



Distribution of pesticides in agroecosystem food webs differ among trophic groups and between annual and perennial crops

Radek Michalko¹ · Luboš Purchart¹ · Jakub Hofman² · Ondřej Košulič³

Accepted: 29 January 2024
© The Author(s) 2024

Abstract

Pesticides threaten biodiversity, but we know little about how they permeate food webs. Few studies have investigated the number, concentration, and composition of pesticides in agroecosystem food webs even though agroecosystems cover one-third of Earth's land area. We conducted a pioneering study on the distribution of pesticides across local (i.e., on farm) and meta food webs (i.e., regional pool of local food webs) within both perennial ($N = 8$) and annual crops ($N = 11$), examining four trophic groups—soil (primary resource), plants (primary producers), rodents (herbivores), and spiders (predators)—for the presence of multiple residues, and comparing these findings to pesticides applied by farmers in recent years. We also undertook interviews with farmers to obtain the most precise information about pesticide applications in their fields. We detected a wide spectrum of pesticides in both annual and perennial crop types. Pesticides applied by farmers represented only a small proportion of all detected pesticides, indicating that pesticides entered local food webs from surrounding landscapes. Some detected pesticides had been banned by the European Union several years ago, which is highly alarming. Trophic group mobility and crop type drove pesticides number at local scale, as mobile groups contained larger numbers of pesticides (probably from encountering wider spectra of pesticides). At a meta scale, spiders contained the highest number of detected pesticides in perennial crops but lowest diversity in annual crops. This might be explained by how spiders' functional traits are selected in different crops. Insecticides and fungicides concentrations mostly increased with trophic level, indicating bioaccumulation. Herbicides concentration were highest in plants suggesting (bio)degradation. As bioaccumulation outweighed (bio)degradation, pesticides increased overall with trophic level. Therefore, the distribution of pesticides in agroecosystem food webs was affected simultaneously by several mechanisms and depended upon trophic group, crop type, and, probably, surrounding landscape.

Keywords Bioaccumulation · Fungicide · Herbicide · Insecticide · Plant · Rodent · Soil · Spider

1 Introduction

Pesticides application is among the major factors responsible for ongoing biodiversity loss (Sánchez-Bayo and Wyckhuys 2019; Li et al. 2020; Raven and Wagner 2021) and also constitutes a serious threat to human health (Shelton et al. 2014). Although pesticides are strictly regulated in the European Union, these regulations fail to conserve biodiversity, mostly because they are based on outdated principles of environmental risk assessment (Brühl and Zaller 2019; Topping et al. 2020). For example, no study to date has investigated the simultaneous distribution of multiple pesticides in agroecosystem food webs. Such studies are necessary to disentangle pesticides' direct and indirect effects (Stark and Banks 2003; Desneux et al. 2007) on biodiversity (Bernhardt et al. 2017; Bakker et al. 2022).

✉ Ondřej Košulič
ondrej.kosulic@mendelu.cz

¹ Department of Forest Ecology, Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemědělská 3, Brno 61300, Czech Republic

² RECETOX, Faculty of Science, Masaryk University, Kamenice 753/5, Brno 625 00, Czech Republic

³ Department of Forest Protection and Wildlife Management, Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemědělská 3, Brno 61300, Czech Republic

Several studies have investigated bioaccumulation of pesticides in off-crop aquatic food webs (e.g., Coat et al. 2011; Verhaert et al. 2017; Tongo et al. 2022). Usually, these have followed the fate of one or a few preselected pesticides to test their ability to penetrate off-crop ecosystems while not gathering data on these residues' concentration levels (Brühl et al. 2021). Studies are also needed, however, to investigate how pesticides permeate directly into terrestrial food webs of agroecosystems. Inasmuch as agroecosystems cover 38% of the global land surface (FAO 2021), pesticide impact on biodiversity in agroecosystems will strongly affect biodiversity at the global scale. Moreover, because multiple pesticides are applied repeatedly during the season, in order to obtain a more realistic picture and investigate factors affecting distribution of pesticides in food webs, we need to screen food webs for multiple pesticides simultaneously during whole seasons rather than just one time and for a few preselected pesticides that are repeatedly applied by farmers (Brühl and Zaller 2019).

Several interacting factors can potentially affect the distribution of pesticides in agroecosystem food webs, including differences in concentration, composition, and number of pesticides among different trophic groups. Pesticides concentrations in food webs can increase with trophic level due to bioaccumulation (Coat et al. 2011; Verhaert et al. 2017), but, because some pesticides are also relatively easily degradable or biodegradable (Rodríguez-Cruz et al. 2006; Zabaleta et al. 2018), their concentrations may theoretically be highest in the target pest organisms and lower in other trophic levels. For example, concentrations of easily (bio) degradable insecticides may be highest in herbivores but lower in predators, plants, and soil. Another organismal functional trait that may potentially affect the distribution of pesticides in crop food webs is the mobility of organisms (Knapp et al. 2023). Sedentary organisms and relatively stationary entities such as plants and soil will most likely mirror the locally applied spectrum of pesticides while mobile organisms that move across the agricultural landscape may encounter wider spectra of pesticides (Drouillard 2008; Knapp et al. 2023). On the other hand, mobile animals are often able to hide during pesticide applications and laboratory experiments suggest that some animals can actively avoid surfaces containing residues of some pesticides (Pekár 2012). These abilities may reduce direct contact with pesticides and, consequently, the spectra and concentrations of pesticides in the bodies of such animals.

In addition to the functional traits of organisms, environmental factors, too, can influence the distribution of pesticides in food webs as they affect the bioaccumulation and (bio)degradation potentials of pesticides (Svobodová et al. 2018; Fritsch et al. 2022; Sabzevari and Hofman 2022). Perennial and annual crops may, theoretically, show different patterns in distribution of pesticides within their food webs

as perennial crops have more stable environments. Food webs in annual crops are greatly disturbed by complete crop removal and are influenced by recolonization of organisms every year (Mestre et al. 2018; Knapp et al. 2022), whereas various organisms overwinter, reproduce, and have viable populations within perennial crops (Michalko et al. 2022). Food webs from annual crops may contain more diverse spectra of pesticides than do food webs in perennial crops as higher proportions of organisms spill over from surrounding landscape to annual than to perennial crops. Therefore, these organisms might come into contact with more diverse spectra of pesticides (Drouillard 2008). On the other hand, pesticides may have more time to bioaccumulate during several animal generations within the food webs of perennial crops than within those of annual crops. Theoretically, therefore, organisms in perennial crops might contain higher concentrations and greater diversity of pesticides.

It is also important to take spatial scale into account, as the patterns may hypothetically change between local food webs (i.e., on farm scale) and meta food webs (i.e., regional pool of local food webs). Highly mobile organisms may contain large numbers of pesticides at the scale of a local field (i.e., alpha diversity), but, due to their movement among local fields, the between-fields differences (i.e., beta diversity) might be small. Just the opposite might be true for sedentary organisms (i.e., small alpha but large beta diversity). Consequently, the overall number of pesticides across meta food webs (i.e., gamma diversity) might be similar between mobile and sedentary organisms.

In the present study, we investigated the concentration, diversity, and composition of pesticides in food webs of perennial and annual crops. The crops were distributed across the main areas of intensive agriculture landscapes of Czechia, and we sampled them through two full growing seasons during 2020 and 2021. We systematically compiled data on pesticide treatments from farmers in their fields, commencing in 2018 and extending through the subsequent sampling years. We screened four groups representing four trophic levels within a food web—soil as the primary resource, plants to represent primary producers, herbivorous small terrestrial mammals (rodents) representing herbivores, and spiders representing predators (not preying on rodents). We screened these four groups for multiple residues of pesticides and compared them to the applied pesticides. We tested five broader hypotheses: H1—landscape hypothesis, according to which we expect to detect pesticides that were not directly applied by farmers. H2—bioaccumulation hypothesis, according to which concentration will increase with trophic level. H3—(bio)degradation hypothesis, according to which concentration will be highest at the trophic level of a pest to which a pesticide is targeted (e.g., herbicides will have concentration high in plants but lower in soil, rodents, and spiders). H4—exposure

hypothesis (H4a—direct vulnerability hypothesis, according to which highest proportion of applied agrochemicals will be found in stationary organisms/entities (i.e., plants and soil), because they cannot hide during agrochemical applications. H4b—movement hypothesis, according to which rodents will contain the highest number of pesticides and the pesticides applied by local farmers will represent lower proportions of overall pesticide spectrum inside their bodies than in other less mobile trophic groups. This is because rodents are largest and have the broadest home ranges of the studied trophic groups. Therefore, they are exposed to a more diverse spectrum of pesticides.) H5—habitat hypothesis, according to which organisms in annual crops will have broader diversity of pesticides than will those in perennial crops due to the greater importance of spillover of organisms from surrounding landscapes in annual than in perennial crops.

2 Material and methods

2.1 Studied regions and agroecosystems

The study was conducted in typical European agricultural landscape within the Czech Republic's Southern and Central Moravia and Central Bohemia regions. The studied regions

well represent Czech arable lowland landscapes characterized by homogeneous large-scale agriculture fields, including various annual crops (mostly cereals and oilseeds) and perennial crops (mostly orchards). Most of these areas have been quite intensively cultivated since the 1950s. Surrounding landscapes consist mostly of various small-scale, non-production habitat types, such as meadows, shrub lands, and deciduous and coniferous forest stands. Within the studied regions, we selected agriculture fields at 19 different locations and including 11 locations with annual and 8 locations with perennial crops (Table 1). Perennial crops were represented by 2 sites consisting of vineyards, 3 sites at pear orchards, and 1 site at each an apricot, a plum, and a peach orchard. As the research was conducted during a 2-year study, annual crops changed in each of the 11 locations according to each farmer's crop rotation system (Fig. 1). The following annual crops were grown during the 2 years of the study years (number of fields shown in parentheses): oilseed rape (5), wheat (9), barley (2), pea (2), mustard (1), and sugar beet (3). The selection of study areas reflected the need to focus on the widest possible spectrum of crop types and with it the related spectrum of pesticides used for plant protection.

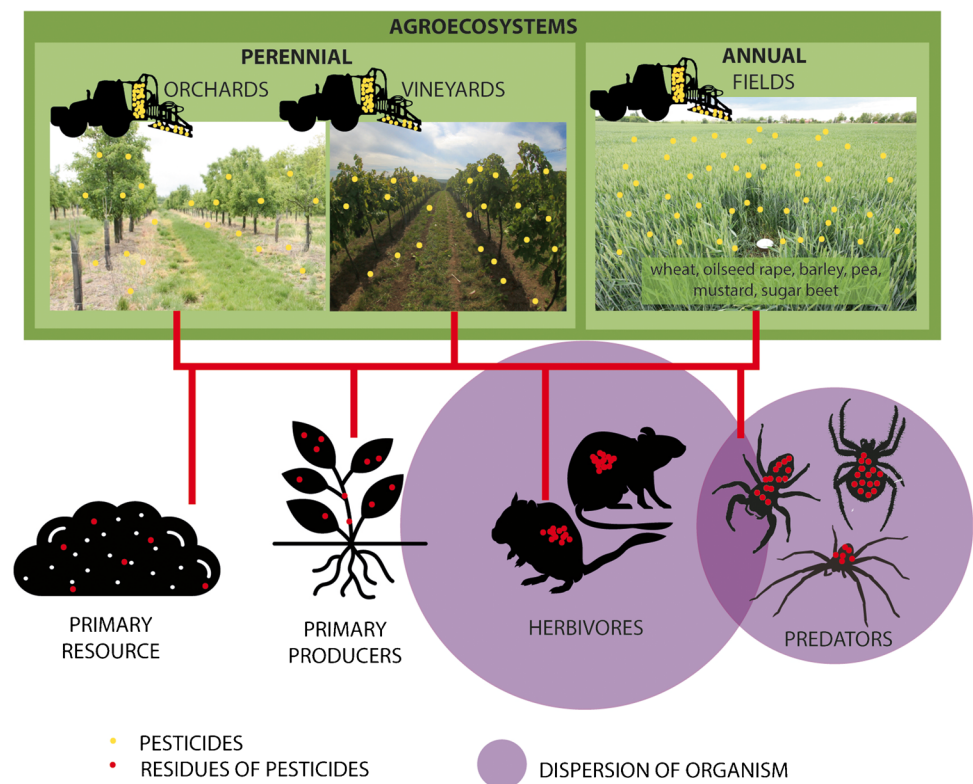
All study sites were treated with various types and amounts of pesticides according to the specific crop system, farming company, and year of application in each case.

Table 1 Characteristics of individual study sites (agroecosystems) located across South Moravia and Central Bohemia in the Czech Republic. Annual crops underwent changes at each of the 11 loca-

tions over the 2-year sampling period, in accordance with the crop rotation systems employed by individual farmers.

No. of location	Agroecosystem type	Crop type		Region	Cadastre	GPS	Altitude (m.a.s.l)
		2020	2021				
1	Annual	Wheat	Sugar beet	South Moravia	Polkovice	49°23'5.49"N 17°15'42.67"E	208
2	Annual	Barley	Wheat	South Moravia	Polkovice	49°21'19.84"N 17°14'12.26"E	235
3	Annual	Oilseed rape	Wheat	South Moravia	Polkovice	49°23'10.92"N 17°16'6.78"E	244
4	Annual	Sugar beet	Barley	South Moravia	Polkovice	49°23'6.04"N 17°13'17.27"E	249
5	Annual	Sugar beet	Wheat	South Moravia	Polkovice	49°22'45.26"N 17°15'1.67"E	226
6	Annual	Oilseed rape	Wheat	Central Bohemia	Dobrovíz	50°7'23.00"N 14°13'2.54"E	343
7	Annual	Wheat	Pea	Central Bohemia	Dobrovíz	50°6'24.59"N 14°12'24.58"E	359
8	Annual	Wheat	Pea	Central Bohemia	Chleby	50°13'52.94"N 15°5'8.11"E	198
9	Annual	Oilseed rape	Wheat	Central Bohemia	Chleby	50°14'10.61"N 15°4'35.39"E	202
10	Annual	Oilseed rape	Mustard	Central Bohemia	Klíčany	50°12'17.87"N 14°25'17.24"E	252
11	Annual	Wheat	Oilseed rape	Central Bohemia	Klíčany	50°11'58.44"N 14°27'19.96"E	279
12	Perennial	Plum	Plum	South Moravia	Litenčice	49°12'34.93"N 17°11'55.40"E	445
13	Perennial	Apricot	Apricot	South Moravia	Kobylí	48°55'59.32"N 16°55'33.28"E	281
14	Perennial	Peach	Peach	South Moravia	Kobylí	48°56'38.55"N 16°55'1.79"E	198
15	Perennial	Pear	Pear	South Moravia	Velké Bílovice	48°50'48.52"N 16°55'6.40"E	196
16	Perennial	Pear	Pear	South Moravia	Velké Bílovice	48°51'32.70"N 16°55'31.87"E	225
17	Perennial	Pear	Pear	South Moravia	Lužice	48°50'0.22"N 17°4'33.96"E	164
18	Perennial	Vineyard	Vineyard	South Moravia	Morkůvky	48°58'17.44"N 16°51'9.68"E	223
19	Perennial	Vineyard	Vineyard	South Moravia	Hustopeče	48°55'51.33"N 16°45'51.24"E	198

Fig. 1 An illustrative overview of the study's conceptual framework designed to elucidate the distribution of pesticide residues and their bioaccumulation across four trophic levels within both perennial and annual agroecosystems, spanning the agricultural landscape of the Czech Republic. Graphic vector elements downloaded from <https://www.vecteezy.com/>.



Generally, farmers applied considerably larger amounts of various pesticides to annual than to perennial crops. This is mainly due to the high-input requirements of conventional farming in arable fields. The earliest application of pesticides was at the beginning of the season during March/April and the latest was at the end of the growing season in September/October. As we aimed to screen all possible pesticide residues in our study locations, we conducted a questionnaire survey among farmers managing the studied locations. We gathered information on pesticide treatments (trade names, active ingredients, and dates of application) since 2018 inclusive of the sampling years 2020 and 2021. The data utilized for comparing farmers' application practices with detected pesticide residues are publicly available on the Zenodo data repository (Michalko et al. 2023).

2.2 Sampling design

We focused on four parts of food webs: soil (primary resource), primary producers (represented by plant vegetation) and consumers with small terrestrial mammals (rodents) as herbivores and spiders as arthropod predators (Fig. 1).

Sampling was conducted during the 2020 and 2021 vegetation seasons. The exact periods and sampling techniques differed according to the target groups. Because we were in direct communication with farmers, we gathered information

about exact planned application dates in their crops for both sampling years. Accordingly, all samples were collected at least 10 days after pesticide application during the entire growing/sampling seasons at all study sites. We were particularly cautious not to collect samples immediately after the application of agrochemicals in the fields. Our aim was to prevent the deposition of recently sprayed pesticides, ensuring an unbiased interpretation of the bioaccumulation of residues in the target groups. After each field collection, samples were transferred to the laboratory and stored in a freezer ($-20\text{ }^{\circ}\text{C}$) until screening for pesticide residue compounds.

Soil samples were collected from the 0–25 cm layer by steel spade, which is the depth most relevant for the occurrence of pesticides (Pose-Juan et al. 2015). Five subsamples of the same weight (about 1.5 kg) were taken randomly across each agroecosystem and mixed together. In annual crops, sampling was performed about 2 weeks before harvesting (usually in June, depending on the annual crop) after lapse of the protective preharvest interval from the final pesticide application in each year. Soil samples in perennial crops were collected in August of both years, usually corresponding to a time after the last pesticide applications of the season according to the type of crop.

For plant samples, 15 subsamples of agricultural crops (in arable fields of annual crops) or herb vegetation (perennial crops—vineyards and orchards) were taken equally and randomly across each agroecosystem, then mixed together.

Together, the combined subsamples weighed at least 1 kg. Plants were collected at the same time as were the soil samples in all studied agroecosystems for both years. To ensure consistency in the collection of plant samples for residual analysis in both types of agroecosystems, only the above-ground parts of plants—specifically, stems with leaves were gathered. The similarity in plant species composition between the vineyard and orchard agroecosystems was attributed to regular mowing and occasional mulching, which promoted habitat homogeneity.

Small mammals were snap-trapped in each agroecosystem during May–June and November in both years. These times cover periods of rodents' greatest activity in agroecosystems (Suchomel et al. 2012). Wood mouse (*Apodemus sylvaticus*) and common vole (*Microtus arvalis*) were sampled with a maximum 10 specimens of each species per locality and year. To ensure sufficient data for statistical analysis, both species were categorized as herbivores, given their predominant herbivorous trophic ecology (Abt and Bock 1998; Jacob et al. 2014). Altogether 340 rodent specimens were collected. Sampling was conducted at least 100 m from the edge of the field in the direction of the center of the pesticide-treated agroecosystem in order to avoid the edge effect of surrounding habitats. To measure the content of pesticides residues in small mammal bodies, kidneys and livers were dissected from each rodent specimen in the laboratory. To acquire the minimum weight needed for analyses, dissected tissues were mixed depending on the weight (5 g for liver and 1.5 g for kidney), usually 3–5 samples together.

To obtain the required sample weight (3 g) per location and year, spiders were collected intensively in both years during May–June and August–September, which months correspond to spiders' greatest activity in European agroecosystems (Samu and Szinetár 2002). Due to the large amount of arthropod biomass required for pesticide residue analyses, sampling was carried out by three intensive methods simultaneously with the aim of collecting sufficient amounts within a wide spectrum of functionally different groups of spiders. Dry pitfall trapping and sweep-netting were used as sampling methods to collect ground-dwelling and vegetation-dwelling spiders, respectively. Beating of lower branches over a cloth tray was employed as an additional method to collect foliage-dwelling spiders in perennial crops of fruit orchards. The species composition was remarkably similar within each of sampling method across the agroecosystems studied, characterized by a predominance of species typical for both perennial agroecosystems of orchards and vineyards (Buchar and Růžička 2002). Additionally, typical agrobiont spiders were dominant in annual crops (Michalko and Birkhofer 2021). Therefore, selected methods were employed to capture different functional groups that would best reflect pesticide bioaccumulation in spiders. Sampling of spiders was conducted at the same spatial scale in pesticide-treated

agroecosystems as was sampling of small mammals. Collected spider specimens were kept alive separately in jars, transported to the laboratory, then stored in a freezer.

2.3 Analysis of pesticide residues

The occurrence of pesticide residues including their metabolites in collected samples was determined by a method targeting 300 analytes selected in accordance with a preliminary sample screening, the questionnaire-based survey of farmers, and the Registration Database of Plant Protection Products in the Czech Republic (Central Institute for Supervising and Testing in Agriculture 2023). Further, a wide scope screening method was used to quantify other types of chemical analytes, such as older and now forbidden pesticides including their metabolites. The detected metabolites were incorporated into the chosen category of insecticides, herbicides, and fungicides for further analysis, given their common occurrence within food webs (Goutte et al. 2020). Limits of quantification (LOQs) for all analytes and their metabolites were ≤ 10 ng/g, except that these were ≤ 50 ng/g for glyphosate and its metabolite aminomethylphosphonic acid. The extraction procedure was based on the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method (Yang et al. 2010; Mei et al. 2011; Kosubová et al. 2020). For all samples, liquid chromatography–high-resolution mass spectrometry (LC/HRMS) analysis was performed on a Q Exactive Focus high-resolution mass spectrometer. An UltiMate 3000 liquid chromatograph and Zorbax Eclipse XDB-C18 separation column (2.1×150 mm, $5 \mu\text{m}$; Agilent) were used for component separation. The subsequent analytical process varied based on the given entity/trophic group (see Supplementary Material 1 for detailed information.)

2.4 Statistical analyses

2.4.1 Diversity of pesticides

The analyses were performed in R (R Development Core Team 2023) and EstimateS 9.1.0 (Colwell 2019). At the local food web scale, we compared the number of detected pesticides among the four groups by generalized mixed effects models (GLMM) with Poisson error structure (GLMM-p) and log link because several measurements were taken in a same field and data were counts (Zuur et al. 2015). There was no problem with data dispersion in the GLMM-ps ($\varphi < 1.25$, $p > 0.100$). The field IDs served as random effects while the factor group (levels: soil, plants, rodents, and spiders), the factor year (levels, 2020, 2021), and their interactions acted as the fixed effects. Significance was tested by log-ratio likelihood. We analyzed the perennial and annual crop food webs separately to avoid computational problems, and therefore, we compare only the differences in qualitative patterns between the crop types at

local scale. We used the R package `glmmTMB` (Brooks et al. 2017) to run all GLMMs.

At the meta food web scale, we compared the numbers of detected pesticides among the groups and within the groups between the crop types by sample-based rarefaction curves based on 1000 iterations and overlap of their 95% confidence intervals (Colwell 2019). A sample was represented by a field within a year.

2.4.2 Proportional representation of applied pesticides

First, we compared what proportion of all locally applied pesticides was detected within a group. We then compared what proportion the locally applied pesticides that were detected represented in the overall spectrum of locally detected pesticides. We analyzed both by GLMM with beta error structure (GLMM-beta) because the data were bounded within the (0,1) interval (Zuur et al. 2015). Because some values reached 0 or 1, however, which is not allowed in beta distribution, we transformed 0s to 0.025 and 1s to 0.975. The structure of the random and fixed effects was the same as described above (i.e., $\text{group} \times \text{year} + (1|\text{field})$); see subsection 2.4.1).

2.4.3 Concentration of pesticides

First, we analyzed overall concentrations of pesticides and then mean concentration of pesticides. This is because some organisms may contain many pesticides but in low concentrations while other organisms may contain few pesticides but in high concentrations. After that, we analyzed concentrations of particular pesticides types (i.e., insecticides, herbicides, and fungicides) separately for each crop type. We analyzed each pesticide type separately to avoid computational problems. In annual crop food webs, there was not enough data (not found or no samples were collected) for insecticides, fungicides, and herbicides to analyze the 2 years separately, so we analyzed only the effect of trophic group. In rodents, where livers and kidneys were analyzed, we used the average amount between these two organs. We used GLMMs with gamma error structure and log link (GLMM-g) because the data were continuous and heteroscedastic (Zuur et al. 2015). For perennial crops, the fixed and random effects were the same as described above (i.e., $\text{group} \times \text{year} + (1|\text{field})$); see subsection 2.4.1). For the annual crops, we did not analyze the effect of year but only the effect of the factor group (i.e., $\text{group} + (1|\text{field})$).

3 Results and discussion

3.1 Overview

During the years 2018–2021, farmers applied 46 pesticides in the perennial crops and 80 pesticides in the annual crops

(Michalko et al. 2023, Zenodo data repository). Across the sampled target trophic groups (soil, plants, rodents, and spiders) and studied annual and perennial crops, a total of 84 pesticide compounds were detected, including 31 fungicides, 30 herbicides, and 23 insecticides. Several pesticide metabolites, such as aminomethylphosphonic acid (AMPA) from the herbicide glyphosate and ketocarbifuran from the insecticide carbofuran, were detected throughout both years of the study. The total number of substances detected above the LOQs for all entities made up 28% of all substances for which analytical screening was performed. The diversity of detected pesticides was notably high, encompassing substances recently registered for plant protection systems and many prohibited agrochemicals that are not permitted for use (Kosubová et al. 2020). For a comprehensive list of applied and detected pesticide compounds, including their metabolites, please refer to the dataset available on the Zenodo data repository (Michalko et al. 2023).

In soil of the perennial crops, we detected 13 pesticides, and the pesticide detected most frequently was the fungicide boscalid (56%, $N = 9$). In soil of the annual crops, we detected 30 pesticides, and the pesticides detected most frequently were the fungicide epoxiconazole (41%, $N = 9$), the herbicide chloridazon (27%, $N = 6$), and the fungicide tebuconazole (27%, $N = 6$). All other pesticides in soil were detected at low frequencies (< 13%).

In the perennial crops, we detected 22 pesticides overall in plants and the pesticides detected most frequently were the herbicide tripropindan (88%, $N = 14$), the fungicide spiroxamine (31%, $N = 5$), as well as the insecticide pyrethrin, the herbicide glyphosate, and the fungicide fluopyram (19%, $N = 3$). In the annual crops, we detected 33 pesticides overall in plants and the 3 pesticides detected most frequently were the herbicide tripropindan (85%, $N = 17$), the herbicide phenmedipham (40%, $N = 8$), and the fungicide spiroxamine (35%, $N = 7$).

We detected 16 pesticides in rodents from the perennial crops and the 3 pesticides detected most frequently were the fungicide spiroxamine (63%, $N = 10$), the herbicide tripropindan (50%, $N = 8$), and the insecticide/rodenticide carbofuran (50%, $N = 8$). We detected 28 pesticides in rodents in the annual crops and the 3 pesticides detected most frequently were the herbicide tripropindan (85%, $N = 11$), the insecticide/rodenticide carbofuran (85%, $N = 11$), and the fungicide spiroxamine (69%, $N = 9$).

In the perennial crops, 29 pesticides were detected in spiders overall, and the 3 pesticides detected most frequently were the insecticide thiacloprid (19%, $N = 3$), the fungicide boscalid (19%, $N = 3$), and the fungicide difenoconazole (19%, $N = 3$). In annual crops, 12 pesticides were detected in spiders overall, and the 3 pesticides detected most frequently were the insecticide thiacloprid (62%, $N = 9$), the fungicide

pyrametostrobin (54%, $N = 7$), and the insecticide malathion (38%, $N = 5$).

Although we detected broad diversity of pesticides, those applied by farmers represented only a minor portion of the whole spectrum of pesticides that were detected. The wide spectrum of pesticides detected in local food webs that had not been applied directly can be explained by transfer from the surrounding landscape. Notably, different trophic groups exhibited distinct pesticide compositions, indicating varied exposure pathways for different organisms. An illustrative example is the widely used herbicide glyphosate, and its metabolites, predominantly detected in perennial crops due to frequent applications in orchards and vineyards (Duke 2018). On the other hand, residues of glyphosate were also frequently found in locations without recent use, suggesting potential transport from surrounding landscapes into local food webs and accumulation within broader meta-food webs. This instance serves as a typical illustration highlighting the complexities of pesticide transport and accumulation in ecosystems (Pose-Juan et al. 2015).

We detected several legacies as well as pesticides never approved and that are prohibited in the European Union or in Great Britain. The occurrence of the prohibited substances can be explained by illegal application of these pesticides, introduction and application of falsified pesticides, and persistence of these pesticides (Brühl and Zaller 2019; Fritsch et al. 2022). Among the prohibited pesticides, for example, carbofuran and its metabolites is strongly poisonous also to humans (Sharma et al. 2012) and it was regularly detected in rodents among most of the perennial and annual crops during the two consecutive years of sampling. This indicates long persistence in the ecosystem and/or ongoing illegal application of this pesticide. Indeed, several deaths

among birds of prey caused by carbofuran are reported every year in Czechia (Novotný et al. 2011), and these deaths are often linked to direct consumption of carbofuran through a poisoned bait. Another significant discovery of prohibited pesticide was methoxychlor, an organochloride insecticide that is listed as the second generation of DDT. This agrochemical has been banned in Europe since 2002 and in the USA since 2003. It is a substance well-known for strong bioaccumulation and persistence in the environment, may be carcinogenic, and has various negative effects on beneficial insects, birds and humans (Safford and Jones 1997; Qi et al. 2022). Also detected were pyrametostrobin and tripropindan, which have never been registered in European, Great Britain, or US markets. It may be that these substances are brought in and sold illegally from Asian countries where they are commonly used as a strobilurin fungicide (Huang et al. 2017).

3.2 Number of pesticides in local food webs and meta food webs

3.2.1 Local food webs

In the perennial agroecosystems, the mean number of agrochemicals detected differed among the trophic groups (GLMM-p, $\chi^2_3 = 21.7$, $p < 0.001$; Fig. 2A) and a higher number of agrochemicals was detected in 2020 than in 2021 (GLMM-p, $\chi^2_1 = 28.9$, $p < 0.001$; Fig. 2A). Overall, there was a pattern suggesting support for the movement hypothesis, as the number of agrochemicals detected tended to be higher in mobile rodents and spiders than in sedentary soil and plants (Fig. 2A). The lowest mean number of agrochemicals was found in soil ($p < 0.008$; Fig. 2A) while similar numbers of agrochemicals were found in the other three

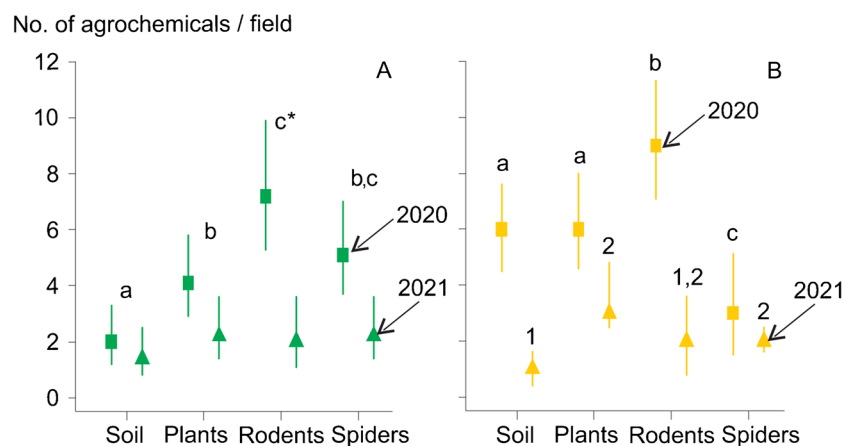


Fig. 2 Diversity (number) of pesticides detected in four groups representing four trophic levels (soil: base resource, plants: primary producers, herbivorous rodents: herbivores, and spiders: predators) in perennial and annual crops at local food web (A and B, respectively).

The points are estimated means and the whiskers indicate CI 95%. Different lowercase letters and numbers indicate statistical differences within a year. The asterisk in panel A indicates a marginal difference ($p = 0.054$).

groups ($p > 0.230$; Fig. 2A). A marginally lower number of agrochemicals was nevertheless found in plants than in rodents ($p = 0.054$).

In the annual agroecosystems, there was significant interaction between year and group (GLMM-p, $\chi^2_3 = 23.5$, $p < 0.001$; Fig. 2B). Rodents had the highest mean number of agrochemicals ($p < 0.028$), followed by soil and plants, which did not differ significantly ($p = 0.836$). Spiders contained the significantly lowest mean number of agrochemicals ($p < 0.023$). In 2021, the lowest mean number of agrochemicals was found in soil ($p < 0.005$), which mean was not significantly different from that in rodents ($p = 0.154$). The highest mean number of agrochemicals was found in plants, which did not differ from spiders ($p = 0.234$).

The diversity of pesticides in local food webs of perennial and annual crops in 2020 supported the mobility hypothesis because the greatest diversity was detected in rodents, which are the most mobile (Vukicevic-Radic et al. 2006; Wang 2013). Mobile organisms have higher probability encounter larger spectra of pesticides (Drouillard 2008; Knapp et al. 2023). Although the diversity of pesticides in local food webs within annual crops in 2021 differed among the groups, there was no pattern supporting any of the bioaccumulation, biodegradation, direct vulnerability, or mobility hypotheses.

3.2.2 Meta food webs

Overlapping 95% confidence intervals (CI 95%) of rarefaction curves show there to have been clear differences among groups in both crop types and also that the patterns differed between the crop types (Fig. 3A, B). Except for plants, the number of detected pesticides differed also between the crop types within the groups (Fig. 3C–F). The patterns at meta food web scale support only our habitat hypothesis. In the perennial crops, the highest number of pesticides was detected in spiders and plants, while in the annual crops the highest number was detected in rodents and plants (Fig. 3A, B). Spiders in annual crops had the lowest number of detected pesticides (Fig. 3B). Moreover, more pesticides were detected in soil (Fig. 3C) and rodents (Fig. 3E) in the annual than perennial crops while in spiders it was the opposite (Fig. 3F).

Overall, the patterns at the meta food web scale did not follow the pattern at the local food web scale. The change in the patterns between spatial scales indicates that high beta-diversity of pesticides (i.e., between-fields differences) in plants and soil compensated for the lower alpha diversity (within field number) of pesticides.

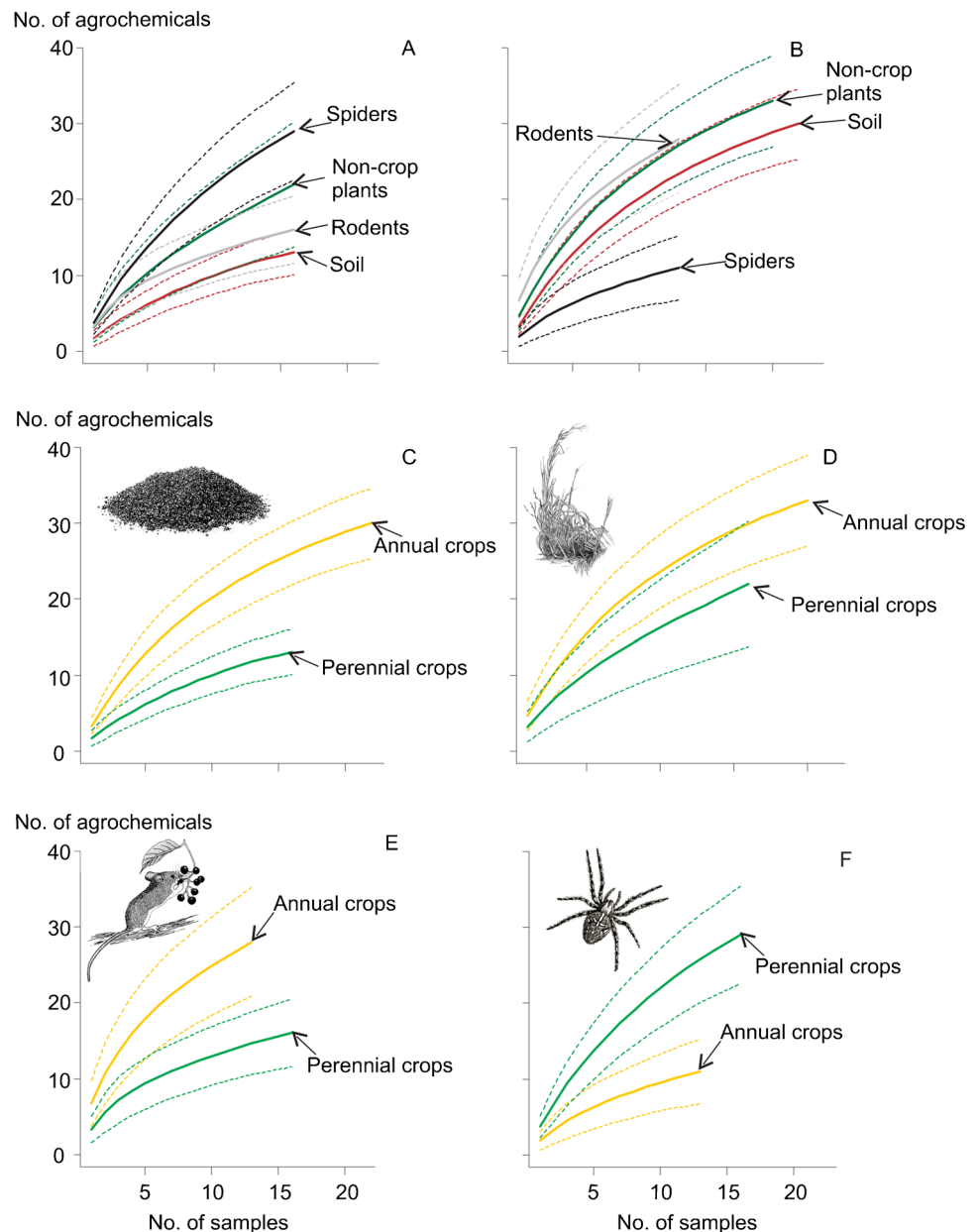
At the meta food web scale, rodents and soil (and with some trend also plants) contained greater pesticides diversity in annual than perennial crops. Although this partly supports our habitat hypothesis, the greater diversity in annual than perennial crops within these groups at meta

food web scale can be explained by the more diverse spectrum of pesticides applied by farmers in annual crops. Even though the applied pesticides represented only a small proportion of those detected, it is possible that a more diverse spectrum of pesticides is also applied in annual crops within the surrounding landscapes. This can be caused, too, by high persistence of particular agrochemicals (e.g., boscalid and epoxiconazole), which probably were used for plant protection in different crop types in previous years due to the rotation system followed in annual agroecosystems. As we have time data showing zero applications by farmers of these pesticides in the past 4 years, this can confirm quite long persistence of these particular analytes in soil environments (Sabzevari and Hofman 2022). On the other hand, spiders, surprisingly, contained greater diversity of pesticides in perennial than in annual agroecosystems. In annual crops, spiders even contained the least diversity of pesticides from the four groups. This difference may theoretically arise because the perennial and annual crops select for different functional traits in spiders (Michalko and Birkhofer 2021). With regard to the large spider species that we mainly collected for the purpose of pesticide screening, there are proportionally more species using aerial webs to capture prey in perennial than annual agroecosystems (Michalko and Birkhofer 2021). Spiders using aerial webs are better dispersers than are large ground-dwelling cursorial spider species (Blandenier 2009) and they prey also on more mobile prey, in particular flying insects (Michalko and Pekár 2016). Cursorial spiders may theoretically hide during pesticide applications whereas pesticides can cling on spider webs (Samu et al. 1992). Several web-building spiders recycle their webs daily by consuming them (Foelix 2011), and that can expose them to more pesticides (Pekár 2012). Overall, the web-building spiders that are proportionally more dominant in perennial than annual crops (Birkhofer et al. 2013; Michalko and Birkhofer 2021) may be exposed to more diverse spectra of pesticides than are cursorial spiders. Indeed, we have observed that in perennial crops the diversity of detected pesticides increases with growing proportional biomass of web-building spiders (Michalko et al. unpubl.). Therefore, further research is necessary to disentangle how functional traits of organisms other than their trophic levels and mobility also affect the distribution of pesticides in food webs.

3.3 Proportional representation of applied pesticides

Generally, only small proportions of the pesticides applied by farmers were detected in the food webs (perennial crops: mean \pm SD = 0.09 ± 0.120 , range 0–0.44; annual crops: mean \pm SD = 0.05 ± 0.061 , range = 0–0.18) and there was

Fig. 3 Diversity (number) of pesticides detected in four groups representing four trophic levels (soil: base resource, plants: primary producers, herbivorous rodents: herbivores, and spiders: predators) in perennial and annual crops at meta food web scales. **A, B** Comparisons among groups within the two crop types (perennial and annual). **C–F** Comparisons between agroecosystem types for soil, plants, rodents, and spiders, respectively. The solid thick lines in panels show the estimated number of pesticides per given number of samples (i.e., number of fields) and the slender broken lines show the corresponding CI 95%.



no significant difference among the groups in either perennial (GLMM-beta; $\chi^2_3 = 4.4$, $p = 0.223$) or annual (GLMM-beta; $\chi^2_3 = 3.4$, $p = 0.337$) crops.

Although the pesticides applied by farmers represented only small proportions of all detected pesticides (mean \pm SD = 0.12 ± 0.358 ; range = 0–1), there were significant differences among the groups in the perennial agroecosystems (GLMM-beta; $\chi^2_3 = 8.6$, $p = 0.035$; Fig. 4). The importance of applied agrochemicals decreased with rising trophic level, although the plants, rodents, and spiders did not differ significantly ($p > 0.613$). The effect of year was not significant (GLMM-g, $\chi^2_1 = 0.3$, $p = 0.574$). The differences among groups were not significant in the annual agroecosystems (GLMM-beta; $\chi^2_3 = 4.6$, $p = 0.203$; mean \pm SD = 0.29 ± 0.314 , range = 0–1).

3.4 Concentration of pesticides

3.4.1 Overall concentration

In the perennial agroecosystems, the overall concentrations of agrochemicals differed among the groups (GLMM-g, $\chi^2_3 = 35.4$, $p < 0.001$; Fig. 5A) and overall concentration of agrochemicals was higher in 2020 than in 2021 (GLMM-g, $\chi^2_1 = 20.1$, $p < 0.001$; Fig. 5A). The pattern supports the bioaccumulation hypothesis, as the overall concentration of agrochemicals increased with trophic level but not in a completely linear fashion. Rodents, in fact, had slightly lower concentration than did plants, although that difference was not statistically significant ($p = 0.334$; Fig. 5A).

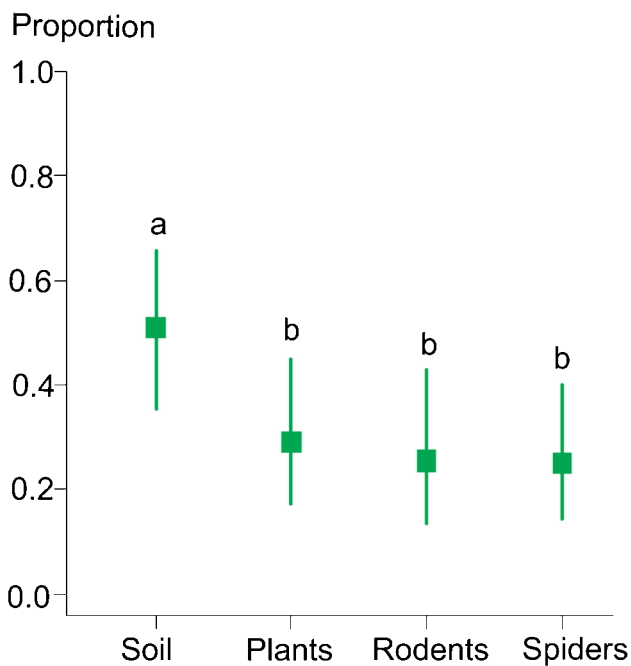


Fig. 4 Comparing proportional representation of pesticides applied by farmers in perennial agroecosystems detected within each group out of all pesticides detected within that group. The four groups represented four trophic levels (soil: base resource, plants: primary producers, herbivorous rodents: herbivores, and spiders: predators). The points are estimated means and the whiskers show CI 95%. Different lowercase letters indicate statistical differences.

In the annual agroecosystems, there was a significant interaction between year and group (GLMM-g, $\chi^2_3 = 14.4$, $p = 0.002$; Fig. 5B) but the patterns provide no support for any of our hypotheses. Although all other groups had higher concentrations of agrochemicals in 2020 than did soil, this was only marginally significant in plants ($p = 0.050$) and not significant in rodents and spiders ($p > 0.216$). In 2021, by contrast, spiders contained the significantly highest concentration of pesticides ($p < 0.013$), followed by plants ($p < 0.002$). Although rodents had higher concentration than did soil, this difference was not significant ($p = 0.447$).

In the perennial agroecosystems, the average concentration per pesticide differed among the groups (GLMM-g, $\chi^2_3 = 49.7$, $p < 0.001$; Fig. 5C) and the average concentration of agrochemicals was higher in 2020 than in 2021 (GLMM-g, $\chi^2_1 = 4.4$, $p = 0.035$; Fig. 5C). The pattern in mean concentration per agrochemical supports the bioaccumulation hypothesis, because the mean concentration increased with trophic level, albeit not linearly (Fig. 5C). Plants had higher mean concentration per agrochemical than did rodents ($p = 0.030$; Fig. 5C), but spiders had the highest concentration ($p < 0.001$; Fig. 5C).

In the annual agroecosystems, the mean concentrations per pesticide differed among the groups (GLMM-g, $\chi^2_3 = 59.0$, $p < 0.001$; Fig. 5D) and were higher in 2020 than

in 2021 (GLMM-g, $\chi^2_1 = 21.1$, $p < 0.001$; Fig. 5D). The pattern was very similar to that of the perennial agroecosystems, partially supporting the bioaccumulation hypothesis because the overall concentration of agrochemicals increased with trophic level but not linearly so, as plants had higher mean concentrations per agrochemical than did rodents ($p < 0.005$; Fig. 5D). Spiders had the highest concentrations per agrochemical ($p < 0.001$; Fig. 5D).

3.4.2 Pesticide functional groups

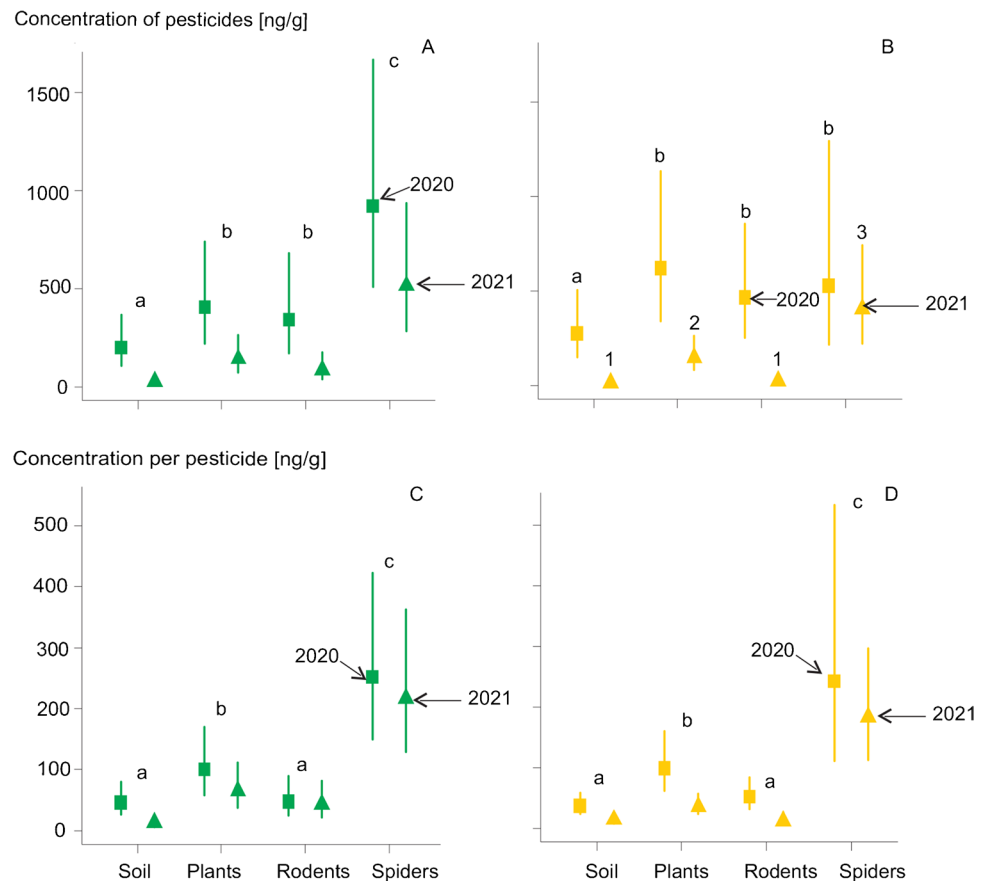
In soil of the perennial crops, insecticides were detected only twice and in just one field during the 2 years of the study (2020: 10 ng/g, 2021: 20 ng/g). There was a significant interaction between group and year for insecticides in the perennial crops (GLMM-g, $\chi^2_2 = 16.0$, $p < 0.001$; Fig. 6A). In 2020, the pattern supported the (bio)degradation hypothesis as the concentration of insecticides was highest in the targeted trophic level (herbivores) (Fig. 6A). Herbivorous rodents contained the highest concentration of insecticides, although this was only marginally greater than in plants ($p = 0.055$). Concentrations of insecticides did not differ significantly between plants and spiders ($p = 0.215$). By contrast, in 2021, the pattern supported the bioaccumulation hypothesis as the highest concentration was detected in spiders ($p < 0.001$). The concentration of insecticides was higher in plants than in rodents but not significantly ($p = 0.093$).

The concentrations of insecticides differed among the groups in annual crops and the pattern supported the bioaccumulation hypothesis, as it increased linearly with trophic level (GLMM-g, $\chi^2_2 = 10.8$, $p = 0.004$; Fig. 6B). Spiders had the highest concentration of insecticides ($p < 0.001$), but this did not differ significantly from that of rodents ($p = 0.627$; Fig. 6B).

In the perennial crops, the concentrations of herbicides differed among the four groups (GLMM-g, $\chi^2_3 = 27.9$, $p < 0.001$; Fig. 6C) and lower herbicide concentrations were detected in 2021 than in 2020 (GLMM-g, $\chi^2_1 = 17.5$, $p < 0.001$; Fig. 6C). The pattern followed the (bio)degradation hypothesis inasmuch as the concentration was highest in the trophic level at which the herbicides were aimed (i.e., weeds; Fig. 6C). The highest concentration was detected in plants ($p < 0.001$), followed by that in rodents, which was not significantly different from that in soil ($p = 0.460$). The lowest concentration of herbicides was detected in spiders ($p < 0.006$; Fig. 6C).

In the annual crops, group significantly affected the concentration of herbicides (GLMM-g, $\chi^2_3 = 27.9$, $p < 0.001$; Fig. 6D). The pattern corresponds to the (bio)degradation hypothesis and the highest concentration was found in plants ($p < 0.014$), but it was only marginally higher than in rodents ($p = 0.062$). The lowest concentration of herbicides was in spiders ($p < 0.001$; Fig. 6D).

Fig. 5 Comparing **A, B** overall concentration and **C, D** mean concentration per pesticide among four groups representing four trophic levels (soil: base resource, plants: primary producers, herbivorous rodents: herbivores, and spiders: predators) in **A, C** perennial and **B, D** annual crops. The points are estimated means and the whiskers show CI 95%. Different lower-case letters indicate statistically significant differences.



In the perennial crops, group and year interactively affected the concentration of fungicides but during both years the pattern can support the bioaccumulation hypothesis (GLMM-g, $\chi^2_3 = 12.9$, $p = 0.005$; Fig. 6E). In 2020, the highest concentration of fungicides was in spiders ($p < 0.001$; Fig. 6E), followed by soil ($p = 0.010$). The lowest concentration was detected in plants, while concentration of fungicides in rodents was between that in soil and plants ($p > 0.153$). In 2021, the highest concentration was in rodents and spiders ($p < 0.035$), between which the concentrations did not differ significantly ($p = 0.907$). The concentration of fungicides was a little bit lower in plants than in soil, but this difference was not significant ($p = 0.522$).

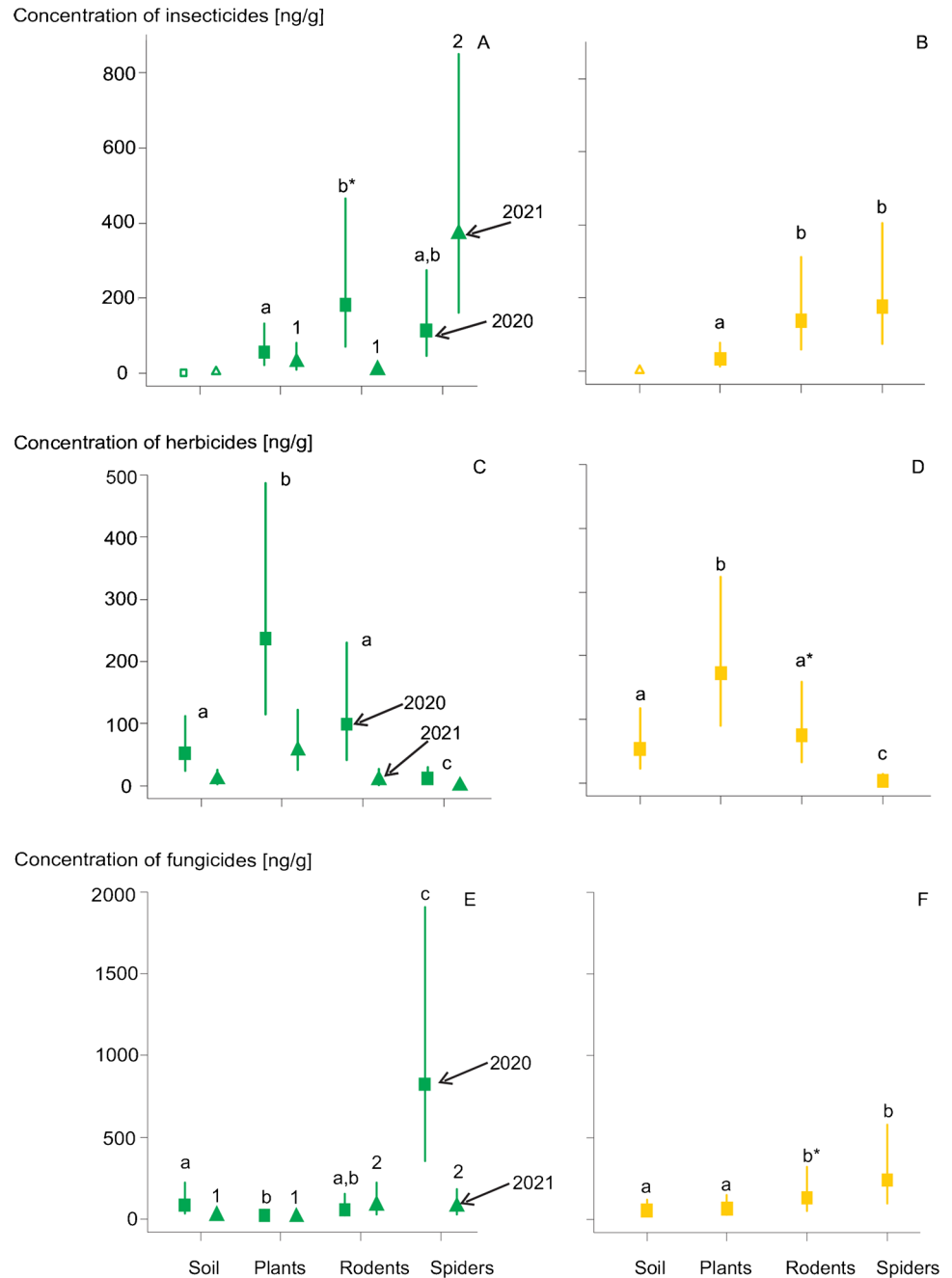
The concentrations of fungicides differed among the groups in annual crops (GLMM-g, $\chi^2_3 = 11.0$, $p = 0.012$; Fig. 6F), and the pattern supports the bioaccumulation hypothesis inasmuch as the concentration linearly increased with trophic level (Fig. 6F). The highest concentration was detected in spiders ($p < 0.010$) but it was not significantly different from that in rodents ($p = 0.226$). The lowest concentration was detected in soil, which value did not differ significantly from that in plants ($p = 0.666$) and was marginally lower than in rodents ($p = 0.067$).

Our data are in agreement with those studies showing that both bioaccumulation (e.g., Coat et al. 2011) as well as (bio) degradation (e.g., Rodriguez-Cruz et al. 2006; Zabaleta et al. 2018) of pesticides can affect distribution of pesticides in food webs. Nevertheless, inasmuch as the overall concentration of pesticides generally increased with trophic level, the process of bioaccumulation overrode the (bio)degradation process. Our results also agree with the studies showing a positive relationship between trophic level and concentration of pesticides (e.g., Coat et al. 2011; Verhaert et al. 2017; Fritsch et al. 2022).

The differences between years in patterns of insecticides concentration in the perennial crops might be caused by differences in weather conditions that are known to affect bioaccumulation potential of pesticides (Li 2020a, b). The bioaccumulation of pesticides seems to be stronger in warm and dry weather, at least in plants and vertebrate herbivores (Li 2020a, b). Indeed, the year 2020, when the concentration and diversity of pesticides were higher, was warmer and drier than was 2021 (Czech Hydrometeorological Institute 2023).

An explanation for the high concentrations of insecticides and fungicides in the herbivorous rodents is relatively

Fig. 6 Comparing concentrations of **A, B** insecticides, **C, D** herbicides, and **E, F** fungicides among four groups representing four trophic levels (soil: base resource, plants: primary producers, herbivorous rodents: herbivores, and spiders: predators) in **A, C, E** perennial and **B, D, F** annual crops. The points are estimated means and the whiskers show CI 95%. Different lower-case letters indicate statistical differences among groups within **A, C, E** a year or **B, D, F** among groups. The asterisk indicates only a marginal difference.



straightforward, and it most likely was because of their consuming plants with pesticide residues. The high concentration of insecticides in spiders is likely because herbivorous insects, the target of insecticides, comprise their dominant prey (Michalko and Pekár 2016). The most frequently detected insecticide in spiders, thiacloprid, is a neonicotinoid and is used against a variety of sap-feeding insects that constitute the dominant part of spiders' diets in agroecosystems (Birkhofer et al. 2022). Thiacloprid has been reported to have lethal (Pekár et al. 2021) as well as various

negative sublethal effects on spiders (Řezáč et al. 2019). The high concentration of fungicides in spiders is likely because spiders in agroecosystems utilize collembolans and other detritus channel-based animals (Roubinet et al. 2017; Birkhofer et al. 2022) that are often fungivorous (Potapov et al. 2022). Although nothing is known about the direct effects of the frequently detected fungicides (pyrimetostrobin, difenconazole, boscalid) on spiders, laboratory (Pekár 2012) and field studies (Michalko and Košulič 2020; Pekár et al. 2021) indicate negative effects of related fungicides on spiders.

3.5 Implications for sustainable development and further studies

Our data empirically show that the documented negative impacts of pesticides on biodiversity in agroecosystems (e.g., Raven and Wagner 2021; Bakker et al. 2022) can be attributed not only to indirect effects but to some extent also to direct effects of pesticides on non-target organisms. This is especially the case when highly toxic substances that were long ago prohibited continue to be detected in food webs, probably due to ongoing illegal application. The direct effects can be due to lethal and various sublethal effects on physiology and behavior affecting fitness of organisms (Stark and Banks 2003; Desneux et al. 2007).

The highest concentration of pesticides in spiders does not automatically mean that spiders constitute the most endangered group of organisms. Nevertheless, spiders are often more susceptible to direct pesticide effects than are the target organisms (Pekár 2012). Pesticides' toxicity depends on organisms' physiology, and organisms may be tolerant to high concentrations of pesticides, for example because they deposit the toxic compounds in tissues serving for waste deposition (Drouillard 2008). Moreover, each studied group contained a different mix of pesticides that can have different impacts on the fitness of individuals and populations due to additive and synergistic effects (Siviter et al. 2021). Nevertheless, due to the high concentration of pesticides in spiders and because they represent important prey for other predators such as lizards, birds, bats, and insectivores (Pekár and Raspotnig 2022), spiders expose their predators to a high dietary dosage of pesticides.

Although some pesticides were shared by the studied groups, the composition of pesticides differed among the groups, meaning that the groups incorporated the pesticides from the same as well as different sources even though there are clear connections among the groups, except between spiders and rodents. Moreover, the concentration and diversity of pesticides in the studied food webs were affected by organisms' trophic group and mobility. Our results therefore provide direct empirical evidence for the argument (Brühl et al. 2021; Topping et al. 2020) that we need to study distribution of multiple pesticides in food webs rather than to study only the fate of one or a few preselected pesticides in a few model species. Only such a comprehensive approach will enable us to fully understand the impacts of pesticides on biodiversity and develop effective principles of ecological risk assessment (Brühl and Zaller 2019; Topping et al. 2020).

The fact that the pesticides applied by farmers represented only a small part of the overall spectrum of pesticides detected shows that pesticides enter local food webs in crops from surrounding landscapes. Further studies should therefore address also how landscape characteristics affect

pesticides' permeation of local food webs not only in habitats but also in crops.

3.6 Study limitations

The fact that spiders do not prey on rodents in Central Europe constitutes a certain limitation for our study because it includes no clear link between herbivorous prey and predators. Nevertheless, inasmuch as the rodents and spiders are not phylogenetically related and spiders do not consume rodents, the results show a general pattern across trophic levels and not a specific pattern within a trophic module. Ideally, further studies should combine herbivores and predators in ways that would provide both clear trophic links but also no such links so that pathways of pesticides could be clearly demonstrated but general patterns also could be tested.

Another limitation is that pesticides in plants and spiders were detected from whole individuals while pesticides in rodents were detected from livers and kidneys. Organs within an organism can differ in their concentrations of pesticides (Drouillard 2008). This is a common limitation with other studies that, for example, investigate food webs in lakes using invertebrate and fish species. This limitation will be difficult to overcome also in future studies, as it is basically impossible to extract organs from small-bodied arthropods to obtain amounts sufficient for chemical analyses. On the other hand, homogenization of whole vertebrate individuals also is not plausible due to the presence of hard tissues.

It also could be argued that sampling during the main part of the farming season can be affected by fresh applications that are followed by material collecting and/or by cumulative effects of residues at the end of the farming season (Brühl et al. 2021). We principally aimed in our multiple residue analyses, which required that large amounts of fresh biomass be collected across the whole season, to see the bioaccumulation patterns in meta food webs of the agricultural landscape instead of to observe acute residue effects after spraying. We also minimized the effect of fresh residues by suitably timing our sampling dates to be well after pesticide applications in crops during the whole season.

4 Conclusion

Our study is the first to investigate the distribution of multiple pesticides in food webs of perennial and annual crops using a wider spectrum of organisms as is necessary for the sustainable development of modern ecological risk assessment practices (Brühl and Zaller 2019; Jacquet et al. 2022). We detected a broad spectrum of pesticides applied in perennial and annual crops in the Czech Republic, but the pesticides applied by farmers in recent years represented only a minor proportion of

all those pesticides detected. Some of the detected pesticides had long ago been banned by the European Union for their high toxicity, and some had not yet been even registered in European and/or U.S. markets, which is highly alarming. The large proportion of those pesticides detected that had not been applied by the farmers indicates that pesticides had entered the local food webs from surrounding landscapes, which is in accordance with our landscape hypothesis.

The diversity of pesticides was driven by a combination of organism/entity mobility and of crop type, which agrees with our mobility and habitat hypotheses. The concentration of pesticides, on the other hand, was driven by bioaccumulation and (bio)degradation processes. However, bioaccumulation prevailed over (bio)degradation and, consequently, the overall concentration of pesticides increased with trophic level, which is in accordance with our bioaccumulation hypothesis. Overall, we show for the first time that the distribution of pesticides in agroecosystem food webs depends on mobility and trophic group of organisms, crop type (i.e., annual vs. perennial), and probably also on landscape characteristics. The study therefore provides a base for further studies investigating those factors affecting pesticide distribution in the environment, and we argue that we need to combine a wide range of approaches from analytical chemistry, ecotoxicology, and ecology, fields which to date have worked largely in isolation from one another.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13593-024-00950-y>.

Acknowledgments The authors would like to express their gratitude to Tomáš Hamřík, Martin Ernst, and Petra Hulejová for their valuable assistance during fieldwork and laboratory processing of samples. We extend our heartfelt thanks to Michal Bartoš, Jiří Kwiecien, and Eva Eglyová from the Research Institute for Organic Synthesis in Rybitví and Zuzana Bílková from Masaryk University in Brno for their insightful discussions on chemical analyses of pesticide residues. Special appreciation goes to the farmers and stakeholders who generously allowed us to conduct research on their properties; their cooperation, which included providing information on their pesticide application within the last years, was crucial to the success of this study. Additionally, we appreciate the journal editors and the two anonymous reviewers for their constructive comments and valuable suggestions on the manuscript. Furthermore, we wish to acknowledge Katerina Sam for her comments on the initial version of manuscript, and we are grateful for the English proofreading assistance provided by Gale A. Kirking.

Authors' contributions RM, OK, and LP contributed to the study conception and design. Material sampling was performed by LP and OK. Statistical analyses were performed by RM. First draft of manuscript was written by RM, second draft was supplemented and reviewed by OK and JH. All authors read and approved the final version of manuscript.

Funding Open access publishing supported by the National Technical Library in Prague. The study was financially supported by the Technology Agency of the Czech Republic, program BETA2 (TITSMZP833).

Data availability The dataset generated during the current study is available at Zenodo data repository (Michalko et al. 2023).

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Abt KF, Bock WF (1998) Seasonal variations of diet composition in farmland field mice *Apodemus* spp. and bank voles *Clethrionomys glareolus*. *Acta Theriol* 43:379–389. <https://doi.org/10.4098/AT.arch.98-49>
- Bakker L, van der Werf W, Bianchi FJ (2022) Sweep netting samples, but not sticky trap samples, indicate beneficial arthropod abundance is negatively associated with landscape wide insecticide use. *J Appl Ecol* 59:942–952. <https://doi.org/10.1111/1365-2664.14106>
- Bernhardt ES, Rosi EJ, Gessner MO (2017) Synthetic chemicals as agents of global change. *Front Ecol Environ* 15:84–90. <https://doi.org/10.1002/fee.1450>
- Birkhofer K, Djoudi EA, Schnerch B, Michalko R (2022) Climatic conditions and functional traits affect spider diets in agricultural and non-agricultural habitats worldwide. *Ecography* 2022:e06090. <https://doi.org/10.1111/ecog.06090>
- Birkhofer K, Entling MH, Lubin Y (2013) Trait composition, spatial relationships, trophic interactions. *Spider Research in the 21st Century: Trends and Perspectives* (D Penney ed). Siri Scientific Press, Rochdale, United Kingdom, 200–229. ISBN 978-0-9574530-1-2
- Blandenier G (2009) Ballooning of spiders (Araneae) in Switzerland: general results from an eleven-year survey. *Arachnology* 14:308–316. <https://doi.org/10.13156/arac.2009.14.7.308>
- Brooks ME, Kristensen K, Van Benthem KJ, Magnusson A, Berg CW, Nielsen A et al (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J* 9:378–400. <https://doi.org/10.3929/ethz-b-000240890>
- Brühl CA, Bakanov N, Köthe S, Eichler L, Sorg M, Hörren T et al (2021) Direct pesticide exposure of insects in nature conservation areas in Germany. *Sci Rep* 11:1–10. <https://doi.org/10.1038/s41598-021-03366-w>

- Brühl CA, Zaller JG (2019) Biodiversity decline as a consequence of an inappropriate environmental risk assessment of pesticides. *Front Environ Sci* 177. <https://doi.org/10.3389/fenvs.2019.00177>
- Buchar J, Růžička V (2002). Catalogue of spiders of the Czech Republic. Prague: Peres publishers. ISBN 80-86360-25-3
- Central Institute for Supervising and Testing in Agriculture (2023) Registration database of plant protection products. Ministry of Agriculture (Czech Republic). <https://eagri.cz/public/app/eagri/app/POR/>. Accessed 15 Apr 2023
- Coat S, Monti D, Legendre P, Bouchon C, Massat F, Lepoint G (2011) Organochlorine pollution in tropical rivers (Guadeloupe): role of ecological factors in food web bioaccumulation. *Environ Pollut* 159:1692–1701. <https://doi.org/10.1016/j.envpol.2011.02.036>
- Colwell RK (2019) EstimateS ver. 9.1.0. statistical estimation of species richness and shared species from samples. Available: <https://www.robertkcolwell.org/pages/1407-estimates>. Accessed 20.1.2023
- Czech Hydrometeorological Institute (2023) <https://www.chmi.cz>. Accessed: 12.2.2023
- Desneux N, Decourtye A, Delpuech JM (2007) The sublethal effects of pesticides on beneficial arthropods. *Annu Rev Entomol* 52:81–106. <https://doi.org/10.1146/annurev.ento.52.110405.091440>
- Drouillard KG (2008) Biomagnification. In Jorgensen SE and Fath BD (eds) *Encyclopaedia of Ecology*. Elsevier, Amsterdam, NE. ISBN 978-0-080-45405-4
- Duke SO (2018) The history and current status of glyphosate. *Pest Manag Sci* 74:1027–1034. <https://doi.org/10.1002/ps.4652>
- Foelix RF (2011) *Biology of spiders*, 3rd ed. Oxford University Press, NY, USA. ISBN 978-0-19-973482-5
- Food and Agriculture Organization of the United Nations (2021) <http://www.fao.org/home/en/>. Accessed 10 Jan 2023
- Fritsch C, Appenzeller B, Burkart L, Coeurdassier M, Scheiffer R, Raoul F et al (2022) Pervasive exposure of wild small mammals to legacy and currently used pesticide mixtures in arable landscapes. *Sci Rep* 12:15904. <https://doi.org/10.1038/s41598-022-19959-y>
- Goutte A, Alliot F, Budzinski H, Simonnet-Laprade C, Santos R, Lachaux V et al (2020) Trophic transfer of micropollutants and their metabolites in an urban riverine food web. *Environ Sci Technol* 54:8043–8050. <https://doi.org/10.1021/acs.est.0c01411>
- Huang C, Zhu H, Yu Y, Ding Q, Jia F, Zhao B et al (2017) Simultaneous determination of pyrametostrobin and its two metabolites in cucumber and soil using a quick, easy, cheap, effective, rugged, and safe method with HPLC–MS/MS. *J Sep Sci* 40:2335–2343. <https://doi.org/10.1002/jssc.201700109>
- Jacob J, Manson P, Barfknecht R, Fredricks T (2014) Common vole (*Microtus arvalis*) ecology and management: implications for risk assessment of plant protection products. *Pest Manag Sci* 70:869–878. <https://doi.org/10.1002/ps.3695>
- Jacquet F, Jeuffroy MH, Jouan J, Le Cadre E, Litrico I, Malausa T, Reboud X, Huyghe C (2022) Pesticide-free agriculture as a new paradigm for research. *Agron Sustain Dev* 42:8. <https://doi.org/10.1007/s13593-021-00742-8>
- Knapp M, Štrobl M, Ventura A, Seidl M, Jakubíková L, Tajovský K et al (2022) Importance of grassy and forest non-crop habitat islands for overwintering of ground-dwelling arthropods in agricultural landscapes: a multi-taxa approach. *Biol Conserv* 275:109757. <https://doi.org/10.1016/j.biocon.2022.109757>
- Knapp JL, Nicholson CC, Jonsson O, de Miranda JR, Rundlöf M (2023) Ecological traits interact with landscape context to determine bees' pesticide risk. *Nat Ecol Evol* 7:547–556. <https://doi.org/10.1038/s41559-023-01990-5>
- Kosubová P, Škulcová L, Poláková Š, Hofman J, Bielská L (2020) Spatial and temporal distribution of the currently-used and recently-banned pesticides in arable soils of the Czech Republic. *Chemosphere* 254:126902. <https://doi.org/10.1016/j.chemosphere.2020.126902>
- Li Z (2020a) Spatiotemporal pattern models for bioaccumulation of pesticides in common herbaceous and woody plants. *J Environ Manag* 276:111334. <https://doi.org/10.1016/j.jenvman.2020.111334>
- Li Z (2020b) Spatiotemporal pattern models for bioaccumulation of pesticides in herbivores: an approximation theory for North American white-tailed deer. *Sci Total Environ* 737:140271. <https://doi.org/10.1016/j.scitotenv.2020.140271>
- Li Y, Miao R, Khanna M (2020) Neonicotinoids and decline in bird biodiversity in the United States. *Nat Sustain* 3:1027–1035. <https://doi.org/10.1038/s41893-020-0582-x>
- Mei M, Du ZX, Chen Y (2011) QuEChERS-ultra performance liquid chromatography-tandem mass spectrometry for determination of 5 currently used herbicides. *Chin J Anal Chem* 39:1659–1664. [https://doi.org/10.1016/S1872-2040\(10\)60482-3](https://doi.org/10.1016/S1872-2040(10)60482-3)
- Mestre L, Schirmel J, Hetz J, Kolb S, Pfister SC, Amato M et al (2018) Both woody and herbaceous semi-natural habitats are essential for spider overwintering in European farmland. *Agric Ecosyst Environ* 267:141–146. <https://doi.org/10.1016/j.agee.2018.08.018>
- Michalko K, Birkhofer K (2021) Habitat niches suggest that non-crop habitat types differ in quality as source habitats for Central European agrobiont spiders. *Agric Ecosyst Environ* 308:107248. <https://doi.org/10.1016/j.agee.2020.107248>
- Michalko R, Košulič O (2020) The management type used in plum orchards alters the functional community structure of arthropod predators. *Int J Pest Manag* 66:173–181. <https://doi.org/10.1080/09670874.2019.1601292>
- Michalko R, Pekár S (2016) Different hunting strategies of generalist predators result in functional differences. *Oecologia* 181:1187–1197. <https://doi.org/10.1007/s00442-016-3631-4>
- Michalko R, Gajski D, Košulič O, Khum W, Michálek O, Pekár S (2022) Association between arthropod densities suggests dominance of top-down control of predator-prey food-webs on pear trees during winter. *Food Webs* 33:e00261. <https://doi.org/10.1016/j.fooweb.2022.e00261>
- Michalko R, Purchart L, Hofman J, Košulič O (2023) Supplementary Material related to: Distribution of pesticides in agroecosystem food webs differ among trophic groups and between annual and perennial crops. Zenodo. <https://doi.org/10.5281/zenodo.10254628>
- Novotný L, Misík J, Honzlová A, Ondráček P, Kuča K, Vávra O, Rachač V, Chloupek P (2011) Incidental poisoning of animals by carbamates in the Czech Republic. *J Appl Biomed* 9:157–161. <https://doi.org/10.2478/v10136-009-0035-3>
- Pekár S (2012) Spiders (Araneae) in the pesticide world: an ecotoxicological review. *Pest Manag Sci* 68:1438–1446. <https://doi.org/10.1002/ps.3397>
- Pekár S, Raspotnig G (2022) Defences of Arachnids: diversified arsenal used against range of enemies. *Entomol Gen* 42:663–679. <https://doi.org/10.1127/entomologia/2022/1531>
- Pekár S, Rezáč M, Kocourek F, Stará J (2021) Effect of three pest-control management systems on abundance, species richness, and selected traits of spiders (Araneae) assemblage in apple orchard. *Int J Pest Manag*:1–13. <https://doi.org/10.1080/09670874.2021.1969471>
- Pose-Juan E, Sanchez-Martin MJ, Soledad Andrades M, Sonia Rodriguez-Cruz M, Herrero-Hernandez E (2015) Pesticide residues in vineyard soils from Spain: spatial and temporal distributions. *Sci Total Environ* 514:351–358. <https://doi.org/10.1016/j.scitotenv.2015.01.076>
- Potapov AM, Beaulieu F, Birkhofer K, Bluhm SL, Degtyarev MI, Devetter M, Scheu S et al (2022) Feeding habits and multifunctional classification of soil-associated consumers from protists to vertebrates. *Biol Rev* 97:1057–1117. <https://doi.org/10.1111/brv.12832>

- Qi SY, Xu XL, Ma WZ, Deng SL, Lian ZX, Yu K (2022) Effects of organochlorine pesticide residues in maternal body on infants. *Front Endocrinol* 13:890307. <https://doi.org/10.3389/fendo.2022.890307>
- R Development Core Team (2023) R. A language and environment for statistical computing. Available: R Foundation for Statistical Computing, Vienna. <http://www.R-project.org/> Accessed 1 Jan 2023
- Raven PH, Wagner DL (2021) Agricultural intensification and climate change are rapidly decreasing insect biodiversity. *PNAS* 118:e2002548117. <https://doi.org/10.1073/pnas.2002548117>
- Řezáč M, Řezáčová V, Heneberg P (2019) Contact application of neonicotinoids suppresses the predation rate in different densities of prey and induces paralysis of common farmland spiders. *Sci Rep* 9:1–9. <https://doi.org/10.1038/s41598-019-42258-y>
- Rodríguez-Cruz MS, Jones JE, Bending GD (2006) Field-scale study of the variability in pesticide biodegradation with soil depth and its relationship with soil characteristics. *Soil Biol Biochem* 38:2910–2918. <https://doi.org/10.1016/j.soilbio.2006.04.051>
- Roubinet E, Birkhofer K, Malsher G, Staudacher K, Ekbom B, Traugott M, Jonsson M (2017) Diet of generalist predators reflects effects of cropping period and farming system on extra- and intraguild prey. *Ecol Appl* 27:1167–1177. <https://doi.org/10.1002/eap.1510>
- Sabzevari S, Hofman J (2022) A worldwide review of currently used pesticides' monitoring in agricultural soils. *Sci Total Environ* 812:152344. <https://doi.org/10.1016/j.scitotenv.2021.152344>
- Safford RJ, Jones CG (1997) Did organochlorine pesticide use cause declines in Mauritian forest birds? *Biodivers Conserv* 6:1445–1451. <https://doi.org/10.1023/A:1018350016327>
- Samu F, Szinetár C (2002) On the nature of agrobionts spiders. *J Arachnol* 30:389–402. <https://doi.org/10.1636/0161-8202>
- Samu F, Matthews GA, Lake D, Vollrath F (1992) Spider webs are efficient collectors of agrochemical spray. *Pest Manag Sci* 36:47–51. <https://doi.org/10.1002/ps.2780360108>
- Sánchez-Bayo F, Wyckhuys KA (2019) Worldwide decline of the entomofauna: a review of its drivers. *Biol Conserv* 232:8–27. <https://doi.org/10.1016/j.biocon.2019.01.020>
- Sharma RK, Jaiswal SK, Siddiqi NJ, Sharma B (2012) Effect of carbofuran on some biochemical indices of human erythrocytes in vitro. *Cell Mol Biol* 58:103–109. <https://doi.org/10.1170/T927>
- Shelton JF, Geraghty EM, Tancredi DJ, Delwiche LD, Schmidt RJ, Ritz B et al (2014) Neurodevelopmental disorders and prenatal residential proximity to agricultural pesticides: the CHARGE study. *Environ Health Perspect* 122:1103–1109. <https://doi.org/10.1289/ehp.1307044>
- Siviter H, Bailes EJ, Martin CD, Oliver TR, Koricheva J, Leadbeater E, Brown MJ (2021) Agrochemicals interact synergistically to increase bee mortality. *Nature* 596:389–392. <https://doi.org/10.1038/s41586-021-03787-7>
- Stark JD, Banks JE (2003) Population-level effects of pesticides and other toxicants on arthropods. *Annu Rev Entomol* 48:505–519. <https://doi.org/10.1146/annurev.ento.48.091801.112621>
- Suchomel J, Purchart L, Čepelka L (2012) Structure and diversity of small-mammal communities of lowland forests in the rural central European landscape. *Eur J for Res* 131:1933–1941. <https://doi.org/10.1007/s10342-012-0644-3>
- Svobodová M, Šmídová K, Hvězdová M, Hofman J (2018) Uptake kinetics of pesticides chlorpyrifos and tebuconazole in the earthworm *Eisenia andrei* in two different soils. *Environ Pollut* 236:257–264. <https://doi.org/10.1016/j.envpol.2018.01.082>
- Tongo I, Onokpasa A, Emerure F, Balogun PT, Enuneku AA, Erhunmwunse N et al. (2022) Levels, bioaccumulation and biomagnification of pesticide residues in a tropical freshwater food web. *Int J Environ Sci Technol*:1–16. <https://doi.org/10.1007/s13762-021-03212-6>
- Topping CJ, Aldrich A, Berny P (2020) Overhaul environmental risk assessment for pesticides. *Science* 367:360–363. <https://doi.org/10.1126/science.aay1144>
- Verhaert V, Newmark N, D'Hollander W, Covaci A, Vlok W, Wepener V et al (2017) Persistent organic pollutants in the Olifants River Basin, South Africa: bioaccumulation and trophic transfer through a subtropical aquatic food web. *Sci Total Environ* 586:792–806. <https://doi.org/10.1016/j.scitotenv.2017.02.057>
- Vukicevic-Radic O, Matic R, Kataranovski D, Stamenkovic S (2006) Spatial organization and home range of *Apodemus flavicollis* and *A. agrarius* on Mt. Avala, Serbia. *Acta Zool Acad Sci Hung* 52:81–96
- Wang M (2013) From home range dynamics to population cycles: validation and realism of a common vole population model for pesticide risk assessment. *Integr Environ Assess Manag* 9:294–307. <https://doi.org/10.1002/ieam.1377>
- Yang XB, Ying GG, Kookana RS (2010) Rapid multiresidue determination for currently used pesticides in agricultural drainage waters and soils using gas chromatography-mass spectrometry. *J Environ Sci Health Part B* 45:152–161. <https://doi.org/10.1080/03601230903472165>
- Zabaleta I, Bizkarguenaga E, Nunoo DB, Schultes L, Leonel J, Prieto A et al (2018) Biodegradation and uptake of the pesticide sulfluramid in a soil–carrot mesocosm. *Environ Sci Technol* 52:2603–2611. <https://doi.org/10.1021/acs.est.7b03876>
- Zuur AF, Hilbe JM, Ieno EN (2015) A beginner's guide to GLM and GLMM with R. A frequentist and Bayesian perspective for ecologists. Highland Statistics Ltd. ISBN 978-0-9571741-3-9

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.