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Estimated decline in global earthworm population size caused by pesticide residue in soil

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ABSTRACT

Pesticides are potent chemical agents for protecting crops and agricultural production but can have secondary adverse effects on soil biodiversity that can propagate through all dimensions of soil security. Earthworms are among the most important actors in making soil healthy because they facilitate nutrient turnover, improve aeration, facilitate water infiltration into the root zone, and control soil-borne diseases. However, earthworms have been shown to be affected by the presence of pesticide residue, with a median survival to some highly toxic active substances concentrations as small as 4 mg/kg-soil. Here we have used the estimated pesticide residue of 87 active ingredients in nine different cropping systems globally, and we have developed the corresponding dose-response curve of earthworms to estimate the percent earthworm population decline and its global distribution caused by residues in the top soil. We found that vegetable and fruits, and orchards and grapes cropping systems are leading to the greatest percent decline in earthworms population in some areas of South America, and East and South East Asia. The decline in soybean, rice, and orchards and grape in boreal regions were the greatest. The maximum decline across the cropping systems ranged between 10 and 20% in about 1.2% of the agricultural locations under consideration, but it was less than 1% in about 66% of agricultural locations. These findings call for further scrutiny of the contamination by pesticide residue in soil and long-term consequences on soil security.

1. Introduction

We are arguably experiencing a silent dichotomy when it comes to the existing knowledge of pesticide ecotoxicity to non-target organisms and the accounting of pesticides in the metrics that determine environmental health and quality. On the one hand, there is a substantial body of knowledge around the mechanisms of interaction between some active substances in pesticides and the biota that eventually lead to the loss of lifely biodiversity niches. On the other hand, the indicators of sustainability typically account only for the water and CO_2 footprint, and land use, but nowhere reference is made to contamination by pesticides, their footprint, and their repercussion on the soil security and related environmental health. Strikingly, active substances in pesticides are used at an annual rate in excess of 4.1 million tonnes (Maggi et al., 2019), corresponding to about 0.5 kg per person and about 1 kg/ha (including pastures) of agricultural land every year globally. Substantially all ecosystems and living organisms of this planet are directly or indirectly exposed to some pesticides including in remote regions of Antarctica (Tatton and Ruzicka, 1967). It appears therefore that there is no escape to the fact that the Earth's surface is somehow contaminated by active xenobiotics used in agriculture.

What implications do these substances have when they accumulate in soil? There is a wide spectrum of implications in the biota response depending on specific organisms. These implications, however, have much wider repercussions on soil security and it is worthwhile expanding along this line. Soil security is defined by functions that include "biomass production", "storing, filtering and transforming of nutrients, substances and water", and "biodiversity pool" (McBratney et al., 2014). Active substances in pesticides can reshape the soil microbial community and reduce microbial growth and their enzymatic activity (Sannino and Gianfreda, 2001; Puglisi, 2012; Thiour-Mauprivez et al., 2019; Singh et al, 2002). Ammonia oxidising bacteria, sulphur oxidising bacteria, and archaea can undergo a decline in their abundance in pesticide-contaminated soil samples (Karas et al., 2018; Wan et al., 2014; Feld et al., 2015), while the symbiotic efficiency of nitrogen-fixing rhizobia bacteria can be reduced (Fox et al., 2007). Likewise, earthworms are variably susceptible to active substances; recent studies in van Hoesel et al., (2017) show that the concomitance of diverse substances such as insecticides and fungicides can decrease the average community activity without changing the population size if exposure is acute and not chronic, while survival experiments under chronic exposure with single, binary, ternary, and quaternary mixtures of various active substances in Chen et al., (2014), Yang et al., (2017), Wang et al., (2015, 2016) Cang et al., (2017) and Yu et al., (2019) show that earthworms mortality increases. The effects can therefore imply a reduced degradation rate of soil particulate organic matter, reduced

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nutrient turnover and availability to microbiota and plants, altered soil structure and aggregate formation, and altered soil hydraulics and heat conductivity. These and possibly other effects influence to some extent the three soil functions named above. Yet, the whole picture of the response of soil biota to pesticides is far from drawn. The soil microbial biodiversity is far too wide and the response of soil organisms to pesticide residue is little studied. For example, insects that spend part of their life cycle in soil are not tested against the toxicity of pesticides. It is therefore evident that the knowledge required to assess the implications of pesticide residue to soil biota and their repercussions on soil security is far from being complete, letting a wide gap in the way regulations for pesticide approval and policies on environmental health are formulated. These arguments are enough to justify the concern about pesticide threats on soil security in view of the UN Convention on Biological Diversity of Rio de Janeiro, (Convention on biological diversity 1992), which declares the ["*conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species*"] and the ["*sustainable use … of components of biological diversity .. does not lead to the long-term decline of biological diversity*"] at Article 2. Agrochemical contamination does not explicitly appear in the Convention, but the recent Lancet Commission on pollution and health indicates the persistence of pesticides in soils, wetlands and groundwater, all environmental compartments that are habitats for biodiversity (Landrigan et al., 2018). Amongst the indicators proposed to measure biodiversity are: the population richness, size, spatial distribution and genetic diversity (Luck et al., 2003).

The aim of this work is to achieve an estimate of the loss in the "biodiversity pool size" and changes in "population distribution" indicators that define Soil Security as a result of the accumulation of pesticide active substances in agricultural land. Specifically, we address earthworms because these are important actors in determining the soil biological vitality and functions. To this aim, we analysed the soil residue concentration of 87 active substances in nine cropping systems globally from Tang and Maggi (2021) and we linked the residues to the median dose-response curve for earthworms (Chou and Talalay, 1984) using median lethal concentrations (LC_{50}) available from the PPDB database (Lewis et al., 2016). This approach has allowed us to determine the potential decline fraction in earthworms population caused by the exposure to multiple residue mixtures. The assessment was conducted globally in the agricultural land (excluding pastures) at a resolution of $0.5^\circ \times 0.5^\circ$ (approximately 55 km at the equator). Targetting other soil organisms along with earthworms would be ideal but this task is currently impossible due to a lack of standard measures of dose-response parameters across the soil biome taxonomy.

2. Methods

2.1. Residue of pesticide active substances

We used the PEST-CHEMGRIDS v1.01 database (Maggi et al., 2019) for the calculation of the residue of pesticide active substances in soil. PEST-CHEMGRIDS reports the 20 most used active substances in 10 cropping systems, totalling 95 active substances across different functional (i.e., target organism) and chemical (i.e., molecular structure) groups, estimated based on the data provided by the USGS Pesticide National Synthesis Project (Baker, 2018) and conditioned to the country-specific pesticide use data reported in FAOSTAT (2019) as well as biotechnology adoption of genetically modified crops in ISAAA (2018) and governance relative to bans in Watts (2019). Of the 10 cropping systems, we excluded pasture and hays because these receive generally limited pesticide treatments and we focused on six dominant (i.e., alfalfa, corn, cotton, rice, soybean, and wheat) and three aggregated (i.e., vegetable and fruits, orchards and grapes, and other crops) cropping systems. These cover about 11.85 million km^2 of croplands (excluding pastures) based on the harvested area estimated in (Monfreda et al., 2008). These data were used with other georeferenced global data sets of soil properties, hydroclimatic variables, and agricultural practices to feed the general-purpose multi-phase and multi-component bioreactive transport simulator (BRTSim v4.0e, Maggi 2019), which mechanistically describes the water, gas, and heat flow along a one dimensional variably-saturated soil column, the transport of aqueous and gaseous chemical species, pesticide volatilization, adsorption, and microbial degradation moderated by biological activity, soil moisture content, temperature, pH, and organic carbon content. Soil residues estimated in this way (Tang and Maggi, 2021) accounted for a total of 87 pesticide active substances in the topsoil (i.e., the top 30 cm) at a spatial resolution of $0.5^\circ \times 0.5^\circ$ (approximately 55 × 55 km at the equator) and over a time scale of 48 years at annual pesticide application rates of year 2015 to reach a stationary state. The soil residue concentration $C_{n,c,g}$ of an active substance n in cropping system c and in grid cell g was ultimately calculated as the average soil concentration in the last five years of the simulation. The estimated residues were benchmarked against field measurements reported in Silva et al. (2019) as largely described in Tang and Maggi (2021). From the original data distributed in Tang and Maggi (2021), we have further filtered out active substances by considering only those with residue concentrations above 0.01 mg/kg-soil because the detectable limit by high-performance liquid chromatography (HPLC) methods is about 0.02 to 0.03 mg/kg-dry soil (Silva et al., 2018). This leads to mixtures consisting of a number of pesticides that varied across the spatial grid of the computational domain and ranging from a minimum of 1 to a maximum of 18 depending on the cropping system (Table 1).

2.2. Median dose-response curve

The median dose-response equation of a test organism to exposure to a single toxic active substance can be used to estimate the affected population fraction f as (Chou and Talalay, 1984)

$$\frac{f}{1-f} = \left(\frac{C}{LC_{50}}\right)^m, \tag{1}$$

where LC_{50} is the median lethal concentration for that active substance, C is its actual concentration, and m is an empirical parameter characterising the steepness of the dose-response curve. The dose-response curve to a mixture of n active substances can be written in a similar way as in Eq. (1) but with C_{mix} and $LC_{50,mix}$ in place of C and LC_{50}, respectively, which are defined as

$$C_{mix} = \sum_n C_n, \tag{2a}$$

$$LC_{50,\ mix} = CI\left(\sum_n \frac{f_n}{LC_{50,n}}\right)^{-1}, \tag{2b}$$

where C_n and $LC_{50,n}$ are the actual concentration and median lethal concentration of active substance n, respectively, $f_n = C_n/C_{mix}$ is the mass fraction of active substance n, and CI is the combination index describing whether the active substances in the mixture have additive effects (CI = 1), antagonistic effects (CI >1), or synergistic effects (CI < 1). If $n = 1$ active substance is considered in Eq. (2b), $LC_{50,\ mix}$ becomes the LC_{50} of that substance, and C_{mix} becomes its actual concentration, with CI = 1 because there is no explicit interaction with other substances. The values of LC_{50}, m and CI are specific to the exposed organism and active substance, either individual or in a mixture of substances.

2.3. Identification of test organisms and parameters sourcing

For the purpose of this work, we have selected the epigeic earthworm *Eisenia foetida* as a model organisms following the recommendations by the OECD guidelines for ecotoxicity risk assessments (OECD, 1984).

Table 1
Pesticide active substances specific to each of the nine cropping systems assessed in this work. Columns 2 and 3 list the type and number of substances in the pesticide residue mixture assessable in the computational domain, while column 4 gives the number of substances eventually assessed in the pesticide mixture after filtering for residues with an environmental concentration above the detectable limit of 0.01 mg/kg-dry soil.

Cropping system	Assessable active substances	Number of assessable substances	Number of assessed substances
Alfalfa	2,4-d, 2,4-db, Bromoxynil, Carbaryl, Chlorpyrifos, Clethodim, Cyhalothrin-lambda, Dimethoate, Diuron, Eptc, Glyphosate, Hexazinone, Indoxacarb, Malathion, Metribuzin, Paraquat, Pendimethalin, Phosmet, Sethoxydim, Trifluralin.	20	1 to 17
Corn	2,4-d, Acetochlor, Alachlor, Atrazine, Azoxystrobin, Chlorpyrifos, Clopyralid, Dicamba, Dimethenamid(-p), Glufosinate, Glyphosate, Isoxaflutole, Mesotrione, Metolachlor(-s), Paraquat, Pendimethalin, Propargite, Pyraclostrobin, Simazine, Terbufos.	20	1 to 18
Cotton	2,4-d, Acephate, Acetochlor, Bifenthrin, Chlorpyrifos, Dicamba, Dichloropropene, Dicrotophos, Diuron, Fluometuron, Fomesafen, Glufosinate, Glyphosate, Imidacloprid, Metolachlor (-s), Msma, Paraquat, Pendimethalin, Prometryn, Trifluralin.	20	1 to 18
Orchards and grapes (OrcGra)	2,4-d, Captan, Chloropicrin, Chlorothalonil, Chlorpyrifos, Copper hydroxide, Copper sulfate tribasic, Copper sulfate, Dichloropropene, Diuron, Glufosinate, Glyphosate, Mancozeb, Methyl bromide, Oxyfluorfen, Paraquat, Pendimethalin, Ziram.	18	1 to 16
Rice	2,4-d, Acifluorfen, Azoxystrobin, Bentazone, Clomazone, Clothianidin, Copper sulfate, Cyhalofop, Cyhalothrin-lambda, Glyphosate, Halosulfuron, Imazethapyr, Pendimethalin, Propanil, Propiconazole, Quinclorac, Saflufenacil, Thiobencarb, Triclopyr, Trifloxystrobin.	20	1 to 16
Soybean	2,4-d, Acephate, Acetochlor, Acifluorfen, Chlorpyrifos, Clethodim, Dicamba, Dimethenamid(-p), Flumioxazin, Fomesafen, Glufosinate, Glyphosate, Metolachlor(-s), Metribuzin, Paraquat, Pendimethalin, Pyraclostrobin, Pyroxasulfone, Sulfentrazone, Trifluralin.	20	1 to 17
Vegetables and fruits (VegFru)	Bensulide, Bentazone, Captan, Chloropicrin, Chlorothalonil, Copper hydroxide, Dichloropropene, Eptc, Ethalfluralin, Ethoprophos, Glyphosate, Mancozeb, Metam potassium, Metam, Methyl bromide, Metolachlor(-s), Pendimethalin, Thiophanate-methyl.	18	1 to 18
Wheat	2,4-d, Atrazine, Azoxystrobin, Bromoxynil, Chlorpyrifos, Clopyralid, Dicamba, Dimethoate, Fluroxypyr, Glyphosate, Mcpa, Metconazole, Paraquat, Pinoxaden, Propiconazole, Prothioconazole, Pyraclostrobin, Tebuconazole, Thiophanate-methyl, Tri-allate.	20	1 to 16
Other crops	2,4-d, Acetochlor, Atrazine, Bromoxynil, Chloropicrin, Chlorothalonil, Chlorpyrifos, Dicamba, Dichloropropene, Dimethenamid(-p), Flutolanil, Glyphosate, Metam, Metolachlor(-s), Metribuzin, Paraquat, Pendimethalin, Phorate, Propazine.	19	1 to 18

However, *E. foetida* is not widely present globally and does not normally occupy cropping ecosystems. We included therefore other earthworms that better represent the biodiversity in cropping systems, including the anecic *Lumbricus terrestris*, the endogeic *Lumbricus rubellus*, the endogeic *Allolobophora chlorotica* (Pelosi et al., 2013), and the endogiec *Aporrectodea calignosa* (Plaas et al., 2019).

In the dose-response curve of Eq. (1), the values of m and CI are unknown, while values of $LC_{50,n}$ for the individual active substance n are accessible for the model organisms *E. fetida* in the PPDB pesticide database in Lewis et al. (2016) but not for other species. Ideally, experiments with all possible combinations and proportions of active substances should be conducted to determine LC_{50} for an organism and be able to calculate CI according to the original definition in Chou and Talalay (1984) and fit m. However, experiments have been conducted only for a number of active substances well below the ideal case of the 87 substances of interest in this work and, to the best of our knowledge, up to quinquenary mixtures for *E. foetida* (4 pesticides and 1 metal, Yu et al., 2019), and only for single mixtures for a few other earthworms. From a survey of the existing literature (Yang et al., 2019; Chen et al, 2014; Wang, An et al., 2016a; Wang, Cang et al., 2016b; Cang et al, 2017; Wang et al., 2015; and Sterensen 1979), we collected or estimated values of m and CI relative to *E. foetida* from experimental observations of the survival fraction f in various mixtures. Similarly, we estimated values of m from observations of f in single pesticide solution relative to *L. terrestris* (Cathey 1982 and Basley and Goulson 2017), and *L. rubellus, A. calignosa* and *A. chlorotica* (Sterensen 1979).

Data summarized in Table 2 show that both m and CI generally decrease with an increasing number of substances in the mixture, with CI < 1 implying a synergistic effect. This observation is relative to *E. foetida*, for which data are available. However, we note that m values for single mixtures in *L. terrestris, L. rubellus, A. calignosa*, and *A. chlorotica* are not substantially different from those of *E. foetida* with the exception of data in Basley and Goulson (2017) relative to *L. terrestris*. After inspection, we have identified biases in the observations in Basley and Goulson (2017), and we consider the estimated m values as outlier to be excluded from our analyses. Because of the similarity in the steepness of the dose-response curve between *E. foetida* and the other species for single pesticide solutions, we hypothesize that m

Table 2
Summary of experimentally derived values of *m* and CI for the survival of *E. foetida, L. terrestris, L. rubellus, A. caligнosa, and A. chlorotica* exposed to various pesticide mixtures. * Active substances accounted for in our assessment. [(a)] the seven active substances were tested in combinations of three but we report the average and standard deviation across all tests. [(b)] Data not used for poor quality. [(c)] parameters estimated in this work by fitting Eq. (1). N/P stands for data not provided.

Reference	Species	Tested substances	Exposure period	N. of active substances per test	*m*	CI
Yang et al., (2017)	*E. foetida (epigeic)*	*Chlorpyrifos, *clothianidin, *acetochlor, Fenobucarb	2 weeks	1	3.28 ± 0.41	1 ± 0
				2	2.90 ± 0.47	0.881 ± 0.344
				3	2.73 ± 0.22	0.7925 ± 0.45
				4	2.14 ± 0	0.17 ± 0
Chen et al., (2014)	*E. foetida (epigeic)*	*Atrazine, butachlor, *lambda-Cyhalothrin	2 weeks	1	4.47 ± 0.17	1±0
				2	4.97 ± 2.14	1.44±1.43
				3	4.30 ± 0	0.88±0
Wang et al., (2016a)	*E. foetida (epigeic)*	*Atrazine, *chlorpyrifos, *lambda-Cyhalothrin, *imidacloprid	2 weeks	2	N/P	1.02±0.73
				3	N/P	0.82±0.6587
				4	N/P	0.87±0
Wang et al., (2016b)	*E. foetida (epigeic)*	Butachlor, *chlorpyrifos, *lambda-Cyhalothrin, phoxim	2 weeks	2	N/P	0.43±0.313
				3	N/P	0.103±0.02
				4	N/P	0.09±0
Cang et al., (2017)	*E. foetida (epigeic)*	*Imidacloprid, *chlorpyrifos, *lambda-cyhalothrin, phoxim	2 weeks	2	N/P	1.66±0.15
				3	N/P	0.85±0.28
				4	N/P	0.46±0
Wang et al., (2015)	*E. foetida (epigeic)*	*Chlorpyrifos, *atrazine, butachlor, *lambda-cyhalotrin, *imidacloprid, avermectin, phoxim	2 weeks	1	6.22 ± 2.3	1±0
				[(a)]3	3.75 ± 0.67	0.87±0.75
Stenersen (1979)	*E. foetida (epigeic)*	Aldicarb, paraoxon, PHMD	2 weeks	1	[(c)]3.92 ± 2.15	1±0
Cathey (1982)	*L. terrestris (anecic)*	Aldrin, *carbaryl, endrin, parathion	6 weeks	1	[(c)]1.64 ± 0.88	1±0
[(b)]Basley and Goulson (2017)	*L. terrestris (anecic)*	*Chlothianidin	16 weeks	1	[(c)]0.13 ± 0.0	1±0
Stenersen (1979)	*L. rubellus (endogeic)*	Aldicarb, PHMD	2 weeks	1	[(c)]5.97 ± 3.28	1±0
Stenersen (1979)	*A. caligosa (endogeic)*	Aldicarb, carbofuran, paraoxon, PHMD	2 weeks	1	[(c)]3.71 ± 2.84	1±0
Stenersen (1979)	*A. chlorotica (endogeic)*	Aldicarb, *carbaryl, carbofuran, PHMD	2 weeks	1	[(c)]5.41 ± 2.32	1±0

values for *L. terrestris, L. rubellus, A. caligнosa* and *A. chlorotica* follow a trend similar to that for *E. foetida* against an increasing number of active substances in the mixture. We use the same hypothesis for CI values in mixtures with increasing number of active substances.

Under the hypotheses above, data in Table (2) allow us to fill in some practicality gaps in the assessment of *m* and CI in cropping systems for which we have assessed the concentration of mixtures of up to 18 active substances (Table 1). To fill in this experimental gap, we first fitted a linear ($y = a\,x + b$) and a power law ($y = a\,x^b$) function to experimental *m* and CI values in the experimental mixtures in Table 2, and we next extrapolated *m* and CI values as a function of the number of substances up to $n = 18$ from the average of the fitting functions.

2.4. Stochastic analysis of dose-response parameters

The hypotheses in Section 2.3 used to parameterize the general dose-response curve for multiple earthworms imply a level of tolerance associated to the organisms and the type of active substances. To quantify the uncertainty associated with these hypotheses, we assessed the dose-response curve using a stochastic approach by extracting 2,000 independent values of the parameters *a* and *b* of the linear and power law regressions describing *m* and CI. Parameters were extracted from a normal probability distribution function with average and standard deviation obtained from fitting against experimental *m* and CI values and limited to the 95% confidence interval. With stochastic values of *a* and *b*, we calculated 2,000 independent linear and power law regressions, and averaged them for both *m* and CI and for *n* ranging from 1 to 18. Next, we used the average ($\overline{m}$ and $\overline{\mathrm{CI}}$) of the 2,000 stochastic replicas of *m* and CI for our assessments of the affected earthworm population globally and their standard deviation (m_σ and CI_σ) to characterize the assessment uncertainty.

2.5. Application of the dose-response curve to the assessment domain

The extrapolated $\overline{m}(n_{c,g})$ and $\overline{\mathrm{CI}}(n_{c,g})$ average values are specific to the mixture with $n_{c,g}$ substances in each of the nine cropping systems *c* and grid cells *g* because grid cells may occasionally have residues of some active substances below the detectable concentration and because some countries have applied bans on some active substances. In either of these cases, the active substance is excluded from the mixture. Hence, Eqs. (1) and (2) can be rewritten with the subscripts of interest as

$$\frac{f_{c,g}}{1-f_{c,g}} = \left(\frac{\sum_n C_{n,c,g}}{\overline{\mathrm{CI}}\left(n_{c,g}\right)\left(\sum_n \frac{f_{n,c,g}}{\mathrm{LC}_{50,n}}\right)^{-1}}\right)^{\overline{m}\left(n_{c,g}\right)}, \tag{3}$$

Using Eq. (3) with the the values of $C_{n,c,g}$ estimated as described in Section 2.1 allows us to estimate the fraction $f_{c,g}$ of the affected earthworm population in each cropping system *c* and grid cell *g*. This allows us to identify the geographic location of a cropping system in which the greatest decline *f* occurs and the average decline for that cropping system globally. The overall decline in a grid cell *g* where multiple cropping systems exist was also calculated as the maximum decline (precautionary principle) across the nine cropping systems as

$$f_{max,g} = \max\{f_{c,g}\}\,\text{with}\, c = \{1,..,9\}. \tag{4}$$

The geographic distribution of $f_{max,g}$ calculated as in Eq. (4) was further analysed by climatic regions (equatorial, arid, temperate, and boreal) using the aggregation in the Koppen-Geiger climatic classification (Koppen and Geiger, 1936) to identify whether the earthworm population decline is concentrated in specific climates and cropping systems.

3. Results and discussion

3.1. Estimated m and CI values

The data relative to experimentally-retrieved values of m and CI available from the literature and summarized in Table 2 are represented in Fig. 1 as a function of the number of active substances in pesticide mixtures. Data suggest that an increasing number of active substances produces smaller m and CI values. While small m means that the steepness of the response curve in Eq. (3) is generally more or less accentuated depending on whether the exposure concentration is greater or smaller than $LC_{50,\mathrm{mix}}$, small CI values imply synergistic effects of the mixture, that is, the exposure dose of the mixture has to be smaller to produce the same effect of a single solution mixture. The trade-off between these two trends and their effect on the affected earthworm population fraction f cannot be anticipated in a straightforward way.

To infer values of m and CI for mixtures with more than four active substances, we used an extrapolation via linear ($y = ax + b$) and power law ($y = ax^b$) regressions (see Fig. 1). The fitting parameters a and b of both regressions are reported with their goodness of fit R (Pearson's coefficient) and RMSE (root mean square error) in Table 3. On the one hand, the linear regression provided a better fit than the power law for m (R = 0.41 versus R = 0.38) and for CI (R = 0.59 versus R = 0.48) but with the disadvantage that m crosses the zero for mixtures with more than 9 active substances and CI crosses zero for more than 7 substances. On the other hand, the power law has a slightly worse fit against experimental data but has the advantage to never cross the zero. To balance the choice of using one regression only, we averaged the linear and power law regressions, with the linear extrapolation limited to positive or zero values ($y = max\{ax + b, 0\}$). This leads to a discontinuity point of the first order.

The stochastic analysis conducted on the parameters m and CI of the dose-response curve highlights a smooth average trend as a function of the number of active substances in the mixture (Fig. 1c and d). We note that the discontinuity of the first order does not introduce any specific

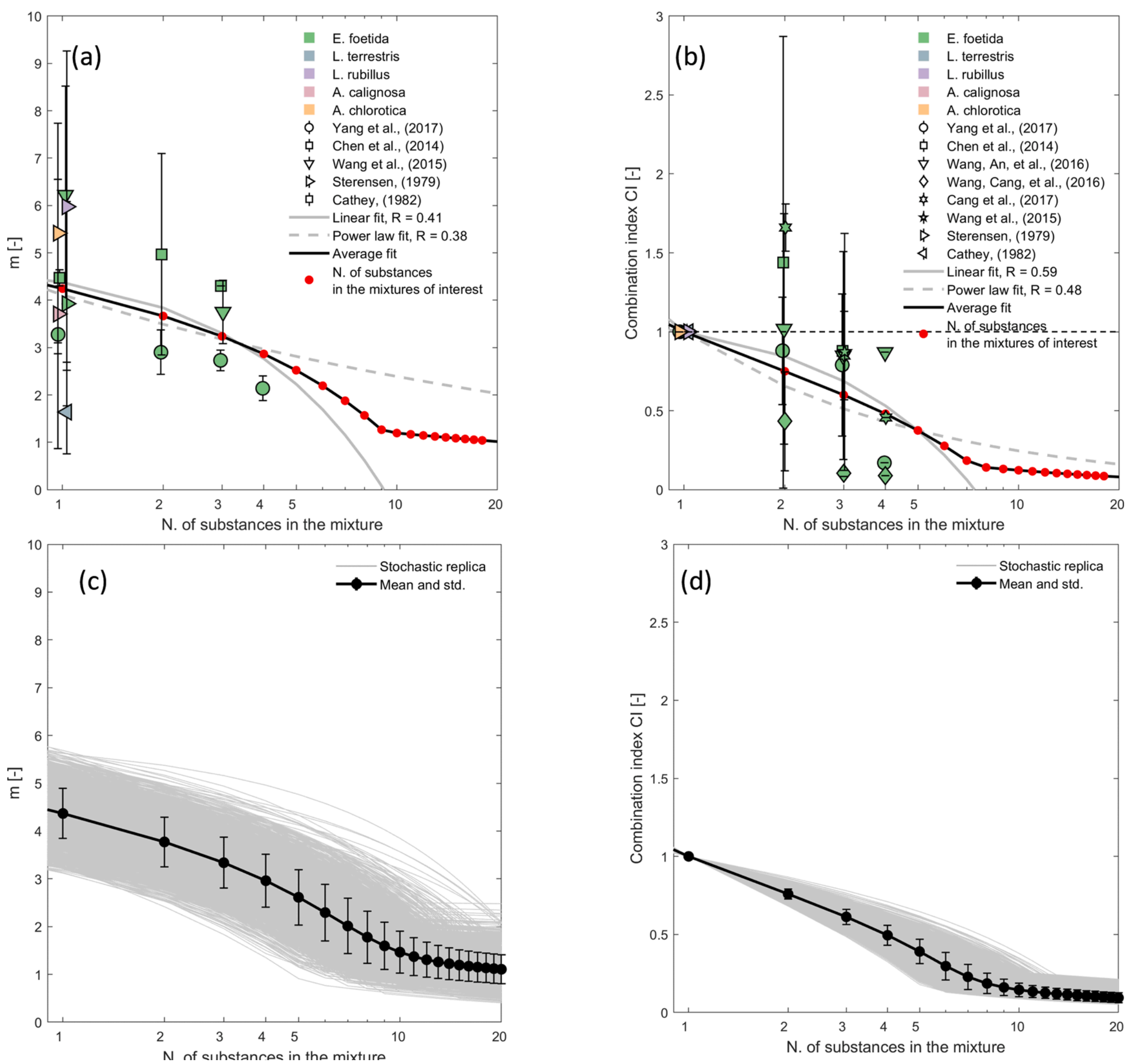

Fig. 1. Experimental values and regressions of (a) the parameter m and (b) the combination index CI used in the dose-response curve for various earthworms as a function of the number of active substances in the mixture. Parameters of linear ($y = ax + b$) and power law ($y = ax^b$) regressions for m and CI are reported in Table 3. The average extrapolation was calculated with the linear regression limited to positive or zero values. (c) and (d) represent the average and standard deviation of the ensamble of the parameters m and CI obtained by stochastic sampling of the parameters a and b of the corresponding regressions and are reported in Table 4.

Table 3
Fitting parameters of the linear and power law regressions against data reported in Table 2 and represented in Fig. 1a and b. Parameters are given with the best fit and the 95% confidence intervals in parantheses. [(a)] constraint to force the linear regression function to CI = 1 for $n = 1$ active substances in the mixture. [(b)] constraint to force the power law regression function to CI = 1 for $n = 1$ active substances in the mixture.

Regression type		Linear ($y = ax + b$)	Power law ($y = ax^b$)
m	*a*	-0.537 (-1.29, 0.22)	4.111 (3.36, 5.372)
	b	4.913 (3.36, 6.46)	-0.235 (-0.6539, 0.1616)
	R	0.41	0.59
	RMSE	1.31	0.31
CI	*a*	-0.1562 (-0.24, -0.076)	[(b)]1
	b	[(a)] (1-a)	-0.609 (-0.758, -0.28032)
	R	0.38	0.48
	RMSE	1.32	0.33

disadvantage in the stochastic assessments of m and CI and it rather disappears in the averages $\overline{m}$ and $\overline{\mathrm{CI}}$. The average values of $\overline{m}$ and $\overline{\mathrm{CI}}$ and their standard deviations m_σ and CI_σ are reported in Table 4 and are used in our next assessments.

3.2. Crop-specific $LC_{50,mix}$ in cropping systems

Using the extrapolated values of $\overline{\mathrm{CI}}(n_{c,g})$ and the residue concentration $C_{n,c,g}$ specific to each cropping system c and grid cell g, we calculated the median lethal concentration of the mixture $\mathrm{LC}_{50,mix}$ (Fig. 2). The smaller the $\mathrm{LC}_{50,mix}$ the higher the toxicity of the mixture. Practically all cropping systems showed that $\mathrm{LC}_{50,mix}$ can reach values as small as in the range between 1 and 10 mg/kg-soil. We attribute this high toxicity to the estimated small values of the combination index $\overline{\mathrm{CI}}(n_{c,g})$, which sits at about or below 0.2 for mixtures with more than 7 active substances (Fig. 1d and Table 4). These low values of $\overline{\mathrm{CI}}(n_{c,g})$ imply that the synergistic effects of the mixture are very strong as compared to additive effects occurring when $\overline{\mathrm{CI}}(n_{c,g}) = 1$, but also indicates that assessments that only emphasize single substances produce a substantial bias of the toxicity level as compared to when multiple pesticides are used.

All cropping systems show geographic locations with at least moderate toxicity, but the cropping systems with the widest fraction of grid cells with moderate to high toxicity are cotton, vegetables and fruits (VegFru), and orchards and grapes (OrcGra) (Fig. 2d, e, and g). The geographic areas subject to the greatest toxicity are recurring across the cropping systems and are located in the Americas, Europe, and East Asia.

3.3. Earthworm population decline by cropping systems

We calculated the percent population decline f of earthworms for each cropping system and grid cell using Eq. (3). Analyses show that the percent decline is widely limited to a fraction mostly not exceeding 1% in most cropping systems globally (Fig. 3). However, cropping of vegetables and fruits, and orchards and grapes (VegFru, OrcGra Fig. 3e and g) can lead up to about 20% decline in some geographic areas located mainly in Asia and South America. Corn, soybean, wheat, rice, and alfalfa lead to a decline generally not exceeding 5% in earthworm population size. Africa and Oceania generally appear to have negligible decline in earthworm population with the exception of small areas in central Africa for corn, VegFru and OrcGra.

3.4. Earthworm population decline by climatic region

It is interesting to analyse if a climatic pattern exists that is associated to the earthworm population decline, which could be inferred in instances in which particularly high pesticide application rates are used such as in warm and humid regions to combat weeds, insects, and fungi attacking crops. In the four climatic regions of interest (equatorial, arid, temperate, and boreal, Koppen and Geiger, 1936), we found that the greatest average decline fraction occurs in the boreal climates in soybean (about 5%), rice (about 8%) and orchards and grapes (about 5%) (Fig. 4). In other cropping systems and climatic regions, the average decline fraction rarely exceeds 3%. However, we note that the 95th percentile of grid cells can frequently exceed 5% and sometime reach 10% decline in earthworm population in some cropping systems and climatic regions except in alfalfa, with the 95th percentile below 5%. Exceptionally, the 95th percentile can be as high as 20% decline in soybean, rice, and orchards and grapes cropping systems.

3.5. Global geography of earthworm population decline

The estimated maximum earthworm population percent decline $f_{max,g}$ in each geographic grid cell can range widely from less than 1% in about 66% of the grid cells and up to more than 50% in no more than 0.3% of grid cells (Fig. 5). The distribution, however, is relatively skewed toward small percent values with a decline between 2 and 5% occurring in about 5% of grid cells, and a decline between 10 and 20% occurring in about 1.2% of grid cells.

In spite of our assessment in Fig. (5) may suggest that the overall earthworm population decline may be considered minor, it is worth noting that the most affected areas are in regions with a generally high biodiversity, such as South America, Central Asia, and South East Asia. In addition, a decline greater than of equal to 50%, even if limited to 0.3% of grid cells, may induce a loss in population size that can cause also a loss in biodiversity if loss in fertility are substantial. Although this aspect is not investigated directly in this work, we suggest that further assessments should be undertaken to produce improved estimates and assess possible consequences of steep declines in earthworm population size (see also Discussion).

4. Discussion

Results proposed in this work bring to light with quantitative analyses the overall estimated toxicity effect of the mixture of pesticide residue on the population count and geographic distributions of earthworms, estimated on the basis of the median dose-effect response for *E. foetida, L. terrestris, L. rubellus, A. caligNosa*, and *A. chlorotica* as the model earthworm species.

We acknowledge limitations in this work. The median dose-effect

Table 4
Average and standard deviation of the parameters m and CI obtained by stochastic sampling as a function of the number n of active substances in the mixture.

n	$\overline{m}$	m_σ	$\overline{\mathrm{CI}}$	CI_σ	n	$\overline{m}$	m_σ	$\overline{\mathrm{CI}}$	CI_σ
1	4.3681	0.5307	1.0000	0	10	1.4854	0.4553	0.1440	0.0432
2	3.7746	0.5351	0.7588	0.0305	11	1.3903	0.4097	0.1333	0.0383
3	3.3418	0.5545	0.6121	0.0469	12	1.3219	0.3771	0.1253	0.0359
4	2.9671	0.5786	0.4944	0.0620	13	1.2736	0.3540	0.1192	0.0349
5	2.6231	0.6040	0.3899	0.0774	14	1.2374	0.3382	0.1143	0.0344
6	2.3063	0.6177	0.2965	0.0875	15	1.2080	0.3281	0.1101	0.0340
7	2.0295	0.6015	0.2281	0.0792	16	1.1843	0.3221	0.1063	0.0337
8	1.7992	0.5607	0.1854	0.0643	17	1.1649	0.3186	0.1029	0.0333
9	1.6190	0.5084	0.1598	0.0517	18	1.1480	0.3164	0.0998	0.0330

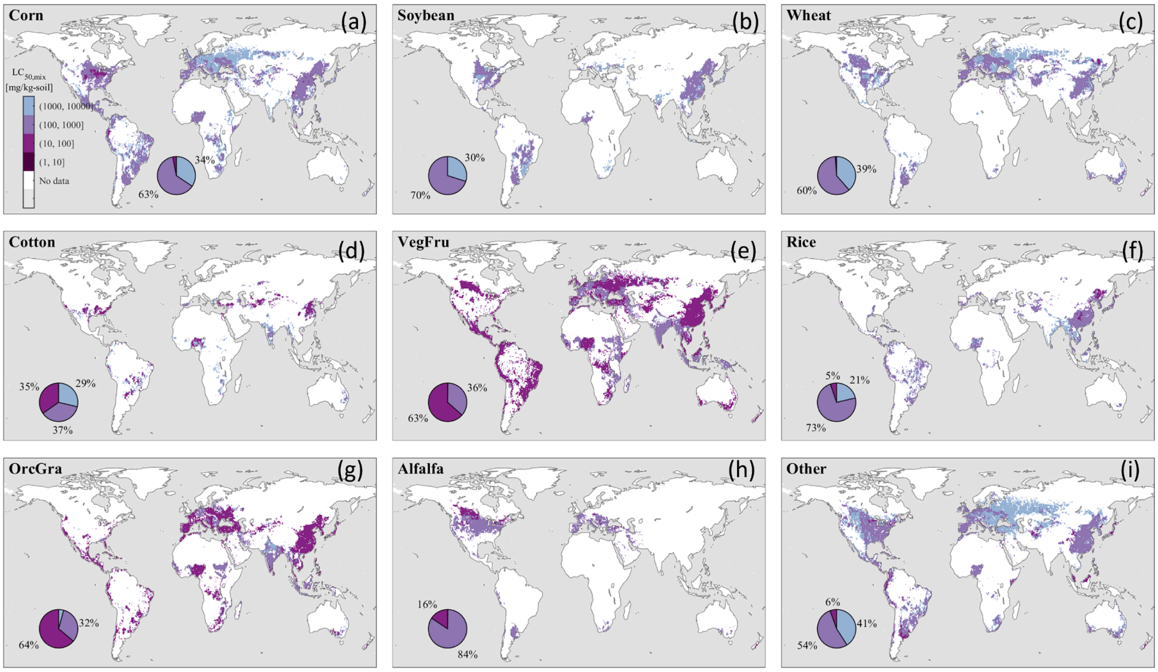

Fig. 2. Calculated median lethal concentration $LC_{50,mix}$ for mixtures of pesticide active substances in the soil of the nine cropping systems investigated in this work. $LC_{50,mix}$ is calculated as in Eq. (2b). The pie chart represents the fraction of grid cells with the color mapped to the legend of $LC_{50,mix}$.

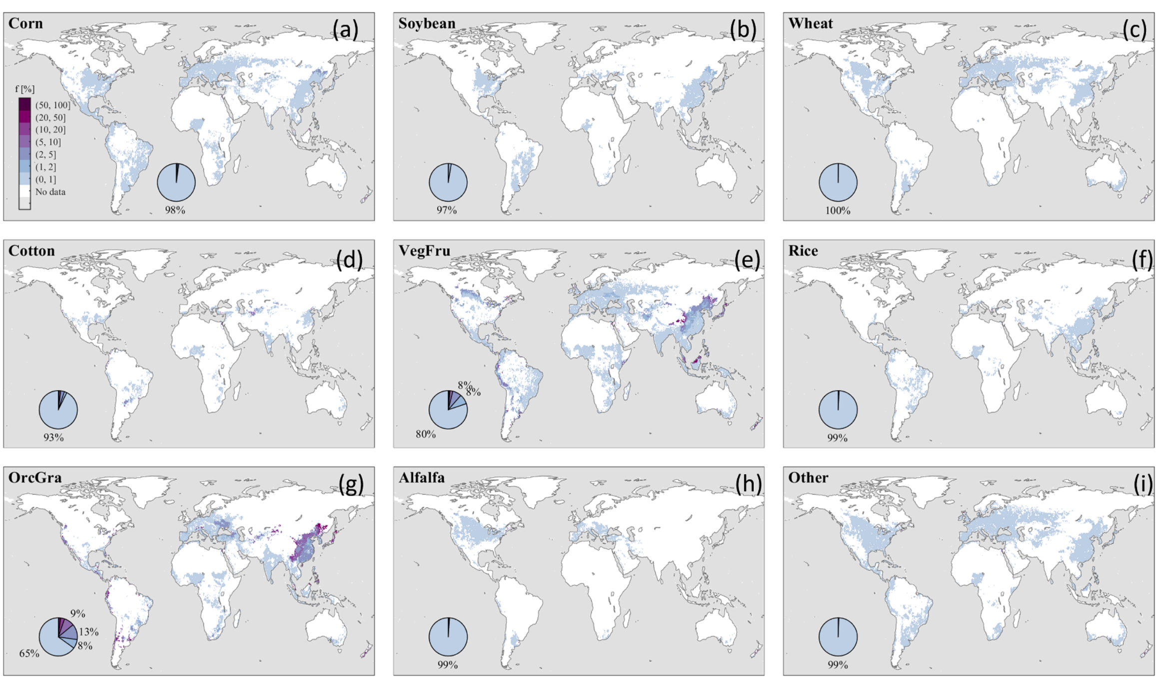

Fig. 3. Calculated percent decline in earthworm population specific to each cropping system in response to mixtures of pesticide residue.

response approach provides a measure of survival of individuals of the same generation but does not provide an indication on whether other adverse effects exist such as on growth and reproduction. These are equally important in determining the dynamics of earthworm populations on a term longer than the natural life expectancy of individual earthworms. For example, even when the survival is not substantially affected by exposure to pesticides, damages to reproductive organs may affect the population size in the next generation. We have not explicitly accounted for these effects in this work, and we recommend here that an improved accounting of the earthwork population dynamics should include growth stages and reproduction of individuals.

Another limitiation of this work is the accounting of the species in the determination of the parameters of the median dose-effect response equation. For this study, we have relied on a small number of existing literature that reports key parameters. Most of the literature refers to *E. foetida* because this is the recommended model organisms by the OECD (1984), while a limited literature was found about other earthworm species. *E. feotida*, however, is present only in some temperate climates, mostly not in agricultural soil (Pelosi et al., 2013; Plaas et al., 2019, Gavinelli et al., 2018). In contrast, data used in this work relative

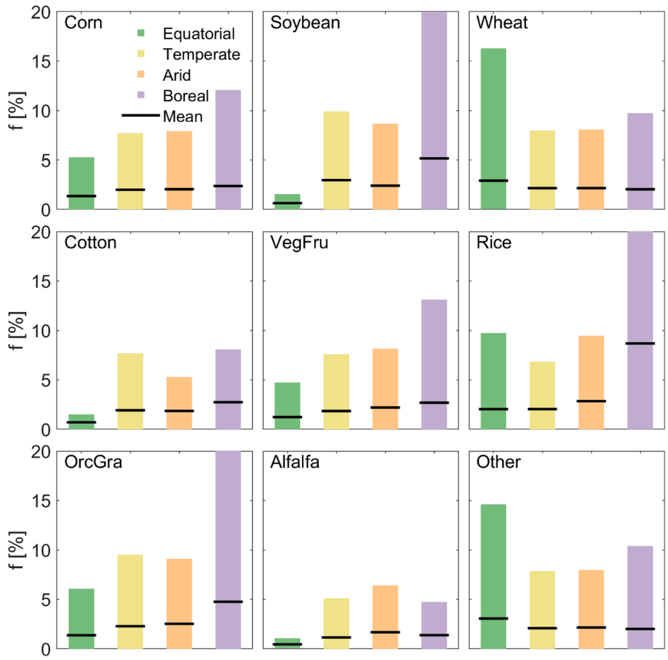

Fig. 4. Calculated percent decline in earthworm population in response to mixtures of pesticide residue by cropping system and climatic region. Vertical bars represent the range between the 5th and 95th percentiles, while the black lines represent the average.

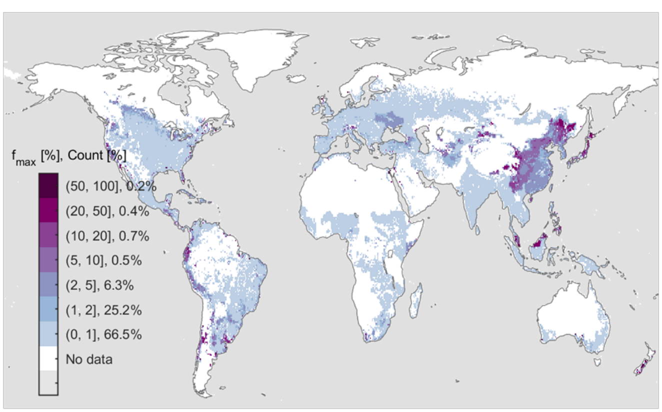

Fig. 5. Calculated maximum percent decline in earthworm population in response to mixtures of pesticide residue in soil. Labels in the colour bar report both the percent decline and the percent of grid cells corresponding to that decline.

to other earthworms are not exhaustive, leaving parameter estimation subject to uncertainty. In the analyses presented here, we found that the parameters *m* relative to *L. terrestris, L. rubellus, A. calignosa*, and *A. chlorotica* were somehow different from those of *E. foetida*, and therefore assessments proposed here may be skewed towards the median dose-effect response of *E. foetida*. However, we incorporated uncertainty in *m* by using a stochastic approach to derive an average parametric representation of the median dose-effect response. The uncertainty was quantified by statistical dispersion (standard deviation) of randomized sampling of parameters describing *m* and CI (Fig. 2) and we deemed this approach reliable to achieve a first assessment of the possible decline in earthworm population globally. Rather, we found that the most limiting aspect in accomplishing this assessment is the limited number of studies conducted for the exposure of earthworms to multiple active substances in combinations. It is known that any drugs can have synergistic or antagonistic effects when in combination with other drugs. We expect therefore that the uncertainty associated with a lack of knowledge of median dose-effect response to mixture may produce some underestimation of the population decline predicted in our assessment.

Additional limitations include the limited number of active substances accounted for here and the availability of reliable data for the application rates and soil residues of all active substances present in pesticides used in agriculture. Pesticide use (by commercial name or active substance) is still largely unreported or not publically accessible, an aspect that causes the greatest uncertainty and difficulty in conducting large-scale exposure assessments.

The soil biological vitality, and its health as a consequence, is often associated with earthworms. This work substantiates therefore the possible threats caused by pesticides on soil health and in particular on three functions that define soil security, namely “biomass production”, “storing, filtering and transforming of nutrients, substances and water”, and “biodiversity pool” (McBratney et al., 2014), and that are measured by the population size and spatial distribution as recommended indicators suggested in Luck et al., (2003). Despite this achievement, we consider this quantification far from sufficient to adequately quantify soil security. We believe that this limitation is not just a lack of this work but, rather, the consequence of a lack of a standard to comprehensively characterize pesticide effects on the biomes in their entirety (this is a difficult task) or at least partially, beyond earthworms. An example to infer the state of the art in soil security is to associate to or incorporate in soil security measures other environmental and socio-economic indicators that have direct or indirect repercussions on soil health. We recommend, as examples to this aim, the Environmental Performance Index (EPI), which ranks all countries by using a number of metrics of measurable quantities of influence to the environment health (Wendling et al., 2020). Similarly, the Risk Score (RS) is an appropriate indicator particularly relevant for soil security because it measures the environmental impact of agrochemicals contamination in soil, surface waters, groundwater, and atmosphere (Tang et al 2021). Currently, there is no consensus on a conceptual framework, neither is there a consensus on the metrics to be used for this purpose. The key ingredient to make a transformational step into measuring soil security is that an international organization such as the United Nations takes a brave initiative to enact strict policies that member countries undertake as a commitment to guarantee the safety of the planet to the next generations.

5. Concluding remarks

In this work, we used earlier estimates of the residue of pesticide applications in six dominant (i.e., alfalfa, corn, cotton, rice, soybean, and wheat) and three aggregated (i.e., vegetable and fruits, orchards and grapes, and other crops) cropping systems to determine the environmental concentration of the mixture of pesticide residue. We integrated these estimates and the combined lethal concentration of pesticide mixtures to determine the median effect dose-response curve of *Eiseina foetida, Lumbricus terrestris, Lumbricus rubellus, Allolobophora chlorotica*, and *Aporrectodea calignosa* as the model earthworms to the toxicity of the mixtures expressed as the percent decline in population size. The assessment was conducted at global scales at a resolution of $0.5^\circ \times 0.5^\circ$ degree (approximately 55 km at the equator). We found that the decline in population size of earthworms was globally less than about 5% but with spikes of up to more than 50% in geographic regions in the South America, and East and South East Asia mostly in vegetables and fruits and in orchards and grapes cropping systems. Surprisingly, the decline in soybean, rice and orchards and grapes were the greatest in boreal climates. Overall, the maximum decline was less than 1% in about 66% of the assessed agricultural locations, between 2 and 5% in about 5% of locations, and between 10 and 20% in about 1.2% of locations.

Data distribution

We distribute data of calculated $LC_{50,\ mix}$ (represented in Fig. 2), the count of active scubstances in the residue mixture, and the percent decline *f* in earthworm population size (represented in Fig. 3) by cropping system as geotiff formatted data (.TIF). These data are distributed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution, and reproduction in any medium or format, as long as you give appropriate credit to the original authors and the source, provide a link to the Creative Commons license, and indicate if changes were made. Distribution data and a Technical Documentation are available at the *figshare* repository https://figshare.com/articles/dataset/_/16543968.

Author contributions

FM and FHMT have conceptualized the work. FHMT has constructed the database of soil residue and collected literature data instrumental for this work. FM has conducted the analysis and produced the figures. FM and FHMT have written the manuscript.

Declaration of interests

The authors declare no competing interests.

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