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Pollinators in a changing World: long term variations and pesticide pressure in agricultural systems

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“Per fer les coses bé cal:
primer, l’amor a elles;
segon, la tècnica”

-Antoni Gaudì

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Pollinators in a changing World: long term variations and pesticides pressure in agricultural systems

Abstract

Insect pollinators play a fundamental role in sustaining biodiversity and human well-being by providing pollination services to nearly 90% of flowering plant species. However, their populations are increasingly threatened by environmental changes driven by human activities, such as urbanization, agricultural intensification, and pollution. Among insect pollinators, bees are the most important group in terms of abundance and effectiveness, ensuring the pollination of approximately 75% of crops.

Despite their crucial ecological and economic importance, significant knowledge gaps remain for 14% of bee species, while 10% of them are recognised as threatened. Long-term studies are therefore essential to investigate species distribution across time and space. In this context, entomological collections represent a valuable resource for obtaining information on species occurrence and for assessing changes in functional traits, as highlighted by several studies. Alongside, many studies have highlighted the role of pesticides in the decline of pollinators. Among these, fungicides and insecticides, either alone or in combination, are toxic to many of them, while herbicides reduce the availability of food resources. Undoubtedly, orchards and other crop systems are the environments where pollinators are mostly exposed to pesticides, although these compounds can spread through the environment and contaminate natural resources. In this regard, the role of pesticide exposure via wild plants growing near cultivated areas remains unclear. At the same time, information concerning pollination deficits, as a consequence of pollinator decline, is limited to few crops.

The aims of this thesis are: to evaluate the variation of pollinator community, in terms of species richness and functional diversity, in northern Italy through a comparison with a historical entomological collection, (Chapter 1); to assess the pesticide contamination in crop and wildflowers in apple orchard, a widespread European crop system, as well as the potential risk posed to pollinators (Chapter 2); to assess pollination deficits in an important and understudied European crop, *Pyrus communis*, in relation to pollinator communities, landscape characterization and pesticide risk (Chapter 3).

In Chapter 1, we present the digitization of the Guido Grandi Apoidea collection of the University of Bologna, and the repeated sampling of 4 well represented sites, with the aim to assess whether bee communities have changed over almost a century. We also measured body size for some species and analysed whether differences in bee communities were related to their ecological traits. The results revealed important changes in species composition and diversity, as past and present communities presented a high species turnover, with significant reduction of species in the city area. By contrast, ecological traits were not affected by the environment across time periods, as differences in trait frequencies were not significant.

In Chapter 2, we report the assessment of pesticide contamination and risk in crop and wildflowers in apple orchards across Europe. In fact, the study involved 4 countries (Italy, Spain, Poland and Sweden) and a total of 27 orchards, where flowers were collected in spring and summer, inside and outside orchards. Multiresidue analysis was performed, 90 different compounds were detected, and the related risk was assessed. The aim was to understand if pesticide contamination and risk differed between crop and wildflowers and between seasons, and if it was related to landscape characterization. Our results show that overall pesticide contamination was pervasive across flower samples, as just 8.8% of the 170 samples analysed was free of pesticides. Moreover, risk was higher in spring, during apple bloom, but did not disappear in summer, after crop bloom, due to the increased insecticides application. Overall, our results raise concerns about the management of agroecosystems, where the availability of floral resources is essential to attract pollinators and consequently sustain pollination services.

In Chapter 3 we explored if *Pyrus communis*, an important self-incompatible crop which is understudied respect to other crops, experienced pollination deficits and how this related to pollinator communities, landscape characteristics and pesticide risk. To achieve these objectives, pollination treatments (open, supplementary and pollinator exclusion) and pollinator surveys were performed; pear flowers were analysed for pesticides residues, and a risk index was calculated. Additionally, the proportion of pollinator-friendly landscape was assessed at three spatial scales and its effect on pollinator's visitation rates was tested. The results reported widespread pollination deficits and confirmed the high dependence on pollinators of *P. communis*. We also found that all flowers were contaminated with at least 4 pesticides, and the visitation rate of wild bees on pear flowers was positively related to pollinator-friendly landscape at 1.5 km. Moreover, the most common managed pollinators, *Apis mellifera* and *Bombus terrestris*, had no or negative effect on seed set, respectively. Our results therefore confirm the importance of natural and semi-natural areas surrounding crops to enhance pollination services and raise concerns about the management of pollination in agricultural systems.

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1. General Introduction

1.1 The importance of pollinators and pollination services

Ecosystem services are the benefits that nature provide to humans, and pollination is among the most important (IPBES 2016; Potts et al. 2016; Liere et al. 2017). Pollination is the transfer of pollen from male to female structures of plants - anthers and pistils in Angiosperms, respectively- representing the first step towards fertilization. Pollination can occur within the same flower (*autogamy*), between flowers of the same plant (*geitonogamy*) or between flowers of different plants (*allogamy*). This process can be mediated by wind and water; however, the majority of wild and cultivated plants relies on animals for pollination. Among them, insects play a fundamental role: nearly 90% of flowering plants, including 75% of crops, depend on insect pollinators, which thereby promote biodiversity and ensure food security (Klein et al. 2007; Potts et al. 2010; Ollerton 2017). Consequently, pollinators ensure one third of global food production and contribute to agriculture for $235–$577 billion annually (IPBES 2016; Tscharntke et al. 2024). Moreover, it has been demonstrated that animal pollination increases crop yield, seed production and fruit quality (Gazzea et al. 2023; Siopa et al. 2024; Eeraerts et al. 2025). At the same time, the area dedicated to pollinator-dependent crops has increased considerably over recent decades (Aizen et al. 2019). Several of the most important crops, such as apples, almonds, coffee and pears, are self-incompatible; therefore, their dependence on pollinators is remarkable (Delaplane and Mayer 2000; Klein et al. 2007). Self-incompatibility is a genetic mechanism that prevents inbreeding and thus cross-pollination among inter-compatible cultivars is required to set seed and fruit. For this reason, managed honeybees (*Apis mellifera*) are employed to supply pollination services, even if they are not so efficient in self-incompatible crops (Saez et al. 2022). In light of their fundamental role in the provision of ecosystem services, it becomes of primary importance to preserve diverse and abundant pollinator communities.

Animal pollinators are mostly insects, but at some latitudes they can also include birds and mammals (Ollerton 2017). Insect pollinators are namely Hymenoptera (especially bees), Diptera (especially syrphids) and Lepidoptera. Bees, with almost 20,000 species described worldwide, represent the most relevant group of pollinators in terms of abundance and relevance both for wild plants and crop species in most ecosystems, with managed honeybees and bumblebees playing an important role in Europe and North America (Klein et al. 2018). Bees are considered excellent pollinators because they are the only group that rely almost completely on floral resources both as adults and larvae, while syrphids only visits flowers in their adult stage (Ollerton 2017; Dunn et al. 2020; Larkin and Stanley 2021). Bees visit flowers for nectar, which is their primary source of sugars, and pollen, which is the main source of protein during the life cycle of all bee species; furthermore, pollen is collected to feed larvae, therefore becoming an important resource for offspring development (Fisogni et al. 2017). Bees also exhibit considerable variation in ecological traits, such as nesting habits, foraging behaviour, feeding specialization, sociality and flight range. In addition, they are extremely diverse in morphological traits such as body size and hairiness, which influence their ability to touch floral reproductive structures and thus determine their effectiveness as pollinators (Kendall et al. 2018; Chloe et al. 2019; Roquer-Beni et al. 2022). Moreover, body size affects the quantity of pollen they can load on collection structures (e.g. corbiculae, ventral scopae) with repercussion on the offspring (Chloe et al. 2019), as well as the number of pollen grains they can move between flowers (Kendall et al. 2018; Herrera et al. 2023). Pollinator effectiveness is determined also by the quality of pollen they carry; indeed, for self-incompatible plants it is fundamental to receive pollen from other compatible cultivars (Quinet and Jacquemart 2017). The strict relationship that exists between plants and their pollinators is the wider example of coevolution, a concept that was developed by Federico Delpino in 1873-74 (Waser et al. 2011). Coevolution can be defined as reciprocal evolutionary change in interacting species (Thompson 1982), where each of the species involved exerts selective pressure on the other. Therefore, the disruption of these interactions poses at risk the

resilience of ecosystem functions (Zimmermann et al. 2023). Indeed, there is increasing evidence that pollinator communities are facing important decline.

1.2 Pollinator decline: trend, causes and consequences

Biodiversity and ecosystem services are under threat, with pollination services being particularly at risk; in fact, global human population has increased sharply in recent decades, leading to a growing demand for food production (Potts et al. 2010; Aizen et al. 2019). Yet mounting evidence indicates that insect pollinators are undergoing a steady decline (Sánchez-Bayo and Wyckhuys 2019; Scharnhorst et al. 2025).

The main drivers of pollinators decline include land use change due to agricultural expansion and urbanization, agricultural intensification and climate change (Potts et al. 2010; LeBuhn and Vargas Luna 2021; Brasil et al. 2023). Agricultural expansion and urbanization lead to the loss of natural habitats, reducing the availability of nesting sites and food resources for pollinators. On the other side, intensive agriculture directly affects pollinators through the massive use of pesticides, which are toxic to many species, alone or in combination with other chemicals (Tosi et al. 2018; Rondeau and Raine 2022), but also indirectly by reducing the availability of food resources with herbicides (Liere et al. 2017). At the same time, modern agriculture is namely based on monocultures, which represent a pulse of food just for a limited time, thus reducing the diversity of floral resources in agroecosystems (De Palma et al. 2015; Hass et al. 2018). Likewise, urbanization led to habitat fragmentation, with consequent reduction in availability of nesting sites (Fauviau et al. 2022) but also limiting dispersion of species with a reduced flight range (Brasil et al. 2023). For this reason, natural and semi-natural areas are recognized as necessary to provide food and nesting sites to pollinators (De Palma et al. 2015). Some studies have also highlighted the positive effect of such landscapes on the provision of pollination services to important crops (e.g. cherry, apple, blueberry) (Holzschuh et al. 2012; Blaauw and Isaacs 2014; Tamburini et al. 2020; Eeraerts 2023). Another driver is climate change, with a clear increase in temperatures and frequency of extreme events, impacts the phenology of species and may cause mismatches in plant–pollinator interactions (Burkle et al. 2013; Rakosy et al. 2022; Reeves et al. 2022). Notwithstanding the importance of pollinators and the ecosystem services they provide, substantial knowledge gaps remain. In 2014, more than 50% of bee species were classified as “Data Deficient” (Bartomeus et al. 2013), whereas this proportion has currently decreased to 14% (IUCN 2025). However, over the same period, the proportion of threatened bee species has more than doubled, compared to the 9% reported in Nieto et al. 2014.

As a direct consequence of pollinator decline, production shortfalls are expected and have been already documented for many important crops like apples and other self-incompatible crops (e.g. kiwifruit, coffee) (Garratt et al. 2021; Castro et al. 2021; Garratt et al. 2022; Bordoni et al. 2024; Turo et al. 2024). Sub-optimal production is defined as “pollination deficit”, which derive from insufficient quantity and quality of pollen deposited on the stigmas (Vaissière et al. 2011). Pollination deficits are also related to weak pollinator community and mismatches between flowering season and pollinator activity (Polce et al. 2014; Reilly et al. 2020). Pollinators are crucial also for wild plants, as lower pollinator diversity is related to lower plant diversity, as reported by the study of Ramos-Jilberto et al. (2020). Given that almost 90% of plants rely on insect pollinators, their decline would impact the stability of natural and agricultural ecosystems.

Anthropogenic environmental changes, such as intensive agriculture, pollution, and urbanization, have occurred rapidly over recent decades. Consequently, significant knowledge gaps remain regarding the long-term effects of these changes on species, especially pollinating insects. In this context, entomological collections, which are repositories of spatial and temporal distribution of species, represent a valuable resource of information (Rakosy et al. 2022). The loss of certain species could disrupt key plant–pollinator interactions; therefore, assessing the functional diversity of pollinator communities is also of great importance (Fisogni et al. 2025). The first objective of this study is to evaluate long-term changes in bee communities in northern Italy through comparison with an entomological collection (Chapter 1).

The functional traits of pollinators play a crucial role in determining how these organisms respond to toxic substances such as pesticides (Basu et al. 2024). However, in the field of ecotoxicology, considerable uncertainty remains regarding how pollinators become exposed to pesticides in the field, and the degree of toxicity of these widespread compounds. For this reason, the second objective of this work is to investigate the impact of pesticides by focusing on the exposure pathway through flowers within agroecosystems, precisely where pollinators are essential to provide pollination services and ensure food security (Chapter 2).

Finally, although several studies have examined the effects of pollinator decline on the production of major crops such as apple, cherry, and blueberry (Holzschuh et al. 2012; Eeraerts et al. 2020; Garratt et al. 2022; Graham et al. 2024), relatively few have focused on other relevant crops, such as pear. The third objective of this research is to assess pollination deficits in pear orchards in relation to pollinators and landscape context (Chapter 3).

1.3 Use of entomological collections to study pollinators communities across time

Entomological collections provide important ecological information about the past, containing data that in some cases span decades to centuries and allowing researchers to determine insect responses to environmental changes over time (Kharouba et al. 2018; Davis et al. 2022). Although museum records are often biased by collectors' interests and sampling effort is usually unknown, specimen labels provide information on species occurrence at specific times and locations, thereby documenting their geographical and temporal distribution (Kharouba et al. 2018; Rakosy et al. 2022). Several studies have analysed trends in pollinator communities over time using historical collections, documenting changes both in species richness and in functional traits (Bartomeus et al. 2013; Burkle et al. 2013; Zimmermann et al. 2023; Graham et al. 2024; Fisogni et al. 2025). Functional traits include sociality, dietary specialization, tongue length, flight range, voltinism and nesting habits. Other studies have focused on morphological traits, particularly body size, which is strongly influenced by the environment. For instance, body size is determined during larval development, meaning that disturbances during this stage, or reduced quantity or quality of food provision, can directly affect it (Theodorou et al. 2020). Moreover, the quantity of pollen that bees can load in their collection structures (e.g. corbiculae, ventral scopae) is directly linked to body size (Chloe et al. 2019). Conversely, body size is also related to pollinator effectiveness, as it influences the ability of individuals to contact floral reproductive structures and thus to ensure pollination (Ollerton 2017; Kendall et al. 2018; Roquer-Beni et al. 2022). In addition, pollen loads preserved on specimens' bodies have been analysed to assess plant–pollinator interactions across time (Kleijn and Raemakers 2008; Gous et al. 2018; de Araújo Romeiro et al. 2023). In recent years, increasing attention has been devoted to the digitization of historical collections, which promotes collaboration and facilitates data sharing within the scientific community (Cobb et al. 2019).

In the first chapter of this work, we present the digitization of the Apoidea preserved in the Guido Grandi collection of the University of Bologna, which comprises more than 6,000 specimens housed in 114 entomological boxes collected between the 1920s and 1960s. We then used this data to assess variation in bee communities over almost a century in Italy.

1.3.1 A tribute to the entomologist Guido Grandi

The entomologist and professor Guido Grandi (1886-1970) was the founder of the Entomological Institution of the University of Bologna in 1926, and in 1928 of an entomological journal now named "Bulletin of Insectology" (https://bulletinofinsectology.org/). Guido Grandi produced more than 200 works concerning the biology of Hymenoptera Aculeata, of which the most important is the "*Introduction to the study of entomology*" consisting of 2,300 pages.

Even though at that time entomological collections were considered a prerogative of amateurs, Grandi believed that "*a well-structured and rationally organized collection is fundamental for any kind of entomological study*", thus conferring a high teaching and scientific value to collections. In fact, he

pledged to build a consistent collection as described by himself in 1959: "*Insects, fortunately, both because of their size and their nature as arthropods, are particularly well suited to being collected and preserved in large quantities and in excellent condition…. Leaving no opportunity unused, neither during the numerous journeys and excursions undertaken, nor during summer holidays, nor on any other occasion, knowing what we sought to achieve and where we needed to search, always keeping a watchful eye for the appearance of any species and an attentive ear for every report, never losing sight of particular biotopes, making use of the most varied methods of research, hunting, and capture, and acquiring interesting material whenever the opportunity arose, we have managed, without in any way straining our scientific activity, to assemble, prepare, and have identified over one million insects, as well as to discover a remarkable number of species new to science*." Grandi was a passionate researcher and teacher, to the point that he took action to save the entomological collection from the bombings of the Second World War, as he himself wrote: "*The responsibility that I had, regarding the conservation of these treasures, was big, but bigger was the love that tied me to them and the desire rooted in me to save them whatever the cost".*

His work and research were continued by his student Maria Matilde Principi (1915-2017) and then from Giorgio Celli (1935-2011) and colleagues. This work is part of the ongoing activity of the group led by professors Giovanni Burgio, Maria Luisa Dindo and Fabio Sgolastra.

1.4 The role of pesticides in pollinator decline

Pesticides are among the principal anthropogenic pressures on insect pollinators (Basu et al. 2024). Large amount of pesticides are applied to boost agricultural production to meet the growing demand for food. Pesticides include several classes of agrochemicals, such as insecticides, fungicides, herbicides, with insecticides being undoubtedly the most harmful for pollinators. Among them, neonicotinoids, pyrethroids and some organophosphates are considered particularly hazardous to bees. Great attention has been posed to neonicotinoids, a class of systemic insecticides developed in the early 1990s, due to their high toxicity to honeybees, their persistence and mobility across the environment (Lonkg and Krupke 2016; Sgolastra et al. 2020). Many studies have reported the presence of neonicotinoids in different environmental matrices, such as soils, water and wildflower plants (Goulson 2013; Botìas et al. 2015; David et al. 2016; Garcìa-Valcàrcel et al. 2022; Ward et al. 2022). In fact, pesticides applied to crops may spread in the surrounding landscape via aerial drift or rain runoff, thus contaminating non-target plants (Zioga et al. 2022). Pollinators can therefore be exposed to pesticides through different routes (e.g. nectar, pollen, water), raising concerns for chronic exposures. Indeed, some studies have found multiple compounds in bees or honeybee collected pollen (David et al. 2016; Botìas et al. 2017; Tosi et al. 2018; Main et al. 2020). Moreover, the concurrent exposure to different compounds may lead to synergistic effects. For instance, fungicides, generally considered relatively safe to bees, have been shown to increase toxicity of other compounds (Sgolastra et al. 2018; Tosi and Nieh 2019). Although many of these compounds are not lethal to bees, they can cause sublethal effects that alter bee physiology and behaviour, with potential consequences for species fitness and plant–pollinator interactions (Siviter et al. 2018). In addition, pesticides risk assessment is usually based on the honeybee *Apis mellifera*, while other bee species may respond differently according to their life history traits (Arena and Sgolastra 2014; Jütte et al. 2023; Basu et al. 2024; Catania et al. 2025). To mitigate pesticides impacts on pollinators, at the European level it has been proposed to restore field edges and establish floral strips, as well reduce mowing to provide pollinators with food resources and nesting sites (Blaauw and Isaacs 2014; Rundlöf et al. 2022). However, these measures, highly attractive to pollinators, may become harmful if contaminated by pesticides as demonstrated by several studies (Botìas et al. 2015; David et al. 2016; Peterson et al. 2021; Ward et al. 2022; Zioga et al. 2023; Graham et al. 2024).

In the second chapter of the thesis pesticide contamination of the target crop and wildflowers collected in a widespread European crop, *Malus domestica*, was assessed with the aim to evaluate the potential risk for insect pollinators in the agroecosystem.

1.5 Pollination deficits as a consequence of pollinator decline

Declines in pollinator communities raise serious concerns about the reduction of pollination services, with negative consequences for crop productivity and farmers' economic incomes (IPBES 2016). Indeed, several studies reported shortfalls in production of many important crops (e.g., apples, kiwifruit, pears) (Gianni and Vania 2018; Holland et al. 2020; Castro et al. 2021; Garratt et al. 2021; Hünicken et al. 2021; Li et al. 2022; Bordoni et al. 2024; Siopa et al. 2024; Turo et al. 2024). Production shortfalls as a consequence of low pollination services are defined as *pollination deficit*, which can result from inadequate quantity and quality of pollen deposited on the stigmas, flowering asynchrony between cultivars and pollinators, and weak pollinator communities (Vaissière et al. 2011; Polce et al. 2014; Reilly et al. 2020). Pollination deficits are measured on initial and final fruit set, which are the metrics for pollination and production, respectively (Garratt et al. 2022), and seed set, which influence fruit quality (Webber et al. 2020; Olhnuud et al. 2022). The evaluation of pollination services is particularly relevant for self-incompatible crops, that require cross pollination between compatible cultivars (Goldway et al. 2019). Therefore, it becomes important the design of the orchards to facilitate pollen transfer (Quinet and Jacquemart 2017). Moreover, maintaining diverse pollinator communities is essential, as pollinator effectiveness varies greatly among species (Eeraerts et al. 2020; Vassvik et al. 2025). For instance, effectiveness is strongly influenced by foraging behavior and morphological traits such as body size and hairiness (Roquer-Beni et al. 2022). Honeybees, for example, often move along tree rows rather than between rows, which can hinder cross-pollination among cultivars (Monzón et al. 2004; Quinet and Jacquemart 2017). Conversely, some wild bees, such as *Osmia* spp., have been shown to be more effective pollinators, transferring larger quantities of pollen per flower visit and spending more time on flowers (Monzón et al. 2004; Eeraerts et al. 2020; Roquer-Beni et al. 2022). Pollination deficits have been thoroughly studied in an important crop such as apples (*Malus domestica*) (Garratt et al. 2021, 2022; Roquer-Beni et al. 2021; Olhnuud et al. 2022) but there are other relevant crops, such as pears (*Pyrus communis*), which are less studied and present low pollination services due to low attractiveness of flowers. In the third chapter of the thesis the evaluation of pollination deficit and pollination services in European pear was performed in several orchards of Emilia-Romagna region (northern Italy).

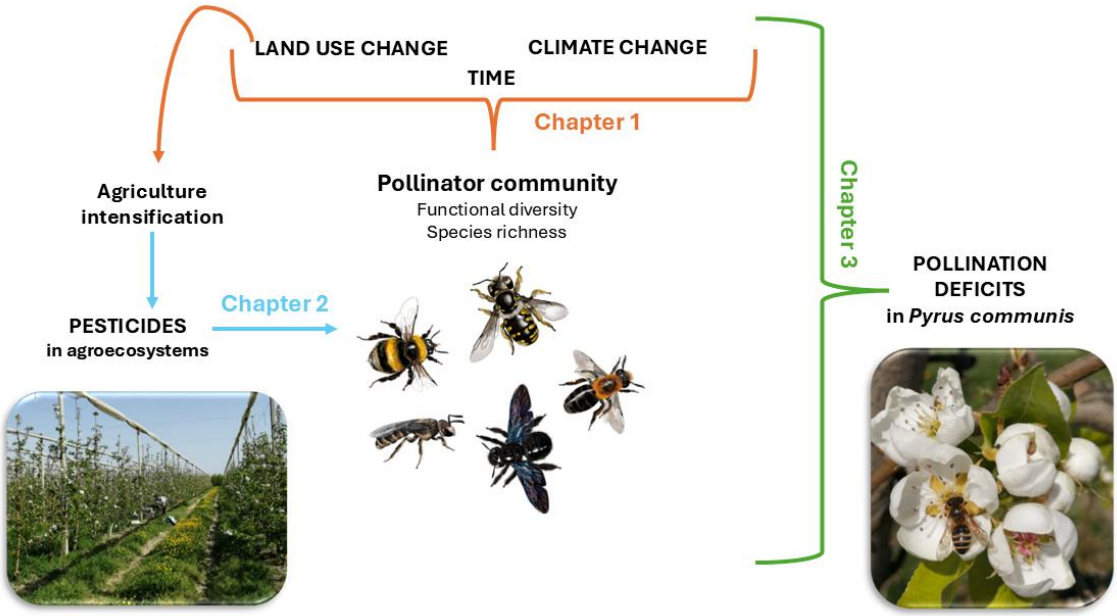

Figure 1. Conceptual framework of the thesis. Chapter 1 regards long term population dynamics of pollinators; chapters 2 and 3 regard pressures from pesticides to pollinators and pollination services, respectively.

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Chapter 1

Long-term variation in bee species diversity composition and ecological traits in northern Italy.

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Abstract

Bees are the most relevant group of pollinators, providing pollination service to almost 90% of flowering plants. However, there is evidence of their decline and, at the same time, around 14% of bee species is still categorized as *data deficient* in the European Red List. Entomological collections are a source of information about species distribution across time and space, but also on how they respond to environmental changes, therefore they can be used to fill these knowledge gaps. In the present study, we digitized the Guido Grandi Apoidea collection of the University of Bologna (Italy) that preserves more than 6,000 specimens collected between the 20s and 60s of the past century, and resampled in 2021 and 2022 four of the most represented sampling sites, two in the city of Bologna and two in the Apennines. Beta diversity of bee communities, in terms of presence/absence, between past and present was measured through the Sørensen index and rarefaction curves were realized to compare species richness. Data on ecological traits, including nesting habits, sociality level, lecty and voltinism, were collected (through literature and dedicated websites) to analyze the ecological diversity of bee communities. At the same time, body size was measured as ITD (Inter Tegular Distance) for species that presented at least 3 individuals per sex per period, accounting for 234 bees measured belonging to 36 species. The results reveal an overall decline in species richness, significantly in the city macro area, as well as changes in bee diversity between periods with low species similarity between communities. However, we did not observe significant differences in ecological traits and body size.

Keywords: *species richness, historical collections, bee community, body size*

1. Introduction

Insects represent more than half of all species described and provide crucial ecosystem services that are essential for humans, including pollination of 87% of flowering plants (Ollerton et al. 2011). However, an increasing body of evidence reports widespread insect decline across many regions of the World, posing at risk food security but also natural ecosystems stability (Potts et al. 2010). Bees are considered the most relevant group of pollinators thanks to their abundance, with almost 20,000 species worldwide, and pollination efficiency respect to other pollinators (Bartomeus et al. 2018; Klein et al. 2018). Indeed, bees rely completely on floral resources (nectar and pollen) both as adults and larvae, hence their life is strictly linked to flowers. Bees also display a wide variety of ecological traits such as sociality level, nesting habit and feeding specialization, known as *lecty,* that can influence their interactions with the environment (Michener 2007). Climate changes and landscape alterations due to human activities (e.g., urbanization, agricultural intensification, industrialization) have been identified as two of the most important drivers of bee decline worldwide (Dicks et al. 2021). However, notwithstanding their ecological and economical importance, there is still a relevant lack of information how the occurrence and distribution of bees have changed overtime and how the ecological traits of different bee species influence their response to environmental changes (Marshall et al. 2024; Sentil et al. 2026). Entomological collections provide important ecological information of the past, containing data which cover decades to centuries in some case, and allow to determine insects' responses to environmental changes over time (Kharouba et al. 2018; Davis et al. 2022). After all, they are important data source on species occurrence and geographical distribution, as well as repository of morphological and ecological traits (e.g. wing size, body size, pollen load) which can be used to study long-term variation (Meineke et al. 2018; Rakosy et al. 2022; Fisogni et al. 2025). In recent years, entomological collections have received great attention from the scientific community, for which one of the primary goals is to make these data easily accessible thorough digitization (Ariño 2010; Blagoderov and Smith 2012; Nooten and Rehan 2019; Davis et al. 2022). The digitization process includes different stages, where the first one is the transcription of the labels. For instance, labels usually report the taxon name, date and place of collection, thus representing the geographical and temporal distribution of the species (Hedrick et al. 2020; Rakosy et al. 2022). Although museum records may be biased by collectors' interests and sampling effort is often unknown, several studies have analyzed trends in pollinator communities over time using historical collections, documenting changes both in species richness and in functional traits (Bartomeus et al. 2013; Burkle et al. 2013; Zimmermann et al. 2023; Graham et al. 2024; Fisogni et al. 2025). Other research has focused on morphological traits, particularly body size, which is strongly influenced by the environment (Gérard et al. 2020; Nooten and Rehan 2020; Herrera et al. 2023). For instance, body size is determined during larval development, meaning that disturbances (e.g. fluctuating temperatures) during this stage, or reduced quantity or quality of food provision, can directly affect it (Radmacher and Strohm 2010; Oliveira et al.2016; Theodorou et al. 2020). Moreover, the quantity of pollen that bees can load into their collection structures (e.g. corbiculae, ventral scopae) to feed the larvae is directly linked to body size (Chloe et al. 2019; Cullen et al. 2021). Conversely, body size is also related to pollinator effectiveness, as it influences the ability of individuals to contact floral reproductive structures and thus to ensure pollination (Ollerton 2017; Kendall et al. 2018; Roquer-Beni et al. 2022).

The Mediterranean Basin is considered a hot spot for bee diversity with the highest percentage of endemism, and Italy is one of the richest countries hosting more than 1,000 species described (Revertè et al. 2023; Cornalba et al. 2024). A thorough and modern revision of the Italian bee fauna is still missing, even though the country's biogeographical setting at the crossroads of several faunal regions makes it exceptionally diverse. This lack of synthesis likely reflects both the limited historical interest of specialists in the Italian fauna, due to its relatively low level of endemism respect to other Mediterranean countries, and the scarcity

of national experts on Hymenoptera Anthophila during much of the twentieth century (Cornalba et al. 2024). An important contribution to the knowledge of Italian wild bee fauna, was derived from the entomologist Guido Grandi (1886-1970) who, in the 1926 founded the Institute of Entomology at the University of Bologna; his entomological collection holds 114 boxes of Apoidea, with more than 6,000 specimens belonging to 535 species collected between the 20s and 60s of the past century. The majority of specimens derives from Italy, but other 11 European and 4 African countries are also represented. However, most specimens were collected in the Emilia-Romagna region (northern Italy), namely near the city of Bologna were Grandi lived and taught, and in the Apennines where he used to spend holidays.

The aims of this study were to i) study long-term variation in species diversity composition, to ii) study variation in ecological traits between past and present bee communities, and iii) to assess variation of body size in some representative species. To achieve these objectives, four well represented sites in the Guido Grandi Apoidea collection were selected and were resampled over two consecutive years (2021-2022) using standardized protocols. Based on species presence/absence data, community composition was compared across two time periods. Variations in ecological traits were evaluated to understand how bee community responds to environment changes. In addition, a subset of 36 species was selected for body size measurement to assess temporal changes in this key morphological trait.

2. Materials and Methods

2.1. Historical specimen's digitization and selection

All Apoidea specimens of the Guido Grandi collection (hereafter called "IEGG", Flaminio et al. 2026) were digitized by transcribing the label data; a dataset has thus been created for all 6,391 specimens, including family, genus, species, sampling date and georeferenced location. The initial dataset included 747 species, but all specimens were taxonomically revised and the dataset resulted in 535 species. The number of species was reduced due to wrong identifications (N= 610 specimens), but also due to changes in the taxonomy (e.g. incorporation of subspecies into other species). The full dataset of bee species in the IEGG is available on request.

To assess variation in species composition between past and present, historical data were filtered according to the most represented sampling sites of the Emilia-Romagna region. Then, given that many years in the IEGG had very few specimens (N<10), we identified two pairs of consecutive years with the highest sampling intensity (1925–1926 and 1934–1935) and considered as the "past" period. These periods were selected to ensure temporal comparability with the contemporary dataset and to minimize biases associated with uneven historical sampling effort (Zimmermann et al. 2023; Scharnhorst et al. 2025). *Apis mellifera* was excluded from the dataset because its presence is strictly related to human activity.

2.2. Study site and bee surveys

Four sites were selected within two macro areas in the Emilia-Romagna region (northern Italy): two located in the urban area of Bologna (44°29'59"N, 11°21'09"E and 44°28'38"N, 11°19'14"E) defined as "City" and two in the Apennines mountain chain (44°14'58"N, 11°08'20"E and 44°20'41"N, 10°59'42"E) defined as "Apennines" (Figure 1). For the Apennine sites, sampling locations could not be precisely identified, therefore broader areas were reconstructed from label descriptions and are representative of semi-natural habitats between 500-600 meters of elevation. These areas were mostly arable land and experienced a depopulation after Second World War (Figure S1).

The urban sites correspond to the Botanical Garden of the University and a city park (Villa Ghigi), both characterized by management of vegetation within an increasing population and urbanized matrix, as shown

by the orthophotos from 1944 and 2021 (Figure S2). For these sites, we were able to determine exact locations based on detailed information from specimen labels.

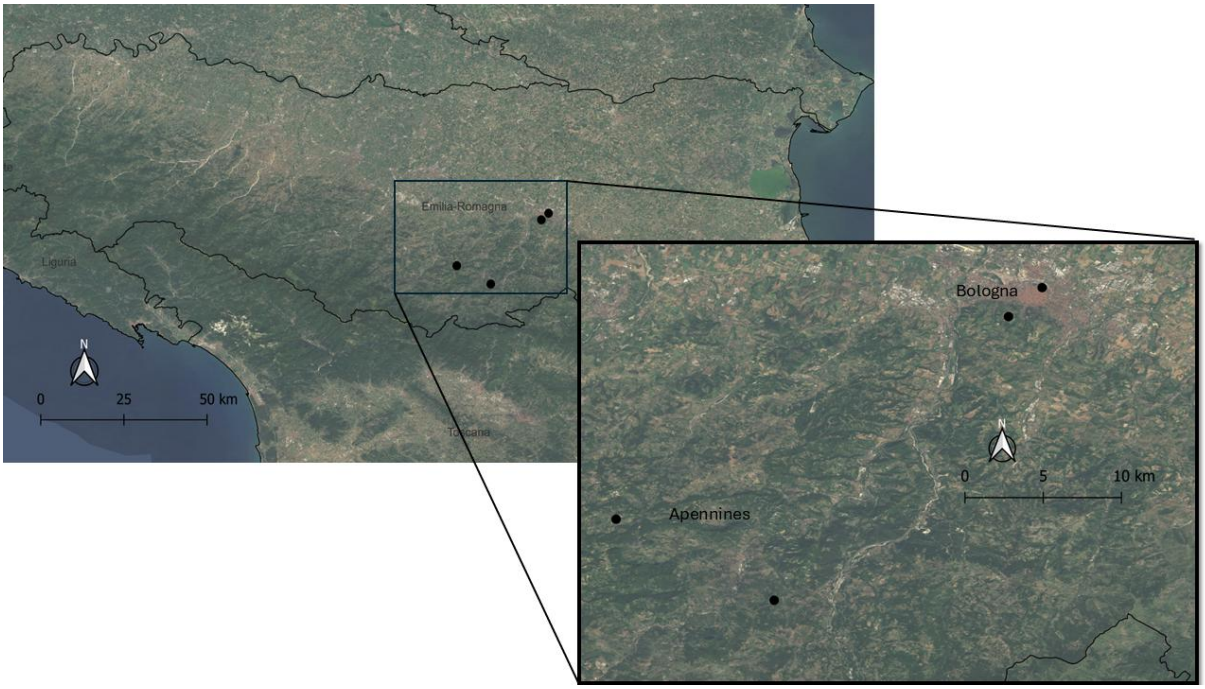

Figure 1. Map showing the study areas, which include two sites in the city of Bologna and two sites in the Apennines.

In each site, pollinator surveys were performed once a month from April to September in 2021 and 2022, during central hours of the day with adequate weather conditions (T≥ 15°C, no wind/cloud), by slowly walking transects of 1.30 hours. Using a sweep net, around 20-30 different individuals per site were collected in each survey, put in falcon tubes with ethyl-acetate and kept at -20°C until pinning and identification.

2.3. Ecological traits

To study variation of bee community in terms of ecological traits, the following traits were selected: sociality, nesting habit, lecty and voltinism. Information on traits was gathered from the literature (Westrich 1989; Michez et al. 2019) and from web platforms BeeTraits (https://beetraits.linnaeus.naturalis.nl/linnaeus_ng), Global Biodiversity Information Facility (GBIF) and Atlas Hymenoptera (http://www.atlashymenoptera.net). Since some traits can include multiple categories (e.g. primitively eusocial for sociality; cavity-nesting for nesting), we standardized them into binary classes in order to allow consistent comparisons across species and time periods: social/solitary, above/below ground nesting, polylectic/monolectic, monovoltine/bivoltine (Fauviau et al. 2022). Brood parasite species were assigned the nesting and lecty traits of their host species (Fisogni et al. 2025); when this information was not available, they were not considered in the analysis.

2.4. Body size measurement

To assess changes in body size, species with at least 3 specimens/sex/period were selected, resulting in 36 species and 234 bees (150 ♀ and 84 ♂). Body size was measured using ITD (Inter Tegular Distance) under a light microscope with a micrometre following Cane (1987). The list of species measured is reported in table S1.

2.5. Data analysis

All analyses were conducted in R version 4.4.1 (R Development Core Team, 2024).

2.5.1. Long-term variation in species diversity composition

To compare species diversity of bee communities, the Sørensen similarity index was calculated:

$$\text{Sørensen} = \frac{2i}{a+b}$$

Where *i* is the number of species in common between past and present, *a* is the number of species in the past and b is the number of species in the present. The index ranges from 0 (no species in common) to 1 (all species in common) (Fauviau et al. 2022; Zimmermann et al. 2023).

The Sørensen index was calculated at family level for the overall dataset and each macro area, city and Apennines.

Species richness was compared between the two sampling periods ("Past" and "Present") using rarefaction and extrapolation cures with a Hill number of order q = 0 (species richness), using the package iNEXT version 3.0.1 (Hsieh et al. 2016). Sample coverage (SC) was used to assess sampling completeness, and 95% confidence intervals were computed to evaluate the statistical significance of differences between assemblages. The analysis was performed for the general dataset and for each macro area (City and Apennines).

2.5.2. Variation in ecological traits between past and present bee communities

To analyse differences of bee communities in relation to ecological traits, we first "cleaned" the dataset removing repeated specimens to obtain species presence/absence. We then calculated relative abundances of each trait and performed Non-metric Multidimensional Scaling (NMDS) using a binary Bray–Curtis distance matrix and PERMANOVA (999 permutations) implemented in the *vegan* package (Oksanen et al. 2024). PERMANOVA is sensitive to differences in dispersion among groups, therefore we first tested for homogeneity of dispersion using the *betadisper* function in the *vegan* package. Trait frequencies were calculated across the two time periods (past vs. present) and macro-areas (City vs. Appennines) and graphically visualized. Subsequently, we performed trait-specific analyses to test whether the frequency of individual ecological trait categories (nesting, sociality, lecty and voltinism) differed between periods and macro-areas. Due to the limited number of spatial replicates (two sites per macro-area), we assessed shifts in trait proportions between the past and present for each macro-area (City and Appennine) using Fisher's Exact Tests.

2.5.3. Variation of body size

To assess variation in body size, Generalized Linear Mixed Models (GLMMs) were fitted with the lme4 package (Bates et al. 2015), where ITD was the response variable while *period*, *sex*, as well as their interaction (*period*sex*), and *macro-area* were included as fixed effect predictors, and *bee species* was included as random effect (following Herrera et al. 2023). Model assumptions were evaluated using residual diagnostics, including visual inspection of residual plots and simulated residuals implemented in the DHARMa package (Hartig, 2025).

3. Results

3.1. Long-term variation in species diversity composition

The historical dataset comprised 1,485 bee specimens belonging to 37 genera and 215 species. In contrast, with the recent surveys we captured 895 specimens from 37 genera and 152 species. Of these, 89 species were present in both periods, and the Sørensen index was 0.49.

In the Apennines macro area, the past period included 663 specimens, 26 genera, and 112 species, whereas the present period recorded 432 specimens, 26 genera, and 104 species. The Sørensen index was 0.38 and ranged from 0 (Melittidae) to 0.54 (Halictidae). The family Andrenidae had the second lowest index (0.23).

In the city macro area, the past dataset consisted of 822 specimens, 28 genera, and 154 species, while the present recording comprises 463 specimens, 30 genera, and 105 species. The Sørensen index was 0.39 and ranged from 0.23 (Andrenidae) to 0.5 (Melittidae).

The relative abundance of species within families for each period in the Apennine and City area are reported in Figure 2*a* and *b*, respectively.

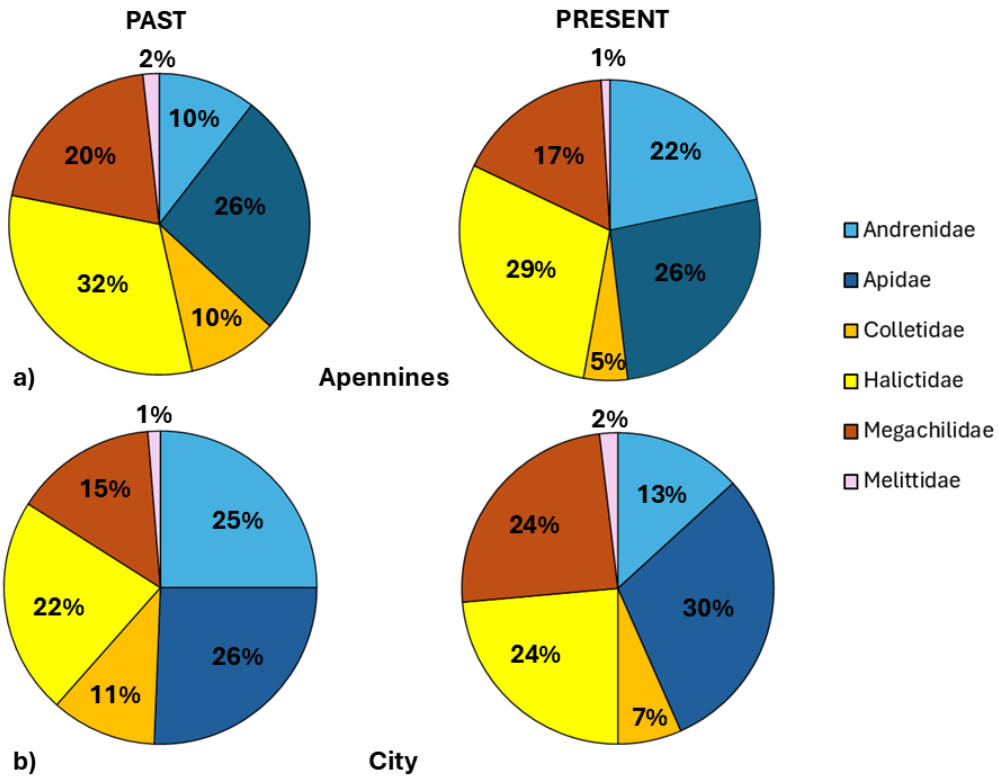

Figure 2a, b. Relative abundance of species within the six bee families between past (left) and present (right) for the Apennines (a) and the City (b).

Overall, species richness was significantly different between the two periods, with lower species richness in the present period (Figure S3).

In the Apennines macro area, species richness was not significantly different between past and present, since 95% CI of accumulation curves did overlap (Figure 3a). By contrast, the city macro area shows a significant reduction in species richness (Figure 3b).

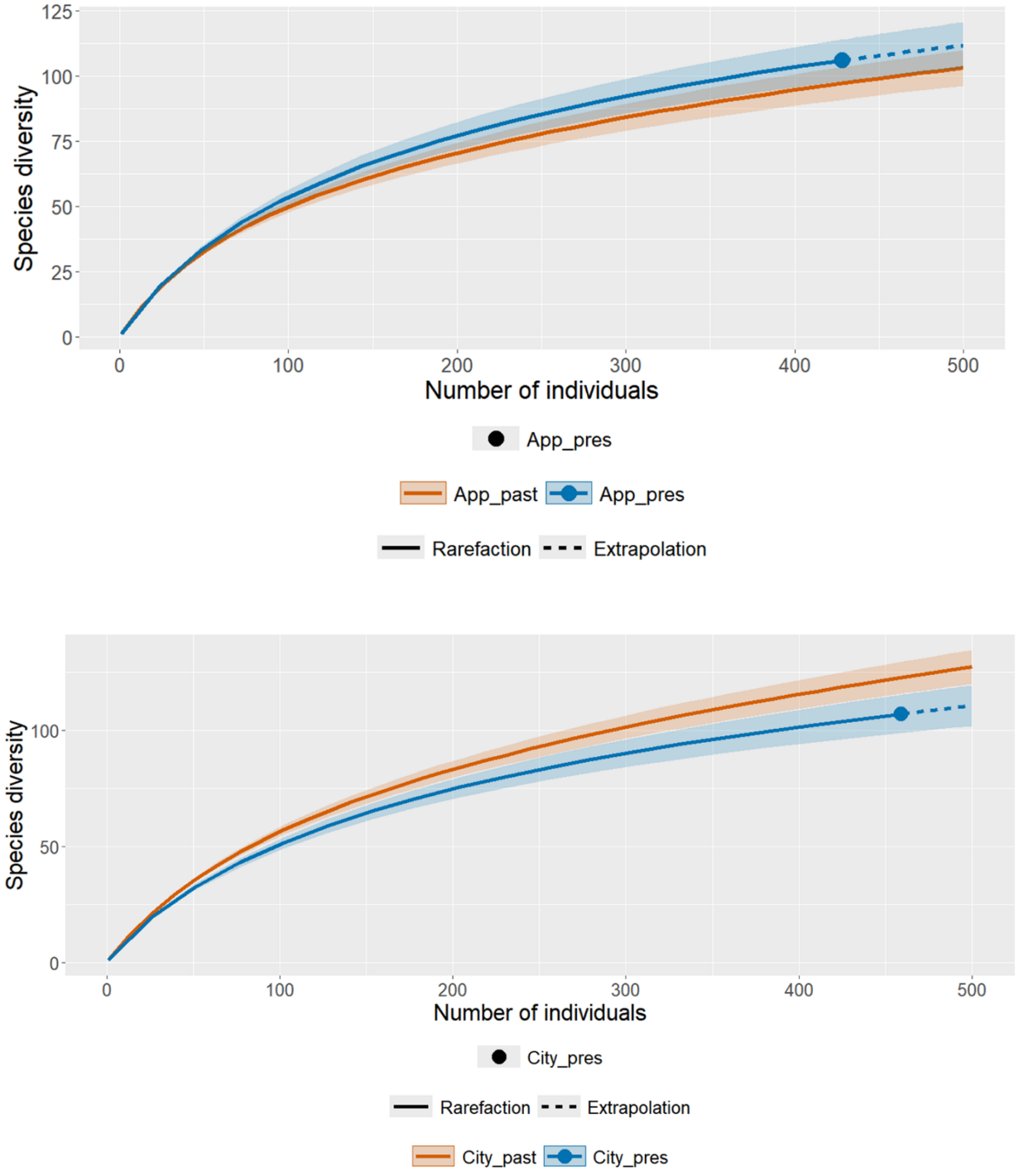

Figure 3 a, b. Diversity accumulation curves (and 95% confidence intervals) compared between past (1925-1926 and 1934-1935) and present (2021 and 2022), based on Hill number q=0, of the Apennines (a) and city (b).

3.2. Variation in ecological traits between past and present bee communities

The NMDS ordination based on Bray–Curtis dissimilarities showed a very low stress value (stress < 0.05), indicating a good fit between the reduced ordination space and the original distance matrix. The ordination revealed substantial overlap between the two sampling periods (Figure 4). The PERMANOVA analysis indicated no significant differences in trait composition between periods ($F = 0.043$, $R^2 = 0.007$, $p = 1$). The

test for homogeneity of multivariate dispersions (*betadisper*) was non-significant (F = 3.21, p = 0.12), confirming that group dispersions were comparable.

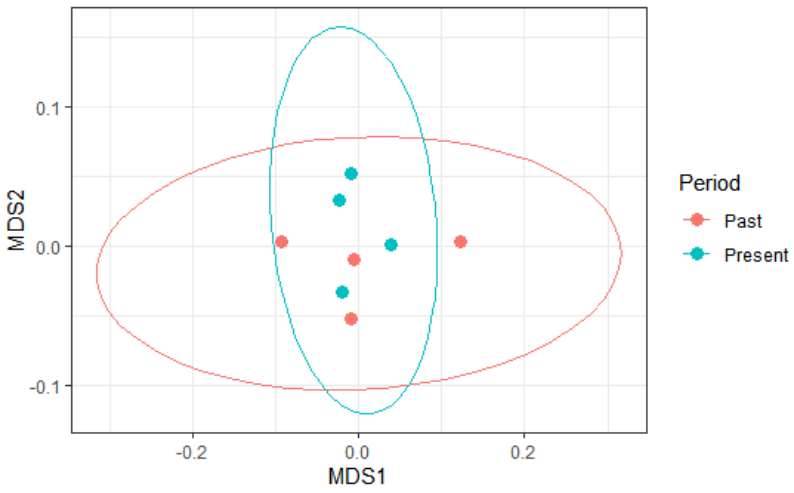

Figure 4. Non-metric Multidimensional Scaling Plot for ecological traits of bee communities. Individual dots, coloured by period, represent sampling sites and ellipses indicate the 95% confidence intervals.

Visual inspection of trait frequencies highlighted more fluctuations within the City macro-area compared to the Appennines (Figure 5). The total number of species recorded for each ecological trait, time period, and macro-area is summarized in Table S2. However, trait frequencies did not differ significantly across time periods per each macro-area (p>0.05) ; all results of Fisher's Exact Tests per each trait category are reported in Table S3.

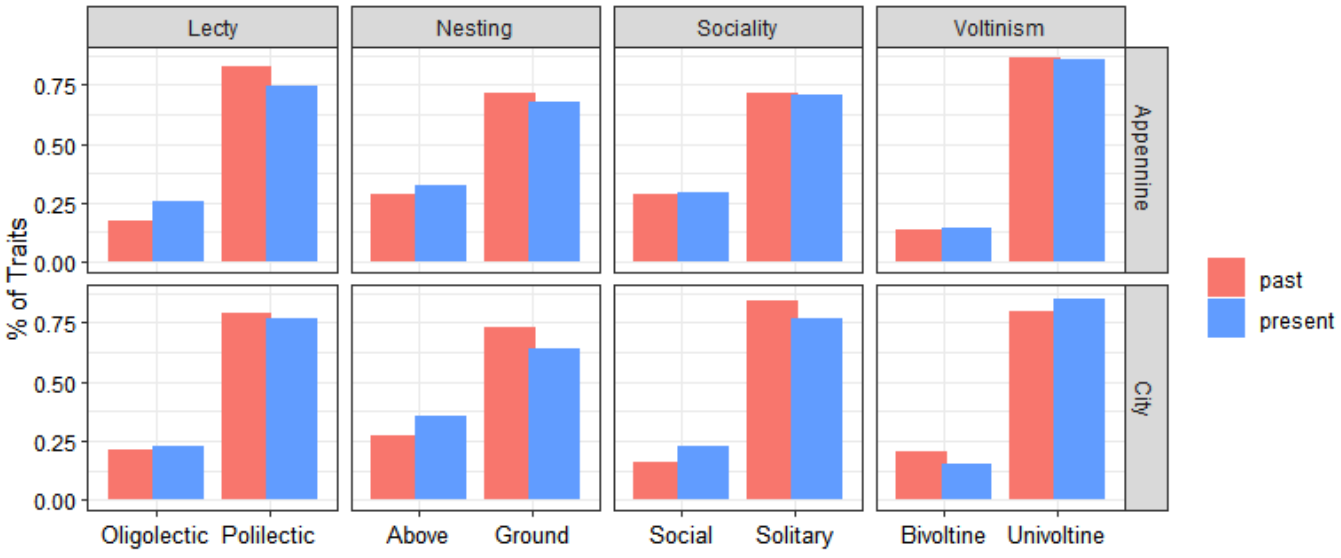

Figure 5. Frequencies of ecological traits (Lecty, Nesting, Sociality, Voltinism) across time periods and macro-areas (City and Appenine).

3.3. Variation of body size

ITD did not significantly differ between past and present (estimate= 0.06, SE= 0.05, df= 195.80, t= 1.22, p= 0.223), and no sex-by-period interaction was detected (estimate= 0.07, SE= 0.08, df= 195.64, t= 0.78, p=

0.437). Also, the macro area had no effect on ITD (estimate= 0.01, SE= 0.06, df= 198.95, t= 0.16 p= 0.87). As expected, we found significant differences between sexes, with males being consistently smaller than females (estimate= -0.24, SE= 0.09, df= 211.82, t= -2.68, p= 0.008). Trend of average body size within-species per sex and period is reported in Figure 6.

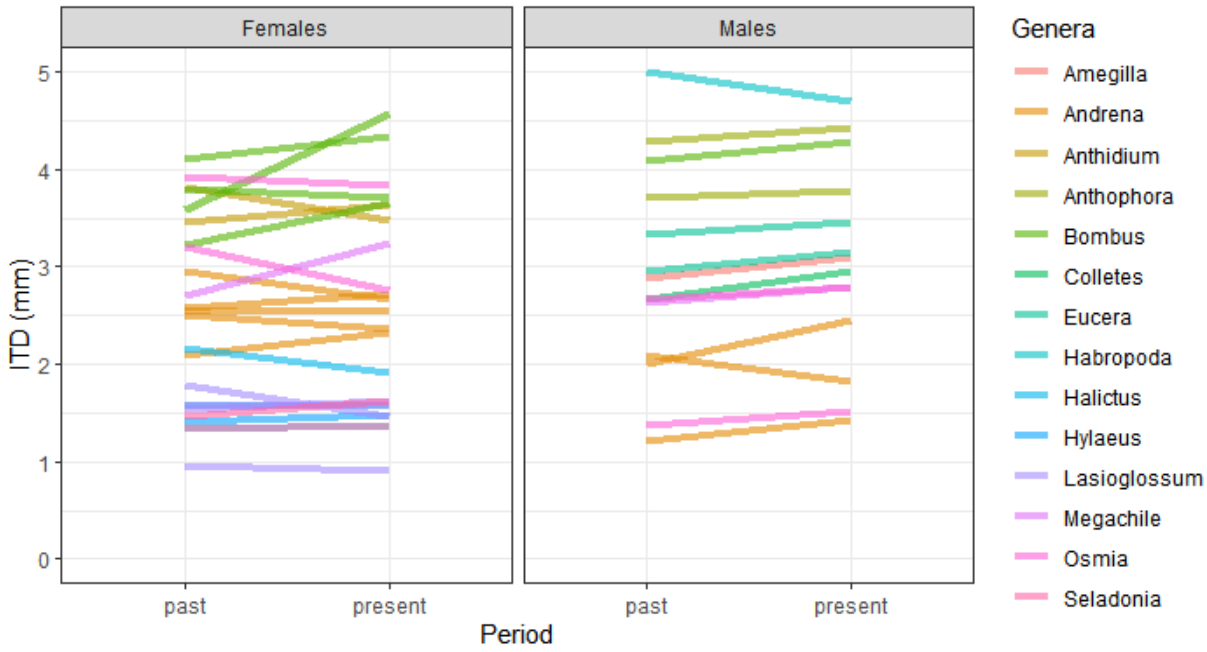

Figure 6. Average ITD comparison within-species for sex per species per period (females: 9 genera, 24 species, 150 individuals; males: 9 genera, 14 species, 84 individuals). Each line connects the past and present averages for the same species, coloured by genera.

4. Discussion

The aim of this study was to assess temporal trends in bee communities using an important entomological collection of northern Italy. We wanted to explore if species richness, as well as ecological traits, had changed after almost a century. Our results revealed that bee communities have changed, with a significant reduction of species richness, especially in the city area. However, we did not observe variations in ecological traits between past and present, indicating that bee responses to environmental changes have not been influenced by their specific traits analysed in this study. Surprisingly, even body size, that in bees is strongly linked to food quality and availability during larval stage, did not change overtime.

4.1. Long-term variation in bee diversity composition

The comparison between past and present bee communities revealed marked changes in species composition and richness. Very low similarity values, expressed through the Sorensen index, were found between the two periods in both City and Apennines macro-areas, indicating a high temporal species turnover, as reported also by other long-term studies (Zimmermann et al. 2023; Graham et al. 2024; Fisogni et al. 2025). Moreover, contrasting trends were observed among bee families, especially within Andrenidae which doubled in the Apennines (from 10% to 22%) and almost halved in the City area (from 25% to 13%). As this family is typically ground nesting, its decline in the city area may be related with reduced availability of nesting surface due to urban cover (Cardoso and Gonçalves 2018). This pattern may also reflect species displacement towards cooler climates, as the Apennines are at 500-600 meters a.s.l. (Magurran 2016). The Colletidae family decreased in both macro areas, which halved in the Apennines (from 10% to 5%) and declined in the City (from 11% to 7%). By contrast, the family Megachilidae increased in the City area from 15% to 24%, which is line with studies that found a positive association between urbanization and cavity-nesting bees (Buchholz et al. 2020;

Fauviau et al. 2022). Although urban areas may represent a refuge for some bee species due to the presence of green areas (Hall et al. 2017), urbanization has been proven to reduce species richness (Cardoso and Gonçalves 2018; Fauviau et al. 2022; Scharnhorst et al. 2025). Accordingly, we found a significant reduction in species richness in the City area as indicated by rarefaction curves (Figure 3b), showing a decline in taxonomic diversity over time.

4.2. Variation in ecological traits between past and present bee communities

After assessing the variation in species composition of the bee community, we examined whether these changes were also reflected in ecological traits. We considered four traits: sociality, nesting behaviour, diet breadth (lecty) and voltinism, classified using binary classification. The NMDS revealed that past and present communities overlap according to ecological traits; this means that even though species from the two periods are different, they show similar ecological traits. We observed some differences in trait frequencies within city macro-area, although these were not corroborated by statistical significance. Specifically, a decline was noted in ground-nesting and solitary species, while above-ground nesting and social species showed an increase. This pattern is consistent with our results on species diversity, where an increase in Megachilidae (typically above-ground nesters) and a reduction in Andrenidae (typically ground-nesters) were observed. Overall, these findings suggest that the environmental pressures characterizing the study sites did not exert a significant selective impact on the ecological traits of the community. Although we found differences in community composition and a significant reduction in species richness in the City area, the same pattern was not observed for ecological traits. This phenomenon is recognized as functional redundancy which occurs when different species occupy the same ecological niche (Moretti et al. 2008; Ricotta et al. 2016). Therefore, our results suggest that the study areas experienced taxonomic but not ecological turnover (Cantwell-Jones et al. 2022). According to some works on bumblebee communities (Pradervand et al. 2014; Fourcade et al. 2021; Cantwell-Jones et al. 2022), changes in taxonomy are not always associated with those in ecological or functional traits. However, the decline in species richness that we found should not be disregarded, as the loss of some species may still have repercussions on plant–pollinator interactions that were not assessed in our study.

4.3. Variation of body size

According to the increasing temperature experienced by the study area (Sabatani et al. 2024) and to the Bergmann's rule, we expected to find body size shrinking in wild bees (Osorio-Canadas et al. 2016; Gérard et al. 2018; Chloe et al. 2019). This decrease could have been further exacerbated by the reduction in floral resources caused by changes in land use. Indeed, fewer nutritional resources during larval development should lead to reduced body size in wild bees (Radmacher and Strohm 2010; Theodorou et al. 2020). However, we did not observe significant differences of ITD between past and present, in contrast to recent studies (Gérard et al. 2019; Nooten and Rehan 2020; Herrera et al. 2023). This result is in line with the previous results on ecological traits, suggesting that bee responses to environmental changes were not influenced by their ecological traits analysed in the current study. Our findings may also suggest that the habitats of the sampled sites have remained relatively stable over time, possibly as a result of management practices promoting green spaces. This highlights the role of urban gardens and parks in sustaining ecological resilience and functional stability within cities (Hall et al. 2017; Buchholz et al. 2020; Cohen et al. 2022). Nevertheless, it must be noticed that our analysis relies on a low sample size (3 individuals/species/sex/period), because of the high species turnover limited the number of comparable taxa. Regarding sexual differences, our study confirms the well described sexual dimorphism in wild bees (Oliveira et al. 2016; Osorio-Canadas et al. 2016; Fitzgerald et al. 2022; Herrera et al. 2023), with males consistently smaller than females.

5. Conclusions

Our results indicate temporal changes in wild bee communities, particularly a significant decline in species richness in the City area, thus confirming the dramatic decline of bee species. These findings emphasize the importance of long-term monitoring and the potential of entomological collections for assessing and monitoring biodiversity change. Although no significant differences were detected in ecological traits, including body size, further studies are needed, especially including plant-pollinator interactions to detect potential nested fluctuations in ecosystem services.

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Chapter 2

Pervasive pesticide contamination of crop flowers and wildflowers in apple agroecosystems

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Abstract

Insect pollination is an ecosystem service that support reproduction of almost 90% of flowering plants, including 75% of crops. However, there is wide evidence for pollinators' decline, namely driven by anthropic factors such as intensive agriculture with high pesticide inputs. In agricultural landscapes, pollinators are routinely exposed to a plethora of pesticides during their foraging bouts. Linked to pesticide drift and use, exposure via flowers can occur in-field and off-field and both from crop flowers and wildflowers. Apart from specific biological traits of each pollinator species, pesticide exposure is influenced by farm management and landscape characteristics. However, it is currently unclear what the pesticide residue levels of crop flowers and wildflowers are, and to what extent landscape and farm management influence these contaminations. The aim of this study was to measure pesticide residues on flowers of a focal widespread crop, *Malus domestica*, and wildflowers inside and outside orchards characterized by a different proportion of semi-natural areas in the landscape and assess the risk posed to pollinators. This study involved 4 European countries: Italy, Spain, Poland and Sweden, for a total of 27 apple orchards. Flowers were sampled in spring during apple bloom and summer, totaling 170 samples; wildflowers were sampled within apple rows and at the orchards' edges with different distances (0-5 and 5-10 meters). The chemical analysis revealed widespread pesticide contamination, with just 8.8% of samples free of pesticides, and detected 90 different compounds. The number of pesticides was significantly lower in summer and decreased with increasing proportion of natural areas in the landscape. The risk was higher inside orchard both in spring, where no difference was observed between crop flowers and wildflowers, and summer, although seasons did not differ in the risk. Moreover, risk was negatively influenced by higher proportion of natural areas and increased by higher proportion of cropland. Overall, the results confirm the widespread pesticide contamination in agroecosystem and highlight the importance of preserving natural areas surrounding crops to mitigate the risk posed to pollinators. Furthermore, the results draw attention to pest management, as the risk was consistent across the season, effectively threatening the health and stability of pollinator populations.

Keywords: *Risk assessment, pesticide residues, agroecosystem, pollinators*

1. Introduction

Agroecosystems are dominated by intensive cropland, usually based on monoculture and high pesticides input. Therefore, in agroecosystems pollinators are routinely exposed to a plethora of pesticides during their foraging bouts (Main et al. 2020). Several studies have shown that honeybee-collected pollen, such as beebread (pollen stored in the hive) and corbicular pollen from returning foragers, are often contaminated with several pesticides (David et al. 2016; McArt et al. 2017; Rundlöf et al. 2022; Sgolastra et al. 2024). This bee matrix is a mix of pollen and nectar collected from different flowers which is considered a good metric for assessing pesticide contamination in the environment and the level of pesticide exposure (Carlson et al. 2025; Rundlöf et al. 2025). However, from this matrix is difficult to know the origin of the contamination. In fact, exposure via flowers can occur in-field and off-field and both from crop flowers (Graham et al. 2022) and wildflowers (Botìas et al. 2015; Park et al. 2015; Long and Krupke 2016). Pesticides can spread into the environment through aerial drift during spray application, reaching also non-target plants. While it can be expected to find pesticide residues in crop flowers, towards which pesticides application is directed, wildflowers should be safeguarded by pesticide contamination. This is particularly relevant within the context of the European CAP (Common Agricultural Policy) Strategic Plan (European Commission, 2023), where it has been proposed to restore field edges and establish floral strips in homogeneous cropland-dominated landscapes to support pollinators and halt the dramatic decline of their populations (Rundlöf et al. 2022; Ward et al. 2022; Gebhardt et al. 2025). Therefore, the use of wildflower strips surrounding crop fields or promote the presence of spontaneous plants should be considered as "pollinator-friendly" strategies; however, these measures can be transformed in a "mortal trap" if contaminated with pesticides. Some studies have already reported high pesticide contamination in wildflowers, analyzing the pollen and nectar (Botìas et al. 2015; David et al. 2016; Peterson et al. 2021; Zioga et al. 2023) or the whole flowers collected directly in the field (Ward et al. 2022; Graham et al. 2024). These studies support the hypothesis that wildflowers represent an important route of pesticide exposure for insect pollinators comparable to crop flowers. However, they focused on a restricted number or type of pesticides (e.g. neonicotinoids).

Apart from specific biological traits of each pollinator species, pesticide exposure is influenced by agricultural practices and landscape characteristics. According to Park et al. (2015), landscapes with higher proportion of natural areas surrounding crops have been proven to buffer the pesticide risk posed to wild bees, while other studies suggested a mitigation of toxic effects on pollinators directly by diluting the source of contamination and indirectly by providing higher nutritive provisions (Centrella et al. 2020; Rundlöf et al. 2022). Moreover, according to Nicholson and Williams (2021), diversified landscapes are associated with a reduced pesticide application, both in terms of frequency and intensity. However, it is currently unclear what are the pesticide residue levels of crop flowers and wildflowers and to what extent landscape surrounding agroecosystems and farm management influence these contaminations. Understanding pesticide levels in the landscape can be useful to better assess the risk and to identify effective mitigation measures.

The aim of this study is to measure pesticide residues on flowers of a focal widespread crop, *Malus domestica*, and wildflowers inside and outside orchards characterized by a different proportion of semi-natural areas in the landscape. The study involved 7 macro-areas including four countries (Italy, Spain, Poland and Sweden) and spread along a longitudinal and latitudinal gradient from South to North and from East to West Europe.

Apple is one of the most valuable fruits globally, with a production of 97.3 million tons in 2023 (FAOSTAT 2024) and an area of 4.6 million hectares worldwide. Europe, with 9,000 hectares dedicated to this crop, is the second largest producer after China, where Poland, Italy and France are the leading countries. Apple, like most Rosaceae species, is a self-incompatible crop and therefore has a great dependence on pollinators to set fruit (Klein et al. 2007; Sheffield, Ngo & Azzu 2016; Osterman et al. 2021). However, apple orchards are

generally intensive crops and receive high pesticides input to control pests (Heller et al. 2020; Joshi et al. 2020), threatening pollinators and pollination services (Granata et al. 2025).

Specifically in our study we aimed: i) to assess the overall pesticides residues detected in both crop flowers and wildflowers in apple agroecosystem; ii) to analyze whether crop and wildflowers inside apple orchards differ in the level of pesticides contamination, in terms of number of active ingredients and estimated risk for pollinators; iii) to analyze whether wildflowers inside and outside apple orchards differ in the level of pesticides contamination between seasons (during and after apple bloom); iv) to analyze the effect of landscape composition, in terms of percentage of cropland and natural/semi-natural area, on pesticide contamination.

2. Materials and methods

2.1 Study sites

The study was carried out in 7 macro-areas across 4 European countries: 4 in Italy with 17 orchards across the regions Trentino Alto-Adige, Veneto, Emilia-Romagna and Sicilia; 1 in Poland with 3 sites in the province of Malopolska; 1 in Spain, with 4 sites in Catalunya, and 1 in Sweden with 3 sites in the county Skåne, for a total of 27 sites (Figure S1). In each area, orchards were managed conventionally or according to the IPM guidelines and were distant at least 2 km from each other. Macro-area consists in groups of at least 3 orchards characterized by the same landscape and climatic features corresponding to regions or county.

2.2 Flower sampling

During apple bloom, between April and May 2024, around 10 grams of flowers were collected inside apple orchards from apple trees (ca 60 flowers/tree) and from inter-rows wild plants, and outside the orchards from the adjacent hedge (between 0 and 5 meters) and from hedges between 5 and 10 meters apart; hereafter, samples will be called FCC, WFC, WF0 and WF5, respectively (Figure 1). The number of wildflowers collected varied according to the size. The sampling of wildflowers was repeated in summer, after apple bloom. Flower samples (N=170) were placed in sterile plastic bags and kept at -20°C until analysis.

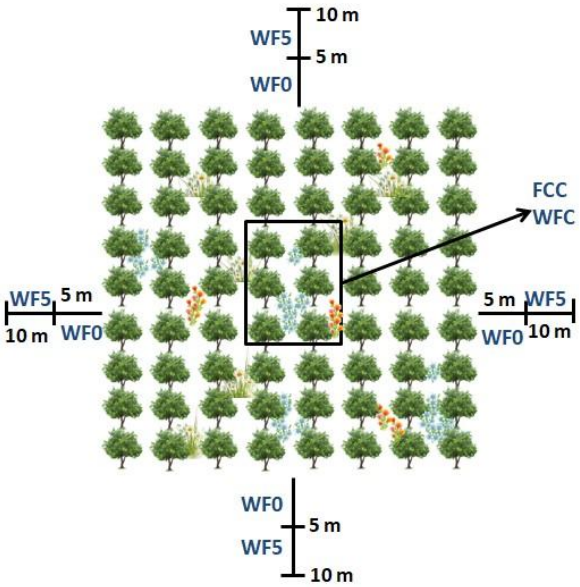


Figure 1. Sampling diagram for flower collection in each apple orchard. FCC: Focal crop, WFC: Wildflowers inside the crop, WFO: Wildflowers near the edges (0-5 meters), WF5: Wildflowers far from the edges (5-10 meters)

2.3 Level of pesticides contamination and risk

The multi-residue analysis, based on the detection of 775 active ingredients, was carried out by Agriparadigma (Ravenna, Italy) using UNI EN 15662:2018 (with GC/MS/MS and LC/MS/MS) method and the CVUA EU RL-SRM QuPPe Vers 12 met 1.3 2021 (with LC/MS/MS) method for glyphosate.

With the residue results, a *risk index* (RI), hereafter called *risk*, was calculated as the sum of the "toxic units" of the active ingredients found in the flowers from each orchard. The index was calculated as the sum of each active ingredient (Ri) divided by its LD50 based on *Apis mellifera*. Both oral and contact LD50s were considered, and the lowest value was selected, thus representing the worst-case scenario (Favaro et al. 2019; Sgolastra et al. 2024):

$$RI = \sum_{i=1}^{n} \frac{Ri}{LD50i}$$

Where *Ri* is the residue of compound *i* expressed in mg/Kg found in the pear flower sample; LD50*i* is the acute oral or contact lethal dose of compound *i* expressed in µg/bee (obtained from the PPBD Pesticide Properties Database).

RI was calculated for each flower sample.

2.4 Landscape characterization

Landscape was characterized at 0.5 and 1.0 km buffers from the centre of the orchards with QGIS software (https://qgis.org/). The proportion of natural and semi-natural areas was assessed using Copernicus Data Space Ecosystem (https://dataspace.copernicus.eu/) and the proportion of cropland according to LPIS (Land Parcel Identification System, European Commission). Landscape proportions were arcsine transformed for statistical analysis.

2.5 Statistical analysis

Two sets of analysis were performed. The first included only spring data (apple bloom period) and considered crop and wildflowers samples collected inside orchards. The second set of models included only wildflower samples (WFC, WF0, WF5) from both spring and summer; in this case, Spain region was excluded because flower samples were gathered only in spring due to technical issues.

To address objective (ii), (iii) and (iv), data were analysed using mixed generalized linear models (GLMMs), where the dependent variables were "number of pesticides" and "risk" (risk index).

For objective (ii) and (iv), only spring data were considered, with "sample" (2 levels: WFC and FCC) and "landscape cover" (natural area or cropland) as fixed predictors. For objective (iii) and (iv), only wildflowers data were considered, with "sample" (3 levels: WFC, WF0 and WF5), "season" (2 levels: spring and summer) and "landscape cover" (natural area or cropland) as predictors. Because the two landscape variables where highly correlated (Pearson's correlation -0.87), only one was included in each model. In addition, we tested two spatial scales for each landscape variable in each model (0.5 km and 1 km buffers). In all analyses, "macro-area" was added as random factor.

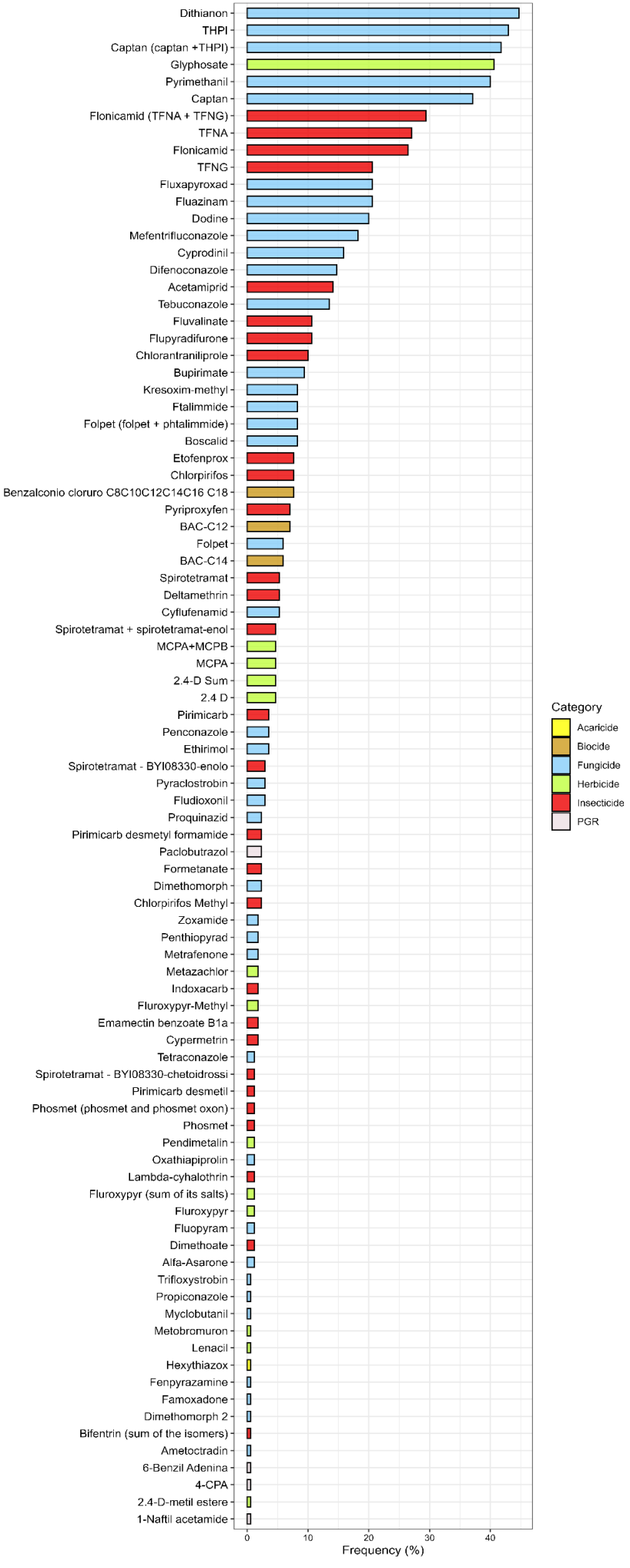
The most suitable distribution was selected using the fitdistrplus package (Delignette-Muller & Dutang, 2015); Negative Binomial distribution was chosen for "number of pesticides" and the Gamma distribution for "risk". The "risk" was analysed using zero inflated generalized linear models (ZI-GLM) due to over dispersion caused by excess zeros. All analyses and figures were carried out using R version 4.4.2 (R Core Team, 2024). GLM were fitted using the lme4 package (Bates et al., 2015) and ZI-GLM using glmmTMB package (Brooks et al.,

2017). Model selection was based on maximum likelihood estimation, and chi-squared tests were conducted using the anova function from the stats package (R Core Team, 2024). More complex models included the interaction term, whereas simpler models contained only the random effect. The effects of independent variables were estimated using the ggeffects package (Lüdecke, 2018), and visualizations were created with ggplot2 (Wickham, 2016). When appropriate, estimated marginal means were calculated for the variable "Sample" using the emmeans package (Lenth, 2024) and pairwise comparisons among the levels of Sample were performed with Tukey's adjustment to account for multiple testing.

3. Results

3.1 Overall pesticide residues in crop flowers and wildflowers

Of the 170 flower samples analysed for the occurrence of more than 700 pesticides, a total of 90 active ingredients were detected, the majority of which were represented by fungicides (39), insecticides (29) and herbicides (13). One compound was DEET, the active ingredient of mosquitos' repellent, presumably deriving from operators, therefore it was excluded from the analysis. The list of all pesticides detected, their residue levels, LD50 and detection frequencies are reported in Table S1 (Supplementary). Only 15 samples, corresponding to 8.8% of the total, were free of pesticides. Within fungicides, Dithianon, Captan (plus its metabolites) and Pyrimethanil were the most frequent compounds. Flonicamid and its metabolites (TFNA and TFNG) were the most frequent insecticide, followed by Acetamiprid. The most frequent herbicide was Glyphosate, being detected in 40.6% of samples. The frequency of detection of each compound is reported in Figure 2.


Dithianon
THPI
Captan (captan +THPI)
Glyphosate
Pyrimethanil
Captan
Flonicamid (TFNA + TFNG)
TFNA
Flonicamid
TFNG
Fluxapyroxad
Fluazinam
Dodine
Mefentrifluconazole
Cyprodinil
Difenoconazole
Acetamiprid
Tebuconazole
Fluvalinate
Flupyradifurone
Chlorantraniliprole
Bupirimate
Kresoxim-methyl
Ftalimmide
Folpet (folpet + phtalimmide)
Boscalid
Etofenprox
Chlorpirifos
Benzalconio cloruro C8C10C12C14C16 C18
Pyriproxyfen
BAC-C12
Folpet
BAC-C14
Spirotetramat
Deltamethrin
Cyflufenamid
Spirotetramat + spirotetramat-enol
MCPA+MCPB
MCPA
2.4-D Sum
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Penconazole
Ethirimol
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Pyraclostrobin
Fludioxonil
Proquinazid
Pirimicarb desmetyl formamide
Paclobutrazol
Formetanate
Dimethomorph
Chlorpirifos Methyl
Zoxamide
Penthiopyrad
Metrafenone
Metazachlor
Indoxacarb
Fluroxypyr-Methyl
Emamectin benzoate B1a
Cypermetrin
Tetraconazole
Spirotetramat - BYI08330-chetoidrossi
Pirimicarb desmetil
Phosmet (phosmet and phosmet oxon)
Phosmet
Pendimetalin
Oxathiapiprolin
Lambda-cyhalothrin
Fluroxypyr (sum of its salts)
Fluroxypyr
Fluopyram
Dimethoate
Alfa-Asarone
Trifloxystrobin
Propiconazole
Myclobutanil
Metobromuron
Lenacil
Hexythiazox
Fenpyrazamine
Famoxadone
Dimethomorph 2
Bifentrin (sum of the isomers)
Ametoctradin
6-Benzil Adenina
4-CPA
2.4-D-metil estere
1-Naftil acetamide
0
10
20
30
40
Frequency (%)
Category
Acaricide
Biocide
Fungicide
Herbicide
Insecticide
PGR

Figure 2. Frequency of detection of the total of 89 pesticides found, coloured according to pesticides categories. PGR= plant growth regulators.

3.2 Level of pesticides contamination according to flower samples and season

Overall, the average (±SD) number of pesticides was 7.22 (± 5.26) with a mean (±SD) concentration of 45.72 (± 296.53) mg/kg per sample. The average number of pesticides and concentration of each flower sample in spring and summer is reported in Table 1. In spring, the highest concentration (1930 mg/kg) was recorded for Captan (plus its metabolites) in FCC from Poland. In summer, the highest concentration (210 mg/kg) was again related to Captan (plus its metabolites) in wildflowers from the edges at 0 meters (WF0) in the Italian region Veneto.

Altogether, the risk ranged from 0 to 653.99 (mean ± SE= 11.92 ± 4.70, median= 0.07); the highest risk was detected in WF0 in the Italian region Veneto in summer, and was driven by Deltametrin, due to its toxicity (LD50 contact= 0.0015 µg/bee) rather than its concentration (0.975 mg/kg). The average risk of each flower sample in spring and summer is reported in Table 1. In spring, the maximum risk was related to Deltametrin detected in WFC from Spain (0.348 mg/kg).

Table 1. Number of samples analysed for each flower sample (FCC= focal crop; WFC=wildflowers inside the crop; WF0=wildflowers outside between 0-5 meters; WF5=wildflowers outside between 5-10 meters), season and summary statistics (range (min-max), mean ± standard deviation, median) of number of pesticides, concentrations and risk.

SAMPLE	N	SEASON	Summary statistics	N° of Pesticides	Concentration (mg/kg)	Risk
FCC	27	Spring	range mean ± SD median	0-19 10.7 ± 6.35 13	0-3791.49 167.59 ± 725.03 17.53	0-49.24 7.65 ± 13.59 0.77
WFC	27	Spring	range mean ± SD median	0-17 9.19 ± 5.36 12	0-503.82 44.56 ± 116.69 4.73	0-237.1 10.51 ± 45.42 0.27
WF0	27	Spring	range mean ± SD median	0-21 8.22 ± 5.55 8	0-262.21 17.93 ± 50.69 2.45	0-8.81 0.78 ± 1.77 0.05
WF5	24	Spring	range mean ± SD median	0-14 6 ± 4.03 4.5	0-34.18 3.73 ± 7.56 0.70	0-13.06 1.4 ± 3.28 0.01
WFC	22	Summer	range mean ± SD median	0-13 5.45 ± 3.74 6	0-222.87 23.55 ± 60.03 1.38	0-235.5 21.62 ± 69.02 0.08
WF0	23	Summer	range mean ± SD median	0-12 5.04 ± 3.79 5	0-400.35 27.29 ± 86.74 0.78	0-653.99 31.54 ± 136.41 0.02
WF5	20	Summer	range mean ± SD median	0-14 4.4 ±4.03 3.5	0-159.49 16.23 ± 39.55 0.28	0-225.35 14 ± 51.05 0.02

3.3 Effects of landscape composition on the level of pesticides contamination between crop flowers and wildflowers inside orchards

In spring, the number of pesticides in flowers within the orchard (FCC and WFC) decreased with increasing proportion of natural area at 0.5 km (estimate = –1.92, $p < 0.001$) and 1 km (estimate = –1.99, $p < 0.001$) (Figure 3A, B). The opposite trend was observed for cropland, where the number of pesticides in the orchard flowers increased with the proportion of surrounding cropland (estimate = 1.85, $p < 0.05$ at 0.5 km; estimate = 1.60, $p < 0.01$ at 1 km), although with a gentler slope and lower significance compared to its decrease with natural areas (Figure 3 C, D). Overall, there were no significant differences between crop and wildflowers collected inside the orchard during spring (estimate= -0.149, p= 0.359).

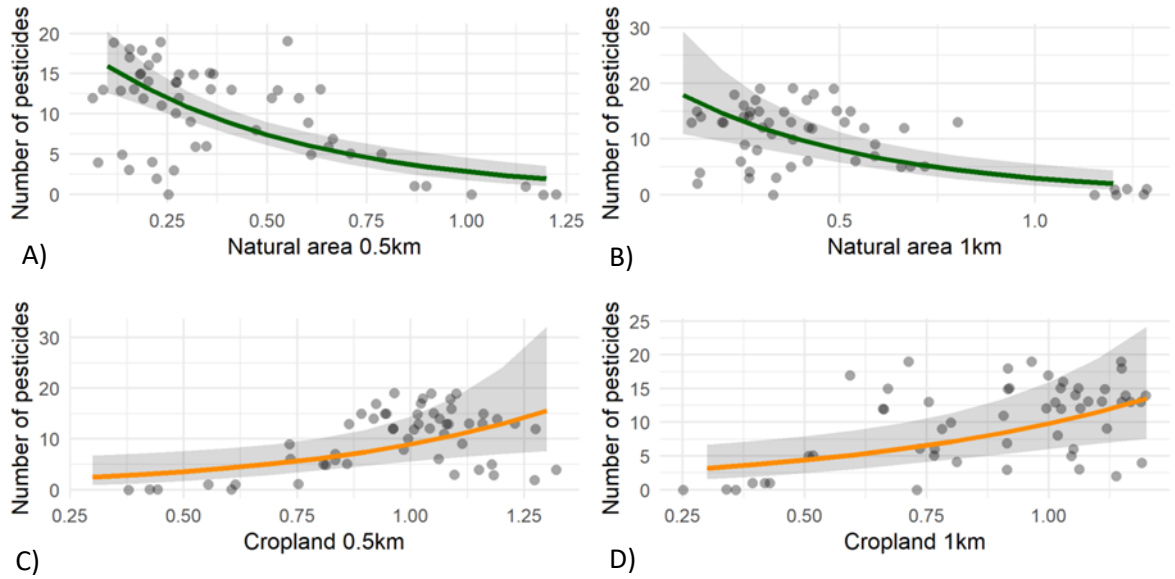

Figure 3. Effects of landscape (natural area and cropland arcsine transformed) on number of pesticides detected from flowers inside orchards. A. Effects of natural area in the landscape at 0.5 km buffer B. Effects of natural area in the landscape at 1 km buffer C. Effects of cropland in the landscape at 0.5 km buffer D. Effects of cropland in the landscape at 1 km buffer.

Concerning the risk, the Zero-inflated model reported that the probability of observing zero risk increased with increasing proportion of natural area both at 0.5 km (estimate = 6.123, $p < 0.01$) and 1 km (estimate = 4.795, $p < 0.001$) (Figure 4 A, B). The natural area was not significant on the risk at 0.5 km (estimate= -3.800, p= 0.204) but at 1 km (estimate= -6.956, p= 0.01) in the conditional model (Figure 4 C, D). The opposite trend was observed for cropland on the probability of having zero risk both at 0.5 km (estimate: -1.434, $p < 0.01$ 0.5) and 1 km (estimate: -7.17, $p < 0.05$) (Figure 4 E, F). As for natural area, cropland was not significant on the risk at 0.5 km (estimate= 1.788, p= 0.76) but at 1 km (estimate= 8.130, p< 0.001) in the conditional model.

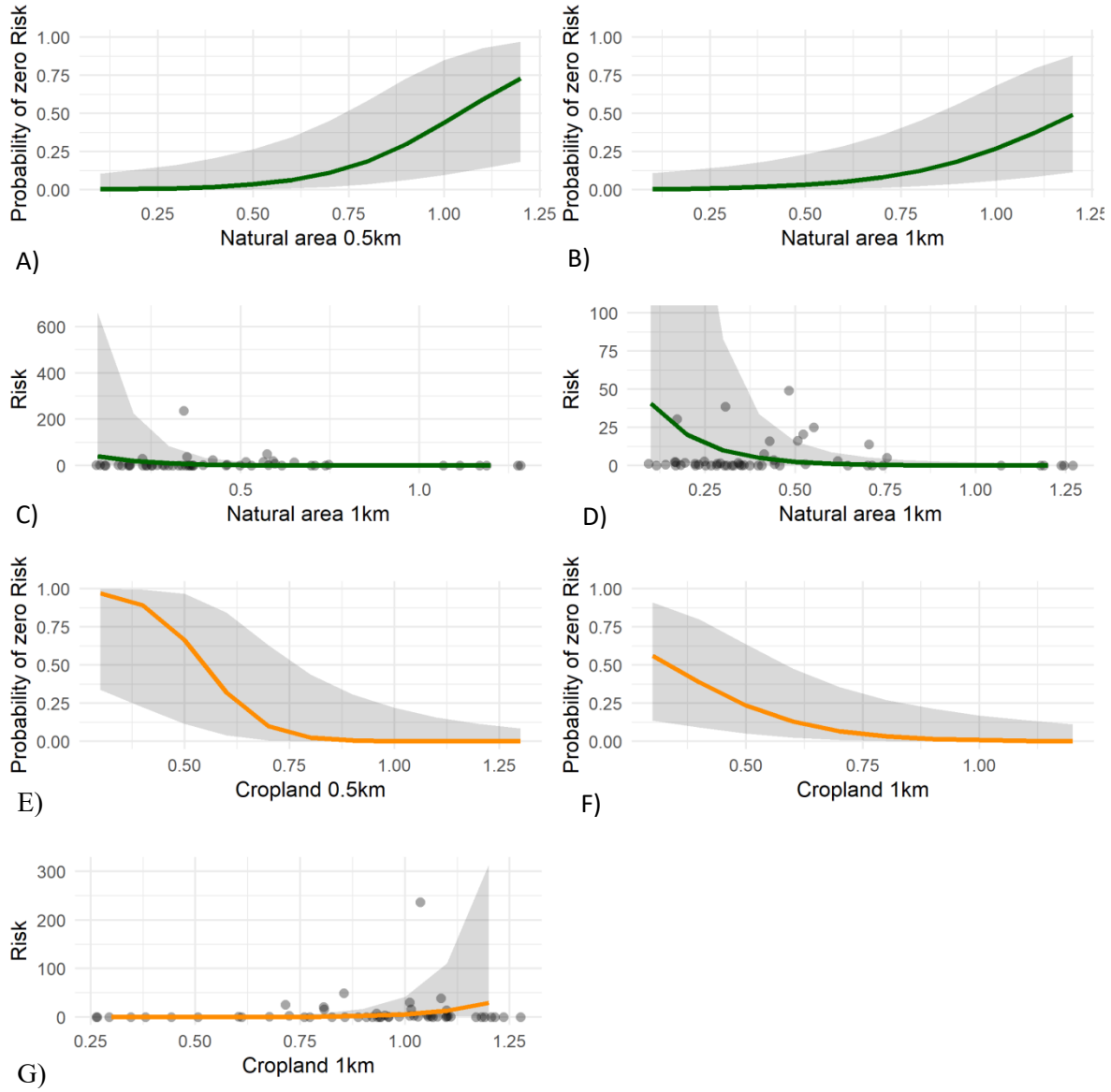

Figure 4. Effects of landscape (natural area and cropland arcsine transformed) on risk from flowers inside orchards. A. Effects of natural area at 0.5 km buffer on probability of risk=0. B. Effects of natural area at 1 km buffer on probability of risk=0 C. Effects of natural area at 1 km buffer on risk. D. As B, but with the zoom in the y-axis. E. Effects of cropland at 0.5 km buffer on probability of risk=0. F. Effects of cropland at 1 km buffer on probability of risk=0. G. Effects of cropland at 1 km buffer on the risk. Note in ZI-GLMMs of models we have two different models results, probability of risk=0 (Zero-inflation model) and the risk values (conditional model). Only significant results are shown.

3.4 Effects of landscape composition and season on level of pesticides contamination between wildflowers

The number of pesticides was significantly influenced only by the season with a greater number of pesticides detected in spring than summer ($p < 0.001$) (Figure 5A). The amount of natural area and cropland within the two buffers analysed did not have significant effects, although natural areas within the 1 km buffer was marginally significant ($p = 0.055$) (Figure 5B).

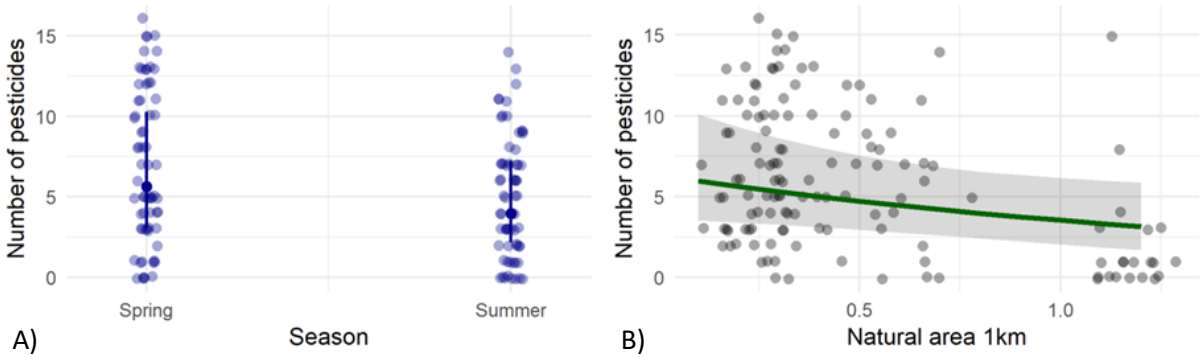

Figure 5. Effects of season and landscape in wildflowers on number of pesticides detected through chemical analyses. A. Effects of season (P< 0.001). B. Effects of natural area in the landscape at 1 km buffer (p=0.055).

Concerning the risk, season had marginal effect (estimate= 1.108, p= 0.069), while significant differences were found among wildflowers: WFC exhibited a significantly higher contamination risk than WF0 (estimate: 1.927, p< 0.001), but not compared to WF5 (estimate: 0.977, p=0.19) (Figure 6A). The probability of observing zero risk increased with the natural areas at both buffers (estimate: 4.52, p< 0.001 for 0.5km buffer; estimate: 3.9597, p< 0.001 for 1km buffer) with a stronger effect at the 0.5 km buffer (Figure 6 B, C) and decreased with cropland at both buffers (estimate: -4.78, p< 0.01 for 0.5km buffer; estimate: -4.09, p< 0.001 for 1km) (Figure 6 D, E). Season had no effect on the probability of observing zero risk (estimate= 1.08, p= 0.128).

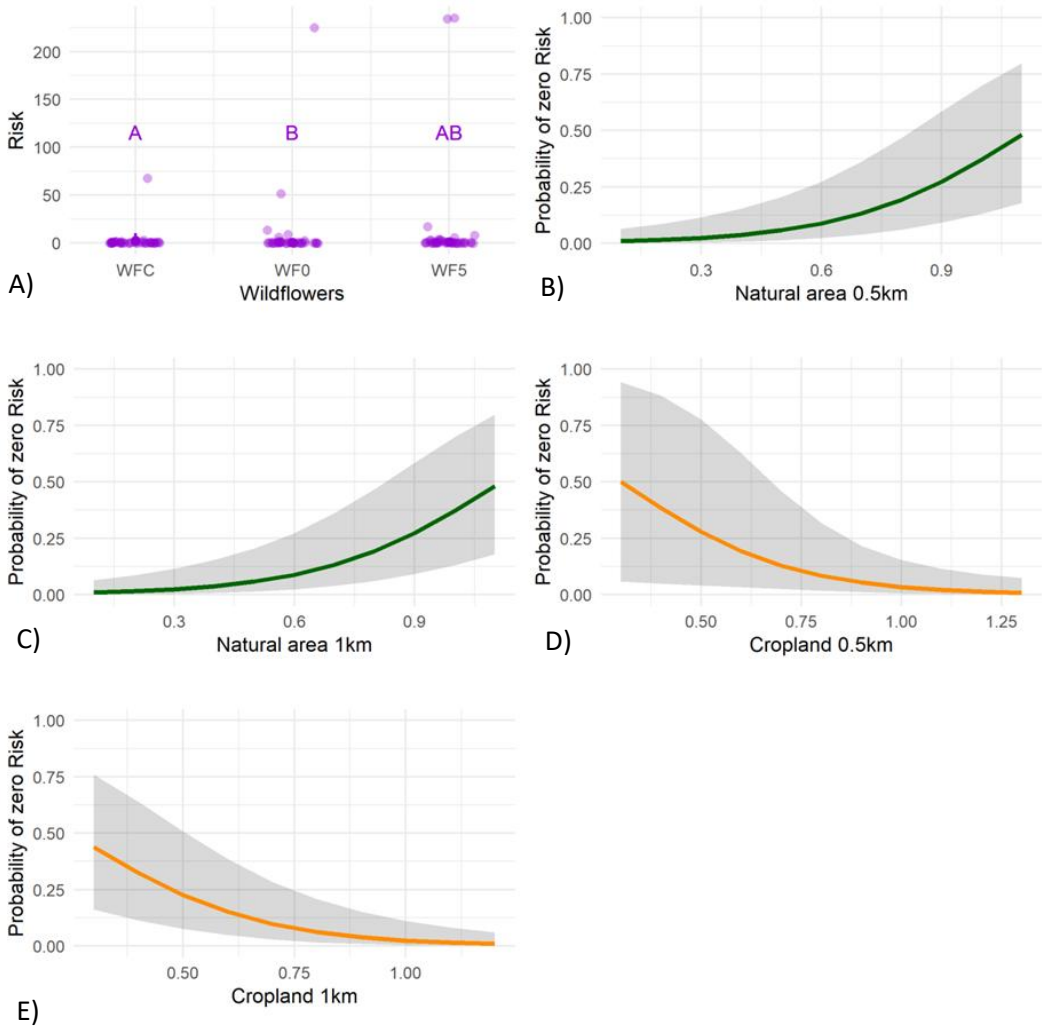

Figure 6. Effects of wildflower sample and landscape on risk. A. Effects of sample wildflower, with pairwise comparisons indicated by letters ($p < 0.001$). B. Effects of natural area at 0.5 km buffer on probability of risk=0. C. Effects of natural area at 1km buffer on probability of risk=0. D. Effects of cropland at 0.5 km buffer on probability of risk=0. E. Effects of cropland at 1 km buffer on probability of risk=0. Only significant results are shown.

4. Discussion

The aim of our study was to assess pesticide contamination and the associated risk in both crop flowers and wildflowers within a widespread agroecosystem, apple orchard. Moreover, we wanted to examine the influence of landscape characterization on the level of pesticides contamination and risk. Our results revealed a widespread contamination of all flowers, inside and outside orchards, both in terms of number of pesticides and risk, which persist also after apple bloom. Moreover, we found important effect of landscape composition on pesticide contamination and risk.

4.1 Overall level of pesticides contamination across flower samples and season

We analysed a total of 170 flower samples for pesticides residues, and we found 89 different pesticides, with around 7 compounds per sample on average, while only 15 samples were free of pesticides. Most of pesticides were fungicides, namely Dithianon, Captan and Pyrimethanil, followed by insecticides, mostly Flonicamid, its metabolites (TFNA, TFNG) and Acetamiprid. Within the 13 herbicides detected, the most frequent was Glyphosate. Insecticides were found both in spring, during apple bloom when their application

is prohibited, and summer, suggesting that they persist after pre-bloom applications (Heller et al. 2020) while their use after crop bloom should be managed in order to avoid contamination of wild floral resources, namely by aerial drift or runoff (Zioga et al. 2022). Our results are in line with other studies that assessed pesticide contamination in non-crop flowers or pollen (Botìas et al. 2015; Ward et al. 2022; Zioga et al. 2023; Graham et al. 2024), reporting a widespread contamination in agroecosystems. Moreover, our study confirms that pollinators are exposed to different compounds simultaneously, thus raising concerns about possible synergistic effects (Sgolastra et al. 2017; Tosi et al. 2022). Indeed, while some pesticides are considered relatively non-toxic for bees, such as fungicides which application is allowed during bloom, their toxicity may be increased by the presence of small residues from other compounds (Sgolastra et al. 2018; Albacete et al. 2024).

Based on pesticides residues and the LD50 (for *Apis mellifera*) of each compound, we calculated a risk index for each flower sample. Overall, risk was higher inside orchards, including both crop flowers and wildflowers inside, as well as for wildflowers inside respect to wildflowers outside orchards. The maximum values of risk were found in in wildflowers inside the orchard (WFC, 237.1) in spring, in wildflowers inside (WFC, 235.5) and outside orchard (WF0, 653) in summer. In all cases, the risk was driven by deltamethrin, which is an insecticide extremely toxic to bees (LD50 contact= 0.0015 µg/bee). Deltamethrin is considered highly toxic also to non-*Apis* bees, such as *Megachile rotundata* (Piccolomini et al. 2018), *Osmia bicornis* and *Bombus terrestris* (PPDB, 2025), even though its use is still authorized by the European Commission.

4.2 Effects of landscape composition on the level of pesticides contamination between crop flowers and wildflowers inside orchards

The results show that the number of pesticides detected did not differ significantly among crop flowers and wildflowers inside orchard; this aspect should be considered when planning pest management practices in apple orchards. For example, the study of Graham et al. 2024 reported no differences in number of pesticides between flowers collected inside the blueberry crop and field margins, but residues were significantly lower in un-sprayed farms. Nevertheless, level of pesticide contamination in our study was negatively influenced by higher proportion of natural areas at the two buffers analysed (0.5 km and 1 km). This effect may be explained by the fact that natural and semi-natural areas are not subjected to pesticide applications, thus their presence helps to reduce overall contamination in the landscape (Park et al. 2015). In addition, fields surrounded by higher portion of natural and semi-natural areas show a lower pest pressure for the greater presence of natural enemies (Veres et al. 2013). By contrast, pesticide contamination was positively associated with higher proportion of cropland, in line with other studies (Cappellari et al. 2024; Shi et al. 2024). The same trend was observed for the risk; indeed, the probability of observing a risk equal to 0 was significantly increased by natural area and decreased by cropland at both buffers. As for pesticide contamination, risk did not differ between crop flowers and wildflowers highlighting the importance to remove the wildflowers inside the crop fields during pesticide applications (Granata et al. 2025).

4.3 Effects of landscape composition and season on level of pesticides contamination between wildflowers

The number of pesticides in wildflowers significantly decreased in summer but we did not find differences between flowers samples collected inside and outside (5-10 m from the edge) the orchards, suggesting that all flowers in the agroecosystem harbour similar number of pesticides. No significant effects of the landscape, both natural and cropland, were found in the number of pesticide contamination in wildflowers, except for a marginal effect of natural areas at the 1 km scale. By contrast, risk was significantly influenced by landscape, with increasing probability of having risk equal to 0 due to increasing proportion of natural area. This landscape effect can be explained by drift and runoff of pesticides from different fields. While the number of

pesticide residues decreased from spring to summer, no significant difference occurred in pesticide risk between seasons. This may depend by the lower pesticide applications in summer but with more toxic compounds such as insecticides (Favaro et al. 2019). The presence of floral resources in summer is important for the stability of pollinators populations and thus for pollination services (Samnegård et al. 2019; Granata et al. 2025) because it provides fundamental nutritional resources when focal crop is not in bloom (Rundlöf et al. 2022). For this reason, it is necessary to draw attention on pest management and treatments' applications to avoid the contamination of these floral sources.

5. Conclusions

Our results reported a pervasive pesticide contamination in apple agroecosystem in space and time thus confirming that pollinators are constantly exposed to pesticides. However, the risk may be limited by increasing the proportion of natural areas in the landscape surrounding crops, which provide more nutritive resources free of pesticides to pollinators, as well as possible increase in other beneficial organisms, with positive repercussions on overall ecosystem services. Therefore, as an effective strategy for pollinator protection at the local scale, careful management of pesticide applications is essential, including the use of less toxic compounds whenever possible and/or using anti-drift nozzles. At the broader landscape level, the preservation of natural areas represents an effective strategy to reduce pesticide exposure and sustaining pollinator populations.

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Chapter 3

Widespread pollination deficits in pear (*Pyrus communis* L.) orchards: the role of pollinators, landscape context and pesticide risk

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Abstract

European pear (*Pyrus communis* L.) is an important entomophilous crop, and most varieties are self-incompatible, therefore strongly dependent on insect pollinators for fruit set. However, harsh conditions during bloom and low sugar content of nectar compared to other co-flowering fruit trees often lead to low pollinator visitation rates, causing shortfalls in production. Pollination deficits and the contribution of pollinator communities to pollination services have been well studied in apples, but we know much less about pollination services in pear. The aim of this study was to assess pollination services and detect pollination deficits in pear orchards, and to analyze the effects of local factors (pesticide load and other orchard management, such as irrigation, fertilization and hormones application) and landscape factors ("pollinator friendly" cover) on pollinators and pollination services. Despite the effect of orchard management on fruit retention, our results confirm the dependence of fruit set on pollination (mean: 37%; range: 0%-80%). Our results also report significant pollination deficits across pear orchards (mean: 31%, range: 0%-74%), and low pollination service (mean: 17%, range: 0%-60%). The most abundant flower visitors were honeybees, followed by Diptera Muscidae, while wild bees were the least abundant group. However, "pollinator friendly" cover at 1.5 km had significant positive effect on wild bees' visitation rate. Managed (honeybees and bumblebees) and wild pollinators (all other pollinators) had no effect on pollination deficit, but higher bumblebees' visits negatively affected seed set. Chemical analysis of pear flowers revealed high levels of pesticide residues, as all flower samples were contaminated with at least four different pesticides, and pesticide risk had a negative effect on fruit set. Our results indicate insufficient pollination services in pear orchards and raise concerns about the management of pollination provision, highlighting the importance of semi-natural areas to boost wild bee visits and to reduce pesticide pressure on pollinators.

Keywords: *Pollinator dependence; Pollination Service; Wild bees; Pesticides*

1. Introduction

Almost 90% of wild angiosperms and 75% of the main agricultural crops rely on animals, mostly insects, for pollination (Klein et al. 2007; Ollerton 2011; IPBES 2016). Importantly, an important fraction of crop pollination services is provided by wild pollinators (Garibaldi et al. 2013), which are experiencing dramatic declines, largely driven by intensive agriculture, resulting in habitat loss and pesticide contamination (Ollerton 2011; Bartomeus et al. 2013; Ramos-Jilberto et al. 2020).

Agricultural intensification, exemplified by mass-flowering monoculture farms, is one of the major drivers of pollinator declines, mediated by the reduction of seminatural ecosystem cover, habitat degradation and decreased floral resources (Scheper et al. 2014). Natural habitats are essential to preserve nesting sites and diversified floral resources, thereby shaping diverse pollinator communities. Numerous studies have documented the positive effect of natural and seminatural landscape cover surrounding crops on arthropods, thus enhancing the provision of ecosystem services, including pollination (Ganser et al. 2018; Hass et al. 2018; Samnegård et al. 2018; Roquer-Beni et al. 2020; Tamburini et al. 2020; Belien et al. 2021). For example, Holzschuh et al. (2012) showed that an increase in the proportion of habitat suitable for bees increased fruit set in cherry orchards. Moreover, landscape heterogeneity has been suggested to have a mitigation effect on overall pesticide contamination (Nicholson and Williams, 2021) and pollinator exposure to pesticides (Rundlöf et al. 2022; Shi et al. 2024), another factor associated with agricultural intensification. Insecticides may have direct negative impacts on pollinator populations by affecting immature development and reducing adult survival, and herbicides may have indirect impacts by reducing floral resources (Liere et al. 2017). Even fungicides, which are considered relatively non-toxic for bees, may lead to physiological and behavioural changes and reduce bee survival when they interact with other chemicals (Rondeau and Raine 2022). In addition, various studies have reported impairments in plant-pollinator interactions as a consequence of pesticide exposure. Pesticides can induce alterations in plant metabolism or physiology, with repercussions on attractiveness to pollinators (Klatt et al. 2023; Tatarko et al. 2025). Other studies have reported alterations in bumblebee visitation rate following pesticide application (Stanley et al. 2015; Avila et al. 2023), as well as reduced pollen deposition on the stigmas (Tamburini et al. 2021), thereby affecting pollination services.

Within this context, reports of pollination deficits in highly pollinator-dependent crops are increasing (Polce et al. 2014; Gianni and Vania 2018; Holland et al. 2020; Castro et al. 2021; Garratt et al. 2021; Siopa et al. 2024; Turo et al. 2024). Pollination deficits are defined as the inadequate quantity and/or quality of pollen deposited on the stigma (Vaissière et al. 2011), resulting in low crop yields (Webber et al. 2020). Suboptimal crop pollination can be triggered by several factors such as low pollinator diversity and abundance, asynchrony between the crop and its pollinators, asynchrony between inter-compatible cultivars (Polce et al. 2014), and inadequate availability or distribution of pollinizer cultivars (Quinet and Jacquemart 2017; Reeves et al. 2022). Successful pollination may be estimated in terms of initial fruit set (Garratt et al 2021), which is often related to final yield, or in terms of seed set (Webber et al. 2020), which is often related to fruit quality and shape (Olhnuud et al. 2022).

Pears are one of the most important crops globally. Most of the global production (82.2%) occurs in Asia (mainly Asian pear species, *P. pyrifolia*, *P. sinkiangensis*) (Wu et al. 2018), followed by Europe (8.4%), and the Americas (5.9%) (*Pyrus communis*) (FAOSTAT 2023). Like most Rosaceae species, pear presents a mechanism of genetic self-incompatibility that prevents inbreeding, even though some varieties exhibit parthenocarpy, and thus rely on cross-pollination among inter-compatible cultivars for reproduction (Fountain et al. 2019). For this reason, pear orchards should present at least two compatible cultivars with overlapping flowering periods (Quinet and Jacquemart, 2017; Goldway et al. 2019). Pear flowers are visited by a diverse array of bees and flies, but flower visitation rates are usually rather low (Quinet et al. 2016; Fountain et al. 2019). Several factors contribute to explaining these low visitation levels. First, pear flowers have a low sugar content (<25%) compared to other co-blooming crops (Quinet and Jacquemart 2017). Second, pear blooms early in the year, when temperatures may be too low for insect activity (Vicens and Bosch 2000). Freezing

temperatures in spring may also lead to flower frost damage and cause necrosis of the seed embryos (Musacchi et al. 2021; Reeves et al. 2022). Finally, the widespread application of pesticides in intensively managed orchards (Belien et al. 2021) can negatively affect visitation rates (Bloom et al. 2021; Tamburini et al. 2021). To overcome low pollination services, growth regulators are often applied to boost early fruit retention (Musacchi et al. 2021) even though seedless fruits are likely to exhibit shape deficiencies (Goldway et al. 2019); moreover, pears produced by parthenocarpy are usually smaller respect to those resulting from cross-pollination (Claessen et al. 2019). For all these reasons, pollination is regarded as an essential factor limiting yields in *P. communis* (Hünicken et al. 2021; Siopa et al. 2024).

Pollination deficits and the contribution of pollinator communities to pollination services have been well studied in apples (e.g., Garratt et al. 2014a; 2014b; 2021; Roquer-Beni et al. 2020; Webber et al. 2020; Olhnuud et al. 2022; Eeraerts et al. 2025) and other pollinator-dependent crops such as blueberry and kiwifruit, for example (Castro et al. 2021; Bordoni et al. 2024; Turo et al. 2024). By comparison, we know much less about pollination services in pear. In this study, we conducted pollination experiments, surveyed pollinators, and assessed pesticide levels and landscape composition in 16 pear orchards in Italy. Our objectives were to: i) assess pollination services and detect potential pollination deficits; and ii) assess the role of local (farm) factors such as pesticide risk and orchard management, and landscape factors ("pollinator-friendly" cover) on pear pollinator guilds and pollination services.

2. Materials and Methods

2.1. Study sites

The study was conducted in 2023, from flowering season between March and April to harvesting in late August, in 16 pear orchards located in three provinces in the Emilia-Romagna region of Northern Italy (44°29'37"N 11°20'35"E). Emilia-Romagna is the leading Italian region for fruit production, both in terms of cultivated area (23% of the total national area) and production (30-40%). The region is characterized by a temperate subcontinental climate, with hot and humid summers and harsh, cold winters. 2023 was the hottest year since 1961, both for medium and highest temperatures; however, in early April, the minimum temperature registered was -2.6 °C and the maximum was 23°C; the average temperature during pear bloom was 12.5°C. Moreover, precipitation in 2023 was above average from March to May, with May recording a 230% increase compared to the mean of the period (ARAPE 2024); such conditions may have an impact on overall production by causing physiological stress in plants (Beillouin et al. 2020; Belien et al. 2021). The study orchards were all managed according to Integrated Pest Management guidelines and were at least 1.5 km apart from each other. In all orchards, *Abate Fétel* was the main variety, with *Williams* serving as the pollinating variety. In all orchards except one, where *Williams* trees were along the rows, disposition of pear varieties was into separate blocks, adjacent or alternate (Table S1).

2.2. Pollinator survey

In early April 2023, pear pollinators were monitored around midday during peak bloom with adequate weather conditions (sunny days, T ≥ 10°C, low wind speed). One observer walked along a transect (400 - 700 m, depending on the orchard) between tree central rows for 30 minutes on two different days, totalling 1 hour, and noted all insects observed visiting pear flowers on both sides of the transect. Because we expected low pollinators abundance, we did not use the sweep net to avoid potential disturbance to flower visitors. Pollinators were classed into broad functional groups, following Garratt et al. (2022) and were honeybees, bumblebees, wild bees, syrphids, muscoid flies and other pollinators (i.e. wasps, Coleoptera and Lepidoptera). To estimate the number of flowers sampled in each transect, we counted the number of inflorescences in 3 trees and multiplied this number by the average number of open flowers (estimated from 10 inflorescences of 3 random trees) and by the number of trees per transect. The number of flowers surveyed was used to calculate pollinator visitation rates (number of individual pollinators observed per flower).

All bumblebees recorded were *Bombus terrestris*. While in most of the orchards were completely absent, their numbers were higher only in two farms, especially in one of them, in which commercial colonies were present. Therefore, we assumed that bumblebees (and honeybees) were managed in these two farms, and for the other orchards we included the few bumblebees into the wild bees group. In the statistical analysis regarding the effect of pollinators on production and pollination deficit, three groups of pollinators were considered: honeybees, bumblebees, considered as two groups of managed pollinators due to their different behaviour as flower visitors (Lee et al. 2017), and "wild pollinators" (wild bees, syrphids, muscoid flies and other pollinators).

2.3. Pollination experiments

To assess pollination levels and fruit set, 10 trees per orchard were randomly selected along three central rows within each orchard. Before flowering, 3 branches on each tree were marked for one of three pollination treatments: Pollinator exclusion (E), Open pollination (O) and Supplementary cross-pollination (S). Branches of the E treatment were covered with a tulle mesh bag (diameter=1 mm) until petal fall. Pollinator exclusion treatment provides data on the plant's ability to set fruit without pollination, and is aided by irrigation, fertilization and hormones applications, which together represent fruit retention management. Branches of the O treatment were left to be freely visited by pollinators; branches of the S treatment were hand-pollinated with pollen from the pollinizer variety (*Williams*) and left open. To obtain *Williams* pollen, flower buds were collected at the balloon stage. Anthers were cut in the laboratory and placed at 37°C for 24 hours to enhance pollen release. Hand-pollinations were performed on two different days during full bloom.

On each branch, we assessed initial and final fruit set. Initial fruit set (percentage of flowers developing into fruitlets) is a good indicator of successful pollination and was assessed in May. Final fruit set was assessed in August, after the natural thinning occurred at the end of May. Seed set (the number of seeds per fruit) was established on 2 fruits per branch/treatment. However, because fruit set was low (see results), only 182 pears from the experimental branches (E=9, O=51, S=122) could be collected. For this reason, to increase the sample size for seed set under open pollination, 20 additional fruits per orchard were randomly collected from other trees (N=224).

2.3.1 Pollinator dependence, pollination service and pollination deficit

To assess the role of pollinating insects in pear production, we calculated two indices - pollination service (*PSer*) and pollinator dependence (*PDep*). Each index was calculated twice: once for the initial fruit set and the other for the final fruit set.

Pollination service (*PSer*) is an index that provides the effective contribution of pollinators to fruit set (Garratt et al. 2021), and it was calculated as:

$$PSer = \frac{fO - fE}{fO}$$

Where *f*O is the fruit set from the open pollination treatment and *f*E is the fruit set from the exclusion treatment.

Pollinator dependence (*PDep*) index denotes the dependence of the variety on pollinator visitation for fruit set (Garratt et al. 2021) and was calculated as:

$$PDep = \frac{fS - fE}{fS}$$

Where *f*S is the fruit set from the supplementary pollination treatment and *f*E is the fruit set from the exclusion treatment.

When *fO* or *fS* were 0, the *PSer* and the *PDep* indexes, respectively, were considered equal to 0.

Pollination deficit (*PDef*) measures the shortage in fruit production compared to the maximum potential fruit set when pollination is not limiting; pollination deficit was calculated as:

$$PDef = \frac{fS - fO}{fS}$$

When the fruit set achieved under open pollination was greater than under supplementary pollination, the deficit was considered equal to 0 (Garratt et al. 2014a; Castro et al. 2021).

Pollination service, pollinator dependence and pollination deficit indexes can also be calculated based on seed set. However, given the very low number of fruits obtained from the exclusion treatment (N=9), and that we included fruits from other plants for open seed set, they were not calculated in our study.

2.4. Pesticide load and risk

At the peak of bloom, around 40 newly opened pear flowers (ca. 10 gr) were collected in each orchard and kept at -20°C for multi-residue analysis. This analysis, based on the detection of 775 active ingredients, was carried out by Agriparadigma (Ravenna, Italy) using the UNI EN 15662:2018 method (with gas chromatography tandem mass spectrometry ,GC/MS/MS, and liquid chromatography tandem mass spectrometry, LC/MS/MS,) and the method CVUA EU RL-SRM QuPPe (Quick Method for the Analysis of Highly Polar Pesticides) Version 12 method 1.3 2021 (with LC/MS/MS) for glyphosate. With the residue data, a *risk index* (RI) was calculated as the sum of the "toxic units" of the active ingredients found in the flowers from each orchard. The index was calculated as the sum of each active ingredient (R) divided by its LD50 (for *Apis mellifera,* PPDB- Pesticide Properties Database https://sitem.herts.ac.uk/aeru/ppdb/). We examined both oral and contact LD50s and selected the lowest value, thus representing the worst-case scenario (Favaro et al. 2019; Sgolastra et al. 2024):

$$RI = \sum_{i=1}^{n} \frac{Ri}{LD50i}$$

Where *Ri* is the residue of compound *i,* expressed in mg/Kg found in the sample; LD50*i* is the acute oral/contact lethal dose of compound *i,* expressed in µg/bee.

According to the Phytosanitary Notebook (register of pesticides applied in each farm), insecticides were applied in March, before bloom, while plant growth regulators and fungicides were applied also during bloom, when flowers were collected for chemical analysis. The application of herbicides was not reported.

2.5. Landscape characterization

Land use composition was assessed at 0.5, 1.0 and 1.5 km buffers from the center of the orchard with CORINE Land Cover (CLC, Coordination of Information on the Environment) reference year 2018. CLC land use types were grouped into 3 categories: "Agricultural", "Urban" and "Pollinator-friendly"; the latter includes all land use types providing suitable flower and nesting resources for pollinators (CLC categories 1.4, artificial non-agricultural vegetated areas; 2.3 and 2.4, pastures and heterogeneous agricultural areas; 3.1and 3.2, forests and shrubs and/or herbaceous vegetation associations; and 4, wetlands). Complete information on landscape characterization is available in Figure S1 (Supplementary). The "pollinator-friendly" category was considered the main landscape factor that could positively influence pollinator abundance and pollination services. Landscape analyses were carried out with QGIS 3.22 version (https://qgis.osgeo.org).

2.6. Statistical analyses

One orchard (RA2) was already in bloom when the study started. For this reason, we were unable to perform the second pollinator survey and the pollinator exclusion treatment. This orchard was excluded from analyses of pollinator guilds and pollination services but was used in the pollination deficit and pesticide analyses.

2.6.1. Pollination services and pollination deficits

To test the effect of different pollination treatments on fruit set and seed set, Generalized Linear Mixed Models (GLMMs) were performed, where either initial or final fruit set or seed set were the dependent variables, and experimental treatment (Exclusion, Open and Supplementary) was the independent variable; orchard identity was added as a random factor. When treatment was significant, a Tukey post-hoc test was performed to identify differences between levels (Exclusion, Open and Supplementary). GLMM was also used to test the relationship between initial fruit set, a metric for pollination, and final fruit set, a metric for production, adding orchard identity and experimental treatment as random factors (Garratt et al. 2021).

One sample t-test was performed to test if pollination deficit, both for initial and final fruit set, across orchards differed significantly from 0.

Seed set is dependent on pollination (Feuerbacher et al. 2024; Siopa et al. 2024) and may be an easier variable to assess than fruit set, even if seed set in pear is considerably variable across cultivars (Nyéki & Soltész, 1998). For this reason, we used GLMM models to test the relationship between seed set and fruit set under open pollination, and between seed set and pollination deficit (Webber et al. 2020), with orchard identity included as a random factor.

GLMMs were performed using the *lme* function from the nlme package (Pinheiro et al. 2025) and post-hoc tests using the *emmeans* function from the emmeans package (Lenth, 2024).

2.6.2. Effects of environmental variables on pollinator visitation

We tested for the effects of landscape composition and pesticide exposure on pollinators (Table S2). Linear models were performed with the visitation rate of each pollinator group as the response variable (one model per pollinator group), and the pesticide risk index and the proportion of pollinator-friendly landscape (arcsine-transformed), as explanatory variables. These analyses were repeated three times, with the percentages of pollinator-friendly landscapes at the three buffers (0.5, 1.0 and 1.5 km), given that the three radii were not highly correlated (Table S4).

2.6.3. Effect of pollinator visitation, pesticide risk and fruit retention management on pollination performance and fruit set

Fruit and seed set in pears are influenced by pollinator visitation (Hünicken et al. 2021), but also by fruit retention management practices (e.g. irrigation, hormone applications, fertilization) (Musacchi et al. 2018). In our study, the latter can be assessed through the ability of the cultivar to set fruit without pollinator visitation (fruit set in pollinator exclusion treatment). In addition, pesticide exposure may alter the foraging behaviour of pollinators, ultimately resulting in decreased pollination services (Klatt et al. 2023; Stanley et al. 2015). We performed a series of linear models in which the response variable was a measure of pollination performance (pollination service index, pollination deficit index) or a measure of fruit set (initial fruit set, final fruit set, seed set in open pollination branches). The explanatory variables were the visitation rates of the three pollinator groups, honeybees, bumblebees and wild pollinators, fruit set in the pollinator exclusion treatment (as a proxy of fruit retention management) and risk index (Table S2).

In all models, the response variables were log10 transformed when normality assumptions were not met.

To identify the environmental and pollinator visitation variables affecting pear pollination and production, we applied a model selection approach based on information theory in all analyses described in sections 2.6.2 and 2.6.3 that included more than one explanatory variable (Table S3). We began with a full model including all explanatory variables of interest and used the *dredge* function from the MuMIn package to generate and compare all possible nested models. Model selection was based on Akaike's Information Criterion corrected for small sample sizes (AICc) (Burnham & Anderson, 2002). Models with a ΔAICc < 2 were considered to have substantial support. When the null model (i.e., the model without explanatory variables) was among the best models (ΔAICc < 2), we interpreted that none of the predictors had a meaningful effect. In most cases where the null model was not among the best models, only one top-ranking model emerged. We then reran this selected model separately to test the statistical significance of its predictors, adopting a significance threshold of $\alpha = 0.05$. In one case, three models were sorted as best models (ΔAICc < 2); therefore, we ran a model that included all variables selected across models (Table S3). To further assess model fit, we used AICc weights to evaluate the relative likelihood of the selected model in comparison to others. We also visually inspected quantile-quantile plots and residuals vs. fitted values plots to verify normality and homoscedasticity of residuals.

All analyses were conducted in R version 4.4.1 (R Development Core Team, 2024).

3. Results

3.1. Pollinator diversity and abundance

A total of 1762 insects were observed visiting pear flowers. The majority were honeybees (*Apis mellifera*, 46%), followed by muscoid flies (35%), syrphids (10%), bumblebees (*Bombus terrestris*, 4%), wild bees (3%) and other pollinators (wasps, Coleoptera and Lepidoptera, 2%). The abundance of each pollinator functional group across the orchards is reported in Figure S2. Visitation rates were normalized for 10,000 flowers, and the mean (± standard error, SE) values were 2.84 (± 0.67), 2.27 (± 0.26), 0.62 (± 0.11), 0.15 (± 0.11), 0.24 (± 0.06) and 0.03 (±0.02) for honeybees, muscoid flies, syrphids, bumblebees, wild bees and other pollinators, respectively (Figure 1).

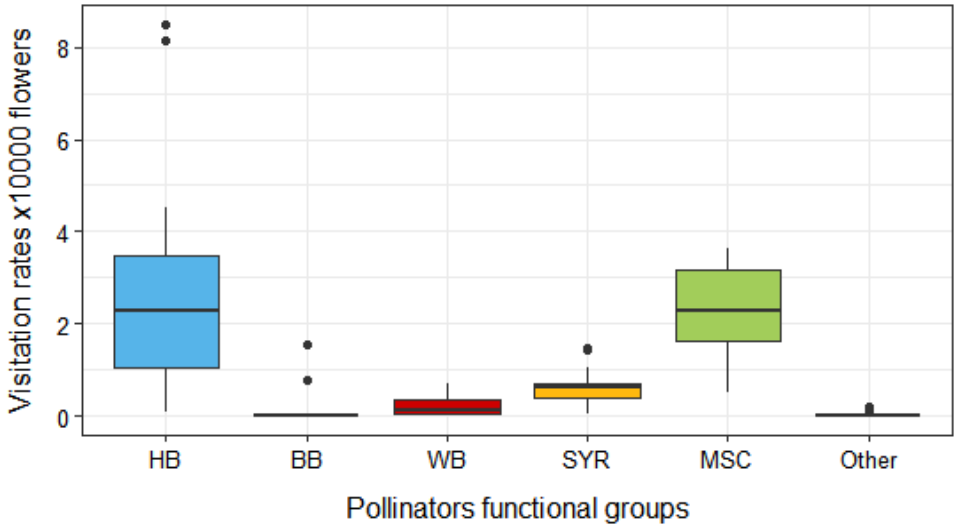

Figure 1. Visitation rates (n° of individuals per n° of flowers in the transect, normalized for 10,000 flowers) of pollinators functional groups. HB= Honeybees, BB= Bumblebees, WB= Wildbees, SYR= Syrphids, MSC= Muscoid flies.

3.2. Pollination experiments

Pollination treatment significantly affected initial ($\chi2=38.2$; df=2; $p<0.001$) and final fruit set ($\chi2=50.2$; df=2; $p<0.001$) (Figure 2a, b). Open pollination significantly increased initial and final fruit set compared to

pollinator exclusion, revealing a high dependence of both variables on pollinator visitation. Supplementary pollination significantly increased initial and final fruit set compared to open pollination, indicating a pollination deficit. Initial and final fruit set at the orchard level were highly related (R^2m=0.73, R^2c= 0.77, t= 34.78, p<0.0001).

Seed set also differed significantly across the three pollination treatments (GLM: $\chi 2$=71.19; df=2; p<0.001) (Figure 2c). It was significantly higher in fruits from supplementary pollination (mean ± SE=3.28 ± 0.19) than in fruits from the open pollination (1.96 ± 0.11) and than in fruits from the pollinator exclusion treatment (0.67 ± 0.55) *(Tukey test;* p *< 0.05)*.

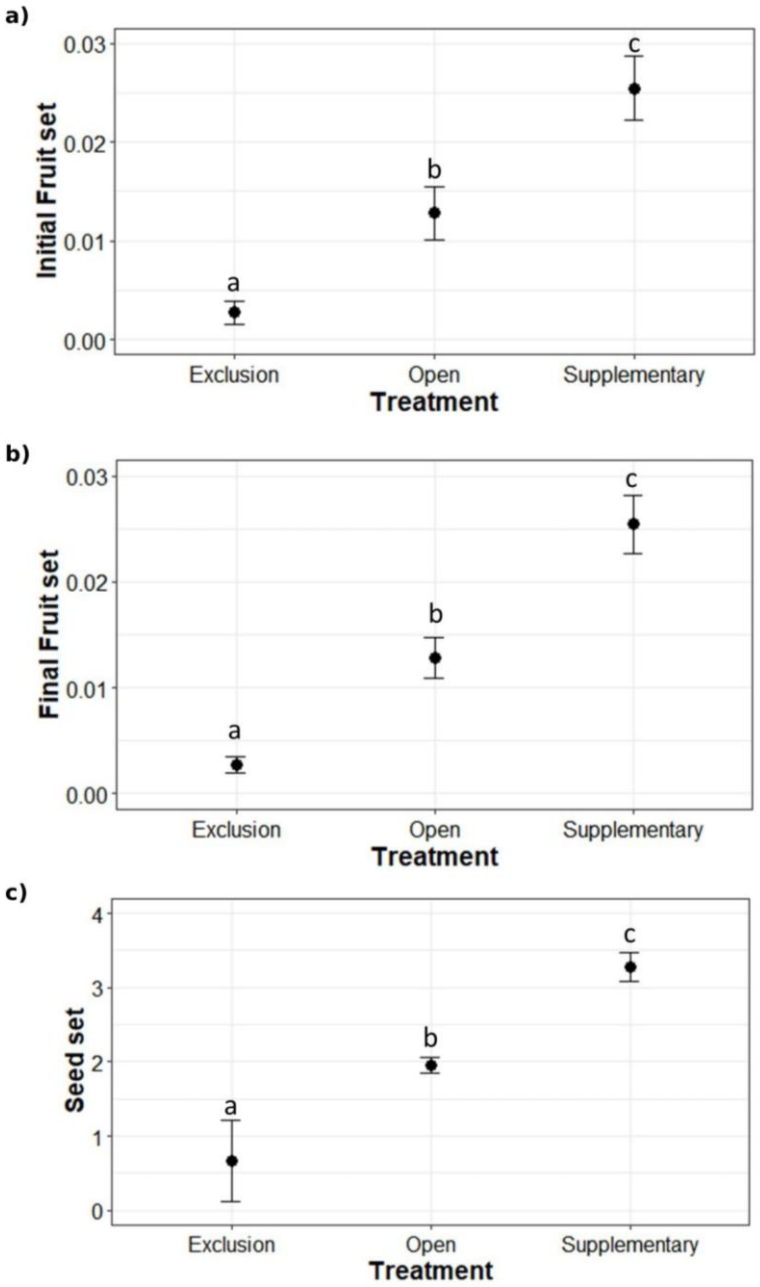

Figure 2. Mean (±SE) initial (a) and final fruit set (b), expressed as the proportion of flowers that set fruit, and seed set (c) in branches of the pollination experiment (pollinator exclusion, open pollination, and supplementary pollination) in 15 pear orchards. Different letters denote significantly different means (Tukey test; p < 0.05).

Seed set was significantly correlated to final fruit set (R^2m= 0.29, R^2c= 0.91, t= 2.50, p= 0.025) and marginally significant to pollination deficit (R^2m= = 0.20, R^2c= 0.90, t= -1.91, p=0.077). (Figure S3a, b).

3.2.1 Pollinator dependence, pollination service and pollination deficit

Mean (±SE) pollinator dependence (*PDep*) index was 0.40±0.06 (range: 0-0.8) for initial fruit set and 0.33± 0.06 (range: 0-0.8) for final fruit set.

Mean (±SE) pollination service (*PSer*) index was 0.18 ± 0.04 (range: 0-0.6) for initial fruit set and 0.17± 0.04 (range: 0-0.5) for final fruit set.

Pollination deficit was significant both at initial (t-test: t=5.36, df=14, p <0.001) and final fruit set (t-test: t=5.8, df=14, p <0.001), with mean (±SE) values of 0.31 ± 0.06 (range: 0.00–0.74) and 0.33 ± 0.06 (range: 0.00–0.74), respectively. The 95% confidence intervals were 0.19-0.44 and 0.21-0.45 for initial and final fruit set, respectively.

3.3. Pesticides and landscape composition

The multi-residue analysis on the pear flowers revealed the presence of 39 pesticides (Table S5) that were grouped into different categories: insecticides, fungicides, herbicides, plant growth regulators, and acaricides. The number of compounds per orchard ranged from 4 to 15 (mean ± SD= 9.93 ± 3.47), and total residue concentrations ranged from 0.011 mg/kg to 264 mg/kg (mean ± SE= 69.76 ± 36.0 mg/kg) (Figure 3a, b). Fungicides were the most abundant pesticide group (52.3%), with Dithianon and Pyrimethanil being the most frequent active ingredients, and Captan and its metabolites reaching unusually high levels (264 mg/kg) in one orchard. Insecticides accounted for 25.64% of the residue levels, with Pyriproxyfen detected in all orchards except one. Also noteworthy is the finding of the herbicide Glyphosate in 75% of the orchards. The list of all pesticides detected, and their residue level is reported in Table S5. The risk index ranged from 0.02 to 11.75 (mean ± SE=1.41 ± 0.71, median= 0.64); the highest risk index was detected in the orchard with high levels of Captan (MO2, Figure 3c).

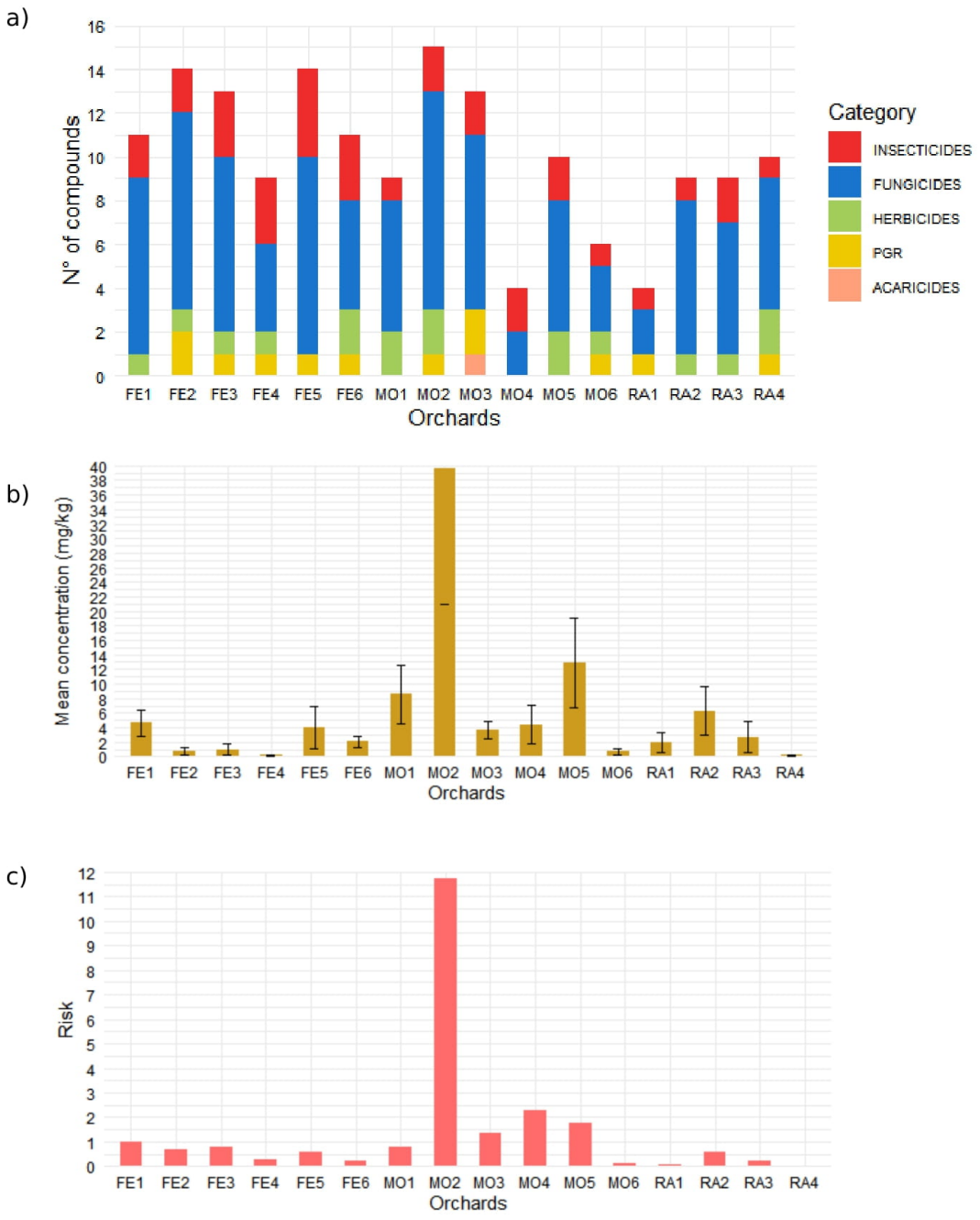

Figure 3. Number of compounds grouped by pesticides categories across orchards (a); mean (± SE) concentration (mg/kg) of pesticides across orchards (b) and Risk index in each orchard (c). PGR = Plant Growth Regulators.

The landscape was rather homogeneous across sites, with a predominance of agricultural land (mean ± SD = 84.2 ± 7.5%, 83.3 ± 2% and 82.6±6.6%) and a low percentage of "pollinator-friendly" areas (mean ± SD= 4.09 ± 3.6%, 4.86 ± 3.08% and 5.2±2.9%) at 0.5, 1.0 and 1.5 km, respectively.

3.4. Effects of pesticides and landscape composition on pollinators

For honeybee, bumblebee, syrphid and muscoid fly visitation rates, the null model was consistently among the best-supported models; therefore, risk index or pollinator-friendly landscape at the 3 radii were not relevant. However, for wild bees' visitation rate model selection highlighted pollinator-friendly landscape at

1.5 km as an explanatory variable, and it resulted as significant (estimate = 8.30e-05, SE = 3.48e-06, t = 2.39, p = 0.033) (Figure 4).

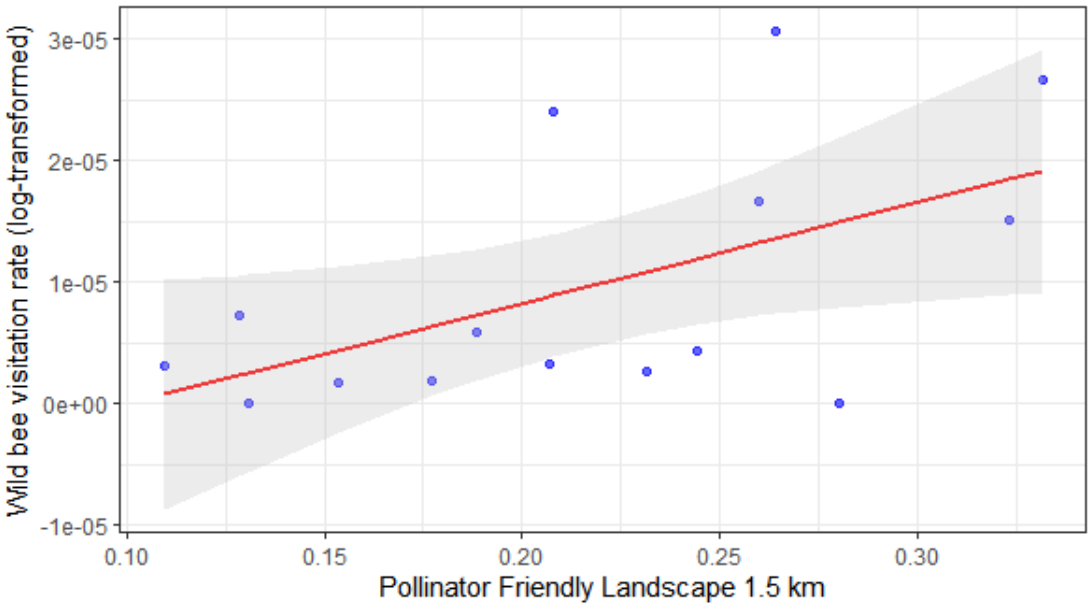

Figure 4. Significant relationship between Pollinator-friendly landscape cover at 1.5 km (arcsin-transformed) and visitation rate of Wild bees (log-transformed).

3.5 Effects of pollinator visitation, fruit retention management, and pesticide risk on pollination services, pollination deficits and fruit production

The model selection for initial fruit set identified fruit retention management and risk index as relevant explanatory variables (Table S3). Initial fruit set increased with the ability of the cultivar to initiate fruits without pollinators (a proxy of fruit retention management) (estimate = 2.08, SE = 0.23, t = 8.97, p <0.001) and decreased with increasing Risk index (estimate= -0.002, SE=0.00, t= -5.07, p< 0.001). Since orchard MO2 was identified as an outlier for the risk index, we re-run the model without it. Model selection retained fruit retention management but not risk index, and it was still significant (estimate = 2.08, SE = 0.24, t = 8.60, p <0.001) (Figure S3). For the final fruit set open, the null model was among the best-supported models (Table S3), indicating no relevant effects of the three explanatory variables.

For seed set, three best supported models emerged and included bumblebee visitation rate, fruit retention management and honeybee visitation rate, so we ran a model with these explanatory variables (Table S3). Surprisingly, the model unveiled a negative effect of bumblebees' visits on seed set (estimate= -8069.57, SE=3251, t=-2.48, p= 0.028) (Figure S5). Orchard FE1, which had the highest number of bumblebees, was identified as an outlier. We reran the model without this orchard, and the model selection reported the null model among the best models (ΔAICc <2); then the effect of bumblebee visitation was no longer relevant.

Model selection showed that, for pollination service and deficit in initial and final fruit set, the null model was consistently among the best models, indicating that pollinators and orchard management were not relevant for these response variables (Table S3).

4. Discussion

The aim of this study was to assess pollination services and pollination deficits in pears, an important self-incompatible crop. Additionally, we wanted to explore how landscape context and pesticide exposure influenced pollinator visitation and pear fruit production. Our results confirmed the high dependence of *P. communis* on pollinators and revealed low levels of pollination services and widespread pollination deficits. At the same time, our study confirmed the positive effect of pollinator-friendly landscapes on wild bee visitation to pear flowers. Alongside, we found a negative effect of pesticide risk on initial fruit set and a negative effect of bumblebees' visits on seed set, even if driven by one orchard in both cases.

4.1. Pollinator guilds

In line with other pear studies (Quinet et al. 2016; Fountain et al. 2019), we found a low overall pollinator visitation rate. In addition to the low attractiveness of pear flowers (Quinet and Jacquemart, 2017), in our study, this was probably exacerbated by the harsh weather conditions experienced during bloom, with temperatures often below 13°C during the day and below 0°C at night, which is a common condition for crops blooming in early spring like *P. communis* (Belien et al. 2021; Reeves et al. 2022).

The pollinator community was dominated by the honeybee *Apis mellifera*, in line with the results of Fountain et al. (2019). However, we did not observe any effect of honeybees' visitation on fruit set, as well as for other pollinators, as also reported by other studies (Eeraerts et al. 2025 and references therein). Although much less abundant than honeybees, we found a negative effect of bumblebee visitation on seed set. Even though bumblebees are considered efficient pollinators of *P. communis* (Jacquemart et al. 2006; Zisovich et al. 2012), some studies reported negative effect of *Bombus terrestris*' visits to flowers. For instance, the study of Smessaert et al. (2021) documented lower fruit set and seed set in *Conference* pear after bumblebee visits, that were attributed to flower damage caused during visitation. Also, Mayer and Lunden (1997) observed that bumblebees tended to work newly opened pear flowers with unripe anthers, therefore, not effectively transferring pollen. Moreover, Saez et al. (2014) showed that increased bumblebees' visitation damage raspberry pistils, therefore compromising drupelet set. These mechanisms may provide possible explanation for our result, although further studies are needed to clarify the effectiveness of these managed pollinators in pear orchards. Many studies show that wild bees are more effective pear pollinators than honeybees (Monzón et al. 2004; Garibaldi et al. 2013; Fountain et al. 2019; Reilly et al. 2020; Belien et al. 2021; Hünicken et al. 2021), but in our study, wild bees accounted for just 3% of the visits recorded. The study of Monzón et al. (2004) shows the beneficial effects of solitary bee visitation on pear seed set even against a strong background of honeybees. Therefore, enhancing wild bee communities near pear orchards would likely improve fruit quality and provide stability in terms of pollination services.

4.2. Pollination dependence, service and deficit

Pear is known to be a strong pollinator-dependent crop because of genetic self-incompatibility (Delaplane & Mayer, 2020; Quinet and Jaquemart, 2017; Goldway et al. 2019; Belien et al. 2021), and our results confirm the dependence of *P. communis* on pollinators, reporting a dependence for the cultivar *Abate Fétel*. Indeed, the pollinator dependence index was around 40% and 33% for initial and final fruit set, respectively. According to Siopa et al. (2024), *P. communis* has an average pollinator dependence of 74%, but with variations across cultivars, ranging from a minimum of 15% to a maximum of 100%.

As expected, based on the low visitation rates observed in our study and in others (Quinet et al. 2016; Fountain et al. 2019; Hünicken et al. 2021), pollination services in our pear orchards were rather low (around 17%). Pollination deficits were pervasive across the study orchards, with mean values around 31% and reaching 74% in one orchard. Pollination deficits may be influenced by insufficient compatible pollen

deposition; in fact, it is a common practice to place honeybee hives in pear orchards to boost cross-pollination (Goldway et al. 2019), but in our study, we did not observe a beneficial effect of honeybee visits on fruit set or pollination deficit reduction. This may be because honeybees tend to move along tree rows rather than between rows (Monzón et al. 2004; Quinet and Jaquemart 2017), hindering cross-pollination between cultivars. This aspect may also be exacerbated by the layout of pear cultivars in the orchards (e.g., pollinisers planting design and density), which were primarily organized into blocks (Quinet and Jaquemart 2017). We reported significant pollination deficits, although we observed a significant effect of the ability of the plant to set fruit without pollination, probably influenced by management practices based on hormone application, fertilization and other practices that promote fruit retention. Given that the cultivar resulted dependent on pollinators and experienced a pollination deficit, it would benefit from an increase in pollination service (Hünicken et al. 2021; Saez et al. 2022).

However, our results indicate that the two most commonly managed species for pear pollination—honeybees and bumblebees—are not particularly effective, raising concerns about the current management of pollination services.. Other managed pollinators, such as *Osmia spp*. have proven to be more efficient because they are attracted by pear flowers and are active under lower temperatures (Maccagnani et al. 2003; Monzón et al. 2004; Fountain et al. 2019; Goldway et al. 2019).

4.3. Pesticide risk and landscape characterization

We evaluated pesticide contamination and characterized the landscape surrounding the orchards to evaluate the effect of local and landscape factors on pollinator guilds and pollination services. We detected 39 different compounds in pear flowers, including 10 insecticides, reflecting their persistence from pre-bloom sprays. The Risk index ranged from 0.02 to 11.75 to (mean=1.41). One orchard, MO2, had a very higher risk index, mostly due to high levels of the fungicide Captan, which was applied during bloom. We found no effect of the Risk index on pollinator visitation rate. However, the Risk index had a negative effect on initial fruit set, which is the metric for pollination, even if this effect was driven by the MO2 orchard. Pesticide exposure may affect the behaviour of pollinators as reported by different studies (Stanley et al. 2015; Tamburini et al. 2021; Avila et al. 2023; Klatt et al. 2023), therefore, even if the risk did not affect pollinators visitation rates it may have interfered indirectly with the pollination process. Indeed, Tamburini et al. (2021) reported a reduction in pollen deposition per flower visit by bumblebees after pesticide exposure, therefore, compromising the pollination process Moreover, Stanley et al. (2015) observed a reduced seed set in apple orchards with bumblebee colonies previously exposed to pesticides, even if individuals showed an increased foraging activity, thus suggesting a behaviour's alteration. Our results confirm the high level of pesticide contamination in agricultural areas (Sgolastra et al. 2024), with high numbers of compounds, some of which could interact synergistically (Sgolastra et al. 2017). The study was conducted in an intensively farmed area, with low cover of the landscape suitable for pollinators. Nevertheless, we observed a significant positive effect of pollinator-friendly cover at 1.5 km on wild bee visitation rate to pear flowers. The proportion of this pollinator-friendly cover was around 5%, likely too low to maintain larger populations of wild bees that are able to provide an effective contribution in pollination service; the study of Eeraerts (2023) suggests that a proportion up to 15% of semi-natural areas have a positive effect on pollination services to cherry crop, while Holzschuh et al. (2012) found a significant effect in a context with more than 18% of high-diversity habitats. Indeed, increasing the availability of pollinator-friendly habitat would enhance wild bees visitation to pear orchards and possibly promote the stability of their populations, thus improving pollination (Ganser et al. 2018; Magrach et al. 2022; Rundlöf et al. 2022) and other ecosystem services (Tamburini et al. 2020).

5. Concluding remarks

Our study reports on pollinator visitation rates and widespread pollination deficits in *Pyrus communis*, on which there are currently few studies, in a leading production area. Importantly, these deficits are not offset through the use of phytohormone applications to enhance fruit retention. Our results also suggest that the two most abundant (managed) pollinator species are not significantly contributing to pear pollination services in the study orchards and that enhancing semi-natural areas to boost wild bees' visits to pear flowers would be a more effective strategy. Our study also reveals that pear orchards are heavily contaminated with multiple pesticides, which may have contributed over the years to a significant decline in wild bee populations (as suggested by their low frequency in the study areas), ultimately resulting in negative impacts on pollination services. The main limitation of our study is that it was carried out under critical weather conditions, which may have exacerbated pollination deficit. Future research should be done under more favourable weather conditions and focus on a deeper investigation on pear pollinator community by identifying the contribution of different pollinator species to pollination service. Alongside, the restoration of semi-natural habitats surrounding pear orchards and the reduction of pesticide applications would enhance diverse wild pollinator communities and may reduce pollination deficits in this understudied crop.

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Conclusions

This research aimed to provide a scientific contribution in assessing trend, causes and consequences of pollinator decline with a special focus on the agroecosystem. For this purpose, we first analysed pollinator communities over a broad temporal scale in Northern Italy, using an historical entomological collection, to assess changes in species composition and ecological traits. Second, since pesticides are among the main causes of pollinator declines with land use, we investigated the pathways of pesticide exposure in the apple agroecosystem, which is highly dependent on insect pollinators, and the role of landscape in shaping pesticide contamination. Finally, we investigated the pollination deficit in another pollinator-dependent crop, *Pyrus communis*, to understand how anthropic pressures, namely landscape composition and pesticide exposure, in interaction with the pollinator community, influence fruit production.

In the first chapter, we analysed a bee community from Northern Italy based on the Guido Grandi historical collection, in order to assess changes in species composition, diversity and ecological traits over time. The results showed a substantial shift in species composition, with differences in the bee community compared to the historical collection. The observed species turnover was associated with a general reduction in species diversity, particularly in urban areas, thus confirming urbanization as a pressure on biodiversity. When analysing ecological diversity, based on traits such as *nesting habit, sociality, lecty* and *voltinism*, and body size (measured as ITD), no significant differences emerged. This phenomenon, known as functional redundancy, suggests that ecosystem functions remain stable despite species turnover; however, the loss of species diversity could still compromise ecosystem interactions. Therefore, further studies are needed, especially integrating the study of plant-pollinator interactions, in order to adopt adequate strategies to protect the overall ecosystem. This chapter highlights the importance of conducting long-term research to better understand spatio-temporal pollinator population dynamics.

In the second chapter, we assessed the level of pesticide contamination in different flowers occurring in the apple agroecosystem. The results reported a widespread contamination in all flowers, inside and outside orchard and across the seasons, spring (apple bloom) and summer (post-bloom). Overall, our results showed no significant differences in pesticide contamination between crop flowers and wildflowers inside the orchards, as well as between wildflowers collected inside and outside the orchards. Interestingly, while the number of pesticides decreased in summer, the risk did not follow the same trend, presumably due to more toxic compounds used after apple bloom. Moreover, the results confirmed the importance of natural areas surrounding orchards in reducing pesticides contamination, as well as increasing the probability of 0 risk. The findings of this study should be considered in the planning of orchard pest management strategies, with the aim of minimizing risks to pollinators and thereby enhancing pollination services.

In the third chapter, we assessed the pollination deficit in *Pyrus communis* and analysed the effect of landscape characterization, pesticide risk and pollinators on pollination performance. The results confirmed the high dependence of *P. communis* on pollinators, and a significant pollination deficit. We also found a pervasive pesticide contamination on pear flowers, as all samples contained at least 4 compounds. We observed a low pollination service and overall low pollination visitation rates. In addition, we found negative effect of bumblebees' visits on seed set. However, we observed increased wild bees' visits to pear flowers thanks to higher proportion of natural landscape at 1.5 km. Since wild bees have been demonstrated to be efficient pollinators for pear, their presence in pear orchards should be enhanced. Our results reported a negative effect of pesticide risk on initial fruit set, suggesting possible interferences in the pollination process.

This thesis provides further evidence of the ongoing decline in pollinators and highlights the major role of anthropogenic activities, especially urbanization and pesticide use, in driving this trend. By integrating long-term changes in pollinator communities with contemporary assessments of pesticide exposure and

pollination performance in crop systems, our results show how pollination services in agroecosystems are affected. Our findings demonstrate that common agricultural systems are pervasively contaminated by pesticides, resulting in constant exposure for pollinators, with potential consequences for pollinator activity and crop pollination. Moreover, we consistently identified the importance of surrounding natural landscapes and their role in mitigating contamination and enhancing the delivery of one of the most essential ecosystem services: pollination. Taken together, these results emphasize that effective conservation and management strategies should rely on spatio-temporal approaches, and promote landscape-scale planning, reduced chemical inputs and the conservation of functionally diverse pollinator communities in both natural and agricultural systems.

Appendix 1

Supplementary Materials-Chapter 1

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Table S1. List of the 36 species analysed for body size. Mean ± SE ITD for each species, sex, Macroarea and period are reported.

Species	Sex	Macroarea	ITD (mm) past	ITD (mm) present
Andrena truncatilabris	F	City	2.96 ± 0.15	2.67 ± 0.04
Anthidium florentinum	F	City	3.81 ± 0.05	3.48 ± 0.09
Anthidium manicatum	F	City	3.46 ± 0.15	3.64 ± 0.04
Anthophora plumipes	M	City	4.29 ± 0.08	4.43 ± 0.14
Bombus pascuorum	F	City	3.57 ± 0.76	3.73 ± 0.08
Eucera nigrescens	M	City	3.33 ± 0.05	3.45 ± 0.21
Habropoda tarsata	M	City	5 ± 0.07	4.70 ± 0.11
Lasioglossum marginatum	F	City	1.79 ± 0.04	1.46 ± 0.18
Lasioglossum politum	F	City	0.96 ± 0.04	0.90 ± 0.05
Megachile centuncularis	M	City	2.63 ± 0.13	2.79 ± 0.22
Osmia bicornis	M	City	2.67 ± 0.08	2.79 ± 0.08
Bombus humilis	F	Apennine	3.58 ± 0.23	4.57 ± 0.36
Bombus humilis apenninus	F	Apennine	3.79 ± 0.22	3.71 ± 0.25
Bombus pascuorum	F	Apennine	2.86 ± 0.14	3.57 ± 0.08
Halictus simplex	F	Apennine	2.17 ± 0.15	1.90 ± 0.26
Lasioglossum interruptum	F	Apennine	1.54 ± 0.11	1.62 ± 0.05
Lasioglossum malachurum	F	Apennine	1.58 ± 0.11	1.57 ± 0.08
Andrena afzeliella	F	Both	2.08 ± 0.18	2.33 ± 0.21
Andrena decipiens	F	Both	2.58 ± 0.08	2.71 ± 0.08
Andrena flavipes	F	Both	2.54 ± 0.08	2.55 ± 0.10
Andrena minutula	F	Both	1.33 ± 0.08	1.38 ± 0.05
Andrena senecionis	F	Both	2.5 ± 0.07	2.36 ± 0.13
Bombus pratorum	F	Both	4.11 ± 0.27	4.29 ± 0.16
Halictus maculatus	F	Both	1.58 ± 0.04	1.57 ± 0.08
Hylaeus gibbus	F	Both	1.42 ± 0.04	1.48 ± 0.05
Lasioglossum villosulum	F	Both	1.33 ± 0.11	1.38 ± 0.05
Osmia aurulenta	F	Both	3.21 ± 0.04	2.76 ± 0.47
Osmia cornuta	F	Both	3.92 ± 0.04	3.83 ± 0.17
Seladonia subaurata	F	Both	1.46 ± 0.11	1.62 ± 0.05
Amegilla albigena	M	Both	2.33 ± 0.21	3.10 ± 0.05
Andrena flavipes	M	Both	2.71 ± 0.08	1.83 ± 0.04
Andrena minutula	M	Both	2.55 ± 0.10	1.43 ± 0.14
Andrena senecionis	M	Both	1.38 ± 0.05	2.45 ± 0.17
Anthophora crinipes	M	Both	2.36 ± 0.13	3.79 ± 0.15
Bombus lapidarius	M	Both	4.29 ± 0.16	4.29 ± 0.00
Colletes hederae	M	Both	1.57 ± 0.08	2.95 ± 0.24
Eucera clypeata	M	Both	1.48 ± 0.05	3.14 ± 0.22
Osmia scutellaris	M	Both	1.38 ± 0.05	1.52 ± 0.05

Table S2. Number of species according to ecological traits, time periods and macro-areas.

Category	**Trait**	**Period**	**Macroarea**	**N° of species**
Nesting	Ground	past	City	110
Nesting	Ground	present	City	65
Nesting	Ground	past	Appennine	78
Nesting	Ground	present	Appennine	67
Nesting	Above	past	City	41
Nesting	Above	present	City	36
Nesting	Above	past	Appennine	31
Nesting	Above	present	Appennine	32
Lecty	Oligolectic	past	City	31
Lecty	Oligolectic	present	City	23
Lecty	Oligolectic	past	Appennine	19
Lecty	Oligolectic	present	Appennine	25
Lecty	Polilectic	past	City	115
Lecty	Polilectic	present	City	77
Lecty	Polilectic	past	Appennine	90
Lecty	Polilectic	present	Appennine	73
Sociality	Solitary	past	City	121
Sociality	Solitary	present	City	74
Sociality	Solitary	past	Appennine	78
Sociality	Solitary	present	Appennine	68
Sociality	Social	past	City	23
Sociality	Social	present	City	22
Sociality	Social	past	Appennine	31
Sociality	Social	present	Appennine	28
Voltinism	Univoltine	past	City	121

Voltinism	Univoltine	present	City	86
Voltinism	Univoltine	past	Appennine	97
Voltinism	Univoltine	present	Appennine	85
Voltinism	Bivoltine	past	City	31
Voltinism	Bivoltine	present	City	15
Voltinism	Bivoltine	past	Appennine	15
Voltinism	Bivoltine	present	Appennine	14

Table S3. Fisher's Exact Test results for proportional shifts in bee ecological traits across time periods and macro-areas (City vs. Appennine). 95% confidence intervals and p-values are shown.

Macro-area	**Trait category**	**95% CI**	**p-value**
City	Lecty	0.469-1.754	0.756
City	Nesting	0.831-2.647	0.165
City	Sociality	0.770-3.161	0.182
City	Voltinism	0.321-1.395	0.319
Appennine	Lecty	0.296-1.272	0.176
Appennine	Nesting	0.636-2.268	0.55
Appennine	Sociality	0.539-1.984	1
Appennine	Voltinism	0.448-2.519	1

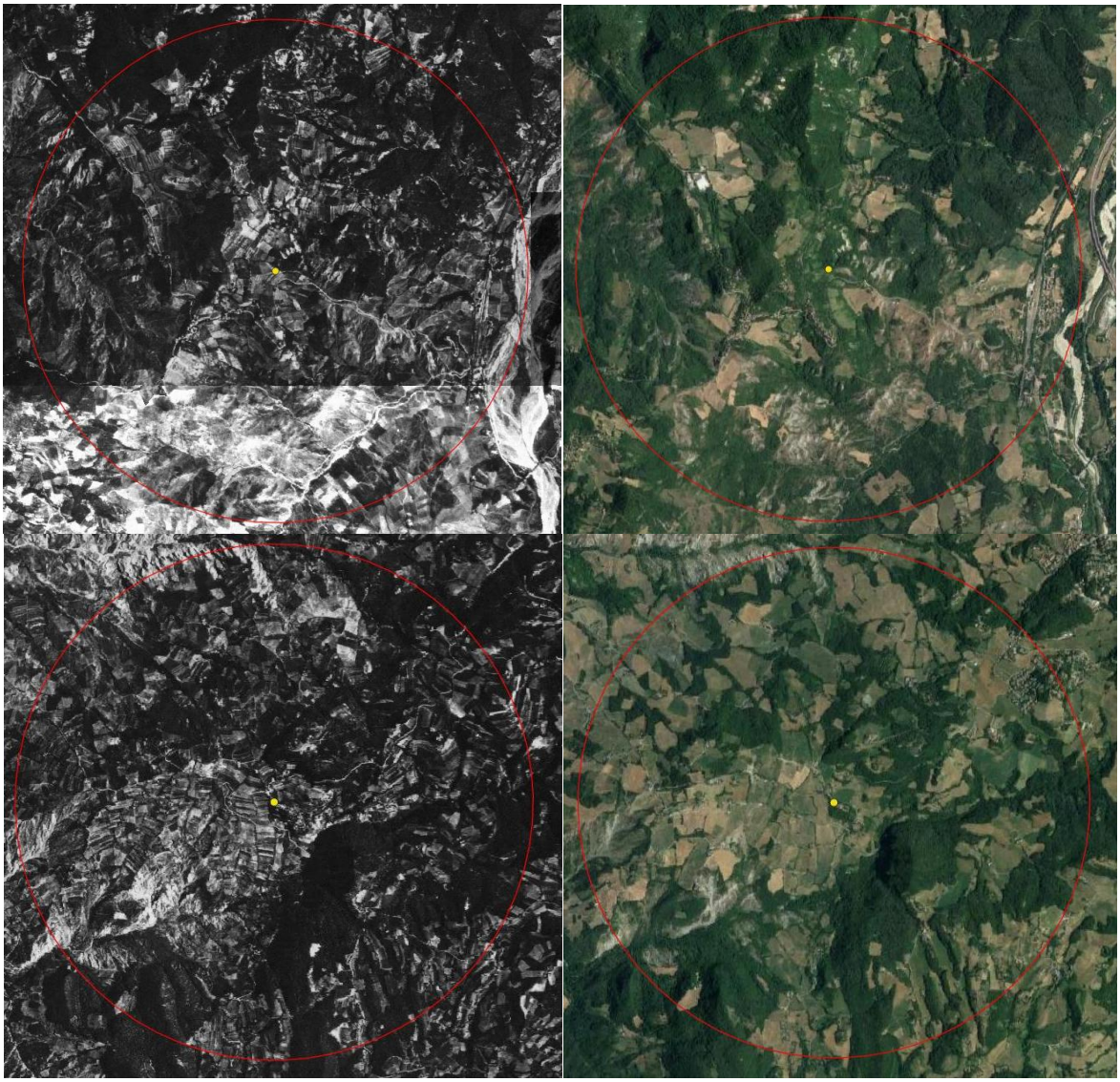

Figure S1. Orthophotos of the two study sites (2 km radius) in the Apennines from 1944 (left) and 2021 (right).

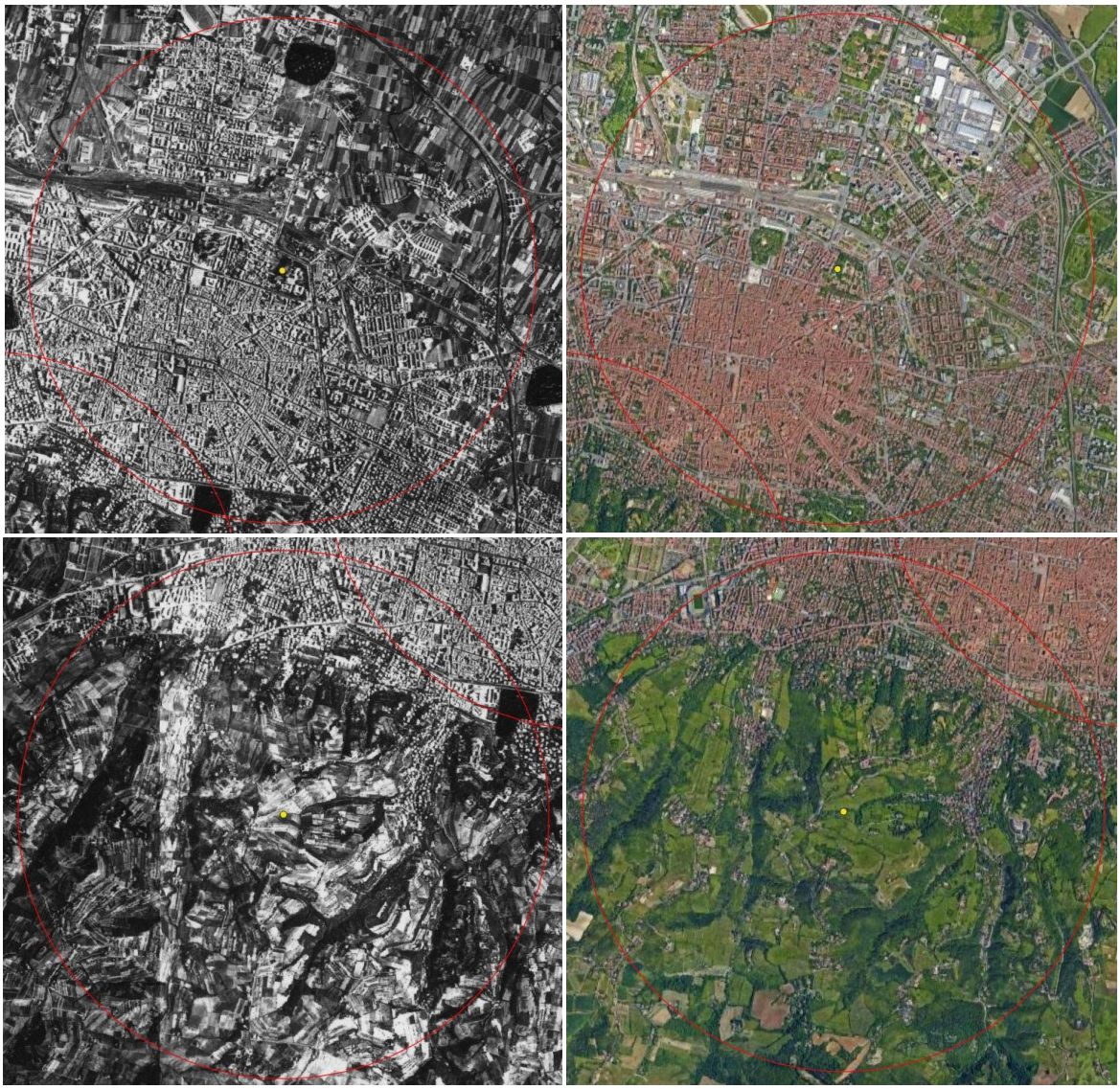

Figure S2. Orthophotos of the two study sites (2 km radius) in the City of Bologna from 1944 (left) and 2021 (right).

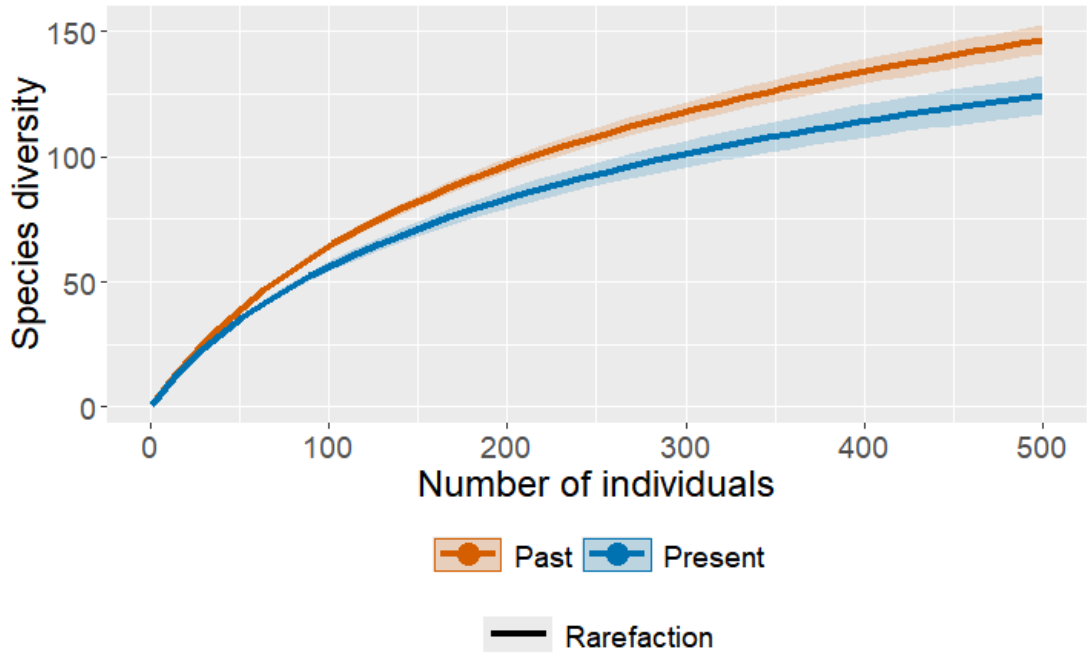

Figure S3. Diversity accumulation curves (and 95% confidence intervals) compared between past (1925-1926 and 1934-1935) and present (2021 and 2022), based on Hill number q=0, based on the overall dataset.

Appendix 2

Supplementary Materials-Chapter 2

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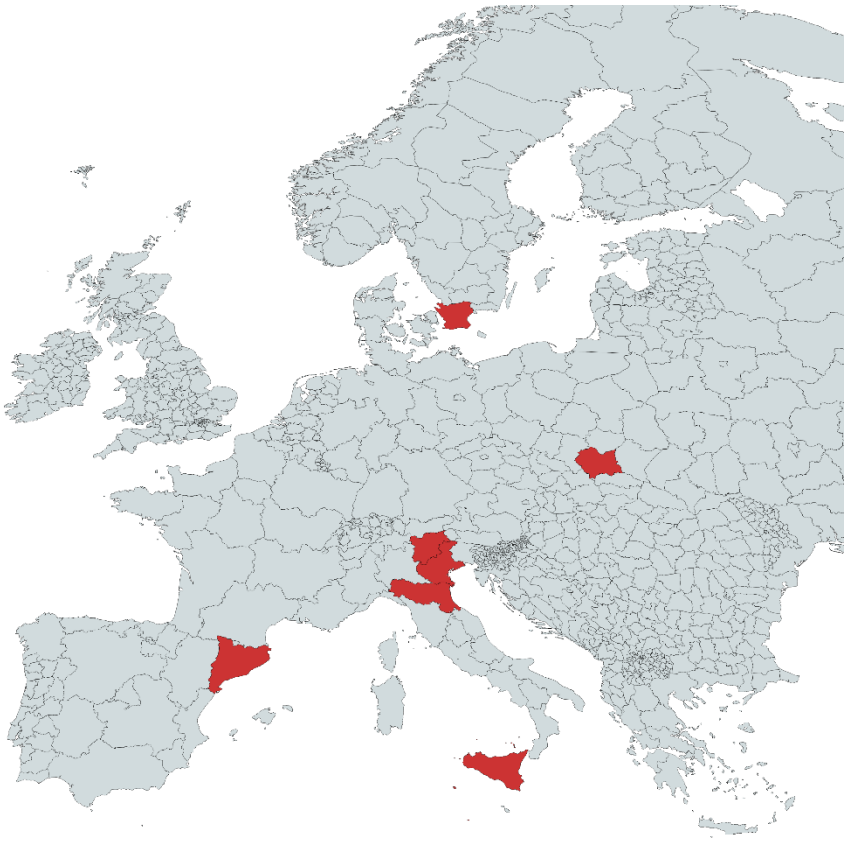

Figure S1. Map of the 7 macro areas including the 27 sampling sites: Catalunya (N=4) in Spain; Sicilia (N=3), Emilia-Romana (N=8), Veneto (N=3) and Trentino Alto-Adige (N=3) in Italy; Malopolska (N=3) in Poland; Skåne (N=3) in Sweden. Map created with *mapchart.net.*

Table S1. List of the 89 pesticides detected, category of belonging, minimum, maximum and mean residue levels (mg/kg), LD50 (based on *Apis mellifera*; oral in red) and frequency of detection. PGR= Plant Growth Regulators

#	Product	Category	LD50 µg/bee (oral/contact)	Min	Max	Mean	Frequency
1	1-Naftil acetamide	PGR	100	0.031	0.031	0.031	0.59%
2	2.4 D	Herbicide	94	0.016	84.5	10.61	4.71%
3	2.4-D (Sum of 2.4-D. its salts, esthers and conjugates, expressed as 2.4-D)	Herbicide	94	0.016	84.5	10.61	4.71%
4	2.4-D-metil estere	Herbicide	100	0.17	0.17	0.17	0.59%
5	4-CPA	PGR	NA	0.38	0.38	0.38	0.59%
6	6-Benzil Adenina	PGR	58.73	0.061	0.061	0.061	0.59%
7	Acetamiprid	Insecticide	8.09	0.012	6.03	0.84	14.12%
8	Alfa-Asarone	Fungicide	NA	0.017	0.502	0.26	1.18%
9	Ametoctradin	Fungicide	100	0.012	0.012	0.012	0.59%
10	Benzalconio cloruro (miscela di cloruri di alchilbenzildimetilammonio con catene alchiliche aventi una lunghezza di C8. C10. C12. C14. C16 e C18)	Biocide	NA	0.019	0.197	0.067	7.65%
11	Bifentrin (sum of the isomers)	Insecticide	0.016	0.018	0.018	0.018	0.59%
12	Boscalid	Fungicide	166	0.01	0.088	0.025	8.24%
13	Bupirimate	Fungicide	50	0.01	4.7	0.666	9.41%
14	Captano	Fungicide	100	0.02	1780	43.45	37.06%
15	Captano (Somma di captano e tetraidroftalimmide (THPI) espressa come captano)	Fungicide	100	0.026	1930	49.96	41.76%
16	Chlorantraniliprole	Insecticide	4	0.011	2.54	0.504	10%
17	Chlorpirifos	Insecticide	0.068	0.011	0.138	0.044	7.65%
18	Chlorpirifos Methyl	Insecticide	0.148	0.018	0.098	0.057	2.35%
19	Cloruro di benzildimetildodecilammonio (BAC-C12)	Biocide	NA	0.019	0.146	0.054	7.06%
20	Cloruro di benzildimetiltetradecilammonio (BAC-C14)	Biocide	NA	0.011	0.051	0.019	5.88%
21	Cyflufenamid (somma di ciflufenamid (isomero Z) e del relativo isomero E. espressa come ciflufenamid)	Fungicide	100	0.021	2	0.580	5.29%
22	Cypermetrin. included other mixtures of isomers (sum of the isomers)	Insecticide	0.023	0.016	0.051	0.029	1.76%
23	Cyprodinil	Fungicide	75	0.01	28.9	2.374	15.88%
24	Deltametrin	Insecticide	0.0015	0.013	0.975	0.239	5.29%
25	Difenoconazole	Fungicide	100	0.01	7.62	0.732	14.71%
26	Dimethomorph	Fungicide	32.4	0.017	0.226	0.077	2.35%

27	Dimetomorf (somma degli isomeri)	Fungicide	32.4	0.013	0.013	0.013	0.59%
28	Dimetoate	Insecticide	0.1	0.018	0.02	0.019	1.18%
29	Dithianon	Fungicide	25.4	0.01	20.2	2.326	44.71%
30	Dodine	Fungicide	145	0.022	5.45	0.863	20%
31	Emamectin (Emamectin benzoate B1a)	Insecticide	0.0036	0.184	0.841	0.421	1.76%
32	Ethirimol	Fungicide	1.6	0.02	0.361	0.162	3.53%
33	Etofenprox	Insecticide	0.038	0.02	1.73	0.284	7.65%
34	Hexythiazox (qualsiasi % di isomeri costituenti)	Acaricide	112	0.026	0.026	0.026	0.59%
35	Famoxadone	Fungicide	1	0.012	0.012	0.012	0.59%
36	Fenpyrazamina	Fungicide	100	0.398	0.398	0.398	0.59%
37	Flonicamid	Insecticide	60.5	0.01	10.4	0.743	26.47%
38	Flonicamid (somma di Flonicamid. TFNA e TFNG espressa in flonicamid)	Insecticide	60.5	0.01	12.4	0.888	29.41%
39	Fluazinam	Fungicide	100	0.01	7.88	0.556	20.59%
40	Fludioxonil	Fungicide	100	0.011	0.113	0.042	2.94%
41	Fluopyram	Fungicide	100	0.013	0.031	0.022	1.18%
42	Flupyradifurone	Insecticide	1.2	0.01	1.3	0.123	10.59%
43	Fluroxypyr	Herbicide	37.1	0.028	0.75	0.389	1.18%
44	Fluroxipir (somma di fluroxipir. i suoi sali. gli esteri e i coniugati. espressa come fluroxipir)	Herbicide	37.1	0.028	0.75	0.389	1.18%
45	Fluroxypyr-1-metileptil estere	Herbicide	100	0.014	1.53	0.557	1.76%
46	Fluvalinate (somma di isomeri) risultante dall'impiego di tau-fluvalinate	Insecticide	12	0.01	32.6	2.26	10.59%
47	Fluxapyroxad	Fungicide	100	0.011	2.72	0.690	20.59%
48	Folpet	Fungicide	100	0.028	25.2	2.884	5.88%
49	Folpet (somma di folpet e phtalimmide espressa in folpet)	Fungicide	100	0.117	42	3.519	8.24%
50	Formetanato (somma di formetanato e relativi sali. espressa in cloridrato di formetanato).	Insecticide	0.16	0.034	0.321	0.150	2.35%
51	Ftalimmide	Fungicide	100	0.02	8.56	0.853	8.24%
52	Glyphosate	Herbicide	100	0.01	87.5	1.539	40.59%
53	Indoxacarb (somma di indoxacarb e del suo enantiomero R)	Insecticide	0.08	0.072	1.07	0.515	1.76%
54	Kresoxim-methyl	Fungicide	100	0.01	3.6	0.379	8.24%
55	Lenacil	Herbicide	206.2	0.012	0.012	0.012	0.59%
56	Lambda-cyhalothrin	Insecticide	0.038	0.016	0.495	0.256	1.18%
57	MCPA	Herbicide	200	0.02	2.21	0.629	4.71%
58	MCPA. MCPB e relativi sali. esteri e coniugati espressi come MCPA	Herbicide	81.8	0.02	2.21	0.629	4.71%
59	Mefentrifluconazole	Fungicide	100	0.011	11.7	1.339	18.24%
60	Metazachlor	Herbicide	72.2	0.017	0.037	0.027	1.76%
61	Metobromuron	Herbicide	119.1	0.024	0.024	0.024	0.59%
62	Metrafenone	Fungicide	114	0.019	0.051	0.039	1.76%

63	Myclobutanil (somma degli isomeri costituenti)	Fungicide	33.9	0.01	0.01	0.01	0.59%
64	Oxathiapiprolin	Fungicide	40.26	0.011	0.073	0.042	1.18%
65	Paclobutrazolo (Somma degli isomeri costituenti)	PGR	2	0.038	0.274	0.160	2.35%
66	Penconazolo (sum of the isomers)	Fungicide	3	0.011	0.052	0.026	3.53%
67	Pendimetalin	Herbicide	100	0.098	0.13	0.114	1.18%
68	Penthiopyrad	Fungicide	500	0.083	2.55	0.968	1.76%
69	Phosmet	Insecticide	0.22	0.036	0.158	0.097	1.18%
70	Phosmet (phosmet and phosmet oxon)	Insecticide	0.22	0.036	0.158	0.097	1.18%
71	Pirimicarb	Insecticide	4	0.026	1.89	0.565	3.53%
72	Pirimicarb desmetil	Insecticide	4	0.247	0.36	0.304	1.18%
73	Pirimicarb desmetyl formamide	Insecticide	4	0.011	0.077	0.035	2.35%
74	Propiconazolo (somma degli isomeri)	Fungicide	100	0.012	0.012	0.012	0.59%
75	Proquinazid	Fungicide	125	0.021	11.3	3.50	2.35%
76	Pyriproxyfen	Insecticide	74	0.012	0.495	0.112	7.06%
77	Pyraclostrobin	Fungicide	100	0.011	0.038	0.019	2.94%
78	Pyrimethanil	Fungicide	100	0.01	43.7	2.496	40%
79	Spirotetramat	Insecticide	100	0.011	7.72	1.085	5.29%
80	Spirotetramat - BYI08330-chetoidrossi	Insecticide	100	0.219	0.312	0.266	1.18%
81	Spirotetramat - BYI08330-enolo	Insecticide	100	0.015	2.74	0.783	2.94%
82	Spirotetramat and spirotetramat-enol (sum of).expressed as spirotetramat	Insecticide	100	0.011	11.1	1.821	4.71%
83	Tebuconazole	Fungicide	83.05	0.011	0.513	0.058	13.53%
84	Tetraconazole (somma degli isomeri costituenti)	Fungicide	63	0.01	0.015	0.013	1.18%
85	Tetraidroftalimmide THPI	Fungicide	100	0.011	97.4	5.692	42.94%
86	TFNA	Insecticide	60.5	0.011	0.394	0.077	27.06%
87	TFNG	Insecticide	60.5	0.01	1.63	0.223	20.59%
88	Trifloxystrobin	Fungicide	200	0.01	0.01	0.01	0.59%
89	Zoxamide	Fungicide	100	0.059	0.086	0.070	1.76%

Appendix 3

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Table S1. General description of the 16 studied orchards with information on orchard management, mean visitation rate of all pollinators, productive metrics (mean Initial fruit set, mean final fruit set and mean seed set under open pollination) and pollination deficits.

ORCHARD	Polliniser distribution	Hormones application	Managed pollinators	Mean visitation rate	Initial fruit set	Final fruit set	Seed set	PDef initial fruit set	PDef final fruit set
FE1	Alternate blocks	No	Yes, BB	0.00017	0.0078	0.0046	0.95	0.60	0.56
FE2	Adjacent block	Yes	No	0.00011	0.0798	0.0393	2.91	0.28	0.30
FE3	Adjacent block	Yes	No	0.00014	0.0097	0.0052	2.00	0.00	0.00
FE4	Adjacent block	No	No	0.00010	0.0000	0.0024	1.88	0.23	0.23
FE5	Adjacent block	Yes	No	0.00011	0.0081	0.0081	1.41	0.74	0.74
FE6	Adjacent block	Yes	No	0.00024	0.0000	0.0000	1.10	0.20	0.40
MO1	Adjacent block	Yes	No	0.00017	0.0057	0.0029	1.47	0.30	0.30
MO2	Alternate blocks	No	No	0.00016	0.0143	0.0100	2.00	0.11	0.11
MO3	Adjacent block	No	No	0.00003	0.0036	0.0036	2.55	0.63	0.56
MO4	Adjacent block	No	No	0.00007	0.0040	0.0011	2.05	0.17	0.17
MO5	Adjacent block	No	No	0.00005	0.0016	0.0000	2.65	0.00	0.00
MO6	Adjacent block	Yes	No	0.00025	0.0061	0.0102	2.14	0.30	0.20
RA1	Adjacent block	Yes	No	0.00003	0.0210	0.0210	1.70	0.46	0.56
RA2	Alternate blocks	No	Yes, HB	NA	0.0360	0.0626	3.14	0.40	0.28
RA3	Alternate blocks	No	No	0.00011	0.0076	0.0048	1.70	0.18	0.30
RA4	Along the rows	Yes	Yes, HB and BB	0.00010	0.0000	0.0071	0.85	0.50	0.50

Table S2. Summary of the analysis performed. First block represents the effect of environmental variables (landscape and pesticide risk) on pollinators visitation rate; second block represents the combined effect of local variables (pesticide risk and orchard management practices, identified by output under pollinator exclusion) and pollinators visitation rate on pear reproductive success. PF0.5/1.0/1.5 km= Pollinator friendly landscape at 0.5, 1.0 and 1.5 km radii, each tested separately; HB=Honeybees, BB= Bumblebees, Wild pollinators= Wild bees + Syrphids + Muscoid flies.

		RESPONSE VARIABLES	EXPLANATORY VARIABLES
Environment	*Pollinators visitation rate*	Honeybees	Risk + PF0.5/1.0/1.5 km
		Bumblebees	Risk + PF0.5/1.0/1.5 km
		Wild bees	Risk + PF0.5/1.0/1.5 km
		Syrphids	Risk + PF0.5/1.0/1.5 km
		Muscoid flies	Risk + PF0.5/1.0/1.5 km
Reproductive success	*Production*	Initial Fruit set	HB + BB + Wild pollinators + Pollinator Exclusion initial fruit set + Risk
		Final Fruit set	HB + BB + Wild pollinators + Pollinator Exclusion final fruit set+ Risk
		Seed set	HB + BB + Wild pollinators + Pollinator Exclusion initial fruit set + Risk
	Pollination service and deficit	Pollination Service (initial Fruit set)	HB + BB + Wild_pollinators + Risk + Pollinator Exclusion initial fruit set
		Pollination Service (final Fruit set)	HB + BB + Wild_pollinators + Risk + Pollinator Exclusion final fruit set
		Pollination Deficit initial Fruit set	HB + BB + Wild_pollinators + Pollinator Exclusion initial fruit set
		Pollination Deficit final Fruit set	HB + BB + Wild_pollinators + Pollinator Exclusion final fruit set

Table S3. Best-supported models (ΔAICc<2) analyzing i) the effect of environment (landscape and pesticide risk) on pollinators visitation rate; ii) effect of pollinators visitation rate, pesticide risk and orchard management (pollinator exclusion) on pear production, pollination service and pollination deficit. PF0.5, PF1, PF1.5= Pollinator friendly landscape at 0.5, 1.0 and 1.5 km, respectively; Risk= pesticide risk index; PS= Pollination service; PD_FLS= pollination deficit initial fruit set; PD_FS= pollination deficit final fruit set; FLS_ex= Initial Fruit set exclusion; FS_ex= Final Fruit set exclusion; HB= Honeybees, BB= Bumblebees, WB= Wild bees, SYR= Syrphids, MSC= Muscoid flies; Wild_mix= Wild pollinators. Blank spaces refer to null model; variables in bold were also significant.

Model	Rank	Variables selected	df	AICc	ΔAICc	Weight	adjR^2
HB_log ~ Risk + PF0.5 km	1		2	-284.7	0.00	0.579	0
	2	PF0.5 km	3	-283.1	1.57	0.264	0
HB_log ~ Risk + PF1.0 km	1		2	-284.7	0.00	0.685	0
HB_log ~ Risk + PF1.5 km	1		2	-284.7	0.00	0.685	0

BB_log ~ Risk + PF0.5 km	1		2	-338.7	0.00	0.675	0
BB_log ~ Risk + PF1.0 km	1		2	-338.7	0.00	0.609	0
BB_log ~ Risk + PF1.5 km	1		2	-338.7	0.00	0.674	0
WB_log ~ Risk + PF0.5 km	1		2	-356.1	0.00	0.455	0
	2	PF0.5	3	-355.0	1.12	0.260	0
	3	Risk	3	-354.3	1.82	0.183	0
WB_log ~ Risk + PF1.0 km	2	PF1.0	3	-357.4	0.00	0.507	0
	1		2	-356.1	1.33	0.260	0
WB_log ~ Risk + PF1.5 km	2	**PF 1.5**	3	-358.4	0.00	0.577	0
SYR ~ Risk + PF0.5 km	1		2	-314.1	0.00	0.494	0
	2	PF0.5	3	-313.5	0.67	0.353	0
SYR ~ Risk + PF1.0 km	1		2	-314.1	0.00	0.569	0
	2	PF1.0	3	-312.7	1.47	0.273	0
SYR ~ Risk + PF1.5 km	1		2	-314.1	0.00	0.495	0
	2	PF1.5	3	-313.4	0.70	0.350	0
Muscoid ~ Risk + PF0.5 km	1		2	-285.5	0.00	0.634	0
Muscoid ~ Risk + PF1.0 km	1		2	-285.5	0.00	0.630	0
Muscoid ~ Risk + PF1.5 km	1		2	-285.5	0.00	0.616	0
FLS_Open_log ~ FLS_ex + HB + BB + Wild_mix + Risk	11	**FLS_ex + Risk**	4	-121.0	0.00	0.671	-9.493e-04
FS_Open_log ~ FS_ex + HB + BB + Wild_mix + Risk	3	FS_ex	3	-118.7	0.00	0.375	-8.680e-05
	1		2	-116.8	1.97	0.140	0
Seed ~ HB + BB + Wild_mix+ FLS_ex + Risk	2	**BB**	2	41.1	0.00	0.244	0.38750
	4	BB+ FLS_ex	4	41.2	1.44	0.119	0.50720
	6	BB + HB	4	41.9	1.62	0.109	0.49900
PS_FLS ~ HB + BB + Wild_mix+ FLS_ex + Risk	11	FLS_ex + Risk	4	-40.0	0.00	0.259	-2.440e-02
	3	FLS_ex	3	-39.0	0.95	0.171	-1.203e-02
	1		2	-38.7	1.30	0.136	0
PS_FS ~ HB + BB + Wild_mix+ FS_ex + Risk	1		2	-42.9	0.00	0.248	0
	5	FS_ex	3	-42.1	0.75	0.169	-6.418e-03
	9	HB	3	-41.2	1.64	0.180	-4.189e-03
PD_FLS ~ HB + BB + Wild_mix+ FLS_ex + Risk	2	BB	3	1.8	0.00	0.222	-0.8757
	1		2	1.9	0.16	0.205	0
	18	BB + Wild_mix	4	3.6	1.88	0.087	-1.3020
PD_FS ~ HB + BB + Wild_mix+ FS_ex + Risk	1		2	1.1	0.00	0.247	0
	2	BB	3	2.1	1.01	0.149	-0.44860
	9	Risk	3	2.4	1.36	0.125	-0.38100

Table S4. Correlation between pollinator-friendly landscape (arcsin-transformed) at 0.5, 1.0 and 1.5 km radii

Variable 1	Variable 2	r	p-value
Friendly_0.5	Friendly_1.0	0.731	0.001
Friendly_0.5	Friendly_1.5	0.460	0.073
Friendly_1.0	Friendly_1.5	0.754	<0.001

Figure S1. *Legend of Corine Land Cover categories grouped in the 3 main categories "Agricultural" (2.1 and 2.2), "Urban" (1.1, 1.2 and 1.3) and "Pollinator friendly" (1.4, 2.3, 2.4, 3.1, 3.2 and 4).*

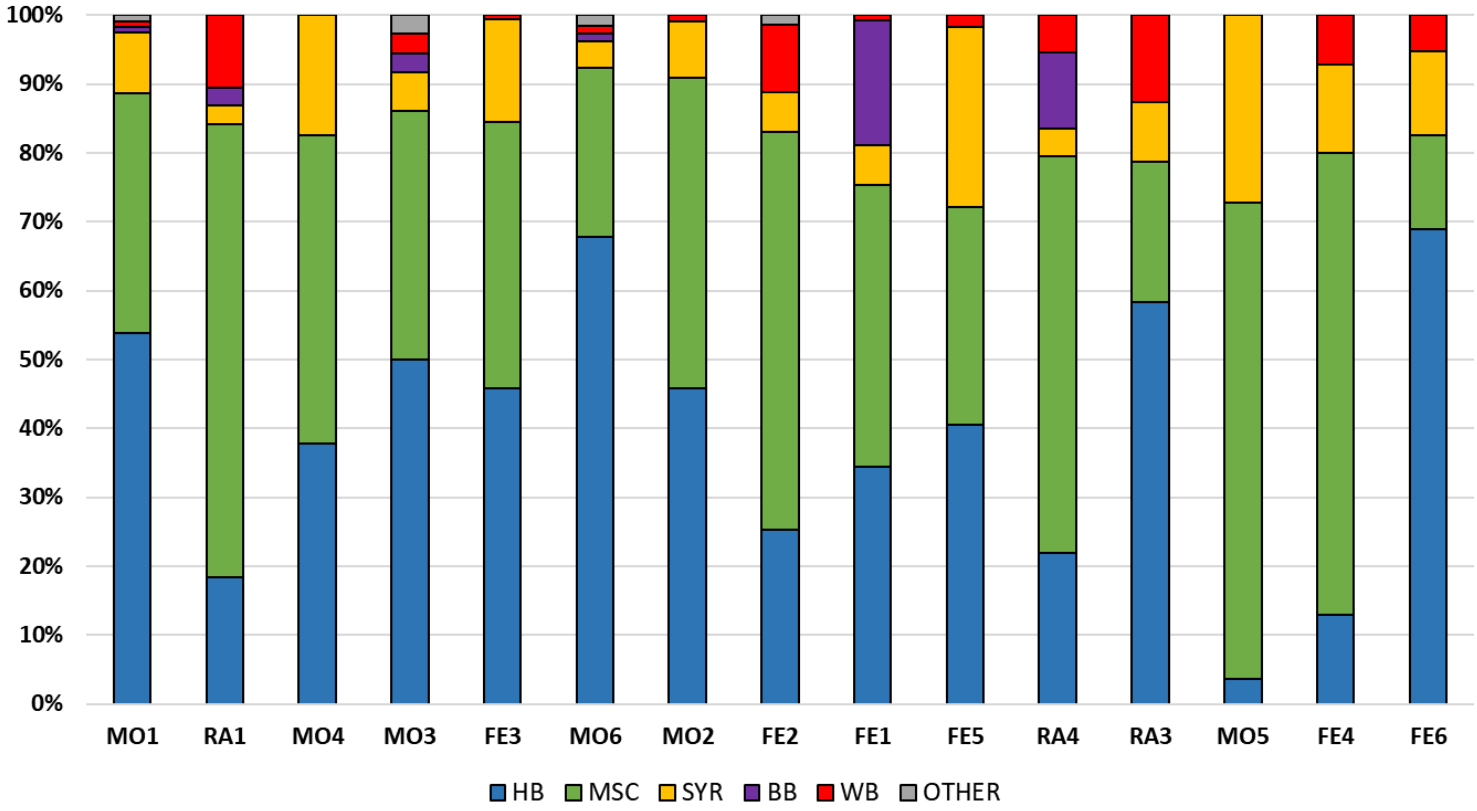

Figure S2. Abundances (%) of pollinators across the studied orchards, ordered by increasing proportion of pollinator-friendly landscape at 1.5 km. HB= Honeybees; MSC= Muscoid flies; SYR= Syrphids; BB= Bumblebees; WB= Wild bees.

Table S5. List of the 39 active ingredients detected in pear flowers; pesticide type, LD50 (µg/bee) and residues (mg/kg) and their frequency of detection across the 16 orchards are reported. LD50 in bold refer to oral toxicity.

#	Compound	Pesticide type	LD50 (µg/bee)	Residues (mg/Kg)				Frequency of detection (% orchards)
				Mean	Median	Min	Max	
1	1-Naftil acetamide	Planth Growth Regulator	100	0.086	0.086	0.086	0.086	6.3%
2	1-naftilacetammide and acid 1-naftilacetic (sum of 1-naftilacetammide and 1-naftilacetic and its salts, expressed as acid 1-naftilacetic)	Planth Growth Regulator	**78.6**	0.086	0.086	0.086	0.086	6.3%
3	6-Benziladenine	Planth Growth Regulator	58.73	0.024	0.023	0.012	0.039	56.3%
4	Acetamiprid	Insecticide	8.09	2.677	1.875	0.038	6.92	25%
5	Acibenzolar acid	Fungicide	100	0.338	0.338	0.338	0.338	6.3%
6	Acibenzolar-s-methyl	Fungicide	100	0.013	0.013	0.013	0.013	6.3%

7	Acibenzolar-S-methyl (sum of acibenzolar-Smethyl and acibenzolar acid)	Fungicide	100	0.375	0.375	0.375	0.375	6.3%
8	Gibberellic Acid	Planth Growth Regulator	**25**	0.237	0.237	0.237	0.237	6.3%
9	Boscalid	Fungicide	**166**	0.015	0.015	0.013	0.017	12.5%
10	Captan	Fungicide	100	32.55	67.335	0.018	162	37.5%
11	Captan (Sum of captan and THPI)	Fungicide	100	41.80	0.377	0.074	264	50%
12	Carbendazim (Sum of benomyl and carbendazim)	Fungicide	**50**	0.034	0.034	0.034	0.034	6.3%
13	Chlorpyrifos	Insecticide	**0.068**	0.029	0.033	0.018	0.037	18.8%
14	Chlorpyrifos Methyl	Insecticide	0.148	0.027	0.027	0.027	0.027	6.3%
15	Cyprodinil	Fungicide	75	13.71	3.46	0.014	59	56.3%
16	Difenoconazole	Fungicide	100	2.08	1.98	0.014	4.84	56.3%
17	Diflufenican	Herbicide	100	0.015	0.015	0.015	0.015	6.3%
18	Dithianon	Fungicide	**25.4**	1.896	0.64	0.022	10.5	68.8%
19	Dodine	Fungicide	**145**	0.022	0.022	0.014	0.03	12.5%
20	Flonicamid	Insecticide	**60.5**	0.012	0.012	0.012	0.012	6.3%
21	Flonicamid (sum of Flonicamid, TFNG e TFNA)	Insecticide	**60.5**	0.012	0.012	0.012	0.012	6.3%
22	Fluazinam	Fungicide	**100**	22.98	20.6	0.011	41.8	31.3%
23	Fludioxonil	Fungicide	**100**	9.052	3.85	2.64	19.3	31.3%
24	Fluopyram	Fungicide	100	1.542	1.542	0.024	3.06	12.5%
25	Flupyradifurone	Insecticide	**1.2**	4.645	4.645	2.48	6.81	12.5%
26	Fluxapyroxad	Fungicide	100	6.257	2.13	1.34	15.3	18.8%
27	Glyphosate	Herbicide	100	0.023	0.016	0.011	0.063	75%
28	Iprodione	Fungicide	100	0.024	0.025	0.019	0.029	18.8%
29	Mefentrifluconazole	Fungicide	100	7.635	0.1135	0.013	30.3	25%
30	Metazaclor	Herbicide	**72.2**	0.061	0.084	0.013	0.087	18.8%
31	Pendimetalin	Herbicide	100	0.026	0.026	0.026	0.026	6.3%
32	Pyriproxifen	Insecticide	74	0.227	0.094	0.013	0.799	93.8%
33	Pyrimethanil	Fungicide	100	7.51	8.145	0.033	14.5	75%
34	Spirodiclofen	Acaricide	**196**	0.153	0.153	0.153	0.153	6.3%
35	Spirotetramat - BYI08330-chetoidrossi	Insecticide	100	0.033	0.033	0.033	0.033	6.3%
36	Tau Fluvalinate	Insecticide	12	0.028	0.012	0.01	0.061	18.8%
37	Tebuconazole	Fungicide	**83.05**	4.733	0.098	0.031	14.4	31.3%
38	Tetraidroftalimmide (THPI)	Fungicide	100	7.903	0.211	0.037	51.2	56.3%
39	Triflumuron	Insecticide	200	0.101	0.101	0.101	0.101	6.3%

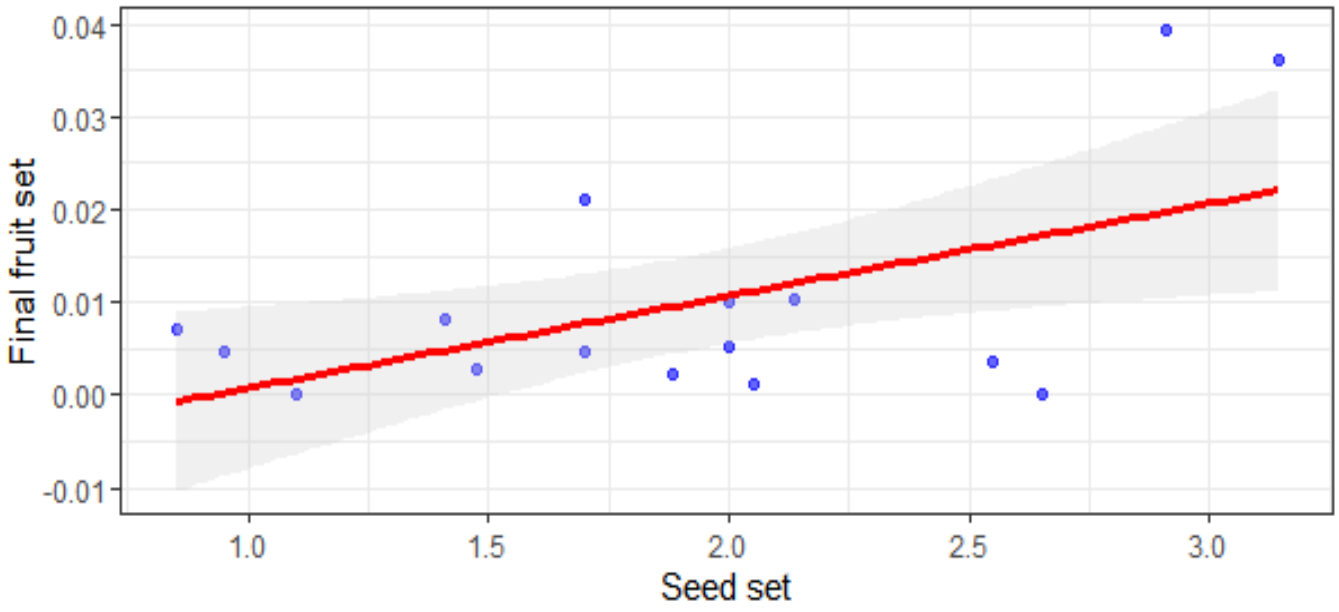

Figure S3a. Positive relationship between seed set and final fruit set open branches (p=0.025); each dot represents an orchard, the red line represents the fitted regression line and the grey shaded area represent the 95% confidence intervals. Final fruit set= final fruit set derived from open pollination.

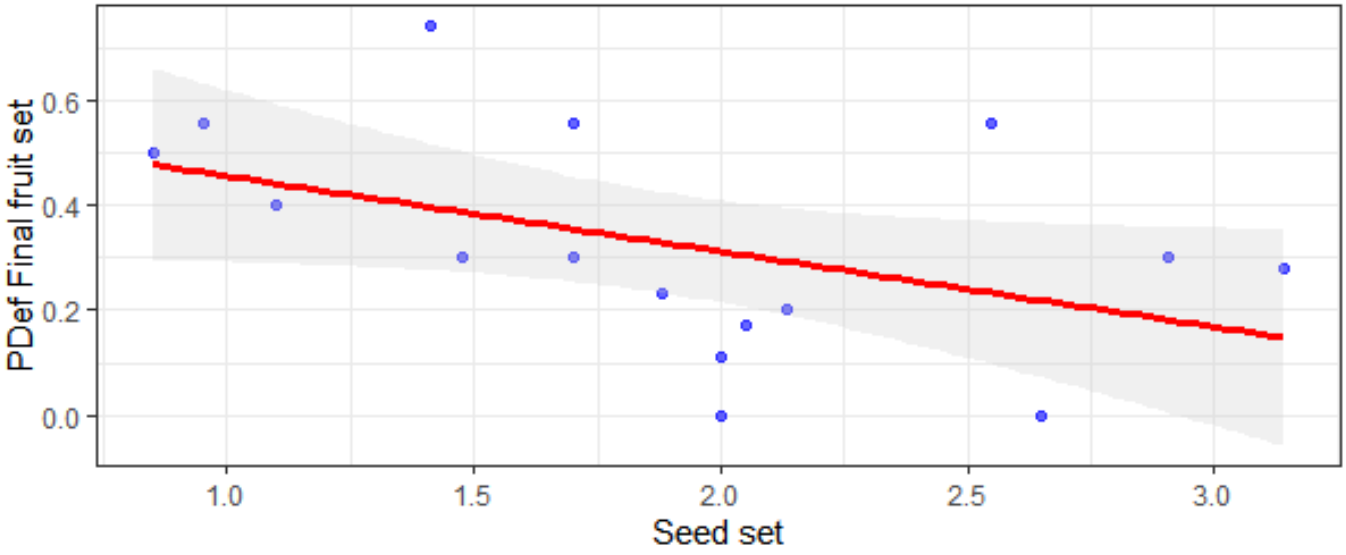

Figure S3b. Negtive trend between seed set and pollination deficit of final fruit set (p=0.077); each dot represents an orchard, the red line represents the fitted regression line and the grey shaded area represent the 95% confidence intervals. PDef fruit set= pollination deficit of final fruit set.

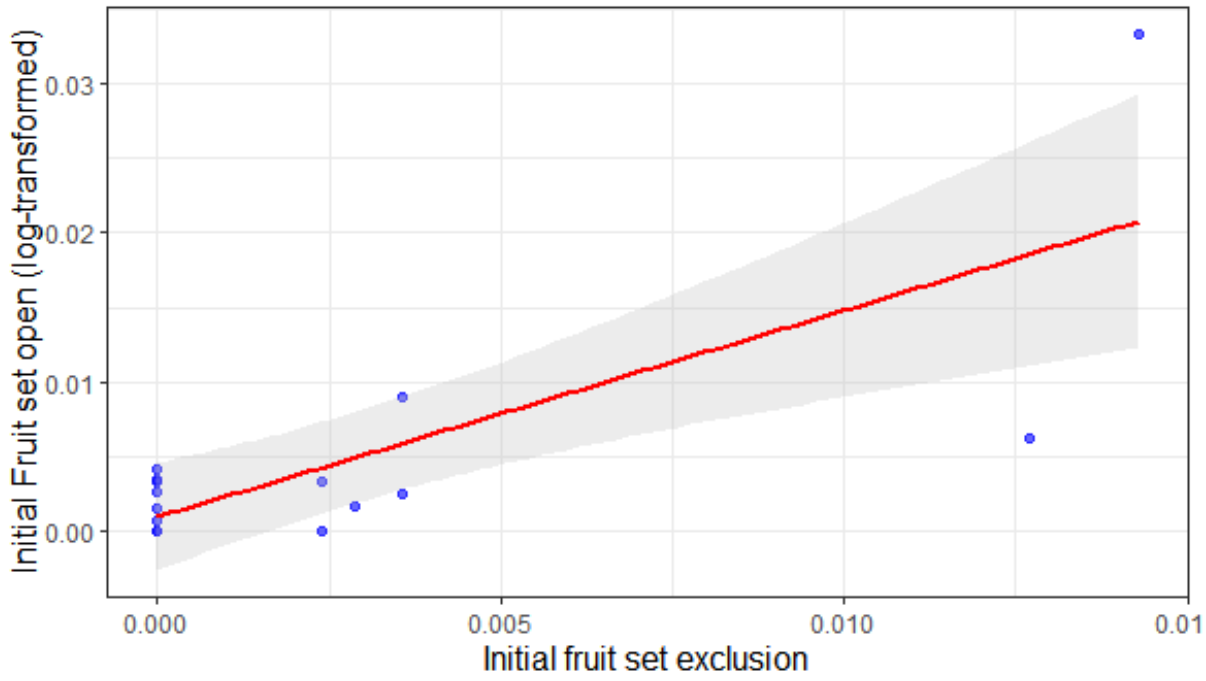

Figure S4. *Positive relationship between initial fruit set open branches and initial fruit set exclusion branches (p< 0.001); each dot represents an orchard, the red line represents the fitted regression line and the grey shaded area represent the 95% confidence intervals.*

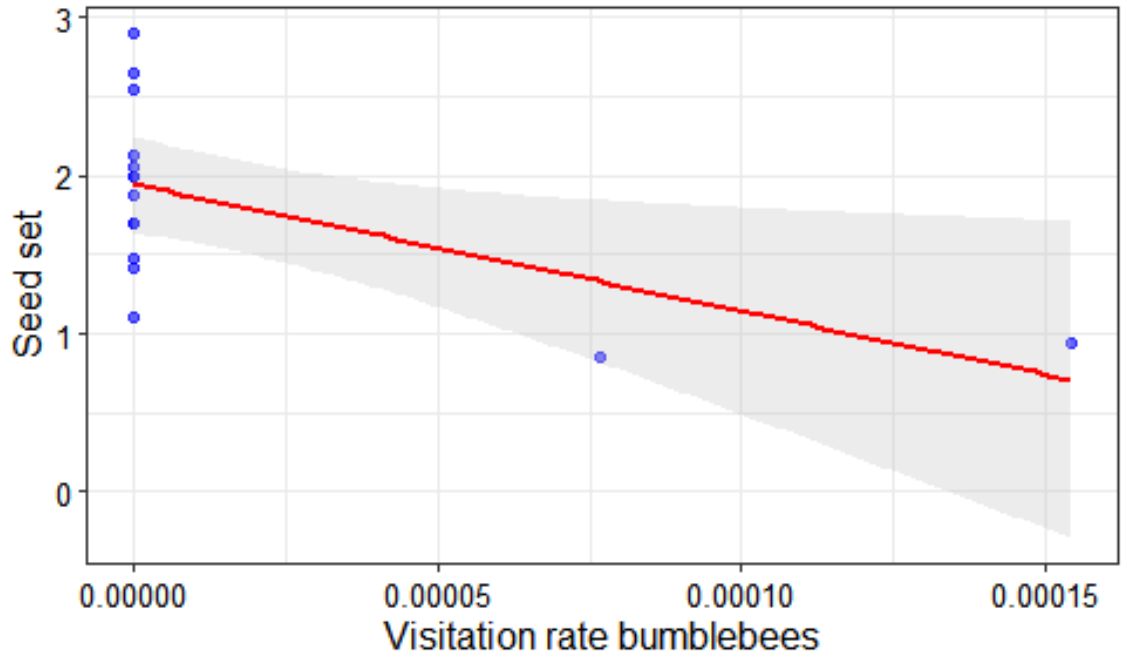

Figure S5. *Negative relationship between bumblebees' visitation rate (n° of bumblebees per n° of flowers in the transect) and seed set (p=0.028); blue dots represent the orchards, the red line represents the fitted regression line and the grey shaded area represent the 95% confidence intervals*