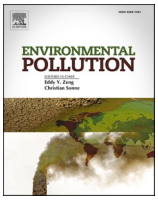
Environmental Pollution 305 (2022) 119290

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol



# Field mixtures of currently used pesticides in agricultural soil pose a risk to soil invertebrates☆

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ARTICLE INFO

*Keywords:*
Field ecotoxicology
Cropping systems
Plant protection products (PPPs)
Oligochaetes
Arthropods
Mollusks

ABSTRACT

Massive use of pesticides in conventional agriculture leads to accumulation in soil of complex mixtures, triggering questions about their potential ecotoxicological risk. This study assessed cropland soils containing pesticide mixtures sampled from conventional and organic farming systems at La Cage and Mons, France. The conventional agricultural field soils contained more pesticide residues (11 and 17 versus 3 and 11, respectively) and at higher concentrations than soils from organic fields (mean 6.6 and 10.5 versus 0.2 and 0.6 μg $kg^{-1}$, respectively), including systemic insecticides belonging to neonicotinoids, carbamate herbicides and broad-spectrum fungicides mostly from the azole family. A risk quotient ($RQ_i$) approach evaluated the toxicity of the pesticide mixtures in soil, assuming concentration addition. Based on measured concentrations, both conventional agricultural soils posed high risks to soil invertebrates, especially due to the presence of epoxiconazole and imidacloprid, whereas soils under organic farming showed negligible to medium risk. To confirm the outcome of the risk assessment, toxicity of the soils was determined in bioassays following standardized test guidelines with seven representative non-target invertebrates: earthworms (*Eisenia andrei*, *Lumbricus rubellus*, *Aporrectodea caliginosa*), enchytraeids (*Enchytraeus crypticus*), Collembola (*Folsomia candida*), oribatid mites (*Oppia nitens*), and snails (*Cantareus aspersus*). Collembola and enchytraeid survival and reproduction and land snail growth were significantly lower in soils from conventional compared to organic agriculture. The earthworms displayed different responses: *L. rubellus* showed higher mortality on soils from conventional agriculture and large body mass loss in all field soils, *E. andrei* showed considerable mass loss and strongly reduced reproduction, and *A. caliginosa* showed significantly reduced acetylcholinesterase activity in soils from conventional agriculture. The oribatid mites did not show consistent differences between organic and conventional farming soils. These results highlight that conventional agricultural practices pose a high risk for soil invertebrates and may threaten soil functionality, likely due to additive or synergistic "cocktail effects".

## 1. Introduction

Worldwide, around 5 million tons of chemical pesticides are used every year (FAO, 2019), belonging to more than 100 classes with various modes of action (Bernhardt et al., 2017). These substances can contaminate the soil, water and atmosphere, and may adversely affect biota (Yera et al., 2020; Silva et al., 2019; Gilliom, 2007). Due to their massive use in conventional agriculture and the persistence of some, multiple pesticide residues were found in European agricultural soils (Silva et al., 2019). Many of the pesticides detected by Silva et al. (2019) have been reported as persistent, bioaccumulative, and toxic, and listed as potential candidates for substitution and as priority pollutants (EC,

☆ This paper has been recommended for acceptance by Montes Marques.

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https://doi.org/10.1016/j.envpol.2022.119290
Received 20 January 2022; Received in revised form 17 March 2022; Accepted 8 April 2022
Available online 15 April 2022

2015).

Similar findings of pesticide residues in soils were recently reported by other authors (e.g., Chiaia-Hernandez et al., 2017; Neuwirthová et al., 2019). Pelosi et al. (2021) sampled 180 soils from France and detected 27 currently used pesticides among the 31 analyzed. Each soil sample contained residues of at least one pesticide. More and higher concentrations of pesticides were found in soils from conventional agriculture than from organic farming plots. Although, in general residue levels were fairly low, with few exceptions, soils always contained mixtures of insecticides, herbicides, and fungicides (Pelosi et al., 2021). This triggered the question of the ecotoxicological risk of mixtures of pesticide residues to non-target soil living organisms and the potential consequences for the whole ecosystem.

Among pesticide residues frequently found in soils, the herbicide glyphosate and its metabolite AMPA, the insecticide imidacloprid, the fungicide boscalid, and some azole fungicides like epoxiconazole and tebuconazole were measured at the highest concentrations (Pelosi et al., 2021; Karasali et al., 2019; Silva et al., 2019). Concentrations of these compounds occasionally exceeded their predicted environmental concentrations (PEC) in soil but were below their respective toxic concentrations measured for different endpoints on soil invertebrates (Silva et al., 2019). However, when such pesticides are present in mixtures, they may present an environmental risk to soil life at concentrations lower than those established upon single-chemical exposures. Based on the concentrations measured in agricultural top soils in Czech Republic, Vašíčková et al. (2019) showed that 35% of the studied sites presented mixture levels that could pose a risk to soil fauna. Pelosi et al. (2021) used a mixture toxicity approach based on the pesticide concentrations measured in soils sampled in different cropland habitats in France to assess the risks to earthworms. They reported a high risk of chronic toxicity to earthworms in 46% of the soils, while none of the soils from conventional cereal plots presented a low or negligible risk to earthworms. Although the legislation of active substances covers pesticides currently or formerly used in agriculture, the regulation is supported only by single-chemical risk assessment approaches. The recent findings of mixtures of pesticides in soils reaching levels likely to harm non-target fauna strongly suggest that they should be systematically monitored and that their risks to non-target organisms should be carefully considered.

Suitable indicators for the effects of pesticides in soil include earthworms (Bart et al., 2019; Pelosi et al., 2014; Paoletti, 1999), enchytraeids (Pelosi & Römbke, 2016), Collembola (Fountain & Hopkin, 2005), land snails (Druart et al., 2011, 2012; 2017) and oribatid mites (Fajana et al., 2019), which are broadly used in soil ecotoxicity tests. These soil invertebrates have been shown to be exposed to pesticides in the field (Daniele et al., 2018), but different soil invertebrates, as well as different species within the same taxonomic group, are not equally sensitive to pesticides (Pelosi et al., 2013; de Lima e Silva et al., 2017; Frampton et al., 2006). For example, for earthworms, Pelosi et al. (2013) found *Lumbricus terrestris* and *Aporrectodea caliginosa* to be more sensitive to pesticides and metabolites than *Eisenia fetida*. de Lima e Silva et al. (2017) showed that *Eisenia andrei* and the springtail *Folsomia candida* were the most sensitive to neonicotinoids among five species tested also including enchytraeids, oribatid mites and isopods. Biochemical biomarkers are currently used to better assess the toxicity of mixtures of pollutants as early and sensitive responses of organisms (Fontanetti et al., 2011). Carboxylesterase (CbEs), glutathione-S-transferase (GST) and acetylcholinesterase (AChE) are specific target enzymes able to highlight the sensitivity of organisms for organophosphate and carbamate poisoning. Thus, using a battery of test species, extended with some biochemical assays, will give an indication of their ecotoxicological responses to mixtures of toxic substances in the environment. It will also allow getting insight into the variability of sensitivities that could be explained by the differences in species physiology and environmental exposure conditions.

Predicting the response of organisms exposed simultaneously to mixtures, so to more than one chemical is one of the major current challenges in ecotoxicological risk assessment (Heys et al., 2016). Different suitable and ecologically relevant tools have already been proposed (e.g. risk quotient, toxic equivalent factor approach, toxic unit summation, hazard index) (Scholze et al., 2014). The toxic unit (TU) approach, first proposed by Sprague and Ramsay (1965) and developed by Höss et al. (2011), allows to evaluate the toxicity of complex mixtures in sediments for different key test organisms based on the concentration addition model (Broderius and Kahl, 1985; Sprague, 1970). Nevertheless, there are still doubts due to the suitability of those indicators for assessing the mixture toxicity and if the predicted or no observed effects could be matched to the effect in field-contaminated soils. For these reasons, it is important to couple these approaches with laboratory or field studies.

The aim of this study was to assess the toxicity to soil invertebrates of field-contaminated soils containing pesticide mixtures, using the first two lines of evidence (chemistry, toxicity) of the TRIAD approach for assessing contaminated soils (Jensen and Mesman, 2006). We first measured the concentrations of pesticides in two field soils under conventional farming (i.e. using pesticides) and two field soils under organic farming (i.e. with no pesticide treatment) from two agricultural areas in France. A risk quotient approach (Vašíčková et al., 2019) was applied, calculating the total summed risk quotient ($RQ_i$), to predict the toxicity of the soils from the measured pesticide concentrations. To confirm the outcome of this risk assessment, toxicity of the soils was assessed using seven invertebrate species representative of different non-target taxa of soil organisms: earthworms (*Eisenia andrei*, *Lumbricus rubellus, Aporrectodea caliginosa*), enchytraeids (*Enchytraeus crypticus*), Collembola (*Folsomia candida*), oribatid mites (*Oppia nitens*) and snails (*Cantareus aspersus*). We hypothesized that soils from the fields under conventional agriculture would (i) contain more pesticides and at higher concentrations, (ii) show a higher risk to soil invertebrates based on RQi, and (iii) be more toxic to soil organisms in bioassays than the soil collected in organic fields. We also expected that measured toxicity would confirm the calculated risk.

## 2. Material and methods

### 2.1. Sampling sites and soil analyses

The test soils were sampled (0–20 cm depth) in July 2018 from two different sites in France. The experimental trial La Cage was set up in 1997 at Versailles INRAE center, 15 km south-west of Paris (48°48′28.6″N 2°04′55.1″E). The site was under conventional agriculture before this date. The soil was a deep luvisol (FAO classification). Mons was situated at the Estrées-Mons INRAE experimental center (49°52N, 3°00E). The soil was a haplic luvisol (FAO classification). At both sites, the soil was taken from two cropping systems, a conventional system and an organic one. The crop rotation for both sites ISO, 2018 is reported in Table 1. A year before the sampling (2017), the conventional soil at La Cage was under pea cultivation and the organic one under wheat, while at Mons the conventional soil was under maize cultivation and the organic one under sugar beet. In 2018, on all sampled fields wheat was grown. The organic system was managed following the rules of the AB France label, without any use of synthetic pesticides or mineral fertilizers. In the conventional system, weeds and pests were controlled with pesticides; an overview of pesticide use on both fields in the year before sampling is given in Table SI-1 in the Supplementary Information. Since all soils were managed by research institutes, there is no evidence of historical contamination with metals or other chemicals.

Soils were dried at room temperature, disaggregated and homogenized before being sieved at 2 mm. A 500 g subsample was sent to the INRAE Arras soil laboratory for determination of soil properties, using standard methods (see the Supplementary Information). Based on the particle size distribution, soil types were determined using the texture triangle.

Pesticide residues were analyzed by a modified QuEChERS (Quick

**Table 1**
Main physicochemical characteristics of the soils from organic (ORG) and conventional (CONV) farming systems from the sampling sites of La Cage and Mons. Also mentioned is the crop rotation on the sampled farms. WHC = water holding capacity, OM = organic matter content.

| Site | La Cage | | Mons | |
|---|---|---|---|---|
| Farming system | ORG | CONV | ORG | CONV |
| Crop rotation | wheat, barley/ pea, alfalfa, wheat | pea, wheat, oilseed rape, wheat | corn, wheat, sugar beets, wheat, corn, wheat | winter barley, green beans, rapeseed, triticale, sugar beet, wheat |
| Clay (%) | 14.3 | 17.5 | 21.0 | 18.1 |
| Fine silt (%) | 21.0 | 21.8 | 28.6 | 28.5 |
| Coarse silt (%) | 40.7 | 39.5 | 44.2 | 46.3 |
| Fine sand (%) | 21.0 | 19.1 | 5.3 | 6.4 |
| Coarse sand (%) | 3.0 | 2.1 | 0.9 | 0.7 |
| pH ($H_2O$) | 6.95 | 7.35 | 7.74 | 7.66 |
| WHC (%) | 35.8 | 35.0 | 38.5 | 35.0 |
| OM (%) | 1.84 | 1.77 | 1.87 | 1.68 |
| C/N | 10.6 | 10.2 | 9.2 | 10.0 |

Easy Cheap Effective Rugged and Safe) extraction, followed by liquid chromatography coupled to tandem mass spectrometry (LCMS/MS) (Pelosi et al., 2021). Soil samples were analyzed for 31 pesticides (Table 2).

### 2.2. Risk characterization using the $RQ_i$ approach

To assess the potential risk of pesticide residues detected in the test soils, risk quotients ($RQ_i$) were calculated dividing the measured concentration ($MEC_{soil}$) of each pesticide by the predicted no-effect concentration in soil (PNEC). The calculation of PNEC was based on the most sensitive endpoint (NOEC for earthworm toxicity, or if not available $LC_{50}$) divided by an assessment factor according to Vašíčková et al. (2019). See the Supplementary Information for more details on the PNEC derivation. For each site the summed risk quotient ($\sum RQ_{site}$) was calculated by summing all $RQ_i$s assuming concentration addition (CA) in the pesticide mixture. According to Sanchez-Bayo et al. (2002), the $\sum RQ_{site}$ was classified into four levels: negligible (RQ < 0.01), low (0.01≤RQ < 0.1), medium (0.1≤RQ < 1) and high risk (RQ>). Finally, the contribution of each pesticide to the summed RQ was estimated.

### 2.3. Ecotoxicological assays

In the ecotoxicological tests, organic and conventional farming soils within the same site were compared. The natural standard LUFA 2.2 soil (Lufa Speyer, Germany), having approximately 1.6% organic carbon, and $pH_{CaCl2}$ between 5.03 and 5.87, was used a control to check for the health of the test organisms, and validity of the bioassays, notably the standardized ones. The field soils were tested at a moisture content of 65% of their WHC, the LUFA 2.2 soil at 45–50% of its WHC.

Seven bioassays were carried out, assessing survival, growth and/or reproduction of: (i) *Eisenia andrei* following OECD Guideline 222 (OECD, 2004a); (ii) *Lumbricus rubellus* following a modification of OECD Guideline 222 as described by Vijver et al. (2005); (iii) *Aporrectodea caliginosa* following OECD Guideline 222 with the modification suggested by Bart et al. (2018); (iv) *Enchytraeus crypticus* following OECD Guideline 220 (OECD, 2004b) with the modification suggested by Castro-Ferreira et al., 2012 (v) *Folsomia candida* following OECD Guideline 232 (OECD, 2009); (vi) *Oppia nitens* following a draft test guideline developed by Environment and Climate Change Canada (2018, 2019; see also Fajana et al., 2019); and (vii) *Cantareus aspersus* following ISO guideline 15952 (ISO, 2018).

*E. andrei*, *E. crypticus*, *F. candida* and *O. nitens* were obtained from pesticide-free laboratory cultures at the Department of Ecological Science, Vrije Universiteit Amsterdam. Adult earthworms of the species *L. rubellus* were acquired from a field collected source (Lasebo, V.O.F., The Netherlands), *A. caliginosa* from a breeding culture at Versailles INRAe center, and *C. aspersus* from the standardized rearing in the Chrono-Environnement laboratory.

The bioassays with *E. andrei*, *E. crypticus*, *F. candida*, and *O. nitens* were performed in a climate-controlled room at 20 ± 1 °C and a 12:12h light/dark photoperiod and illumination of 400–800 lux in the area of the test. The test with *L. rubellus* was carried out at 15 ± 1 °C with the same photoperiod and illumination conditions, the test with *A. caliginosa* at 15 ± 1 °C in the dark as recommended by Bart et al. (2018). The test with *C. aspersus* was performed at 20 °C, a humidity of 70%, and a photoperiod of 16/8h light/darkness.

A brief description of the bioassays follows herein; for full details, refer to the Supplementary Information.

#### 2.3.1. Earthworm tests

Earthworms (*Eisenia andrei, Lumbricus rubellus* and *Aporrectodea caliginosa*) with a fully developed clitellum were acclimated in LUFA 2.2 and weighted individually before being introduced randomly into glass jars with moist soil (800 mL jars + 300 g moist soil for 10 *E. andrei* or 5 *L. rubellus*; 1 L jars +500 g moist soil for 5 *A. caliginosa*). Four replicates were used for each test soil. The earthworms were fed with moist horse manure collected from horses that did not receive any veterinary pharmaceuticals for more than 3 months. After 4 weeks, all the surviving adults were removed manually from each jar, counted and weighed

**Table 2**
Pesticide residue concentrations (in μg $kg^{-1}$ d.w.) detected above limit of detection (LOD) in soils from organic (ORG) and conventional (CONV) farming systems from the sites of La Cage and Mons in France. LOQ: limits of quantification; ND: not detected.

| | LOD | LOQ | La Cage | | Mons | |
|---|---|---|---|---|---|---|
| | (μg $kg^{-1}$) | (μg $kg^{-1}$) | ORG | CONV | ORG | CONV |
| *Herbicides* | | | | | | |
| Napropamide | 0.02 | 0.05 | ND | 0.7 | 0.3 | 0.2 |
| Diflufenican | 0.02 | 0.06 | 0.3 | 28.2 | 0.3 | 1.1 |
| Acetochlor | 0.53 | 0.53 | ND | ND | ND | 1.9 |
| Dimethachlor | 0.02 | 0.08 | ND | 0.3 | ND | ND |
| Aclonifen | 0.77 | 2.50 | ND | 6.3 | <LOQ | 3.4 |
| Clomazone | 0.02 | 0.02 | ND | 0.3 | 0.1 | 46.3 |
| Metazachlor | 0.01 | 0.07 | ND | 0.2 | 0.2 | 0.3 |
| S-Metolachlor | 0.02 | 0.12 | <LOQ | <LOQ | <LOQ | 3.4 |
| CloquintocetMexyl | 0.02 | 0.03 | ND | ND | ND | ND |
| Cycloxydim | 0.12 | 0.14 | ND | ND | ND | ND |
| Pyroxsulam | 0.01 | 0.01 | ND | ND | ND | ND |
| Pendimethalin | 0.86 | 5.50 | ND | <LOQ | ND | <LOQ |
| *Fungicides* | | | | | | |
| Cyproconazole | 0.05 | 0.05 | ND | ND | 0.1 | 40.3 |
| Epoxiconazole | 0.00 | 0.14 | ND | 4.9 | 3.1 | 11.4 |
| Metconazole | 0.06 | 0.12 | ND | 3.3 | ND | 0.5 |
| Prochloraz | 0.00 | 0.03 | ND | ND | 0.4 | 0.9 |
| Propiconazole | 0.01 | 0.01 | ND | ND | <LOQ | 0.8 |
| Boscalid | 0.00 | 0.06 | 0.2 | 21.3 | 1.2 | 8.9 |
| Pyraclostrobin[a] | 0.01 | 0.03 | ND | ND | <LOQ | 0.2 |
| Fenpropidin | 0.08 | 0.36 | ND | ND | ND | ND |
| Metrafenone | 0.02 | 0.12 | <LOQ | <LOQ | <LOQ | ND |
| Fluoxastrobin | 0.02 | 0.06 | ND | ND | ND | ND |
| *Insecticides* | | | | | | |
| Pirimicarb | 0.00 | 0.01 | 0.1 | 0.3 | 0.2 | 0.2 |
| Imidacloprid | 0.02 | 0.41 | <LOQ | 7.6 | 0.6 | 58.5 |
| Thiamethoxam | 0.01 | 0.03 | <LOQ | ND | ND | 0.2 |
| Thiacloprid | 0.00 | 0.01 | ND | ND | ND | ND |
| Cypermethrin | 0.66 | 0.66 | ND | ND | ND | ND |
| Bifenthrin | 0.86 | 0.86 | ND | ND | ND | ND |
| LambdaCyhalothrin | 3.00 | 3.00 | ND | ND | ND | ND |
| TauFluvalinate | 0.03 | 0.43 | ND | ND | ND | ND |
| Deltamethrin | 0.42 | 2.70 | ND | ND | ND | ND |

[a] Fungicide and plant growth regulator.

individually to assess weight change. Surviving *A. caliginosa* were frozen for biomarker measurements.

The soil of each test jar was carefully returned into the jars and incubated for another 4 (*E. andrei*) or 6 weeks (*L. rubellus*, *A. caliginosa*) in the same conditions to allow the hatching of the cocoons. After this period, for *E. andrei* and *L. rubellus*, the jars were placed in a water bath (Julabo TW12) at 60 °C to extract and count the juveniles emerging from the soils. For *A. caliginosa*, after 6 weeks, juveniles were hand sorted and the soil of each box was wet sieved through a 1-mm mesh size in order to retain the cocoons, as recommended by Bart et al. (2018). The collected cocoons were incubated on wet filter paper in petri dishes at 20 °C until hatching. The number of juveniles is the sum of the hand-sorted individuals and the hatchlings in the petri dishes.

Since according to the literature, *A. caliginosa* was thought to be the most sensitive among the three tested earthworm species (Pelosi et al., 2013), biomarkers were tested only on this species. For that purpose, in surviving *A. caliginosa*, the activities of B-esterases (acetylcholinesterase: AChE, and carboxylesterases: CbEs) and glutathione-S-transferase (GST) were measured following the methods detailed in the Supplementary Information.

#### 2.3.2. Enchytraeid tests

For each test soil, five replicates were tested containing 10 sexually mature *Enchytraeus crypticus* (with a clearly visible white clitellum) randomly assigned to a 100 mL glass jar with approximately 30 g moist soil. Individuals were fed adding few grains of oat flakes (Instant, Brinta, Netherlands) to each jar. After 3 weeks, ethanol (96%) was added to each test jar to fix the animals, and 1% Bengal rose solution in ethanol for staining the organisms. After 160 μm sieving, the pink colored adults and juveniles of enchytraeids were counted from photographs using ImageJ2.

#### 2.3.3. Collembola test

For each test soil, five replicates were prepared, each containing ten juvenile *Folsomia candida* randomly assigned to a 100 mL glass jar filled with approximately 30 g moist soil, and a few grains of dry baker's yeast added for food. After 4 weeks of exposure, demineralized water was added to the test jars to transfer their content to a plastic beaker, allowing the animals to float and to be photographed with a camera. All animals were counted from the photographs using ImageJ2.

#### 2.3.4. Oribatid mite test

For each test soil, five replicates were prepared, each containing fifteen adult age-synchronized mites (*Oppia nitens*) mites in approximately 20 g moist soil in a 40 mL polyethylene test jar. A few grains of dry baker's yeast were provided for food. After 5 weeks of incubation, all mites were extracted from the test soil using a Tullgren apparatus, operated with a temperature gradient of 30 °C in the upper and 5 °C in the lower compartment. After 2 days extraction, the number of adults, sub-adults and nymphs were counted.

#### 2.3.5. Land snail test

Three replicate experimental boxes consisting of two transparent polystyrene containers (one top and one bottom to ensure enough space for the growing individuals) with 1 cm layer of moist test soil at the bottom (250 g of dry soil) were prepared for each test soil. Juvenile *Cantareus aspersus* of around 1 g were woken up from estivation and fed with Helinove® Biaucomplet B. After 24 h, the food was removed so the snails emptied their gastrointestinal tract. An initial number of twelve snails were weighted, measured and introduced in each box and food Helinove® Biaucomplet B was provided *ad libitum*.

The experiment was performed under static conditions (ISO, 2018). Three times a week the food was renewed, the sides of the boxes were cleaned and the feces removed. Soil moisture content was maintained by spraying water when necessary. To test for effects on growth, individual snails were weighted to the nearest 0.01 mg and the maximum shell diameter was measured weekly. The exposure lasted for 28 days.

#### 2.3.6. Statistical analyses

Toxicity data were checked for their Gaussian distribution with Shapiro-Wilk's test. The unpaired *t*-test was performed to evaluate, for each sampling site, the statistical significance of differences in the endpoints measured (i.e. survival, reproduction, weight change, biomarker responses) on the test organisms between soils collected from the organic and conventional farming plots.

For the land snails, differences in the increase of fresh body mass or shell diameter between sites or types of farming were analyzed using generalized additive mixed models (GAMM) (Zuur et al., 2009). The models were fitted using the Gaussian family with identity link on the log-transformed fresh mass or shell diameter data to meet assumptions of normality and homoscedasticity. The variables "site" (La Cage or Mons), "type of farming" (conventional or organic) and "time" (duration of exposure in days) were used as fixed explanatory factors with interaction between "time" and "type of farming", and the experimental box of exposure was added as a random factor. A smooth term was applied on time with the parameter K limited to 5. The interactions between the factors "time" and "type of farming" were used because we assumed that growth pattern might be modulated by pesticides regardless of the site. The models were fitted using the "gamm4" function and the marginal $R^2$ was computed using the function "r2beta" in the software "R" version 4.0.3 (packages gamm4, mgcv, lme4, r2glmm) (R Core Team 2020). Differences were considered statistically significant when $p < 0.05$.

## 3. Results

### 3.1. Soil characteristics and pesticides residues

The soils from organic and conventional farming sampled within the same site had similar properties and texture, although pH was slightly higher in the conventional compared to the organic farming soil at La Cage (Table 1). Among the thirty-one pesticides analyzed, eighteen were detected, eleven were not detected and two were under LOQ in all the soils (Table 2). Among the eighteen pesticides detected, two were systemic insecticides belonging to neonicotinoids (imidacloprid, thiamethoxam), one carbamate (pirimicarb), eight herbicides (clomazone, dimethachlor, aclonifen, acetochlor, napropamide, metazachlor, S-metolachlor, diflufenican) and seven were broad spectrum fungicides mostly from the azole family (cyproconazole, metconazole, epoxiconazole, propiconazole, boscalid, prochloraz, pyraclostrobin). Conventional agricultural soils contained more pesticide residues and at higher concentrations than soils from organic fields (Table 2). At La Cage, three and eleven active substances were detected in organic (mean concentration 0.2 μg $kg^{-1}$ d. w.) and conventional soils (mean 6.6 μg $kg^{-1}$ d. w.), respectively. Ten active substances were detected in organic soils from Mons (mean 0.6 μg $kg^{-1}$ d. w.) and seventeen in conventional soils from the same site (mean 10.5 μg $kg^{-1}$ d. w.).

Among the active substances detected, cyproconazole, epoxiconazole, clomazone, boscalid, S-metolachlor and imidacloprid, showed the highest concentrations in soil from Mons, while metaconazole, epoxiconazole, boscalid, diflufenican and aclonifen showed the highest concentration in soil from La Cage (Table 2). Other active substances were present at low levels (<1 μg $kg^{-1}$) at both investigated sites (Table 2).

### 3.2. RQ approach for soils risk assessment

Table SI-2 shows the Predicted No-Effect Concentrations (PNECs) that were used to derive RQ values for the risk of the detected pesticides in the field soils. The resulting RQ values for each pesticide and each soil are reported in Table 3. The soil from organic farming fields represented a negligible risk ($\sum RQ_{site} = 0.002$) at La Cage and a medium risk ($\sum RQ_{site} = 0.358$) at Mons, while both soils under conventional faming

represented a high risk (= 1.11 at La Cage, $\sum RQ_{site}$ = 5.65 at Mons) (Table 3). The fungicides epoxiconazole (44.2%) and boscalid (16.0%), together with the insecticide imidacloprid (38.5%) contributed to 98.7% of the overall risk for soil under conventional farming from La Cage. Epoxiconazole (86.6%) and imidacloprid (9.42%) contributed to 96% of the overall risk for soil under organic farming from Mons. The insecticides clomazone (10.2%) and imidacloprid (58.2%) and the fungicides cyproconazole (9.52%) and epoxiconazole (20.2%) contributed to 98.1% of the overall risk for soil under conventional farming from Mons. The other compounds had only a minor or negligible contribution to the $\sum RQ_{site}$.

### 3.3. Ecotoxicological assays

The bioassays in the natural control LUFA 2.2 soil showed that all test animals were healthy as their performance met the validity criteria set by the OECD guidelines (Figs. 1–6). For L. *rubellus* and *A. caliginosa* such a check was not possible as no guideline is available for these species; nevertheless, control survival in natural LUFA soil 2.2 was 100%.

#### 3.3.1. Earthworm toxicity tests

No mortality was observed for *E. andrei* and *A. caliginosa* in any of the test soils. *L. rubellus* showed 5% mortality in both soils from organic farming systems and 35% and 50% for La Cage and Mons soils under conventional farming, respectively (Fig. 1); due to the large variation between replicates this difference was not statistically significant. At the end of the test *L. rubellus* showed a mean weight gain of 6.5% in LUFA 2.2 soil, but considerable weight loss in the soils from both organic and conventional farming with no significant differences between soils ($p > 0.05$; Fig. 1). No juvenile was produced in any of the test soils.

After 28 days of exposure, *E. andrei* showed a mean (±SD; n = 4) weight gain of 15 ± 4.0% in the LUFA 2.2 soil (Fig. 2). Significant differences ($p < 0.001$) in mean weight gain were detected between organic and conventional farming soils for both sites with a small weight gain (+4–5%) in the organic farming soil, and a strong weight loss (24–30%) in conventional farming soils (Fig. 2).

Juvenile numbers of *E. andrei* were significantly higher in both organic farming soils compared to the conventional ones. The mean (±SD; n = 4) numbers of juveniles were 25 ± 4.6 and 14 ± 1.5 for La Cage ($p < 0.05$) and 26 ± 2.1 and 15 ± 1.9 for Mons ($p < 0.01$) for soils under organic and conventional farming, respectively (Fig. 2).

*A. caliginosa* showed a mean (±SD; n = 4) weight gain of 16.6 ± 8.4% in LUFA 2.2 soil. In all soils, the weight of *A. caliginosa* increased during 4 weeks, and with no significant differences ($p > 0.05$) between the two farming systems for both sites (Fig. 2). No differences were found in the number of juveniles between the two farming systems for both sites ($p > 0.05$; Fig. 2).

Four-week exposure of *A. caliginosa* to organic or conventional soil from La Cage or Mons did not significantly affect AChE or GST activity (Figure SI-1). However, a lower (14.7 ± 1.3%; $p < 0.05$) total CbE activity was observed after exposure to the conventional soil from Mons compared to the organic soil, while no significant decrease (6.8 ± 0.7%) was noted after exposure to the conventional soil from La Cage (Figure SI-1).

#### 3.3.2. Enchytraeid, Collembola, oribatid mite and snail toxicity tests

After 21 days, survival of *E. crypticus* was significantly lower in the soil from conventional than from organic farming for Mons ($p < 0.05$) but not for La Cage ($p > 0.05$). Juvenile numbers were significantly higher in soils from organic farming for both sites ($p < 0.01$ for La Cage and $p < 0.001$ for Mons) (Fig. 3).

After 28 days, survival of *F. candida* was significantly higher in soils from organic compared to conventional farming for both La Cage ($p <$

**Table 3**
Total estimated risk of soils from organic (ORG) and conventional (CONV) farming systems from the sites of La Cage and Mons in France. The summed risk quotient ($\sum RQ_{site}$) was calculated by summing up the risk quotients for the pesticides quantified in each soil ($RQ_i$). For each compound the contribution to $\sum RQ_{site}$ (in %) is provided in brackets, with values > 10% of contribution to the estimated risk reported in bold. The calculated $\sum RQ_{site}$ values were classified into four risk levels according to Sanchez-Bayo et al. (2002): high risk ($\sum RQ_{site}$ >1), medium risk ($0.1 \leq \sum RQ_{site} < 1$), low risk ($0.01 \leq \sum RQ_{site} < 0.1$) and negligible risk ($\sum RQ_{site} \leq 0.01$). ND = pesticide not detected. The values of predicted no effect concentration (PNEC) are given in mg/kg dry soil. See Table SI-2 and the SI for the derivation of PNEC values.

| Site | | La Cage | | | | Mons | | | |
|---|---|---|---|---|---|---|---|---|---|
| Farming system | | ORG | | CONV | | ORG | | CONV | |
| | PNEC | RQi | (%) | RQi | (%) | RQi | (%) | RQi | (%) |
| **Herbicides** | | | | | | | | | |
| Napropamide | 3.0 | ND | (0) | 0.000 | (0.02) | 0.000 | (0.03) | 0.000 | (0) |
| Diflufenican | 4.86 | 0.000 | (0) | 0.006 | (0.52) | 0.000 | (0.02) | 0.000 | (0) |
| Acetochlor | 1.06 | ND | (0) | ND | (0) | ND | (0) | 0.002 | (0.03) |
| Dimethachlor | 0.7 | ND | (0) | 0.000 | (0.04) | ND | (0) | ND | (0) |
| Aclonifen | 4.5 | ND | (0) | 0.001 | (0.13) | ND | (0) | 0.001 | (0.01) |
| Clomazone | 0.08 | ND | (0) | 0.004 | (0.34) | 0.001 | (0.35) | 0.579 | (10.2) |
| Metazachlor | 5.0 | ND | (0) | 0.000 | (0) | 0.000 | (0.01) | 0.000 | (0) |
| S-Metolachlor | 0.254 | ND | (0) | ND | (0) | ND | (0) | 0.013 | (0.24) |
| **Fungicides** | | | | | | | | | |
| Cyproconazole | 0.075 | ND | (0) | ND | (0) | 0.001 | (0.37) | 0.537 | (9.52) |
| Epoxiconazole | 0.01 | ND | (0) | 0.490 | (44.2) | 0.310 | (86.6) | 1.140 | (20.2) |
| Metconazole | 2.0 | ND | (0) | 0.002 | (0.15) | ND | (0) | 0.000 | (0) |
| Prochloraz | 0.42 | ND | (0) | ND | (0) | 0.001 | (0.27) | 0.002 | (0.04) |
| Propiconazole | 0.0833 | ND | (0) | ND | (0) | ND | (0) | 0.010 | (0.17) |
| Boscalid | 0.12 | 0.002 | (100) | 0.178 | (16.0) | 0.010 | (2.79) | 0.074 | (1.31) |
| Pyraclostrobin[a] | 2.31 | ND | (0) | ND | (0) | ND | (0) | 0.000 | (0) |
| **Insecticides** | | | | | | | | | |
| Pirimicarb | 0.546 | 0.000 | (0) | 0.001 | (0.05) | 0.000 | (0.10) | 0.000 | (0.01) |
| Imidacloprid | 0.0178 | ND | (0) | 0.427 | (38.5) | 0.034 | (9.42) | 3.280 | (58.2) |
| Thiamethoxam | 0.534 | ND | (0) | ND | (0) | ND | (0) | 0.000 | (0.01) |
| $\sum RQ_{site}$ | | 0.002 | | 1.11 | | 0.358 | | 5.65 | |
| Risk level | | Negligible risk | | High risk | | Medium risk | | High risk | |

[a] Fungicide and plant growth regulator.

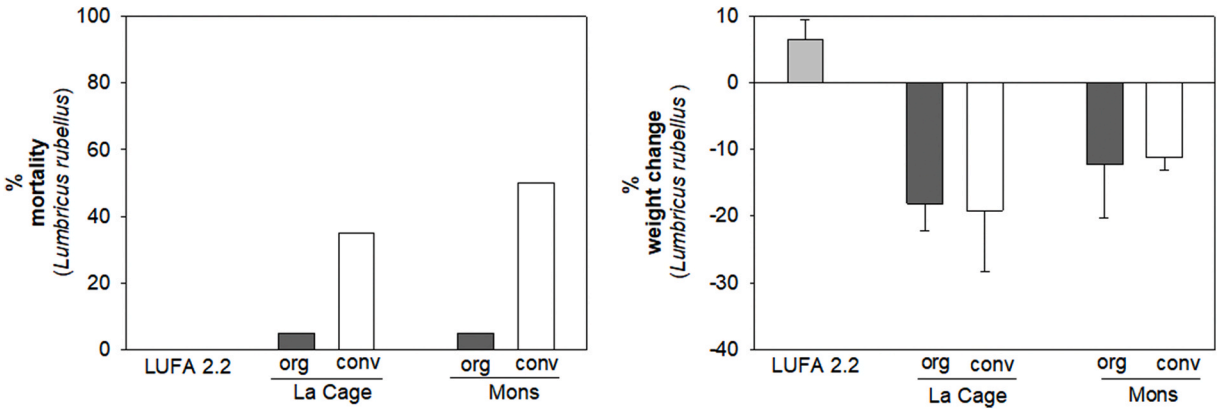


**Fig. 1.** Mortality (%) (left) and mean (±SD; n = 4) weight change (%) (right) of *Lumbricus rubellus* exposed for 28 days to natural LUFA 2.2 soil (LUFA 2.2) and soils from organic (org) and conventional (conv) farming from La Cage and Mons. No statistically significant differences in weight change between the organic and conventional farming soils from the same area were found (unpaired *t*-test; $p > 0.05$).

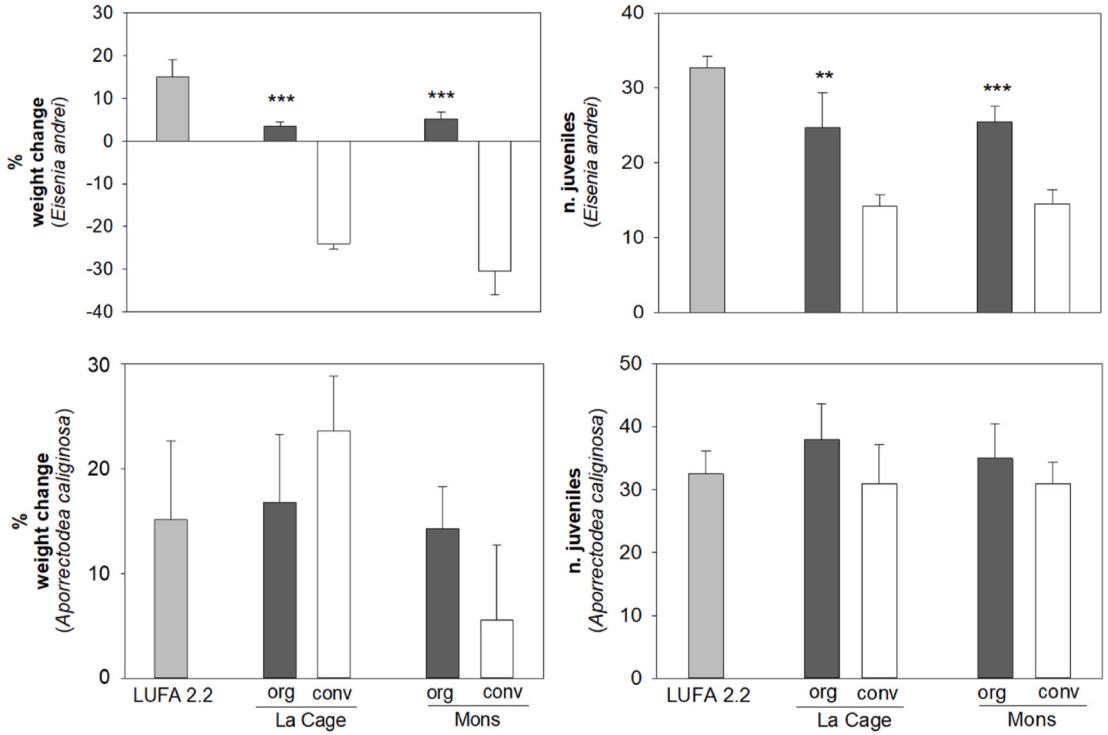


**Fig. 2.** Mean (±SD; n = 4) weight change of surviving adults (%) (left) and number of juveniles (right) of *Eisenia andrei* (top) and *Aporrectodea caliginosa* (bottom) after 28 (+28) days exposure to natural LUFA soil 2.2 (LUFA 2.2), soil from organic (org) and conventional (conv) farming from the areas of La Cage and Mons in France. The asterisks indicate the statistically significant differences between the organic and conventional farming soils from the same area (unpaired *t*-test; **$p < 0.01$; ***$p < 0.001$).

0.001) and Mons ($p < 0.01$). Significantly higher juvenile numbers were also found in the soils from organic compared to conventional farming, for both sites ($p < 0.001$) (Fig. 4).

The oribatid mites developed faster than expected in all soils, with some of the juveniles produced already being adult after 5 weeks of incubation. This hampered assessment of survival as in most test jars more than 15 adult mites were found. Using population size, the sum of all juvenile, sub-adult and adult mites, as endpoint, on average (±SD; n = 5) 204 ± 46.6 mites were found in the LUFA 2.2 soil (Fig. 5). A similar number was found in the La Cage soil from conventional agriculture, but lower numbers in all other soils. The number of mites was significantly lower ($p < 0.005$) in the organic compared to conventional farming soil from La Cage, but no such difference was found for Mons ($p > 0.05$; Fig. 5).

Three snails (2.5%) died during the 28-day exposure, all in La Cage organic soils, their death being due to errors during handling. After 28 days, mean (±SD; n = 3) snail fresh body mass gain was of 3.88 ± 0.36 g in LUFA 2.2 soil, 3.91 ± 0.43 g in conventional soils and 4.11 ± 0.41 g in organic soils (Fig. 6). Corresponding increases in shell diameter (final shell diameter size – initial shell diameter size) were 10.7 ± 0.78, 10.8 ± 0.75 and 11.3 ± 0.78 mm, respectively (Fig. 6). No significant differences in mass or shell diameter growth were detected between the sites Mons or La Cage, while the growth was higher under organic than conventional farming for both mass and shell diameter ($p < 0.001$; Table SI-4, Fig. 6).

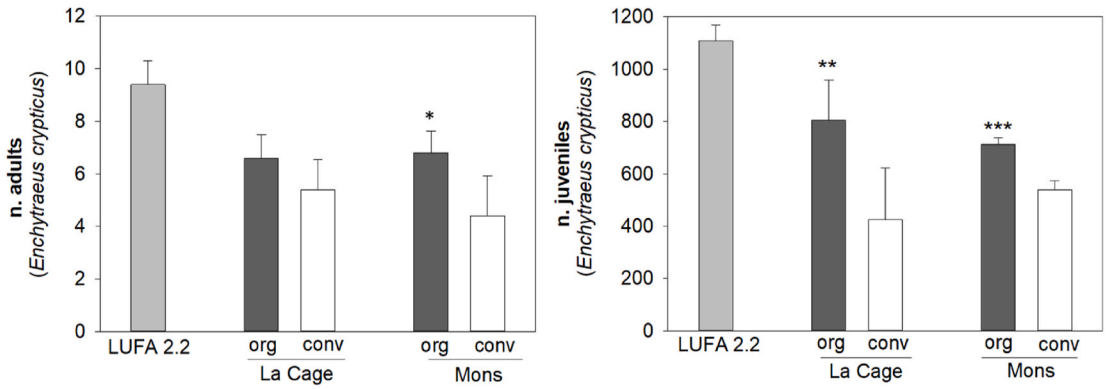


**Fig. 3.** Mean (±SD; n = 5) survival (number of adults out of 10; left) and reproduction (number of juveniles; right) of *Enchytraeus crypticus* after 21 days exposure to natural LUFA 2.2 soil (LUFA 2.2) and soils from organic (org) and conventional (conv) farming from La Cage and Mons. The asterisks indicate the statistically significant differences between the organic and conventional farming soils from the same area (unpaired *t*-test; *p < 0.05; **p < 0.01; ***p < 0.001).

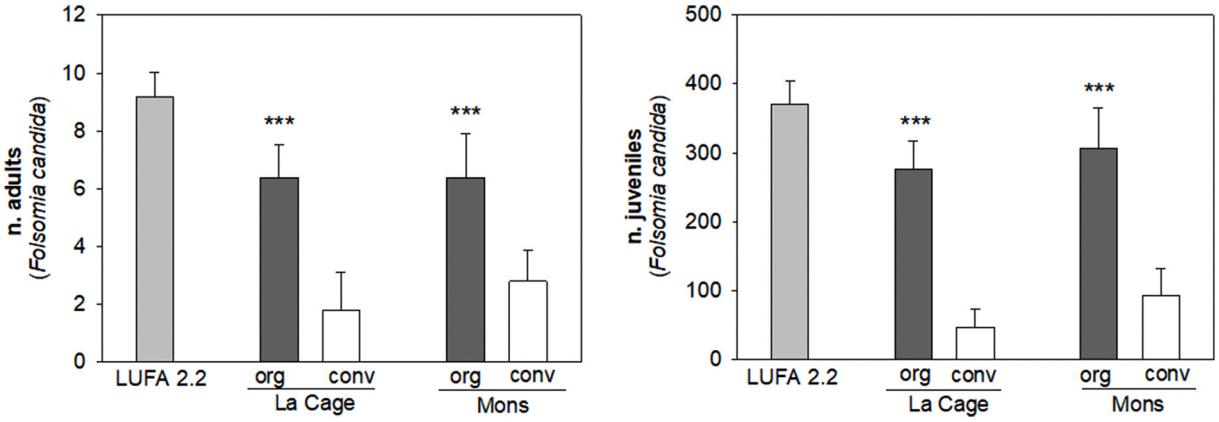


**Fig. 4.** Mean (±SD; n = 5) survival (number of adults out of 10; left) and reproduction (number of juveniles; right) of *Folsomia candida* after 28 days exposure to natural LUFA 2.2 soil (LUFA 2.2); and soils from organic (org) and conventional (conv) farming from La Cage and Mons. The asterisks indicate the statistically significant differences between the organic and conventional farming soils from the same area (unpaired *t*-test; ***p < 0.001).

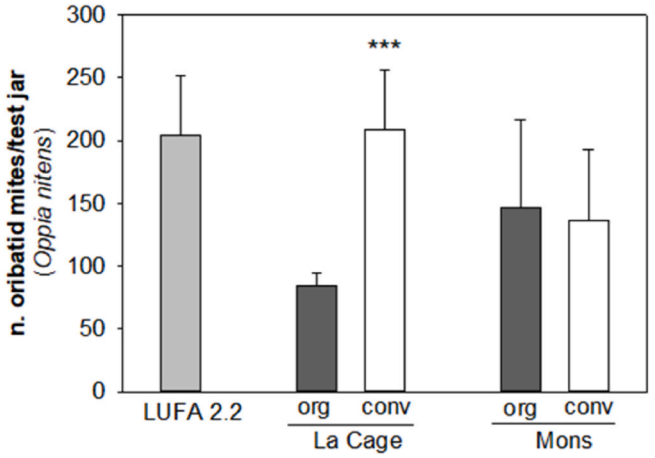


**Fig. 5.** Mean (±SD; n = 5) total numbers of oribatid mites (*Oppia nitens*; sum of adults, sub-adults and juveniles) per test jar after 5 weeks of incubation in LUFA 2.2 soil and soils from organic (org) and conventional (conv) farming from La Cage and Mons. The asterisks indicate the statistically significant differences between the organic and conventional farming soils from the same area (unpaired *t*-test; ***p < 0.005). Tests started with 15 adult mites per test jar.

## 4. Discussion

This study addressed the ecotoxicological effects and risks of agricultural field soils with different management systems using a panel of soil invertebrate toxicity tests. Soils from fields under conventional farming had higher negative effects on survival, growth and reproduction of several soil organisms compared to soils from organic fields. This was in accordance with the outcome of the RQ assessment that showed that the conventional soils may pose a high risk to soil invertebrates whereas soils from organic fields showed a negligible or medium risk level. As demonstrated by the ecotoxicological tests, the higher content of pesticide residues explained the observed negative effects, in particular for soils from the conventional fields.

The pesticide residue levels measured varied greatly. In particular, both conventional and organic soils from Mons showed higher concentrations of pesticide residues than those from La Cage (Table 2). These differences between the two studied sites were probably due to agricultural practices that were used in the two experimental trials. At Mons, the use of pesticides was stopped in 2012 while the trial has been set up in 1997 at La Cage. This could explain why less pesticide residues were found in the organic system at La Cage than at Mons. For the conventional systems, the crop rotations (e.g., sugar beet at Mons) and pesticide applications (sprayed and seed treatment at Mons, only sprayed at La Cage) could explain the differences between the studied sites (see Table SI-1). As illegal pesticide use can be excluded, the residues in the organic farming soil could result from the persistence of pesticides that were applied before the establishment of the trial (Pelosi et al., 2015).

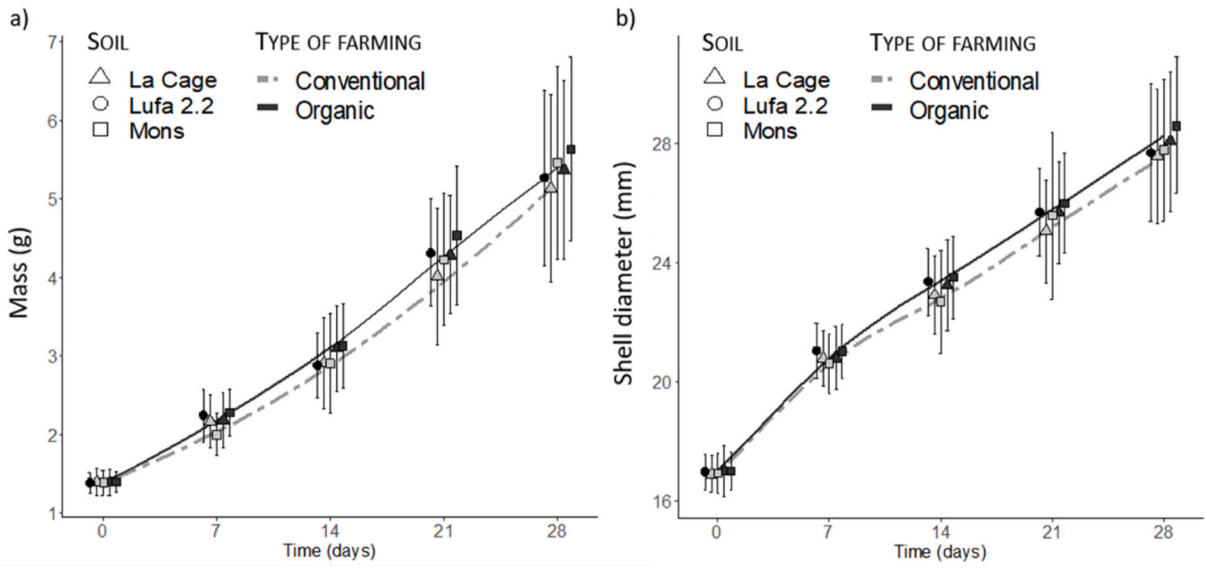


**Fig. 6.** Mean (±SD; n = 834) land snail (*Cantareus aspersus*) body mass (left) and shell diameter (right) during the 28 days exposure to natural LUFA 2.2 soil and soils under conventional and organic farming from La Cage and Mons. The lines for organic and conventional farming were fitted according the modelling on log-transformed mass or shell diameter data where growth significantly differed according to farming type (see Table SI-4 for corresponding statistical analysis).

These residues may also be the result of surface runoff or volatilization of pesticides as well as pesticide drift from wind and soil particles from surrounding treated plots (Bernasconi et al., 2021), as conventional fields were located 50 m from the organic field at Mons and only 5 m at La Cage.

Several soil abiotic properties such as soil texture, organic matter content, pH, soil moisture content and nutrient status can directly influence the bioavailability, degradation and effects of pesticides on soil organisms (Kalia and Gosal, 2011; Gill and Garg, 2014). The soils from conventional and organic farming however, only slightly differed in texture (% clay, silt, sand) and abiotic properties (pH, WHC, OM and C/N) within the areas of Mons or La Cage (Table 1). These differences in soil properties were too small to explain for differences in fauna responses evidenced in this study between conventional and organic farming soils for Mons or La Cage.

The test organisms, selected to represent the major taxonomic groups of soil invertebrates, showed different responses to the conventional farming soils with springtails being more sensitive than enchytraeids, oribatid mites or land snails. Among the earthworms, different responses were observed, with *L. rubellus* showing high mortality on the soils from conventional farming and large body mass loss on all soils. *E. andrei* survived exposure in all soils but showed considerable mass loss and strongly reduced reproduction on the soils from conventional agriculture. *A. caliginosa* showed no effect for the endpoints assessed in all the soils, but did show negative effects on CbE activity. The presence of high concentration of imidacloprid in the conventional soil can explain that result.

The large body mass loss of the earthworms *L. rubellus* on all field soils and the absence of reproduction also in the clean LUFA 2.2 soil suggests their condition or health was not optimal or that test duration and/or feeding conditions were not adequate. These earthworms were obtained from a commercial supplier, who collected them from the field, so it is difficult to determine whether they had already been exposed to contaminants. The earthworms were kept in the laboratory for several weeks at rather high density before being used in the test, which may also have affected their condition. This may have contributed to the high biomass loss in all field soils, and also triggered a higher sensitivity in the soils from conventional farming. In spite of its limitations, the test with *L. rubellus* adds to the conclusion that the soils from conventional farming pose a risk to earthworms.

*A. caliginosa* is reported to be particularly sensitive to contaminants, notably to pesticides (Pelosi et al., 2013) or metals (Khalil et al., 1996; Maity et al., 2018). The mass loss and the absence of reproduction of *L. rubellus* on all field soils make the comparison between both species difficult, although there was a higher mortality on the conventional farming soils. Carter et al. (2016) concluded that chemical uptake by earthworms cannot be generalized between species because the influence of species' traits can vary depending on the nature of the studied chemicals. Our test soils contained mixtures of pesticides, making effects much more difficult to explain as they can be caused by a combination of different factors, including the way the earthworms interact with the soil and encounter the pesticide (bioavailability, uptake kinetics), capacity of biotransformation of different pesticides, etc. As a consequence, it may not be as straightforward to expect *A. caliginosa* to be most sensitive, and this may also explain the differences between species in our study.

According to the RQ approach, the high toxicity of the conventional agricultural soil from Mons and La Cage may partly be explained by the relatively high levels of imidacloprid and epoxiconazole that together contributed most to the estimated risk. Cyproconazole, boscalid and clomazone also added to the risk in these soils (Table 3). These findings match with those of Pelosi et al. (2021), although the soil samples were not the same.

Although the estimated risk of the conventional farming soil from Mons was the highest (Table 3), both soils showed a high toxicity to some of the test species, especially for the springtail *F. candida*. The RQ approach in this study used data on earthworm toxicity, which may be a suitable indicator to assessing the risk to soil invertebrates of fungicides but not necessarily for insecticides. De Lima e Silva et al. (2017, 2020) showed that springtails are more sensitive to neonicotinoids than earthworms. EC10 values for the reproduction toxicity of imidacloprid, pure and in a commercial formulation, to different springtail species ranged between 30 and 750 μg a. s. $kg^{-1}$ dry LUFA 2.2 soil (de Lima e Silva et al., 2021), while for the earthworm *E. andrei* EC10 was 250–300 μg a. s. $kg^{-1}$ dry LUFA 2.2 soil (de Lima e Silva et al., 2020). The lowest EC10 is two times lower than the imidacloprid concentration measured in the conventional farming soil from Mons (Table 2), while that for earthworms is 4 times higher. This confirms the important contribution of imidacloprid to the toxicity of the conventional farming soil from Mons. Imidacloprid concentration in the conventional farming soil from La Cage, however, was almost 10 times lower. So, the high toxicity to springtails of the La Cage soil cannot be explained only from the

presence of imidacloprid, and suggests a "cocktail effect" induced by the mixture of pesticides being additive or synergistic. For thiamethoxam, the EC10 for reproduction toxicity to *F. candida* was 100 μg $kg^{-1}$ dry soil (de Lima e Silva et al., 2020), which is 500 times higher than its concentration in the conventional farming soil from Mons (Table 2). Only limited information is available on the toxicity to springtails of the other pesticides detected.

Very few data are available on the toxicity to enchytraeids of the detected pesticides. The EC20 for the effects of imidacloprid on the reproduction of *E. crypticus* was 1200 μg $kg^{-1}$ (de Lima e Silva et al., 2017), which is much higher than the concentrations measured in the field soils (Table 2). The lack of data on the toxicity of pesticides to enchytraeids makes it hard to explain the high toxicity of the field soils, but additive or synergistic mixture effects cannot be excluded. This needs further investigation.

Despite emerging evidences that new classes of insecticides like neonicotinoids are also involved in AChE reduction (Györi et al., 2017), the absence of inhibition in AChE activity in earthworms can be explained by the fact that no organophosphate insecticides and only low concentrations of the carbamate pirimicarb were detected in the field soils. CbEs are inhibited by organophosphorus insecticides and play an important role in the metabolism of many agrochemicals and pharmaceuticals products (Wheelock et al., 2005). The inhibition of CbE activities occurred in conventional soils with high imidacloprid concentrations. CbE and GST activities have been previously shown to exhibit differential response in *A. caliginosa*, with CbE being inhibited while GST activity was not altered following 28 days exposure to imidacloprid (Wang et al., 2019). The absence of GST response is consistent with previous observations on *A. caliginosa* exposed to a fungicide, a herbicide or their mixture (Givaudan et al., 2014). The results obtained on the biochemical biomarkers support the observations made on survival and reproduction, and suggest that *A. caliginosa* was able to sustain long-term exposure in the soils from La Cage or Mons, regardless the farming practices. Thus, *A. caliginosa* would be insensitive or able to acclimatize (e.g. through metabolism, Givaudan et al., 2014) to the field exposure scenarios under the conventional farming systems. Given species-specific responses and metabolism capabilities, testing on several species is useful to properly assess risk assessment and to adapt management decisions.

Although several studies have determined the toxicity of pesticides to land snails via oral or topical application, only few have assessed these effects via contaminated soils (Coeurdassier et al., 2002; Druart et al., 2011, 2012; 2017; Fritsch et al., 2011; Mazzia et al., 2011). Moreover, the use of land snails for risk assessment studies of currently used pesticides remains scarce. Few studies have addressed the effects of pesticide mixtures on land snails, most of them focused on one single compound. In our study, *C. aspersus* exposed to conventional farming soils exhibited a slightly lower growth, which was unexpected considering the low pesticide concentrations measured in these soils compared to the available toxicity data for land snails. El-Gendy et al. (2019) found a decreased growth of *Theba pisana* snails exposed to food containing 15.69 mg $kg^{-1}$ thiamethoxam. Smina et al. (2016) found reduction in AChE activity for *C. aspersus* fed with lettuce soaked in 100 mg $L^{-1}$ thiamethoxam, and increases in GST and catalase (CAT) activities when exposed to food spiked with 50 and 100 mg $L^{-1}$ thiamethoxam, respectively. All these concentrations by far exceed the thiamethoxam levels in the conventional farming soils ($\leq$0.0002 mg $kg^{-1}$). Radwan and Mohamed (2013) reported modifications in AChE, CAT and GST activities and depletion of lipid and glycogen content of *C. aspersus* when topically exposed to imidacloprid at $\geq$0.021 mg $snail^{-1}$ (48h LD50 = 0.109 mg $snail^{-1}$). Although these alterations may also have an adverse effect on snail growth, exposure is expressed in different units hampering a proper comparison. In all cases, the doses offered through dietary or topical exposure seem high compared to the imidacloprid levels found in our soils under conventional farming (0.0585 mg $kg^{-1}$ in Mons and 0.0076 mg $kg^{-1}$ in La Cage). Based on the low concentrations of imidacloprid and thiamethoxam, as well as the lack of toxicity data for the other pesticides present in the mixture, we are not able to conclude whether the lower body mass and shell diameter gain in snails exposed to conventional soils may be due to (i) the presence of a single or multiple compounds in the soil at toxic concentrations for snails, or (ii) the occurrence of a mixture of several pesticides at low concentrations, supporting the possibility of additive or synergistic effects among multiple compounds as for the other models in this work, which can lead to a higher toxicity of the contaminated matrix (Uwizeyimana et al., 2017).

Also other studies have compared effects of organic and conventional farming systems on soil fauna (see e.g., Harkes et al., 2019). The results often are not so clear as several factors differ between systems under organic and conventional management (e.g. crop rotations, soil tillage, organic matter inputs, surrounding landscape), which can hinder the interpretation of the role of pesticides (Hole et al., 2005; Flohre et al., 2011). Yet, a general trend was found for a higher abundance, diversity and activity of soil organisms in organic than conventional systems, that was related to the absence of artificial fertilizers and pesticides (Bengtsson et al., 2005; Pelosi et al., 2015; Van Diepeningen et al., 2006). This is in accordance with our results.

In this study, 31 compound residues were screened in the soils, and it cannot be excluded that other currently used and/or legacy pesticides as well as other ingredients of the commercial formations not included in the analytical menu occurred in the soils. The comprehensive quantification of the impacts of the use of synthetic pesticides on biodiversity will require a lengthy research work to characterize the actual multi-residue concentrations of pesticides in agricultural soils and the "cocktail effect" arising from such low dose but high diversity mixtures of compounds.

## 5. Conclusion

This study provides insight into the role of pesticides as a driver of biodiversity decline in agricultural ecosystems and into the current risk assessment methods. A risk quotient approach showed that the mixture of pesticide residues detected in conventional farming soils may pose a risk for soil invertebrates, which was confirmed by bioassays using different species in a battery of representative non-target organisms. The mixtures of pesticide residues present in soils from conventional agricultural soils therefore may negatively affect soil organism populations and communities, thus threatening biodiversity and the functioning of these soils. Among the pesticides detected, azole fungicides and the insecticide imidacloprid contributed the most to the risk of soils under conventional agricultural practices. Further investigation is needed considering the possible risk of pesticide residues and interactions for sustainable agriculture.

## Author contribution

Speranza C. Panico: Methodology, Investigation, Formal analysis, Visualization, Writing – original draft; Cornelis A.M. van Gestel: Conceptualization, Formal analysis, Data curation, Writing – review & editing, Supervision, Project administration; Rudo A. Verweij: Methodology, Investigation, Formal analysis: Magali Rault: Methodology, Investigation, Formal analysis, Visualization, Writing – review & editing; Colette Bertrand: Conceptualization, Methodology, Investigation, Writing – review & editing; Carlos A. Menacho Barriga: Methodology, Investigation, Formal analysis, Visualization, Writing – original draft; Michaël Coeurdassier: Investigation, Formal analysis, Visualization, Data curation, Writing – original draft, Writing – review & editing, Supervision; Clémentine Fritsch: Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Supervision; Frédéric Gimbert: Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Supervision; Céline Pelosi: Conceptualization, Formal analysis, Data curation, Writing –

review & editing, Supervision, Project administration.

## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## 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.

## Acknowledgements

This study was performed within the framework of the "PING" research project, funded by the Métaprogramme INRAe SMaCH Call 2017. The study also benefited from results obtained during the "RESCAPE" research project, action led by the Ministry for Agriculture and Food and the Ministry for an Ecological and Solidary Transition, with the financial support of the French Biodiversity Agency on "Resistance and Pesticides" research call, with the fees for diffuse pollution coming from the Ecophyto Plan through the national agency ONEMA. We thank Solène Orrière, Sébastien Darras and Rosemonde Devaux (INRAe, Estrées-Mons) for sampling the soil at the studied sites and providing information on the trials; Véronique Etiévant (INRAe, ECOSYS) for soil preparation; Gaëlle Daniele and Emmanuelle Vulliet (CNRS, ISA) for soil pesticide residues analyses; and Giovanni Caria (INRAe, LAS) for soil characteristics analyses. We also warmly thank Annette de Vaufleury and Dominique Rieffel for the long-term management and maintenance of snail rearing, and the PEA$^2$t platform of the Chrono-Environnement laboratory which manages the equipment used for the snail experiment.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.119290.

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