25	408.175 > 355.2	8
Pethoxamid	ES+	20	296 > 131.1	20	296 > 250.1	14
Phosmet	ES+	30	318 > 160	22	318 > 77	46
Phoxim	ES+	15	299 > 129	13	299 > 153	7
Picoxystrobin	ES+	15	368.1 > 145.1	22	368.1 > 205.1	10
Piperonyl butoxide	ES+	16	356.2873 > 177.109	14	356.2873 > 119.117	34
Pirimicarb	ES+	25	239.1 > 72	18	239.1 > 182.1	15
Pirimicarb-desmethyl	ES+	25	225 > 72	20	225 > 168	16
Pirimiphos-methyl	ES+	25	306.1 > 108.1	32	306.1 > 164.1	22
Prochloraz	ES+	20	376 > 308	15	376 > 266	15
Profenofos	ES+	25	372.9 > 302.6	20	372.9 > 127.9	40
Promecarb	ES+	15	208.1 > 151	10	208.1 > 109	16
Prometon	ES+	30	226.1 > 184.3	28	226.1 > 86.3	18
Prometryn	ES+	27	242 > 200.1	17	NA	24

Propamocarb	ES+	30	189.1596 > 101.9647	16	189.1596 > 74.0084	24
Propanil	ES+	30	218 > 162	16	218 > 127	22
Propaquizafop	NA	NA	444.1 > 299.1	NA	444.1 > 100.04	NA
Propargite	ES+	20	368.22 > 231.21	10	368.22 > 175.15	14
Propham	ES+	5	180 > 138	8	NA	16
Propiconazole	ES+	37	342 > 159	34	342 > 69	22
Propoxur	ES+	12	210 > 111	16	210 > 168	10
Propoxycarbazone	ES+	30	416 > 116	35	416 > 199	15
Propyzamide	ES+	22	256.1 > 190	16	256.1 > 173	23
Proquinazid	ES+	18	373 > 289	22	373 > 272	32
Prosulfocarb	ES+	22	252.1 > 90.9	22	252.1 > 127.9	13
Prosulfuron	ES+	26	420 > 141	20	420 > 167	21
Prothioconazole	ES+	20	344.1 > 326	12	344.1 > 189	20
Prothioconazole-desthio	NA	NA	312 > 70	NA	312 > 125	NA
Pyraclostrobin	ES+	20	388.1 > 163	25	388.1 > 193.9	12
Pyraflufen	ES-	28	383 > 325	16	383 > 274	30
Pyraflufen-ethyl	ES+	40	413 > 339	20	413 > 253	30
Pyrazophos	ES+	33	374 > 222.1	22	374 > 194	32
Pyrethrum (Cinerin I)	ES+	18	317.16 > 149.016	8	317.16 > 106.998	20
Pyrethrum (Cinerin II)	ES+	18	361.16 > 149.011	8	361.16 > 106.995	18
Pyrethrum (Jasmolin I)	NA	20	331.1 > 164	10	NA	NA
Pyrethrum (Jasmolin II)	ES+	38	375.213 > 163.005	14	375.213 > 79.093	40
Pyrethrum (Pyrethrin I)	ES+	18	329.16 > 160.99	10	329.16 > 142.98	16
Pyrethrum (Pyrethrin II)	ES+	20	373.16 > 160.991	10	373.16 > 133.022	18
Pyridaben	ES+	22	365.16 > 147.12	24	365.16 > 309.1	12
Piridafention	ES+	31	341 > 189	22	341 > 92	34
Pyrifenox	ES+	29	295 > 93.1	22	297 > 93	20
Pyrimethanil	ES+	42	200 > 107	24	200 > 82	24
Pyriofenone	ES+	35	366 > 184.1	25	366 > 209	25
Pyriproxyfen	ES+	28	322.16 > 96.06	14	322.16 > 227.1	14
Quinalphos	ES+	15	299 > 96.9	30	299 > 162.9	24
Quinoxyfen	ES+	52	308 > 197	32	308 > 161.9	44
Quizalofop-P-ethyl	ES+	30	373 > 299	22	NA	32
Sedaxane	ES-	35	330.1 > 131	20	330.1 > 91	35
Sethoxydim	ES+	26	328.2 > 178	20	328.2 > 282.1	12
Simazine	ES+	32	202 > 96	22	NA	17
Spinetoram	ES+	40	748.4 > 142.12	32	760.4 > 98.05	52
Spinosad A	ES+	47	732.6 > 142	31	732.6 > 98.1	59
Spinosad D	ES+	42	746.52 > 142	31	746.52 > 98.1	53
Spirodiclofen	ES+	22	411.1 > 313	13	411.1 > 71.2	13
Spirotetramat	ES+	28	374.35 > 216.18	34	374.35 > 302.27	16
Spirotetramat-enol	ES+	35	302.1 > 216.1	28	302.1 > 117	30
Spirotetramat-enol-glucoside	NA	NA	464 > 302	NA	464 > 216	NA

Spirotetramat-ketohydroxy	ES+	20	318.1 > 300.1	12	318.1 > 214.1	28
Spiroxamine	ES+	27	298 > 144	20	298 > 100	32
Sulfotep	ES+	17	323 > 97	32	323 > 171	15
Sulfoxaflor	ES+	25	276 > 213	15	276 > 261	15
Tebuconazole	ES+	31	308 > 125	40	308 > 70.1	22
Tebufenozide	ES+	12	353.1 > 133	20	353.1 > 297.1	8
Tebufenpyrad	ES+	43	334 > 117	34	334 > 145	28
Tebupirimifos	ES+	22	319.1 > 153	29	319.1 > 277	15
Teflubenzuron	ES+	17	380.9 > 158	20	380.9 > 140.9	40
Tembotrione	ES+	30	441 > 261	20	441 > 305	24
Tepraloxydim	ES+	25	342.1 > 250.1	12	342.1 > 166.1	22
Terbufos	ES+	12	289 > 103	8	289 > 57.2	22
Terbumeton	ES+	30	226.2 > 170.1	15	226.2 > 114.1	25
Terbuthylazine	ES+	28	230 > 174	16	NA	28
Terbutryn	ES+	28	242.1 > 186.1	19	NA	20
Tetrachlorvinphos	ES+	30	366.8 > 127	16	366.8 > 240.9	23
Tetraconazole	ES+	32	372 > 159	30	372 > 70.1	20
Tetramethrin	ES+	20	332 > 164	25	332 > 135	15
Thiabendazole	ES+	35	202.1 > 175	28	202.1 > 131	32
Thiacloprid	ES+	32	253 > 126	20	253 > 90.1	40
Thiamethoxam	ES+	19	292 > 211.2	12	292 > 132	22
Thifensulfuron-methyl	ES+	22	388 > 167	15	388 > 56	40
Thiobencarb	ES+	20	258 > 125	15	258 > 89	35
Thiodicarb	ES+	15	355 > 88	20	355 > 108	15
Thiophanate-methyl	ES+	24	343.1 > 151.02	20	343.1 > 311.1	10
Tolclofos-methyl	ES+	30	301.1 > 174.9	29	301.1 > 125	17
Tri-allate	ES+	30	304 > 142.9	28	304 > 86	18
Triadimefon	ES+	22	294.1 > 197.2	15	294.1 > 69.3	20
Triasulfuron	ES+	28	402.05 > 167.05	18	402.05 > 141.05	30
Triazamate	ES+	20	315.1 > 72	20	315.1 > 226.1	11
Triazophos	ES+	22	314.1 > 161.9	18	314.1 > 118.9	35
Trichlorfon	ES+	19	257 > 109	18	257 > 79	30
Triclopyr	ES-	12	256 > 198	15	254 > 196	15
Tricyclazole	ES+	42	190 > 136	27	190 > 163	18
Trifloxystrobin	ES+	25	409 > 186	16	409 > 145	40
Triflumizole	ES+	13	346 > 277.9	10	346 > 73	15
Triflumuron	ES+	23	359 > 156.1	16	359 > 139.1	35
Triticonazole	ES+	15	318.1 > 70.1	18	318.1 > 124.9	40
Tritosulfuron	ES+	30	446 > 145	34	446 > 195	18
Valifenalate	ES+	23	399 > 116	25	399 > 155	25
Vamidothion	ES+	30	288 > 118	28	288 > 146	10
Zoxamide	ES+	27	336 > 187.1	25	336 > 159	38

Table S3.5: Instrument acquisition data for pesticides analysed by GC-MS/MS. The table reports quantification trace (Quant trace), collision energy (CE, expressed as eV), and qualification trace (Qual trace).

Compound	Quant trace	CE	Qual trace	CE
2, 4-DDD	235.0 -> 165.1	25	235.0 -> 200.1	10
2, 4-DDE	248.0 -> 176.2	30	246.0 -> 176.2	30
2, 4-DDT	237.0 -> 165.2	20	235.0 -> 165.2	20
3, 5-dichloroaniline	161.0 -> 99.0	20	161.0 -> 90.0	20
4, 4-DDE	246.1 -> 176.2	30	315.8 -> 246.0	15
4, 4-DDT	237.0 -> 165.2	20	235.0 -> 165.2	20
Acetochlor	146.0 -> 131.1	10	174.0 -> 146.1	10
Acrinathrin	181.0 -> 152.0	30	207.8 -> 181.1	10
Alachlor	188.1 -> 160.1	10	160.1 -> 132.1	15
Aldrin	262.9 -> 192.9	35	254.9 -> 220.0	20
Benfluralin	292.0 -> 264.0	5	292.0 -> 206.0	10
Bifenox	189.1 -> 126.0	20	340.9 -> 309.9	10
Bromophos-ethyl	302.8 -> 284.7	15	358.7 -> 302.8	15
Bromopropylate	185.0 -> 157.0	15	183.0 -> 155.0	15
Captan	263.0 -> 79.0	25	149.0 -> 70.0	15
Chinomethionat	206.0 -> 148.1	15	233.9 -> 206.1	10
Chlorfenapyr	137.0 -> 102.0	15	247.1 -> 227.1	20
Chlorfenson	175.0 -> 111.0	10	111.0 -> 75.0	15
Chlormephos	121.1 -> 65.0	10	153.9 -> 121.1	0
Chlorpropham	127.0 -> 65.0	25	213.0 -> 127.0	10
Chlorpyrifos-methyl	78.9 -> 47.0	10	286.0 -> 93.0	15
Cyfluthrin I	162.9 -> 90.9	15	162.9 -> 127.0	5
Cyfluthrin II {CAS # 68359-37-5}	162.9 -> 90.9	15	162.9 -> 127.0	5
Cyfluthrin III {CAS # 68359-37-5}	162.9 -> 90.9	15	162.9 -> 127.0	5
Cyfluthrin IV {CAS # 68359-37-5}	162.9 -> 90.9	15	162.9 -> 127.0	5
Cyhalofop-butyl	120.1 -> 91.0	15	256.2 -> 120.1	10
Cypermethrin I	163.0 -> 91.0	10	163.0 -> 127.0	5
Cypermethrin II {CAS # 52315-07-8}	163.1 -> 127.1	5	163.1 -> 91.0	15
Cypermethrin III {CAS # 52315-07-8}	163.1 -> 91.0	15	163.1 -> 127.1	5
Cypermethrin IV {CAS # 52315-07-8}	163.1 -> 91.0	15	163.1 -> 127.1	5
Cypermethrin, alpha-	163.0 -> 91.0	15	163.0 -> 127.0	5
Cypermethrin, beta- {CAS# 67375-30-8)	163.0 -> 91.0	15	181.0 -> 152.0	30
Deltamethrin	252.9 -> 93.0	15	181.0 -> 152.1	25
Dichlobenil	171.0 -> 100.0	20	171.0 -> 136.0	20
Dichlofenthion	223.0 -> 204.9	15	279.0 -> 223.0	15
Dichlofluanid	123.0 -> 77.0	20	123.0 -> 51.0	40
Dicloran	206.1 -> 176.0	10	160.1 -> 124.1	10
Dicofol o, p'-	139.0 -> 75.0	30	139.0 -> 111.0	10
Dicofol p, p'-	183.9 -> 169.3	5	183.9 -> 155.0	30
Dieldrin	262.9 -> 193.0	35	277.0 -> 241.0	5
Diflufenican	266.0 -> 238.1	15	266.0 -> 246.1	15
Dioxathion	124.9 -> 96.9	5	152.9 -> 96.9	10
Diphenylamine	169.0 -> 168.2	15	168.0 -> 167.2	15
Disulfoton	88.0 -> 60.0	5	142.0 -> 109.0	5

Endosulfan sulfate	273.8 -> 238.9	15	271.9 -> 237.0	20
Endosulfan alpha	194.9 -> 159.0	5	194.9 -> 160.0	5
Endosulfan beta	194.9 -> 158.9	10	206.9 -> 172.0	15
EPN	169.0 -> 141.1	5	169.0 -> 77.1	25
Ethalfluralin	275.9 -> 202.1	15	315.9 -> 275.9	10
Etridiazole	183.0 -> 140.0	15	211.1 -> 183.0	10
Famoxadone	197.0 -> 141.1	15	223.9 -> 196.2	10
Fenchlorphos	268.9 -> 254.0	15	270.9 -> 256.0	15
Fenitrothion	125.1 -> 47.0	15	125.1 -> 79.0	5
Fenpropathrin	181.1 -> 152.1	25	207.9 -> 181.0	5
Fenson	141.0 -> 77.1	5	267.9 -> 77.1	20
Fenthion-sulfone	124.9 -> 79.0	5	124.9 -> 47.0	10
Fenvalerate I	167.0 -> 125.1	5	208.9 -> 141.1	15
Fenvalerate II {CAS # 51630-58-1}	167.0 -> 125.1	5	208.9 -> 141.1	15
Flucythrinate I	156.9 -> 107.1	15	198.9 -> 157.0	10
Flucythrinate II {CAS # 70124-77-5}	156.9 -> 107.1	10	198.9 -> 157.0	10
Folpet	261.8 -> 130.1	15	259.8 -> 130.1	15
Heptachlor	271.7 -> 236.9	15	273.7 -> 238.9	15
Hexachlorobenzene	283.8 -> 213.9	30	283.8 -> 248.8	15
Hexaflumuron	176.0 -> 148.0	15	277.0 -> 176.0	15
Iprodione	316.0 -> 247.0	10	314.0 -> 271.0	10
Isofenphos	212.9 -> 185.1	5	212.9 -> 121.1	10
Kresoxim-methyl	116.0 -> 89.0	15	116.0 -> 63.0	30
Lambda-cyhalothrin	181.1 -> 152.1	30	208.1 -> 181.1	10
Malathion	126.9 -> 99.0	5	172.9 -> 99.0	15
Methoxychlor o, p'-	121.1 -> 91.1	15	227.1 -> 121.1	15
Methoxychlor p, p'-	227.0 -> 141.1	40	227.0 -> 169.1	25
Ortophenylphenol	170.0 -> 141.0	15	141.0 -> 115.0	15
Paraoxon-methyl	108.9 -> 79.0	5	229.9 -> 106.1	15
Parathion-ethyl	139.0 -> 109.0	5	109.0 -> 81.0	15
Parathion-methyl	125.0 -> 47.0	10	262.9 -> 109.0	10
Phosalone	182.0 -> 111.0	15	121.1 -> 65.0	10
Procymidone	96.0 -> 67.1	10	96.0 -> 53.1	15
Profluralin	318.1 -> 199.1	15	318.1 -> 55.1	15
Prothiofos	113.0 -> 94.9	10	266.9 -> 239.0	5
Quintozene	237.0 -> 119.0	20	295.0 -> 237.0	20
Tecnazene	214.9 -> 179.0	10	260.9 -> 203.0	10
Tefluthrin	177.1 -> 127.1	15	197.0 -> 141.1	10
Tetradifon	158.9 -> 131.0	10	226.9 -> 199.0	15
Thiometon	125.0 -> 47.0	15	125.0 -> 79.0	10
Tolylfluanid	137.0 -> 91.1	20	238.0 -> 137.0	15
Triadimenol	128.0 -> 65.0	25	168.0 -> 70.0	10
Trifluralin	264.0 -> 206.0	5	306.1 -> 264.0	5
Vinclozolin	187.0 -> 124.0	20	197.9 -> 145.0	15

Table S3.6: Validation data of the QuEChERS method (EN 15662:2018) applied on the pollen matrix. Matrix-matched calibration was used to quantify spiked samples (5 concentration levels included in the range shown in the table). Six replicates were prepared for each level added, and recovery data shown represents the average of six replicates. For each compound, the table reports the limit of quantification (LOQ, expressed as µg/kg, estimated according to the SANTE guidelines), limit of detection (LOD, expressed as µg/kg, estimated as one-third of the LOQs), the three concentration levels of added compounds (10, 50, and 200 µg/kg), recovery (Rec, expressed as %) and repeatability (RSD, expressed as %) of the method, matrix effect (expressed as %), and linearity (R^2).

Compound	**LOQ**	**LOD**	**Instrumental technique**	**10 µg/kg**		**50 µg/kg**		**200 µg/kg**		**Calib range**	**Matrix effect**	**R^2**
				Rec	**RSD**	**Rec**	**RSD**	**Rec**	**RSD**			
2, 4-DDD	10	3	GC-MS/MS	88	7	91	4	97	2	10 - 200	-8	0.9902
2, 4-DDE	10	3	GC-MS/MS	89	5	80	5	74	2	10 - 200	17	0.9807
2, 4-DDT	10	3	GC-MS/MS	71	3	80	2	86	2	10 - 200	-23	0.9809
3, 5-dichloroaniline	30	10	GC-MS/MS	65	27	71	10	103	4	30 - 250	18	0.9945
3-hydroxycarbofuran	3	1	LC-MS/MS	87	3	98	2	105	3	3 - 200	-11	0.9979
3-ketocarbofuran	3	1	LC-MS/MS	119	5	111	6	106	6	3 - 200	7	0.9905
4, 4-DDE	10	3	GC-MS/MS	92	10	95	8	103	7	10 - 200	-8	0.9908
4, 4-DDT	10	3	GC-MS/MS	89	7	95	8	96	4	10 - 200	-20	0.9854
6-benzylaminopurine	3	1	LC-MS/MS	111	7	108	6	105	5	3 - 200	14	0.9928
Abamectin	10	3	LC-MS/MS	61	20	85	26	103	6	10 - 200	18	0.9854
Acephate	5	2	LC-MS/MS	98	3	106	2	114	2	5 - 200	-14	0.9867
Acetamiprid	3	1	LC-MS/MS	66	15	84	11	95	2	3 - 200	22	0.9805
Acetochlor	10	3	GC-MS/MS	80	8	90	8	93	6	10 - 200	45	0.9842
Acibenzolar-S-methyl	3	1	LC-MS/MS	82	20	95	12	112	5	3 - 200	1	0.9856
Acrinathrin	10	3	GC-MS/MS	90	7	92	5	96	3	10 - 200	24	0.993
Alachlor	30	10	GC-MS/MS	77	3	86	3	93	3	30 - 250	7	0.9961
Aldicarb (sum)	30	10	LC-MS/MS	96	8	99	6	103	4	10 - 200	18	0.9931
Aldicarb sulfone	3	1	LC-MS/MS	88	20	88	21	94	14	3 - 200	8	0.9827
Aldicarb sulfoxide	5	2	LC-MS/MS	69	18	71	16	77	10	5 - 200	-17	0.9805

Aldrin	10	3	GC-MS/MS	79	2	88	3	99	2	10 - 200	-16	0.9813
Allethrin	10	3	LC-MS/MS	85	3	95	3	106	2	10 - 200	7	0.9847
Ametoctradin	3	1	LC-MS/MS	69	22	77	19	75	20	3 - 200	-4	0.9862
Ametryn	3	1	LC-MS/MS	74	12	90	8	103	5	3 - 200	-14	0.9853
Amidosulfuron	3	1	LC-MS/MS	69	2	84	3	98	4	3 - 200	13	0.9969
Amisulbrom	30	10	LC-MS/MS	96	4	98	3	98	2	10 - 200	-19	0.9848
Amitraz	30	10	LC-MS/MS	77	4	87	4	96	3	10 - 200	-21	0.9974
Atrazine	3	1	LC-MS/MS	73	4	86	4	101	3	3 - 200	2	0.9805
Azaconazole	3	1	LC-MS/MS	76	12	80	25	81	19	3 - 200	14	0.9887
Azinphos-ethyl	30	10	LC-MS/MS	93	12	95	15	98	15	10 - 200	9	0.9859
Azinphos-methyl	10	3	LC-MS/MS	100	15	62	11	70	16	10 - 200	4	0.9951
Azoxystrobin	3	1	LC-MS/MS	69	5	71	4	90	3	3 - 200	11	0.988
Beflubutamid	5	2	LC-MS/MS	110	13	104	7	100	2	5 - 200	14	0.9958
Benalaxyl	3	1	LC-MS/MS	66	20	78	13	84	7	3 - 200	-17	0.9892
Bendiocarb	3	1	LC-MS/MS	76	7	84	5	87	3	3 - 200	22	0.9821
Benfluralin	10	3	GC-MS/MS	60	18	68	21	66	18	10 - 200	-9	0.9967
Benfuracarb	10	3	LC-MS/MS	120	16	115	25	100	10	10 - 200	-6	0.9956
Benomyl	50	20	LC-MS/MS	82	8	96	7	102	8	50 - 400	14	0.994
Bensulfuron-methyl	10	3	LC-MS/MS	65	21	74	12	94	6	10 - 200	-20	0.9846
Benthiavalicarb isopropyl	3	1	LC-MS/MS	92	15	97	9	99	5	3 - 200	-5	0.9901
Benzoximate	10	3	LC-MS/MS	90	7	88	6	85	5	10 - 200	-13	0.9925
Benzoylprop-ethyl	3	1	LC-MS/MS	101	19	93	10	95	17	3 - 200	11	0.9897
Bifenazate	3	1	LC-MS/MS	96	12	90	11	93	19	3 - 200	-12	0.9808
Bifenox	10	3	GC-MS/MS	85	7	93	5	104	3	10 - 200	-22	0.9934
Bifenthrin	3	1	LC-MS/MS	114	6	113	7	113	5	3 - 200	17	0.9858
Bitertanol	10	3	LC-MS/MS	88	11	93	10	100	7	10 - 200	23	0.9938
Boscalid	3	1	LC-MS/MS	109	11	104	9	99	8	3 - 200	7	0.9828
Bromacil	3	1	LC-MS/MS	103	15	98	13	96	11	3 - 200	4	0.9871
Bromophos-ethyl	10	3	GC-MS/MS	71	5	82	4	89	3	10 - 200	3	0.9805
Bromophos-methyl	3	1	LC-MS/MS	75	12	85	6	67	5	3 - 200	25	0.9807

Bromopropylate	10	3	GC-MS/MS	87	12	94	10	98	3	10 - 200	20	0.9909
Bromoxynil	5	2	LC-MS/MS	76	6	86	5	96	3	5 - 200	-17	0.9937
Bromuconazole	3	1	LC-MS/MS	76	3	88	3	97	3	3 - 200	12	0.983
Bupirimate	3	1	LC-MS/MS	87	4	99	3	108	3	3 - 200	-15	0.9817
Buprofezin	3	1	LC-MS/MS	66	21	69	18	69	18	3 - 200	12	0.9959
Cadusafos	3	1	LC-MS/MS	68	4	76	4	84	2	3 - 200	19	0.9968
Captan	30	10	GC-MS/MS	91	7	95	5	100	3	30 - 250	-16	0.9856
Carbaryl	3	1	LC-MS/MS	95	8	95	9	96	6	3 - 200	16	0.9927
Carbendazim	3	1	LC-MS/MS	82	15	85	9	92	4	3 - 200	2	0.9968
Carbofuran	3	1	LC-MS/MS	111	5	106	5	103	4	3 - 200	14	0.9955
Carbosulfan	10	3	LC-MS/MS	121	21	122	15	110	19	10 - 200	-12	0.9806
Carboxin	3	1	LC-MS/MS	77	6	85	5	90	5	3 - 200	1	0.9949
Carfentrazone-ethyl	3	1	LC-MS/MS	107	11	105	10	103	9	3 - 200	21	0.9923
Chinomethionat	10	3	GC-MS/MS	115	12	115	13	97	8	10 - 200	4	0.9906
Chlorantraniliprole	10	3	LC-MS/MS	91	8	96	4	102	2	10 - 200	-11	0.9867
Chlorfenapyr	30	10	GC-MS/MS	109	4	110	5	110	5	30 - 250	17	0.9966
Chlorfenson	10	3	GC-MS/MS	82	19	87	9	84	5	10 - 200	-10	0.9924
Chlorfenvinphos	5	2	LC-MS/MS	104	5	103	5	101	4	5 - 200	13	0.993
Chlormephos	30	10	GC-MS/MS	81	19	86	18	91	13	30 - 250	-11	0.9923
Chlorpropham	30	10	GC-MS/MS	63	10	66	6	71	2	30 - 250	-7	0.9809
Chlorpyrifos	3	1	LC-MS/MS	82	11	98	8	100	3	3 - 200	1	0.9943
Chlorpyrifos-methyl	10	3	GC-MS/MS	129	14	79	13	98	20	10 - 200	-2	0.9902
Chlozolinate	10	3	LC-MS/MS	68	21	70	19	73	16	10 - 200	-24	0.998
Chromafenozide	10	3	LC-MS/MS	108	4	99	4	92	4	10 - 200	14	0.9925
Clethodim Isomer A	30	10	LC-MS/MS	85	4	93	4	105	3	10 - 200	-2	0.9992
Clethodim Isomer B	3	1	LC-MS/MS	85	5	93	4	95	3	3 - 200	-25	0.9853
Clofentezine	10	3	LC-MS/MS	76	15	83	9	85	4	10 - 200	-22	0.9972
Cloquintocet	30	10	LC-MS/MS	60	7	62	16	69	16	10 - 200	-13	0.9941
Cloquintocet-mexyl	3	1	LC-MS/MS	85	5	91	3	92	3	3 - 200	-13	0.9872
Clothianidin	3	1	LC-MS/MS	91	4	95	3	99	2	3 - 200	-18	0.9836

Coumaphos	3	1	LC-MS/MS	115	25	119	24	95	19	3 - 200	2	0.9929
Cyanazine	3	1	LC-MS/MS	115	21	110	20	116	15	3 - 200	14	0.9833
Cyantraniliprole	5	2	LC-MS/MS	105	14	107	11	105	10	5 - 200	8	0.9924
Cyazofamid	3	1	LC-MS/MS	120	12	115	9	74	7	3 - 200	-2	0.9809
Cycloxydim	3	1	LC-MS/MS	71	20	74	19	70	12	3 - 200	-15	0.9932
Cyflufenamid	3	1	LC-MS/MS	121	11	119	12	115	4	3 - 200	15	0.9961
Cyflumetofen	3	1	LC-MS/MS	74	9	78	7	76	5	3 - 200	21	0.9916
Cyfluthrin (sum)	30	10	GC-MS/MS	113	12	118	15	93	20	30 - 250	-8	0.9957
Cyhalofop-butyl	10	3	GC-MS/MS	71	15	85	14	86	27	10 - 200	-1	0.9949
Cymoxanil	10	3	LC-MS/MS	65	12	90	12	93	8	10 - 200	-20	0.9994
Cypermethrin (sum)	30	10	GC-MS/MS	85	19	91	15	90	9	30 - 250	-7	0.982
Cyproconazole	3	1	LC-MS/MS	96	18	100	16	80	10	3 - 200	-18	0.9955
Cyprodinil	3	1	LC-MS/MS	85	14	91	14	80	10	3 - 200	25	0.9831
Deltamethrin	10	3	GC-MS/MS	94	8	97	5	103	2	10 - 200	5	0.9902
Demeton-S-methyl	30	10	LC-MS/MS	104	4	104	5	104	5	10 - 200	16	0.991
Demeton-S-methylsulfone	3	1	LC-MS/MS	85	7	95	7	97	6	3 - 200	19	0.9848
Desethyl-atrazine	5	2	LC-MS/MS	85	7	93	7	95	5	5 - 200	2	0.9913
Desisopropyl-atrazine	3	1	LC-MS/MS	62	8	72	19	85	10	3 - 200	-12	0.9961
Desmedipham	3	1	LC-MS/MS	110	12	97	10	87	5	3 - 200	-13	0.9871
Diazinon	3	1	LC-MS/MS	90	25	102	19	96	18	3 - 200	16	0.9947
Dicamba	50	20	LC-MS/MS	116	3	117	2	112	1	50 - 400	23	0.9888
Dichlobenil	10	3	GC-MS/MS	77	5	86	4	89	4	10 - 200	25	0.9877
Dichlofenthion	10	3	GC-MS/MS	99	9	101	5	106	2	10 - 200	-1	0.9834
Dichlofluanid	30	10	GC-MS/MS	69	7	79	6	88	3	30 - 250	-13	0.9959
Dicloran	30	10	GC-MS/MS	77	6	88	4	96	2	30 - 250	20	0.9904
Dichlorvos	3	1	LC-MS/MS	95	3	100	2	108	1	3 - 200	-15	0.9874
Dicofol (sum)	30	10	GC-MS/MS	69	12	72	12	77	10	30 - 250	8	0.9807
Dicrotophos	3	1	LC-MS/MS	88	8	98	9	99	9	3 - 200	17	0.9882
Dieldrin	30	10	GC-MS/MS	96	9	99	8	101	6	30 - 250	5	0.9936
Diethofencarb	3	1	LC-MS/MS	114	10	112	14	76	13	3 - 200	24	0.9955

Difenoconazole	3	1	LC-MS/MS	118	17	116	13	115	8	3 - 200	-23	0.9832
Diflubenzuron	30	10	LC-MS/MS	110	19	112	3	119	6	10 - 200	6	0.9805
Diflufenican	10	3	GC-MS/MS	78	8	94	5	103	5	10 - 200	-15	0.9841
Dimethoate	3	1	LC-MS/MS	117	15	102	16	80	12	3 - 200	15	0.9893
Dimethomorph	3	1	LC-MS/MS	96	13	98	25	89	15	3 - 200	-6	0.9977
Dimoxystrobin	3	1	LC-MS/MS	84	4	89	3	94	3	3 - 200	-25	0.9801
Diniconazole	3	1	LC-MS/MS	105	7	103	7	98	5	3 - 200	0	0.9808
Dinotefuran	30	10	LC-MS/MS	93	7	100	5	108	3	10 - 200	23	0.9819
Dioxathion	30	10	GC-MS/MS	73	5	79	5	80	4	30 - 250	-25	0.9826
Diphenamid	3	1	LC-MS/MS	130	13	98	10	105	8	3 - 200	18	0.9915
Diphenylamine	30	10	GC-MS/MS	115	11	107	8	103	5	30 - 250	-4	0.9993
Disulfoton	10	3	GC-MS/MS	96	7	101	8	110	3	10 - 200	24	0.9944
Ditalimfos	10	3	LC-MS/MS	79	10	87	7	96	3	10 - 200	-18	0.9868
Diuron	3	1	LC-MS/MS	76	5	91	4	113	2	3 - 200	-4	0.9847
Dodemorph	3	1	LC-MS/MS	79	13	84	8	84	4	3 - 200	20	0.9952
Dodine	10	3	LC-MS/MS	70	15	72	16	82	3	10 - 200	-13	0.9929
Emamectin benzoate	3	1	LC-MS/MS	120	9	127	11	108	9	3 - 200	-17	0.9962
Endosulfan alpha	10	3	GC-MS/MS	82	4	94	3	111	2	10 - 200	12	0.9956
Endosulfan beta	30	10	GC-MS/MS	108	6	114	6	114	5	30 - 250	-16	0.9992
Endosulfan sulfate	30	10	GC-MS/MS	105	11	109	9	112	6	30 - 250	-25	0.9907
EPN	30	10	GC-MS/MS	89	21	78	12	67	5	30 - 250	-19	0.9819
Epoxiconazole	3	1	LC-MS/MS	81	4	92	3	104	3	3 - 200	-22	0.9873
Etaconazole	3	1	LC-MS/MS	68	6	77	8	83	7	3 - 200	25	0.9948
Ethalfluralin	10	3	GC-MS/MS	74	17	91	15	119	8	10 - 200	25	0.9911
Ethion	10	3	LC-MS/MS	63	3	54	10	71	7	10 - 200	-25	0.9804
Ethirimol	3	1	LC-MS/MS	91	9	99	8	101	6	3 - 200	3	0.9915
Ethofumesate	5	2	LC-MS/MS	100	5	101	6	102	4	5 - 200	25	0.9889
Ethoprophos	3	1	LC-MS/MS	102	18	100	10	95	6	3 - 200	-23	0.9874
Ethoxyquin	3	1	LC-MS/MS	98	9	97	16	93	18	3 - 200	-4	0.993
Etofenprox	3	1	LC-MS/MS	99	3	97	3	95	1	3 - 200	-3	0.9911

Etoxazole	3	1	LC-MS/MS	95	8	97	6	99	3	3 - 200	-6	0.9815
Etridiazole	10	3	GC-MS/MS	105	15	109	13	108	7	10 - 200	15	0.9856
Etrimfos	3	1	LC-MS/MS	84	12	90	8	92	3	3 - 200	13	0.9946
Famoxadone	10	3	GC-MS/MS	93	4	92	3	93	3	10 - 200	-23	0.9928
Fenamidone	3	1	LC-MS/MS	71	11	88	7	104	3	3 - 200	1	0.9923
Fenamiphos	3	1	LC-MS/MS	79	13	87	9	96	4	3 - 200	-18	0.9884
Fenarimol	10	3	LC-MS/MS	63	9	78	6	100	2	10 - 200	21	0.9913
Fenazaquin	3	1	LC-MS/MS	69	2	84	3	94	4	3 - 200	-5	0.9996
Fenbuconazole	3	1	LC-MS/MS	75	3	84	3	90	3	3 - 200	8	0.992
Fenbutatin-oxide	3	1	LC-MS/MS	111	12	101	13	93	13	3 - 200	13	0.9927
Fenchlorphos	10	3	GC-MS/MS	90	4	97	3	108	2	10 - 200	24	0.9888
Fenhexamid	5	2	LC-MS/MS	85	5	94	3	102	2	5 - 200	17	0.9978
Fenitrothion	10	3	GC-MS/MS	94	4	91	4	92	3	10 - 200	-8	0.9813
Fenothiocarb	3	1	LC-MS/MS	86	20	92	12	91	4	3 - 200	-7	0.9907
Fenoxaprop	50	20	LC-MS/MS	75	4	84	4	98	4	50 - 400	21	0.9868
Fenoxycarb	3	1	LC-MS/MS	105	8	98	5	96	2	3 - 200	1	0.9986
Fenpropathrin	10	3	GC-MS/MS	82	3	90	3	99	2	10 - 200	15	0.988
Fenpropidin	3	1	LC-MS/MS	103	9	101	14	102	6	3 - 200	-22	0.9911
Fenpropimorph	3	1	LC-MS/MS	90	5	103	10	100	5	3 - 200	6	0.9888
Fenpyrazamine	3	1	LC-MS/MS	78	13	83	9	85	4	3 - 200	7	0.9905
Fenpyroximate	3	1	LC-MS/MS	125	3	119	5	112	4	3 - 200	-15	0.9858
Fenson	10	3	GC-MS/MS	121	9	114	6	95	3	10 - 200	-25	0.9944
Fenthion	3	1	LC-MS/MS	65	19	62	18	70	7	3 - 200	22	0.9821
Fenthion-sulfone	30	10	GC-MS/MS	89	5	91	4	91	3	30 - 250	-30	0.9951
Fenthion-sulfoxide	3	1	LC-MS/MS	111	6	102	5	97	3	3 - 200	-40	0.9903
Fenvalerate (sum)	10	3	GC-MS/MS	92	4	84	8	81	7	10 - 200	-23	0.9998
Fipronil	5	2	LC-MS/MS	80	4	86	7	93	7	5 - 200	16	0.9891
Fipronil-sulfone	3	1	LC-MS/MS	83	18	88	18	92	19	3 - 200	23	0.997
Flazasulfuron	10	3	LC-MS/MS	72	3	82	3	85	3	10 - 200	-5	0.9849
Flonicamid	3	1	LC-MS/MS	110	6	105	6	101	5	3 - 200	21	0.9918

Florasulam	3	1	LC-MS/MS	95	8	93	8	97	8	3 - 200	21	0.9974
Fluazifop	10	3	LC-MS/MS	118	10	116	9	119	7	10 - 200	-17	0.9891
Fluazifop-P-butyl	3	1	LC-MS/MS	87	4	98	3	106	3	3 - 200	-3	0.9859
Fluazinam	5	2	LC-MS/MS	119	6	118	6	115	4	5 - 200	9	0.9971
Flubendiamide	30	10	LC-MS/MS	123	7	116	5	115	4	10 - 200	-1	0.9851
Flucythrinate (sum)	10	3	GC-MS/MS	91	18	97	9	109	3	10 - 200	5	0.9879
Fludioxonil	10	3	LC-MS/MS	89	9	99	6	107	4	10 - 200	-22	0.9977
Flufenacet	3	1	LC-MS/MS	101	3	98	4	95	4	3 - 200	9	0.9923
Flufenoxuron	3	1	LC-MS/MS	78	3	90	3	95	2	3 - 200	-7	0.9896
Fluopicolide	5	2	LC-MS/MS	71	13	81	11	85	9	5 - 200	-1	0.9978
Fluopyram	3	1	LC-MS/MS	80	4	92	3	106	2	3 - 200	18	0.9911
Flupyradifurone	3	1	LC-MS/MS	95	12	94	7	96	3	3 - 200	1	0.9962
Fluquinconazole	10	3	LC-MS/MS	97	16	100	15	103	12	10 - 200	-14	0.9846
Fluroxypyr	10	3	LC-MS/MS	115	7	110	6	105	4	10 - 200	23	0.9855
Fluroxypyr-1-methylheptyl ester	5	2	LC-MS/MS	112	11	119	8	120	4	5 - 200	-6	0.9859
Flusilazole	3	1	LC-MS/MS	88	23	92	14	99	6	3 - 200	-18	0.9985
Flutriafol	10	3	LC-MS/MS	95	14	98	11	96	9	10 - 200	-9	0.983
Fluvalinate Tau	3	1	LC-MS/MS	103	7	99	5	95	2	3 - 200	-25	0.981
Fluxapyroxad	3	1	LC-MS/MS	74	8	89	4	96	3	3 - 200	1	0.9955
Folpet	30	10	GC-MS/MS	78	16	87	16	91	16	30 - 250	-20	0.9962
Fonofos	3	1	LC-MS/MS	93	15	89	12	84	9	3 - 200	-23	0.9974
Fosthiazate	3	1	LC-MS/MS	82	23	79	17	76	10	3 - 200	-11	0.9865
Fuberidazole	3	1	LC-MS/MS	75	18	86	10	101	5	3 - 200	10	0.9837
Furalaxyl	3	1	LC-MS/MS	64	10	81	7	88	5	3 - 200	23	0.9887
Furathiocarb	3	1	LC-MS/MS	71	4	79	5	83	4	3 - 200	10	0.9965
Heptachlor	10	3	GC-MS/MS	76	11	84	6	80	3	10 - 200	12	0.9835
Heptenophos	3	1	LC-MS/MS	100	11	109	9	115	6	3 - 200	2	0.9987
Hexachlorobenzene	30	10	GC-MS/MS	82	13	87	8	89	3	30 - 250	14	0.9923
Hexaconazole	3	1	LC-MS/MS	65	10	71	18	80	21	3 - 200	-1	0.9857
Hexaflumuron	30	10	GC-MS/MS	75	10	88	7	104	6	30 - 250	-2	0.9851

Hexythiazox	3	1	LC-MS/MS	73	4	80	6	93	4	3 - 200	16	0.9917
Imazalil	3	1	LC-MS/MS	93	4	98	3	105	2	3 - 200	15	0.9958
Imazaquin	10	3	LC-MS/MS	88	10	96	10	105	2	10 - 200	-22	0.9968
Imazosulfuron	10	3	LC-MS/MS	89	3	98	3	109	2	10 - 200	-16	0.9956
Imidacloprid	3	1	LC-MS/MS	67	5	83	4	103	4	3 - 200	0	0.9836
Indoxacarb	3	1	LC-MS/MS	104	5	107	6	104	6	3 - 200	-21	0.9955
Ipconazole	3	1	LC-MS/MS	79	5	90	4	104	3	3 - 200	0	0.982
Iprodione	30	10	GC-MS/MS	89	7	89	8	86	7	30 - 250	-23	0.993
Iprovalicarb	3	1	LC-MS/MS	80	10	86	6	92	2	3 - 200	-18	0.9836
Isofenphos	30	10	GC-MS/MS	115	23	110	15	111	10	30 - 250	7	0.9937
Isofetamid	3	1	LC-MS/MS	75	10	83	6	88	5	3 - 200	14	0.9821
Isopropalin	10	3	LC-MS/MS	87	7	81	9	80	7	10 - 200	23	0.983
Isoproturon	3	1	LC-MS/MS	85	3	87	4	91	5	3 - 200	-30	0.9851
Isopyrazam	3	1	LC-MS/MS	70	7	72	12	80	13	3 - 200	-14	0.9827
Kresoxim-methyl	10	3	GC-MS/MS	118	4	108	3	102	2	10 - 200	-5	0.994
Lambda-cyhalothrin	30	10	GC-MS/MS	113	6	110	4	109	3	30 - 250	-21	0.9884
Lenacil	3	1	LC-MS/MS	78	7	85	5	86	4	3 - 200	5	0.9862
Linuron	3	1	LC-MS/MS	102	10	98	15	89	7	3 - 200	-2	0.9846
Lufenuron	10	3	LC-MS/MS	110	13	104	13	86	6	10 - 200	-18	0.9824
Malaoxon	3	1	LC-MS/MS	77	13	85	11	89	11	3 - 200	18	0.983
Malathion	10	3	GC-MS/MS	78	20	85	15	91	9	10 - 200	8	0.9827
Mandipropamid	3	1	LC-MS/MS	90	8	96	6	102	4	3 - 200	5	0.994
MCPA	30	10	LC-MS/MS	85	14	92	8	101	4	10 - 200	8	0.9915
Mecarbam	3	1	LC-MS/MS	83	3	92	2	101	2	3 - 200	15	0.9849
Mecoprob	30	10	LC-MS/MS	81	6	93	5	104	4	10 - 200	14	0.9804
Mepanipyrim	10	3	LC-MS/MS	110	7	108	6	107	5	10 - 200	-25	0.9809
Mepronil	3	1	LC-MS/MS	81	4	86	3	88	3	3 - 200	-19	0.9858
Meptyldinocap	50	20	LC-MS/MS	78	10	96	20	103	14	50 - 400	22	0.9856
Metalaxyl	3	1	LC-MS/MS	78	4	90	3	103	3	3 - 200	-21	0.9898
Metamitron	3	1	LC-MS/MS	108	5	105	6	107	6	3 - 200	9	0.9833

Metazachlor	3	1	LC-MS/MS	78	9	93	9	107	8	3 - 200	24	0.9825
Metconazole (sum)	3	1	LC-MS/MS	68	10	75	15	78	20	3 - 200	20	0.9883
Methamidophos	3	1	LC-MS/MS	74	12	80	9	85	7	3 - 200	15	0.9923
Methidathion	3	1	LC-MS/MS	61	25	71	17	72	10	3 - 200	18	0.9882
Methiocarb (sum)	3	1	LC-MS/MS	94	15	100	15	113	14	3 - 200	-19	0.9951
Methiocarb-sulfone	3	1	LC-MS/MS	90	6	92	6	91	4	3 - 200	18	0.9839
Methiocarb-sulfoxide	3	1	LC-MS/MS	91	12	90	10	93	10	3 - 200	24	0.9911
Methomyl	3	1	LC-MS/MS	91	10	98	21	97	11	3 - 200	-1	0.9817
Methoxychlor (sum)	10	3	GC-MS/MS	82	6	86	4	85	2	10 - 200	-4	0.9932
Methoxyfenozide	10	3	LC-MS/MS	84	3	89	3	95	3	10 - 200	-10	0.9879
Metolachlor	3	1	LC-MS/MS	81	10	88	6	91	3	3 - 200	-7	0.9904
Metrafenone	3	1	LC-MS/MS	79	3	91	3	101	3	3 - 200	16	0.9976
Metribuzin	3	1	LC-MS/MS	74	8	85	6	91	4	3 - 200	-25	0.9927
Mevinphos	10	3	LC-MS/MS	72	6	81	7	92	7	10 - 200	25	0.9826
Monocrotophos	3	1	LC-MS/MS	61	8	75	14	79	7	3 - 200	22	0.9834
Monolinuron	3	1	LC-MS/MS	79	3	81	3	81	2	3 - 200	0	0.9804
Monuron	3	1	LC-MS/MS	92	3	98	4	104	4	3 - 200	7	0.9822
Myclobutanil	5	2	LC-MS/MS	85	14	95	8	98	6	5 - 200	-1	0.9986
Napropamide	3	1	LC-MS/MS	113	5	86	10	72	10	3 - 200	15	0.9868
Nicosulfuron	3	1	LC-MS/MS	102	14	109	12	118	10	3 - 200	-3	0.9826
Nitenpyram	3	1	LC-MS/MS	91	6	93	4	98	3	3 - 200	-14	0.9978
Nuarimol	3	1	LC-MS/MS	78	9	88	7	96	7	3 - 200	-15	0.9908
Omethoate	5	2	LC-MS/MS	90	6	93	4	96	2	5 - 200	-29	0.9887
Ortophenylphenol	30	10	GC-MS/MS	122	20	109	12	103	5	30 - 250	-17	0.9976
Oryzalin	30	10	LC-MS/MS	78	11	84	6	87	3	10 - 200	3	0.9933
Oxadiazon	3	1	LC-MS/MS	111	4	113	7	110	2	3 - 200	12	0.9864
Oxadixyl	3	1	LC-MS/MS	69	7	84	5	97	4	3 - 200	11	0.9948
Oxamyl	3	1	LC-MS/MS	73	4	84	4	91	4	3 - 200	11	0.9952
Oxathiapiprolin	30	10	LC-MS/MS	109	9	102	10	86	13	10 - 200	15	0.9923
Oxycarboxin	3	1	LC-MS/MS	79	9	88	7	100	4	3 - 200	0	0.9906

Paclobutrazol	5	2	LC-MS/MS	69	7	75	5	81	3	5 - 200	24	0.9966
Paraoxon	3	1	LC-MS/MS	90	6	91	4	93	2	3 - 200	25	0.9902
Paraoxon-methyl	10	3	GC-MS/MS	90	9	92	6	92	4	10 - 200	19	0.9866
Parathion-ethyl	30	10	GC-MS/MS	83	5	94	3	103	2	30 - 250	-18	0.9866
Parathion-methyl	10	3	GC-MS/MS	104	5	112	6	114	5	10 - 200	20	0.9811
Penconazole	3	1	LC-MS/MS	102	12	103	15	103	13	3 - 200	-14	0.9915
Pencycuron	3	1	LC-MS/MS	77	10	86	8	94	4	3 - 200	-9	0.9947
Pendimethalin	10	3	LC-MS/MS	94	4	105	2	115	2	10 - 200	-16	0.9994
Penoxsulam	3	1	LC-MS/MS	80	9	80	5	77	3	3 - 200	-18	0.9846
Penthiopyrad	3	1	LC-MS/MS	86	28	92	21	97	16	3 - 200	6	0.9933
Permethrin	5	2	LC-MS/MS	118	12	106	10	91	10	5 - 200	11	0.994
Pethoxamid	3	1	LC-MS/MS	88	3	94	4	102	4	3 - 200	2	0.997
Phosalone	10	3	GC-MS/MS	81	17	96	21	98	11	10 - 200	11	0.994
Phosmet	10	3	GC-MS/MS	106	7	102	10	103	8	10 - 200	13	0.9915
Phoxim	10	3	LC-MS/MS	113	3	110	6	106	5	10 - 200	-22	0.987
Picoxystrobin	3	1	LC-MS/MS	82	5	90	4	102	3	3 - 200	-11	0.9862
Piperonyl butoxide	3	1	LC-MS/MS	76	5	84	4	89	3	3 - 200	15	0.983
Pirimicarb	3	1	LC-MS/MS	71	16	76	11	85	5	3 - 200	18	0.986
Pirimicarb-desmethyl	3	1	LC-MS/MS	126	9	119	9	114	5	3 - 200	-13	0.9985
Pirimiphos-methyl	3	1	LC-MS/MS	68	5	83	4	100	2	3 - 200	-11	0.9926
Prochloraz	3	1	LC-MS/MS	79	6	88	4	96	2	3 - 200	-21	0.9995
Procymidone	10	3	GC-MS/MS	89	8	113	6	110	3	10 - 200	-23	0.9974
Profenofos	3	1	LC-MS/MS	116	8	91	9	117	4	3 - 200	-22	0.9834
Profluralin	10	3	GC-MS/MS	105	7	106	8	103	5	10 - 200	5	0.9859
Promecarb	3	1	LC-MS/MS	95	8	111	13	86	7	3 - 200	-15	0.9849
Prometon	10	3	LC-MS/MS	119	5	84	15	119	6	10 - 200	20	0.9804
Prometryn	3	1	LC-MS/MS	115	13	110	10	93	8	3 - 200	7	0.998
Propamocarb	3	1	LC-MS/MS	72	22	92	6	96	12	3 - 200	18	0.999
Propanil	3	1	LC-MS/MS	108	10	81	4	104	7	3 - 200	-17	0.981
Propaquizafop	3	1	LC-MS/MS	82	7	92	3	105	4	3 - 200	6	0.9955

Propargite	3	1	LC-MS/MS	68	6	99	2	91	3	3 - 200	10	0.9962
Propham	30	10	LC-MS/MS	89	3	75	7	96	2	10 - 200	-3	0.9974
Propiconazole	3	1	LC-MS/MS	92	3	107	7	111	2	3 - 200	20	0.9857
Propoxur	3	1	LC-MS/MS	63	11	85	4	93	3	3 - 200	-8	0.9865
Propoxycarbazone	50	20	LC-MS/MS	105	7	110	11	105	5	50 - 400	-19	0.9837
Propyzamide	10	3	LC-MS/MS	77	7	83	9	89	3	10 - 200	-21	0.9887
Proquinazid	3	1	LC-MS/MS	123	17	101	3	103	6	3 - 200	-6	0.9965
Prosulfocarb	3	1	LC-MS/MS	62	14	94	2	104	7	3 - 200	21	0.9835
Prosulfuron	10	3	LC-MS/MS	92	5	94	5	114	2	10 - 200	25	0.9987
Prothioconazole	30	10	LC-MS/MS	91	4	89	14	96	1	10 - 200	17	0.9923
Prothioconazole-desthio	5	2	LC-MS/MS	81	5	93	5	111	3	5 - 200	-12	0.9857
Prothiofos	10	3	GC-MS/MS	85	14	85	4	91	14	10 - 200	-12	0.9851
Pyraclostrobin	3	1	LC-MS/MS	82	8	63	27	103	3	3 - 200	-12	0.9917
Pyraflufen	30	10	LC-MS/MS	75	4	99	4	93	3	10 - 200	8	0.9958
Pyraflufen-ethyl	3	1	LC-MS/MS	85	10	86	4	71	14	3 - 200	-7	0.9968
Pyrazophos	3	1	LC-MS/MS	97	7	102	15	105	3	3 - 200	9	0.9923
Pyrethrum (Cinerin I)	10	3	LC-MS/MS	77	6	91	10	93	3	10 - 200	-1	0.9906
Pyrethrum (Cinerin II)	5	2	LC-MS/MS	125	16	88	3	95	11	5 - 200	-13	0.9966
Pyrethrum (Jasmolin I)	30	10	LC-MS/MS	95	13	84	3	86	5	10 - 200	-25	0.9902
Pyrethrum (Jasmolin II)	30	10	LC-MS/MS	82	4	75	6	92	3	10 - 200	23	0.9866
Pyrethrum (Pyrethrin I)	3	1	LC-MS/MS	73	4	69	12	93	3	3 - 200	6	0.9866
Pyrethrum (Pyrethrin II)	5	2	LC-MS/MS	97	9	95	6	87	2	5 - 200	4	0.9811
Pyridaben	3	1	LC-MS/MS	80	19	107	8	69	13	3 - 200	-12	0.9915
Piridafention	10	3	LC-MS/MS	85	9	91	4	99	4	10 - 200	-15	0.9947
Pyrifenox	3	1	LC-MS/MS	107	13	58	10	105	5	3 - 200	17	0.9994
Pyrimethanil	3	1	LC-MS/MS	81	4	90	6	109	4	3 - 200	-4	0.9846
Pyriofenone	3	1	LC-MS/MS	100	10	90	5	83	16	3 - 200	1	0.9933
Pyriproxyfen	3	1	LC-MS/MS	79	10	84	16	97	2	3 - 200	-25	0.9865
Quinalphos	3	1	LC-MS/MS	82	6	97	3	101	2	3 - 200	-18	0.994
Quinoxyfen	3	1	LC-MS/MS	62	24	113	17	96	10	3 - 200	23	0.997

Quintozene	10	3	GC-MS/MS	87	10	78	5	89	17	10 - 200	5	0.994
Quizalofop-P-ethyl	3	1	LC-MS/MS	106	12	90	4	105	15	3 - 200	-4	0.9915
Sedaxane	30	10	LC-MS/MS	82	10	89	3	67	5	10 - 200	24	0.987
Sethoxydim	3	1	LC-MS/MS	77	9	80	3	96	4	3 - 200	22	0.9862
Simazine	30	10	LC-MS/MS	82	7	103	3	99	3	10 - 200	-32	0.9986
Spinetoram	3	1	LC-MS/MS	95	4	85	4	97	2	3 - 200	-21	0.988
Spinosad A	3	1	LC-MS/MS	113	15	101	8	108	19	3 - 200	18	0.9911
Spinosad D	3	1	LC-MS/MS	81	6	88	3	94	5	3 - 200	-20	0.9888
Spirodiclofen	3	1	LC-MS/MS	74	18	104	19	118	3	3 - 200	9	0.9905
Spirotetramat	3	1	LC-MS/MS	79	4	80	3	98	2	3 - 200	-5	0.9858
Spirotetramat-enol	5	2	LC-MS/MS	70	5	72	7	95	2	5 - 200	17	0.9944
Spirotetramat-enol-glucoside	5	2	LC-MS/MS	105	5	80	11	102	2	5 - 200	11	0.9821
Spirotetramat-ketohydroxy	10	3	LC-MS/MS	86	5	77	10	87	4	10 - 200	14	0.9951
Spiroxamine	3	1	LC-MS/MS	101	7	76	11	100	7	3 - 200	5	0.9903
Sulfotep	3	1	LC-MS/MS	77	4	94	5	104	2	3 - 200	-2	0.9998
Sulfoxaflor	10	3	LC-MS/MS	106	10	100	12	106	15	10 - 200	-24	0.9891
Tebuconazole	5	2	LC-MS/MS	76	3	66	4	87	2	5 - 200	13	0.997
Tebufenozide	5	2	LC-MS/MS	91	12	71	8	78	3	5 - 200	1	0.9849
Tebufenpyrad	3	1	LC-MS/MS	85	8	87	7	81	6	3 - 200	22	0.9918
Tebupirimifos	3	1	LC-MS/MS	79	7	78	9	74	6	3 - 200	4	0.9974
Tecnazene	10	3	GC-MS/MS	81	7	98	4	73	7	10 - 200	14	0.9891
Teflubenzuron	30	10	LC-MS/MS	83	7	97	9	100	3	10 - 200	6	0.9859
Tefluthrin	30	10	GC-MS/MS	105	13	102	6	98	12	30 - 250	22	0.9971
Tembotrione	3	1	LC-MS/MS	64	5	84	16	69	1	3 - 200	12	0.9851
Tepraloxydim	30	10	LC-MS/MS	70	4	109	3	75	4	10 - 200	-8	0.9879
Terbufos	30	10	LC-MS/MS	64	12	92	3	102	3	10 - 200	10	0.9977
Terbumeton	3	1	LC-MS/MS	75	6	102	8	82	7	3 - 200	18	0.9939
Terbuthylazine	3	1	LC-MS/MS	92	4	94	4	110	2	3 - 200	15	0.9923
Terbutryn	3	1	LC-MS/MS	101	13	96	7	96	4	3 - 200	-8	0.9896
Tetrachlorvinphos	3	1	LC-MS/MS	106	8	72	9	101	4	3 - 200	16	0.9978

Tetraconazole	3	1	LC-MS/MS	88	13	61	19	87	17	3 - 200	16	0.9911
Tetradifon	10	3	GC-MS/MS	116	4	76	5	100	3	10 - 200	-15	0.9928
Tetramethrin	3	1	LC-MS/MS	82	5	74	3	100	3	3 - 200	17	0.9854
Thiabendazole	3	1	LC-MS/MS	87	6	88	7	101	7	3 - 200	-16	0.9842
Thiacloprid	3	1	LC-MS/MS	70	16	103	4	76	4	3 - 200	-2	0.9856
Thiamethoxam	3	1	LC-MS/MS	80	10	110	6	105	13	3 - 200	-25	0.993
Thifensulfuron-methyl	10	3	LC-MS/MS	85	8	96	3	93	19	10 - 200	-23	0.9961
Thiobencarb	3	1	LC-MS/MS	65	9	75	9	75	2	3 - 200	9	0.9931
Thiodicarb	3	1	LC-MS/MS	84	19	81	15	103	2	3 - 200	-9	0.9827
Thiometon	30	10	GC-MS/MS	101	4	86	3	108	2	30 - 250	15	0.9805
Thiophanate-methyl	3	1	LC-MS/MS	86	11	89	3	89	7	3 - 200	-11	0.9813
Tolclofos-methyl	10	3	LC-MS/MS	118	8	117	3	86	2	10 - 200	16	0.9847
Tolylfluanid	30	10	GC-MS/MS	112	9	70	8	106	4	30 - 250	-25	0.9862
Triadimefon	10	3	LC-MS/MS	61	15	78	9	85	15	10 - 200	-20	0.9969
Triadimenol	30	10	GC-MS/MS	85	25	114	16	105	11	30 - 250	-8	0.9848
Tri-allate	3	1	LC-MS/MS	79	4	81	5	94	2	3 - 200	-6	0.9974
Triasulfuron	10	3	LC-MS/MS	89	5	93	12	88	2	10 - 200	11	0.9805
Triazamate	3	1	LC-MS/MS	119	5	65	18	112	2	3 - 200	-23	0.9887
Triazophos	5	2	LC-MS/MS	65	14	71	10	72	12	5 - 200	-19	0.9859
Trichlorfon	3	1	LC-MS/MS	62	15	87	10	91	4	3 - 200	-22	0.9951
Triclopyr	30	10	LC-MS/MS	65	18	72	13	89	2	10 - 200	-22	0.988
Tricyclazole	3	1	LC-MS/MS	105	7	96	6	120	5	3 - 200	8	0.9958
Trifloxystrobin	3	1	LC-MS/MS	79	7	72	6	100	3	3 - 200	-18	0.9892
Triflumizole	3	1	LC-MS/MS	95	18	93	8	103	8	3 - 200	21	0.9821
Triflumuron	3	1	LC-MS/MS	86	11	87	10	85	14	3 - 200	-7	0.9906
Trifluralin	30	10	GC-MS/MS	85	3	87	3	82	3	30 - 250	22	0.9966
Triticonazole	3	1	LC-MS/MS	84	15	86	14	86	11	3 - 200	23	0.9902
Tritosulfuron	3	1	LC-MS/MS	90	15	94	2	95	6	3 - 200	22	0.9866
Valifenalate	5	2	LC-MS/MS	88	10	85	4	103	5	5 - 200	-2	0.9866
Vamidothion	3	1	LC-MS/MS	69	9	74	7	81	4	3 - 200	-18	0.9811

Vinclozolin	10	3	GC-MS/MS	89	7	86	5	99	6	10 - 200	23	0.9915
Zoxamide	3	1	LC-MS/MS	82	14	107	9	92	9	3 - 200	14	0.9947

Table S3.7: Validation data of the QuPPe-PO-Method applied to the pollen matrix. Matrix-matched calibration was used to quantify spiked samples (5 concentration levels included in the range shown in the table). Six replicates were prepared for each level added, and recovery data shown represents the average of six replicates. The table reports the limit of quantification (LOQ, expressed as µg/kg, estimated according to the SANTE guidelines), limit of detection (LOD, expressed as µg/kg, estimated as one third of the LOQ), the three concentration levels of added compound (100, 500, and 2000 ug/kg), recovery (Rec, expressed as %) and repeatability (RSD, expressed as %) of the method, matrix effect (expressed as %), and linearity (R^2).

Compound	**LOQ**	**LOD**	**Instrumental technique**	**100 ug/kg**		**500 ug/kg**		**2000 ug/kg**		**Calib range**	**Matrix effect**	**R^2**
				Rec	**RSD**	**Rec**	**RSD**	**Rec**	**RSD**			
Glyphosate	0.1	0.030	LC-MS/MS	90%	10%	91%	6%	97%	5%	100 - 2000	3	0.991

Table S3.8: Results of the linear mixed-effects model testing the effect of the interaction between the sampling month and pesticide category, the interaction between the sampling month and landscape PC1, the interaction between the sampling month and landscape PC2, the interaction between the sampling month and landscape PC3, and the sampling year on the PHQ of pollen samples (ln-transformed). Landscape PC1, PC2 and PC3 were calculated using the regional land-use map categories in 5 km radius buffers around the sampling locations. Values in bold indicate significant effects (p-value < 0.05). Only significant results after a backward stepwise model selection procedure are reported.

	χ2	**df**	***p*-value**
Intercept	0.009	1	0.925
Month	17.571	5	**0.004**
Pesticide category	32.377	2	**<0.001**
Landscape PC1 (5 km)	14.922	1	**<0.001**
Landscape PC2 (5 km)	0.370	1	0.543
Landscape PC3 (5 km)	0.002	1	0.970
Year	1.396	1	0.237
Month x Pesticide category	38.397	10	**<0.001**
Month x Landscape PC1 (5 km)	19.717	5	**0.001**

Table S3.9: Results of the linear mixed-effects model testing the effect of the percentage of annual and perennial crops in 5 km radius buffers around the sampling locations on the PHQ of pollen samples (ln-transformed). Values in bold indicate significant effects (p-value < 0.05).

	value	**SE**	**df**	***t*-value**	***p*-value**
Intercept	0.779	1.061	64	0.734	0.466
Annual crop % (5 km)	6.227	3.825	10	1.628	0.135
Perennial crop % (5 km)	12.690	4.620	10	2.747	**0.021**

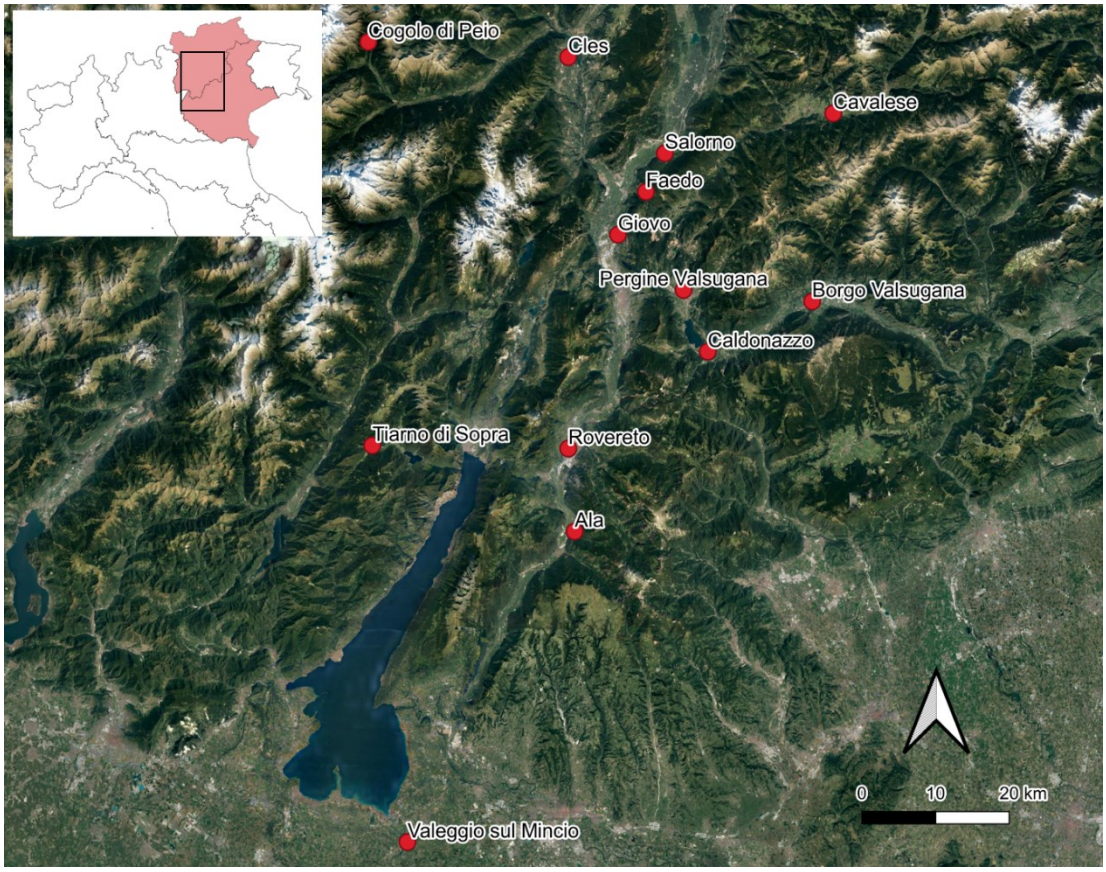

Figure S3.1: Map of the 13 sampling locations. Imagery © 2023 TerraMetrics, Map data © 2023 Google.

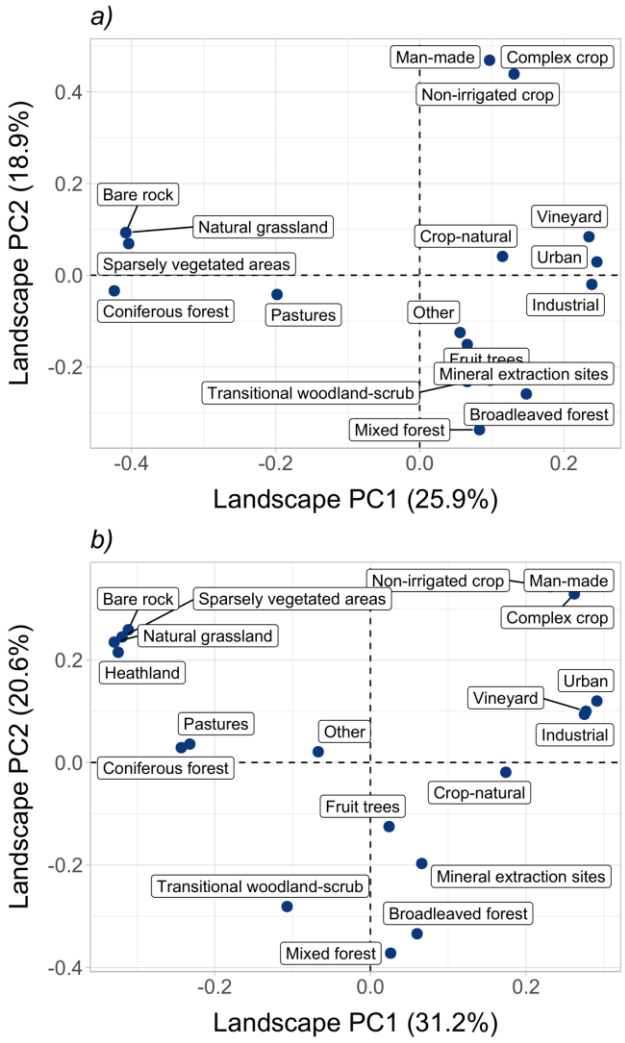

Figure S3.2: Principal Component Analysis loading plots showing landscape PC1, landscape PC2, and the regional land-use map categories at *a)* 3 km radius buffers around the sampling locations and *b)* 5 km radius buffers around the sampling locations.

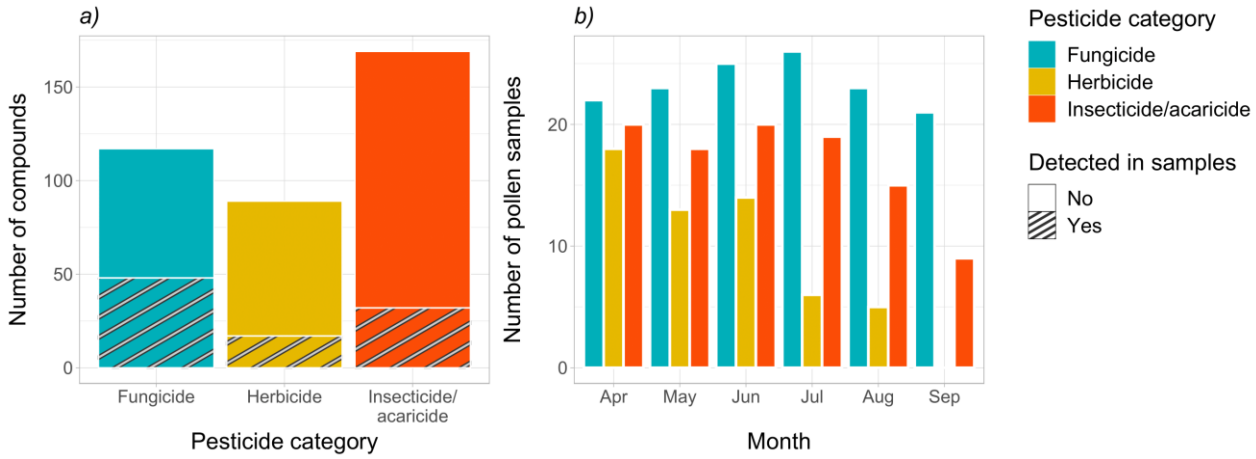

Figure S3.3: Plots showing *a)* the number of searched and detected compounds for each pesticide category and *b)* the number of pollen samples containing each pesticide category found for each sampling month.

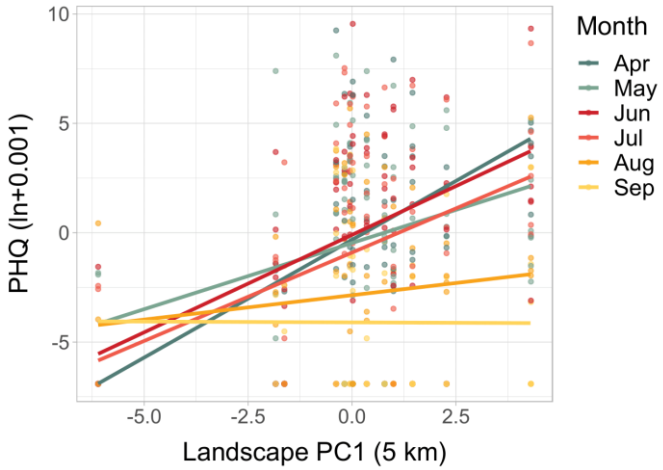

Figure S3.4: Plot showing the effect of the interaction between the sampling period and landscape PC1 on PHQ of pollen samples (ln-transformed). Landscape PC1 was calculated using the regional land-use map categories in 5 km radius buffers around the sampling locations. Points represent raw data points and lines represent model estimates.

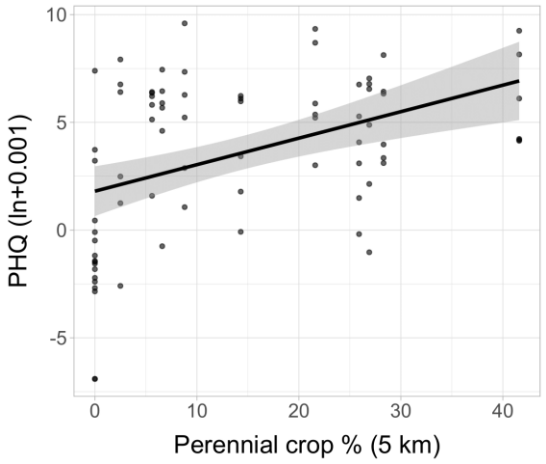

Figure S3.5: Plot showing the effect of the cover of perennial crops (fruit trees and vineyards) in 5 km radius buffers around the sampling locations on PHQ of pollen samples (ln-transformed). Points represent raw data points, the line represents model estimate, and the shaded area represents the 95% confidence interval.

CHAPTER 4

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Table S4.1: List of the 36 sampling sites with information on coordinates (WGS84, decimal degrees), distance from the city centre (km), area of open habitat in a 500 m buffer (ha), and surface temperature (°C).

Site ID	Lat (N)	Lon (E)	Distance from city centre	Open habitat	Temp
A	41.91781	12.40884	7.6	24.9	42.2511
B	41.84717	12.47192	5.1	33.8	36.9242
C	41.81964	12.44892	8.6	27.3	39.5111
D	41.87496	12.50697	2.1	34.4	40.7396
E	41.91042	12.48148	2.4	23.4	36.6633
F	41.83737	12.43618	7.5	34.8	42.0457
G	41.89109	12.42198	5.8	29	38.5319
H	41.82944	12.48466	6.8	18.8	38.299
I	41.89424	12.56649	6.2	9	40.7699
J	41.8567	12.49564	3.7	18.8	41.5258
K	41.92947	12.52897	5.3	20.5	39.4605
L	41.90908	12.42125	6.3	8.1	39.7145
M	41.88127	12.38622	8.8	34.8	40.4957
N	41.93898	12.55273	7.4	19.2	39.377
O	41.8919	12.4581	2.8	41.3	34.7341
P	41.93897	12.49161	5.3	31.5	40.3586
Q	41.93688	12.45631	6	3.7	39.2041
R	41.91835	12.43294	5.8	28	35.378
S	41.95461	12.44354	8.2	18.6	34.102
T	41.92894	12.50487	4.4	7.9	36.9358
U	41.96494	12.50347	8.4	41.4	40.7492
V	41.94426	12.38364	10.8	24.6	37.5948
W	41.9382	12.51657	5.7	34.8	37.4956
X	41.93949	12.57683	8.9	30.8	41.8364
Y	41.931	12.60127	10.1	19.8	41.307
Z	41.92021	12.51727	3.9	10.9	37.7365
AA	41.91173	12.54171	4.8	26.3	41.944
AB	41.90247	12.53001	3.4	26.1	40.7863
AC	41.88369	12.49458	0.7	34.9	36.194
AD	41.87573	12.54309	4.5	17.2	42.967
AE	41.86517	12.47121	3.3	13.8	41.5534
AF	41.8585	12.57989	8.1	39.2	40.6268
AG	41.83896	12.50837	5.8	26.7	40.6211
AH	41.83125	12.56888	9.1	43	40.3188
AI	41.81884	12.41284	10.3	53.8	38.3601
AJ	41.81038	12.43725	10	32.7	38.4801

Table S4.2: List of collected wild bee species with information on their abundance (Ab) and functional group assignments. Body size (Body) is the mean value in mm of the measured specimens. For social behaviour (Social), social species comprise semi-social, social, and eusocial bees. For nesting strategy (Nest), categories were collapsed to below-ground and above-ground nesting (which include those species which build their nests in stems or pre-existing cavities). For diet breadth (Diet), oligolectic bees (oligo) are specialized to forage on one specific plant taxon.

Species	Ab	Body	Social	Nest	Diet
Amegilla quadrifasciata	1	12.5	solitary	below-ground	poly
Andrena aeneiventris	2	6.5	solitary	below-ground	poly
Andrena decipiens	1	12.5	solitary	below-ground	poly
Andrena flavipes	11	11.5	solitary	below-ground	poly
Andrena pellucens	48	8.5	solitary	below-ground	poly
Andrena sp. 1	1	9	solitary	below-ground	NA
Anthidiellum strigatum	2	6.5	solitary	above-ground	poly
Anthidium florentinum	4	15.5	solitary	above-ground	poly
Anthidium manicatum	4	14	solitary	above-ground	poly
Bombus humilis	1	14.5	social	below-ground	poly
Bombus pascuorum	13	14.5	social	below-ground	poly
Bombus sylvarum	3	14.5	social	below-ground	poly
Bombus terrestris	2	16.6	social	below-ground	poly
Ceratina cucurbitina	9	8	solitary	above-ground	poly
Ceratina cyanea	14	7	solitary	above-ground	poly
Ceratina dallatorreana	10	5.5	solitary	above-ground	poly
Ceratina dentiventris	10	6.5	solitary	above-ground	poly
Ceratina parvula	4	3.5	solitary	above-ground	poly
Chelostoma campanularum	4	6	solitary	above-ground	oligo
Chelostoma rapunculi	1	9	solitary	above-ground	oligo
Dasypoda hirtipes	5	14	solitary	below-ground	oligo
Epeolus sp. 1	1	8	kleptoparasitic	NA	NA
Epeolus sp. 2	7	8.5	kleptoparasitic	NA	NA
Eucera nigrifacies	1	10	solitary	below-ground	oligo
Halictus asperulus	18	8.2	social	below-ground	poly
Halictus brunnescens	1	17.5	solitary	below-ground	poly
Halictus fulvipes	53	11.5	social	below-ground	poly
Halictus gruenwaldti	1	17.5	solitary	below-ground	poly
Halictus langobardicus	2	8.5	solitary	below-ground	poly
Halictus maculatus	8	8.2	social	below-ground	poly
Halictus quadricinctus	3	18.2	solitary	below-ground	poly
Halictus rubicundus	1	11.5	social	below-ground	poly
Halictus scabiosae	33	13.9	social	below-ground	poly
Heriades rubicola	6	7	solitary	above-ground	oligo
Heriades truncorum	1	7	solitary	above-ground	oligo
Hoplitis leucomelana	3	7.5	solitary	above-ground	poly

Hylaeus angustatus	5	5.7	solitary	above-ground	poly
Hylaeus brevicornis	3	4.6	solitary	above-ground	poly
Hylaeus cf. *confusus*	3	7.2	solitary	above-ground	poly
Hylaeus cf. *imparilis*	2	5.2	solitary	above-ground	poly
Hylaeus gibbus	2	6.4	solitary	above-ground	poly
Hylaeus punctatus	2	5.2	solitary	above-ground	oligo
Hylaeus taeniolatus	6	4.7	solitary	above-ground	poly
Lasioglossum albocinctum	10	11.25	solitary	below-ground	poly
Lasioglossum brevicorne	17	6.5	solitary	below-ground	poly
Lasioglossum discus	13	8.5	solitary	below-ground	poly
Lasioglossum glabriusculum	456	4.5	social	below-ground	poly
Lasioglossum griseolum	197	4.5	solitary	below-ground	poly
Lasioglossum interruptum	4	7.2	social	below-ground	poly
Lasioglossum laticeps	4	7.2	social	below-ground	poly
Lasioglossum leucozonium	52	7.9	solitary	below-ground	poly
Lasioglossum limbellus	1	6.5	solitary	below-ground	poly
Lasioglossum malachurum	897	8.2	social	below-ground	poly
Lasioglossum minutissimum	13	4.25	solitary	below-ground	poly
Lasioglossum morio	220	6.2	social	below-ground	poly
Lasioglossum nigripes	1	10	social	below-ground	poly
Lasioglossum nitidulum	201	6.5	solitary	below-ground	poly
Lasioglossum pauperatum	6	5	social	below-ground	poly
Lasioglossum pauxillum	19	6.25	social	below-ground	poly
Lasioglossum politum	118	5.1	social	below-ground	poly
Lasioglossum punctatissimum	1	5.4	solitary	below-ground	poly
Lasioglossum pygmaeum	3	5.5	solitary	below-ground	poly
Lasioglossum sp. 1	1	5.2	NA	below-ground	poly
Lasioglossum sp. 2	1	4.4	NA	below-ground	poly
Lasioglossum transitorium	5	6.2	solitary	below-ground	poly
Lasioglossum truncaticolle	4	7	solitary	below-ground	poly
Lasioglossum villosulum	19	6.25	solitary	below-ground	poly
Lithurgus chrysurus	3	11.8	solitary	above-ground	oligo
Lithurgus cornutus	1	15.2	solitary	above-ground	oligo
Megachile apicalis	9	8.5	solitary	above-ground	poly
Megachile centuncularis	1	11	solitary	above-ground	poly
Megachile cf. *rotundata*	2	7.5	solitary	above-ground	poly
Megachile ericetorum	1	13.5	solitary	above-ground	oligo
Megachile pilidens	17	8.1	solitary	above-ground	poly
Megachile pusilla	7	8	solitary	above-ground	poly
Nomada sp. 1	1	7.5	kleptoparasitic	NA	NA
Nomada sp. 2	1	6	kleptoparasitic	NA	NA
Nomada sp. 3	1	5.8	kleptoparasitic	NA	NA
Nomada sp. 4	1	5	kleptoparasitic	NA	NA
Nomiapis diversipes	2	8	solitary	below-ground	poly
Nomioides facilis	9	3.7	solitary	below-ground	poly
Osmia andrenoides	2	7.2	solitary	above-ground	poly
Osmia caerulescens	12	8.75	solitary	above-ground	poly

Osmia cf. *notata*	1	10	solitary	above-ground	poly
Osmia latreillei	1	11.2	solitary	above-ground	oligo
Osmia ligurica	2	8.5	solitary	above-ground	oligo
Osmia niveata	1	10.7	solitary	above-ground	oligo
Osmia scutellaris	1	7	solitary	above-ground	oligo
Osmia spinulosa	7	7.5	solitary	above-ground	oligo
Panurgus calcaratus	7	7.8	solitary	below-ground	oligo
Rhodanthidium septemdentatum	5	14.2	solitary	above-ground	poly
Seladonia gemmea	275	6.7	social	below-ground	poly
Seladonia smaragdula	191	5.75	social	below-ground	poly
Seladonia subaurata	94	6.9	social	below-ground	poly
Seladonia vestita	49	6.75	solitary	below-ground	poly
Sphecodes gibbus	1	11.3	kleptoparasitic	NA	NA

Table S4.3: Results of the linear models testing the effect of woody cover (km^2) on abundance (ln-transformed), species richness (ln-transformed), and community evenness of *a)* all wild bee species and *b)* wood nesting wild bee species.

	Response variable	**Estimate**	**SE**	***t* value**	***p* value**
a) All wild bee species	Wild bee abundance (ln)	-0.748	0.835	-0.895	0.377
	Wild bee richness (ln)	-0.203	0.375	-0.541	0.592
	Community evenness	0.1978	0.161	1.227	0.228
b) Wood nesting wild bee species	Wild bee abundance (ln)	-0.694	0.431	-1.611	0.125
	Wild bee richness (ln)	-0.526	0.360	-1.464	0.160
	Community evenness	0.219	0.746	0.294	0.778

Table S4.4: Results of the multi-model inference analysis testing the effects of distance from the city centre (Dist), open habitat cover (Open), temperature (Temp) and their interactions on *a)* wild bee abundance (ln-transformed), *b)* wild bee richness (ln-transformed), and *c)* community evenness. The table reports the estimate for each variable, the ΔAICc, and the Akaike weight (w) for each model of the set with ΔAICc < 4.

Response variable	Ranking	Intercept	Dist	Open	Temp	Dist × Open	Dist × Temp	Open × Temp	Dist × Open × Temp	ΔAICc	w
a) Wild bee abundance (ln)	1	-0.131	-	-	0.114	-	-	-	-	0	0.344
	2	0.237	-	-0.01	0.111	-	-	-	-	1.195	0.189
	3	-0.129	0.003	-	0.113	-	-	-	-	2.535	0.097
	4	3.296	-	-0.125	0.033	-	-	0.003	-	3.456	0.061
	5	0.278	0.015	-0.011	0.108	-	-	-	-	3.761	0.052
b) Wild bee richness (ln)	1	1.155	-	-0.007	0.049	-	-	-	-	0	0.310
	2	0.888	-	-	0.051	-	-	-	-	1.158	0.174
	3	2.326	-	-0.051	0.019	-	-	0.001	-	2.355	0.095
	4	1.154	0	-0.007	0.049	-	-	-	-	2.709	0.080
	5	1.788	-0.069	-0.022	0.044	0.002	-	-	-	3.439	0.056
	6	0.882	-0.008	-	0.053	-	-	-	-	3.476	0.055
c) Community evenness	1	1.123	-	-	-0.016	-	-	-	-	0	0.260
	2	0.509	-	-		-	-	-	-	0.709	0.182
	3	1.081	-	0.001	-0.015	-	-	-	-	2.129	0.090
	4	1.121	-0.003	-	-0.015	-	-	-	-	2.407	0.078
	5	0.476	-	0.001	-	-	-	-	-	2.584	0.071
	6	0.539	-0.005	-	-	-	-	-	-	2.717	0.067
	7	0.168	0.167	-	0.009	-	-0.004	-	-	3.516	0.045

Table S4.5: Results of the multi-model inference analysis testing the effects of distance from the city centre (Dist), open habitat cover (Open), temperature (Temp) and their interaction on CWMs for *a)* body size, *b)* nesting strategy, *c)* diet breadth and *d)* social behaviour. The table reports the estimate for each variable, the ΔAICc, and the Akaike weight (w) for each model of the set with ΔAICc < 4.

Response variable	**Intercept**	**Dist**	**Open**	**Temp**	**Dist × Open**	**Dist × Temp**	**Open × Temp**	**Dist × Open × Temp**	**ΔAICc**	**w**
a) CWM body size	10.919	0.095	-	-0.115	-	-	-	-	0	0.351
	6.915	0.810	-	-0.011	-	-0.018	-	-	1.561	0.161
	10.892	0.094	0.001	-0.114	-	-	-	-	2.704	0.091
	10.848	-	-	-0.098	-	-	-	-	3.176	0.072
	6.512	0.079	-	-	-	-	-	-	3.833	0.052
b) CWM nesting strategy (above ground)	0.044	-	-	-	-	-	-	-	0	0.282
	0.061	-	-0.001	-	-	-	-	-	0.183	0.258
	0.051	-0.001	-	-	-	-	-	-	2.090	0.099
	0.029	-	-	0.000	-	-	-	-	2.376	0.086
	0.063	0.000	-0.001	-	-	-	-	-	2.704	0.073
	0.063	-	-0.001	-	-	-	-	-	2.741	0.071
c) CWM diet breadth (polylecty)	0.524	-	0.002	0.023	-	-	-	-	0	0.270
	0.616	-	-	0.022	-	-	-	-	0.708	0.189
	-0.244	-	0.031	0.042	-	-	-0.001	-	1.569	0.123
	0.620	0.006	-	0.021	-	-	-	-	2.332	0.084
	0.534	0.003	0.002	0.022	-	-	-	-	2.399	0.081
d) CWM social behaviour (sociality)	0.601	-	0.004	-	-	-	-	-	0	0.176
	-0.061	-	0.004	0.017	-	-	-	-	0.016	0.175
	0.713	-	-	-	-	-	-	-	1.461	0.085
	0.556	0.009	0.004	-	-	-	-	-	1.602	0.079
	-1.169	-	0.046	0.045	-	-	-0.001	-	1.753	0.073
	0.108	-	-	0.015	-	-	-	-	1.931	0.067
	0.631	0.013	-	-	-	-	-	-	2.020	0.064
	-0.042	0.007	0.004	0.015	-	-	-	-	2.196	0.059

1.767	-0.283	-	-0.029	-	0.008	-	-	2.609	0.048
0.117	0.011	-	0.013	-	-	-	-	3.083	0.038
1.254	-0.218	0.003	-0.018	-	0.006	-	-	3.218	0.035

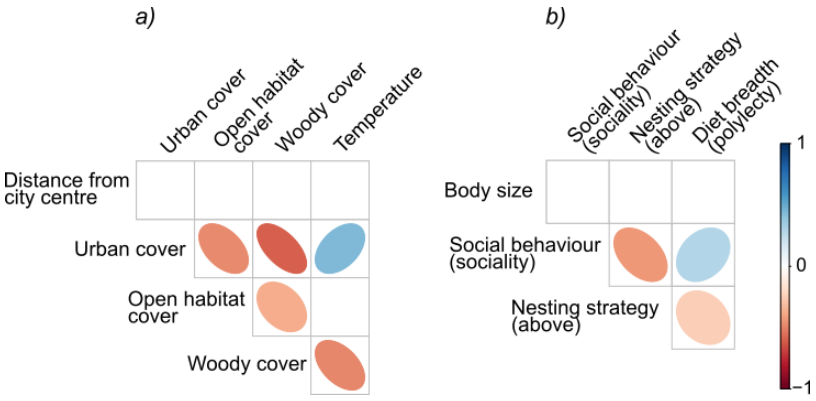

Figure S4.1: Correlation matrices for *a)* predictors and *b)* wild bee functional traits based on Pearson's correlation. Right-oriented blue ellipses indicate positive correlations, while left-oriented red ellipses indicate negative correlations. Narrower ellipses indicate stronger correlations. Only significant correlations (p value < 0.05) are displayed.

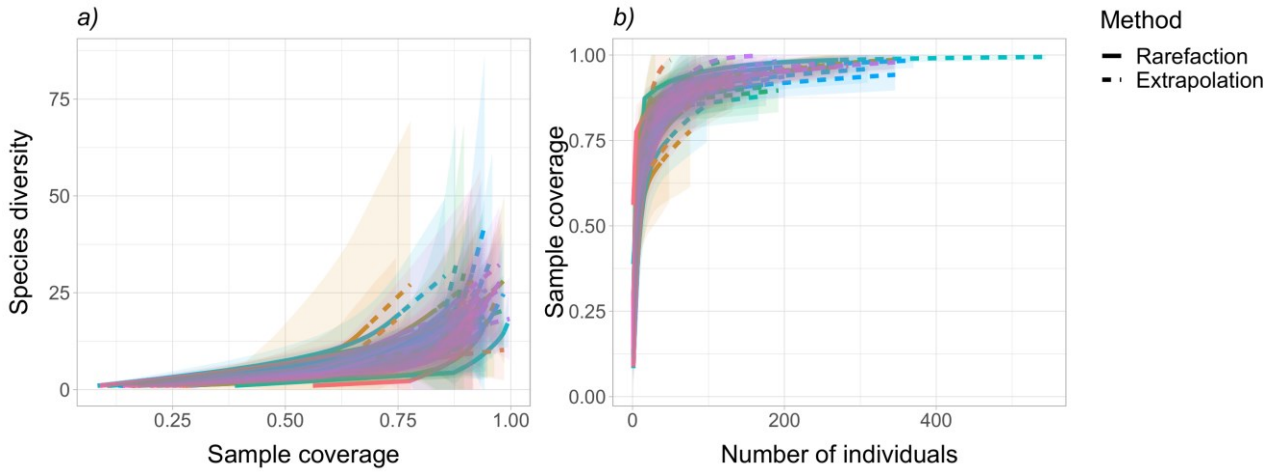

Figure S4.2: Species rarefaction and extrapolation curves: *a)* sample completeness curve per site and *b)* coverage-based sampling curve per site. Each site is shown in a different colour.

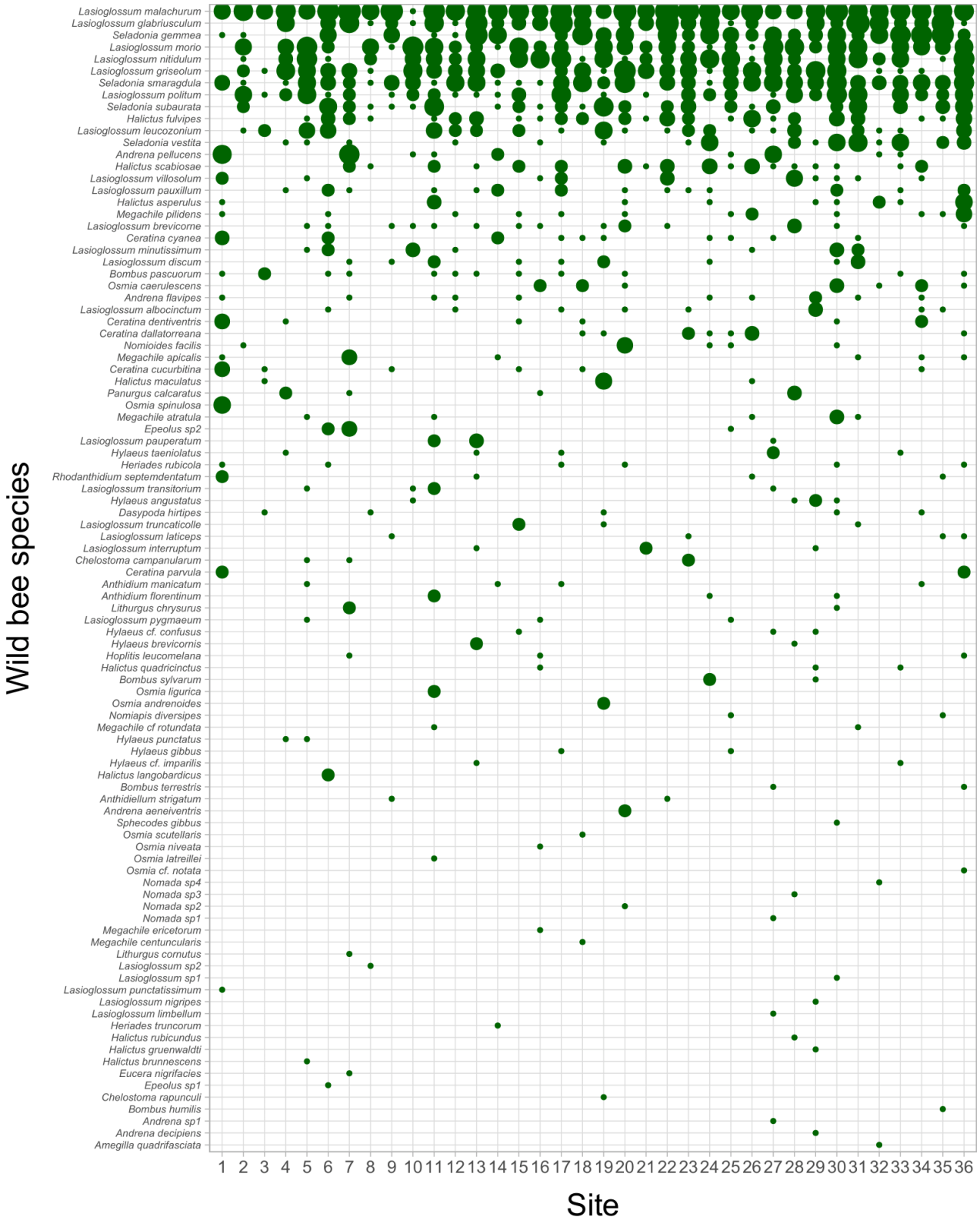

Figure S4.3: Wild bee species abundance per site. The size of each point is proportional to logarithmic transformed abundance. Sites are ordered according to increasing surface temperature, while species are ordered according to their overall abundance.

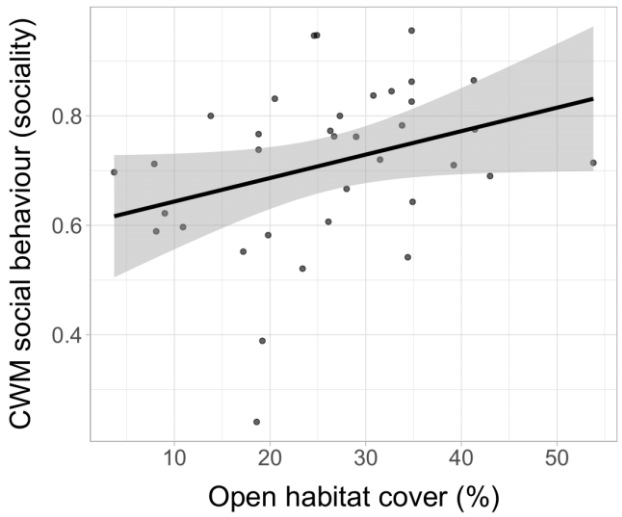

Figure S4.4: Plot showing the effect of open habitat cover on CWM social behaviour (sociality). Points represent raw data points, the line represents model estimate, and the shaded area represents the 95% confidence interval.

CHAPTER 5

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Table S5.1: List of the 51 sampling sites with information on coordinates (WGS84, decimal degrees), elevation (m a.s.l.), recorded air temperature (°C), number of flowering plant species (Plant rich), number of pollinator species (Poll rich), abundance of honey bees (Honey bee ab), and abundance of wild pollinators (Wild poll ab).

Site ID	Lat (N)	Lon (E)	Elev	Temp	Plant rich	Poll rich	Honey bee ab	Wild poll ab
A	45.587007	11.468313	60	27.67	20	31	5	111
AA	46.487789	12.035109	2005	25.29	26	22	768	73
AB	46.490416	12.066126	1982	23.24	28	12	109	16
AC	46.518712	12.007109	2090	22.36	34	19	145	39
AD	46.533594	11.986739	2111	27.05	25	13	282	43
AE	46.565153	12.243253	1658	22.37	21	30	34	65
AF	46.548764	12.261756	1500	26.72	19	16	22	29
AG	46.396688	12.104436	1450	25.43	27	49	274	119
AH	46.420795	12.105355	1780	20.95	28	30	31	150
AI	46.484398	11.831885	2008	19.63	22	14	2	93
AJ	45.82542	12.180076	175	27.99	13	27	44	63
AK	45.808239	12.111145	320	30.23	11	14	80	25
AL	46.485461	11.788397	1915	19.52	34	34	101	110
AM	46.549767	11.813354	2055	19.02	35	30	116	78
AN	46.339973	11.804519	2032	20.95	13	19	9	130
AO	46.195026	11.42058	1430	22.72	18	26	34	51
AP	46.348829	11.845274	1600	22.33	18	16	25	61
AQ	46.139193	11.472058	1250	24.49	16	24	149	332
AR	46.147208	11.771577	910	30.98	15	17	4	31
AS	46.172563	11.441852	2040	19.65	16	22	227	64
AT	45.880301	11.794101	1665	21.37	18	24	2	75
AU	45.755492	10.874539	1500	18.05	11	10	3	25
AV	45.756764	10.920759	640	24.63	10	9	108	29
AW	45.803576	12.049459	200	35.54	8	28	201	67
AX	45.868023	12.009987	170	30.91	10	13	24	45
AZ	45.694425	10.926014	130	32.84	10	12	55	16
B	45.583447	11.463828	150	27.37	22	29	25	119
C	45.747647	11.329589	670	24.04	19	35	113	109
D	45.755546	11.363854	630	24.65	17	29	64	69
E	45.711296	11.68899	76	30.6	15	32	302	94
F	45.760339	11.699603	106	36.89	12	25	508	73
G	45.595876	11.467499	150	37.89	22	22	19	121
H	45.622053	11.415189	165	33.93	16	19	19	103
I	45.862562	11.762562	1251	21.02	19	27	353	57
J	45.649502	11.7196	50	28.19	11	9	31	25
K	45.979623	12.730447	35	27.88	20	25	179	72
L	45.839134	11.736687	1000	25.27	15	17	550	51
M	45.848196	11.452165	943	26.37	29	29	119	93
N	45.824215	11.455581	1170	23.79	19	18	31	71
O	45.760665	11.39284	1215	19.51	22	34	21	119

P	46.137372	11.502687	1170	23.54	20	20	44	114
Q	46.121675	11.49488	920	27.36	19	22	66	61
R	46.154322	11.424568	1530	25.61	17	22	19	36
S	46.068526	11.431498	870	26.67	24	29	306	77
T	46.006465	11.404734	890	28.29	22	16	266	60
U	46.012846	11.432452	825	29.46	31	21	215	41
V	45.670237	11.056824	1430	24.46	32	35	5	191
W	46.196241	12.658885	870	21.16	25	29	150	112
X	45.758992	11.192283	1000	23.27	30	38	309	143
Y	45.769546	11.136029	860	24.26	20	34	115	109
Z	45.637685	11.720541	40	30.6	16	22	35	63

Table S5.2: Functional traits of flowering plant species: flower colour (six classes: blue, brown, pink/purple, red, white, yellow/orange), flower type (nine classes: bell/funnel/lip, brush, hidden disc, open disc, flag, flower head, NA, pollen, and stalk disc flower), and flower corolla length (mm).

Species	Flower color	Flower type	Corolla length
Achillea clavennae	white	flower head	6
Achillea millefolium	white	flower head	4.7
Aconitum degenii	blue	bell/funnel/lip	26
Aconitum lycoctonum	yellow/orange	bell/funnel/lip	25.5
Aconitum napellus	blue	bell/funnel/lip	11.5
Adenostyles alpina	pink/purple	stalk disc	13
Aegopodium podagraria	white	open disc	1
Agrimonia eupatoria	yellow/orange	pollen	0.05
Allium carinatum	pink/purple	hidden disc	5.5
Allium schoenoprasum	pink/purple	hidden disc	12
Angelica sylvestris	white	open disc	1
Anthriscus sylvestris	white	open disc	0.4
Anthyllis vulneraria	yellow/orange	flag	12
Aristolochia clematitis	yellow/orange	bell/funnel/lip	30
Arnica montana	yellow/orange	flower head	17.5
Asperula cristata	pink/purple	bell/funnel/lip	3
Aster alpinus	pink/purple	flower head	8
Astragalus glycyphyllos	yellow/orange	flag	7
Astrantia major	white	flower head	6
Bellis perennis	white	flower head	4
Betonica alopecurus	yellow/orange	bell/funnel/lip	10
Buddleja davidii	pink/purple	stalk disc	10
Buphthalmum salicifolium	yellow/orange	flower head	8
Calluna vulgaris	pink/purple	bell/funnel/lip	5
Calystegia sepium	white	bell/funnel/lip	33.3
Campanula barbata	pink/purple	bell/funnel/lip	16
Campanula carnica	pink/purple	bell/funnel/lip	11
Campanula glomerata	pink/purple	bell/funnel/lip	13
Campanula patula	pink/purple	bell/funnel/lip	11
Campanula persicifolia	pink/purple	bell/funnel/lip	18
Campanula rapunculoides	pink/purple	bell/funnel/lip	17
Campanula rapunculus	pink/purple	bell/funnel/lip	15
Campanula rotundifolia	pink/purple	bell/funnel/lip	15
Campanula scheuchzeri	pink/purple	bell/funnel/lip	15
Campanula spicata	pink/purple	bell/funnel/lip	11
Campanula trachelium	pink/purple	bell/funnel/lip	26.5

Carduus defloratus	pink/purple	flower head	17
Carduus nutans	pink/purple	flower head	12
Carduus personata	pink/purple	flower head	22
Carum carvi	white	open disc	0.3
Centaurea jacea	pink/purple	flower head	20
Centaurea nervosa	pink/purple	flower head	23.5
Centaurea nigrescens	pink/purple	flower head	12.2
Centaurea scabiosa	pink/purple	flower head	13.5
Centaurea stoebe	pink/purple	flower head	10
Centaurea triumfetti	blue	flower head	12
Cerastium arvense	white	hidden disc	4
Cerastium holosteoides	white	hidden disc	2
Chaerophyllum hirsutum	white	open disc	1
Cichorium intybus	blue	flower head	0.05
Cirsium arvense	pink/purple	flower head	16
Cirsium erisithales	yellow/orange	flower head	23.3
Cirsium heterophyllum	pink/purple	flower head	33
Cirsium montanum	pink/purple	flower head	7
Cirsium oleraceum	yellow/orange	flower head	23
Clematis vitalba	white	pollen	0.05
Clinopodium acinos	pink/purple	bell/funnel/lip	9
Clinopodium alpinum	pink/purple	bell/funnel/lip	9
Clinopodium nepeta	pink/purple	bell/funnel/lip	9.5
Clinopodium vulgare	pink/purple	bell/funnel/lip	11
Convolvulus arvensis	white	bell/funnel/lip	16.3
Conyza canadensis	white	flower head	5
Crepis aurea	yellow/orange	flower head	13.5
Crepis biennis	yellow/orange	flower head	8.5
Crepis foetida	yellow/orange	flower head	6
Crepis paludosa	yellow/orange	flower head	11
Crepis vesicaria	yellow/orange	flower head	8
Cruciata laevipes	yellow/orange	open disc	0.05
Daucus carota	white	open disc	0.05
Delosperma sp.	pink/purple	flower head	5
Dianthus superbus	pink/purple	stalk disc	30
Diplotaxis tenuifolia	yellow/orange	hidden disc	4
Doronicum austriacum	yellow/orange	flower head	4.5
Dorycnium pentaphyllum	white	flag	4
Dryas octopetala	white	hidden disc	0.05
Echium vulgare	pink/purple	bell/funnel/lip	9.3
Epilobium angustifolium	pink/purple	hidden disc	0.05

Epilobium dodonaei	pink/purple	hidden disc	0.05
Epilobium hirsutum	pink/purple	hidden disc	0.05
Epilobium montanum	pink/purple	hidden disc	7
Erigeron annuus	white	flower head	1
Eupatorium cannabinum	pink/purple	flower head	5
Euphrasia rostkoviana	white	bell/funnel/lip	10
Euphrasia salisburgensis	white	bell/funnel/lip	6
Euphrasia sp.	white	bell/funnel/lip	6.5
Filipendula vulgaris	white	pollen	0.05
Fragaria vesca	white	hidden disc	0.05
Galeopsis pubescens	pink/purple	bell/funnel/lip	17
Galeopsis speciosa	yellow/orange	bell/funnel/lip	22
Galeopsis tetrahit	pink/purple	bell/funnel/lip	13
Galium lucidum	white	open disc	0.5
Galium mollugo	white	open disc	1
Galium saxatile	white	open disc	1
Galium verum	yellow/orange	open disc	0.3
Genista tinctoria	yellow/orange	flag	7.7
Gentiana cruciata	blue	bell/funnel/lip	14.5
Gentianella rhaetica	pink/purple	bell/funnel/lip	17
Geranium columbinum	pink/purple	hidden disc	0.05
Geranium molle	pink/purple	hidden disc	0.05
Geranium phaeum	pink/purple	hidden disc	0.05
Geranium pyrenaicum	pink/purple	hidden disc	0.05
Geranium robertianum	pink/purple	hidden disc	6.5
Geranium sylvaticum	pink/purple	hidden disc	0.05
Geum rivale	red	bell/funnel/lip	7
Gymandenia conopsea	pink/purple	bell/funnel/lip	6
Gypsophila repens	white	bell/funnel/lip	7
Hedysarum hedysaroides	pink/purple	flag	8
Helianthemum nummularium	yellow/orange	pollen	0.05
Heracleum sphondylium	white	open disc	0.1
Hieracium bifidum	yellow/orange	flower head	13.3
Hieracium glaucum	yellow/orange	flower head	12
Hieracium picroides	yellow/orange	flower head	11
Hieracium pilosella	yellow/orange	flower head	11
Hieracium sp.	yellow/orange	flower head	8.5
Hieracium valdepilosum	yellow/orange	flower head	20
Horminum pyrenaicum	pink/purple	bell/funnel/lip	14
Hypericum maculatum	yellow/orange	pollen	0.05
Hypericum perforatum	yellow/orange	pollen	0.05

Hypochaeris uniflora	yellow/orange	flower head	22
Impatiens glandulifera	pink/purple	bell/funnel/lip	23
Inula salicina	yellow/orange	flower head	7.5
Inula sp.	yellow/orange	flower head	9
Jacobaea alpina	yellow/orange	flower head	7.5
Knautia arvensis	blue	flower head	7.3
Knautia drymeia	pink/purple	flower head	7
Knautia longifolia	pink/purple	flower head	9
Lamium album	white	bell/funnel/lip	9.5
Lamium galeobdolon	yellow/orange	bell/funnel/lip	9
Lamium orvala	pink/purple	bell/funnel/lip	14
Lathyrus pratensis	yellow/orange	flag	13.7
Lathyrus sylvestris	pink/purple	flag	12.5
Leontodon hispidus	yellow/orange	flower head	11.7
Leucanthemum vulgare	white	flower head	4.8
Ligustrum lucidum	white	bell/funnel/lip	3
Ligustrum vulgare	white	bell/funnel/lip	3
Lilium bulbiferum	yellow/orange	bell/funnel/lip	0.05
Lilium martagon	pink/purple	bell/funnel/lip	10
Linaria alpina	pink/purple	bell/funnel/lip	8
Loncomelos brevistylus	white	hidden disc	0.05
Lotus corniculatus	yellow/orange	flag	5.5
Lupinus polyphyllus	blue	flag	9
Lychnis flos cuculi	pink/purple	stalk disc	10.5
Lysimachia arvensis	yellow/orange	pollen	0.05
Lysimachia vulgaris	yellow/orange	pollen	0.05
Lythrum salicaria	pink/purple	bell/funnel/lip	7
Malva sylvestris	pink/purple	hidden disc	0.05
Matricaria chamomilla	white	flower head	7
Medicago falcata	yellow/orange	flag	5
Medicago lupulina	yellow/orange	flag	2.5
Medicago sativa	pink/purple	flag	6
Melampyrum italicum	yellow/orange	bell/funnel/lip	18
Melampyrum sp.	yellow/orange	bell/funnel/lip	10
Melilotus albus	white	flag	3.3
Mentha arvensis	pink/purple	bell/funnel/lip	4
Mentha longifolia	pink/purple	bell/funnel/lip	3
Minuartia recurva	white	hidden disc	0.05
Myosotis sp.	blue	stalk disc	2.3
Myosoton aquaticum	white	hidden disc	0.05
Oenothera biennis	yellow/orange	stalk disc	17

Onobrychis montana	pink/purple	flag	11.7
Onobrychis viciifolia	pink/purple	flag	11.5
Ononis spinosa	pink/purple	flag	10
Origanum vulgare	pink/purple	bell/funnel/lip	6
Ornithogalum pyrenaicum	white	hidden disc	0.05
Ornithogalum umbellatum	white	hidden disc	1
Oxalis articulata	pink/purple	hidden disc	3
Oxytropis montana	pink/purple	flag	11.5
Papaver rhoeas	red	pollen	0.05
Parnassia palustris	white	open disc	0.05
Pastinaca sativa	yellow/orange	open disc	1
Pedicularis comosa	yellow/orange	bell/funnel/lip	17
Pedicularis palustris	pink/purple	bell/funnel/lip	14
Persicaria bistorta	pink/purple	bell/funnel/lip	2
Petrorhagia saxifraga	pink/purple	bell/funnel/lip	4.7
Phyteuma betonicifolium	blue	NA	4.8
Phyteuma orbiculare	blue	NA	4
Picris hieracioides	yellow/orange	flower head	12.3
Pimpinella major	white	open disc	1.3
Pimpinella saxifraga	white	open disc	0.05
Plantago lanceolata	white	NA	0.05
Plantago media	white	NA	0.05
Polygonum viviparum	white	bell/funnel/lip	2
Potentilla argentea	yellow/orange	hidden disc	0.05
Potentilla erecta	yellow/orange	hidden disc	0.05
Potentilla nitida	pink/purple	hidden disc	0.05
Potentilla reptans	yellow/orange	hidden disc	0.05
Prenanthes purpurea	pink/purple	flower head	11.5
Prunella grandiflora	pink/purple	bell/funnel/lip	13
Prunella laciniata	white	bell/funnel/lip	6
Prunella vulgaris	pink/purple	bell/funnel/lip	8.4
Pyrola minor	white	bell/funnel/lip	0.05
Ranunculus acris	yellow/orange	hidden disc	0.05
Ranunculus platanifolius	white	hidden disc	0.05
Ranunculus polyanthemophyllus	yellow/orange	hidden disc	0.05
Ranunculus repens	yellow/orange	hidden disc	0.05
Rhinanthus alectorolophus	yellow/orange	bell/funnel/lip	22.5
Rhinanthus freynii	yellow/orange	bell/funnel/lip	18
Rhinanthus minor	yellow/orange	bell/funnel/lip	16
Rhododendron hirsutum	pink/purple	bell/funnel/lip	10.5
Rorippa sylvestris	yellow/orange	hidden disc	2

Rosa canina	pink/purple	pollen	0.05
Rubus sp.	white	hidden disc	0.05
Salvia glutinosa	yellow/orange	bell/funnel/lip	20
Salvia pratensis	blue	bell/funnel/lip	9
Saponaria officinalis	pink/purple	stalk disc	23
Saxifraga rotundifolia	white	open disc	0.05
Scabiosa columbaria	pink/purple	flower head	5.5
Scabiosa gramuntia	pink/purple	flower head	6.5
Scabiosa lucida	pink/purple	flower head	6
Scabiosa triandra	pink/purple	flower head	6.4
Scrophularia nodosa	red	bell/funnel/lip	6
Securigera varia	pink/purple	flag	11
Sedum acre	yellow/orange	hidden disc	1.75
Sedum album	white	hidden disc	1.5
Senecio inaequidens	yellow/orange	flower head	8
Senecio nemorensis	yellow/orange	flower head	13
Senecio squalidus	yellow/orange	flower head	10.3
Sherardia arvensis	pink/purple	bell/funnel/lip	2.5
Silene alba	white	stalk disc	16
Silene dioica	pink/purple	stalk disc	15.3
Silene nutans	white	stalk disc	13
Silene saxifraga	white	stalk disc	5.5
Silene vulgaris	white	stalk disc	18
Solanum tuberosum	white	pollen	2
Solidago gigantea	yellow/orange	flower head	6
Solidago virgaurea	yellow/orange	flower head	10.7
Stachys alpina	pink/purple	bell/funnel/lip	10
Stachys officinalis	pink/purple	bell/funnel/lip	9
Stachys recta	white	bell/funnel/lip	8
Stachys sylvatica	pink/purple	bell/funnel/lip	11
Stellaria graminea	white	hidden disc	0.05
Stellaria nemorum	white	hidden disc	2.5
Symphytum officinale	pink/purple	bell/funnel/lip	15
Tanacetum corymbosum	white	flower head	8
Tanacetum vulgare	yellow/orange	flower head	5
Taraxacum officinale	yellow/orange	flower head	8
Teucrium chamaedrys	pink/purple	bell/funnel/lip	8
Thalictrum aquilegifolium	pink/purple	brush	0.05
Thalictrum lucidum	yellow/orange	brush	0.05
Thymus sp.	pink/purple	bell/funnel/lip	3.5
Torilis arvensis	white	open disc	0.1

Tragopogon pratensis	yellow/orange	flower head	12.5
Trifolium badium	yellow/orange	flag	5.5
Trifolium medium	pink/purple	flag	13
Trifolium montanum	white	flag	8.5
Trifolium pratense	pink/purple	flag	11.3
Trifolium repens	white	flag	4
Valeriana montana	pink/purple	bell/funnel/lip	5
Valeriana officinalis	pink/purple	bell/funnel/lip	3.7
Verbascum alpinum	yellow/orange	bell/funnel/lip	8
Verbascum chaixii	yellow/orange	bell/funnel/lip	5
Verbascum densiflorum	yellow/orange	bell/funnel/lip	10
Verbascum lychnitis	yellow/orange	bell/funnel/lip	10
Verbascum nigrum	yellow/orange	bell/funnel/lip	3
Verbena officinalis	pink/purple	bell/funnel/lip	5.3
Veronica anagallis-aquatica	pink/purple	bell/funnel/lip	0.05
Veronica chamaedrys	blue	bell/funnel/lip	0.05
Veronica persica	blue	bell/funnel/lip	0.05
Vicia cracca	pink/purple	flag	7.6
Vicia sepium	pink/purple	flag	10
Vicia sylvatica	white	flag	13
Vicia villosa	pink/purple	flag	11

Table S5.3: Functional traits of pollinator species: taxonomic family, proboscis length (mm; mean value of the measured specimens), body size in mm (mm; mean value of the measured specimens), and type of foraging range (*C* central-place forager, *NC* non-central-place forager).

Species	Family	Proboscis	Body	Foraging
Abia fasciata	Cimbicidae	0.8	12	NC
Amegilla quadrifasciata	Apidae	8.2	13	C
Andrena aeneiventris	Andrenidae	1.1	7	C
Andrena alfkenella	Andrenidae	0.95	5.75	C
Andrena cineraria	Andrenidae	2	12	C
Andrena haemorrhoa	Andrenidae	1.6	9	C
Andrena hattorfiana	Andrenidae	3.5	14	C
Andrena intermedia	Andrenidae	2.3	11	C
Andrena labialis	Andrenidae	2	11.5	C
Andrena lathyri	Andrenidae	2.7	10	C
Andrena limata	Andrenidae	1.85	11	C
Andrena pandellei	Andrenidae	1.95	9.5	C
Andrena schenckii	Andrenidae	2.8	12	C
Andrena sp. 1	Andrenidae	1.6	9	C
Andrena sp. 2	Andrenidae	1.45	9	C
Andrena sp. 3	Andrenidae	1.5	8	C
Andrena sp. 4	Andrenidae	1.03	6.75	C
Andrena sp. 5	Andrenidae	1.15	10	C
Andrena sp. 6	Andrenidae	1.2	10	C
Andrena sp. 7	Andrenidae	1.25	8	C
Andrena subopaca	Andrenidae	1	5.5	C
Andrena wilkella	Andrenidae	1.95	9.5	C
Anthidiellum strigatum	Megachilidae	3.2	7	C
Anthidium florentinum	Megachilidae	4	13	C
Anthidium oblongatum	Megachilidae	2.7	7	C
Anthidium punctatum	Megachilidae	3.3	8	C
Anthophora balneorum	Apidae	12	15	C
Anthophora crinipes	Apidae	6	12	C
Anthophora furcata	Apidae	8.25	11	C
Anthophora plumipes	Apidae	9	14	C
Apis mellifera	Apidae	5	12	C
Argogorytes mystaceus	Crabronidae	1.1	13	C
Athalia rosae	Tenthredinidae	0.5	6.5	NC
Billaea triangulifera	Tachinidae	2.3	10	NC
Bombus argillaceus	Apidae	10.7	15.67	C
Bombus barbutellus	Apidae	7.15	17	NC

Bombus bohemicus	Apidae	6.35	17.75	NC
Bombus campestris	Apidae	6.2	15	NC
Bombus gerstaeckeri	Apidae	16	18	C
Bombus hortorum	Apidae	12.53	16.83	C
Bombus humilis	Apidae	8.33	15	C
Bombus hypnorum	Apidae	7.25	13.5	C
Bombus inexspectatus	Apidae	7	15	NC
Bombus lapidarius	Apidae	7.27	15.33	C
Bombus lucorum	Apidae	5.5	16	C
Bombus mendax	Apidae	9.5	16	C
Bombus mesomelas	Apidae	8.5	15.5	C
Bombus monticola	Apidae	5.5	13	C
Bombus pascuorum	Apidae	8.25	15	C
Bombus pratorum	Apidae	7.67	14.67	C
Bombus pyrenaeus	Apidae	5.13	13.33	C
Bombus ruderarius	Apidae	7.13	13.25	C
Bombus rupestris	Apidae	7.4	18	NC
Bombus sichelii	Apidae	6.75	15.5	C
Bombus soroeensis	Apidae	6.35	17	C
Bombus sylvarum	Apidae	8.8	16	C
Bombus sylvestris	Apidae	6.5	16	NC
Bombus terrestris	Apidae	6.5	14.67	C
Bombus vestalis	Apidae	8.4	22	NC
Bombus wurflenii	Apidae	7.75	17	C
Callicera aurata	Syrphidae	1.5	12	NC
Ceratina chalybea	Apidae	3.5	9.5	C
Ceratina cucurbitina	Apidae	3.5	8.5	C
Ceratina cyanea	Apidae	2.5	7.5	C
Ceratina dallatorreana	Apidae	2.6	6	C
Ceratina gravidula	Apidae	4.7	11	C
Cerceris rubida	Crabronidae	1.6	7.5	C
Cerceris sabulosa	Crabronidae	1.6	9	C
Cheilosia aerea	Syrphidae	1	8	NC
Cheilosia albipila	Syrphidae	1	10	NC
Cheilosia antiqua	Syrphidae	1	7	NC
Cheilosia canicularis	Syrphidae	1	12	NC
Cheilosia frontalis	Syrphidae	1	8.5	NC
Cheilosia illustrata	Syrphidae	1	10	NC
Cheilosia laticornis	Syrphidae	1	9	NC
Cheilosia latifrons	Syrphidae	1	9	NC
Cheilosia longula	Syrphidae	1	7.5	NC

Cheilosia mutabilis	Syrphidae	1	6.5	NC
Cheilosia nigripes	Syrphidae	1	7	NC
Cheilosia pagana	Syrphidae	1	6.5	NC
Cheilosia personata	Syrphidae	1	9.5	NC
Cheilosia proxima	Syrphidae	1	8	NC
Cheilosia ranunculi	Syrphidae	1	8	NC
Cheilosia scutellata	Syrphidae	1	8.5	NC
Cheilosia soror	Syrphidae	1	8.5	NC
Cheilosia urbana	Syrphidae	1	7	NC
Cheilosia vernalis	Syrphidae	1	6	NC
Cheilosia vulpina	Syrphidae	1	9	NC
Chelostoma campanularum	Megachilidae	2.9	6.5	C
Chelostoma distinctum	Megachilidae	1.8	7	C
Chelostoma florisomne	Megachilidae	2.3	9.5	C
Chelostoma rapunculi	Megachilidae	2.7	9	C
Chrysosomoxys macrocercus	Tachinidae	2.2	7.5	NC
Chrysotoxum bicinctum	Syrphidae	1	10.5	NC
Chrysotoxum intermedium	Syrphidae	1	12	NC
Chrysotoxum vernale	Syrphidae	1	11.5	NC
Chrysotoxum verralli	Syrphidae	1	11.5	NC
Coelioxys conoideus	Megachilidae	4.1	10	NC
Conops flavipes	Conopidae	4.2	11	NC
Conops quadrifasciatus	Conopidae	3.3	8.5	NC
Corynis crassicornis	Cimbicidae	0.9	8	NC
Crossocerus cinxius	Crabronidae	0.65	7	C
Crossocerus leucostoma	Crabronidae	0.6	8	C
Cylindromyia brassicaria	Tachinidae	2.4	9	NC
Dasysyrphus albostriatus	Syrphidae	1	9	NC
Didea alneti	Syrphidae	1	14	NC
Didea erratica	Syrphidae	1	12.5	NC
Dinera carinifrons	Tachinidae	2.3	7.5	NC
Dinera ferina	Tachinidae	3.5	12	NC
Dufourea alpina	Halictidae	1.3	5.5	C
Ectemnius borealis	Crabronidae	0.95	7	C
Ectemnius continuus	Crabronidae	1.4	10	C
Ectophasia crassipennis	Tachinidae	3.3	9.75	NC
Entomognathus brevis	Crabronidae	0.4	4.5	C
Epistrophe grossulariae	Syrphidae	1	13	NC
Epistrophe melanostoma	Syrphidae	1	11	NC
Epistrophe nitidicollis	Syrphidae	1	11.5	NC
Episyrphus balteatus	Syrphidae	1	11.5	NC

Eriozona syrphoides	Syrphidae	1.5	14	NC
Eristalinus taeniops	Syrphidae	1.5	12.5	NC
Eristalis arbustorum	Syrphidae	1	10	NC
Eristalis horticola	Syrphidae	1.5	12	NC
Eristalis interrupta	Syrphidae	1	12	NC
Eristalis jugorum	Syrphidae	1.5	12.5	NC
Eristalis pertinax	Syrphidae	1.5	14	NC
Eristalis rupium	Syrphidae	1	11.5	NC
Eristalis similis	Syrphidae	1.5	14	NC
Eristalis tenax	Syrphidae	1.5	15	NC
Erycia fatua	Tachinidae	2.1	8.5	NC
Eucera longicornis	Apidae	6.25	14.5	C
Eucera nigrescens	Apidae	8.25	13	C
Eulabidogaster setifacies	Tachinidae	2.1	7	NC
Eupeodes corollae	Syrphidae	1	8	NC
Eupeodes lapponicus	Syrphidae	1	8	NC
Eupeodes latifasciatus	Syrphidae	1	10	NC
Eupeodes luniger	Syrphidae	1	9.5	NC
Eupeodes tirolensis	Syrphidae	1	10	NC
Exorista rustica	Tachinidae	2.2	12	NC
Exorista tubulosa	Tachinidae	2.4	8.5	NC
Gorytes quinquecinctus	Crabronidae	0.7	10	C
Gymnosoma clavatum	Tachinidae	1.9	7	NC
Gymnosoma nitens	Tachinidae	1.7	5	NC
Gymnosoma rotundatum	Tachinidae	2.4	7.75	NC
Gymnosoma sp.	Tachinidae	2	7	NC
Halictus compressus	Halictidae	2.55	9.5	C
Halictus langobardicus	Halictidae	2.1	8.5	C
Halictus maculatus	Halictidae	1.6	8.5	C
Halictus rubicundus	Halictidae	2.23	10.5	C
Halictus scabiosae	Halictidae	3.65	13	C
Halictus simplex	Halictidae	2.75	9.75	C
Helophilus pendulus	Syrphidae	1	12	NC
Helophilus trivittatus	Syrphidae	1	15.5	NC
Heriades rubicola	Megachilidae	1.8	6	C
Heriades truncorum	Megachilidae	2.2	8	C
Hoplitis adunca	Megachilidae	4.5	9	C
Hoplitis sp. 1	Megachilidae	3.9	7.5	C
Hoplitis villosa	Megachilidae	4.8	12	C
Hyalurgus cruciger	Tachinidae	1.1	5.5	NC
Hylaeus brevicornis	Colletidae	0.75	4.75	C

Hylaeus communis	Colletidae	0.75	4.75	C
Hylaeus confusus	Colletidae	1	5.5	C
Hylaeus gibbus	Colletidae	1.1	6.75	C
Hylaeus hyalinatus	Colletidae	0.8	5.3	C
Hylaeus imparilis	Colletidae	0.55	4	C
Hylaeus punctatus	Colletidae	0.7	5	C
Hylaeus taeniolatus	Colletidae	0.6	4	C
Hylaeus tyrolensis	Colletidae	0.6	5	C
Hylaeus variegatus	Colletidae	1	6.25	C
Lasioglossum albipes	Halictidae	1.5	8	C
Lasioglossum angusticeps	Halictidae	1.8	7	C
Lasioglossum calceatum	Halictidae	2.2	10	C
Lasioglossum discum	Halictidae	2.6	8.75	C
Lasioglossum fulvicorne	Halictidae	1.3	7.5	C
Lasioglossum glabriusculum	Halictidae	1	4.5	C
Lasioglossum lativentre	Halictidae	1.7	8	C
Lasioglossum leucozonium	Halictidae	2.25	8	C
Lasioglossum malachurum	Halictidae	1.8	9	C
Lasioglossum morio	Halictidae	1.1	7	C
Lasioglossum nigripes	Halictidae	2.45	9.75	C
Lasioglossum parvulum	Halictidae	1.3	6.5	C
Lasioglossum politum	Halictidae	0.93	5.25	C
Lasioglossum punctatissimum	Halictidae	1.3	6	C
Lasioglossum pygmaeum	Halictidae	1	6	C
Lasioglossum rufitarse	Halictidae	1.3	6	C
Lasioglossum sp. 1	Halictidae	1.2	6.5	C
Lasioglossum sp. 2	Halictidae	2.5	8	C
Lasioglossum villosulum	Halictidae	1.65	6	C
Lasioglossum zonulum	Halictidae	2.5	8.5	C
Leucostoma simplex	Tachinidae	2.2	7	NC
Leucozona lucorum	Syrphidae	1	11.5	NC
Lindenius albilabris	Crabronidae	0.7	6	C
Linnaemya impudica	Tachinidae	4.3	13	NC
Linnaemya lithosiophaga	Tachinidae	1.7	7	NC
Linnaemya picta	Tachinidae	3.3	12	NC
Linnaemya zachvatkini	Tachinidae	3	12	NC
Macrophya montana	Tenthredinidae	0.95	11.25	NC
Macropis europaea	Melittidae	1.2	9	C
Masistylum arcuatum	Tachinidae	2.8	8.25	NC
Megachile circumcincta	Megachilidae	3.2	12	C
Megachile lagopoda	Megachilidae	6.5	18	C

Megachile leachella	Megachilidae	3.7	9	C
Megachile ligniseca	Megachilidae	4.5	14	C
Megachile melanopyga	Megachilidae	3.6	11	C
Megachile nigriventris	Megachilidae	6.2	15	C
Megachile pilidens	Megachilidae	3.7	8.75	C
Megachile sculpturalis	Megachilidae	5.65	20	C
Megachile sp. 1	Megachilidae	4.8	15	C
Megachile sp. 2	Megachilidae	4.1	12.5	C
Megachile versicolor	Megachilidae	4	9	C
Megachile willughbiella	Megachilidae	4.45	12.25	C
Megalodontes sp. 1	Megalodontesidae	2.3	12	NC
Megalodontes sp. 2	Megalodontesidae	2.2	12	NC
Melangyna compositarum	Syrphidae	1	10	NC
Melanogaster nuda	Syrphidae	0.6	5.5	NC
Melanostoma mellinum	Syrphidae	0.6	6	NC
Melanostoma scalare	Syrphidae	0.6	8.5	NC
Meliscaeva auricollis	Syrphidae	1	9.5	NC
Meliscaeva cinctella	Syrphidae	1	10	NC
Merodon aeneus	Syrphidae	1	8.5	NC
Merodon cinereus	Syrphidae	1	9	NC
Merodon costans	Syrphidae	1	11	NC
Merodon equestris	Syrphidae	1	13	NC
Merodon funestus	Syrphidae	1	9	NC
Merodon sp.	Syrphidae	1	10	NC
Mintho rufiventris	Tachinidae	1.4	7	NC
Myathropa florea	Syrphidae	1	12	NC
Neoascia podagrica	Syrphidae	0.6	5.5	NC
Nomada armata	Apidae	3.5	9	NC
Nomada flavopicta	Apidae	3.1	8	NC
Nomada sexfasciata	Apidae	4.7	11	NC
Nomiapis diversipes	Halictidae	2.3	8.25	C
Nowickia ferox	Tachinidae	4.45	14.5	NC
Nowickia marklini	Tachinidae	4.3	13	NC
Osmia aurulenta	Megachilidae	4	12	C
Osmia bicolor	Megachilidae	5.2	11.5	C
Osmia caerulescens	Megachilidae	4.9	7.5	C
Osmia leaiana	Megachilidae	4	9	C
Osmia rufohirta	Megachilidae	4	7	C
Oxybelus mucronatus	Crabronidae	0.8	5	C
Oxybelus trispinosus	Crabronidae	1	5.5	C
Panurginus montanus	Andrenidae	1.4	7.5	C

Panzeria vivida	Tachinidae	2.7	8.5	NC
Paragus bicolor	Syrphidae	0.6	6.5	NC
Paragus constrictus	Syrphidae	0.6	5	NC
Paragus haemorrhous	Syrphidae	0.6	6.5	NC
Paragus sp.	Syrphidae	0.6	6	NC
Paragus tibialis	Syrphidae	0.6	6.5	NC
Parasyrphus lineolus	Syrphidae	1	9	NC
Peleteria iavana	Tachinidae	2.6	9	NC
Phasia aurulans	Tachinidae	2.1	6	NC
Phasia obesa	Tachinidae	1.6	5	NC
Physocephala rufipes	Conopidae	5	13	NC
Physocephala vittata	Conopidae	4.7	10.5	NC
Pipiza austriaca	Syrphidae	0.6	8.5	NC
Pipiza lugubris	Syrphidae	0.6	8	NC
Pipiza sp.	Syrphidae	0.6	8	NC
Pipizella divicoi	Syrphidae	0.6	6.5	NC
Pipizella sp.	Syrphidae	0.6	6	NC
Pipizella viduata	Syrphidae	0.6	6.5	NC
Platycheirus albimanus	Syrphidae	0.6	8.5	NC
Platycheirus scutatus	Syrphidae	0.6	8.5	NC
Platycheirus sp.	Syrphidae	0.6	8.5	NC
Platycheirus tarsalis	Syrphidae	0.6	8.5	NC
Polistes associus	Vespidae	1.5	14	C
Polistes biglumis	Vespidae	1.65	13	C
Polistes dominula	Vespidae	1.6	14	C
Polistes gallicus	Vespidae	1.6	14	C
Polistes nimpha	Vespidae	1.4	13.5	C
Polistes semenowi	Vespidae	1.5	13	NC
Prosena siberita	Tachinidae	5.9	8	NC
Psenulus pallipes	Crabronidae	0.5	6.5	C
Pseudoanthidium scapulare	Megachilidae	3.75	6.5	C
Rhingia campestris	Syrphidae	3.5	9.5	NC
Rhodanthidium septemdentatum	Megachilidae	4.5	12.5	C
Rhogogaster picta	Tenthredinidae	0.6	12	NC
Scaeva pyrastri	Syrphidae	1.5	12.5	NC
Scaeva selenitica	Syrphidae	1.5	13.5	NC
Scolia hirta	Scoliidae	4.1	19	NC
Seladonia subaurata	Halictidae	1.85	7	C
Sicus ferrugineus	Conopidae	5.8	11.25	NC
Siphona flavifrons	Tachinidae	3.7	5.5	NC
Siphona geniculata	Tachinidae	3.05	5	NC

Solieria vacua	Tachinidae	1.75	6.75	NC
Sphaerophoria infuscata	Syrphidae	0.6	8.5	NC
Sphaerophoria interrupta	Syrphidae	0.6	8.5	NC
Sphaerophoria scripta	Syrphidae	0.6	11.5	NC
Sphaerophoria sp.	Syrphidae	0.6	9.5	NC
Sphaerophoria taeniata	Syrphidae	0.6	9	NC
Sphecodes gibbus	Halictidae	1.7	8	NC
Sphecodes monilicornis	Halictidae	2.1	9	NC
Sphecodes pellucidus	Halictidae	1.5	8	NC
Sphecodes schencki	Halictidae	1.7	8	NC
Sphegina clunipes	Syrphidae	0.6	6.5	NC
Sphegina sp.	Syrphidae	0.6	6.5	NC
Stelis punctulatissima	Megachilidae	3.1	8.5	NC
Strongygaster globula	Tachinidae	1	6	NC
Syritta pipiens	Syrphidae	0.6	8	NC
Syrphus ribesii	Syrphidae	1	11	NC
Syrphus torvus	Syrphidae	1	11.5	NC
Syrphus vitripennis	Syrphidae	1	9.5	NC
Tachina fera	Tachinidae	4.8	14	NC
Tachina magnicornis	Tachinidae	3.45	12.5	NC
Tenthredo arcuata	Tenthredinidae	1	10.5	NC
Tenthredo crassa	Tenthredinidae	1.05	13	NC
Tenthredo koehleri	Tenthredinidae	1.7	11.5	NC
Tenthredo olivacea	Tenthredinidae	0.9	11	NC
Tenthredo rubricoxis	Tenthredinidae	0.8	12	NC
Tenthredo sp. 1	Tenthredinidae	0.9	10.5	NC
Tenthredo sp. 2	Tenthredinidae	1	11	NC
Tenthredo zonula	Tenthredinidae	0.68	8.5	NC
Tenthredopsis sp. 1	Tenthredinidae	0.9	13	NC
Tenthredopsis tischbeinii	Tenthredinidae	0.9	11	NC
Tetralonia dentata	Apidae	4.3	12	C
Tetralonia salicariae	Apidae	3	8	C
Thecophora atra	Conopidae	3	4.8	NC
Thecophora distincta	Conopidae	3.4	5.2	NC
Trachusa byssina	Megachilidae	4.45	10.5	C
Trichopoda pennipes	Tachinidae	2.4	8.5	NC
Volucella bombylans	Syrphidae	1.5	13	NC
Volucella pellucens	Syrphidae	1.5	15.5	NC
Xylocopa iris	Apidae	6	19	C
Xylocopa violacea	Apidae	7.85	22.5	C
Xylota jakutorum	Syrphidae	1	11	NC

Zodion cinereum	Conopidae	3.4	6	NC
Zophomyia temula	Tachinidae	2.7	11	NC

Table S5.4: Functional traits of pollinator families: number of collected specimens (N), proboscis length (mm; mean and SD values of the measured specimens), body size (mm; mean and SD values of the measured specimens), and percentage of central and non-central forager species (*C* central-place foragers, *NC* non-central-place foragers).

Family	N	Proboscis	Body	C species	NC species
Andrenidae	86	1.73 (0.67)	9.33 (2.17)	100	-
Apidae	8045	6.88 (2.66)	14.1 (3.56)	78	22
Cimbicidae	2	0.85 (0.07)	10 (2.83)	-	100
Colletidae	37	0.79 (0.19)	5.13 (0.88)	100	-
Conopidae	43	4.1 (0.99)	8.78 (3.12)	-	100
Crabronidae	40	0.92 (0.4)	7.62 (2.38)	100	-
Halictidae	258	1.84 (0.62)	7.87 (1.73)	88	12
Megachilidae	116	3.9 (1.11)	10.31 (3.32)	94	6
Megalodontesidae	3	2.25 (0.07)	12 (0)	-	100
Melittidae	8	1.2 (NA)	9 (NA)	100	-
Scoliidae	4	4.1 (NA)	19 (NA)	-	100
Syrphidae	2000	0.98 (0.38)	9.66 (2.49)	-	100
Tachinidae	102	2.68 (1.07)	8.74 (2.72)	-	100
Tenthredinidae	81	0.91 (0.29)	10.9 (1.76)	-	100
Vespidae	16	1.54 (0.09)	13.58 (0.49)	83	17

Table S5.5: Summary of the six global models. Abbreviated explanatory variables are honey bee abundance (*Apis*, ln-transformed), temperature (Temp), standardized functional richness of plant community (FRic), functional dispersion of plant community (FDis), trait similarity between wild pollinator community and the honey bee (TSim), proboscis length category (Prob), body size category (Body), type of foraging range (For), and taxonomic family (Fam). The continuous explanatory variables were scaled to mean 0 and standard deviation 1 to make slopes comparable.

Model nr	Type of model	Response variable	Explanatory variables	Random effect
Model 1	Linear model	CWM resource overlap between wild pollinators and the honey bee (one value per network)	*Apis* + Temp + FRic + TSim + *Apis* × FRic + *Apis* × TSim + FRic × TSim + *Apis* × FRic × TSim	NA
Model 2	Linear model	CWM resource overlap between wild pollinators and the honey bee (one value per network)	*Apis* + Temp + FDis + TSim + *Apis* × FDis + *Apis* × TSim + FDis × TSim + *Apis* × FDis × TSim	NA
Model 3	Linear mixed-effect model	CWM resource overlap between wild pollinators and the honey bee (one value per network per trait category, *i.e.*, proboscis shorter, similar, and longer than the honey bee)	*Apis* × Prob + Temp	Network
Model 4	Linear mixed-effect model	CWM resource overlap between wild pollinators and the honey bee (one value per network per trait category, *i.e.*, smaller, similar, and larger than the honey bee)	*Apis* × Body + Temp	Network
Model 5	Linear mixed-effect model	CWM resource overlap between wild pollinators and the honey bee (one value per network per trait category, *i.e.*, central forager and non-central forager)	*Apis* × For + Temp	Network
Model 6	Linear mixed-effect model	CWM resource overlap between wild pollinators and the honey bee (one value per network per trait category, *i.e.*, Andrenidae, Apidae, Colletidae, Conopidae, Crabronidae, Halictidae, Megachilidae, other families, Syrphidae, Tachinidae, Tenthredinidae, and Vespidae)	*Apis* × Fam + Temp	Network

Table S5.6: Results of the multi-model inference analysis testing the effects on CWM resource overlap of honey bee abundance (*Apis*, ln-transformed), temperature (Temp), standardized functional richness of plant community (FRic), trait similarity between wild pollinator community and the honey bee (TSim), and the following interactions: *Apis* × FRic, *Apis* × TSim, FRic × TSim, and *Apis* × FRic × TSim. The table reports the estimates for each variable, the ΔAICc, the R^2, and the Akaike weight (w) for each model with ΔAICc < 6. All the explanatory variables were scaled to mean 0 and standard deviation 1.

Ranking	**Intercept**	***Apis***	**Temp**	**FRic**	**TSim**	***Apis* × FRic**	***Apis* × TSim**	**FRic × TSim**	***Apis* × FRic × TSim**	**ΔAICc**	**R^2**	**w**
1	-1.471	-0.087	-	-0.010	0.143	-0.193	-	-	-	0.000	0.290	0.286
2	-1.474	-0.084	-	-0.008	0.155	-0.201	-0.058	-	-	1.917	0.300	0.110
3	-1.458	-0.087	-	0.000	0.159	-0.190	-	0.043	-	2.290	0.295	0.091
4	-1.472	-0.089	0.007	-0.007	0.144	-0.191	-	-	-	2.682	0.290	0.075
5	-1.472	-0.087	-	-0.056	-	-0.189	-	-	-	3.373	0.202	0.053
6	-1.517	-	-	-	0.158	-	-	-	-	3.511	0.120	0.049
7	-1.517	-0.084	-	-	0.152	-	-	-	-	3.884	0.154	0.041
8	-1.458	-0.083	-	0.006	0.177	-0.199	-0.069	0.055	-	4.065	0.310	0.037
9	-1.474	-0.084	0.000	-0.007	0.155	-0.201	-0.058	-	-	4.741	0.300	0.027
10	-1.517	-	-	-0.061	0.139	-	-	-	-	4.931	0.136	0.024
11	-1.459	-0.089	0.007	0.003	0.160	-0.188	-	0.043	-	5.099	0.295	0.022
12	-1.517	-	0.048	-	0.159	-	-	-	-	5.226	0.131	0.021
13	-1.517	-0.093	0.061	-	0.152	-	-	-	-	5.270	0.172	0.021
14	-1.517	-0.075	-	-0.043	0.139	-	-	-	-	5.890	0.161	0.015
15	-1.471	-0.084	-0.015	-0.062	-	-0.194	-	-	-	5.894	0.203	0.015

Table S5.7: Results of the multi-model inference analysis testing the effects on CWM resource overlap between wild pollinators and the honey bee of honey bee abundance (*Apis*, ln-transformed), temperature (Temp), functional dispersion of plant community (FDis), trait similarity between wild pollinator community and the honey bee (TSim), and the following interactions: *Apis* × FDis, *Apis* × TSim, FDis × TSim, and *Apis* × FDis × TSim. The table reports the estimates for each variable, the ΔAICc, the R^2, and the Akaike weight (w) for each model with ΔAICc < 6. All the explanatory variables were scaled to mean 0 and standard deviation 1.

Ranking	Intercept	*Apis*	Temp	FDis	TSim	*Apis* × FDis	*Apis* × TSim	FDis × TSim	*Apis* × FDis × TSim	ΔAICc	R^2	w
1	-1.517	-	-	-	0.171	-	-	-	-	0.000	0.141	0.144
2	-1.517	-0.082	-	-	0.165	-	-	-	-	0.436	0.173	0.116
3	-1.517	-	-	-0.082	0.143	-	-	-	-	0.635	0.169	0.105
4	-1.517	-0.087	-	-0.088	0.134	-	-	-	-	0.842	0.205	0.095
5	-1.517	-	0.038	-	0.170	-	-	-	-	1.944	0.148	0.055
6	-1.517	-0.090	0.052	-	0.161	-	-	-	-	2.128	0.185	0.050
7	-1.519	-0.081	-	-	0.168	-	-0.023	-	-	2.793	0.174	0.036
8	-1.527	-	-	-0.067	0.140	-	-	-0.030	-	2.857	0.173	0.035
9	-1.519	-0.079	-	-0.084	0.135	-0.059	-	-	-	2.967	0.212	0.033
10	-1.517	-0.100	-	-0.134	-	-	-	-	-	2.981	0.130	0.033
11	-1.517	-	0.021	-0.077	0.144	-	-	-	-	2.982	0.171	0.032
12	-1.517	-0.092	0.034	-0.080	0.135	-	-	-	-	3.097	0.210	0.031
13	-1.527	-0.088	-	-0.072	0.130	-	-	-0.031	-	3.135	0.210	0.030
14	-1.518	-0.086	-	-0.087	0.137	-	-0.019	-	-	3.344	0.206	0.027
15	-1.517	-	-	-0.131	-	-	-	-	-	3.344	0.083	0.027
16	-1.518	-0.089	0.050	-	0.164	-	-0.016	-	-	4.650	0.186	0.014
17	-1.518	-0.092	-	-0.131	-	-0.056	-	-	-	5.077	0.136	0.011
18	-1.517	-0.104	0.030	-0.127	-	-	-	-	-	5.205	0.134	0.011
19	-1.528	-	0.026	-0.058	0.140	-	-	-0.034	-	5.245	0.176	0.010
20	-1.530	-0.093	0.040	-0.059	0.130	-	-	-0.039	-	5.377	0.217	0.010
21	-1.529	-0.079	-	-0.069	0.131	-0.058	-	-0.031	-	5.390	0.216	0.010
22	-1.517	-0.096	-	-	-	-	-	-	-	5.456	0.044	0.009

23	-1.522	-0.076	-	-0.082	0.140	-0.069	-0.032	-	-	5.464	0.215	0.009
24	-1.517	-	-	-	-	-	-	-	-	5.477	0.000	0.009
25	-1.518	-0.083	0.023	-0.079	0.135	-0.047	-	-	-	5.535	0.214	0.009
26	-1.517	-	0.015	-0.128	-	-	-	-	-	5.646	0.084	0.009
27	-1.530	-0.087	-	-0.070	0.134	-	-0.025	-0.034	-	5.710	0.212	0.008
28	-1.518	-0.091	0.032	-0.080	0.137	-	-0.015	-	-	5.747	0.211	0.008

Table S5.8: Results of the multi-model inference analysis for single traits of pollinators, testing the effects on CWM resource overlap between wild pollinators and the honey bee of honey bee abundance (*Apis*, ln-transformed), temperature (Temp), and *a)* proboscis length (Prob) and the interaction between honey bee abundance and proboscis length (*Apis* × Prob), *b)* body size (Body) and the interaction between honey bee abundance and body size (*Apis* × Body), *c)* type of foraging range (For) and the interaction between honey bee abundance and type of foraging range (*Apis* × For), and *d)* taxonomic family (Fam) and the interaction between honey bee abundance and taxonomic family (*Apis* × Fam). The table reports the estimate for each continuous variable or the presence of each categorical variable in the model, the ΔAICc, the R^2, and the Akaike weight (w) for each model of the set with ΔAICc < 6. All the continuous explanatory variables were scaled to mean 0 and standard deviation 1.

***a)* Proboscis length**								
Ranking	**Intercept**	***Apis***	**Temp**	**Prob**	***Apis* × Prob**	**ΔAICc**	**R^2**	**w**
1	-1.675	-	-	+	-	0.000	0.117	0.487
2	-1.674	-0.032	-	+	-	1.918	0.119	0.187
3	-1.676	-	0.025	+	-	2.017	0.118	0.178
4	-1.675	-0.036	0.030	+	-	3.911	0.120	0.069
5	-1.672	-0.099	-	+	+	4.893	0.128	0.042
6	-1.673	-0.104	0.032	+	+	6.916	0.129	0.015
***b)* Body size category**								
Ranking	**Intercept**	***Apis***	**Temp**	**Body**	***Apis* × Body**	**ΔAICc**	**R^2**	**w**
1	-1.987	-	0.087	+	-	0.000	0.249	0.339
2	-1.986	-	-	+	-	0.533	0.236	0.260
3	-1.985	-0.061	0.096	+	-	0.811	0.257	0.226
4	-1.985	-0.048	-	+	-	1.899	0.240	0.131
5	-1.987	-0.047	0.096	+	+	5.025	0.258	0.027
6	-1.986	-0.034	-	+	+	6.085	0.241	0.016
***c)* Type of foraging range**								
Ranking	**Intercept**	***Apis***	**Temp**	**For**	***Apis* × For**	**ΔAICc**	**R^2**	**w**
1	-1.394	-0.092	-	+	-	0.000	0.135	0.343
2	-1.394	-	-	+	-	0.653	0.110	0.248
3	-1.395	-0.098	0.041	+	-	1.674	0.140	0.149
4	-1.394	-0.085	-	+	+	2.236	0.135	0.112
5	-1.395	-	0.027	+	-	2.624	0.112	0.092
6	-1.395	-0.091	0.041	+	+	3.959	0.140	0.047
***d)* Taxonomic family**								
Ranking	**Intercept**	***Apis***	**Temp**	**Fam**	***Apis* × Fam**	**ΔAICc**	**R^2**	**w**
1	-1.987	-0.074	0.081	+	-	0.000	0.239	0.393
2	-1.993	-	0.071	+	-	1.049	0.230	0.232
3	-1.988	-0.065	-	+	-	1.401	0.230	0.195
4	-1.992	-	-	+	-	1.583	0.223	0.178

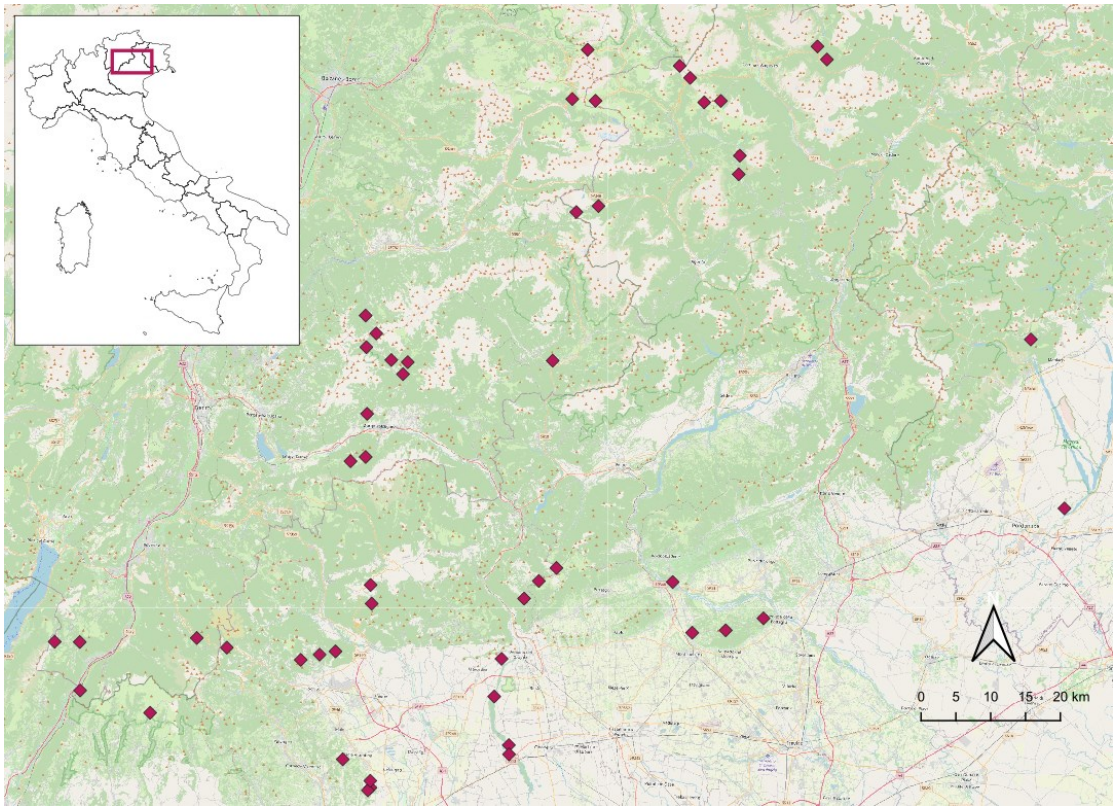

Figure S5.1: Map of the 51 sampling sites. Map credit: © OpenStreetMap contributors.

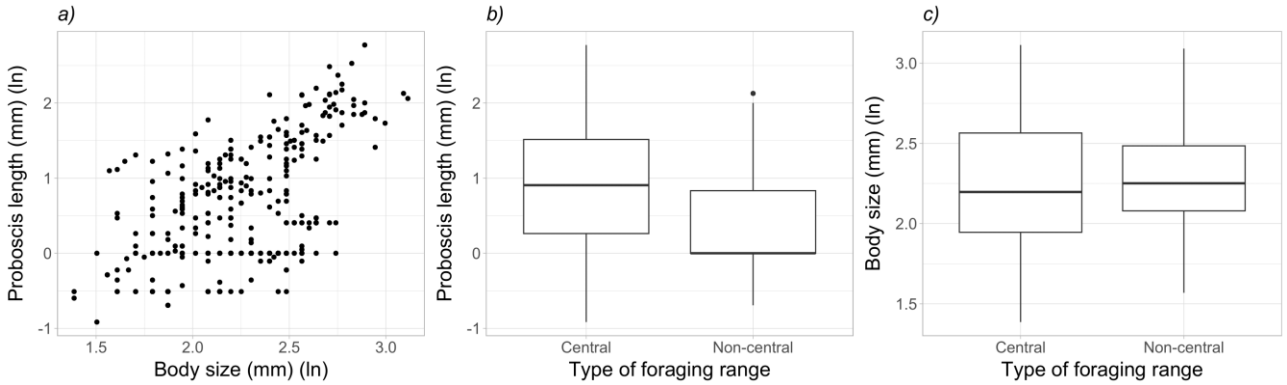

Figure S5.2: Plots showing the relationships between functional traits of pollinators, *i.e.*, *a)* proboscis length and body size, *b)* type of foraging range and proboscis length, and *c)* type of foraging range and body size. In all plots, proboscis length and body size were ln-transformed.

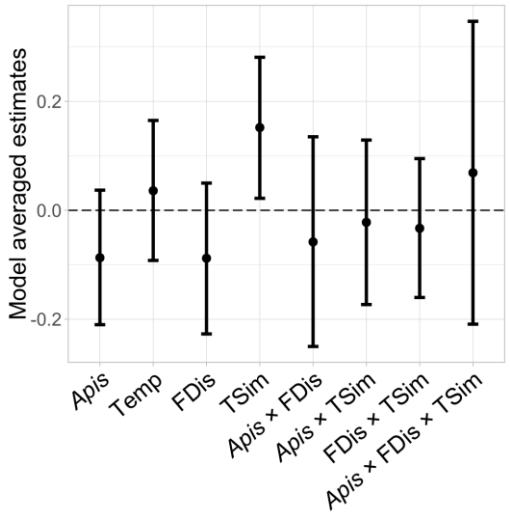

Figure S5.3: Model estimates from the model-averaging procedure based on the set of models including all functional traits of both plants and pollinators. Explanatory variables of the global model are honey bee abundance (*Apis*, ln-transformed), temperature (Temp), functional dispersion of plant community (FDis), trait similarity between wild pollinator community and the honey bee (TSim), and the following interactions: *Apis* × FDis, *Apis* × TSim, FDis × TSim, and *Apis* × FDis × TSim. All explanatory variables were scaled to mean 0 and standard deviation 1. Points represent model estimates and bars represent the 95% confidence intervals. The variable effect is supported when the confidence interval does not include zero.

CHAPTER 6

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Table S6.1: List of the 96 sampling sites with information on coordinates (WGS84 decimal degrees) and habitat type.

Region ID	Site ID	Lat (N)	Lon (E)	Habitat type
A	A_01	45.578072	10.257650	Crop field margin
A	A_02	45.592457	10.132257	Crop field margin
A	A_03	45.616016	10.196582	Crop field margin
A	A_04	45.572702	10.132798	Crop field margin
A	A_05	45.562111	10.173978	Crop field margin
A	A_06	45.577428	10.222978	Crop field margin
A	A_07	45.582009	10.254944	Crop field margin
A	A_08	45.594660	10.220166	Crop field margin
A	A_09	45.610087	10.219113	Semi-natural patch
A	A_10	45.609861	10.191606	Semi-natural patch
A	A_11	45.572787	10.193279	Semi-natural patch
A	A_12	45.631952	10.185443	Semi-natural patch
A	A_13	45.618859	10.341904	Semi-natural patch
A	A_14	45.717467	10.230152	Semi-natural patch
A	A_15	45.591956	10.257321	Semi-natural patch
A	A_16	45.568304	10.270196	Semi-natural patch
A	A_17	45.608140	10.214333	Urban green area
A	A_18	45.650008	10.204661	Urban green area
A	A_19	45.587637	10.290779	Urban green area
A	A_20	45.578976	10.233252	Urban green area
A	A_21	45.595429	10.243125	Urban green area
A	A_22	45.600659	10.187303	Urban green area
A	A_23	45.600026	10.217907	Urban green area
A	A_24	45.643470	10.269344	Urban green area
B	B_01	45.429625	10.556370	Crop field margin
B	B_02	45.441183	10.512542	Crop field margin
B	B_03	45.460481	10.488349	Crop field margin
B	B_04	45.443564	10.437596	Crop field margin
B	B_05	45.498061	10.500970	Crop field margin
B	B_06	45.485867	10.508788	Crop field margin
B	B_07	45.450325	10.560798	Crop field margin
B	B_08	45.427781	10.495385	Crop field margin
B	B_09	45.453070	10.587704	Semi-natural patch
B	B_10	45.438428	10.559610	Semi-natural patch
B	B_11	45.435161	10.538063	Semi-natural patch
B	B_12	45.407612	10.492146	Semi-natural patch
B	B_13	45.421973	10.485619	Semi-natural patch
B	B_14	45.518309	10.406367	Semi-natural patch
B	B_15	45.536879	10.481359	Semi-natural patch
B	B_16	45.473110	10.515111	Semi-natural patch
B	B_17	45.427676	10.549209	Urban green area
B	B_18	45.443631	10.508984	Urban green area
B	B_19	45.460408	10.478585	Urban green area
B	B_20	45.487254	10.472554	Urban green area

B	B_21	45.522115	10.492482	Urban green area
B	B_22	45.484442	10.502942	Urban green area
B	B_23	45.457225	10.572286	Urban green area
B	B_24	45.481495	10.406795	Urban green area
C	C_01	45.677446	11.734262	Crop field margin
C	C_02	45.694669	11.796586	Crop field margin
C	C_03	45.701099	11.705786	Crop field margin
C	C_04	45.705140	11.856183	Crop field margin
C	C_05	45.741244	11.734336	Crop field margin
C	C_06	45.715621	11.734044	Crop field margin
C	C_07	45.675655	11.806411	Crop field margin
C	C_08	45.698590	11.758165	Crop field margin
C	C_09	45.805377	11.811259	Semi-natural patch
C	C_10	45.676237	11.690087	Semi-natural patch
C	C_11	45.723906	11.692958	Semi-natural patch
C	C_12	45.699973	11.689300	Semi-natural patch
C	C_13	45.749033	11.704326	Semi-natural patch
C	C_14	45.776125	11.702758	Semi-natural patch
C	C_15	45.765021	11.672584	Semi-natural patch
C	C_16	45.803253	11.744679	Semi-natural patch
C	C_17	45.765316	11.715554	Urban green area
C	C_18	45.734517	11.717818	Urban green area
C	C_19	45.721509	11.700172	Urban green area
C	C_20	45.724172	11.748767	Urban green area
C	C_21	45.679331	11.813076	Urban green area
C	C_22	45.692089	11.768549	Urban green area
C	C_23	45.679638	11.780690	Urban green area
C	C_24	45.732640	11.773673	Urban green area
D	D_01	45.330593	11.880736	Crop field margin
D	D_02	45.302054	11.905265	Crop field margin
D	D_03	45.324089	11.829982	Crop field margin
D	D_04	45.270746	11.914408	Crop field margin
D	D_05	45.325905	11.776464	Crop field margin
D	D_06	45.354301	11.821223	Crop field margin
D	D_07	45.300539	11.831199	Crop field margin
D	D_08	45.268157	11.795260	Crop field margin
D	D_09	45.294496	11.773620	Semi-natural patch
D	D_10	45.292375	11.722253	Semi-natural patch
D	D_11	45.283420	11.702152	Semi-natural patch
D	D_12	45.285935	11.867935	Semi-natural patch
D	D_13	45.249354	11.744417	Semi-natural patch
D	D_14	45.271092	11.745190	Semi-natural patch
D	D_15	45.355303	11.754242	Semi-natural patch
D	D_16	45.306372	11.758257	Semi-natural patch
D	D_17	45.365198	11.878347	Urban green area
D	D_18	45.391019	11.783634	Urban green area
D	D_19	45.331776	11.880167	Urban green area
D	D_20	45.357843	11.777367	Urban green area

D	D_21	45.313625	11.885229	Urban green area
D	D_22	45.352749	11.899804	Urban green area
D	D_23	45.286736	11.871270	Urban green area
D	D_24	45.270335	11.858052	Urban green area

Table S6.2: Comparison of AICcs among models testing the effect on wild pollinator α-diversity and averaged EMF of flower cover and habitat type (Model 1), of the percentage of semi-natural habitats in 250 m radius buffers from the sampling sites (Model 2), and of flower cover, habitat type, and the percentage of semi-natural habitats in 250 m radius buffers from the sampling sites (Model 3).

Response variable	**ΔAICc Model 1**	**ΔAICc Model 2**	**ΔAICc Model 3**
Wild pollinator α-diversity	0	10.95	1.38
Averaged EMF	0	11.3	2.13

Table S6.3: List of sampled wild pollinator species.

Family	Species
Andrenidae	*Andrena dorsata*
	Andrena flavipes
	Andrena fulva
	Andrena hattorfiana
	Andrena hesperia
	Andrena humilis
	Andrena labialis
	Andrena labiata
	Andrena minutula
	Andrena minutuloides
	Andrena nigroaenea
	Andrena pastellensis
	Andrena sp. 1
	Andrena sp. 2
	Andrena sp. 3
	Andrena sp. 4
	Andrena wilkella
	Panurgus calcaratus
Apidae	*Anthophora plumipes*
	Bombus hortorum
	Bombus lapidarius
	Bombus pascuorum
	Bombus terrestris
	Ceratina cucurbitina
	Ceratina cyanea
	Ceratina dallatorreana
	Eucera sp.
	Melecta albifrons
	Melecta obscura
	Nomada sp. 1
	Nomada sp. 2
	Nomada sp. 3
	Tetralonia malvae
	Xylocopa violacea
Colletidae	*Hylaeus annularis*
	Hylaeus brevicornis
	Hylaeus gibbus
	Hylaeus gredleri
Halictidae	*Halictus compressus* group
	Halictus langobardicus
	Halictus maculatus
	Halictus scabiosae
	Lasioglossum albocinctum
	Lasioglossum angusticeps
	Lasioglossum calceatum
	Lasioglossum discum

	Lasioglossum glabriusculum
	Lasioglossum griseolum
	Lasioglossum interruptum
	Lasioglossum laevigatum
	Lasioglossum laticeps
	Lasioglossum leucozonium
	Lasioglossum lucidulum
	Lasioglossum malachurum
	Lasioglossum marginatum
	Lasioglossum medinai
	Lasioglossum mesosclerum
	Lasioglossum minutissimum
	Lasioglossum minutulum
	Lasioglossum morio
	Lasioglossum nigripes
	Lasioglossum nitidulum
	Lasioglossum pauxillum
	Lasioglossum politum
	Lasioglossum puncticolle
	Lasioglossum pygmaeum
	Lasioglossum transitorium
	Lasioglossum tricinctum
	Lasioglossum villosulum
	Lasioglossum zonulum
	Nomiapis diversipes
	Nomioides facilis
	Seladonia confusa
	Seladonia smaragdula
	Seladonia subaurata
	Sphecodes alternatus
	Sphecodes ephippius
	Sphecodes gibbus
	Sphecodes longulus
	Sphecodes monilicornis
	Sphecodes niger
	Sphecodes scabricollis
	Sphecodes sp.
	Systropha curvicornis
Megachilidae	*Anthidium manicatum*
	Anthidium oblongatum
	Chelostoma campanularum
	Chelostoma emarginatum
	Coelioxys elongatus
	Heriades rubicola
	Hoplitis adunca
	Hoplitis cf. *papaveris*
	Hoplitis leucomelana
	Lithurgus chrysurus

	Megachile apicalis
	Megachile centuncularis
	Megachile circumcincta
	Megachile ericetorum
	Megachile flabellipes
	Megachile pilidens
	Megachile pusilla
	Megachile rotundata
	Osmia andrenoides
	Osmia aurulenta
	Osmia bicornis
	Osmia caerulescens
	Osmia erythrogastra
	Osmia latreillei
	Osmia rufohirta
	Osmia sp. 1
	Osmia sp. 2
	Osmia spinulosa
	Osmia submicans
Melittidae	*Melitta haemorrhoidalis*
Syrphidae	*Chalcosyrphus nemorum*
	Episyrphus balteatus
	Eristalinus sepulchralis
	Eristalis arbustorum
	Eristalis interrupta
	Eristalis similis
	Eristalis tenax
	Eumerus funeralis
	Eumerus ornatus
	Eumerus uncipes
	Eupeodes corollae
	Eupeodes latifasciatus
	Helophilus pendulus
	Heringia heringi
	Melanostoma mellinum
	Merodon albifrons
	Merodon equestris
	Merodon rufus
	Myathropa florea
	Neoascia podagrica
	Paragus pecchiolii
	Pipiza noctiluca
	Pipizella sp.
	Pipizella viduata
	Sphaerophoria infuscata
	Sphaerophoria scripta
	Syritta pipiens
	Syrphus ribesii

Syrphus vitripennis
Xylota segnis

Table S6.4: Summary table of the variables measured in the three habitat types (mean and SD). Abbreviations are: "Wild poll α-div" for wild pollinator α-diversity, "Flower cover" for flowering plant cover, "Flower α-div" for flowering plant α-diversity, "Honey bee ab" for managed honey bee abundance (honey bee-related ESs), "Ground arth ab" for ground-dwelling arthropod abundance (ground-dwelling arthropod-related ESs), "Cat pred rate" for dummy caterpillar predation rate (pest control), "Seed pred rate" for seed predation rate (seed predation), "Mosq egg ab (ref)" for Asian tiger mosquito egg abundance (reflected) (disease control), "TBI S" for soil stabilisation factor S and "TBI k" for soil decomposition rate k (soil nutrient cycling), and "Inf rate" for water infiltration rate in soil (flood control).

Habitat type	Wild poll α-div	Flower cover	Flower α-div	Honey bee ab	Ground arth ab	Cat pred rate	Seed pred rate	Mosquito egg ab (ref)	TBI S	TBI k	Inf rate
Crop field	8.94	6.44	7	4.31	317.56	0.26	0.68	92.22	0.23	0.02	34.63
margin	(3.83)	(3.19)	(3.16)	(4.41)	212.32	(0.19)	(0.18)	(79.24)	(0.06)	(0.01	(39.67)
Semi-natural	8.66	19.09	12.53	23.31	223.06	0.38	0.71	70.72	0.26	0.02	51.81
patch	(4.8)	(12.9)	(4.48)	(39.04)	160.24	(0.16)	(0.18)	(104.49)	(0.05)	(0.01)	(53.37)
Urban green	6	13.34	9.75	20.28	140.34	0.2	0.51	114.34	0.26	0.02	40.94
area	(3.37)	(7.17)	(3.7)	(22.6)	87.73	(0.14)	(0.21)	(91.02)	(0.07)	(0.01)	(34.5)

Table S6.5: Model outputs for the multiple threshold analysis, testing the effect of habitat type, between *a)* crop field margins and semi-natural patches, *b)* crop field margins and urban green areas, and *c)* semi-natural patches and urban green areas. Values in bold indicate significant thresholds (*p* value < 0.05).

	Threshold	Estimate	SE	Statistic	*p* value
a) Crop field margin - semi-natural patch					
	1	0.281	0.137	2.054	**0.044**
	2	0.281	0.137	2.047	**0.045**
	3	0.344	0.143	2.408	**0.019**
	4	0.438	0.142	3.091	**0.003**
	5	0.375	0.172	2.186	**0.033**
	6	0.406	0.162	2.511	**0.015**
	7	0.469	0.158	2.970	**0.004**
	8	0.469	0.165	2.843	**0.006**
	9	0.531	0.169	3.150	**0.003**
	10	0.469	0.175	2.680	**0.009**
	11	0.344	0.179	1.922	**0.059**
	12	0.250	0.178	1.404	0.165
	13	0.188	0.183	1.025	0.309
	14	0.125	0.182	0.689	0.494
	15	0.344	0.192	1.787	0.079
	16	0.313	0.197	1.589	0.117
	17	0.313	0.196	1.593	0.116
	18	0.281	0.198	1.419	0.161
	19	0.250	0.198	1.265	0.211
	20	0.250	0.206	1.216	0.229
	21	0.281	0.204	1.380	0.173
	22	0.219	0.230	0.951	0.345
	23	0.188	0.232	0.810	0.421
	24	0.219	0.244	0.895	0.374
	25	0.281	0.243	1.157	0.252
	26	0.219	0.246	0.890	0.377
	27	0.219	0.243	0.900	0.372
	28	0.281	0.242	1.161	0.250
	29	0.438	0.235	1.863	0.067
	30	0.500	0.233	2.144	**0.036**
	31	0.469	0.241	1.948	**0.056**
	32	0.531	0.234	2.270	**0.027**
	33	0.594	0.245	2.425	**0.018**
	34	0.563	0.243	2.313	**0.024**
	35	0.500	0.240	2.087	**0.041**
	36	0.500	0.252	1.984	**0.052**
	37	0.469	0.249	1.879	0.065
	38	0.438	0.248	1.766	0.082
	39	0.469	0.255	1.839	**0.071**
	40	0.438	0.247	1.769	0.082

41	0.438	0.237	1.842	0.070
42	0.500	0.247	2.026	**0.047**
43	0.531	0.254	2.092	**0.041**
44	0.469	0.252	1.862	0.067
45	0.500	0.268	1.863	0.067
46	0.406	0.262	1.549	0.126
47	0.375	0.273	1.375	0.174
48	0.406	0.288	1.411	0.163
49	0.563	0.270	2.082	0.041
50	0.500	0.268	1.869	0.066
51	0.531	0.270	1.969	0.053
52	0.531	0.265	2.006	**0.049**
53	0.563	0.267	2.107	**0.039**
54	0.563	0.265	2.119	**0.038**
55	0.656	0.282	2.328	**0.023**
56	0.594	0.278	2.137	**0.037**
57	0.500	0.284	1.763	0.083
58	0.438	0.271	1.613	0.112
59	0.500	0.265	1.886	0.064
60	0.469	0.264	1.776	**0.081**
61	0.563	0.267	2.107	**0.039**
62	0.594	0.245	2.420	**0.018**
63	0.656	0.254	2.587	**0.012**
64	0.563	0.244	2.309	**0.024**
65	0.375	0.247	1.515	0.135
66	0.375	0.237	1.585	0.118
67	0.438	0.235	1.859	0.068
68	0.469	0.228	2.058	**0.044**
69	0.531	0.230	2.310	**0.024**
70	0.531	0.220	2.413	**0.019**
71	0.563	0.219	2.574	**0.012**
72	0.531	0.222	2.391	**0.020**
73	0.500	0.227	2.207	**0.031**
74	0.438	0.228	1.922	0.059
75	0.438	0.234	1.869	0.066
76	0.469	0.234	2.000	0.050
77	0.406	0.217	1.874	0.066
78	0.438	0.226	1.935	0.057
79	0.406	0.237	1.714	0.091
80	0.438	0.236	1.852	0.069
81	0.438	0.236	1.852	0.069
82	0.500	0.235	2.123	**0.038**
83	0.500	0.235	2.123	**0.038**
84	0.500	0.228	2.196	**0.032**
85	0.500	0.228	2.189	**0.032**
86	0.625	0.228	2.738	**0.008**
87	0.594	0.208	2.853	**0.006**
88	0.531	0.200	2.655	**0.010**

	89	0.531	0.200	2.655	**0.010**
	90	0.531	0.200	2.655	**0.010**
	91	0.375	0.189	1.982	**0.052**
	92	0.375	0.189	1.982	**0.052**
	93	0.375	0.176	2.132	**0.037**
	94	0.375	0.172	2.175	**0.033**
	95	0.344	0.164	2.090	**0.041**
	96	0.219	0.159	1.375	0.174
	97	0.281	0.145	1.946	0.056
	98	0.281	0.144	1.952	0.055
	99	0.250	0.144	1.742	0.087
b) Crop field margin – urban green area					
	1	0.219	0.127	1.724	0.090
	2	0.344	0.134	2.572	0.013
	3	0.250	0.145	1.718	0.091
	4	0.219	0.168	1.303	0.197
	5	0.250	0.170	1.469	0.147
	6	0.281	0.177	1.589	0.117
	7	0.375	0.183	2.050	0.045
	8	0.313	0.226	1.380	0.173
	9	0.313	0.236	1.323	0.191
	10	0.219	0.240	0.912	0.365
	11	0.156	0.235	0.665	0.509
	12	0.063	0.243	0.258	0.798
	13	0.063	0.254	0.246	0.806
	14	0.000	0.252	0.000	1.000
	15	0.125	0.268	0.466	0.643
	16	0.031	0.268	0.117	0.907
	17	0.000	0.266	0.000	1.000
	18	-0.031	0.259	-0.121	0.904
	19	0.031	0.251	0.124	0.901
	20	-0.125	0.261	-0.479	0.633
	21	-0.156	0.249	-0.627	0.533
	22	-0.281	0.245	-1.149	0.255
	23	-0.438	0.254	-1.724	0.090
	24	-0.500	0.264	-1.896	0.063
	25	-0.531	0.259	-2.054	0.044
	26	-0.594	0.260	-2.281	0.026
	27	-0.531	0.261	-2.033	0.046
	28	-0.500	0.270	-1.850	0.069
	29	-0.469	0.271	-1.733	0.088
	30	-0.344	0.258	-1.333	0.187
	31	-0.500	0.277	-1.807	0.076
	32	-0.531	0.278	-1.910	0.061
	33	-0.469	0.273	-1.718	0.091
	34	-0.469	0.284	-1.651	0.104
	35	-0.469	0.284	-1.651	0.104

36	-0.438	0.273	-1.603	0.114
37	-0.469	0.271	-1.731	0.088
38	-0.563	0.278	-2.021	0.048
39	-0.500	0.280	-1.783	0.079
40	-0.469	0.279	-1.681	0.098
41	-0.438	0.268	-1.631	0.108
42	-0.531	0.285	-1.861	0.067
43	-0.500	0.300	-1.669	0.100
44	-0.375	0.265	-1.417	0.162
45	-0.375	0.265	-1.417	0.162
46	-0.375	0.265	-1.417	0.162
47	-0.438	0.271	-1.613	0.112
48	-0.375	0.280	-1.342	0.185
49	-0.406	0.276	-1.472	0.146
50	-0.406	0.276	-1.472	0.146
51	-0.375	0.278	-1.348	0.183
52	-0.406	0.274	-1.483	0.143
53	-0.344	0.274	-1.255	0.214
54	-0.344	0.272	-1.262	0.212
55	-0.219	0.280	-0.780	0.438
56	-0.219	0.280	-0.780	0.438
57	-0.156	0.276	-0.566	0.573
58	-0.250	0.252	-0.994	0.324
59	-0.219	0.249	-0.880	0.382
60	-0.188	0.240	-0.781	0.438
61	-0.250	0.254	-0.985	0.329
62	-0.219	0.251	-0.873	0.386
63	-0.219	0.241	-0.909	0.367
64	-0.219	0.216	-1.012	0.315
65	-0.219	0.219	-0.999	0.322
66	-0.313	0.209	-1.494	0.140
67	-0.344	0.223	-1.541	0.128
68	-0.281	0.225	-1.248	0.217
69	-0.375	0.219	-1.710	0.092
70	-0.438	0.215	-2.033	0.046
71	-0.438	0.220	-1.991	0.051
72	-0.375	0.224	-1.675	0.099
73	-0.375	0.223	-1.679	0.098
74	-0.375	0.223	-1.679	0.098
75	-0.406	0.219	-1.855	0.068
76	-0.313	0.212	-1.478	0.145
77	-0.219	0.201	-1.088	0.281
78	-0.156	0.206	-0.757	0.452
79	-0.125	0.201	-0.621	0.537
80	-0.125	0.203	-0.617	0.539
81	-0.125	0.200	-0.624	0.535
82	-0.250	0.198	-1.265	0.211
83	-0.250	0.198	-1.265	0.211

	84	-0.250	0.198	-1.265	0.211
	85	-0.313	0.184	-1.702	0.094
	86	-0.250	0.171	-1.464	0.148
	87	-0.250	0.165	-1.515	0.135
	88	-0.281	0.158	-1.782	0.080
	89	-0.281	0.158	-1.782	0.080
	90	-0.250	0.152	-1.644	0.105
	91	-0.281	0.151	-1.864	0.067
	92	-0.281	0.151	-1.864	0.067
	93	-0.250	0.145	-1.729	0.089
	94	-0.156	0.145	-1.078	0.285
	95	-0.156	0.145	-1.078	0.285
	96	-0.188	0.142	-1.325	0.190
	97	-0.094	0.124	-0.757	0.452
	98	-0.125	0.122	-1.027	0.308
	99	-0.156	0.115	-1.360	0.179
c) Semi-natural patch – Urban green area					
	1	0.063	0.114	0.549	0.585
	2	0.031	0.122	0.257	0.798
	3	0.063	0.155	0.403	0.688
	4	-0.063	0.183	-0.341	0.734
	5	0.031	0.201	0.155	0.877
	6	0.031	0.205	0.152	0.879
	7	-0.031	0.206	-0.151	0.880
	8	-0.063	0.196	-0.320	0.750
	9	-0.250	0.204	-1.223	0.226
	10	-0.406	0.228	-1.784	0.079
	11	-0.313	0.229	-1.367	0.177
	12	-0.344	0.234	-1.472	0.146
	13	-0.406	0.240	-1.695	0.095
	14	-0.375	0.247	-1.516	0.135
	15	-0.281	0.244	-1.152	0.254
	16	-0.375	0.240	-1.562	0.123
	17	-0.406	0.225	-1.803	0.076
	18	-0.406	0.230	-1.769	0.082
	19	-0.500	0.235	-2.123	**0.038**
	20	-0.563	0.247	-2.274	**0.026**
	21	-0.656	0.236	-2.782	**0.007**
	22	-0.656	0.233	-2.817	**0.006**
	23	-0.750	0.248	-3.026	**0.004**
	24	-0.844	0.239	-3.536	**0.001**
	25	-0.875	0.235	-3.725	**<0.001**
	26	-0.906	0.238	-3.807	**<0.001**
	27	-0.906	0.243	-3.737	**<0.001**
	28	-0.875	0.280	-3.130	**0.003**
	29	-0.906	0.287	-3.155	**0.002**
	30	-1.031	0.303	-3.401	**0.001**

31	-1.156	0.311	-3.722	**<0.001**
32	-1.156	0.301	-3.837	**<0.001**
33	-1.156	0.301	-3.837	**<0.001**
34	-1.156	0.301	-3.839	**<0.001**
35	-1.125	0.302	-3.731	**<0.001**
36	-1.031	0.302	-3.420	**0.001**
37	-0.969	0.296	-3.272	**0.002**
38	-1.125	0.287	-3.925	**<0.001**
39	-1.156	0.284	-4.066	**<0.001**
40	-1.188	0.286	-4.151	**<0.001**
41	-1.156	0.280	-4.124	**<0.001**
42	-1.219	0.293	-4.155	**<0.001**
43	-1.250	0.300	-4.163	**<0.001**
44	-1.281	0.262	-4.899	**<0.001**
45	-1.219	0.264	-4.622	**<0.001**
46	-1.188	0.248	-4.781	**<0.001**
47	-1.125	0.248	-4.530	**<0.001**
48	-1.094	0.253	-4.320	**<0.001**
49	-1.094	0.252	-4.341	**<0.001**
50	-1.063	0.252	-4.220	**<0.001**
51	-1.063	0.254	-4.185	**<0.001**
52	-1.094	0.244	-4.477	**<0.001**
53	-1.125	0.241	-4.673	**<0.001**
54	-1.063	0.244	-4.359	**<0.001**
55	-1.063	0.246	-4.323	**<0.001**
56	-1.063	0.242	-4.399	**<0.001**
57	-1.125	0.232	-4.844	**<0.001**
58	-1.031	0.229	-4.500	**<0.001**
59	-0.938	0.215	-4.357	**<0.001**
60	-0.844	0.231	-3.660	**0.001**
61	-0.969	0.238	-4.069	**<0.001**
62	-0.969	0.230	-4.207	**<0.001**
63	-0.969	0.235	-4.115	**<0.001**
64	-1.031	0.232	-4.442	**<0.001**
65	-0.906	0.227	-3.988	**<0.001**
66	-1.000	0.218	-4.594	**<0.001**
67	-1.063	0.237	-4.477	**<0.001**
68	-1.000	0.238	-4.209	**<0.001**
69	-1.063	0.228	-4.669	**<0.001**
70	-1.125	0.217	-5.186	**<0.001**
71	-1.156	0.233	-4.957	**<0.001**
72	-1.125	0.223	-5.036	**<0.001**
73	-1.125	0.205	-5.500	**<0.001**
74	-1.000	0.201	-4.980	**<0.001**
75	-1.000	0.214	-4.672	**<0.001**
76	-0.969	0.224	-4.327	**<0.001**
77	-0.969	0.227	-4.269	**<0.001**
78	-0.906	0.229	-3.950	**<0.001**

79	-0.906	0.225	-4.023	**<0.001**
80	-0.969	0.228	-4.258	**<0.001**
81	-1.000	0.228	-4.378	**<0.001**
82	-0.938	0.215	-4.357	**<0.001**
83	-0.938	0.215	-4.357	**<0.001**
84	-0.906	0.215	-4.216	**<0.001**
85	-0.938	0.204	-4.590	**<0.001**
86	-0.906	0.206	-4.406	**<0.001**
87	-0.906	0.199	-4.558	**<0.001**
88	-0.906	0.191	-4.735	**<0.001**
89	-0.906	0.191	-4.735	**<0.001**
90	-0.844	0.193	-4.363	**<0.001**
91	-0.750	0.178	-4.213	**<0.001**
92	-0.750	0.178	-4.213	**<0.001**
93	-0.688	0.166	-4.134	**<0.001**
94	-0.594	0.155	-3.820	**<0.001**
95	-0.500	0.155	-3.219	**0.002**
96	-0.438	0.151	-2.893	**0.005**
97	-0.438	0.150	-2.913	**0.005**
98	-0.406	0.135	-3.008	**0.004**
99	-0.406	0.133	-3.061	**0.003**

Table S6.6: Model output for the multiple threshold analysis testing the effect of flower cover on the number of functions maximized at each threshold. Values in bold indicate significant thresholds (p value < 0.05).

Threshold	Estimate	SE	Statistic	*p* value
1	0.018	0.006	3.068	0.003
2	0.016	0.006	2.627	**0.010**
3	0.017	0.007	2.338	**0.022**
4	0.016	0.008	2.005	**0.048**
5	0.018	0.009	2.028	**0.045**
6	0.012	0.009	1.385	0.169
7	0.013	0.008	1.524	0.131
8	0.013	0.009	1.426	0.157
9	0.015	0.010	1.527	0.130
10	0.014	0.010	1.373	0.173
11	0.010	0.010	0.953	0.343
12	0.013	0.010	1.260	0.211
13	0.013	0.011	1.258	0.212
14	0.013	0.011	1.206	0.231
15	0.014	0.011	1.269	0.208
16	0.020	0.011	1.804	0.074
17	0.019	0.010	1.855	**0.067**
18	0.015	0.010	1.448	0.151
19	0.015	0.010	1.478	0.143
20	0.014	0.011	1.286	0.202
21	0.007	0.011	0.657	0.513
22	0.011	0.011	0.984	0.328
23	0.007	0.012	0.546	0.586
24	0.005	0.012	0.441	0.660
25	0.004	0.012	0.298	0.766
26	0.003	0.012	0.236	0.814
27	0.000	0.012	0.021	0.984
28	-0.002	0.013	-0.155	0.877
29	-0.004	0.013	-0.342	0.733
30	-0.006	0.013	-0.491	0.625
31	-0.004	0.013	-0.280	0.780
32	-0.001	0.013	-0.058	0.954
33	0.003	0.013	0.235	0.815
34	0.007	0.013	0.574	0.568
35	0.008	0.013	0.600	0.550
36	0.006	0.013	0.479	0.633
37	0.007	0.012	0.559	0.577
38	0.005	0.013	0.368	0.713
39	0.002	0.013	0.131	0.896
40	0.001	0.013	0.101	0.919
41	0.004	0.013	0.285	0.776
42	0.004	0.013	0.281	0.779
43	0.008	0.014	0.549	0.584
44	0.006	0.012	0.491	0.624

45	0.003	0.012	0.252	0.801
46	0.005	0.012	0.436	0.664
47	0.007	0.012	0.581	0.563
48	0.010	0.013	0.797	0.428
49	0.009	0.013	0.681	0.497
50	0.012	0.012	0.963	0.338
51	0.013	0.013	1.069	0.288
52	0.012	0.012	0.944	0.348
53	0.011	0.012	0.884	0.379
54	0.015	0.012	1.229	0.222
55	0.021	0.013	1.640	0.104
56	0.021	0.012	1.736	0.086
57	0.023	0.012	1.904	0.060
58	0.013	0.011	1.138	0.258
59	0.016	0.011	1.444	0.152
60	0.017	0.011	1.578	0.118
61	0.020	0.011	1.774	0.079
62	0.018	0.011	1.543	0.126
63	0.019	0.011	1.712	0.090
64	0.025	0.010	2.377	**0.020**
65	0.021	0.011	2.032	**0.045**
66	0.021	0.010	2.041	**0.044**
67	0.022	0.011	2.014	**0.047**
68	0.023	0.011	2.156	**0.034**
69	0.021	0.010	2.019	**0.046**
70	0.020	0.010	2.023	**0.046**
71	0.013	0.010	1.231	0.222
72	0.009	0.010	0.877	0.383
73	0.009	0.010	0.911	0.365
74	0.005	0.010	0.502	0.617
75	0.002	0.010	0.239	0.812
76	0.007	0.010	0.694	0.490
77	0.007	0.010	0.695	0.489
78	0.007	0.010	0.634	0.528
79	0.008	0.011	0.740	0.461
80	0.009	0.011	0.840	0.403
81	0.009	0.010	0.884	0.379
82	0.011	0.010	1.081	0.282
83	0.011	0.010	1.081	0.282
84	0.013	0.010	1.323	0.189
85	0.009	0.010	0.941	0.349
86	0.006	0.009	0.676	0.501
87	0.005	0.009	0.561	0.576
88	0.007	0.009	0.801	0.425
89	0.007	0.009	0.801	0.425
90	0.007	0.008	0.885	0.378
91	0.012	0.008	1.455	0.149
92	0.012	0.008	1.455	0.149

93	0.012	0.007	1.632	0.106
94	0.013	0.007	1.880	0.063
95	0.009	0.007	1.323	0.189
96	0.006	0.007	0.864	0.390
97	0.008	0.006	1.303	0.196
98	0.009	0.006	1.549	0.125
99	0.010	0.006	1.766	0.081

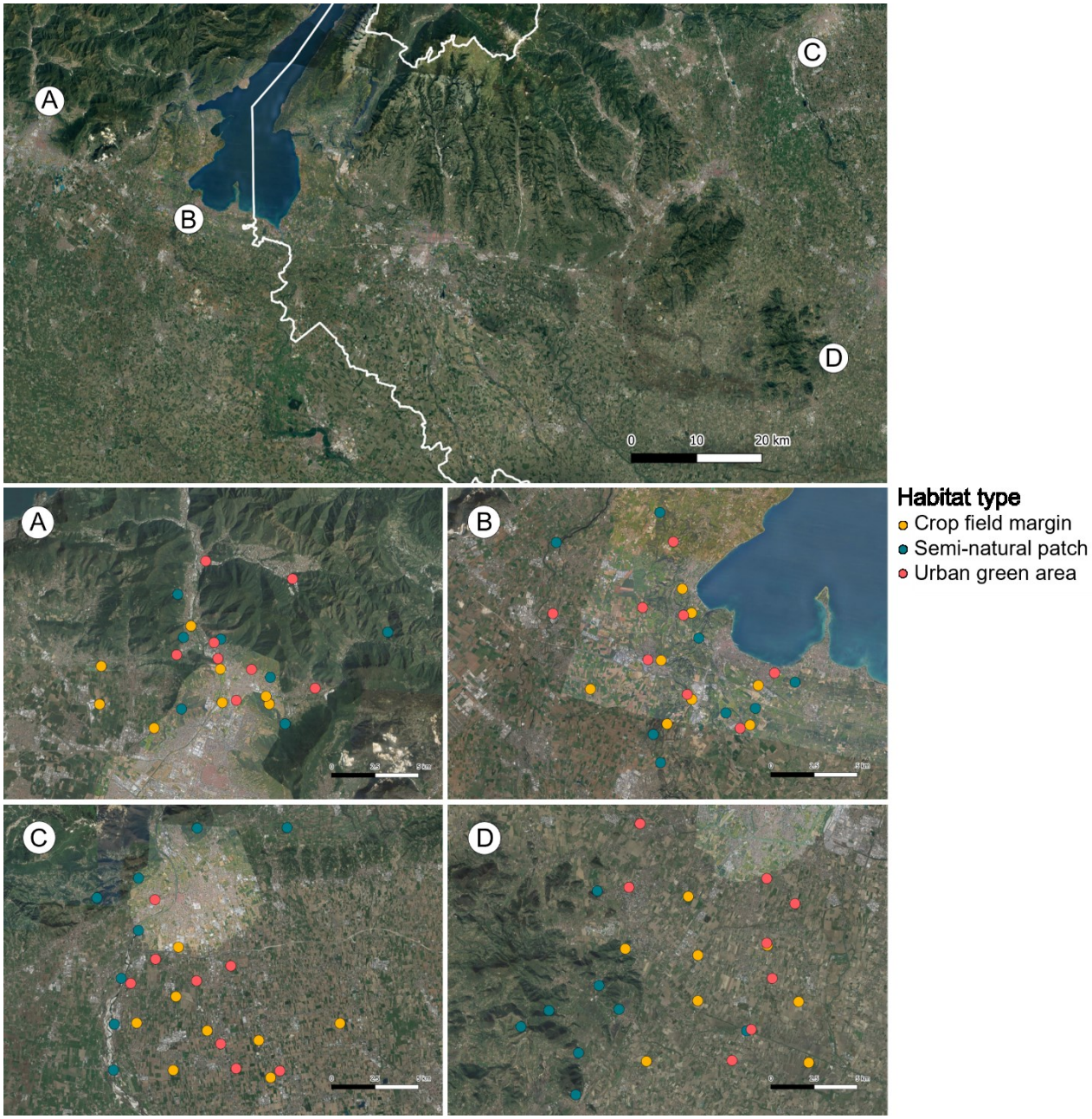

Figure S6.1: Map of the 96 sampling sites. Imagery © 2023 TerraMetrics, Map data © 2023 Google.

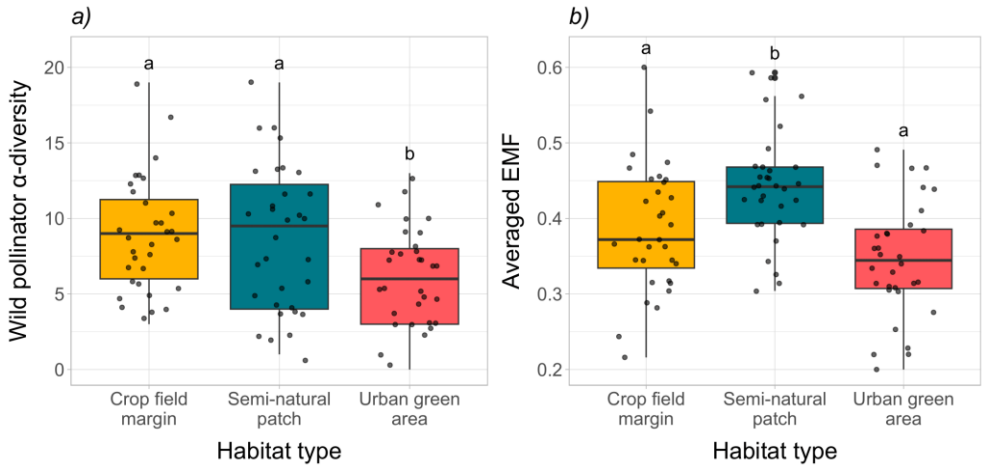

Figure S6.2: Boxplots showing the relationships between habitat type and *a)* wild pollinator α-diversity and *b)* averaged EMF. Points represent raw data points.

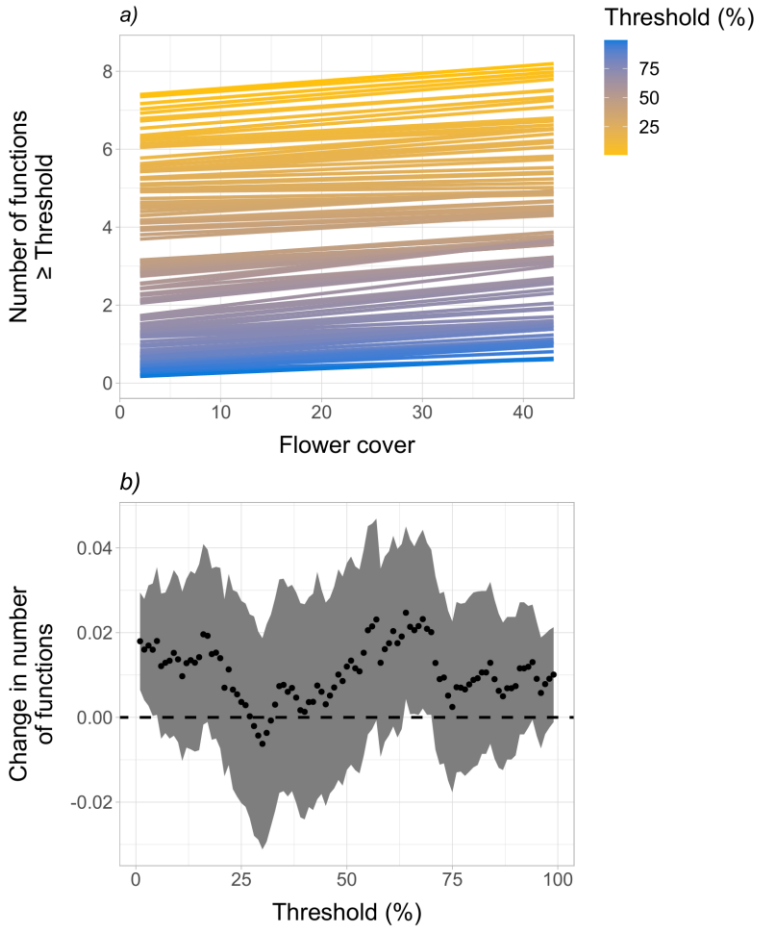

Figure S6.3: Effect of flower cover on multiple threshold EMF. Panel *a)* shows the relationship between flower cover and the number of functions that performed higher than a certain threshold. We considered the full range of thresholds, from 1% to 99%, and each line represents a given threshold. Panel *b)* shows the slope of the relationship between flower cover and the number of functions reaching a certain threshold. Black points represent fitted values and the shaded areas represent the 95% confidence intervals. For each threshold, the relationship with flower cover is significant only if the confidence interval does not overlap 0.

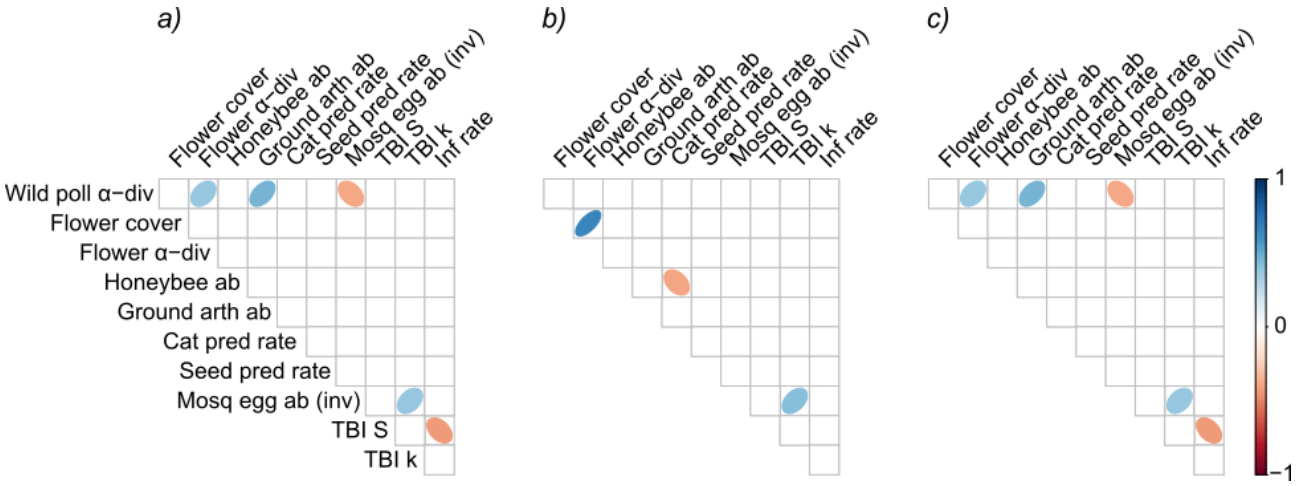

Figure S6.4: Correlation matrices for pollinator α-diversity, flower cover, flowering plant α-diversity and ES indicators based on Pearson's correlation in the three habitat types, *i.e.*, *a)* crop field margins, *b)* semi-natural patches, and *c)* urban green areas. Right-oriented blue ellipses indicate positive correlations, while left-oriented red ellipses indicate negative correlations. Narrower ellipses indicate stronger correlations. Only significant correlations (p value < 0.05) are displayed. Abbreviations are: "Wild poll α-div" for wild pollinator α-diversity, "Flower cover" for flowering plant cover, "Flower α-div" for flowering plant α-diversity, "Honey bee ab" for managed honey bee abundance (honey bee-related ESs), "Ground arth ab" for ground-dwelling arthropod abundance (ground-dwelling arthropod-related ESs), "Cat pred rate" for dummy caterpillar predation rate (pest control), "Seed pred rate" for seed predation rate (seed predation), "Mosq egg ab (ref)" for Asian tiger mosquito egg abundance (reflected) (disease control), "TBI S" for soil stabilisation factor S and "TBI k" for soil decomposition rate k (soil nutrient cycling), and "Inf rate" for water infiltration rate in soil (flood control).

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