



Quantitative synthesis on key groups of agrochemicals in bees under semi-field and field conditions

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Summary

Our understanding of how bee communities are affected by agrochemicals under field conditions in real agricultural landscapes, and how exposure to agrochemicals may interact with other components of agricultural intensification is limited. Exposure to agrochemicals and their interactive effects with further drivers may act as environmental filters that impair and homogenize bee communities and, as a consequence, potentially also their pollination services in crops. In bees, pesticide exposure and susceptibility may depend on traits such as body size, dietary breadth, nesting mode or sociality. Identifying the role of such traits in driving impacts of agrochemicals and the filtering of bee communities should contribute to a better mechanistic understanding of the impact of agrochemicals on bee communities and to better predictions of their risks.

In this manuscript (D7.2) we report the findings of a quantitative global synthesis of empirical data which was reanalyzed to study the impacts of agrochemicals and their interactions with the loss of semi-natural habitat in agricultural landscapes as potential key drivers of bee community composition under natural field conditions (conducted as part of PoshBee Task 7.2: Assessing single and combined effects of key agrochemicals and other stressors on different model bee species under semi-field conditions across multiple European sites). This synthesis complements synthesis work of findings on the impacts of exposure to agrochemicals and interactions with further stressors from semi-field experiments reported in D7.1: Manuscript on single and combined effects of key chemical and other stressors on bees under semi-field conditions. Consequently, here we focus on studies conducted under field conditions. We analysed the primary data from 27 field studies from Europe, North America and Africa and analysed data from 764 sites (fields) including 6,989 records of pesticide applications and 80,779 recorded bee specimens from 887 wild bee species. A hazard quotient for local pesticide use based on application rates and oral bee toxicity of applied pesticides was calculated. For the recorded wild bee species, we compiled information on five key life-history traits (body size, lecty (pollen dietary specialization), nesting location and mode, as well as sociality and cleptoparasitism, that were expected to modulate pesticide exposure and susceptibility to the loss of semi-natural habitats in bees. We found that both pesticide exposure and loss of semi-natural habitats in agricultural landscapes resulted in a loss of wild bees, resulting in decreased bee abundance and species richness in crop fields, as well as homogenization with negative effects on their functional diversity. No clear evidence was found, however, that a high proportion of semi-natural habitats in landscapes would mitigate negative effects of pesticides on bees in crops. These findings show that the protection of wild bee pollinators in agricultural landscapes requires both the conservation and restoration of semi-natural habitats and protection from exposure to high pesticide hazards. Moreover, they suggest that loss of bee abundance and impaired functional bee diversity in crop fields may negatively affect pollination services.

1. Introduction

Bees are the most important group of pollinators worldwide and contribute to the pollination service of entomophilous plant species, including more than 75% of the most important global crops (Klein et al., 2007; Potts, Imperatriz Fonseca, et al., 2016). Pesticide exposure, the loss of floral resources and nesting sites accompanying agricultural intensification, and the loss of semi-natural habitats are considered major drivers of wild bee decline (Potts, Imperatriz-Fonseca, et al., 2016). However, empirical knowledge of how bee communities are affected by pesticide exposure under field

conditions in real agricultural landscapes, the relative importance of impacts of pesticide exposure and loss of semi-natural habitats, and potential interactive effects of these two major components of agricultural intensification is limited and scattered across single case studies (e.g., Park et al., 2015). Recent attempts to achieve a more general understanding are primarily based on expert knowledge assessments (e.g. Dicks et al., 2021), but a quantitative synthesis of empirical data is currently lacking.

As the susceptibility of bee species to these drivers can depend on their traits, they can be expected to act as environmental filters that may impair trait diversity and homogenize bee communities (Grab et al., 2019; McLean et al., 2021). In bees, specific traits such as body size, sociality, or nesting mode can modulate the response of bees to pesticide exposure and habitat loss (Brittain and Potts, 2011; De Palma et al., 2015; Forrest et al., 2015). Impacts of pesticide exposure may be mitigated by high amounts of semi-natural habitat providing high-quality food and nesting sites. Consequently, pesticide exposure and habitat loss may interact synergistically to impair bee communities in crop fields. Alternatively, homogenisation of communities through stressors such as semi-natural habitat loss, resulting in assemblages that are dominated by fewer species with similar traits, may result in a reduced response to impacts of further stressors such as pesticide exposure. Such impacts on functional trait diversity of bee pollinator communities should have important functional consequences for the delivery of pollination services (Woodcock et al., 2019). However, the role of both local pesticide use in crop fields and the composition of surrounding landscapes in environmental filtering of bee communities remain poorly understood, and synthetic knowledge is lacking. Such knowledge is important for a better mechanistic understanding of the impact of agrochemicals on bee communities and for an accurate assessment of their risks for bees in real agroecosystems.

Here, we report the findings of a quantitative synthesis of available empirical data to explore the consequences of pesticide exposure and loss of semi-natural habitat on abundance, species richness, functional trait diversity, and phylogenetic diversity of bee communities in crop fields. We also examined how pesticide exposure and habitat loss interact in potentially enhancing or mitigating each other. This synthesis complements findings on the impacts of exposure to agrochemicals and interactions with further stressors from semi-field experiments reported in D7.1: Manuscript on single and combined effects of key chemical and other stressors on bees under semi-field conditions. Consequently, here we focus on studies conducted under field conditions.

2. Methods

2.1. Dataset

A systematic Web of Science search was performed using the search terms wild bee abundance OR bee diversity OR bee species richness AND pesticide use OR agrochemicals. This search yielded a total of 170 potentially relevant studies. These were checked for eligibility based on the following criteria for inclusion in the analysis: (1) the study contains information on pesticide use (e.g., number of applications, products applied, information on hazard quotients etc.); (2) this information on pesticide use was collected in the local crop field for which quantitative information about the composition of wild bee communities is also available; (3) wild bee communities were quantified to species or similar high-resolution taxonomic level (morphospecies); (4) information about the proportion of semi-natural habitat in the agricultural landscapes surrounding the focal fields is available. These criteria applied to 36 potentially suitable studies that were considered for the analysis, and for which

corresponding authors were contacted to request primary data. Additionally, corresponding authors were asked for potentially available unpublished data collected by the authors or other researchers of their institution fulfilling the above mentioned criteria. From the collected studies, four had to be excluded because of insufficient sampling of bees, with less than 10 individuals per site on average. This yielded in a total of 27 primary studies that could be included in the analysis, covering 764 sites from Europe, North America and Africa (Fig. 1). Of these, 20 studies (585 sites) contained information on spray protocols during the years of bee collection, and 22 studies (655 sites) contained simple information about overall high vs. low pesticide use associated with a production system (i.e., conventional vs organic production system) (Table 1). In all subsequent analyses these two sets of studies were analysed separately. Furthermore, to ensure comparability of pollinator communities, studies that collected bee communities in different crops at different sites were sub-divided accordingly. Similarly, the data of one study that sampled different sites during different years (Carrie 2017, see Table 1) was sub-divided into different datasets by year. This resulted in a total of 39 study-year-crop combinations (hereafter datasets) that were used as the highest hierarchical unit in the analyses (Table 1; see e.g. Dainese et al., 2019 for an identical approach).

2.2. Hazard quotient of local pesticide use

Pesticide hazard of each site was calculated based on the farmers spray records (provided in personal interviews or in databases), considering oral exposure of insecticides, fungicides and herbicides as:

$$HQ_{oral} = \log \sum_{n=1}^N \frac{\text{Application rate (kg product per ha)} \times \% \text{ Active ingredient in product}}{LD_{50}}$$

The HQ_{oral} sums up all N applications of a field considering the application rate of the active ingredient and the toxicity of the pesticide (estimated based on oral acute toxicity (LD_{50}) for honeybees, *Apis mellifera* L.). Log-transformation was used to account for the non-linear dose-response relationships. Besides the HQ_{oral} as described above, hazard quotients considering contact exposure and chronic oral exposure were calculated, but their performance in terms of predictive power and goodness of fit (i.e., explained variation in abundance, species richness and functional dispersion) was lower than that of oral acute toxicity, and the latter was therefore used for all subsequent analyses.

The information about the concentrations of active ingredients in the applied products were gathered from the national product labels. Application rates were mostly provided by farmer interviews and, where missing, the application rate as recommended by the national product label for the according crop and development stage was used. Oral LD_{50} values for both synthetic and biopesticides for honey bees were obtained from the Pesticide Properties Database (PPDB) and the Bio Pesticides Database (BPDB), respectively (Lewis et al., 2016). For active ingredients with unbounded estimates (“>”; mostly fungicides and herbicides) the minimal LD_{50} was used. For active ingredients where no oral LD_{50} value could be obtained, the contact LD_{50} was used as a proxy.

For single applications for which only the pesticide type (e.g., herbicide) was provided, without more detailed information on the product, the HQ_{oral} was calculated as the mean of HQs of the same pesticide type and production system (conventional or organic) within the same study or, if only pesticide types were provided for all applications in the whole study (one study), means were obtained

across all studies in the dataset. In total, we compiled information on 6989 pesticide applications including 277 active ingredients.

2.3. Bee taxonomic and trait data

In total, we compiled life-history traits of 887 species and morphospecies (80,779 specimens) from six bee families (Andrenidae, Apidae, Colletidae, Halictidae, Megachilidae, Melittidae). To avoid any synonymies, taxonomy of all species was checked using ITIS (Integrated Taxonomy Information System) (ITIS, 2022). We used life-history traits that have been hypothesized to modulate bees' pesticide exposure and sensitivity towards semi-natural habitat loss (Table 2). Data on traits were mostly provided by dataset holders. Where missing, species traits were supplemented from existing bee trait databases (Baldock et al., 2018; Wilson & Jamieson, 2019; Andrej, 2022; Müller, 2022) and from the literature (Benachour et al., 2007; Bouseman & LaBerge, 1978; Bzdyk, 2012; Cane et al., 2007; Cross, 2017; Eardley, 1988; Eickwort, 1981; Gérard et al., 2018; Gibbs, 2010; Gonzalez & Griswold, 2013; Gusenleitner & Schwarz, 2002; Hobbs & Lilly, 1954; Hurd Jr et al., 1980; Kasperek, 2019; Lanner et al., 2020; Lerman & Milam, 2016; Litman, 2012; Müller, 2002, 2018a, 2018b; O'Brien, 2007; Ornos et al., 2007; Praz et al., 2008; Praz, 2017; Risch, 1999; Roberts, 1973; Sedivy et al., 2013; Shebl, 2016; Sheffield et al., 2011; Tadauchi, 2008; Torchio et al., 1967; Wcislo & Engel, 1996; Westrich, 2008; Wood et al., 2020; Wood & Roberts, 2017).

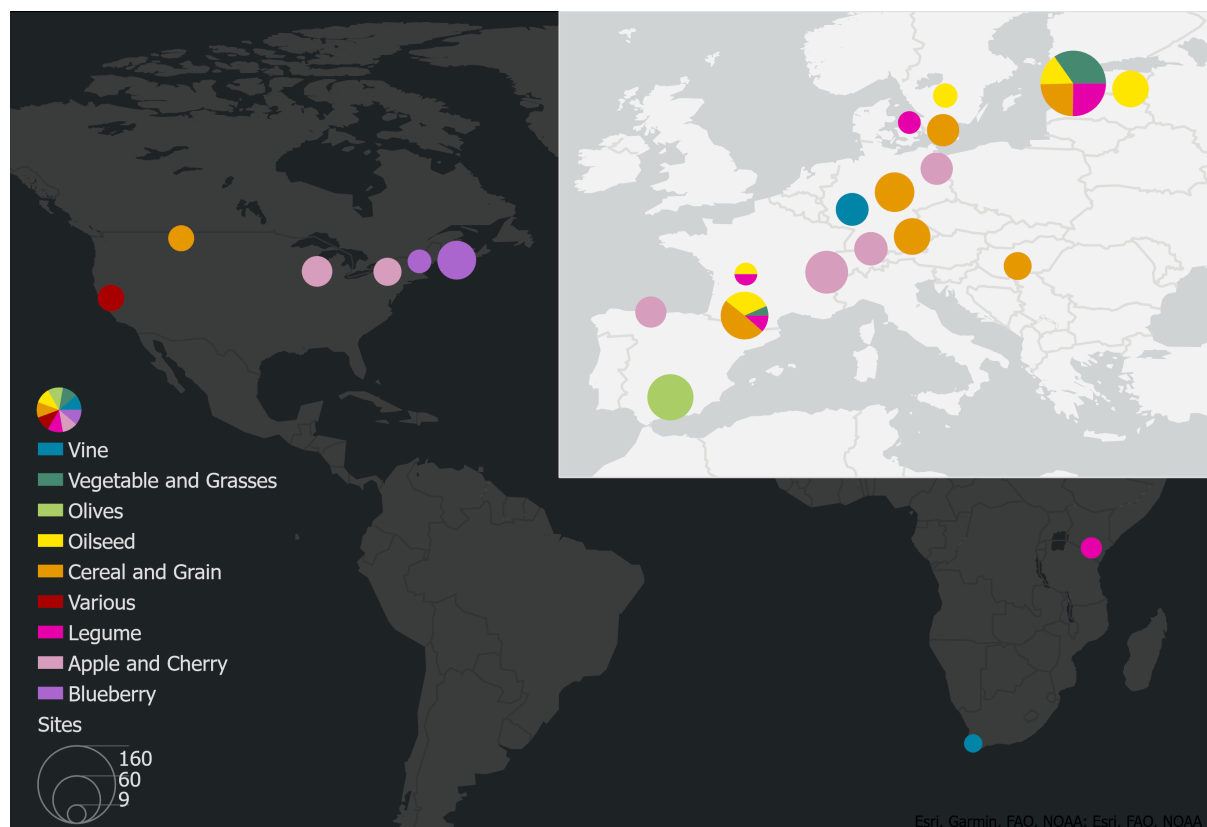


Figure 1: Geographic distribution of study regions and crop types represented by sites within studies considered in the analysis (globally and within Europe (upper right map)). The size of dots represent the number of sites per region (see Table 1). Studies that were carried out in the same region are illustrated in the same dot (France: Carr. 2017, Rive. unpub and Quin. unpub; Spain: Mart. 2019 and Cano unpub).

Table 1: Overview of the studies considered in the quantitative synthesis.

Data-set	Study	Sites per study	Country	Spray protocols available for HQ calculation	Crop type	Year(s)	Variation in management
1	Adhi. 2019	18	USA	yes	cereal	2013 - 2015	yes
2	Ande. unpub	28	Sweden	no	cereal	2008	yes
3	Bush. 2015	40	USA	yes	blueberry	2010 - 2012	yes
4	Cano unpub	18	Spain	no	olive trees	2018	yes
5	Carr. 2017	26	France	yes	corn	2013	no (only conv.)
6					oilseed	2014	
7						2013	
8						2014	
9	Goni. unpub	15	USA	yes	various around hedgerow	2006 - 2014	yes
10	Happ. 2018	36	Germany	yes	cereal	2013	yes
11	Happ. 2019	30	Germany	no	apple	2015	yes
12	Holzschuh. 2007	42	Germany	yes	cereal	2003	yes
13	Kehi. 2014	9	South Africa	no	vine	2009	yes
14	Knap. unpub	14	Sweden	yes	legume	2019	yes
15	Kova. 2011	21	Hungary	yes	cereal	2005	yes
16	Lues. 2014	14	France	yes	legume	2010	yes
17			France		sunflower		
18	Mall. 2015	25	USA	yes	apple	2010	yes
19						2012-2013	
20	Marj. unpub	115	Estonia	yes	arable crop	2011	yes
21					cereal		
22					grasses		
23					legume		
24					canola		
25	Mart. 2019	39	Spain	no	olive trees	2017	yes
26	Mina. 2018	26	Spain	yes	apple	2015	yes
27	Nich. 2017	13	USA	no	blueberry	2013 - 2015	yes
28	Otie. 2015	12	Kenya	yes	legume	2009	yes
29	Ouin. unpub	23	France	yes	cereal	2017 - 2018	yes
30					grasses		
31					legume		
32					sunflower		
33	Park 2013	21	USA	yes	apple	2009 - 2012	yes
34	Rive. unpub	21	France	yes	cereal	2016	no (only conv.)
35	Rund. 2015	16	Sweden	yes	canola	2013	no (only conv.)
36	Samn. unpub	28	Sweden	no	apple	2015	yes
37	Sutt. unpub	49	Switzerland	yes	apple and cherry	2018	no (only conv.)
38	Uzma. 2020	29	Germany	yes	vine	2016	yes
39	Vero. unpub	36	Estonia	yes	canola	2014	no (only conv.)

2.4. Species loss along gradients of pesticide exposure and semi-natural habitat loss

To test for an additive or interactive disassembly process caused by pesticide exposure and the loss of semi-natural habitat in the agricultural landscape, for which we hypothesized impaired communities would represent nested subsets of less impaired communities, we used abundance weighted NODF (Nestedness of communities based on Overlap and Decreasing Fill) (Almeida-Neto & Ulrich, 2011). NODF was calculated across comparable communities, separately for each dataset (Table 1). To account for potential sampling biases across sites, the relative abundance per sample (pan trap or transect) was calculated per single species. Abundance data from different years were subsequently averaged. To test for environmental filtering by pesticide exposure, community matrices were ordered

by increasing HQ_{oral} to calculate NODF with the `nestednodf` function from the `vegan` package (Oksanen et al., 2013). For each dataset, we additionally created 99 “null communities” by random permutation of the site by species matrix with the `swap` algorithm from the `bipartite` package (Dormann et al., 2008), which keeps the matrix fill and marginal totals constant. From these null communities the mean NODF was obtained for each dataset. Finally, a paired t-test was used to test NODF values from observed against null community NODFs.

To test for environmental filtering by the proportion of semi-natural habitat in the landscape, the same procedure as described above was used, but matrices were ordered by decreasing proportion of semi-natural habitat.

We also tested whether filtering by pesticide exposure interacted with filtering by loss of semi-natural habitat. To this end, the community matrices were ordered by low and high proportion of semi-natural habitat, where low was defined as sites with semi-natural habitat proportion values below the median of the respective dataset, and high as sites above this median, respectively. Within each of these categories, sites were additionally ordered by HQ_{oral} (Fig. 2). We also ordered the matrices the opposite way (first by high proportion of semi-natural habitat, followed by low proportion; Fig. 2).

2.5. Effect of pesticide exposure and semi-natural habitat loss on bee abundance and diversity

All indices of bee abundance and diversity were calculated separately for each site and year and subsequently measures from different years were averaged for sites that were sampled over multiple years. Measures from different sampling methods (pan trap and observation) were averaged per site to avoid any bias from unbalanced designs. Bee communities were characterized using bee abundance, species richness, Simpson diversity index, functional diversity (measured as functional dispersion based on species occurrence (FD_{occ}) and weighted by species abundance (FD_{abu})) and phylogenetic diversity measured as the mean phylogenetic distance (MPD). These diversity metrics have been used as proxies to assess community characteristics in response to drivers (Weekers et al., 2022), and are considered important determinants of pollination service provisioning (Woodcock et al., 2019).

To control for variation in sampling effort across sites within some studies, we calculated the relative bee abundance per sample (e.g. pan trap or transect) and used individual-based rarefaction and extrapolation to calculate species richness and the Simpson index using the `iNEXT` function in R (Hsieh et al., 2016). Functional dispersion (i.e., the mean distance of individual species to the centroid of all species in the community with respect to a suite of traits) was selected as measure of functional diversity as it is less correlated with species richness than other proxies (Laliberté & Legendre, 2010). The `FD` package (Laliberté et al., 2014) was used to calculate functional dispersion, excluding specimens for which more than two traits could not be assessed (e.g., if bee identification was done on morpho-species rather than species level in the primary study) to avoid bias through certain traits. To achieve a more uniform contribution of traits, `FD` was calculated on the Gower multi-trait dissimilarity matrix obtained with the `gawdis` function (de Bello et al., 2021) incorporating grouped nesting traits (nesting site and nesting mode; see Table 2). To test whether potential effects of pesticide exposure and habitat loss were driven by phylogenetic distance rather than dispersion in traits we calculated mean phylogenetic distance (MPD). MPD per site was calculated using the `Picante`

package (Kembel et al., 2010) using Grafen branch lengths from the phylogeny that was built using the species' taxonomic relationships. As for FD, MPD was calculated with both species occurrence matrices and abundance-weighted data. As the results were qualitatively similar for the two indices, only the abundance-weighted MPD was considered for the final analysis.

Linear mixed effect models were used to test how pesticide exposure in local fields (HQ_{oral}) and the proportion of semi-natural habitat in the surrounding landscape affected bee abundance and the above mentioned diversity descriptors as response variables. In all models, these two variables and their interaction were included as fixed explanatory variables, and dataset ID (Table 1) as a random intercept. HQ_{oral} and semi-natural habitat were not correlated ($r = -0.009$). To fit these linear mixed effect models we used the lmer function from the lme4 package (Bates et al., 2014).

To account for differences in sampling methods, sampling effort etc., all continuous explanatory and response variables were scaled (z-transformation) prior to statistical analysis (e.g. Garibaldi et al., 2013; Dainese et al., 2019; Albrecht et al., 2020).

P-values were obtained by the Kenward-Roger method (Luke, 2017) and model assumptions were checked graphically (Zuur et al., 2009). Where necessary, transformation of response variables (log or sqrt) was done prior to scaling. All statistical analyses were performed in R version 4.1.2 (R Core Team, 2014).

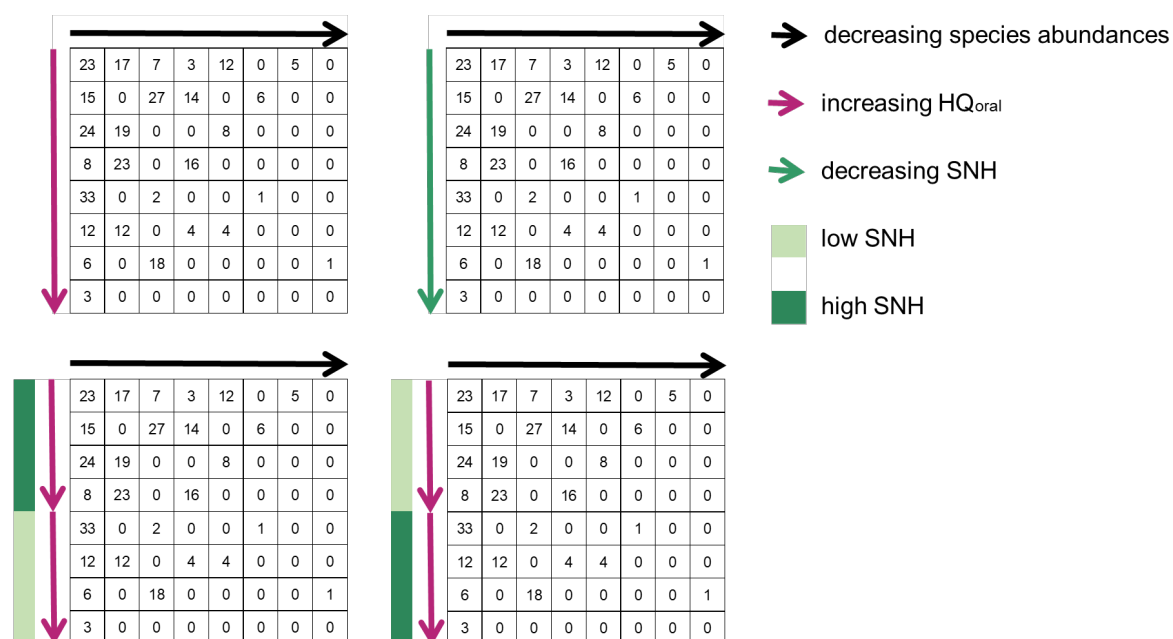


Figure 2: Ordering of species by site matrices for the calculation of abundance-weighted nestedness (NODF). Rows represent sites, and columns species. HQ_{oral} : acute oral hazard quotient; SNH: proportion of semi-natural habitat in the surrounding landscape around focal fields

Table 2: Considered bee life-history traits and hypotheses regarding their role in shaping impacts of pesticide exposure and loss of semi-natural habitat on bee communities.

Trait	Scale	Measurement	Expected impact on pesticide exposure and of the loss of SNH
Body size	Continuous	Intertegular distance (ITD)	Pesticides: Small bees have smaller foraging ranges (Greenleaf et al., 2007) and therefore have smaller dilution effects by surrounding landscapes. SNH: Because of their smaller foraging ranges, small bees are more affected by isolation from suitable nesting sites or complementary food sources in SNH (De Palma et al., 2015).
Lecty	Binary	Oligolectic Polylectic	Pesticides: Oligolectic bees experience higher exposure, especially orally, when specialized on the sprayed crop compared to polylectic crop visitors that may have a diluted effect when also visiting other plants. Lowest exposure can be expected in oligolectic species specialized on plants other than the crop itself, but visiting accompanying flora, e.g. in field margins (Brittain & Potts, 2011). SNH: As oligolectic bees are less flexible in switching to alternative food sources, they may suffer more from a decline in plant diversity accompanying the loss of SNH (De Palma et al., 2015).
Sociality	Ordinal	Social Semisocial Solitary	Pesticides: Social bee species may compensate for temporary negative effects of agrochemicals at the colony level at a later point in time while negative effects should directly impair the reproductive output in solitary bee species (Sgolastra et al., 2019; Straub et al., 2015). SNH: SNH can be important in providing complementary food sources during flower scarce periods in arable land. As social bees can compensate for temporary food scarcity with their food stores, they are less susceptible to loss of SNH (De Palma et al., 2015).
Nesting site	Ordinal	Above Flexible Below	Pesticides: If ground nests are built in the surrounding of crop fields, the brood can be exposed to water soluble pesticides through run off. Nests in stems or twigs can be affected via drift, but are typically not directly adjacent to crop fields (Brittain & Potts, 2011). SNH: SNH can provide important nesting habitats to bees. Especially cavity-nesting bees depend on dead wood or insect burrows mainly found in SNH (De Palma et al., 2015).
Nesting strategy	Binary	Renter Excavator	Pesticides: Excavators can be exposed to pesticides if the excavated material is contaminated through drift or run off. SNH: Renting structures as insect burrows, snail shells or rodent holes are often provided in SNH (De Palma et al., 2015).
Cleptoparasitic	Binary	No cleptoparasite Cleptoparasite	Pesticides: Females of cleptoparasitic species can be negatively affected by pesticides directly and additionally also indirectly when their host declines because of agrochemicals. SNH: Cleptoparasitic species can be negatively affected by loss SNH directly (e.g. decline of floral resources) but also indirectly when loss of SNH is associated with a decline of their host.

3. Results

3.1. Species loss along gradients of pesticide exposure and semi-natural habitat loss

From the 30 datasets (from 20 studies) that were tested for nestedness along a gradient of increasing HQ_{oral} , 9 datasets showed significantly higher NODF compared to null models (Fig. 3). Across studies, nestedness of communities was higher compared to mean NODF from null models (Fig. 4). Similarly, NODF was increased by a loss of semi-natural habitats in 11 out of 38 tested experimental datasets, resulting in a significant overall effect of the proportion of semi-natural habitat on nestedness across communities. The effect of HQ_{oral} on nestedness of communities also persisted when the gradient of HQ_{oral} was nested within gradients of semi-natural habitats (as illustrated in Fig 2).

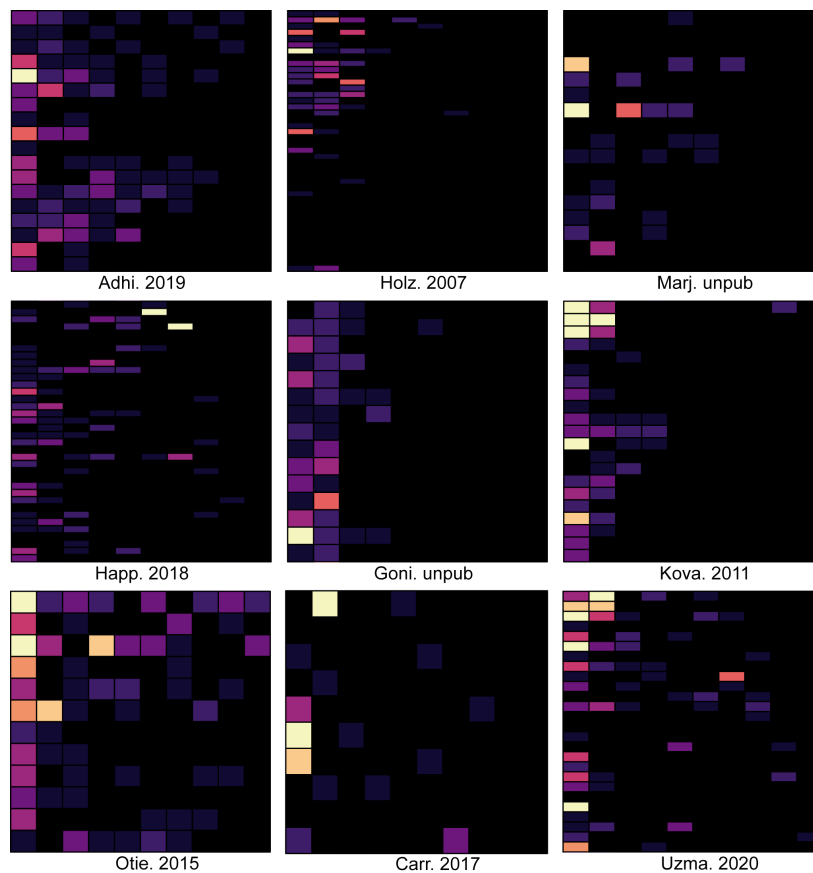


Figure 3: Species by site matrices that showed a significantly higher abundance weighted nestedness (NODF) compared to null models when sites were ordered along gradients of increasing pesticide exposure (acute oral hazard quotient HQ_{oral}).

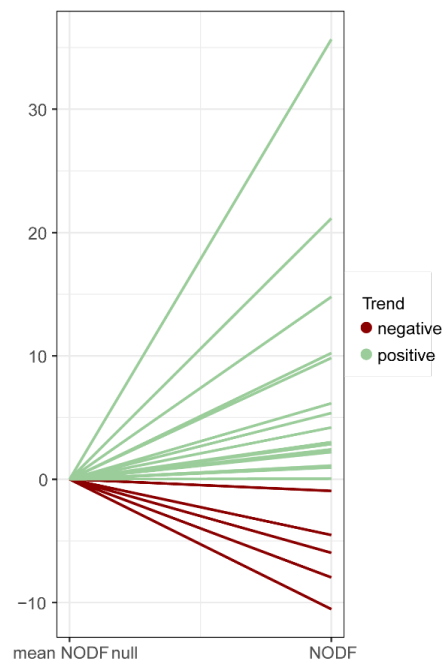


Figure 4: Abundance weighted nestedness (NODF) of bee communities along a gradient of increasing pesticide exposure (acute oral hazard quotient HQ_{oral}) relative to the mean NODF of null models. Each line represents a study.

3.2. Effect of pesticide exposure and semi-natural habitat loss on bee abundance and diversity

The HQ_{oral} as well as conventional management reduced bee abundance, species richness and functional diversity, while the mean phylogenetic distance and the Simpson diversity index were not affected. The proportion of semi-natural habitat had a positive effect on bee abundance and various aspects of diversity, but not on functional diversity, which was only affected by pesticide exposure. Contrary to our hypothesis, we did not find any buffering of pesticide exposure by a high proportion of semi-natural habitat (as would be indicated by a positive interaction term). Instead, the HQ_{oral} had a stronger negative effect on the Simpson diversity index in landscapes with a high proportion of semi-natural habitat (Fig. 5).

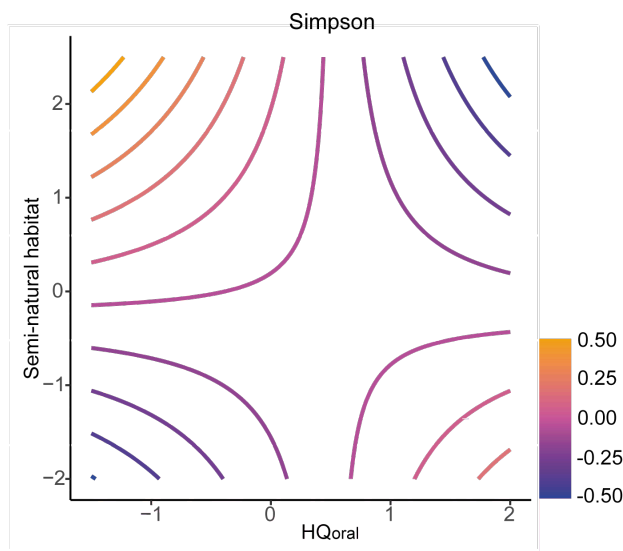


Figure 5: Effect of pesticide exposure (acute oral hazard quotient “ HQ_{oral} ” and the proportion of semi-natural habitat on the Simpson diversity index.

4. Discussion and conclusions

The present quantitative synthesis of 27 primary field studies shows that both pesticide exposure and loss of semi-natural habitats in agricultural landscapes contribute to a loss of wild bee abundance and diversity in crop fields. It has been hypothesized that enhanced response diversity of diverse bee communities in agricultural systems with high amounts of semi-natural habitats may mitigate adverse impacts of pesticide exposure (e.g., Park et al., 2015). However, this quantitative synthesis indicates that high proportions of semi-natural habitats in the landscape generally fail to mitigate negative effects of pesticides on bees in crops. Our results indicate that habitat loss and pesticide exposure impose independent filtering of bee communities, which causes a homogenization of wild bee pollinator communities, and consequently leads to an impairment of functional trait diversity in pollinator communities. In approximately a third of the bee communities, increasing oral hazard quotient and decreasing the proportion of semi-natural habitat also caused species loss that resulted in a more nested structure of the remaining species in the wild bee communities. These findings have important implications for the conservation of pollinators; effective protection of wild bee pollinators in agricultural landscapes requires both the conservation and restoration of semi-natural habitats and the reductions of pesticide hazards. As pollinator abundance and functional diversity have been demonstrated to represent reliable proxies for pollination functions (Woodcock et al., 2019), the loss of bee abundance and impaired functional diversity of bee pollinator communities revealed in this study can be expected to negatively affect crop pollination services.

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