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Residues of currently used pesticides in soils and earthworms: A silent threat?

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A B S T R A C T

Critical knowledge gaps about environmental fate and unintentional effects of currently used pesticides (CUPs) hamper the understanding and mitigation of their global impacts on ecological processes. We investigated the exposure of earthworms to 31 multiclass CUPs in an arable landscape in France. We highlighted the presence of at least one pesticide in all soils (n = 180) and 92 % of earthworms (n = 155) both in treated crops and non-treated habitats (hedgerows, grasslands, and cereals under organic farming). Mixtures of at least one insecticide, one herbicide, and one fungicide (> limit of quantification) contaminated 90 % of soils and 54 % of earthworms at levels that could endanger these nontarget beneficial soil organisms. A high risk of chronic toxicity to earthworms was found (46 % of samples) both in treated winter cereals and nontreated habitats considered as refuges. This may alter biodiversity, hinder recovery, and impair ecosystem functions. These results provide essential insights for sustainable agriculture and CUP regulation, and highlight the potential of pesticides as agents of global change.

1. Introduction

Worldwide, the diversity and quantity per hectare of synthetic pesticides used are increasing, along with an increase in the area of treated surfaces (Bernhardt et al., 2017; DiBartolomeis et al., 2019; Hossard et al., 2017). Global pesticide use (in tons of active ingredients) increased by 80 % worldwide between 1990 (2 285 881 tons) and 2017 (4 113 591 tons) (FAOSTAT, 2019; Zhang et al., 2011), and the total sales of pesticides remained constant in Europe between 2011 and 2018, revealing that there was no reduction in reliance on pesticides (Environmental indicator report, 2018; Eurostat, 2020; FAOSTAT, 2019). Hundreds of thousands of formulated pesticides have been developed since the 1980s (Zhang et al., 2011), and 479 active ingredients are currently used in several thousands of commercial products in the European Union (European Commission, 2020). Consequently, and despite precautions to farmers to limit pesticide losses and efforts to reduce pesticide mobility within the environment, their application leads to unavoidable transfer by spray drift, volatilization, infiltration, and runoff from treated areas (Mottes et al., 2014). These processes potentially result in the contamination of air (Bedos et al., 2002), soil (Silva et al., 2019) and water (Gilliom, 2007) by currently used pesticides (CUPs), with serious concerns regarding their effects on the ecosystem

Abbreviations: CUPs, currently used pesticides; LC50, lethal concentration 50 %; LOD, limits of detection; LOQ, limits of quantification; NOEC, no observed effect concentration; PECs, predicted environmental concentrations in soils; RD, recommended dose; $TER_{earthworm}$, toxicity/exposure ratio for earthworms; ZA-PVS, Zone Atelier Plaine & Val de Sèvre.

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services provided by soils, water systems (Lautenbach et al., 2012) and wildlife (Brühl and Zaller, 2019; Geiger et al., 2010).

While water contamination by pesticides has been extensively studied for approximately 30 years (Gilliom, 2007; Hallberg, 1987), data on the contamination of soils by CUPs *in natura* are surprisingly scarce. However, the restoration or conservation of soils and their quality has been recognized as a key issue, considering the fundamental role of soils in the ecosystem and the economy (BIO Intelligence Service, 2014; EUR-Lex, 2006). Some recent data revealed the high occurrence of mixtures of CUPs in soils of arable fields directly treated with pesticides (Chiaia-Hernandez et al., 2017; Gamón et al., 2003; Hvězdová et al., 2018; Karasali et al., 2016; Marković et al., 2010; Silva et al., 2019; Suszter and Ambrus, 2017; Zhao et al., 2018). However, no information is available on the overall soil multiresidue contamination of farmland at the landscape scale (i.e., including both treated and nontreated areas), except for neonicotinod class (e.g., Main et al., 2020). Indeed, no data can be found on soil contamination by multiclass CUPs in off-field landscape elements corresponding to seminatural habitats (e.g., hedgerows, wooded patches, field margins) or nontreated organic fields. However, it is widely recognized that these habitats favor the presence of beneficial organisms in agricultural landscapes (Bengtsson et al., 2005; Geiger et al., 2010) by playing an important role as a refuge and source of recolonization following pesticide application (EFSA Scientific Committee, 2016). Consequently, when contaminated by pesticides, these nontreated habitats could act as ecological traps for organisms due to a mismatch between habitat attractiveness and quality.

Animals living in close contact with the soil can be directly exposed to pesticides and harmed. It was recently shown that the CUP concentrations in agricultural soils treated with pesticides exceeded the toxicological benchmarks for earthworms or other soil invertebrates in 35 % of the agricultural sites studied (Vašíčková et al., 2019). Earthworms play a key beneficial role in soil structure, functioning and productivity (Liu et al., 2019; van Groenigen et al., 2015) and are important prey for numerous predators (King et al., 2010). Earthworm abundance has been shown to increase when pesticide use decreases (Pelosi et al., 2013a) and to be lower in conventional than organic fields (Pelosi et al., 2015), although it is difficult to isolate the effects of pesticides, due to biotic and abiotic factors operating at the same time. However, there are no available data on the contamination of earthworms by multiclass CUPs *in natura* in either treated or nontreated habitats in arable landscapes. Such data would provide new insight into the pesticide bioaccumulation potential, likely unintentional effects of these chemicals on earthworm populations, and the risks of transfer to their predators.

In this study, we investigated the level of contamination by CUPs in soils and earthworms in treated and nontreated habitats of an intensive agricultural landscape. We checked whether multiclass residues of CUPs might be detected in soils and earthworms, including some compounds that are assumed to be weakly or moderately persistent in the environment, presenting low bioaccumulation potential and/or are used in limited amounts (at a low dose rate, or only on certain crops). We hypothesized that the contamination patterns of soils and earthworms would differ in the different habitats (grasslands, cereal fields, and hedgerows) and according to the agricultural management (treated *vs* nontreated habitats, organic *vs* conventional farming). We expected that the number and the concentrations of the pesticides would be higher in habitats that were treated by CUPs than in seminatural habitats and organic fields that are not directly targeted by pesticides. Based on the available data on the predicted environmental concentrations of pesticides in soils (PEC_s provided in risk assessment documents according to the European regulation) and toxic thresholds for earthworm reproduction (for each pesticide separately, and using a mixture approach based on concentration addition), we also assessed the risks to earthworms.

2. Methods

2.1. Sampling area and design

The sampling of soils and earthworms was conducted in Spring 2016 in the Long-Term Socio-Ecological Site Zone Atelier Plaine & Val de Sèvre (ZA-PVS (Bretagnolle et al., 2018); http://www.za.plainevalsevre.cnrs.fr/). Sixty landscapes of 1 km^2 were selected in which soil and earthworms were sampled in an arable field sown with winter cereals, a grassland and a hedgerow or woody patch edge (as close as possible to the cereal field), for a total of 180 sampling site locations (Table 1). Among the 52 cereal fields where earthworms were sampled, 44, 6, and 2 were sown with winter wheat, winter barley, and einkorn, respectively. The farming practices in the organic cereal fields and grasslands respected the rules of the AB France label and were under organic farming for at least 3 years at the time of sampling. A total of 180 soils and 155 earthworms were therefore analyzed to determine pesticide concentrations (Table 1).

2.2. Collection of soils and earthworms

In each plot, regardless of the size of the sampled habitats (i.e., winter cereal fields, grasslands, hedgerows), three subsamples (0–5 cm depth; Amelung et al., 2007; de Geronimo et al., 2015) were taken using a 5 cm Ø soil auger. They were then combined to obtain one composite sample per site. The depth of 5 cm was chosen because the soils in the sampling area were shallow and rocky, sometimes not allowing to sample at more than 5 cm depth. Moreover, the studied earthworm *A. chlorotica* is an epi-endogeic species that is commonly found in the top 5 cm of the soil (Pelosi et al., 2013a; Le Couteulx et al., 2015). The soils were frozen at −20 °C before being analyzed.

Soil properties were measured at the Laboratoire d'Analyse des Sols of the Institut National de la Recherche Agronomique (Arras, France), which benefits from the COFRAC (French accreditation committee) accreditation of its analytical quality regarding soil characteristics. Briefly, soils were dried at room temperature and then disaggregated and homogenized before being sieved at 2 mm. The following soil characteristics were measured: pH (by water suspension), organic matter and nitrogen contents (by dry combustion, in g kg^{-1}), grain size distribution (clay < 2 μm, silt 2−20 μm, and sand > 20 μm, in g kg^{-1}), total calcium carbonate $CaCO_3$ (in g kg^{-1}), and total phosphorus P2O5 (by ICP-MS spectrometry, in g kg^{-1}).

We focused on the epi-endogeic earthworm species *Allolobophora chlorotica* which is well represented in the different sampled landscape habitats in the ZA PVS. Because pesticides generally accumulate at the soil surface, species living in contact with the soil surface will potentially be more strongly affected than those living deeper (Pelosi et al., 2013a). Regardless of the size of the sampled habitats (i.e., winter cereal fields, grasslands, hedgerows), earthworms were searched for 15−30 min at each site location by superficially digging the soil, allowing to find between 0 and 10 *A. chlorotica* adult individuals. In 25 out of the 180 sampling site locations, *A. chlorotica* could not be found. Before being weighed and frozen at −80 °C, earthworms were individually placed in petri dishes on damp filter paper for 48 h to void their gut contents.

2.3. Analytics for residues of pesticides

The analyzed pesticides (Table S1) were selected based on analytical capabilities as well as their frequency and amount of application over the sampling area recorded in surveys of farmers over the last 5 years before sampling. Thirty-one pesticides (9 insecticides, 10 fungicides, and 12 herbicides, see Tables 2 and 3) were studied, 29 of which were still registered and used at the time of sampling, while 2 were recently banned pesticides (acetochlor and bifenthrine, banned in 2013). They were all referred to as Currently Used Pesticides (CUPs) in this study. For analytical reasons or because they were applied after the sampling date,

Table 1
Number of treated and untreated sampling site locations for soils and earthworms (*Allolobophora chlorotica*). OF means organic farming.

	Soils			Earthworms		
	Cereal crops	Grasslands	Hedgerows	Cereal crops	Grasslands	Hedgerows
Treated	53	34	0	45	30	0
Untreated	7	11 in OF 15 permanent	60	7	10 in OF 12 permanent	51
Total	60	60	60	52	52	51

Table 2
Concentrations of the 31 pesticides in the 180 soils, ordered by decreasing numbers of detections. nd for not detected. OF for organic farming. Recommended doses for cereals *or other crops* (including potential multiapplications) based on e-phy database (https://ephy.anses.fr). For more detail, see Table S1.

Rank	Name	Type	Recommended dose (ng g^{-1})	Number of detected samples	Concentration max (ng g^{-1})	Median concentration by habitat (ng g^{-1})				
						Cereal crops		Grasslands		Hedgerows
						Conventionnal	OF	Conventionnal	OF	
1	Diflufenican	Herbicide	250	162	1360.7	**137.3**	nd	**0.8**	**0.4**	**2.3**
2	Imidacloprid	Insecticide	168	160	160.0	**15.1**	**0.9**	**0.4**	**0.3**	**1.8**
3	Boscalid	Fungicide	467	155	1211.9	**4.7**	**2.0**	**0.7**	**0.3**	**3.2**
4	Epoxiconazole	Fungicide	153	145	283.0	**34.6**	**4.9**	**1.1**	**0.5**	**2.7**
5	Prochloraz	Fungicide	600	96	485.2	**0.6**	nd	**0.2**	nd	nd
6	Napropamide	Herbicide	*1680*	94	19.7	**0.2**	**0.1**	nd	nd	**0.1**
7	Cyproconazole	Fungicide	133	82	245.8	**0.3**	nd	nd	nd	nd
8	Metazachlor	Herbicide	*1333*	75	4.2	**0.2**	nd	nd	nd	nd
9	S-metolachlor	Herbicide	*2000*	65	8.3	nd	nd	nd	nd	nd
10	Metrafenone	Fungicide	200	61	187.1	**0.2**	nd	nd	nd	nd
11	Pendimethalin	Herbicide	1540	57	923.1	nd	nd	nd	nd	nd
12	Pyraclostrobin	Fungicide	221	56	53.9	**0.1**	nd	nd	nd	nd
13	Propiconazole	Fungicide	167	47	87.1	nd	nd	nd	nd	nd
14	Aclonifen	Herbicide	1200	41	34.5	nd	nd	nd	nd	nd
15	Clomazone	Herbicide	*159*	39	1.0	nd	nd	nd	nd	nd
16	Thiamethoxam	Insecticide	*53*	37	2.0	nd	nd	nd	nd	nd
17	Pirimicarb	Insecticide	334	35	1.4	nd	nd	nd	nd	nd
18	Metconazole	Fungicide	120	28	75.2	nd	nd	nd	nd	nd
19	Thiacloprid	Insecticide	83	25	1.4	nd	nd	nd	nd	nd
20	Fluoxastrobin	Fungicide	266	25	8.6	nd	nd	nd	nd	nd
21	Dimethachlor	Herbicide	*1000*	16	1.5	nd	nd	nd	nd	nd
22	Pyroxsulam	Herbicide	25	15	99.1	nd	nd	nd	nd	nd
23	Cloquintocet-mexyl	Herbicide safener	25	14	15.4	nd	nd	nd	nd	nd
24	Acetochlor	Herbicide	*2447*	12	48.8	nd	nd	nd	nd	nd
25	Cypermethrin	Insecticide	33	5	50.9	nd	nd	nd	nd	nd
26	Fenpropidin	Fungicide	1498	3	92.8	nd	nd	nd	nd	nd
27	Tau-fluvalinate	Insecticide	96	2	1.6	nd	nd	nd	nd	nd
28–31	Lambda-cyhalothrin, Bifenthrin, Deltamethrin, Cycloxydim : nd									

some pesticides had been applied over the sampling area but were not measured in this study such as e.g., glyphosate, prothioconazole, metaldehyde, florasulam, pinoxaden, picolinafen, or isoproturon. We also voluntarily limited the number of active substances in the analyses to keep low limits of detection (LOD) and quantification (LOQ).

The recommended dose (RD) of each active substance was calculated considering the commercial formulations currently used on cereal crops, penetration of 5 cm depth, and a soil bulk density of 1.5 (EFSA (European Food Safety Authority), 2017).

An analytical multiresidue method has been implemented and validated (Daniele et al., 2018) to measure 31 pesticides in soils and earthworms. As pesticides are sensitive to temperature, soil samples were air dried at room temperature in the dark during one night. The soil was sieved at 250 μm before extraction. The limits of detection (LOD) and quantification (LOQ) are provided in Table S1. Because LOD and LOQ values were different for each compound, we chose to always consider what was the LOQ for saying positive/negative. Briefly, a modified QuEChERS extraction approach was implemented for individual earthworms (aliquots of 250-mg wet weight, i.e., between 1 and 3 earthworm individuals) using water (6 mL), heptane (3 mL) and two successive extractions were performed with acetonitrile (5 mL), citrate salt and a PSA/C18 clean-up step, followed by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS). For soil analysis, QuEChERS extraction (citrate salt) was conducted for 2.5 g of dried and sieved soils using water (6 mL, containing 0.1 M EDTA), and two successive extractions were performed with 5 mL of acetonitrile in the presence of citrate buffer, followed by dispersive solid-phase extraction with a PSA/C18 phase. The extracts were analyzed by using LC–MS/MS. The instrumental performance and eventual carry-over have been controlled regularly by injecting quality control and analytical blank samples, respectively.

2.4. Risk assessment

The predicted environmental concentrations in soils (PECs) and acute (LC50) or chronic (NOEC reproduction) toxicity thresholds for earthworms (*Eisenia fetida*) were collected from evaluation reports provided according to European Directives regarding the registration of plant protection products under the authority of the "Health & consumer protection directorate-general of the European Commission" and the "European Food Safety Authority" (European Commission, 2003; European Parliament and Council of the European Union, 2009). Toxicity values were checked and updated if necessary based on information from the "Pesticide Properties DataBase" (http://sitem.herts.ac.uk/aeru/ppdb/index.htm). Alternatively, when the parameters of interest were not provided in these sources, other reports of risk assessments (e.g., postregistration, authority of national agencies) and scientific publications were searched.

Table 3
Concentrations of the 31 pesticides in the 155 earthworms (*A. chlorotica*), ordered by decreasing numbers of detections. nd for not detected. < LOQ lower than the limit of quantification.

Rank	Name	Type	Number of detected samples	Concentration max (ng g^{-1})	Median concentration by habitat (ng g^{-1})				
					Cereal crops		Grasslands		Hedgerows
					Conventionnal	OF	Conventionnal	OF	
1	Imidacloprid	Insecticide	122	777.0	**340**	**33.2**	**14.35**	nd	**35.3**
2	Diflufenican	Herbicide	97	3863.0	**68.6**	nd	**<LOQ**	nd	**1.9**
3	Cyproconazole	Fungicide	69	117.0	nd	nd	nd	nd	nd
4	Epoxiconazole	Fungicide	64	203.0	**10.3**	**<LOQ**	nd	nd	nd
5	Thiacloprid	Insecticide	53	42.1	nd	nd	nd	nd	nd
6	Prochloraz	Fungicide	33	1210.0	nd	nd	nd	nd	nd
7	Pendimethalin	Herbicide	24	10765.0	nd	nd	nd	nd	nd
8	Boscalid	Fungicide	20	19.8	nd	nd	nd	nd	nd
9	Propiconazole	Fungicide	18	212.0	nd	nd	nd	nd	nd
10	Metrafenone	Fungicide	17	37.0	nd	nd	nd	nd	nd
11	Pyroxsulam	Herbicide	11	470.0	nd	nd	nd	nd	nd
12	Napropamide	Herbicide	5	24.0	nd	nd	nd	nd	nd
13	Fenpropidin	Fungicide	3	11.8	nd	nd	nd	nd	nd
14	Pyraclostrobin	Fungicide	3	49.7	nd	nd	nd	nd	nd
15	S-metolachlor	Herbicide	2	2.6	nd	nd	nd	nd	nd
16	Metconazole	Fungicide	2	54.6	nd	nd	nd	nd	nd
17	Fluoxastrobin	Fungicide	2	3.7	nd	nd	nd	nd	nd
18	Metazachlor	Herbicide	1	<LOQ	nd	nd	nd	nd	nd
19–31	Pirimicarb, Lambda-cyhalothrin, Cypermethrin, Thiamethoxam, Bifenthrin, Tau-fluvalinate, Deltamethrin, Clomazone, Dimethachlor, Aclonifen, Acetochlor, Cycloxydim, Cloquintocet-mexyl : nd								

The PECs are concentrations expected in agricultural soils under worst case conditions in scenarios of authorized commercial use for each given compound, that are obtained from modeling and/or measured concentrations in trials. The measured concentrations in soils (MECs) are thus supposed to be equal to or lower than the maximum PECs. The values of PECs are used in risk assessment procedures to calculate the toxicity/exposure ratio, which is a crucial endpoint to determine whether a risk to organisms can arise from the use of the compound under allowed practices at recommended doses, and therefore determine the marketing authorization. We here used the PECs values provided in registration documents calculated for the same crop that studied in our dataset (i.e., wheat) when available or similar application scheme on other crops (e.g., general case cereals) at recommended application rates. In order to provide quantitative data about the general patterns of contamination with regards to expected levels in the environment, we compared MECs to PECs for each compound. Indeed, to get further insights into the ecotoxicological significance and the efficiency of risk assessment procedure, comparing MECs to PECs is a way to highlight whether levels of residues in soils occur at "trace levels" both in treated and nontreated plots with regards to potential risk and allowed practices. Since no data about time of application and detailed practices in each plot were available, several PECs values related to "worst cases" and used to calculate toxicity ratio for soil fauna, such as PECs initial after treatment, long term PECs and maximum PECs were considered. The fact that MECs can be higher than PECs in soils where compounds are used under normal scenario or where a compound might not have been applied at all is an important result to enlight the spatial patterns of pesticide contamination in terrestrial environment.

A single-pesticide approach was applied first using the toxicity/exposure ratio for earthworms ($TER_{earthworm}$). This approach follows the risk assessment method for pesticide regulation defined by European legislation and has been used in recent scientific studies (e.g., Vašíčková et al., 2019). The value of $TER_{earthworm}$ was calculated for each soil sample as the ratio between the values of LC50 or NOEC divided by the measured soil concentrations above the limits of detection for each CUP individually. When thresholds were provided as “greater than” values, the given benchmarks were used in the calculations. The risk was considered negligible when the TERearthworm values were above a trigger limit of 10 for acute toxicity and of 5 for chronic toxicity following European regulations. As an example, considering epoxiconazole, the TER for acute toxicity and chronic toxicity were calculated for each sample as a LC50 of 62,500 ng g^{-1} and a NOEC of 84 ng g^{-1}, respectively, divided by epoxiconazole concentration in soil. In case the acute toxicity TER was higher than 10, the risk was considered negligible, which was the case for all samples. In case the chronic toxicity TER was higher than 5, the risk is considered negligible, which was not the case for 52 soil samples in which the calculated TER value was under or equal to this trigger of 5.

Then, a mixture approach was applied to assess the risks related to the presence of several residues in the samples. The risk quotient (RQ), as primarily used by the United States Environmental Protection Agency (2017); Vašíčková et al., 2019, was computed for each single CUP as the ratio between the measured environmental concentrations (when above the limits of detection) divided by predicted no effect concentration (PNEC) for each soil sample. The PNEC values were computed as the most susceptible endpoint, i.e., the NOEC or, if not available, the LC50, divided by the recommended assessment factors (AF). Assessment factors were derived from the instructions of the Environmental Risk Assessment Guidance (European Commission, 2003) using 1000 for the LC50 (AF for short-term toxicity test) and 10 for the NOEC (AF for long-term toxicity tests; since we focused on earthworms, the application of the criteria related to the number of trophic levels of the targets was not performed).

Finally, an additional approach was applied as recommended to assess the multiple toxicity of several pesticides in the guidelines of the European Food Safety Authority for risk assessment for birds and mammals (European Food Safety Authority (EFSA), 2009). Despite some drawbacks of such a use of the concentration addition concept (e.g., synergistic effects are not considered), no alternative reliable and validated method is available or routinely applied. The addition concept is broadly accepted by authorities around the world and used in scientific publications (e.g., Vašíčková et al., 2019). The individual RQ values for every CUP were summed ($\sum RQ$) for each soil sample. Finally, the $\sum RQ$ values were classified into four categories: high risk ($\sum RQ \geq 1$), medium risk ($0.1 \leq \sum RQ < 1$), low risk ($0.01 \leq \sum RQ < 0.1$) and negligible risk ($\sum RQ \leq 0.01$) (United States Environmental Protection Agency, 2017; Vašíčková et al., 2019).

2.5. Statistics

When a pesticide was not detected in a sample (value < LOD), the concentration value was set at 0 when necessary for statistical method application. When a pesticide was detected at a level below the LOQ but above the LOD, the LOD value was attributed.

ANOVA (or the Kruskal-Wallis test, when assumptions regarding the normality and homoscedasticity of variances were not respected) was used to assess the differences in earthworm and soil pesticide variables (i.e., number of pesticides and concentrations) between the three habitats (i.e., cereal fields, grasslands, hedgerows). The *t*-test (or the Wilcoxon test when assumptions regarding the normality and homoscedasticity of variances were not respected) was used to assess the differences in earthworm and soil pesticide variables between the two modalities of pesticide use (treated/nontreated). For the differences between conventional and organic fields and grasslands (T-test or Wilcoxon test), the data from the hedgerows (or woody patches) were removed from the dataset.

Multivariate conditional inference trees were used to cluster soils and earthworms according to the relationships between the patterns of soil or earthworm contamination (response variables: concentrations of CUPs) and four explanatory variables: type of habitat, treated/nontreated by pesticides, organic matter and clay contents. The last two parameters were chosen as they influence the most the fate and accumulation of pesticides in the studied matrices. In addition, they were not correlated with each other. pH was not included in the MRT analyses since 83 % of the values were between 8 and 8.5 and were thus not potentially discriminant in the analysis. The number of variables in the MRT analyses was deliberately kept small to maintain sufficient statistical power. Pesticides that had never been detected were removed from MRT analysis because they were not discriminating. Spearman correlation tests were used to test the relationships between the number of years since the switch to organic farming (in cereal fields and grasslands) and the numbers or concentrations of CUPs for earthworms and soils.

All statistical analyses were performed in RStudio version 3.3.2 using the following packages: partykit (Hothorn and Zeileis, 2015), pgirmess (Giraudoux, 2018), and car (Fox et al., 2018) for the other analysis.

3. Results

3.1. CUPs in soils and earthworms

Among the 31 CUPs analyzed, 27 were detected in soils (Table 2). All the soils contained at least one CUP (n = 180), 83 % exhibited five CUPs or more, and 38 % exhibited ten or more (Fig. 1). The herbicide diflufenican, the insecticide imidacloprid, and the fungicides boscalid and epoxiconazole were found in 90 %, 89 %, 85 %, and 79 % of the soils, respectively (Table 2). The most common mixture consisted of an insecticide (imidacloprid), an herbicide (diflufenican), and a fungicide (boscalid (74 % of the soils), epoxiconazole (71 %), or prochloraz (48 %)). Some pesticides were found at relatively low concentrations (<10 ng g^{-1}) in soils, but others, such as diflufenican, boscalid, and the herbicide pendimethalin, reached concentrations >100 ng g^{-1}, or >500 ng g^{-1} (Table 2, Fig. 2a). For instance, boscalid was measured at a concentration of 1212 ng g^{-1} in a cereal field under conventional farming, corresponding to 2.3 times the recommended dose (RD) (Table S1). For diflufenican (1361 ng g^{-1}, representing 4.9 times the RD) and prochloraz (485 ng g^{-1}, or 0.7 times the RD), the highest concentrations were found in the same cereal field (Table 2, Table S1). The four non-detected pesticides were the herbicide cycloxydim and the pyrethroid insecticides bifenthrin, deltamethrin, and lambda-cyhalothrin, which was consistent with their limited use on the sampled crops and their low persistence in soils (Table S1).

In earthworms, 18 CUPs were detected among the 31 CUPs analyzed (Table 3). The mean number of CUPs per earthworm (3.5 ± 2.2 pesticides per individual, n = 155) was lower than that in soils (8.5 ± 4.1 pesticides per soil sample, n = 180) (Fig. 1), but higher concentrations were measured in earthworms for some pesticides, such as diflufenican and imidacloprid (Fig. 2, Tables 2 and 3). Up to 11 pesticides were found in one earthworm sampled in a winter wheat field (Fig. 1). Ninety-two percent of earthworms contained at least one of the pesticides, and 34 % (n = 52) exhibited five pesticides or more (Fig. 1). Overall, imidacloprid was the most frequently detected CUP regardless of the habitat (cereal fields, hedgerows, grasslands) and farming system (conventional *vs* organic farming), with 79 % of individuals being positive for imidacloprid (Table 2). This insecticide also showed the highest frequency of high concentrations, with 43 % of the earthworms presenting imidacloprid concentrations >100 ng g^{-1} and 8.4 % >500 ng g^{-1} (Fig. 2b). The mean concentration of imidacloprid in earthworms was the highest among all the CUPs analyzed in the three landscape habitats, followed by diflufenican in cereal fields and hedgerows and epoxiconazole in grasslands (Table 4). The highest concentrations in a single individual were found for two herbicides, pendimethalin and diflufenican, one fungicide, prochloraz, and one insecticide, imidacloprid (Table 3). The most frequent mixture in earthworms was the same as that in soils, i.e., imidacloprid, diflufenican and one of two fungicides, epoxiconazole (33 % of the earthworms) or cyproconazole (27 %).

3.2. Patterns of contamination according to habitats and agricultural management

The pesticide contamination patterns of the soils differed first according to habitat type (Fig. 3a). The soil contamination profiles associated with cereals were characterized by a greater number of pesticides, relatively high concentrations of diflufenican, imidacloprid, boscalid, epoxiconazole, prochloraz and pendimethalin, and a high occurrence (number of samples in which the pesticide was detected) of cyproconazole compared to those in soils from grasslands and hedgerows (Fig. 3a). In addition, a greater number of herbicides, fungicides, or insecticides was found in soils from cereal fields than in soils from other habitats (Table 4). The occurrence and concentration of the five most

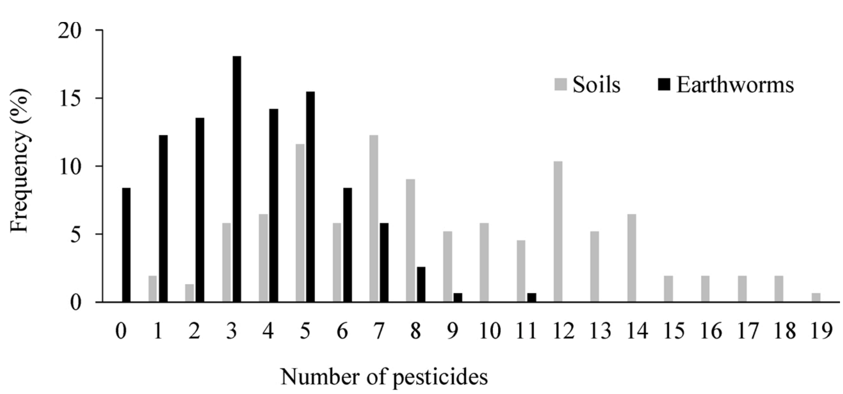

Fig. 1. Frequency of the number of pesticides (all classes) per soil sample and earthworm individual.

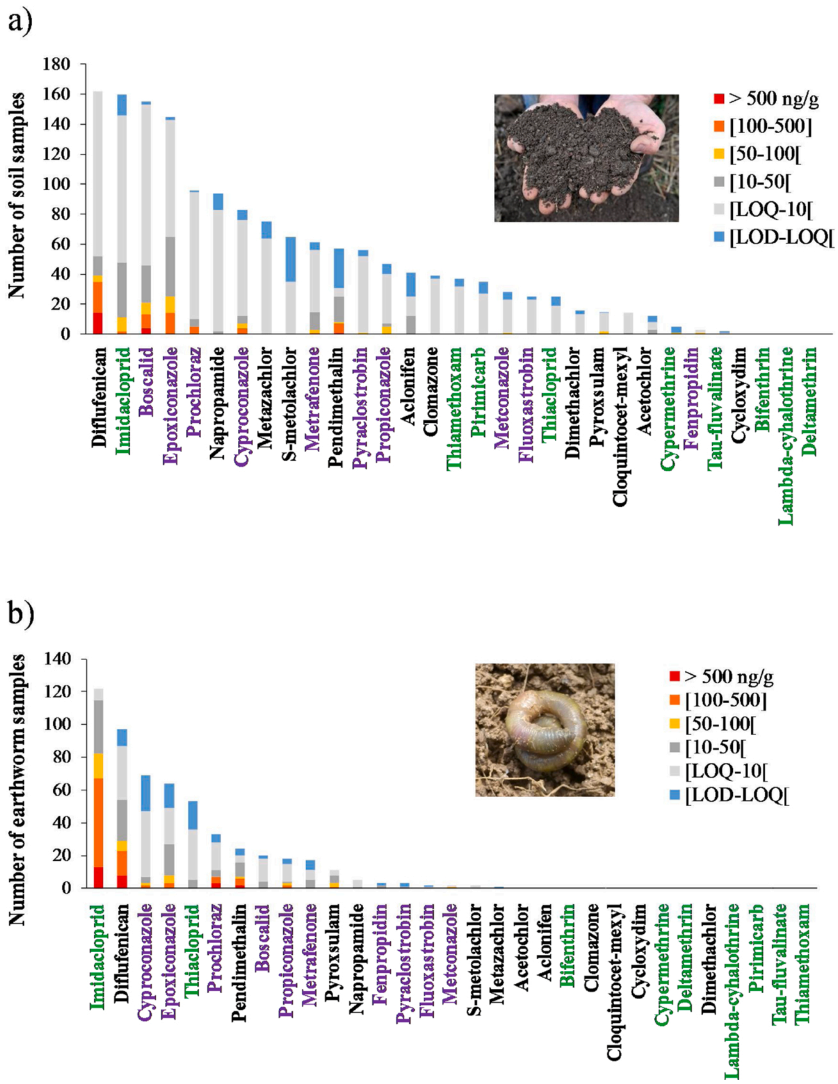

Fig. 2. Concentrations (ng g^{-1} dry weight) of pesticides (herbicides in black, fungicides in purple, and insecticides in green) in a) soils (n = 180) and b) earthworms (n = 155 individuals). LOD: limit of detection, LOQ: limit of quantification (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

frequent pesticides in soils (Table 2) were higher in cereal fields than in hedgerows and grasslands (Table 4). The diflufenican, imidacloprid, boscalid, epoxiconazole, and prochloraz concentrations were 18, 5, 15, 6, and 45 times lower, respectively, in hedgerows and grasslands than in cereal fields.

Regardless of the class of CUPs, the soil of fields treated with pesticides exhibited a greater number of pesticides than those of nontreated habitats (i.e., hedgerows, organic cereal fields or grasslands, and permanent grasslands) (Table 4), although the factor "treated or nontreated" did not shape the patterns of soil contamination (Fig. 3a). The difference was less noticeable for insecticides than for the other pesticides, as 67 %, 56 %, and 29 % greater number of herbicides, fungicides, and insecticides (mean number of pesticides per sample), respectively, were found in soils from treated than nontreated habitats (Table 4, Table S2). Moreover, similar concentrations of boscalid were found in treated/nontreated habitats as well as in organic and conventional fields. Among the 93 soil samples collected in the nontreated habitats, 83 % contained more than 3 pesticides. The comparison of soil contamination between conventional and organic farming revealed that the number of CUPs found in soils from conventional fields was 63 % higher for insecticides, 89 % higher for fungicides, and 68 % higher for herbicides (Table 4, Table S2). However, 83 % of the soils under organic farming exhibited three pesticides or more; 72 % contained imidacloprid (from 0.4–7.7 ng g^{-1}) and 61 % were contaminated by diflufenican (from 0.1–4.2 ng g^{-1}). On average, 6 pesticides per soil were found in cereal fields under organic farming, with three soils (i.e., 43 % of the samples) containing nine pesticides or more. In grasslands under organic farming, 5 pesticides per soil were detected on average, and one of the samples was contaminated by 14 pesticides.

Similar to the results for soils, earthworm contamination profiles differed according to habitat type and they were not segregated by the treated/nontreated factor (Fig. 3b). The profiles associated with cereals were characterized by a greater number of pesticides per earthworm than those in hedgerows and grasslands. As found in soils, greater numbers of total CUPs (all classes), herbicides, or fungicides were found in earthworms from cereal fields than in the other two habitats (Table 4). However, the mean number of insecticides per individual was similar in cereal fields and hedgerows. It was also not significantly different between treated and nontreated habitats (Table 4), with on average 1 (±0.8) and 1 (±0.6) pesticides per earthworm, respectively. The number of CUPs per earthworm was greater in conventional fields than in organic fields for all classes of pesticides (Table 4; Table S2). However, among the seven earthworms from organic cereal fields, four contained between 2 and 6 pesticides, and five exhibited imidacloprid

Table 4
Mean (± SD) number of pesticides (all classes, herbicides, fungicides, and insecticides) and concentrations of the five most frequent pesticides in soils and earthworms according to the habitat (i.e., cereal fields, grasslands, or hedgerows). For cereal fields, n = 7 under organic farming (nontreated). For grasslands, n = 11 (for soils) and 10 (for earthworms) under organic farming, while n = 15 (for soils) and 12 (for earthworms) in permanent grasslands, for total numbers of 26 (for soils) and 22 (for earthworms) nontreated grasslands. Nonparametric Kruskal-Wallis tests were used for all variables, except for the total numbers in soil (ANOVA). Different letters indicate significant differences at p = 0.05 between habitats (one analysis per soil or earthworm variable i.e., number or concentrations of CUPs). For pesticide use and cropping system analyses, NS means not significant; *p < 0.05; *p < 0.01; ***p < 0.001 (Student or Wilcoxon tests).

Soil	Cereal (n = 60)		Grassland (n = 60)		Hedgerow (n = 60)		Pesticide use (treated/untreated)	Cropping system (organic/conventionnal)
Number								
All classes of pesticides (31 analyzed)	10.97 (4.08)	b	7.53 (3.96)	a	7.37 (3.24)	a	***	***
Herbicide (12 analyzed)	4.37 (2.16)	b	2.85 (1.95)	a	2.62 (1.45)	a	***	**
Fungicide (10 analyzed)	4.87 (2.01)	b	3.38 (2.12)	a	3.38 (1.79)	a	***	***
Insecticide (9 analyzed)	1.73 (0.88)	b	1.30 (0.70)	a	1.37 (0.76)	a	**	**
Concentration								
Diflufenican	258.04 (346.44)	c	1.16 (1.33)	a	28.15 (90.27)	b	***	***
Imidacloprid	20.48 (22.97)	c	1.41 (2.72)	a	7.02 (22.13)	b	***	***
Boscalid	88.39 (212.31)	b	2.51 (4.39)	a	8.97 (13.47)	b	NS	NS
Epoxiconazole	51.26 (60.17)	b	6.76 (17.59)	a	9.37 (25.36)	a	***	**
Prochloraz	23.18 (83.22)	b	0.22 (0.34)	a	0.82 (2.85)	a	***	**
Earthworms	Cereal (n = 52)		Grassland (n = 52)		Hedgerow (n = 51)			
Number								
All classes of pesticides (31 analyzed)	5.04 (2.25)	b	2.31 (1.74)	a	3.22 (1.65)	a	***	***
Herbicide (12 analyzed)	1.42 (1.02)	b	0.54 (0.70)	a	0.75 (0.66)	a	***	***
Fungicide (10 analyzed)	2.42 (1.56)	b	1.00 (1.03)	a	1.04 (1.08)	a	***	***
Insecticide (9 analyzed)	1.19 (0.53)	b	0.77 (0.70)	a	1.43 (0.64)	b	NS	**
Concentration								
Imidacloprid	327.55 (212.57)	b	52.76 (81.40)	a	83.81 (130.53)	a	***	***
Diflufenican	306.91 (739.79)	b	4.29 (17.63)	a	20.87 (66.93)	a	***	***
Cyproconazole	5.24 (17.53)	a	0.93 (2.29)	a	3.71 (16.33)	a	NS	*
Epoxiconazole	20.16 (36.88)	b	6.65 (26.17)	a	0.58 (2.33)	a	***	***
Thiacloprid	1.27 (6.03)	ab	0.03 (0.09)	a	1.37 (5.07)	b	NS	NS

concentrations ranging from 27.2–110.0 ng g^{-1} (mean 47.3 ng g^{-1}). Similarly, among the ten earthworms from organic grasslands, two contained 43.3 and 102.0 ng g^{-1} imidacloprid. The other CUPs presented very low concentrations (<10 ng g^{-1}) in the earthworms sampled in organic cereal fields and organic grasslands.

Earthworms from cereal fields were characterized by relatively high concentrations of imidacloprid, diflufenican, and cyproconazole and high occurrences of epoxiconazole, prochloraz and pyroxsulam compared to those in soils from grasslands and hedgerows (Fig. 3b). For instance, the concentrations of imidacloprid, diflufenican, and epoxiconazole were between 3 (epoxiconazole, in cereal fields *vs* grasslands) and 72 times (diflufenican, in cereal fields *vs* grasslands) higher in earthworms sampled in cereal fields (Table 4). These results highlight the considerable weight of diflufenican, imidacloprid, boscalid, epoxiconazole, prochloraz, and cyproconazole in both soil and earthworm contamination patterns. While the load of pendimethalin also shaped the profiles of CUPs in soils from cereal plots and grasslands, the presence of pyroxsulam was discriminant for CUP profiles in earthworms. The pesticides that drove the patterns were thus not necessarily the most frequent ones (Tables 2,3 and Fig. 3). Finally, we tested the correlations between the number of years since the switch to organic farming and the number of CUPs or the concentrations of pesticides in earthworms and soils, but no significant relationships (Spearman correlation) were found.

3.3. *Risk to earthworms exposed to a single CUP or mixture*

The predicted environmental concentrations in soils (PEC_s) were exceeded for 5–11 pesticides (in 14–170 soils, or 8–94% of samples, respectively) depending on the considered type of PEC_s (e.g., the initial concentration after treatment, or the long-term, plateau or maximum concentration; Table 5, Table S3). The main pesticides reaching levels higher than the PEC_s were boscalid, cyproconazole, epoxiconazole, prochloraz (fungicides, up to 5 times higher than the initial PEC_s), diflufenican, pyroxsulam (herbicides, up to 4 times higher than the initial PEC_s), and imidacloprid (insecticide, 1.03 times higher than the initial PEC_s). The initial PEC_s were exceeded for 7 pesticides in 22 % of samples, mostly in soils from conventional cereal plots but also in nontreated soils from hedgerows in 10 % of cases. The maximum PEC_s were exceeded for boscalid, cyproconazole, epoxiconazole, diflufenican and pyroxsulam in 18 % of soil samples (n = 32) collected in conventional cereal plots and in hedgerows (n = 3) (Table 5).

Considering the single pesticide approach based on the toxicity/exposure ratio for earthworms ($TER_{earthworm}$), no acute risk of the measured concentrations in soils was found (Table 5). However, a risk of chronic toxicity was indicated for 4 pesticides, boscalid, cyproconazole, epoxiconazole or imidacloprid, in 42 % of soils (Table 5, Table S3). Seventy-six percent of these soils were sampled in conventional cereal plots, while 12 % came from hedgerows in which epoxiconazole or imidacloprid exceeded toxic levels for earthworm reproduction. Moreover, epoxiconazole was found to potentially alter earthworm reproduction in several grasslands (n = 8) and one organic cereal plot.

Regarding mixture toxicity, a high risk was found in 46 % of the soil samples (Table 6). Considering that the trigger value for the high risk level was set as $\sum RQ \geq 1$, high $\sum RQ$ values were found, up to 38 in cultivated soils and 21 in seminatural habitats (Table 6). The pesticide mixtures in soils posed a negligible or low risk in only 22 % of soils, and no conventional cereal plot presented a low or negligible risk (Table 6). Even nontreated soils from organic cereal fields and hedgerows displayed a high risk in 3 and 22 samples, respectively, representing 43 % and 37 % of the organic cereal field and hedgerow samples. A high risk in soils from grasslands occurred only under conventional farming (5% of all the soils sampled, or 19 % of the grassland soils). Grasslands under organic farming were the only habitat where the CUP mixture in the soils was not classified as high risk. The pesticides in the mixture that mostly contributed to the risk were the same as those under the TER approach (boscalid, cyproconazole, epoxiconazole and imidacloprid), in addition to propiconazole and pyraclostrobin.

Table 5
Environmental risk characterization based on predicted environmental concentrations in soils (PEC_{soil}) and toxicity/exposure ratio (TER) for earthworms.

	Number of pesticides for which $[C]_{soil} > PEC_{soil}$ or $TER_{earthworm} \leq$ trigger value	Number (and %) of samples for which $[C]_{soil} > PEC_{soil}$ or $TER_{earthworm} \leq$ trigger value	Pesticides of concern (number of soil samples containing each pesticide)
PEC_{soil} initial	7	40 (22 %)	Boscalid (4), Cyproconazole (6), Epoxiconazole (8), Prochloraz (2), Diflufenican (17), Pyroxsulam (2), Imidacloprid (1)
PEC_{soil} accumulated/plateau	11	170 (94 %)	Boscalid (2), Cyproconazole (9), Epoxiconazole (4), Prochloraz (2), Propiconazole (5), Cloquintocel-Mexyl* (14), Diflufenican (22), Pendimethalin (5), S-Metolachlore* (65), Cypermethrine* (5), Thiamethoxam* (37)
PEC_{soil} long term (time weighted average 100 days)	5	14 (8%)	Cyproconazole (6), Metrafenone (1), Prochloraz (3), Propiconazole (1), Pyroxsulam (3)
PEC_{soil} maximum	5	32 (18 %)	Boscalid (4), Cyproconazole (5), Epoxiconazole (4), Diflufenican (17), Pyroxsulam (2)
$TER_{earthworm}$ acute	0	0	
$TER_{earthworm}$ chronic	4	75 (42 %)	Boscalid (6), Cyproconazole (3), Epoxiconazole (52), Imidacloprid (14)

PEC_{soil}: predicted environmental concentration in soil. Details of the values and sources are provided in Sup. Mat. Table S3.
PEC_{soil} initial not available for pyraclostrobine, cloquintocet-mexyl, s-metolachlor, cypermethrin, deltamethrin, thiacloprid, and thiamethoxam.
PEC_{soil} accumulated/plateau not available for pyraclostrobine, acetochlor, dimethachlor, metazachlor. For pesticides that were not expected to accumulate in soil, PEC_{soil} accumulated was set as "0″: cloquintocet-Mexyl, s-metolachlor, cypermethrin, deltamethrin, and thiamethoxam. * values of PEC_{soil} accumulated were set at 0.
PEC_{soil} long-term: value obtained from the time-weighted average at 100 days. Not available for boscalid, epoxiconazole, pyraclostrobine, cloquintocet-mexyl, diflufenican, s-metolachlor, cypermethrin, deltamethrin, imidacloprid, lambda-cyhalothrine, tau-fluvanilate, thiacloprid, and thiamethoxam.
PEC_{soil} maximum: value cited as the maximum or used in toxicity/exposure ratios in regulation and risk assessment documents. Not available for fluoxastrobine, metconazole, pyraclostrobine, clomazone, cloquintocet-mexyl, s-metolachlor, cypermethrin, deltamethrin, thiacloprid, and thiamethoxam.
$TER_{earthworm}$ acute: LC50 / $[C]_{soil}$, trigger value = 10; LC50: lethal concentration 50 %.
LC50 acute earthworms available for all pesticides.
$TER_{earthworm}$ chronic: NOEC / $[C]_{soil}$, trigger value = 5. NOEC: no observed effect concentration.
NOECs (reproduction) for earthworms were not available for acetochlor, cloquintocet-mexyl, cycloxydime, dimetachlor, metazachlor, deltamethrin, and pirimicarb.

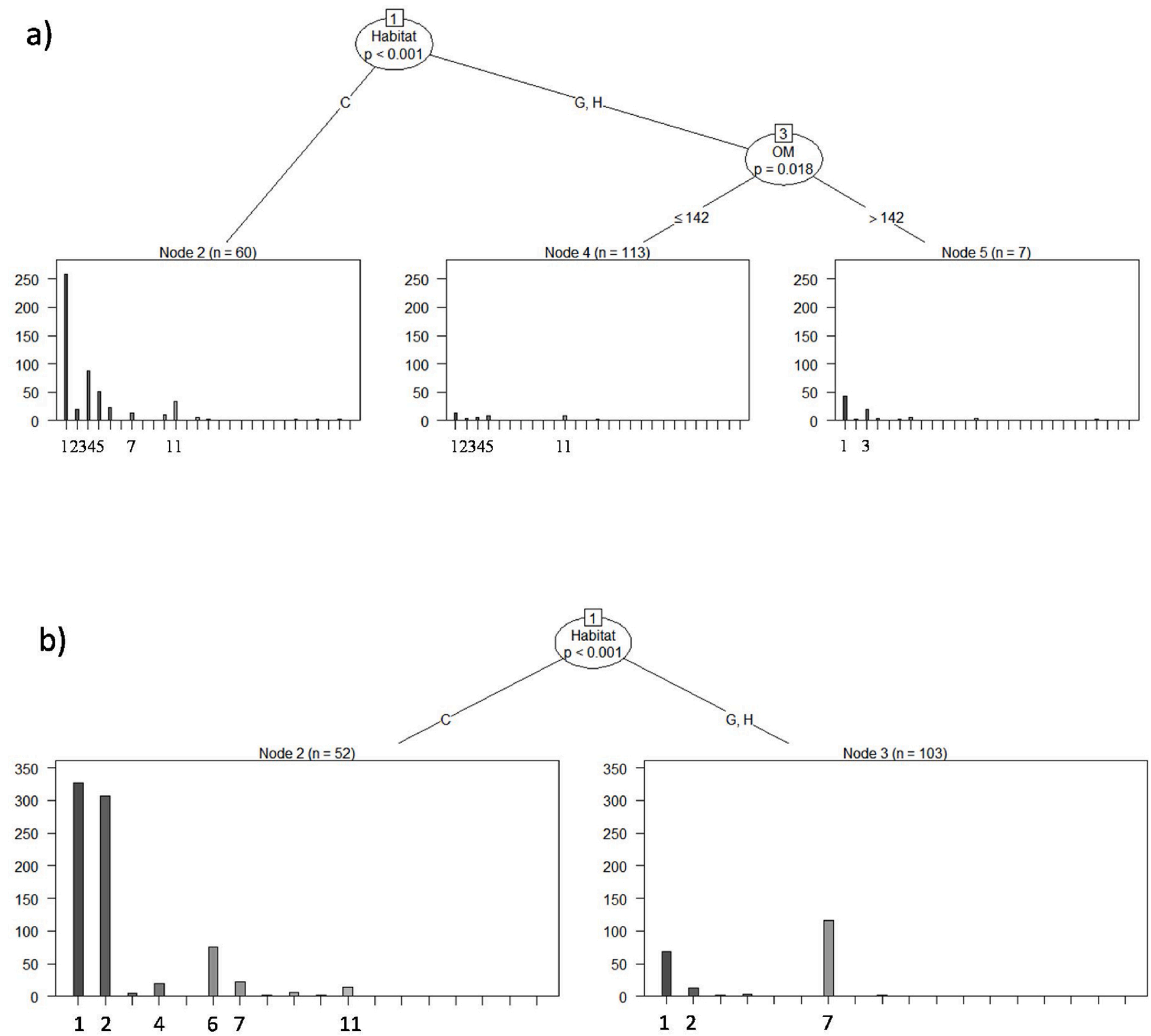

Fig. 3. Multivariate conditional inference trees for the data on pesticide concentrations in a) soils (27 pesticides) and b) earthworms (18 pesticides). Each split is represented graphically as a branch that is labeled with the classification variable; on each branch, the bar plot shows the multivariate means of pesticide concentrations (in ng g^{-1}). Above each histogram, n is the number of sites in the leaf (group). The numbers under each histogram refer to the occurrence rank of the pesticides (see Table 1 for soils and Table 2 for earthworms). C: cereal fields, G: grasslands, H: hedgerows, OM: organic matter content. The y-axis represents the pesticide concentrations in ng g^{-1}.

Table 6
Environmental risk characterization based on the sum of risk quotients for earthworms: number of soil samples showing each risk level for the 180 plots studied, according to the type of habitat and cropping system (CF: conventional farming, OF: organic farming).

	High risk ($\sum RQ \geq 1$)				Medium risk ($0.1 \leq \sum RQ < 1$)	Low risk ($0.01 \leq \sum RQ < 0.1$)	Negligible risk ($\sum RQ \leq 0.01$)
	n	% in high risk class	*mean* $\sum RQ$	*max* $\sum RQ$	*n*	*n*	*n*
Cereal fields	**51**	(85 %)			**8**	**0**	**1**
CF	**48**	(91 %)	*10*	*38*	**5**	**0**	**0**
OF	**3**	(43 %)	*3*	*6*	**3**	**0**	**1**
Grasslands	**9**	(15 %)			**25**	**20**	**6**
CF	**9**	(19 %)	*5*	*12*	**20**	**15**	**4**
OF	**0**				**5**	**5**	**2**
Hedgerows	**22**	(37 %)	*4*	*21*	**26**	**10**	**2**
Total	**82**	(46 %)			**59**	**30**	**9**

4. Discussion

4.1. CUPs in soils

4.1.1. Ubiquity of contamination in soils

The first result of great importance in this study was the wide contamination of soils at the scale of an agricultural landscape, as 100 % of the soils sampled in conventional fields, in plots managed under organic farming and in off-field habitats contained CUPs. Overall, although the levels of most of the pesticides in our soils were within the ranges reported in recent studies (e.g., Chiaia-Hernandez et al., 2017; Karasali et al., 2016; Suszter and Ambrus, 2017), we measured relatively high occurrences and concentrations of CUPs, mainly for diflufenican, imidacloprid, boscalid, and epoxiconazole (i.e., all were found in more than 80 % of the samples, at up to 1361 ng g^{-1}). Among the most notable differences, Silva et al. (2019) reported a maximum value of 410 ng g^{-1} for boscalid while we measured a concentration up to 1211 ng g^{-1} in soil from a cereal field. Similarly, these authors found that imidacloprid was present in 7% of the examined EU topsoil samples, based on a limit of quantification of 10 ng g^{-1}, with a maximum content of 60 ng g^{-1}, while we found imidacloprid in 90 % of soils (or 26 % of soils when considering concentrations above 10 ng g^{-1}), and at concentrations as high as 160 ng g^{-1}. Lower concentrations of imidacloprid (between <0.09 and 10.7 ng g^{-1}) and thiamethoxam (between <0.02 and 1.5 ng g^{-1}) have also been measured in arable soils in England, where neonicotinoids have been used as seed dressings (Jones et al., 2014). Numerous nonexclusive factors related to environmental conditions, type of crop studied, agronomic practices and pesticide properties as well as sampling time (e.g., date since last applications), sampling strategies and analytical methods (e.g., limits of detection and quantification) may drive the differences observed between the present results and the previous studies (Bonmatin et al., 2015). Further investigations will be required to identify and disentangle these factors but the levels of CUPs found here in soils from treated and nontreated habitats suggest higher persistence and/or inputs than expected.

4.1.2. Mixture of pesticides in soils

A striking result of our study was the contamination of soils by a mixture of multiclass CUPs with different chemical characteristics, modes of action and targets, since a mixture consisting of at least one herbicide, fungicide and insecticide was found in 90 % of the soils. However, most of these CUPs are assumed to be weakly or moderately persistent in the environment. It is worth pinpointing that only 31 pesticides were analyzed while about 60 active ingredients were found to be applied in the studied area. In cereal fields, we detected an average of 11 pesticides in the soils (i.e., 35 % of the analyzed pesticide), which was higher than previous observations (e.g., 10 − 15 pesticides per soil in treated fields corresponding to 10–16 % of the analyzed pesticide in Chiaia-Hernandez et al., 2017). Moreover, the percentage of soils containing at least 5 pesticides was 83 %, which was higher than that previously reported for soils collected in arable lands (51 %) (Hvězdová et al., 2018). This is even more striking when considering that we also sampled soils in nontreated habitats. As mentioned in the previous sub-section, numerous factors may explain these differences between studies. We detected diflufenican, imidacloprid, boscalid, and epoxiconazole most frequently (i.e., in >80 % of soils), which was consistent with previous findings in farmland soils indicating that epoxiconazole and diflufenican or boscalid showed the highest occurrence (Hvězdová et al., 2018; Silva et al., 2019). Neonicotinoids (notably imidacloprid) have rarely been measured in arable soils, although these compounds are of high environmental concern regarding their potential negative impacts on biodiversity and ecosystem functioning worldwide (van der Sluijs et al., 2015). Imidacloprid has also been detected at a high frequency in vegetable crop fields in Jordan (Kailani et al., 2019) or in France, where it was detected in 91 % of sampled soils (Bonmatin et al., 2015). In this last study, 97 % of soils seeded with treated seeds 1 or 2 years before sampling were still contaminated by imidacloprid, a neonicotinoid that potentially exhibits long persistence in the environment (Jones et al., 2014; van der Sluijs et al., 2015).

4.1.3. CUPs contaminate soils in both treated and nontreated habitats

One of our main findings was the ubiquity of the CUPs in all habitats of the agricultural landscape, regardless of whether they had been treated with pesticides. Although the concentrations and the number of pesticides were higher in soils sampled in habitats that directly received pesticides, we identified different mixtures of CUPs in nontreated off-field habitats; for instance, an average of 6 pesticides per soil was found in organic cereal fields. To our knowledge, this is the first time that data showing the wide contamination of nontreated habitats have been reported, since previous studies dealing with pesticides in mixture mainly focused on treated cropped fields (e.g., Chiaia-Hernandez et al., 2017; Hvězdová et al., 2018; Silva et al., 2019). The rare studies considering off-field or nontreated areas dealt only with neonicotinoids, and Bonmatin et al. (2005) did not detect any residues of imidacloprid (limit of quantification at 1 ng g^{-1}) in French organic soils. However, repeated and massive use of neonicotinoids in arable landscape may have modified this pattern with time course. The processes explaining the contamination of nontreated habitats measured in our study could include horizontal transfer via air and water from treated to nontreated habitats along with residues of the applied chemicals (Navarro et al., 2007). Jones et al. (2014) detected the neonicotinoids clothianidin, thiamethoxam, and imidacloprid in edges or several fields where these chemicals had not been used in the three previous years and suggested that this may have been due to applications in surrounding fields and dust drift. Similarly, pendimethalin and imidacloprid were found in plots where they were not applied by farmers, which might be partly due to CUP treatments applied in the surrounding fields (Chiaia-Hernandez et al., 2017). Overall, our results suggest that habitat shaped the profiles of contamination more than farming practices (conventional versus organic farming), which implies that the local beneficial effects of organic farming are dampened because of neighboring inputs from large surfaces treated with CUPs.

4.1.4. Risk assessment using PECs

The predicted environmental concentrations in soils (PECs) calculated within the framework of environmental risk assessment methodology in Europe are key criteria for determining whether soil contamination after treatments poses a risk to the soil fauna or not. We here considered « worst case » values since we provided comparisons to initial PECs and maximum PECs, or plateau/long term PEC for compounds that are not supposed to accumulate in soils. This means that even if the soil sampled had been treated the within hours before collection, the MECs should be at maximum equal or lower than the maximum or initial PECs values. As a consequence, according to the marketing authorization of the products, the MECs in plots submitted to applications whatever the time of application and in nontargeted plots should be under the maximum PECs value.

We showed that the measured concentrations in soils exceeded the initial PEC_s by factors of 1.03–5.08 for several herbicides, fungicides and insecticides, even in nontreated habitats, raising two main issues. First, this leads to questions regarding the relevance of the laboratory testing and modeling approaches that are used for regulation to assess degradation and accumulation and to predict the environmental levels of pesticides only in treated plots. Additionally, local environmental conditions influence transfer, bioavailability and persistence and, thus, may alter the fate of pesticides (Navarro et al., 2007), which is not considered in PEC calculation. At the landscape scale, pesticides can be applied repeatedly in a mosaic of fields, leading to the contamination of neighboring habitats by drift or volatilization and run-off. Second, the efficiency of postregistration survey methods needs to be reconsidered (Marković et al., 2010) since residues in soils are rarely considered.

4.2. Mixture of multiclass CUPs over the landscape, a threat to earthworms

Except from insects and especially bees, no data are currently available regarding the accumulation of multiclass CUPs in nontarget fauna or regarding the risk to wildlife arising from soil pesticide mixtures under realistic field conditions. We showed that soil contamination by CUPs led to the accumulation of a mixture of pesticides in 92 % of the earthworms sampled. High concentrations of several CUPs were measured in some earthworms, with the residues of diflufenican, prochloraz or pendimethalin exceeding 1000 ng g^{-1} in 6 individuals. Overall, the ability of CUPs to bioaccumulate in soil organisms remains under question. CUPs are commonly considered to show low to moderate bioaccumulation compared to organochlorine pesticides, which were prohibited several years ago, but empirical evidence (i.e., the measurement of residues in free-living organisms) is lacking. For the earthworm *Eisenia andrei* exposed to field-contaminated soils in laboratory experiments, bioaccumulation was observed only for pendimethalin in one of 4 tested soils but not for epoxiconazole and prochloraz (Neuwirthová et al., 2019). In our study, 3 pesticides that are among the most frequently detected pesticides in *Allolobophora chlorotica*, diflufenican, imidacloprid, and epoxiconazole, exhibited higher concentrations in earthworms than in soils. Neonicotinoids (notably imidacloprid) have rarely been measured in wildlife apart from pollinators, although these compounds are of high environmental concern regarding their potential negative impacts on biodiversity and ecosystem functioning worldwide (van der Sluijs et al., 2015). The only study concerning CUP accumulation in free-living earthworms reported levels of some neonicotinoids in a few individuals sampled opportunistically in two soya bean plots. The authors have detected imidacloprid at concentrations of 25 and 23 ng g^{-1}, and total neonicotinoid concentrations reached 54 and 279 ng g^{-1}, which support our findings about the ability of soil organisms to be exposed to and accumulate neonicotinoids (Douglas et al., 2015). These results along with those reported in our study attest to the bioaccumulation potential of some CUPs, at least under field conditions, suggesting a need for much more field monitoring to complement lab or modeling assessment. As earthworms are the main or occasional prey of numerous wildlife species, the diverse mixture of pesticides that we found in their tissues gives rise to the question of whether they could play a key role as vectors of pesticides in food webs and, thus, contribute to endanger their predators.

Our results emphasized that several single pesticides are present in soils at levels above toxic thresholds for nontarget soil organisms and may therefore present a risk to earthworms. Moreover, in the consideration of potential mixture toxicity, we calculated a high risk for almost half of 180 the soils sampled, including organic fields, grasslands and hedgerows. This was in line with studies revealing negative impacts of pesticides used in cropping systems on earthworm populations and communities (Pelosi et al., 2013a, 2015; Pfiffner and Mäder, 1997). This also reinforced current questions about the relevance of risk assessment procedures to biodiversity (Brühl and Zaller, 2019; Wintermantel et al., 2020). Furthermore, this alarming level of risk over a large extent and various landscape patches is likely to be underestimated since we analyzed only 31 pesticides, and additional CUPs are used and can occur in soils, with potential synergistic deleterious effects. Moreover, the earthworm species *Eisenia fetida* used in risk assessment procedures has been shown to be less sensitive to pesticides than other earthworm species found in cultivated fields (Pelosi et al., 2013b; Tejada et al., 2011), which can underestimate the calculated risks. Finally, PEC values and chronic toxic thresholds were not available for the full set of pesticides studied, which may lower the risk evaluations for several compounds. Neither the toxic threshold related to the long-term exposure of earthworms to similar mixtures of several CUPs nor reference values relating CUP residues in earthworm tissues to toxicological endpoints were available to further assess the potential risk to soil organisms at the individual, population and community levels.

The fact that levels of CUPs in fields under conventional farming never presented low or negligible risk but high risk to earthworms in 91 % of soils seriously questions the sustainability of chemical mainstream agriculture. Moreover, within agricultural landscapes, nontreated habitats such as organic fields, hedgerows or permanent grasslands are assumed to promote biodiversity (EFSA Scientific Committee, 2016; Nienstedt et al., 2012), but our results give rise to the question of whether they could act as ecological traps and harm animals that live there by exposing them to pesticide mixtures at relatively high concentrations. The contamination of these “off-field” habitats by pesticides could affect the resilience of agrosystems at the landscape scale by preventing any possibility of these areas to act as shelters and sources for recolonization. It has been emphasized that the use of neonicotinoids hinders the maintenance of biodiversity and the ecological functions and services the organisms perform (van der Sluijs et al., 2015). Our results regarding off-field habitat contamination by CUPs bolstered this conclusion and indicated that its application should also be broadened to several other pesticides, notably fungicides and herbicides such as epoxiconazole and diflufenican. Agroecological transition and environmental policies encourage the protection and extension of seminatural habitats in agricultural landscapes to promote biodiversity and ecosystem services. We strongly recommend that the potential of these habitats to expose nontarget organisms to CUPs, the associated risk and the mitigation of actual CUP contamination be considered.

Further, to mitigate the contamination of both off-field areas and nontarget arable soils, we advise to view pesticide use reduction at landscape scale i.e., considering the surfaces and location of treated crops versus other land covers within the mosaic.

Bernhardt et al. (2017) showed that the increases in synthetic chemicals (pesticides, pharmaceuticals, and other synthetic chemicals) in terms of their total quantities, diversity, and geographic expansion over the past four decades have exceeded the rate of the increase in most well-recognized drivers of global change, such as rising atmospheric $CO2$ concentrations, habitat destruction, and biodiversity loss. Despite this situation, far less attention has been devoted to studies addressing synthetic chemicals than to studies about other agents of global change, and far less funding has been dedicated to this topic. This represents a critical knowledge gap with regard to scientific advances in global ecology and achieving the goals of sustainable development.

4.3. Author contributions

C.P. and C.F. coordinated the research project and designed the experiments. C.P., C.F., and V.B. organized the sampling design. C.P. carried out the sampling. C.B. managed data curation and extraction of land use metrics from maps of ZA-PVS in Geographic Information Systems. C.B. and C.F. prepared the databases. C.P., C.F., and C.B. analyzed the data. C.P., C.F. and M.C. wrote the first draft of the manuscript. G.D., F.L., and E.V. developed the analytical method for pesticide measurements, performed the analyses and, with P.B and S.N., wrote Appendix S1. S.G. and V.B. supervised the ZA PVS sampling area and provided information on land use and farming practices. All authors contributed to the writing of the final version of the manuscript, which was revised for English by American Journal Experts (AJE®).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agee.2020.107167.

References

Amelung, W., Nikolakis, A., Laabs, V., 2007. Multiresidue determination of pesticides in acid-clay soils from Thailand. J. AOAC Int. 90, 1659–1669.

Bedos, C., Cellier, P., Calvet, R., Barriuso, E., Gabrielle, B., 2002. Mass transfer of pesticides into the atmosphereby volatilization from soils and plants: overview. Agronomie 22, 21–33. https://doi.org/10.1051/agro:2001003.

Bengtsson, J., AhnströM, J., Weibull, A.-C., 2005. The effects of organic agriculture on biodiversity and abundance: a meta-analysis: organic agriculture, biodiversity and abundance. J. Appl. Ecol. 42, 261–269. https://doi.org/10.1111/j.1365-2664.2005.01005.x.

Bernhardt, E.S., Rosi, E.J., Gessner, M.O., 2017. Synthetic chemicals as agents of global change. Front. Ecol. Environ. 15, 84–90. https://doi.org/10.1002/fee.1450.

BIO Intelligence Service, 2014. Soil and Water in a Changing Environment - Final Report Prepared for European Commission (DG ENV), With Support from HydroLogic (Final Report Prepared for European Commission (DG ENV), With Support from HydroLogic).

Bonmatin, J.-M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C., Liess, M., Long, E., Marzaro, M., Mitchell, E.A.D., Noome, D.A., Simon-Delso, N., Tapparo, A., 2015. Environmental fate and exposure; neonicotinoids and fipronil. Environ. Sci. Pollut. Res. - Int. 22, 35–67. https://doi.org/10.1007/s11356-014-3332-7.

Bretagnolle, V., Berthet, E., Gross, N., Gauffre, B., Plumejeaud, C., Houte, S., Badenhausser, I., Monceau, K., Allier, F., Monestiez, P., Gaba, S., 2018. Towards sustainable and multifunctional agriculture in farmland landscapes: Lessons from the integrative approach of a French LTSER platform. Sci. Total Environ. 627, 822–834. https://doi.org/10.1016/j.scitotenv.2018.01.142.

Brühl, C.A., Zaller, J.G., 2019. Biodiversity decline as a consequence of an inappropriate environmental risk assessment of pesticides. Front. Environ. Sci. 7, 177. https://doi.org/10.3389/fenvs.2019.00177.

Chiaia-Hernandez, A.C., Keller, A., Wächter, D., Steinlin, C., Camenzuli, L., Hollender, J., Krauss, M., 2017. Long-term persistence of pesticides and TPs in archived agricultural soil samples and comparison with pesticide application. Environ. Sci. Technol. 51, 10642–10651. https://doi.org/10.1021/acs.est.7b02529.

Daniele, G., Lafay, F., Pelosi, C., Fritsch, C., Vulliet, E., 2018. Development of a method for the simultaneous determination of multi-class pesticides in earthworms by liquid chromatography coupled to tandem electrospray mass spectrometry. Anal. Bioanal. Chem. 410, 5009–5018. https://doi.org/10.1007/s00216-018-1151-2.

de Geronimo, E., Botero-Coy, A.M., Marin, J.M., Aparicio, V.C., Costa, J.L., Sancho, J.V., Hernandez, F., 2015. A simple and rapid analytical methodology based on liquid chromatography-tandem mass spectrometry for monitoring pesticide residues in soils from Argentina. Anal. Methods 7, 9504–9512.

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, e0220029. https://doi.org/10.1371/journal.pone.0220029.

Douglas, M.R., Rohr, J.R., Tooker, J.F., 2015. Neonicotinoid insecticide travels through a soil food chain, disrupting biological control of non-target pests and decreasing soya bean yield. J. Appl. Ecol. 52, 250–260. https://doi.org/10.1111/1365-2664.12372.

EFSA (European Food Safety Authority), 2017. EFSA Guidance Document for predicting environmental concentrations of active substances of plant protection products and transformation products of these active substances in soil. Efsa J. 15 (10), 115. https://doi.org/10.2903/j.efsa.2017.4982, 4982.

EFSA Scientific Committee, 2016. Scientific opinion on recovery in environmental risk assessments at EFSA. EFSA J. 14, 4313–4398. https://doi.org/10.2903/j.efsa.2016.4313.

EUR-Lex, 2006. Communication from the Commission to the Council, the European Parliament, the European Economic and Social Committee and the Committee of the Regions - Thematic Strategy for Soil Protection. URL.

European Commission, 2020. EU-pesticides-database (2020). URL. https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.selection&language=EN (2020)..

EUROPEAN COMMISSION, 2003. Technical Guidance Document on Risk Assessment in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances, Commission Regulation (EC) No 1488/94 on Risk Assessment for Existing Substances, Directive 98/8/EC of the European Parliament and of the Council Concerning the Placing of Biocidal Products on the Market. Part II (No. EUR 20418 EN/2).

European Food Safety Authority (EFSA), 2009. Guidance of EFSA - risk assessment for birds and mammals. EFSA Journal 7, 1438. https://doi.org/10.2903/j.efsa.2009.1438.

European Parliament and Council of the European Union, 2009. Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 Establishing a Framework for Community Action to Achieve the Sustainable Use of Pesticides (Text with EEA Relevance).

Eurostat, 2020. https://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=aei_fm_salpest09&lang=en.

FAOSTAT, 2019. http://www.fao.org/faostat/en/#data/.

Fox, J., Weisberg, S., Fox, J., 2018. An R Companion to Applied Regression, 3rd ed. SAGE Publications, Thousand Oaks, Calif.

Gamón, M., Sáez, E., Gil, J., Boluda, R., 2003. Direct and indirect exogenous contamination by pesticides of rice-farming soils in a Mediterranean Wetland. Arch. Environ. Contam. Toxicol. 44, 141–151. https://doi.org/10.1007/s00244-002-2008-3.

Geiger, F., Bengtsson, J., Berendse, F., Weisser, W.W., Emmerson, M., Morales, M.B., Ceryngier, P., Liira, J., Tscharntke, T., Winqvist, C., Eggers, S., Bommarco, R., Part, T., Bretagnolle, V., Plantegenest, M., Clement, L.W., Dennis, C., Palmer, C., Onate, J.J., Guerrero, I., Hawro, V., Aavik, T., Thies, C., Flohre, A., Hanke, S., Fischer, C., Goedhart, P.W., Inchausti, P., 2010. Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. Basic Appl. Ecol. 11, 97–105. https://doi.org/10.1016/j.baae.2009.12.001.

Gilliom, R.J., 2007. Pesticides in U.S. Streams and groundwater. Environ. Sci. Technol. 41, 3408–3414. https://doi.org/10.1021/es072531u.

Giraudoux, P., 2018. Package ‘pgirmess’, Spatial Analysis and Data Mining for Field Ecologists. URL.

Hallberg, G.R., 1987. The impacts of agricultural chemicals on ground water quality. GeoJournal 15, 283–295. https://doi.org/10.1007/BF00213456.

Hossard, L., Guichard, L., Pelosi, C., Makowski, D., 2017. Lack of evidence for a decrease in synthetic pesticide use on the main arable crops in France. Sci. Total Environ. 575, 152–161. https://doi.org/10.1016/j.scitotenv.2016.10.008.

Hothorn, T., Zeileis, A., 2015. Partykit: a modular toolkit for recursive partytioning in r. J. Mach. Learn. Res. 16, 3905–3909.

Hvězdová, M., Kosubová, P., Košíková, M., Scherr, K.E., Šimek, Z., Brodský, L., Šudoma, M., Škulcová, L., Sáňka, M., Svobodová, M., Krkošková, L., Vašíčková, J., Neuwirthová, N., Bielská, L., Hofman, J., 2018. Currently and recently used pesticides in Central European arable soils. Sci. Total Environ. 613–614, 361–370. https://doi.org/10.1016/j.scitotenv.2017.09.049.

Jones, A., Harrington, P., Turnbull, G., 2014. Neonicotinoid concentrations in arable soils after seed treatment applications in preceding years: neonicotinoid concentrations in arable soil. Pest Manag. Sci. 70, 1780–1784. https://doi.org/10.1002/ps.3836.

Kailani, M.H., Al-Antary, T.M., Alawi, M.A., 2019. Monitoring of pesticides residues in soil samples from the southern districts of Jordan in 2016/2017. Toxin Rev. 1–17. https://doi.org/10.1080/15569543.2019.1580747.

Karasali, H., Marousopoulou, A., Machera, K., 2016. Pesticide residue concentration in soil following conventional and Low-Input Crop Management in a Mediterranean agro-ecosystem, in Central Greece. Sci. Total Environ. 541, 130–142. https://doi.org/10.1016/j.scitotenv.2015.09.016.

King, R.A., Vaughan, I.P., Bell, J.R., Bohan, D.A., Symondson, W.O.C., 2010. Prey choice by carabid beetles feeding on an earthworm community analysed using species- and lineage-specific PCR primers. Mol. Ecol. 19, 1721–1732. https://doi.org/10.1111/j.1365-294X.2010.04602.x.

Lautenbach, S., Maes, J., Kattwinkel, M., Seppelt, R., Strauch, M., Scholz, M., Schulz-Zunkel, C., Volk, M., Weinert, J., Dormann, C.F., 2012. Mapping water quality-related ecosystem services: concepts and applications for nitrogen retention and pesticide risk reduction. Int. J. Biodiversity Sci., Ecosyst. Services & Manag. 8, 35–49. https://doi.org/10.1080/21513732.2011.631940.

Le Couteulx, A., Wolf, C., Hallaire, V., Peres, G., 2015. Burrowing and casting activities of three endogeic earthworm species affected by organic matter location. Pedobiologia 58, 97–103.

Liu, T., Chen, X., Gong, X., Lubbers, I.M., Jiang, Y., Feng, W., Li, X., Whalen, J.K., Bonkowski, M., Griffiths, B.S., Hu, F., Liu, M., 2019. Earthworms coordinate soil Biota to improve multiple ecosystem functions. Curr. Biol. 29, 3420–3429. https://doi.org/10.1016/j.cub.2019.08.045 e5.

Main, A.R., Webb, E.B., Goyne, K.W., Mengel, D., 2020. Reduced species richness of native bees in field margins associated with neonicotinoid concentrations in non-target soils. Agric. Ecosyst. Environ. 287, 106693 https://doi.org/10.1016/j.agee.2019.106693.

Marković, M., Cupać, S., Đurović, R., Milinović, J., Kljajić, P., 2010. Assessment of heavy metal and pesticide levels in soil and plant products from agricultural area of Belgrade. Serbia. Arch Environ Contam Toxicol 58, 341–351. https://doi.org/10.1007/s00244-009-9359-y.

Mottes, C., Lesueur-Jannoyer, M., Le Bail, M., Malézieux, E., 2014. Pesticide transfer models in crop and watershed systems: a review. Agron. Sustain. Dev. 34, 229–250. https://doi.org/10.1007/s13593-013-0176-3.

Navarro, S., Vela, N., Navarro, G., 2007. Review. An overview on the environmental behaviour of pesticide residues in soils. Span. J. Agric. Res. 5, 357. https://doi.org/10.5424/sjar/2007053-5344.

Neuwirthová, N., Trojan, M., Svobodová, M., Vašíčková, J., Šimek, Z., Hofman, J., Bielská, L., 2019. Pesticide residues remaining in soils from previous growing season (s) - Can they accumulate in non-target organisms and contaminate the food web?

Sci. Total Environ. 646, 1056–1062. https://doi.org/10.1016/j.scitotenv.2018.07.357.

Nienstedt, K.M., Brock, T.C.M., van Wensem, J., Montforts, M., Hart, A., Aagaard, A., Alix, A., Boesten, J., Bopp, S.K., Brown, C., Capri, E., Forbes, V., Köpp, H., Liess, M., Luttik, R., Maltby, L., Sousa, J.P., Streissl, F., Hardy, A.R., 2012. Development of a framework based on an ecosystem services approach for deriving specific protection goals for environmental risk assessment of pesticides. Sci. Total Environ. 415, 31–38. https://doi.org/10.1016/j.scitotenv.2011.05.057.

Pelosi, C., Joimel, S., Makowski, D., 2013a. Searching for a more sensitive earthworm species to be used in pesticide homologation tests – a meta-analysis. Chemosphere 90, 895–900. https://doi.org/10.1016/j.chemosphere.2012.09.034.

Pelosi, C., Toutous, L., Chiron, F., Dubs, F., Hedde, M., Muratet, A., Ponge, J.-F., Salmon, S., Makowski, D., 2013b. Reduction of pesticide use can increase earthworm populations in wheat crops in a European temperate region. Agric. Ecosyst. Environ. 181, 223–230. https://doi.org/10.1016/j.agee.2013.10.003.

Pelosi, C., Bertrand, M., Thénard, J., Mougin, C., 2015. Earthworms in a 15 years agricultural trial. Appl. Soil Ecol. 88, 1–8. https://doi.org/10.1016/j.apsoil.2014.12.004.

Pfiffner, L., Mäder, P., 1997. Effects of biodynamic, organic and conventional production systems on earthworm populations. Biol. Agric. Hortic. 15, 2–10. https://doi.org/10.1080/01448765.1997.9755177.

Silva, V., Mol, H.G.J., Zomer, P., Tienstra, M., Ritsema, C.J., Geissen, V., 2019. Pesticide residues in European agricultural soils – a hidden reality unfolded. Sci. Total Environ. 653, 1532–1545. https://doi.org/10.1016/j.scitotenv.2018.10.441.

Suszter, G.K., Ambrus, Á., 2017. Distribution of pesticide residues in soil and uncertainty of sampling. J. Environ. Sci. Health B 52, 557–563. https://doi.org/10.1080/03601234.2017.1316171.

Tejada, M., Gómez, I., del Toro, M., 2011. Use of organic amendments as a bioremediation strategy to reduce the bioavailability of chlorpyrifos insecticide in soils. Effects on soil biology. Ecotoxicol. Environ. Saf. 74, 2075–2081. https://doi.org/10.1016/j.ecoenv.2011.07.005.

United States Environmental Protection Agency, 2017. Technical Overview of Ecological Risk Assessment: Risk Characterization. URL.

van der Sluijs, J.P., Amaral-Rogers, V., Belzunces, L.P., Bijleveld van Lexmond, M.F.I.J., Bonmatin, J.-M., Chagnon, M., Downs, C.A., Furlan, L., Gibbons, D.W., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C., Liess, M., Long, E., McField, M., Mineau, P., Mitchell, E.A.D., Morrissey, C.A., Noome, D.A., Pisa, L., Settele, J., Simon-Delso, N., Stark, J.D., Tapparo, A., Van Dyck, H., van Praagh, J., Whitehorn, P.R., Wiemers, M., 2015. Conclusions of the Worldwide Integrated Assessment on the risks of neonicotinoids and fipronil to biodiversity and ecosystem functioning. Environ. Sci. Pollut. Res. - Int. 22, 148–154. https://doi.org/10.1007/s11356-014-3229-5.

van Groenigen, J.W., Lubbers, I.M., Vos, H.M.J., Brown, G.G., De Deyn, G.B., van Groenigen, K.J., 2015. Earthworms increase plant production: a meta-analysis. Sci. Rep. 4, 6365. https://doi.org/10.1038/srep06365.

Vašíčková, J., Hvězdová, M., Kosubová, P., Hofman, J., 2019. Ecological risk assessment of pesticide residues in arable soils of the Czech Republic. Chemosphere 216, 479–487. https://doi.org/10.1016/j.chemosphere.2018.10.158.

Wintermantel, D., Odoux, J.-F., Decourtye, A., Henry, M., Allier, F., Bretagnolle, V., 2020. Neonicotinoid-induced mortality risk for bees foraging on oilseed rape nectar persists despite EU moratorium. Sci. Total Environ. 704, 135400 https://doi.org/10.1016/j.scitotenv.2019.135400.

Zhang, W., Jiang, F., Ou, J., 2011. Global pesticide consumption and pollution: with China as a focus. Proc. Int. Acad. Ecol. Environ. Sci. 1, 125–144.

Zhao, P., Wang, Z., Li, K., Guo, X., Zhao, L., 2018. Multi-residue enantiomeric analysis of 18 chiral pesticides in water, soil and river sediment using magnetic solid-phase extraction based on amino modified multiwalled carbon nanotubes and chiral liquid chromatography coupled with tandem mass spectrometry. J. Chromatogr. A 1568, 8–21. https://doi.org/10.1016/j.chroma.2018.07.022.