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Prioritization of currently used pesticides in soils of main European cropping systems and an Argentinian cropping system for assessment of mixture toxicity and risk on terrestrial biota

Olukayode Jegede^{a,1,*}, Paula S. Tourinho^{b,*}, Violette Geissen^a, Jakub Hofman^b 

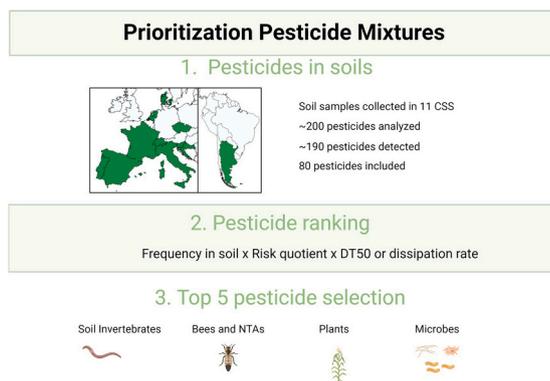
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HIGHLIGHTS

- A score-based approach to prioritize pesticides for mixture risks.
- Fungicides, herbicides and insecticides posed significant risks to invertebrates.
- Herbicides and fungicides posed major risks to plants and microbes respectively.
- Glyphosate/AMPA prioritized over other pesticides in multiple cropping systems.
- Insufficient data may skew pesticide prioritization and underrepresent risks.

GRAPHICAL ABSTRACT



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ABSTRACT

Identifying driving compounds in mixture pressure on terrestrial organisms is crucial to assessing the possible risk of current-use pesticides. This study presents a scoring approach for the prioritization of pesticides occurring in real mixtures. Soil samples associated with eleven crop systems across Europe and Argentina were analyzed for approximately 200 pesticides and metabolites within the H2020-funded project SPRINT (Sustainable Plant Protection Transition: A Global Health Approach). The pesticides were ranked based on the frequency of detection in soil samples, risk quotient (RQ), and degradation rates. RQ was calculated using the regulatory ecotoxicological data, and Predicted Environmental Concentration (PEC) values were extracted from EFSA documents. The prioritization was conducted separately for soil invertebrates, bees and non-target arthropods (NTAs), non-target terrestrial plants, and microbes. To prioritize for ecotoxicity tests, the top five ranked pesticides for each crop scenario were considered as the most relevant environmental mixture for that crop scenario. Overall, pesticides of concern were related to their specific targeted chemistry, e.g. herbicides affected plants the most and fungicides affected microbes the most. This study demonstrated that a scoring approach can be helpful

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in monitoring programs that aim to assess the risk of pesticides occurring in terrestrial ecosystems for environmental protection.

1. Introduction

Pesticides (i.e., plant protection products – PPP) are intensively used in farming systems in Europe to secure crop yields and ensure food safety. From 2011–2020, an average of 350,000 tons of pesticides are used yearly in Europe [1]. These pesticides constitute several thousand different commercial formulations of 455 approved active substances (A.S) [2]. When applied in agriculture, pesticides tend to deposit on plant, soil, and water surfaces through direct spraying or wind drift and subsequent deposition [3,4]. As a result, non-target organisms living in these environmental media can be exposed to pesticides. The exposure of non-target species to pesticides often leads to deleterious effects such as reproduction or growth inhibition, behavioural impairment, and in many cases, mortality [5,6]. The toxic effect of pesticides depends on the dose that the organisms are exposed to [7], and the potency and toxic mode of action affecting distinct taxonomic groups differently [8]. Sublethal effects of pesticides can be due to the low concentration of pesticides relative to the lethal effect. One major driver for exposure is the environmental persistence of the pesticide. The persistence of a pesticide is the measure of how long the pesticide can stay active and/or intact [7]. In many cases, the pesticides could be transformed into metabolites that can be persistent and, in some cases, more toxic [9,10]. For the currently approved substances in the EU, as part of the continuous assessment of their safety in the environment, it is pertinent to know if the concentrations present in the environment lead to acceptable risks. Since pesticides often exist as mixtures in the environment, their safety based on mixtures needs to be accounted for.

Documentary evidence showed that when organisms are exposed to chemical mixtures, they often elicit stronger ecotoxicological effects (due to additivity and/or potentiation) than when exposed to single chemicals [11,12]. Ecotoxicological effects of mixtures can be observed even at concentrations safe for each single chemical exposure [13]. In real agricultural soils, pesticides rarely occur as single substances but often together. Of 317 EU agricultural soil samples taken in the LUCAS monitoring campaign in 2015 across Europe, 83 % had pesticide residues, and 58 % contained pesticide mixtures [14]. This is because i) in several pesticide formulations, more than one A.S. can be present, ii) for some applications, mixing of multiple PPPs can be recommended (tank mix), iii) pesticides can co-occur in the environment due to application of spray series schemes, iv) pesticides co-occur in the environment due to their re-distribution, persistence, and transformation. Therefore, organisms in the environment are potentially exposed to these mixtures. According to the EU Commission on Assessment of Mixtures, many studies have reported that few pesticides found in mixtures (priority pesticides) drive the majority of toxicity potency of the respective mixtures [12]. Therefore, ecotoxicological tests do not need to be carried out with all the pesticides in the mixtures; instead, they should primarily focus on priority pesticides in the mixture that drive the overall toxicity of the mixture.

The persistence of a pesticide is an important characteristic that is often considered in environmental risk assessment. Some authors have argued that high persistence alone can be sufficient to trigger risk mitigation [15]. Based on Knuth et al. [16], the persistence of pesticides has been underestimated, where about 79 % of detected residues during soil sampling were from pesticides that were not applied in that growing season. Therefore, apart from the relative toxicity of the pesticides, persistence should be considered an essential criterion for cumulative mixture risk assessment. Since not all components of a mixture drive the toxicity, this study aims to elaborate a selection procedure for priority pesticides that potentially drive mixture toxicity in ecological receptors (fauna, flora, and microbes) in terrestrial compartments. The aim of this

prioritization is to select the most relevant pesticide mixtures in 11 crop systems, which should be further assessed in ecotoxicological studies for risk assessment of the mixtures on the terrestrial ecosystem. To do this, we selected the pesticides by using the risk quotient of the pesticides, their persistence in the environment, and their frequency of detection as criteria for scoring and attributing equal weight of scoring to the criteria.

2. Methodology

2.1. Pesticide residue assessment

For this procedure, we used the results of the SPRINT (sprint-h2020.eu) monitoring campaign (growing season 2021) on 192 pesticide residues from > 200 agricultural soils across 10 European countries and Argentina [16]. The main European cropping systems (CS) were covered (Table S1), from conventional and organic farms [17,18]. Also, one cropping system (cereal) for Argentina was included, covering wheat, which is exported to Europe for livestock feed. Detailed information on the selection of the 192 pesticide residues, soil sampling, and pesticide analysis are given in Supplementary Material (S1) and comprehensively in Knuth et al. [16]. In total, 97 pesticide residues were quantified (i.e., concentrations higher than the limit of quantitation - LOQ) in the 11 crop systems. The number of times each pesticide was found in samples collected from various farms in each CS was recorded as the frequency of detection or occurrence of these pesticides. These 97 pesticide residues included non-approved pesticides, legacy pesticides, and their metabolites (for example, DDT and metabolites occurred in all crop systems). To focus on the pesticides that are currently approved in Europe during the sampling period, we did not include any pesticides not approved before the beginning of the previous planting season (September 2020). From the 97 residues, 24 pesticides were banned before the sampling period. Therefore 24 pesticides were removed and the remaining 73 pesticides were included in the following steps of the prioritization procedure.

2.1.1. Exposure, toxicity data collection, and risk quotient calculation

2.1.1.1. Exposure data collection. Data on exposure and toxicity of the 73 pesticides and metabolites from the list obtained for the pesticide residue were collected for soil invertebrates, bees and non-target arthropods, plants, and microbes. These ecological receptors from the terrestrial environment were chosen to represent different taxons used in the ecotoxicology tests necessary for the approval of pesticides, including arthropods (bees and non-target insects), soil meso- and macro-fauna (earthworms and other soil invertebrates), non-target plants, and microbes as soil nitrogen transformation [19]. Soil is not generally the main route of exposure to all these organisms; for example, bees and non-target arthropods are mainly exposed via air (i.e., dermal uptake, inhalation) and diet (oral intake of contaminated pollen and other particles) [20], and plants are exposed via air (foliar uptake) [21]. Therefore, for the bees and non-target arthropods, we proposed to use the pesticide dissipation rate in and on the plant matrix from the EFSA conclusions to account for persistence. However, these values were not available for some pesticides. We used the soil pesticide degradation rate (DT50) to account for the persistence of the soil invertebrates and microbes.

The data for exposure were taken from EFSA documents as the highest values found for accumulated predicted environmental concentration (PEC_{accum}), application rate, or predicted environmental rate (PER_{off-field}). Modeled data was chosen over measured concentrations in the crop systems since the soil samples were collected at one time-point, while measured concentrations (MEC) are often used in risk

assessment when they are obtained from long-term monitoring programs [22].

2.1.2. Toxicity data and risk quotient calculation

The ecotoxicological data collected and risk quotient calculation (Fig. 1) for each of the ecological receptors were done as follows:

2.1.2.1. Soil invertebrates. PPDB (<http://sitem.herts.ac.uk/aeru/ppdb/>) [23] and EFSA documents (i.e., conclusions and assessment reports) (<http://www.efsa.europa.eu/>) were first checked for the NOEC/LC50/EC50 values for standard species of soil invertebrates (Earthworm = *Eisenia fetida*, springtail = *Folsomia candida* and predatory soil mite = *Hypoaspis aculeifer*). For the soil invertebrate data, the assessment factors for risk were applied to calculate the predicted no-effect concentration (PNEC). Assessment factors of 10 and 5 were used for LC50/EC50 and NOEC, respectively. The PNEC was derived by dividing the LC50/EC50 or NOEC by the assessment factors (trigger values when using a toxicity vs. exposure ratio approach). The risk quotient (RQ) was calculated as PEC/PNEC for soil invertebrates (Fig. 1).

2.1.2.2. Bees and non-target arthropods. For the bees and non-target arthropods (NTAs), the dose effects on bees and NTAs were used. These dose-effect data were curated from the PPDB/EFSA conclusion reports for active substances. The LD50 data for bees, *Apis mellifera*, *Bombus terrestris*, and Mason bees were used. For the NTAs, the LR50 data for the *Aphidius rhopalosiph* and *Typhlodromus pyri* were used. No assessment factor was applied. The exposure of the bees and NTAs was the application rate of the pesticides on crops. These application rates on crops were derived from the EFSA conclusions for each of the pesticides. The risk quotient was thus calculated as Application rate (g/ha)/LD50 or LR50.

2.1.2.3. Terrestrial plants. For terrestrial plants, ecotoxicological data (ER50 or HC5) were extracted from EFSA documents, except for chloroturon, which data was obtained from the literature [24]. The exposure of terrestrial plants was collected primarily from EFSA conclusions which considers, $PER_{off-field}$, PER_{drift} and application rate. If it was not

provided in the conclusions, the values used in the risk assessment in draft assessment reports or draft renewal assessment reports were adopted. For some metabolites with no data on exposure, the exposure values were calculated using the % formation of the applied dose of the parent compound (also provided in EFSA reports). For example, the maximum AMPA formation is 53.8 % of glyphosate applied dose [25]. Thus, exposure of AMPA was calculated as glyphosate $PER_{off-field}$ multiplied by 0.538. The Risk Quotient (RQ) was calculated by the ratio of exposure ($PER_{off-field}$ or application rate) and PNEC. The same unit was used for exposure and PNEC in the calculation (either as mL/ha, g/ha, or mg/kg).

2.1.2.4. Microbes. For microbes, the effects of pesticides on soil nitrogen transformations were used for the prioritization. Nitrogen transformation was the endpoint used because data on other endpoints (carbon transformation, enzymatic activities) were less available. The data was extracted from EFSA conclusions. The concentration (in mg/kg) showing $\geq 25\%$ deviation from control for effects on nitrogen mineralization was used as the dose effect (also in mg/kg) for calculating the RQ. No assessment factor was applied for microbes since only one endpoint was considered.

2.2. Pesticide ranking and mixture selection (or prioritized pesticides) to be used in ecotoxicological tests

The pesticides were scored and ranked relative to each other within each crop system for each ecological receptor, as follows:

2.2.1. Soil invertebrates

For each pesticide, the risk quotient, DT50, and frequency of detection in soil were obtained, and used as criteria for the ranking of the pesticides.

Table 1 shows an exemplification of scores obtained and their normalization. The score of each pesticide was divided by the highest score obtained in each criterium to normalize all scores to 1. Using the multiplication rule of the probability of two or more events, we multiplied the 3 normalized scores together.

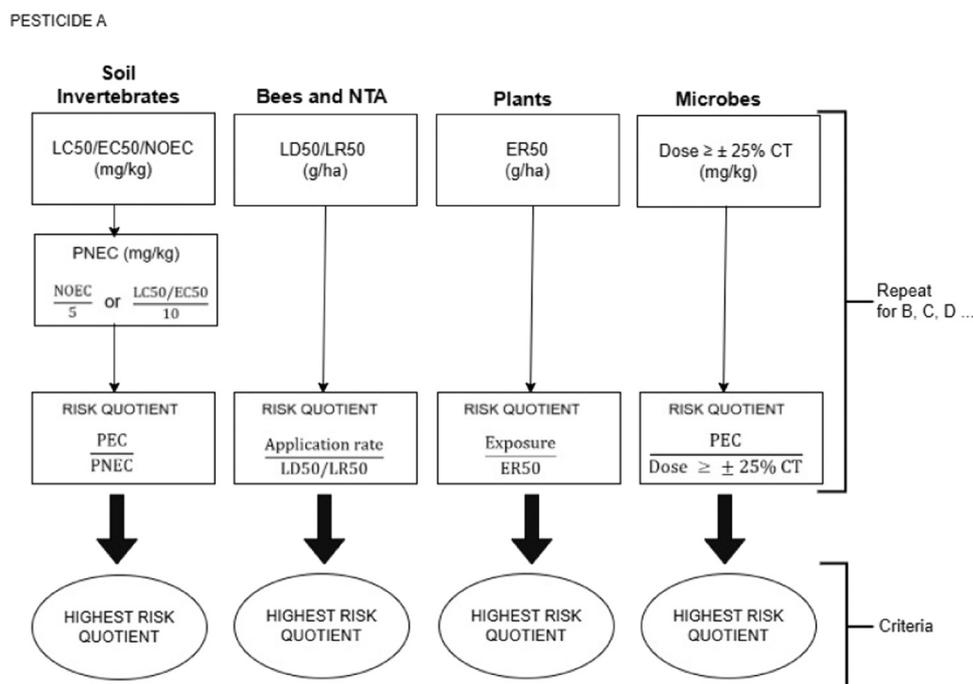


Fig. 1. A conceptual model of the risk quotient calculation for selecting the pesticides of concern in the crop systems for soil invertebrates, bees, and NTAs, plants, and microbes. The exposure for the plants could be $PER_{off-field}$ or PER_{drift} or application rate depending on which is available.

Table 1
Example of pesticide scoring and normalized scores.

A -Pesticide scoring	Risk quotient	DT50 typical (days)	Occurrence (%)	
Pesticide A	5	75	100	
Pesticide B	2.2	100	75	
Pesticide C	2.7	35	62	
Pesticide D	6.1	42	60	
B- Normalization	Normalized risk quotient	Normalized DT50	Normalized Occurrence	Total score
	0.82	0.75	1	0.62
	0.36	1	0.75	0.27
	0.44	0.35	0.62	0.10
	1	0.42	0.60	0.25

Rearranging the pesticides in order of the highest scores to the lowest, the pesticides with the highest scores are pesticides that are prioritized for mixture toxicity testing:

1. Pesticide A
2. Pesticide B
3. Pesticide D
4. Pesticide C

Using this method, the first five pesticides with the highest scores (1–5) were the prioritized pesticides that would make up the mixture toxicity testing for the soil invertebrates.

2.2.2. Bees and non-target arthropods

The pesticides were scored using risk quotient and occurrence. To account for persistence, the half-life of the pesticides (represented by the pesticide dissipation rate in and on the plant matrix) was available in the EFSA guidance document for some pesticides and unavailable for some. Apart from that, the half-lives of the pesticides in/were not significantly different within an order of magnitude and would not change the outcome, so we could not use the half-life of the pesticides in this prioritization. Following the same procedures exemplified with soil invertebrates, the scores of these two criteria (risk quotient and occurrence) were normalized and multiplied. The first five pesticides with the highest scores would make up the mixture toxicity testing for the bees and non-target arthropods.

2.2.3. Plants

For plants, the pesticides were scored based on their risk quotient and occurrence in soil. DT50 was not considered a criterion because foliar uptake is the main route of pesticides in plants. The scores were normalized and multiplied, giving each pesticide a final score. Following the same procedures as exemplified with soil invertebrates, the first five pesticides with the highest scores would make up the mixture toxicity testing for the plants.

2.2.4. Soil microbes

For the soil microbes, the procedure was the same for the soil invertebrates, except that the toxicity to microbes was based on dose $\geq \pm 25\%$ nitrogen transformation. No assessment factor was applied since only one endpoint was considered. The scores of risk quotient, DT50, and occurrence were normalized, multiplied, and ranked. The first five pesticides with the highest scores were the prioritized pesticides that would make up the mixture toxicity testing for the soil microbes.

The [Supplementary Material](#) contains Excel spreadsheets with comprehensive details of the calculations and scoring for all the ecological receptors. Spreadsheets to show a full example of how pesticides were prioritized for soil invertebrates and bees and NTAs in one cropping system was added.

3. Results

3.1. Pesticide residue assessment result

Of the 73 pesticides and metabolites assessed for final prioritization, fungicides were the most common in all the sites, considering the number of fungicide residues detected in soils (Table 2), followed by herbicides and then by insecticides. However, 29 % of the fungicides found in the crop systems were prioritized for soil invertebrates, plants, and microbes, and 34 % were prioritized for insects. Out of a total of 21 herbicides that were detected, about 86 % were prioritized for plants, and out of the total of 15 insecticides included, about 79 % were prioritized for the bees and NTAs. From these 73 pesticides, 59 were included in the final prioritization list (i.e., top 5 scored pesticides in each crop system), considering all included ecological receptors (Table 3). The full list of the 73 pesticides can be assessed (Table S2). And the pesticides that were not included in the final prioritization can be assessed (Table S3).

3.2. Priority pesticide of concern

3.2.1. Soil invertebrates

Twenty-three ($n = 23$) unique pesticides were prioritized for the ecological receptors in all crop systems, two of which are metabolites (AMPA and THPI) of the parent compound (Fig. 2). Fungicides made up the highest proportion of pesticides (52 %), followed by herbicides (35 %) and insecticides (13 %). As criteria for prioritizing pesticides, frequency, and DT50 had higher scores than RQ on average. Relative to the other criteria, the herbicide oxyfluorfen had the highest RQ, metolachlor had the highest frequency, and prochloraz had the highest DT50. Nine pesticides had $RQ > 1$ (Table S4). The pesticides belonged to seventeen ($n = 17$) pesticide groups with conazole, triazole, carboxamide and strobilurin being more common (Table S4). Most of the pesticides belonged to the moderate toxicity class to the earthworm *Eisenia fetida* (Table S4).

3.2.2. Bees and non-target arthropods

Thirty ($n = 30$) unique pesticides of concern were identified from all the crop systems (Fig. 3). Like soil invertebrates, fungicides comprised the highest proportion (47 %), followed by insecticides (33 %). Frequency was more prominent in terms of higher scores for prioritizing the pesticides (Fig. 5). Relative to the other criteria, emamectin had the highest RQ, thiencazabone-methyl had the highest frequency and propamocarb (hydrochloride) had the greater half-life. The pesticides belonged to twenty-five ($n = 25$) pesticide groups (Table S5). Pyrethroid, carbamate, carboxamide, organobromide and triazole were more common ($n = 2$) (Table S5). Three of the pesticides belonged to the highly toxic class, and thirteen pesticides were moderately toxic. The three highly toxic pesticides were all insecticides. However, three insecticides were moderately toxic, six fungicides were also moderately toxic, and three herbicides were moderately toxic (Table S5). Fifteen pesticides had $RQ > 1$. From these 15 pesticides with $RQ > 1$, 63 % were insecticides, 25 % were fungicides, and 12 % were herbicides

Table 2

The total number of each pesticide type (fungicide, herbicide, and insecticide) that are approved and currently used and detected (>LOQ) in all crop systems and the proportion (%) of the prioritized pesticides for the different terrestrial ecological receptors (soil invertebrates, bees and NTAs, plants, and microbes). Bees and NTAs = Bees and non-target arthropods, microbes = soil microbes.

Target classes	Fungicides (Total n = 38)		Herbicides (Total n = 21)		Insecticides (Total n = 14)	
	Ecological receptors	Number of fungicides	Percentage of total Fungicides	Number of herbicides	Percentage of total herbicides	Number of insecticides
Soil Invertebrates	11	28.9 %	8	38.1 %	4	28.6 %
Bees and NTAs	13	34.2 %	6	28.6 %	11	78.6 %
Plants	11	28.9 %	18	76.19 %	3	21.4 %
Microbes	11	28.9 %	6	28.6 %	2	14.2 %

Table 3

Prioritized pesticides (5 pesticides that would be used for mixture toxicity assays) of the 11 cropping systems (CS) for soil invertebrates, bees and non-target arthropods, plants, and microbes.

Cropping systems	Soil invertebrates	Bees/NTAs	Plants	Microbes
Spain (Vegetables)	Oxyfluorfen	Lambda Cyhalothrin	AMPA	Metrafenone
	Chlorantraniliprole	Acetamiprid	Glyphosate	Propamocarb (hydrochloride)
	Difenoconazole	Chlorantraniliprole	Metazachlor	Pendimethalin
	Boscalid	Glyphosate	Propyzamide	Boscalid
	Propyzamide	Propamocarb (hydrochloride)	Oxyfluorfen	Difenoconazole
Portugal (Vineyards)	Chlorantraniliprole	Cyprodinil	Glyphosate	Metrafenone
	Boscalid	Tebuconazole	AMPA	Boscalid
	Difenoconazole	Glyphosate	Iprovalicarb	Fludioxonil
	AMPA	Spirotetramat	Penconazole	Penconazole
	Azoxystrobin	Spiroxamine	Metolachlor oxanilic acid	Tebuconazole
France (Vineyards)	Chlorantraniliprole	Lambda Cyhalothrin	AMPA	Metrafenone
	Boscalid	Trifloxystrobin	Glyphosate	Boscalid
	Difenoconazole	Spiroxamine	Fenbuconazole	Glyphosate
	AMPA	Metrafenone	Metrafenone	Dimethomorph
	Cyflufenamid	Glyphosate	Difenoconazole	AMPA
Switzerland (Orchards)	Difenoconazole	Emamectin	AMPA	AMPA
	Methoxyfenozide	Tebuconazole	Difenoconazole	Difenoconazole
	Myclobutanil	Myclobutanil	Myclobutanil	Methoxyfenozide
	AMPA	Pirimicarb	Emamectin	Tebuconazole
	Pirimicarb	Difenoconazole	Tebuconazole	
Italy (Vegetables)	Chlorantraniliprole	Lambda Cyhalothrin	Propyzamide	Metrafenone
	Oxyfluorfen	Chlorantraniliprole	Boscalid	Boscalid
	Boscalid	Tebuconazole	Lambda Cyhalothrin	Pendimethalin
	Propyzamide	Pendimethalin	Oxyfluorfen	Propamocarb (hydrochloride)
	Difenoconazole	Boscalid	Difenoconazole	Tebuconazole
Croatia (Olives)	Boscalid	Phosmet	Glyphosate	Boscalid
	AMPA	Acetamiprid	AMPA	Tebuconazole
	Dimethomorph	Tebuconazole	Deltamethrin	AMPA
	Tebuconazole	Glyphosate	Trifloxystrobin	Glyphosate
	Glyphosate	Trifloxystrobin	Tebuconazole	Dimethomorph
Slovenia (Maize)	Bixafen	Bixafen	Metolachlor (S)	AMPA
	AMPA	Terbuthylazine	Terbuthylazine	Tebuconazole
	Tebuconazole		Thiencarbazone-methyl	Terbuthylazine
	Terbuthylazine	Tebuconazole	AMPA	Thiencarbazone-methyl
	Metolachlor (S)	Thiencarbazone-methyl	Terbuthylazine desethyl	
Czech republic (Oilseed rape)	Boscalid	Lambda Cyhalothrin	AMPA	Metrafenone
	Prochloraz	Tau-fluvalinate	Diflufenican	Boscalid
	AMPA	Acetamiprid	Mecoprop P	Tebuconazole
	Dimoxystrobin	Flupyradifurone	Dimethenamid (P)	Dimoxystrobin
	Azoxystrobin	Spiroxamine	Deltamethrin	Flupyradifurone
Netherlands (Root crops)	Bixafen	Lambda Cyhalothrin	Metribuzin	AMPA
	Boscalid	Prochloraz BTS 44595	Metobromuron	Boscalid
	Azoxystrobin	Esfenvalerate	AMPA	Fluoxastrobin
	AMPA	Metobromuron	Diflufenican	Metobromuron
	Pirimicarb	Prosulfocarb	Prosulfocarb	Prochloraz BTS 44595
Denmark (Cereals)	Boscalid	Esfenvalerate	AMPA	AMPA
	AMPA	Boscalid	Diflufenican	Boscalid
	Diflufenican	Glyphosate	Fluopyram	Pendimethalin
	Fluopyram	Fluopyram	Pendimethalin	
	Pendimethalin	Pendimethalin	Boscalid	
Argentina (Wheat)	AMPA	Lambda Cyhalothrin	Glyphosate	
	methoxyfenozide	Glyphosate	AMPA	AMPA
	Azoxystrobin	Bixafen	Diflufenican	Glyphosate
	Fluxapyroxad	Fluxapyroxad	2,4-D (free)	Methoxyfenozide
	Bixafen	Folpet	Folpet	

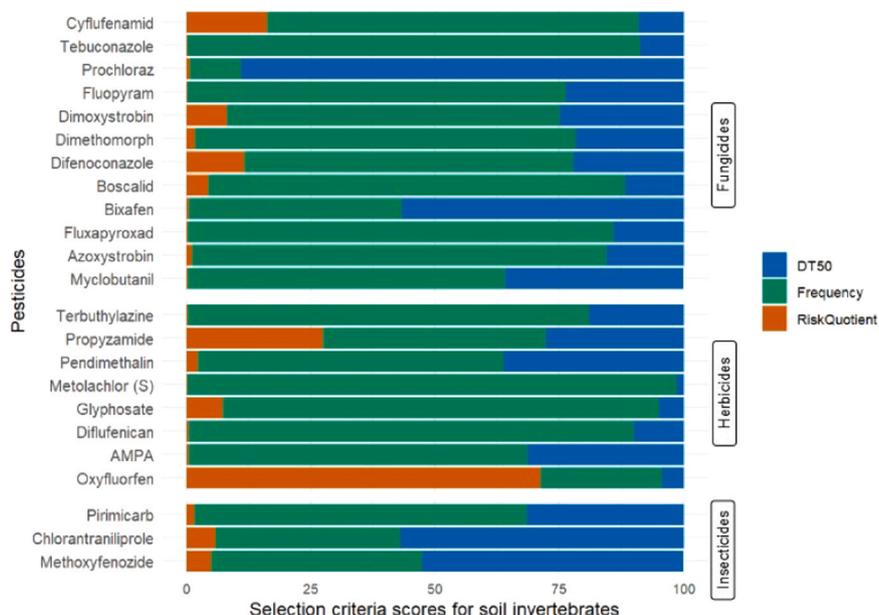


Fig. 2. The prioritized pesticides (n = 23) for soil invertebrates found in eleven (n = 11) crop scenarios showing the percentage of the risk quotient (RQ), frequency and DT50. The longer the bar, the higher the percentage of the criteria for the pesticide. The DT50 = 50 % degradation time in soil (in days).

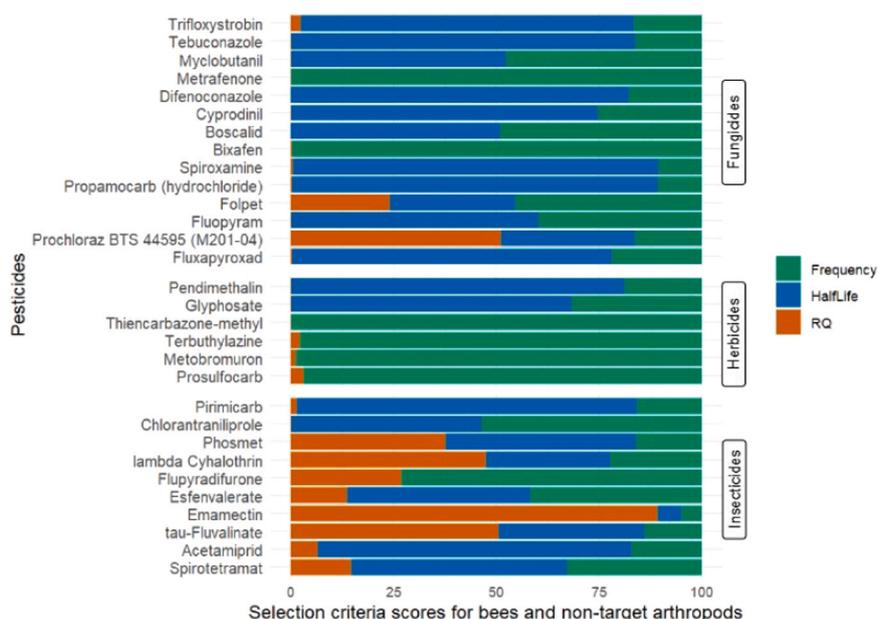


Fig. 3. The prioritized pesticides (n = 30) for bees and non-target arthropods found in eleven (n = 11) crop scenarios, showing the percentage of the risk quotient (RQ), frequency and half-life. The longer the bar, the higher the percentage of the criteria for the pesticide. The half-life = 50 % dissipation rate of the pesticide in crop (in days).

(Table S5).

3.2.3. Terrestrial plants

Thirty unique pesticide residues were selected for the mixture composition of the 5 pesticides from 11 crop systems (Fig. 4). The majority were herbicides (53 %), and the least were insecticides (10 %). They belonged to 22 groups. Triazole (n = 4) was the most common followed by Chloroacetamide (n = 3). Eighteen pesticides had RQ > 1. Of those, 78 % were herbicides, 17 % were fungicides, and 5 % were insecticides. Relative to the other criteria, metolachlor had the highest RQ and boscalid had the highest frequency (Table S6).

3.2.4. Microbes

From the 73 pesticides assessed, 38 pesticides did not show any effects on soil nitrogen transformation (i.e., deviation < 25 % CT) at any time and were, therefore, not included in the prioritization. The data of some compounds were not fully provided in the EFSA reports, only stating that effects were < 25 % after 28 days of exposure. For these compounds, we had to assume that no effect occurred at any other time points (e.g., 7 or 14 days). Finally, 35 out of 73 pesticides were found to affect nitrogen mineralization. After ranking the top 5 pesticides from each crop system, 19 unique pesticides were included in the final prioritization list for effects on microbes (Fig. 5). Of those, 58 % were fungicides, 32 % were herbicides and 10 % were insecticides. They belonged to 16 groups. Phosphonoglycine (n = 2), conazole (n = 2), and

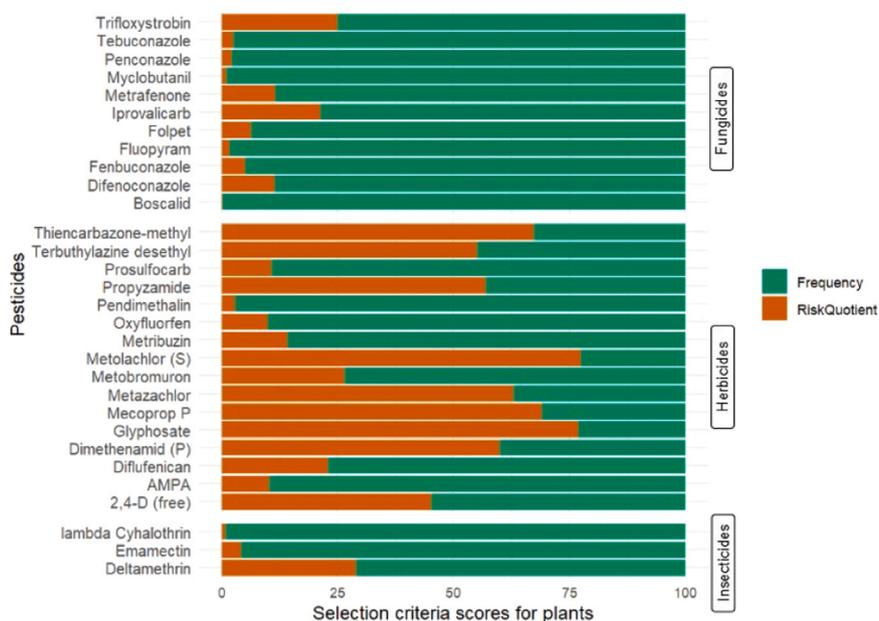


Fig. 4. The prioritized pesticides (n = 30) for plants found in eleven (n = 11) crop scenarios, showing the percentage of the risk quotient (RQ) and frequency. The longer the bar, the higher the percentage of the criteria for the pesticide.

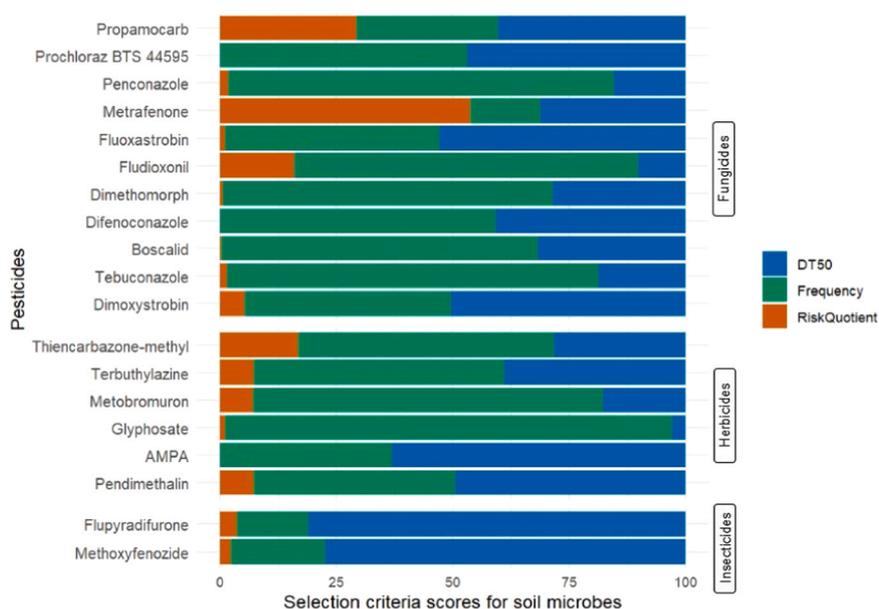


Fig. 5. The prioritized pesticides (n = 19) for soil microbes found in eleven (n = 11) crop scenarios showing the percentage of the risk quotient (RQ), frequency and DT50. The longer the bar, the higher the percentage of the criteria for the pesticide. The DT50 = 50 % degradation time in soil (in days).

triazole (n = 2) were more common of the groups (Table S5). Four pesticides had $RQ \geq 1$, namely the fungicides metrafenone, propamocarb hydrochloride, fludioxonil, and the herbicide thiencarbazono-methyl (Table S7). The DT50 and frequency of the pesticides dominated with higher scores as the criteria for prioritizing the pesticides (Fig. 5).

3.3. Comparison of risk quotients (RQs) and frequency of prioritized pesticides between the ecological receptors

3.3.1. Risk quotients

Considering the median risk quotients (RQs), pesticides were of more concern to the bees and NTAs and plants than to soil invertebrates and microbes (Fig. 6). The microbes were shown to be, on average, least affected by pesticide exposure because this organism class has the lowest

median RQ of the ecological receptors.

3.3.2. Frequency of prioritized pesticides to the ecological receptors

Across all the CS, some pesticides were prioritized for each ecological receptor in at least 4 CS (Table 4). The soil invertebrates had the most (n = 5) frequently used pesticides in the CS compared to other ecological receptors. Two ecological receptors had four frequently used pesticides each except the bees and NTAs, which had three (n = 3), which was the least. Only soil invertebrates and beneficial insects had all the classes of pesticides (fungicides = FU, herbicides = HB, and insecticides = IN) frequently occurring in the CS.

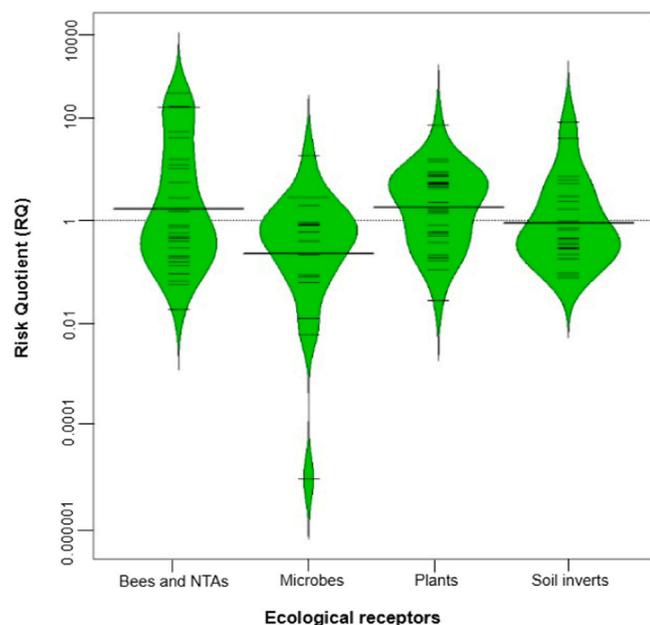


Fig. 6. Bean plot showing the distribution and median of the risk quotient (RQ) of the pesticides in all case study sites per ecological receptor. The dotted horizontal line is the point where the RQ = 1. Bees and NTAs: bees and non-target arthropods. Microbes: microbial activity measured as nitrogen transformation. Plants: terrestrial non-target plants. Soil inverts: soil invertebrates.

4. Discussion

4.1. Pesticide prioritization makes monitoring efficient

Continental-level projects, such as in Europe, have shown that biogeographical differences and the dominating vegetation or cropping systems are important for assessing the risk of pesticides on biodiversity [17,26,27]. As such, residues of pesticides currently used in agriculture are distributed variably across the EU regions, with markedly distinct pesticide mixtures in many cases. On the surface, it is important to assess the risk based on all pesticides found on sites. Studies have repeatedly shown that only a few compounds cause significant risk [28,29]; therefore, prioritizing the pesticides of concern increases the efficiency in monitoring and performing required ecotoxicological tests [30]. Before now, monitoring programs are poorly utilized to support prioritization programs [30]. More recent prioritization programs, although focused on aquatic risk assessment, utilized monitoring data [29–31]. The terrestrial component of SPRINT, making up the present study, used the vast monitoring data collected on pesticide residue to prioritize pesticides of concern. In this paper, we developed a prioritization approach for selecting pesticides of concern found in environmental samples from case study sites representing different crop systems. The quantification of pesticide residues in soil samples from 11 CS was conducted within SPRINT project, and here, we aimed at selecting up to 5 pesticides representing the highest risk to different terrestrial organisms from each CS. A relevant mixture of 5 pesticides was chosen, as

most agricultural soils contain 2–10 pesticide residues [14,32,33]. This prioritization can be a helpful tool to define a mixture of pesticides quantified in environmental samples to be used in ecotoxicological tests and where increased attention can be focused.

4.2. Ecotoxicological data is scarce for many registered pesticides

Before being placed in the European market, the risk assessment of pesticides must be carried out following Regulation No 1107/2009 [34]. Still, the availability of data is considerably variable among pesticides. Partially because these data are often not publicly available or because not all ecological receptors are tested. The lack of available data has been pointed out in risk assessment studies in terrestrial compartments [35,36]. In a study on ERA of pesticides in Czech soils, Vašíčková et al. [36] observed that chronic data of different trophic levels were not available for 30 % of the pesticides and metabolites assessed. Similarly, to our study, Di Nica et al. [35] used a scoring approach to rank veterinary pharmaceuticals, calculating RQ as the ratio between PEC and PNEC. However, due to the lack of ecotoxicological data for some compounds, they found it necessary to use a default score for these missing compounds.

4.3. Class-specific impacts of pesticides on taxonomic groups

Generally, fungicide residues were the most commonly found of all pesticides in all CS. However, fungicides were only more concerning to microbes, and herbicides were more concerning to the non-target plants. This is not surprising because, for new or currently used pesticides, there is an increased focus on targeted chemistry [31]. Glyphosate and/or its metabolite, AMPA, was of concern to all taxonomic groups in more than 4 CS. This shows the spread and intensity of glyphosate use in agroecosystems, which is consistent with many reports [37]. It is known that glyphosate is the most sold, and most used of broad-spectrum herbicides globally and many studies have shown that it can be toxic to microbes, soil invertebrates, and insects [38,39]. The only fungicide that was of concern to plants in more than 4 CS was difenoconazole. Difenoconazole has been implicated as toxic to plants. For example, difenoconazole significantly inhibited wheat (*Triticum aestivum* L.) plant growth [40]. The study showed that difenoconazole caused oxidative stress by generating ROS in the leaves and roots of the wheat seedlings. The pesticides of concern for plants (75 % more herbicides) and microbes (75 % more fungicides) in more than 4 CS was expected because these classes of pesticides were designed to specifically target these two ecological receptors (Table 4).

4.4. Soil invertebrates and beneficial insects were more vulnerable to all classes of pesticides

Soil invertebrates and beneficial insects (i.e., bees and NTAs) were the only receptors that had all the 3 classes of pesticides to be of concern in more than 4 CS (Table 4). This shows the vulnerability of these two ecological receptors to incidences of pesticides, regardless of pesticide type, applied in agroecosystems. This makes a case for testing all pesticides on non-target organisms regardless of their target chemistry. Honeybees ecotoxicological data represented most of the data for the

Table 4

The prioritized pesticides for each of the ecological receptors in \geq four crop systems.

	Ecological receptors			
	Soil Invertebrates	Bees and NTAs	Plants	Microbes
Pesticides that occurred in at least 4 CS	AMPA (HB) Boscalid (FU) Chlorantraniliprole (IN) Difenoconazole (FU) Azoxystrobin (FU)	Tebuconazole (FU) Glyphosate (HB) Lambda Cyhalothrin (IN)	AMPA (HB) Difenoconazole (FU) Diflufenican (HB) Glyphosate (HB)	AMPA (HB) Boscalid (FU) Metrafenone (FU) Tebuconazole (FU)

beneficial insects and are the most sensitive based on RQs. This is consistent with Cech et al. [3], who reported that the pesticide residue hazards to honeybees were higher than that of the earthworms. The earthworm data overwhelmingly represented most of the soil invertebrate data available. For currently used pesticides, there is a reduction in the number of very toxic pesticides to earthworms, but the number of moderately toxic pesticides to earthworms did not change [3]. Microbes were the least sensitive receptors based on the mean RQ. However, it could be a mere consequence of the RQ calculations using a trigger of 1, because the ecotoxicological data of only one endpoint was included. The risk assessment of pesticides in microbes is, in general, overlooked. Several studies have pointed out that other endpoints should be included when assessing the risks of contaminants to microbes so that effects such as abundance and diversity are also taken into consideration [41,42].

4.5. Prioritization of pesticides of concern should always include the potential risks of the pesticides

Compared to the aquatic ecosystems, prioritization of pesticide exercises is scarce for environmental risk assessment of terrestrial ecosystems. For the terrestrial ecosystems, the few ones are targeted towards human health [43]. [43] used a multi-level strategy to prioritize pesticides by scoring pesticides produced based on the likelihood of use in agriculture, the residues in crops with potential for transfer to humans through consumption, intake rates by humans through soil, food, and dermal contact. They suggested that the focus should also be on ecological health, such as in the current study. Anderson et al. [31] prioritized pesticides by factoring pesticide sales volume data and the concentrations of the pesticides detected in the aquatic environment as criteria. However, one limitation of the data used in Anderson et al. [31] is that risk was not considered since the volume of pesticide sales and environmental concentrations does not necessarily mean risk because the associated hazard is not known.

4.6. Persistence and frequency of detection are important for pesticide mixture prioritization

Persistence and frequency of detection were the most important criteria for prioritizing the pesticide mixtures to soil invertebrates and microbes (Fig. 2 & 5), frequency of detection was the most important for beneficial insects (Fig. 3), and the risk quotient was the most important for the plants (Fig. 4). Our (SPRINT) method prioritized pesticide residue for ecotoxicological tests representative of CS by considering hazard risks, persistence, and frequency of occurrence of the pesticides as very important criteria. In agreement with Vryzas et al. [30], SPRINT prioritization was done on a case-by-case basis whereby we prioritized based on the different use patterns peculiar to the main European cropping systems. Although we relied on the regulatory databases for the pesticides DT50 in soil, half-life in plants, PECs in soil, or application rates, not all data was available in a few cases for the prioritization exercise. We also have to acknowledge that the scarcity of toxicity data can be very challenging when carrying out efficient prioritization exercises. Our method of prioritization is an efficient way of prioritizing pesticides for mixture toxicity assessment for environmental risks, considering the scarcity of frequent monitoring data. However, to be more comprehensive, it would be worthwhile to look at prioritization across crops within the same cropping system since each supports more than one type of crop. Doing it this way will give a better picture of the prioritized pesticides for a more robust risk assessment.

4.7. Limitations of our score-based approach for pesticide prioritization and recommendations

This study aimed at providing a scoring approach to help the decision of testing pesticide mixtures when campaign data on the occurrence of pesticides in the agriculture field is conducted; therefore, the scoring

approach was produced as a tool to be used for any dataset from monitoring campaigns and programs. This approach, however, has its limitations, and several factors influenced the final list of pesticides prioritized. Firstly, the selection of the pesticides is conditioned to the list of pesticides analyzed. The soil samples were analyzed for 192 pesticides (parent compounds and metabolites), although the list of current-use pesticides is much larger. For example, over 400 active substances, safeners, and synergists are currently approved in the Europe Union according to the EC database [2]. Some of those substances, such as copper, were not analyzed in the soil samples, although copper-based fungicides are highly used in Europe, including organic crops [44].

The use of the frequency of occurrence as a parameter in the scoring approach is also a limiting factor. The detection of pesticide residues depends on the LOQs, which are subject to the analytical methods and properties of the pesticides. A pesticide can have a higher frequency of occurrence when its LOQ is lower. Therefore, the frequency of occurrence can be biased by the LOQ values. Finally, the data on pesticide occurrence in the soil was based on a one-time sampling; the presence of pesticide residues in soil can be affected by the time of sample collection. For the pesticides applied during the planting season, the duration between the time of application and the time of sampling and the persistence of the pesticides play a significant role in their occurrence.

The prioritization of pesticides for non-target terrestrial plants (NTTPs) has several limitations. One key issue is the heterogeneity in exposure data. The exposure values used were not consistent across all pesticides, as they included both in-field application rates (e.g., maximum application rates) and off-field exposure rates (e.g., drift rates). However, we chose to use the same exposure values as those reported in EFSA risk assessments to maintain consistency with established regulatory frameworks. Additionally, because our prioritization was based on pesticides detected in specific study fields, it is possible that some of the prioritized compounds are not of concern for NTTPs, as for example, pesticides with ER50 values exceeding maximum application rates. The scarcity of toxicity data for some pesticides could also be a limitation. Pesticides with more toxicity data to calculate a risk quotient may influence their selection over pesticides with few or just one data. This is because it is easy to pick a high-risk quotient from a range of risk quotients for a toxicity-data-rich pesticide, compared to just limited to one or few risk quotients for toxicity-data-poor pesticides.

As recommendations to ecotoxicology testing, the testing concentrations should include the environmental measured concentrations, considering that soil is the main route of exposure to the test organism. Other concentrations to be used may include the PEC values or application rates.

5. Conclusion

The findings from this study highlight that current pesticide assessments, often based on single-chemical evaluations, are insufficient given the real-world scenario of cumulative pesticide mixtures. This study then significantly contributes to improving environmental risk assessment by systematically prioritizing pesticides that drive mixture toxicity in various terrestrial ecological receptors across European and Argentinian cropping systems. By prioritizing pesticides based on their ecological risk, persistence, and frequency of detection, we identified the top five pesticides for each crop system that can help streamline ecotoxicity testing and focus on the mixtures that pose the greatest risk to soil invertebrates, beneficial insects, plants, and microbial communities. Results clearly demonstrate that certain pesticide classes disproportionately impact specific ecological groups such as herbicides notably affecting plants, fungicides impacting microbes, and all the three classes of pesticides (insecticide, herbicides and fungicides) posing risk to soil invertebrates and beneficial insects which includes the bees. However, our study identified data availability as a limitation for more efficient prioritization of pesticides for mixture risks and a call for more

data thereby advocated. Nevertheless, our approach is practical and useful for evaluating risk of sites using monitoring data. By prioritizing pesticides based on monitoring data from diverse cropping systems, our approach provides practical tool to improve pesticide management strategies when mixture ecotoxicological tests are more representative of the environment, hence achieve better environmental protection.

Environmental implications

Current risk assessment of pesticides is based on single pesticide test data, but in reality, it should be based on cumulative pesticide mixtures. However, not all mixtures can be tested therefore a need for prioritizing pesticides that will drive mixture toxicity. This study therefore prioritized pesticide mixtures in the terrestrial ecosystem based on three criteria: their ecological risk, persistence, and the frequency of detection. Using real pesticide residue measurement in the environment, the study enhanced monitoring programs by focusing on the most concerning pesticides across major cropping systems to address risk to soil invertebrates, beneficial insects, plants and microbes.

CRedit authorship contribution statement

Jakub Hofman: Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Violette Geissen:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Paula S. Tourinho:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jegede Olukayode Oluwole:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Violette Geissen reports financial support was provided by European Union Horizon 2020. Reports a relationship with that includes: Has patent pending to. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2025.138942](https://doi.org/10.1016/j.jhazmat.2025.138942).

Data availability

Data will be made available on request.

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