



**Manuscript on the factors and processes leading to
contamination, and the effects of multiple stressors on bee
health**

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PoshBee

**Pan-european assessment, monitoring, and mitigation
of stressors on the health of bees**



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Highlights

Based on a comprehensive dataset on bee performance and multiple environmental and anthropogenic pressures in agricultural landscapes across Europe, we found:

- Accumulating effects of multiple pressures on the performance of managed bees (*Apis mellifera*), eusocial wild bees (*Bombus terrestris*), and solitary wild bees (*Osmia bicornis*);
- Consistent negative effects of pesticides across all three bee species under field conditions;
- Strong effects of pesticides on colony growth and smaller effects on reproduction;
- Consistent and strong negative effects of pathogens on eusocial bees (*A. mellifera* and *B. terrestris*);
- Adverse effects acting directly on growth rates and reproduction but also indirectly, e.g., by affecting worker activity and behaviour;
- Negative impacts of *A. mellifera* on the colonisation rates of *O. bicornis*, potentially via competition, and on the performance of *B. terrestris*, potentially via the transfer of pathogens;
- Species-specific effects of the landscape structure, flower diversity and diversity of pollen collected by the bees;
- Consistent negative impacts of warmer temperatures across all three bee species, being particularly strong for *O. bicornis*;
- Compensatory mechanisms in *B. terrestris* by shifting colony investment from growth to reproduction;
- Options for mitigation actions by increasing local flower diversity and quality.

Summary

Given their tremendous ecological and economic importance, current declines of pollinators have raised international awareness, which is reflected in multiple national and international commitments and efforts to halt and reverse these declines. Multiple pressures, such as habitat loss and degradation, resource limitations, increased application and severity of agrochemicals, pathogens and their transfer from managed to wild pollinators, and climate change, have been identified. However, most studies on the effects of such pressures are limited to laboratory or semi-field experiments, e.g., addressing pesticides or pathogens, focus on community level measures, e.g., analysing the impact of landscape-scale pressures, or use only occurrence data, e.g., for large-scale analyses of climatic impacts. Moreover, most studies focus on a single group of pressures and remain on a single spatial scale, while a holistic and more mechanistic picture of how multiple pressures impact the performance of pollinators under field-conditions is currently lacking.

With this deliverable, we are closing this gap and provide a mechanistic understanding of direct and indirect effects of (i) pesticides, (ii) pathogens, (iii) flower resource diversity and quality, (iv) landscape structure, and (v) climate on the local performance of pollinators across Europe.

Our analysis is based on samples and performance measures of three sentinel bee species: the honey bee (*Apis mellifera*), the buff-tailed bumble bee (*Bombus terrestris*) and the red mason bee (*Osmia bicornis*). Samples and measures were obtained from 128 sites of the PoshBee European field site network, located in eight apple orchards and at the border of eight oilseed rape fields in each of eight countries (Estonia, Germany, Ireland, Italy, Spain, Sweden, Switzerland, United Kingdom). We obtained information from farmers about the nature and amount of applied plant protection products. In addition, we screened for 256 active ingredients in pollen stores from the three bee species, 11 honey bee pathogens, and protein and lipid concentrations of sampled pollen stores, and conducted palynological analyses of pollen diversity collected by the three bee species. This dataset was complemented by local flower richness and *A. mellifera* densities from field-based pollinator surveys, GIS-based measures of landscape structure, and climatic data obtained from CHLSA.

Compared to oilseed rape sites, we found considerably higher numbers and toxicity-levels of active ingredients of pesticides, measured in bee pollen stores, in apple sites. For diversity and quality of pollen from pollen stores, measured as protein to lipid ratio, we did not find noticeable differences between the two crops, but we did find differences among the bee species. Interestingly, the pathogen risk was the same for all three bee species, regardless of crop type.

Structural equation models revealed negative effects of pesticides and pathogens consistently across the three sentinel species. In addition to those effects, all analysed pressures had an impact on each of the species but with varying severity. These impacts acted either directly on measures of bee performance or were mediated by other compartments of the system. Our findings highlight the severity of combined pressures for pollinators but also provide options for management actions to compensate the effects of single pressures. For instance, high flower diversity and related diversity of pollen sampled by the bees can directly reduce pathogen risk, potentially by strengthening the immune defence system of the pollinators. It can also facilitate forager efficiency and thus increase the resource supply within a colony, which is otherwise diminished by high pathogen or pesticide risk, and in turn supports colony growth.

We also found internal compensation mechanisms for *B. terrestris*. Under stressful conditions, e.g., high pesticide or pathogen pressure, adverse climatic conditions or suboptimal nutritional conditions, *B. terrestris* colonies invest more into the production of sexuals and in particular in new queens. While this might be seen as a survival strategy, this comes at the expense of worker production. However, such an overall decline in workers contributes to the current declines of pollinators, with likely severe impacts on the provision of pollination services to wild and crop plants.

In general, our results support calls and actions for reducing pressures on bees, in particular of plant protection products and pathogen risks within *A. mellifera* and their assumed transfer to wild pollinators, but also of climate change. Because of their strong beneficial direct and indirect effects, we consider conservation actions aiming at increasing local flower diversity as highly promising to compensate the impacts of other pressures.

1. Objectives

The major aim of this study was to provide tests for hypothesised causal direct and indirect impacts of multiple environmental and anthropogenic pressures on the performance of managed and wild bee species and to disentangle their relative importance under field conditions, using sentinel hives, colonies or nests of the honey bee (*Apis mellifera*), the buff-tailed bumble bee (*Bombus terrestris*) and the red mason bee (*Osmia bicornis*).

In particular, we tested the following hypotheses:

- (i) Pesticide risks from plant protection products applied to two major crops in Europe (apple and oilseed rape) have consistently direct negative effects on bee performance;
- (ii) Pesticide risk has an indirect negative impact on bee performance by increasing pathogen risk, e.g., via weakening the immune defence system;
- (iii) Pesticide risk has an indirect negative effect on bee performance, mediated by protein-to-lipid ratio of pollen collected by bees, since foragers might have been already affected during larval development with subsequent impacts on their foraging behaviour and efficiency;
- (iv) Pathogen risks (presumably driven by *A. mellifera*) have a consistent direct negative impact on bee performance;
- (v) Flowering plant diversity and corresponding diversity of pollen collected by the bees can compensate the negative effects of pesticides and pathogens either directly by increasing growth rates, or indirectly by diluting contaminated pollen from the focal crops, and by lowering pathogen risk, e.g., by strengthening the immune defence system.

- (vi) Flowering plant and respective diversity of pollen collected by bees has an impact on protein-to-lipid ratio of the collected pollen, e.g., by relaxing potential foraging limitations in low-diversity landscapes;
- (vii) Protein-to-lipid ratio of bee-collected pollen is related to pathogen risk, e.g., by increasing the immune defence system via species-specific optimal nutrition;
- (viii) Climatic and landscape structure conditions can compensate or even override negative effects of the other pressures, but vary in importance depending on the ecology of the species.

2. Methods

2.1. Study sites

To allow for a comparison of agro-ecosystems with different landscape structure, habitat quality and field management across Europe, eight countries were selected covering four main biogeographic regions in Europe: Boreal (Sweden and Estonia), Atlantic (Ireland and United Kingdom), Continental (Germany and Switzerland) and Mediterranean (Spain and Italy). Within each of the eight European countries, 16 sites were selected according to a gradient of land use intensity (using percentage of cropland as a proxy), resulting in a total of 128 sites (Figure 1a).

We focused on two major European crops, oilseed rape (*Brassica napus*) and apple (*Malus domestica*) to reflect annual and perennial cropping practices and, therefore, different pest management strategies and pesticide use (Nicholson et al. 2021). These two crops also show contrasting growing systems and pollination biology: apple, a perennial, and self-incompatible crop grown in long standing (5-10 year) orchard plantations and winter-sown oilseed rape, an annual, and self-compatible crop planted irregularly as a break crop in arable rotations. We selected eight sites per crop in each country with a mean distance between two sites of at least 3 km to avoid overlapping landscape buffers and violation of the statistical assumption of spatial independence for subsequent analyses (see Hodge et al. 2022 for more details).

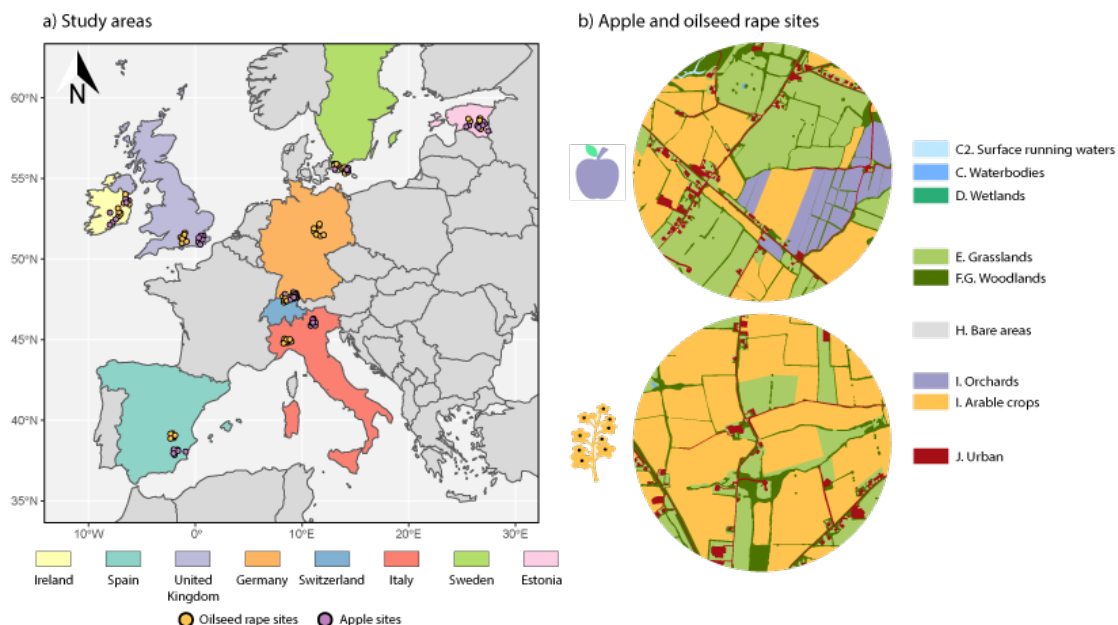


Figure 1: Location of the 128 sites comprising the PoshBee field site network. a) Oilseed rape sites (orange dots) and apple orchard sites (purple dots). b) Examples of mapped land-cover features within a 1-km radius buffer (from Bottero et al. unpublished).

2.2. Sentinel colonies and performance

Following a common protocol, three *A. mellifera* hives, three colonies of *B. terrestris* and three trap nests (each seeded with 100 cocoons) of *O. bicornis* were placed per site, with a minimum distance of

5 m. Since *O. bicornis* does not occur naturally in Ireland and due to purchasing problems in the UK, *O. bicornis* was not deployed in either country. Deployment of the sentinel colonies, i.e., hives, colonies, and nests, started shortly before the focal crop started to bloom and the last measurement of performance was taken shortly after crop flowering ended. Species-specific measures of colony performance were taken according to a standardised protocol (Hodge et al. 2019).

2.2.1. *Apis mellifera* performance

Colony performance measures were taken twice: before and after crop flowering. Following Delaplane et al. (2013) and Sandroock et al. (2014), the surface area of each comb in an *A. mellifera* hive was visually estimated twice, allowing for a proper assessment (to the nearest 5%) of (i) the number of pollen cells as an indication of food availability and foraging activity, (ii) the number of brood cells as a measure of colony strength, and (iii) the number of adult bees, as a measure of colony size. Swarming colonies were disregarded.

2.2.2. *Bombus terrestris* performance

Three measures of *B. terrestris* colony performance were calculated (see [deliverable D1.1](#)). First, colony growth was assessed by weighing all three colonies in each site at three different time points: before deployment, during peak bloom of the focal crop, and when the colonies were retrieved from the sites when blooming ended. Second, as measures of colony performance, the number of produced sexuals (gynes and males) were counted at the time of colony termination by summing up the number of males in the colony, and intact and eclosed queen cells. In addition, the number of pollen storage cells and wax cups used for nectar storage were counted. Finally, as a measure of investment into queen production, the number of intact and eclosed queen cocoons were counted (Rundlöf et al. 2015) and set in to relation to the summed number of intact and eclosed worker and male brood cells.

2.2.3. *Osmia bicornis* performance

At the end of the flowering period of each crop, a fine-meshed netting fabric was placed in front of each trap nest to prevent further nesting by bees and entrance of natural enemies. Trap nests were then stored under ambient conditions until October. As a measure of colonisation rate, the number of sealed and unsealed nesting tubes were recorded for each trap-nest.

2.3. Pesticide use and residue analyses

To assess the impact of pesticide application, we conducted surveys with farmers about their management practices, and analysed pesticide residues from pollen stores.

2.3.1. Field management and pesticide use

Pesticide use data were collected directly from participating farmers using a standardised questionnaire hosted on Qualtrics and translated into local languages, or were obtained by personal visits. The questionnaire was checked by representatives of farming organisations for clarity of language prior to dissemination and approved by the University of Reading Ethics Committee. Alongside demographic questions, the questionnaire asked for the dates of application, and names and amount (l/ha) of applied plant protection products. Only applications between October 2018 and June 2019 (period preceding the final performance measurement) were considered. Since not all farmers were responsive, 29 of the 128 sites remained without information on pesticide application.

2.3.2. Pesticides residues present in pollen stores

Pollen was sampled from pollen stores (beebread from *A. mellifera* hives, pollen cells from terminated *B. terrestris* colonies, pollen samples from *O. bicornis* nesting tubes) towards the end of the flowering period from *A. mellifera* hives (in the field) and from *B. terrestris* colonies (in the laboratory after termination) and during peak flowering from stored *O. bicornis* nest tubes. Samples were homogenized and further split for pesticide residue, palynological and nutritional analyses. Samples were stored at -20°C. Following Kiljanek et al. (2021), a total of 0.3 g of pollen was used to screen for 256 compounds including isomers and metabolites, with a focus on active substances of plant

protection products that were recommended for the protection of oilseed rape and apple (see [deliverable D2.2](#)). Procedural standard calibration and recovery checks were performed in accordance with SANTE/12682/2019 criteria (European Commission 2019).

2.4. Pathogen loads

To assess pathogen loads, bees were sampled at two time points of the flowering period: at the deployment at the sites before crop flowering (for quantification before exposure to field conditions), and after the flowering period (for quantification after exposure). To reduce the impact of the sampling on bee performance before exposure, we minimised the sample size. We collected 12 specimens of *B. terrestris*, and 60 of *A. mellifera* per site and 10 specimens of *O. bicornis* per country, since the cocoons were obtained from a single provider. During full bloom of the focal crops, 18 specimens of female *O. bicornis* were targeted to be sampled per site and at the end or after crop bloom, 60 *A. mellifera* and 30 *B. terrestris* specimens were sampled per site. All samples were stored at -80°C.

Samples were analysed with quantitative molecular methods (harmonised high-throughput real-time qPCR; [deliverable D2.3](#)) (Babin et al. 2022) focusing on 11 predominantly honey bee pathogens and parasites: 6 RNA viruses (acute bee paralysis virus - ABPV, black queen cell virus - BQCV, chronic bee paralysis virus - CBPV, deformed wing virus types A and B - DWV-A and -B, and sacbrood virus - SBV), the 2 bacterial causative agents of honey bee foulbrood (*Paenibacillus larvae* and *Melissococcus plutonius*), and 3 *Vairimorpha* (recently reassigned *Nosema*) microsporidian parasites (*Vairimorpha* (*Nosema*) *apis* and *Vairimorpha* (*Nosema*) *ceranae* historically described in *A. mellifera*, and the bumble bee parasite *Vairimorpha* (*Nosema*) *bombi*).

2.5. Pollen diversity and pollen nutritional quality

To cover the variation in habitat and diet quality, the diversity and the nutritional quality of pollen collected by all three sentinel bee species were assessed. This was evaluated based on pollen stores sampled from *A. mellifera* hives, *B. terrestris* colonies and *O. bicornis* nest tubes, where the samples have been homogenised and split for pesticides residue, palynological and nutritional analyses (see 2.3.2 and [deliverable D2.4](#)).

2.5.1. Pollen diversity

For each homogenized pollen store sample, recognition of pollen identity was based on comparison between the observed pollen forms and those present in the CREA collection of reference slides (213 pollen types; developed using anthers of identified plant species). In most of the cases (76%) an identification to the genus-level at least was possible. Compositae and Labiate were differentiated into five and three morpho-groups, respectively, while the rest were considered at the family level.

2.5.2. Pollen nutritional quality

Nutritional aspects were addressed through the characterisation of total protein, total lipid (both as µg nutrient/mg pollen), and protein-lipid ratios ([deliverable D2.4](#)). Pollen protein concentration was measured using the Bradford assay according to Vaudo et al. (2020) and pollen lipid concentrations were determined using a modified protocol from Vanhandel et al. (1988).

2.6. Flower diversity and *Apis mellifera* density

To assess flower diversity within each of the field sites, we performed a rapid flower survey by assigning floral units to an ordinal scoring system based on a 10-fold geometric sequence (i.e., no floral units = 0; 1–10 floral units = 1; 11–100 floral units = 2; >100 floral units = 3; (D1.1.)). Flower surveys were performed during peak flowering of the focal crop along four transects at the boundaries surrounding the focal crop field. Per transect, we surveyed floral units in three evenly spaced locations within one (horizontal) square survey plot of 1m x 1m in the herbaceous vegetation and one (vertical) in hedgerows or trees when present, resulting in up to 24 floral survey plots per site.

In addition, honey bee density was estimated in each site by conducting four 50 m transect counts. Two transects were placed in the centre of the crop, and two on its margins. Each transect was walked for 5 min and all honey bees within 1 m to the left and to the right were counted. Estimation of honey bee density was only performed during suitable weather conditions, and between 10:00am and 4:00 pm.

2.7. Landscape structure and climatic variables

2.7.1. Landscape structure

To account for the variation of landscape characteristics in each site across countries, we quantified landscape heterogeneity within a 1-km radius centred at the location of sentinel colonies, by manually identifying all land cover features using high-resolution images provided by World Imagery (ESRI) and a Geographical Information Systems Software (ArcGIS Pro, 2.4.1, ESRI) (Figure 1b).

Identified land cover features were then classified into ten final categories: surface running waters, waterbodies, wetlands, grasslands, woodlands and heathlands, bare areas, orchards, cropland, roads and urban areas. Landscape structure was quantified by calculating three independent metrics of landscape composition and configuration (for more details, please see [deliverable D1.2](#)).

As a measure of compositional landscape heterogeneity, we first measured the proportion of woodlands for each landscape. Although, the EUNIS reference offers a detailed classification of each land-cover that best defines ecological habitats, we harmonised and reclassified the land cover categories in accordance with the habitat requirements of flower-visiting insects. Therefore, woodlands and hedgerows were combined into the same land cover class, under the assumption that they both positively benefit flower-visiting insects, by providing potential additional nectar, pollen or nesting resources (Marini et al. 2012). In addition, we calculated the Shannon diversity index as a measure of landscape diversity, using all ten land cover categories.

As a measure of configurational landscape heterogeneity, we measured edge densities of semi-natural habitats by dividing the edge length of semi-natural habitats by the total area of the corresponding landscape.

2.7.2. Climatic variables

For each site, long-term climate parameters (30-year averages from 1981 to 2010; spatial resolution of 30 arc sec, ~1km), related to multi-annual temperature (bio_01) and precipitation variables (bio_12), were extracted from the CHELSA database (v1.2; <https://chelsa-climate.org/downloads/>) to assess overall climatic conditions.

2.8. Statistical analyses

We calculated three measures of performance of *A. mellifera* colonies in terms of growth rates in the numbers of (i) pollen cells, (ii) adults, and (iii) brood cells by taking the log-ratio of measures before and after flowering of the focal crop, e.g., $\log(\text{final number of pollen cells}/\text{initial number of pollen cells})$. Since deployment times differed among the sites, we standardised all growth rates by the number of days of deployment. Four measures of colony performance were calculated for *B. terrestris*. First, foraging activity and food availability were measured as number of pollen cells and number of nectar cells produced. Second, overall colony growth was measured as the log-ratio of final weight after crop bloom to initial weight before deployment. Growth rates were also standardised by the number of days of deployment. Third, the number of produced sexuals (gynes and males) was taken as a measure of investment into reproduction. Fourth, the proportion of new queens produced relative to the number of workers and males was taken as a measure of colony investment into queens. For *O. bicornis*, we used colonisation rate as a measure of performance. All performance measures were calculated per hive, colony or nest and then averaged per site.

We calculated pesticide risks based on summed toxicity-weighted concentrations (TWC) of active ingredients of the plant protection products applied in the field and measured in the pollen stores (Rundlof et al. 2022, Nicholson et al. unpublished). To this aim, for each active ingredient, concentrations for applications in the field (g/ha) and for pollen samples ($\mu\text{g/kg}$) were divided by the average of the acute toxicity endpoint (LD_{50} - the dose required to cause 50% mortality in the test population) for oral and contact application. We rounded LD_{50} down when expressed as 'greater than'. Site-level pesticide risks were then calculated by summing up all toxicity-weighted concentrations. LD_{50} values for each active ingredient found were obtained from the Pesticide Properties DataBase (PPDB; <http://sitem.herts.ac.uk/aeru/ppdb/>; license purchased by UFZ) and refer to adults of *A. mellifera* only, since toxicity data are very incomplete for *B. terrestris* and *O. bicornis*. However, high correlations of LD_{50} values across species indicate the feasibility of using one measure of toxicity for all three species (Arena et al. 2014, DiBartolomeis et al. 2019). Since the levels of pesticide risks covered several orders of magnitude for measures of pollen from pollen stores, we log-transformed them to linearise the relationships with the bee performance measures in subsequent analyses.

In a similar manner, we calculated pathogen risk based on the pathogen quantification obtained from qPCR runs (quantification results were expressed as decimal logarithm) for each tested pathogen divided by a species-specific threshold above which clinical symptoms can be expected (Schurr et al. 2019). This threshold was defined by the frequency distribution of observed pathogen quantifications across all samples (Babin et al. unpublished). In the case of a normal distribution of this frequency, the mean + 1.65 times the standard deviation was taken as the respective threshold. In the case of a bimodal distribution, the threshold was visually assessed by the start of the second peak when moving towards higher abundances. We calculated site-level pathogen risks by subjecting the risk values of all pathogens observed to multiple correspondence analyses and reduced the dimensionality to one.

As a measure of pollen nutritional quality, we used the protein-to-lipid ratio in the samples from bee pollen stores. Diversity of sampled pollen was measured in terms of taxonomic richness (for 76% of the pollen at genus level and at group or family level for the rest). We further calculated floral richness as the average of the flowering plant richness across the flower survey plots per site. *Apis mellifera* densities were calculated by averaging their densities in the transects per site.

After merging the data sets across crop types per species and due to missing values for some of the variables, a total of 70 data points (sites) remained for analyses of *A. mellifera*, 72 for *B. terrestris*, and 55 for *O. bicornis*. Major reasons for missing data were an incomplete response of the farmers providing information on the application of plant protection products (29 sites missing), and the death of all three *B. terrestris* colonies in a few sites.

We assessed differences in pesticide risks measured in pollen stores, pathogen risk, sampled pollen diversity and their protein-to-lipid ratio, and whether these differences alter between samples of oilseed rape and apple sites with linear mixed effects models, including species as fixed effect and the country where the samples took place as a random intercept effect. Differences among species and crop type were then tested on the basis of estimated marginal means.

To provide a holistic and more mechanistic picture of the impact of multiple pressures on bee performance, we identified direct and indirect effects of external environmental and anthropogenic pressures on the measured risks and, together with direct and indirect effects of these risks, on the respective performance measures and their interrelationships per bee species. Therefore, we used a structural equation modelling approach. We standardised all variables to mean zero and unit standard deviation and developed a set of initial linear mixed effects models (one for each risk and performance measure as response variables) with country and crop type as crossed random intercepts. To identify relevant explanatory variables per model of this set, we used a multimodel inference approach by calculating sub-models of all possible variable combinations. For each initial model, we kept all variables occurring in sub-models with a difference in AICc lower than two when compared to the best

model (Burnham et al. 2010). If the intercept-only model was among them, we disregarded this particular response variable. The optimised models were then subjected to structural equation models per species and standardised effect sizes of direct and indirect effects were extracted and visualised. Conditional independence tests among the variables were performed based on Fisher's C statistic and identified dependencies have been added to the respective model when necessary. We calculated conditional (full model) and marginal (fixed effects only) coefficients of determination (Pseudo-R²) based on Nakagawa et al. (2017).

For measures of investment into reproduction (growth rate of brood cells for *A. mellifera*, and production of sexuals and investment into queen production for *B. terrestris*), we did not expect direct effects of the landscape structure, and for *B. terrestris* no direct effect of *A. mellifera* densities was assumed. Thus, these relationships were not considered in the models.

For all three species the same hierarchical effects among the risk measures were expected. Pollen diversity was defined to have an impact on (i) pesticide risks in pollen stores, e.g., via dilution effects by non-crop pollen; (ii) pollen protein-to-lipid ratio, e.g. by relaxing potential foraging limitations in low-diversity landscapes; and (iii) pathogen risk, e.g. by increasing pathogen resistance by optimal nutrition. We expected pesticide risk from pollen in pollen stores to impact (i) pollen protein-to-lipid ratio, since foragers might have been affected already during larval development with impacts on their foraging behaviour and efficiency; and (ii) pathogen risk, e.g., by decreasing the immune defence system. Finally, protein-to-lipid ratio was assumed to be related to pathogen risk, e.g., by increasing the immune defence system via optimal nutrition.

For relationships among the performance measures, we also assumed a hierarchical structure, where the production of pollen cells for *A. mellifera* and of nectar and pollen cells of *B. terrestris* has an impact on colony growth, which in turn impacts the investment into the production of sexuals and queens for *B. terrestris* and brood for *A. mellifera*. Due to incomplete coverage within the data for *O. bicornis*, flower diversity and risk from pesticide application in the field had to be excluded.

3. Results

3.1. Risks across species

The number of active ingredients found in pollen stores and their overall toxicity were considerably higher in apple sites compared to oilseed rape sites, while differences among bee species were minor and only occurred in apple sites (Figure 2). Here, pollen collected by *O. bicornis* had lower numbers of active ingredients, while *B. terrestris* pollen had higher levels of toxicity. In contrast, stored pollen richness differed considerably among the bee species, with particularly low values for *O. bicornis* (Figure 3a). Both *A. mellifera* and *B. terrestris* foraged predominantly in the focal crop. For *A. mellifera*, 50% of the pollen collected in oilseed rape sites was from *Brassica* species. In apple sites, 27% of the pollen stores consisted of *Brassica* species and 19% of *Malus* species. The pollen sampled by *B. terrestris* in oilseed rape fields consisted of 28% *Brassica* species, and of 21% *Malus* species in apple sites. All other plant species were collected with much lower frequencies. In contrast, both focal crops were not the most sampled species of *O. bicornis*. *Osmia bicornis* foraged predominantly on *Quercus robur* (28% in oilseed rape and 47% in apple sites), followed by *Papaver* species in oilseed rape sites (23%) and *Ranunculus* species in apple sites (13%). Focal crops were ranked only third in order with 18% of *Brassica* species in oilseed rape sites and 9% of *Malus* species in apple sites, which, however, was not reflected by an expected dilution effect in pesticide risk (Figure 2b) caused by alternative pollen.

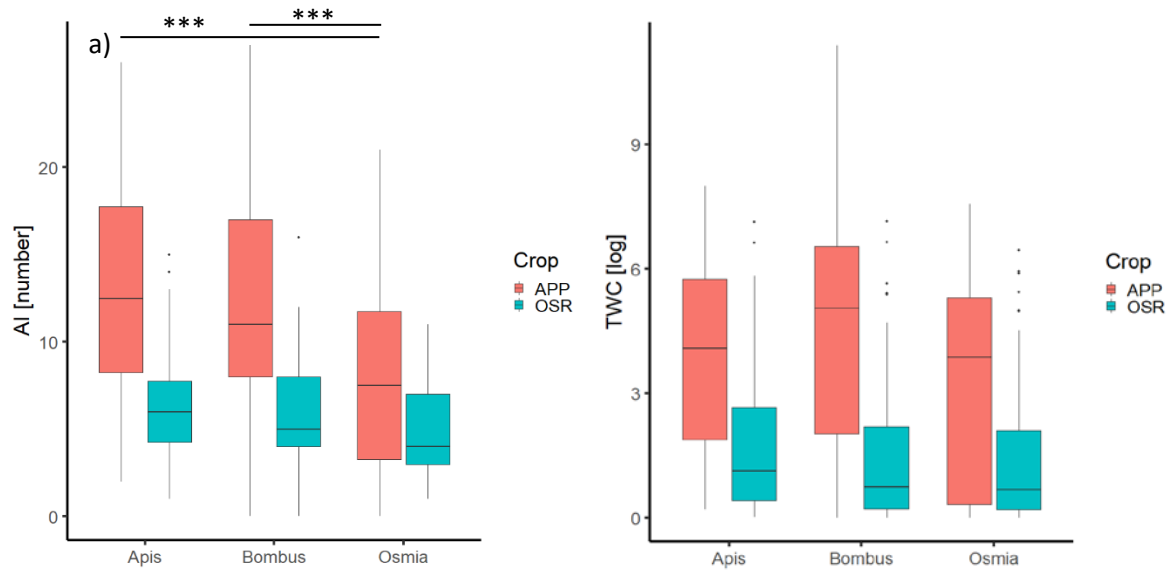


Figure 2: Pesticide risks across three bee species and two crop types. Number of different active ingredients (AI) of plant protection products (a) and their summed toxicity-weighted concentration (TWC; b) found in bee pollen stores. Apple (APP) sites are indicated in red, oilseed rape sites (OSR) are in blue.

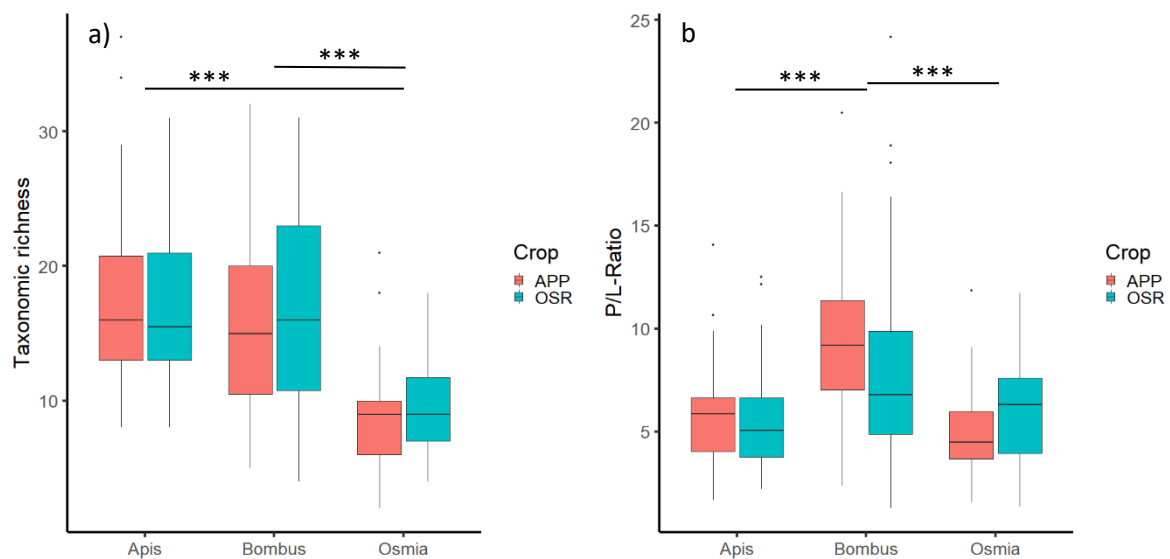


Figure 3: Taxonomic richness (a) and protein-to-lipid ratio (b) of pollen from pollen stores across three bee species and two crop types. Apple (APP) sites are indicated in red, oilseed rape sites (OSR) are in blue. P/L-ratio, protein-to-lipid ratio.

Protein-to-lipid ratios of collected pollen were highest for *B. terrestris* and did not differ between *A. mellifera* and *O. bicornis* (Figure 3b). Interestingly, pathogen risk did not differ between crop types nor between bee species (Tukey post-hoc test, all P-values > 0.27).

3.2. Risk pathways

3.2.1. Pesticides

Bombus terrestris

In general, *B. terrestris* showed the most complex pattern of risk pathways (Figure 4). As expected, pesticide risk measured in pollen stores increased with increasing risk of the applied plant protection products, but also decreased with increasingly wet climates, *A. mellifera* densities and flower diversity in the landscape. It had direct negative effects on pathogen risk, nectar and pollen cells, and most importantly on colony growth. It also had direct positive effects on the production of sexuals and colony investment into new queens and indirect effects on all three measures of colony performance mediated by pathogen risk, number of pollen and nectar cells and the positive impacts of growth on the production of sexuals, and in turn on the investment into new queens (Figure 4).

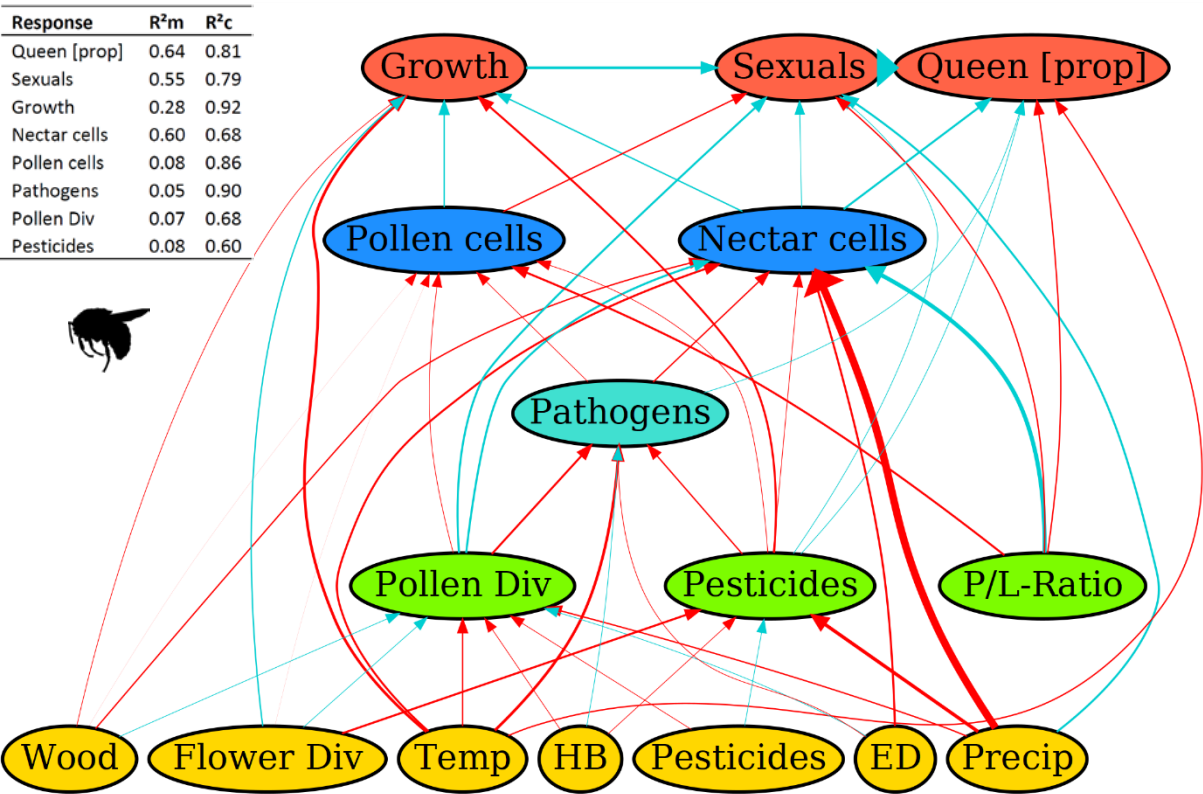


Figure 4: Path diagram showing environmental and anthropogenic pressures on *Bombus terrestris* performance. Arrow thickness is scaled to the standardised effect size of the relationship between the two respective variables. Arrows in red indicate negative relationships. Arrows in blue indicate positive relationships. External variables are in yellow, measures from pollen stores in green, pathogen risk from adults in light blue, number of pollen and nectar cells in dark blue, colony performance measures in red. Marginal (R^2_m ; fixed effects only) and conditional R^2 (R^2_c ; full model) for each response variable are given in the inserted table. Wood, proportion of woody habitats; Flower Div, richness of flowering plant species; Temp, long-term mean annual temperature; HB, *Apis mellifera* density; Pesticides (yellow oval), pesticide risk from the application of plant protection products; ED, edge density of semi-natural habitats; Precip, long-term annual precipitation; Pollen Div, taxonomic richness of pollen in pollen stores; Pesticides (green oval), pesticide risk from pollen in pollen stores; P/L-Ratio, protein-to-lipid ratio from pollen in pollen stores; Pathogens, pathogen risk; Pollen cells, number of pollen cells; Nectar cells, number of nectar cells; Growth, colony growth rate based on weight differences before and after flowering of focal crop; Sexuals, number of sexuals produced; Queen [prop], proportion of new queens relative to produced workers and males.

Apis mellifera

As with *B. terrestris*, pesticide risk from pollen stores increased with increasing risk of the applied plant protection products, and decreased with flower diversity (Figure 5). In addition, pesticide risk was lower under warmer climates, and higher habitat diversity and edge density of semi-natural habitats. It directly increased the number of pollen cells and, most importantly, decreased the number of workers. Indirect effects on the number of brood cells were mediated by the number of workers (Figure 5).

Osmia bicornis

In contrast to *A. mellifera*, pesticide risk from pollen stores of *O. bicornis* increased with warmer climates. It also decreased with increasing proportion of woody habitats. It had an expected negative direct effect on nest colonisation rate, and a positive effect on pathogen risk, which also mediated a further positive indirect effect on colonisation (Figure 6).

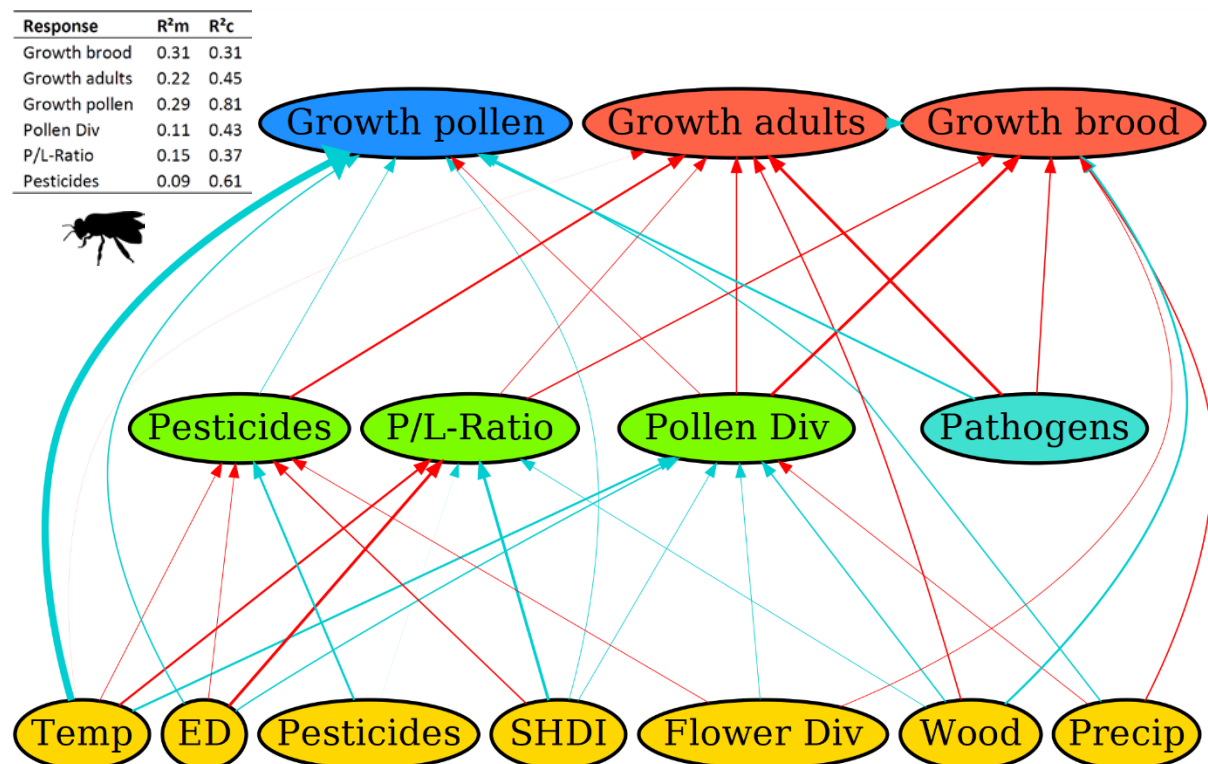


Figure 5: Path diagram showing environmental and anthropogenic pressures on *Apis mellifera* performance. Arrow thickness is scaled to the standardised effect size of the relationship between the two respective variables. Arrows in red indicate negative relationships. Arrows in blue indicate positive relationships. External variables are in yellow, measures from pollen stores in green, pathogen risk from adults in light blue, growth rate of the number of pollen cells in dark blue, colony performance measures in red. Marginal (R^2m ; fixed effects only) and conditional R^2 (R^2c ; full model) for each response variable are given in the inserted table. Wood, proportion of woody habitats; Flower Div, Richness of flowering plant species; Temp, long-term mean annual temperature; SHDI, Shannon habitat diversity index; Pesticides (yellow oval), pesticide risk from the application of plant protection products; ED, edge density of semi-natural habitats; Precip, long-term annual precipitation; Pollen Div, taxonomic richness of pollen in pollen stores; P/L-Ratio, protein-to-lipid ratio from pollen in pollen stores; Pesticides (green oval), pesticide risk from pollen in pollen stores; Pathogens, pathogen risk; Growth pollen, growth rate of pollen cells; Growth adults, growth rate of number of adults; Growth brood, growth rate of brood cells.

3.2.2. Pathogens

Bombus terrestris

Pathogen risk of *B. terrestris* was predominantly climate-driven with lower risks under warmer climates, but higher edge densities of semi-natural habitats also decreased the risk (Figure 4). A likely transfer of pathogens from *A. mellifera* to *B. terrestris* was indicated by a positive relationship between the respective risks for both species. Indirect effects of other external pressures were mediated via collected pollen diversity and pesticide risks from pollen stores. In accordance with our expectations, a high diversity of collected pollen decreased pathogen risk, while in contrast to our expectations, it was also decreased by a high pesticide risk. A high pathogen risk decreased the production of both nectar and pollen cells and increased the proportion of produced queens. Indirect effects on all three levels of performance were mediated via the number of produced nectar and pollen cells (Figure 4).

Apis mellifera

Pathogen risk of *A. mellifera* was not impacted by any of the analysed variables, but it had direct positive effects on pollen growth, and negative effects on growth rates of adults and brood cells (Figure 5).

Osmia bicornis

Pathogen risk of *O. bicornis* decreased with wetter climates and an increasing proportion of woody habitats, but increased with increasing habitat diversity (Figure 6). Other external factors were mediated by pesticide risk, pollen diversity, and protein-to-lipid ratio. We found an expected positive effect of pesticide risk on pathogen risk, but an unexpected positive relationship with the diversity of collected pollen. High pathogen risk had also an unexpected positive effect on colonisation rate (Figure 6).

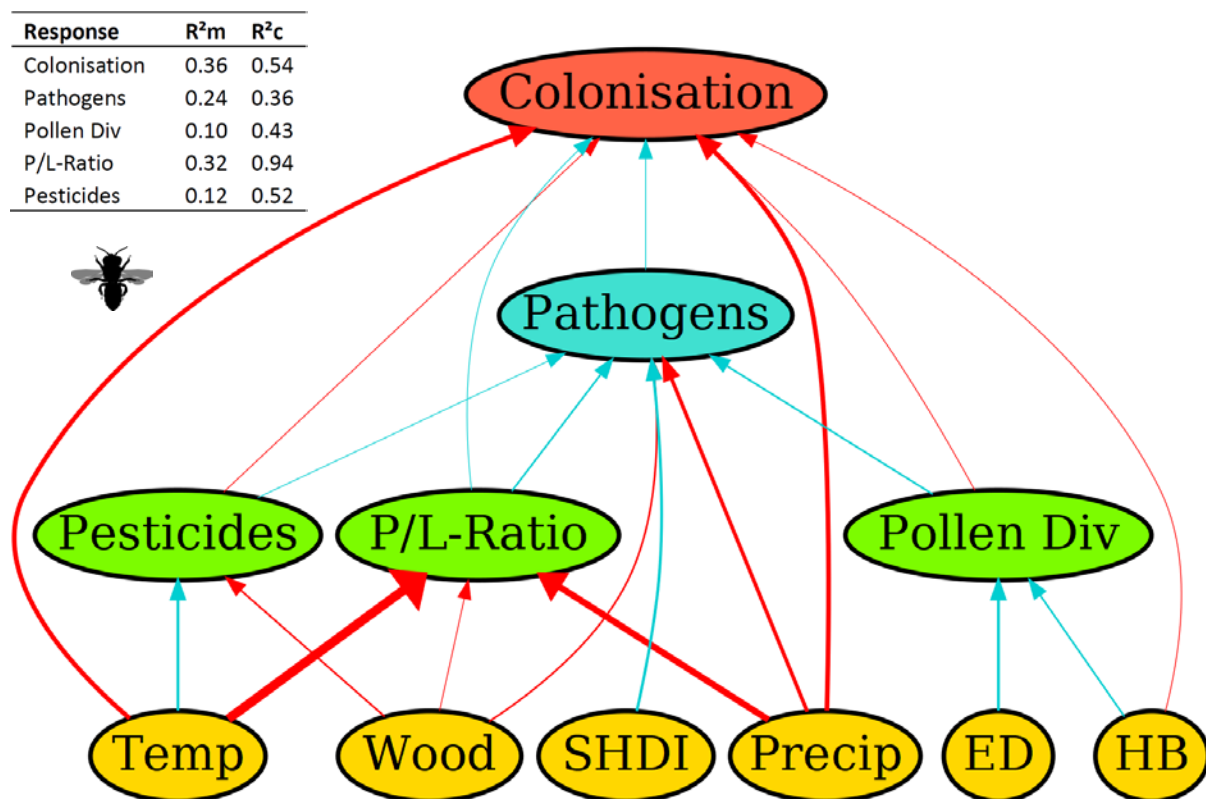


Figure 6: Path diagram showing environmental and anthropogenic pressures on *Osmia bicornis* performance. Arrow thickness is scaled to the standardised effect size of the relationship between the two respective variables. Arrows in red indicate negative relationships. Arrows in blue indicate

positive relationships. External variables are in yellow, measures from pollen stores in green, pathogen risk from adults in light blue, colony performance measures in red. Marginal (R^2_m ; fixed effects only) and conditional R^2 (R^2_c ; full model) for each response variable are given in the inserted table. Wood, proportion of woody habitats; SHDI, Shannon habitat diversity index; Temp, long-term mean annual temperature; HB, *Apis mellifera* density; ED, edge density of semi-natural habitats; Precip, long-term annual precipitation; Pollen Div, taxonomic richness of pollen in pollen stores; Pesticides (green oval), pesticide risk from pollen in pollen stores; P/L-Ratio, protein-to-lipid ratio from pollen in pollen stores; Pathogens, pathogen risk; Colonisation, nest colonisation rate.

3.2.3. Pollen diversity

Bombus terrestris

The diversity of pollen stored by *B. terrestris* was impacted by all external factors with positive effects of woody habitats, flower diversity and edge density of semi-natural habitats, and negative effects of warmer and wetter climates, pesticide risks from field application and densities of *A. mellifera* (Figure 4). As expected, high pollen diversity decreased pathogen risk. It increased the number of nectar cells but decreased the number of pollen cells, with subsequent indirect effects on all three measures of colony performance. It also increased the number of sexuals produced.

Apis mellifera

Similar to *B. terrestris*, pollen diversity stored by *A. mellifera* was affected by all external variables, except for field pesticide applications, and in the same way, except for a positive effect under warmer climates. The effect of pollen diversity was negative for the growth ratios of pollen cells, adults and brood cells (Figure 5).

Osmia bicornis

For *O. bicornis*, stored pollen diversity was only and positively related to edge density of semi-natural habitats and densities of *A. mellifera*. Contrary to our expectations, it had a positive effect on pathogen risk and a negative effect on colonisation rate (Figure 6).

3.2.4. Pollen quality (protein-to-lipid ratio)

Bombus terrestris

Protein-to-lipid ratio of pollen collected by *B. terrestris* was not explained by any of the considered factors. High protein-to-lipid ratios decreased the number of pollen cells, increased the number of nectar cells, and reduced the production of sexuals and the investment in to new queens (Figure 4).

Apis mellifera

Protein-to-lipid ratio of pollen collected by *A. mellifera* increased with habitat diversity and proportion of woody habitats, and decreased with warmer climates and higher edge densities of semi-natural habitats. Similar to *B. terrestris*, high protein-to-lipid ratios had a negative effect on colony growth in terms of both adults and brood cells (Figure 5).

Osmia bicornis

For *O. bicornis*, protein-to-lipid ratio was predominantly defined by climatic conditions with low ratios under warmer and wetter climates and in sites with higher amounts of woody habitats. High protein-to-lipid ratios had a positive effect on pathogen risk and on colonisation rates (Figure 6).

3.2.5. Total effects of environmental and anthropogenic pressures on bee performance

The total effect (combined direct and all indirect effects) of pesticide risks obtained from pollen stores was consistently negative for all three species. It was particularly high for measures of colony growth of *B. terrestris* (Figure 7a) and growth in the number adult *A. mellifera* (Figure 7d), and moderate for the measure of colonisation rate of *O. bicornis* (Figure 7f). However, this effect was reduced for the production of sexuals (Figure 7b) and colony investment into queens of *B. terrestris* (Figure 7c), and for the growth rate of brood cells in *A. mellifera* hives (Figure 7e). In addition to pesticide risk from

pollen stores, pesticide risk calculated on the basis of field application of plant protection products had a minor impact (Figures 7a,b,d,e), indicating a potential combined effect of intoxication by (larval) feeding and direct contact during foraging flights. While the effect of pesticide risk on bee performance was negative in most of the cases, high pesticide risks increased the proportion of *B. terrestris* queens produced.

The effects of pathogen risks are similar to that of pesticide risks. High pathogen risk decreased measures of growth for *B. terrestris* (Figure 7a) and *A. mellifera* (Figure 7d,e), but not for colonisation rates of *O. bicornis* (Figure 7f). It further increased the investment into the production of *B. terrestris* queens (Figure 7c). The impacts of pathogen risk were particularly high for *A. mellifera* and the proportion of *B. terrestris* queens.

Stored pollen diversity and protein-to-lipid ratio had similar effect sizes in most of the cases (Figure 7). The only noteworthy differences were in their effects on the production of sexuals of *B. terrestris* (Figure 7b) and growth rate of *A. mellifera* brood cells (Figure 7e), where pollen diversity was more important. The impact of pollen diversity differed among the bee species. It was negatively related to all performance measures of *A. mellifera* and *O. bicornis*, but positively affected all performance measures of *B. terrestris*. A high protein-to-lipid ratio of stored pollen increased colony growth (Figure 7a), but reduced the production of sexuals and the investment into queens for *B. terrestris* (Figures 7b,c). In contrast, it reduced the growth rates of adults and brood of *A. mellifera*. It also had a positive effect on nest colonisation rates of *O. bicornis*. The observed pattern of protein-to-lipid ratios of *B. terrestris* was reflected by the effects of the number of pollen cells, while the number of nectar cells had a consistently positive effect on all three performance measures. The direct effects of flower diversity at the landscape scale reflected the direct effects of pollen diversity and were even more important for colony growth rates and investment into queens for *B. terrestris* (Figures 7a,c).

The importance of climatic and landscape conditions differed considerably among the species. *Bombus terrestris* and *A. mellifera* were largely driven by pesticide risk, pathogen risk, and measures of flower resource diversity and quality, while *O. bicornis* was most dependent on climatic conditions with strong negative effects of warmer and wetter conditions (Figure 7f). Climatic conditions had generally stronger effects compared to landscape structure variables and both long-term annual temperature and precipitation had a consistent negative impact across all three species, but with different effect sizes.

The effect of *A. mellifera* densities was low for *B. terrestris* but was the third most important effect for *O. bicornis* (Figure 7f).

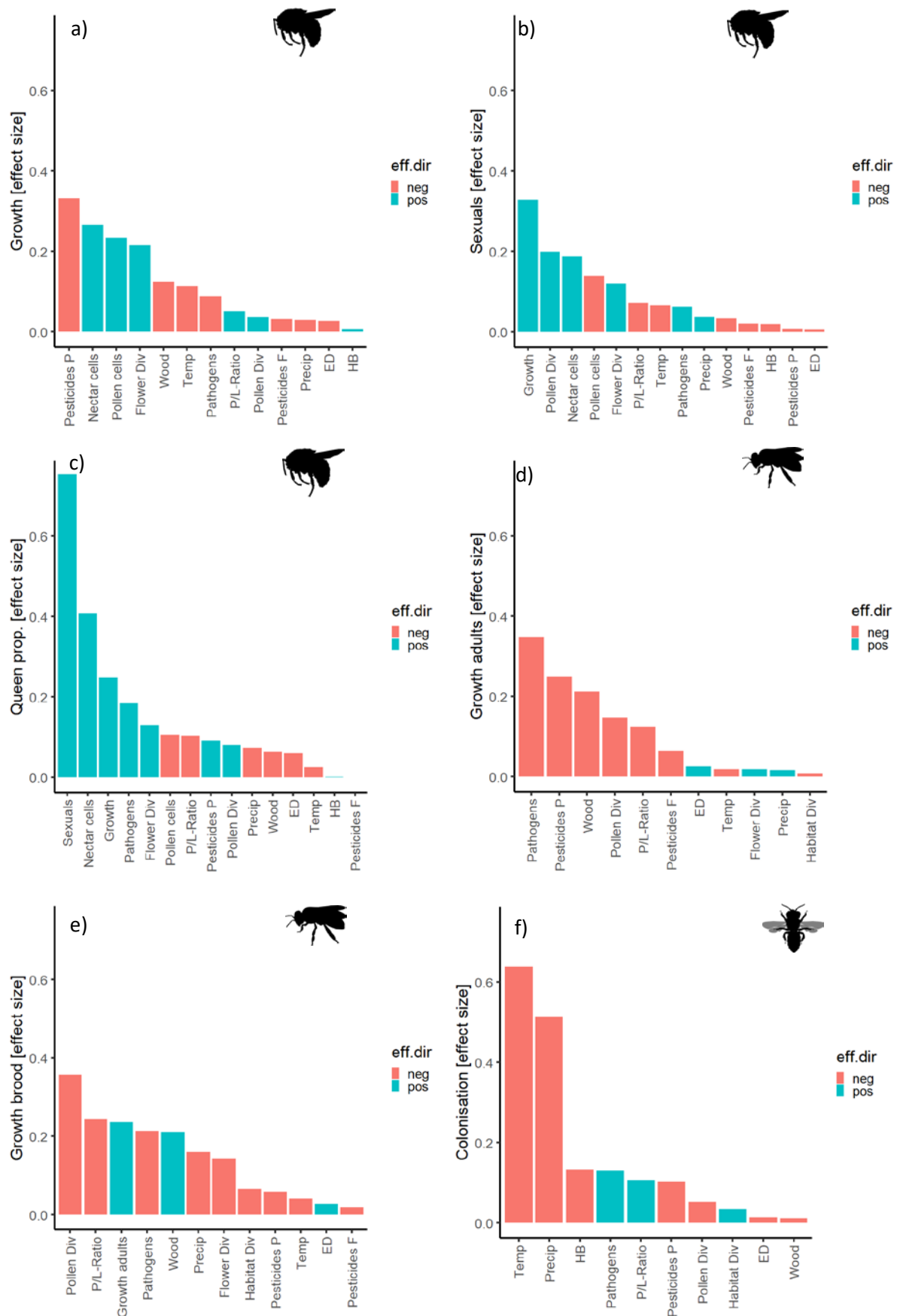


Figure 7: Total effect sizes of environmental and anthropogenic pressures on bee performance. Measures of bee performance are (a) colony growth rate based on weight differences before and after

flowering of focal crop (Growth), (b) the number of sexuals produced (Sexuals), and (c) proportion of new queens relative to produced workers and males (Queen prop) for *Bombus terrestris*; (d) growth rate for the number of adults (Growth adults), and (e) growth rate for the number of brood cells (Growth brood) for *Apis mellifera*; and (f) nest colonisation rate (Colonisation) for *Osmia bicornis*. Effect sizes are standardised and allow for comparisons within and among groups. Positive effects are indicated in red, negative in blue. Variable codes are explained in Figures 4,5, and 6. Pesticides P, pesticide risk from pollen stores; Pesticides F, pesticide risk from field applications of plant protection products. Silhouette images are by Ferran Sayol (*Bombus terrestris*), Mattia Menchetti (*Apis mellifera*), and Melissa Broussard (*Megachile rotundata*).

4. Conclusions

4.1. Pathways of pressure effects

We found strong support for our first hypothesis that pesticide risk has a consistent negative impact on the performance of all three bee species under field conditions. It was particularly high in apple sites compared to oilseed rape sites and generally consistent across the three species. Pesticides acted in two ways on bee performance. They directly decreased colony growth or colonisation rates. In addition, they seemed to affect foraging efficiency of workers as indicated by lower numbers of nectar and pollen cells which in turn decreased colony growth in *B. terrestris*.

While the impact of pesticides on colony growth was strong, this was not translated in a 1:1 manner to reproductive success (as measured in numbers of sexuals produced by *B. terrestris*). As a consequence, high risks from the application of plant protection products can cause drastic reductions in overall pollinator abundances, with potentially severe consequences for the provision of pollination services to wild and crop plants, but taken alone, they may have a smaller impact on the bee reproduction.

We did not find support for our second and third hypothesis of indirect pesticide effects, e.g., by weakening the immune defence system, on pathogen risk, or by impacting worker foraging behaviour in terms of collected pollen quality (protein-to-lipid ratio).

High levels of pathogen risk had a negative impact on the performance of *A. mellifera* and *B. terrestris*, providing support for our fourth hypothesis; and for *B. terrestris* a potential transfer from *A. mellifera* was indicated by a positive relationship of pathogen risk to *A. mellifera* densities. The impact of pathogen risk was mostly indirect, since it mainly affected the number of nectar and pollen cells negatively with subsequent negative effects on colony growth and production of sexuals in *B. terrestris*. But our fourth hypothesis was not fully supported, since *O. bicornis* colonisation rates increased with increasing pathogen risk.

The effects of flower diversity in the landscape and pollen diversity collected by bees were species specific. While *B. terrestris* met our expectations of a positive impact on bee performance according to our fifth hypothesis, *A. mellifera* and *O. bicornis* showed a negative relationship. This might be caused by a higher dietary niche specialisation of *O. bicornis*, indicated by the overall low taxonomic diversity of sampled pollen compared to *A. mellifera* and *B. terrestris* and a particular preference for pollen from *Q. robur*, *Papaver* species, and *Ranunculus* species. A high pollen diversity might be indicative of limitations in the preferred pollen sources and the need to shift to less suitable resources with consequently lower colonisation attempts. *Apis mellifera* is known to optimise its foraging efforts and concentrates on highly rewarding resources such as mass-flowering crops. *Apis mellifera* collected on average 50% of the pollen from both focal crops in comparison to 30% for *B. terrestris*. Higher pollen diversity might be indicative of longer foraging times, consequently leading to lower colony growth rates.

In contrast to expectations from our fifth hypothesis, we did not find modulating effects of pollen diversity on pesticide risks, and modulating effects on pathogen risks were species-specific. There was no impact of pollen diversity on pathogen risk for *A. mellifera*, while it was negative (as expected) for *B. terrestris* and positive for *O. bicornis*. The negative effect for *B. terrestris* indicates a potential empowering of the immune defence system by a diverse larval food and highlights the importance of alternative pollen sources in agricultural landscapes to minimise the risk from pathogens. The positive relationship of pollen diversity and pathogen risk for *O. bicornis* might be caused by a higher likelihood of getting contaminated by pathogens with the need for visiting a diverse set of plant species to collect pollen. This is particularly observed when densities of *A. mellifera* are high, as indicated by a positive relationship of *A. mellifera* densities and pollen diversity collected by *O. bicornis*.

We did not find support for our sixth hypothesis of flowering plant and stored pollen diversity having an impact on the stored pollen protein-to-lipid ratio.

Our seventh hypothesis that the protein-to-lipid ratio of stored pollen is related to pathogen risk was only supported by *O. bicornis*, where high protein-to-lipid ratios increased pathogen risks. Compared to *B. terrestris*, *O. bicornis* prefers pollen with low protein-to-lipid ratios. Thus, higher values might come from pollen sources being suboptimal for *O. bicornis* potentially with consequent negative impacts on the immune defence system.

We also assessed the relationship between *A. mellifera* densities and performance measures of the other two species. This effect was low for *B. terrestris*, but considerably higher for *O. bicornis*. This difference in response might be due to the difference in the level of sociality in both species. As a eusocial species, *B. terrestris* might be less impacted by competition with *A. mellifera*, while solitary species, such as *O. bicornis*, with a considerable overlap of visited plant species might be much more sensitive to competition.

Concerning our eighth hypothesis, climatic conditions, i.e. long-term annual temperature and precipitation, had a consistent negative impact across all three species, while the impact of landscape structure was of lesser importance, e.g. compared to landscape-level flower diversity and collected pollen diversity, and with species-specific impacts and effect sizes. Effects of climatic conditions had by far the strongest effects on *O. bicornis*, indicating that effects of climate change can override negative effects of other pressures.

A particular concern arises from the fact that all types of pressures (climate, landscape structure, flower diversity and pollen resource quality, pathogens and pesticides) affected each of the three bee species. Although pesticides and pathogens have a dominant role, our results indicate that current bee declines are a result of the sum of multiple pressures. On the other hand, this opens opportunities for management actions to compensate for particularly adverse effects of single pressures.

4.2. Compensation options of pressure effects

In general, our results support calls and actions for reducing pressures on bees, in particular of plant protection products and pathogen risks within *A. mellifera* and their likely transfer to wild pollinators, but also of flower resource limitation and climate change.

The only direct link between pressures we found was between pollen diversity collected by bees and pathogen risk, which was negative for *B. terrestris*. This result provides great support for management actions and respective political incentives to increase landscape-level flower diversity to support bee health in general and in particular in terms of resistance to pathogens. Such actions could be particularly targeted to areas with high densities of *A. mellifera*.

Our results also show that in addition to the direct effects of pressures on bee performance, they can be modulated by different compartments of the system. For instance, pesticides and pathogens of *B.*

terrestris affected the number of pollen and nectar cells produced by the colony, which in turn had a considerable impact on colony growth and reproduction, likely by affecting worker foraging behaviour and efficiency. However, the production of pollen and nectar cells is also defined by other variables, such as climate, landscape structure, and flower and pollen diversity, which can be managed in a way to partly compensate the negative effects of the other pressures.

The example of *B. terrestris* also shows the potential of internal compensation mechanisms. Under stressful conditions, such as high pesticide risks, high pathogen risks, low availability of protein (high number of nectar cells or low pollen protein-to-lipid ratio), or suboptimal climatic conditions, the colony invests more into the production of sexuals and in particular new queens, but at the expense on the number of workers. This might be considered as a survival strategy, but a drastically reduced number of workers contributes to the ongoing pollinator declines and can also have substantial impacts on the provision of pollination services to wild and crop plants.

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6. References

- Arena M, Sgolastra F (2014) A meta-analysis comparing the sensitivity of bees to pesticides. *Ecotoxicology* **23**: 324-334.
- Babin A, Schurr F, Delannoy S, Fach O, Albrecht M, Attridge E, Bottero I, Breeze TD, Cini E, Costa C, de la Rúa P, de Miranda JR, di Prisco G, Dominik C, Dzul D, Hodge S, Klein AM, Knapp J, Knauer A, Mänd M, Martínez-López V, Medrzycki P, Pereira Peixoto MH, Potts SG, Raimets R, Rundlöf M, Schwarz JM, Schweiger O, Senapathi D, Serrano J, Stout JC, Tamburini G, Brown MJF, Rivi  re M-P, Chauzat MP, Dubois E (unpublished) Another brick in the wall: spillover of infectious and parasitic agents between bee species in a pan-European assessment.
- Babin A, Schurr F, Rivi  re MP, Chauzat MP, Dubois E (2022) Specific detection and quantification of three microsporidia infecting bees, *Nosema apis*, *Nosema ceranae*, and *Nosema bombi*, using probe-based real-time PCR. *European Journal of Protistology* **86**.
- Bottero I, Dominik C, Schweiger O, Albrecht M, Attridge E, Brown MJF, Cini E, Costa C, de la R  a P, de Miranda JR, di Prisco G, Dzul Uuh D, Hodge S, Ivarsson K, Knauer AC, Klein AM, Mand M, Mart  nez-L  pez V, Medrzycki P, Pereira Peixoto MH, Potts SG, Raimets R, Rund  f M, Schwarz JM, Senapathi D, Tamburini G, Tobajas Talavan E, Stout JC (unpublished) Impact of landscape configuration and composition on pollinator communities across different European biogeographic regions.
- Burnham KP, Anderson DR. 2010. Model selection and multimodel inference: a practical information-theoretic approach. 2nd edition. Springer-Verlag, New York.
- Delaplane KS, van der Steen J, Guzman-Novoa E (2013) Standard methods for estimating strength parameters of *Apis mellifera* colonies. *Journal of Apicultural Research* **52**: Artn 52.1.03.
- DiBartolomeis M, Kegley S, Mineau P, Radford R, Klein K (2019) An assessment of acute insecticide toxicity loading (AITL) of chemical pesticides used on agricultural land in the United States. *Plos One* **14**.
- European Commission. 2019. Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food & Feed. Document No. SANTE/ 12682/2019.
- Hodge S, Schweiger O, Klein A-M, Potts SG, Costa C, Albrecht M, de Miranda JR, Mand M, De la R  a P, Rund  f M, Attridge E, Dean R, Bulet P, Michez D, Paxton RJ, Babin A, Cougoule N, Laurent M, Martel A-C, Paris L, Rivi  re M-P, Dubois E, Chauzat M-P, Arafah K, Askri D, Voisin SN, Kiljanek T,

- Bottero I, Dominik C, Tamburini G, Pereira-Peixoto MH, Wintermantel D, Breeze TD, Cini E, Senapathi D, Di Prisco G, Medrzycki P, Hagenbucher S, Knauer A, Schwarz JM, Raimets R, Martínez-López V, Ivarsson K, Hartfield C, Hunter P, Brown MJF, Stout JC (2022) Design and Planning of a Transdisciplinary Investigation into Farmland Pollinators: Rationale, Co-Design, and Lessons Learned. *Sustainability* **14**: 10549.
- Hodge S, Stout JC (2019) Protocols for Methods of Field Sampling. Deliverable D1.1 PoshBee Project, Grant agreement No. 773921. 2019. Available online: Poshbee.eu.
- Kiljanek T, Niewiadowska A, Malysiak M, Posyniak A (2021) Miniaturized multiresidue method for determination of 267 pesticides, their metabolites and polychlorinated biphenyls in low mass beebread samples by liquid and gas chromatography coupled with tandem mass spectrometry. *Talanta* **235**: ARTN 122721.
- Marini L, Quaranta M, Fontana P, Biesmeijer JC, Bommarco R (2012) Landscape context and elevation affect pollinator communities in intensive apple orchards. *Basic and Applied Ecology* **13**: 681-689.
- Nakagawa S, Johnson PCD, Schielzeth H (2017) The coefficient of determination R² and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. *Journal of the Royal Society Interface* **14**: ARTN 20170213.
- Nicholson CC, Knapp J, Albrecht M, Chauzat MP, Costa C, de la Rúa P, Kiljanek T, Klein AM, Mänd M, Potts SG, Schweiger O, Bottero I, Cini E, de Miranda JR, Di Prisco G, Dominik C, Hodge S, Kaunath V, Knauer A, Laurent M, Martínez-López V, Medrzycki P, Pereira Peixoto MH, Raimets R, Schwarz JM, Senapathi D, Tamburini G, Brown MJF, Stout JC, Rundlöf M (unpublished) Agricultural pesticide use negatively affects bumble bee colonies across Europe.
- Nicholson CC, Williams NM (2021) Cropland heterogeneity drives frequency and intensity of pesticide use. *Environmental Research Letters* **16**: ARTN 074008.
- Rundlöf M, Andersson GKS, Bommarco R, Fries I, Hederström V, Herbertsson L, Jonsson O, Klatt BK, Pedersen TR, Yourstone J, Smith HG (2015) Seed coating with a neonicotinoid insecticide negatively affects wild bees. *Nature* **521**: 77-80.
- Rundlof M, Stuligross C, Lindh A, Malfi RL, Burns K, Mola JM, Cibotti S, Williams NM (2022) Flower plantings support wild bee reproduction and may also mitigate pesticide exposure effects. *Journal of Applied Ecology* **59**: 2117-2127.
- Sandrock C, Tanadini M, Tanadini LG, Fauser-Misslin A, Potts SG, Neumann P (2014) Impact of Chronic Neonicotinoid Exposure on Honeybee Colony Performance and Queen Supersedure. *Plos One* **9**: ARTN e103592.
- Schurr F, Tison A, Militano L, Cheviron N, Sircoulomb F, Riviere MP, Ribiere-Chabert M, Thierry R, Dubois E (2019) Validation of quantitative real-time RT-PCR assays for the detection of six honeybee viruses. *Journal of Virological Methods* **270**: 70-78.
- Vanhandel E, Day JF (1988) Assay of Lipids, Glycogen and Sugars in Individual Mosquitos - Correlations with Wing Length in Field-Collected Aedes-Vexans. *Journal of the American Mosquito Control Association* **4**: 549-550.
- Vaudo AD, Tooker JF, Patch HM, Biddinger DJ, Coccia M, Crone MK, Fiely M, Francis JS, Hines HM, Hodges M, Jackson SW, Michez D, Mu JP, Russo L, Safari M, Treanore ED, Vanderplanck M, Yip E, Leonard AS, Grozinger CM (2020) Pollen Protein: Lipid Macronutrient Ratios May Guide Broad Patterns of Bee Species Floral Preferences. *Insects* **11**: ARTN 132.