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Critical Review

Collembola are Among the Most Pesticide-Sensitive Soil Fauna Groups: A Meta-Analysis

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Abstract: Pesticides are a major concern because of their deleterious impacts on biodiversity and on the ecological functions provided by living organisms. Although earthworms are well studied, smaller-sized organisms, such as Collembola, also contribute to the agroecosystem functioning, and their sensitivity to pesticides makes them good bioindicators of soil quality. Using data from 21 publications, we performed a meta-analysis to compare the pesticide sensitivity of Collembola with other soil invertebrate groups and discuss the relevance of including tests on representatives of this microarthropods group in European regulation tests. We defined a paired observation as the median lethal concentration or the median effect concentration values for both Collembola species and another soil fauna group (Acari, enchytraeids, earthworms, isopods, and nematodes) under a unique combination of author, year, substance, and type of soil (61 and 57 paired observations for reproduction and lethal effects). In some studies, paired comparisons were available for several groups of soil fauna. We demonstrated that Collembola are among the most sensitive soil fauna groups to a variety of pesticides, notably for effects on reproduction, mostly compared with earthworms and enchytraeids. Because there are several modes of exposure and explaining factors, we suggest moving from a single-species study to a food-chain approach integrating different taxonomic groups. Differences between soil fauna groups in sensitivity or response to pesticides could have effects on soil communities and also on soil functions. *Environ Toxicol Chem* 2022;41:2333–2341. © 2022 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Regulation test; Ecotoxicological studies; Soil organisms; Collembola; Meta-analysis; Pesticides

INTRODUCTION

Conventional agriculture uses pesticides for plant protection all over the world, but this is a major concern due to their deleterious impacts on biodiversity and the ecological functions of living organisms. Regulatory ecotoxicity tests implemented in the pesticides regulation process are common and raise questions about which model organisms to use for these tests. Today, the role of earthworms in soils is well studied and described; earthworms are often used as a biological model in tests. Nevertheless, smaller-sized organisms also contribute to agro-ecosystem functioning and their sensitivity to pesticides makes them good bioindicators of soil quality. This is particularly true of Collembola (Cortet et al., 1999). They are numerous in temperate agroecosystems and are an important trophic link in soil communities: on the one hand, they graze on microorganisms, on the other hand, they are consumed by a wide variety of predatory arthropods and by some insectivorous vertebrates. Their distribution and abundance in arable fields therefore influences the nutrient cycle and plant productivity, as well as the spatial disposition and abundance of their predators. Given their trophic position in agroecosystems, it is a significant challenge to maintain Collembola communities.

While Collembola are nontarget organisms of pesticide applications, they are often exposed to them (de Santo et al., 2018). Although less well known than earthworms, their diversity, their abundance in soils, and their ease of maintenance in laboratory conditions (short generation times) justifies their use in ecotoxicology. Although the first ecotoxicological studies on the effects of pesticides on Collembola date back to 1953, it was not until 2005 that the first comparisons were made between different taxa (Belden et al., 2005). Thus, many studies have already shown that Collembola are more sensitive to

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certain pollutants than other soil organisms, such as earthworms, which are most often used for chemical assessments (Alves et al., 2014; Bandeira et al., 2020; de Lima e Silva et al., 2020). Frampton et al. (2006) demonstrated that the Collembola test species, *Folsomia candida*, is among the most sensitive species to pesticides and to a broad range of toxic modes of action (biocide, fungicide, herbicide, and insecticide) in lethality tests. This specific sensitivity to pesticides makes Collembola a good bioindicator of the pesticides ecotoxic effect.

The use of collembolans in toxicity testing is becoming more prevalent and relevant to soil toxicity testing. Experimental conditions in different studies on soil fauna can be similar if they follow the Organisation for Economic Cooperation and Development (OECD) guidelines for testing chemicals (OECD, 2016a) or International Organization for Standardization (ISO 11267:2014, 2014), which standardize the methodologies for ecotoxicological tests. Although *F. candida* is the most prevalent at this time, standardization efforts take into consideration an expansion of collembolan species (e.g., *Folsomia fimetaria*) available for use in ecotoxicity testing that captures other functional traits (e.g., sexual reproduction).

Moreover, earthworms and Collembola are not the only groups used as biological models for ecotoxicity tests. Efforts have also been focused on the diversification of soil fauna groups, including tests conducted on enchytraeids (OECD, 2016b) or Acari such as *Oppia nitens* (oribatid; Environment and Climate Change Canada, 2020) or *Hypoaspis aculeifer* (predatory; OECD, 2016c).

Nevertheless, the interpretation of the effects of pesticides on a soil community due to ecotoxicological tests remains quite complex. The lack of critical information for terrestrial invertebrate species hinders not only the establishment of environment soil quality criteria for contaminants in surface soils, but also a full risk assessment of the soil invertebrate community (Princz et al., 2018).

To promote the diversification of soil organism models for a pesticides ecotoxic effect, we carried out a meta-analysis of the sensitivity of several soil fauna groups to pesticides. The aim was to focus on the sensitivity of Collembola to pesticides compared with other soil invertebrate groups, and we discuss the relevance of this microarthropod in European regulation tests.

MATERIALS AND METHODS

Literature search

A systematic literature review was conducted on the basis of keywords in the ISI Web of Knowledge, using the "All Databases" option, with the following keywords: (springtail* OR folsomia* OR collembol*) AND ("pesticid* OR herbicid* OR insecticid* OR mollusc* OR nematicid*OR fungicid*) AND ecotox* in Topics (from 1955 to 2020).

A selection was made among a corpus of 260 references using titles and abstracts, and if necessary by examining the full text.

Pelosi et al. (2013) illustrate the value of a meta-analysis approach for comparing the sensitivity of different earthworm species to pesticides. Following this previous work on soil fauna sensitivity to pesticides, we only considered publications which provided data on Collembola and any other taxonomic group of soil fauna in the same study to compare the sensitivity of the taxonomic group in similar conditions (e.g., type of substrate, possible addition of organic matter). We only included studies conducted on pesticides (in formulation or active substance) in laboratory tests conducted on natural or artificial soils and we excluded results from filter paper tests. By limiting the study to single-species tests, there were no restrictions on soil fauna groups or species to be included and our analysis was only limited by the availability of data.

It was also decided to focus on lethal effects and reproduction effects, avoiding behavior tests for which there were not enough studies which compared different soil fauna groups. Finally, we obtained a corpus of 21 publications but only 17 were used for data analysis (Supporting Information, Table S1). Indeed, several median lethal concentration (LC50) and/or median effect concentration (EC50) values were not available, either because of the absence of recorded values due to the lack of effect on the reproduction or survival of one or all the tested groups, or because of the absence of comparison under similar conditions.

Data extraction

The data from the 21 selected publications were entered into a database including several variables: author(s), year of the study, taxonomic group, species, active substance name, type of pesticide, pesticide addition method, soil type, and LC50 value or EC50 value with their standard deviation and replicates numbers.

When both standard deviation and confidence interval were missing for LC50 or EC50 values, the missing confidence interval was set equal to the largest one reported for LC50 or EC50 values in the studies selected for the same analysis. According to Pelosi et al. (2013), this approach allowed us to minimize the risk of underestimating the level of uncertainty associated with our calculations.

Data analysis

We defined a paired observation as the LC50 or the EC50 values for both a Collembola species and a species belonging to another soil fauna group under a unique combination of author, year, substance, and type of soil. Thus, when different modalities were reported in the same study, a paired observation was given for each modality to guarantee observation in the same conditions. In some studies, paired comparisons were available for several groups of soil fauna.

Finally, 17 publications were used to provide 61 and 57 paired observations for reproduction and lethal effect, respectively.

Effect sizes and 95% confidence intervals were calculated as the standardized mean difference between Collembola and other group values (i.e., for each paired observation) with a small-sample bias correction using Hedges' g calculation (Hedges & Olkin, 1985). The overall effect size was estimated using a random-effect model. For an effect size to be considered as significantly positive or negative, its confidence interval should not cross the zero threshold. A negative effect size means that the studied soil fauna group is more sensitive to pesticides than Collembola, while a positive effect size indicates that Collembola have a higher sensitivity. The heterogeneity between studies was calculated using the I^2 statistic (Higgins & Thompson, 2002). Subgroup analyses were conducted to compare the sensitivity of Collembola with the sensitivity of others soil fauna groups (Acari, enchytraeids, earthworms, isopods, and nematodes), and for different pesticides (herbicides, insecticides, and fungicides) and soil types (natural and artificial) using relevant paired observations.

We performed all the statistical analyses using R Ver 3.5.0 (R Development Core Team, 2018) and the *metacont* package (Schwarzer, Carpenter, and Rücker, 2015).

RESULTS

Bibliometric analysis

In the 21 publications, the toxicity of pesticides on Collembola was compared mostly to earthworms (17 publications), followed by enchytraeids (10 publications), acari (five publications), and isopods (four publications). With only one publication each, comparisons with nematodes and gasteropods were also recorded. We also noticed that 50% of the studies make multiple comparisons (two, three, or four groups are sometimes compared with Collembola). On the other hand, there is little variability in the model species for each group (Table 1) whereas, except for imidacloprid (4 publications), the majority of pesticides have been tested only once. Most studies focused on the toxicity of insecticides and fungicides (Supporting Information, Table S1). The soil used for the experiments was mostly natural soil (13 publications), and more particularly LUFA soil. The artificial soil was Tropical Artificial Soil (TAS, 3) or OECD soils (6). Tropical artificial soil (pH 6.1, organic matter 5%) is adapted with low organic matter content from the OECD soil which is a loamy sand soil (pH 6.1, organic matter 10%). Tropical artificial soil also has finer sand than OECD soil (75% instead of 70%; de Santo et al., 2018).

TABLE 1: List of species in the 21 publications analyzed for comparison of the sensitivity to pesticides between Collembola and other soil fauna groups

Taxonomic groups	Species	Number of studies
Collembola	*Folsomia candida*	20
	Lobella sokamensis	1
Earthworms	*Eisenia fetida (ss andrei or fetida)*	17
Enchytraeids	*Enchytraeus crypticus*	9
	Enchytraeus albidus	1
Acari	*Hypoaspis aculeifer*	3
	Oppia nitens	2
Isopods	*Porcellio scaber*	3
	Armadillidium sp.	1
Nematodes	*Caenorhabditis elegans*	1
Gasteropods	*Helix aspersa*	1

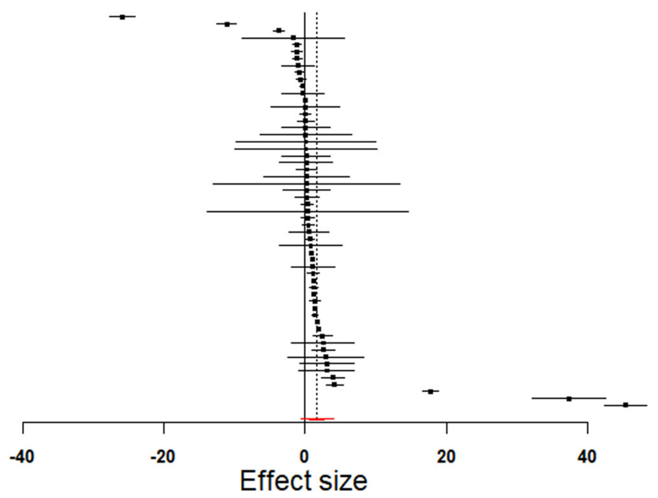

FIGURE 1: Comparison of the sensitivity to pesticides on the lethality of Collembola and other soil fauna groups. Shown is the effect size defined as the difference in median lethal concentration values (black point) and corresponding confidence interval (95%; black line) of all paired observations on lethality effects. Mean effect size (red point) and confidence interval (red line) were calculated from the 57 experimental paired observations on lethality.

Lethal effect

The 95% confidence intervals of the individual effect size frequently overlap the zero threshold (Figure 1), and the overall laped effect size value (computed over all groups) was not significantly positive (1.7, confidence interval = −0.6 to 4.0; Table 2) indicating that Collembola species were not significantly more sensitive to pesticides than other species. A substantial heterogeneity was observed ($I^2 = 98.5\%$).

Due to the high variability, the mean effect sizes and their confidence intervals confirm only that enchytraeids are significantly less sensitive to pesticides than Collembola. No difference in sensitivity is due to soil type (natural or artificial soil). However, we noticed a higher sensitivity of Collembola to insecticides (3.0) than to fungicides (−2.7), with a significant effect size for insecticides only (Table 2). The number of paired

TABLE 2: Comparison of the sensitivity to pesticides on lethality of Collembola and other soil fauna groups

	k	SMD	95% CI
All groups	57	1.7	−0.6 to 4.0
Groups			
Acari	17	2.8	−1.4 to 7
Enchytraeids	7	1.0	0.9–1.1[a]
Earthworms	27	−0.5	−3.0 to 2.0
Pesticides			
Fungicide	13	−2.7	−6.9 to 1.5
Insecticide	44	3.0	0.4–5.7[a]
Soil type			
Natural	45	1.6	−0.3 to 3.4
Artificial	12	1.8	−7.0 to 11.0

[a]Significant difference.
Shown are the number of paired observations (*k*), the standardized mean effect size (SMD) defined as the difference in median lethal concentrations between Collembola and the mentioned soil fauna group, and the corresponding 95% confidence intervals (95% CI).

TABLE 3: Comparison of the sensitivity to pesticides on the reproduction of Collembola and other soil fauna groups

	k	SMD	95% CI
All groups	61	3.5	2.0–5.0[a]
Groups			
Acari	19	1.3	1.0–1.6[a]
Enchytraeids	14	4.1	1.9–6.2[a]
Earthworms	24	4.9	1.3–8.4[a]
Pesticides			
Fungicide	19	7.0	2.5–11.4[a]
Insecticide	34	2.6	2.4–2.9[a]
Soil type			
Natural	47	4.3	0.8–1.5[a]
Artificial	14	1.2	2.4–6.2[a]

[a]Significant difference.
Shown are the number of paired observations (*k*), the standardized mean effect size (SMD) defined as the difference in median effect concentrations between Collembola and the mentioned soil fauna group, and the corresponding 95% confidence intervals (95% CI).

observations was too small for other soil fauna groups (i.e., gasteropods and isopods) or other variables (e.g., active substances) to justify separate analyses.

Reproduction effect

Most of the individual effect sizes were higher than zero (56 paired observations out of 61), and the mean effect size for reproduction effect was 3.5 (confidence interval = 2–5; Table 3), indicating that Collembola species are generally more sensitive to pesticides than the other soil fauna groups. A high heterogeneity was also observed ($I^2 = 98\%$).

The mean effect sizes and their confidence intervals confirm that earthworms and enchytraeids, two common soil fauna groups in ecotoxicological tests, were significantly less sensitive to pesticides than Collembola (Table 3 and Figure 2).

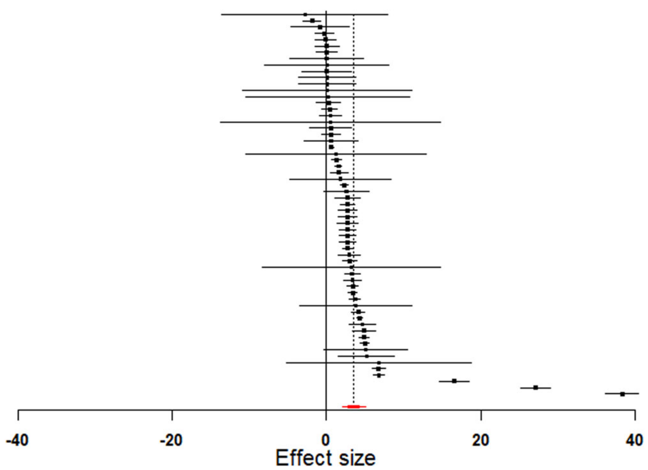

FIGURE 2: Comparison of the sensitivity to pesticides on the reproduction of Collembola and other soil fauna groups. Shown is the effect size defined as the difference in median effect concentration values (black point) and corresponding confidence interval (95%; black line) of all paired observations on reproduction effects. Mean effect size (red point) and confidence interval (red line) were calculated from the 61 experimental paired observations on reproduction.

However, the mean effect size obtained for Acari (soil fauna groups less commonly used in ecotoxicological studies) showed similar sensitivity to pesticides for Collembola. Concerning the effect of other variables, such as pesticide type or soil type, we noticed a higher sensitivity of Collembola in natural soils than in artificial soils and a higher sensitivity to fungicides than to insecticides (confidence interval = 2.5–11.4). The number of paired observations was too small for other soil fauna groups (i.e., gasteropods and nematodes) or other variables (e.g., active substances) to justify separate analyses.

DISCUSSION

The corpus of studies selected for this meta-analysis allowed us to compare the sensitivity of several soil fauna groups to pesticides. While the registration procedure for plant protection products requires data from industry, these are usually confidential. Moreover, because pesticide regulation favors a predictive approach, the data collected were generated in standardized toxicity tests performed under controlled conditions. For these reasons, our corpus consists of only 21 publications. It is nevertheless enough to demonstrate that Collembola are among the most sensitive soil fauna groups to a variety of pesticides, notably with effects on reproduction.

The LC50 data show a higher sensitivity of springtails to insecticides than to fungicides. This is consistent with the literature, which generally describes a high sensitivity of terrestrial invertebrates to insecticides (Pekar, 2012; Pekar & Benes, 2008). However, it should be noted that the ecotoxic effect of insecticides is studied more often than that of fungicides, and our corpus confirms this because 11 papers concern insecticides against only four for fungicides.

However, our EC50 meta-analysis shows a higher sensitivity of springtails to fungicides. This result is consistent with the study of Christensen and Mather (2004), which showed an increase in the surface activity of springtails exposed to fungicide-treated seeds. In contrast, the study led by Daam et al. (2011) tends to describe different results from this analysis. While springtails appear to be more sensitive than the other biological models used in our analysis, Daam et al. describe a higher sensitivity of *Eisenia fetida* to fungicides than springtails. This difference can be explained by several elements related to the study protocol: Daam et al. compared the sensitivities of the biological models based on no-observable effect concentration data and by calculating the Species Sensitivity Distribution. In addition, their dataset was composed of data from the US Environmental Protection Agency ECOTOX database, and included 21 data for insecticides compounds and seven for fungicides. Also, the Collembola data concerned nine different taxa whereas the data collected in our meta-analysis concern mainly the species *F. candida*. Finally, Daam et al. (2011) sought to characterize the difference in sensitivity of *E. fetida* compared with other models of terrestrial invertebrates, whereas our approach is focused on springtails. All these methodological differences greatly limit the comparison of the results obtained by Daam et al. (2011) with our own.

The observed differences in sensitivity of soil fauna to pesticides are difficult to explain (Neuhauser et al., 1986) but could have important consequences for soil functioning (Bart et al., 2017). These factors could be related to their morphology, physiology, or ecological parameters (behavioral characteristics) inducing a higher exposure depending on the characteristics of soil organisms.

A first obvious hypothesis would be to link sensitivity to the size of the organisms. If several authors have put forward that a larger size involves a larger surface responsible for a higher exposure, this hypothesis has often been refuted, especially in the case of the differences in sensitivity found between earthworm species (Ma & Bodt, 1993; Pelosi et al., 2014). Indeed, small organisms have surface/volume ratios higher than larger organisms, which involves a higher exposure due to their low biomass. In both cases, this relationship between size and difference in sensitivity must be questioned because (1) earthworms are larger and less sensitive than Collembola, and (2) within the same-size group (e.g., mesofauna) differences in sensitivity are observed between Collembola and enchytraeids. The size criterion is especially important if the exposure is mainly by contact.

For this reason, another hypothesis, still related to morphology, has been put forward that emphasizes the characteristics specific to each group that influence their mode of exposure. According to Alves et al. (2014), although earthworms may be exposed through more different pathways to contaminants in soil, in comparison to Collembola, the toxicity of the substances can be conditioned not only by the route of exposure, but mainly by its mode of entry. For example, the ventral tube of Collembola, the collophore, would be an additional route of absorption for toxic substances, in addition to ingestion via nutrition (Fountain & Hopkin, 2005). In contrast, soft-bodied organisms (earthworms, enchytraeids) are more likely to be exposed via passive skin penetration of pore water (Belfroid et al., 1994), in addition to ingestion. Depending on their chemical properties (e.g., more or less soluble in water or fat), pesticides may be preferentially found in the solid or aqueous phase of the soil. Because exposure pathways differ between taxonomic groups, these chemical properties may be the reason for preferential exposure of one taxon over another. That explains the differences in sensitivity of organisms to pesticides: in this case, this apparent difference in sensitivity would indirectly reflect the variability of the organisms' exposure routes to pesticides.

In addition, the interspecies variations of pesticide distribution, biotransformation, and elimination are likely involved in the variability of pesticide sensitivity. This difference in absorption route is particularly important because it conditions toxicokinetics and more specifically the metabolization phase of substances, which can be different according to this absorption route. Soil organisms have a well-established enzyme system that detoxifies organic pesticides compounds taken up by the organism but differ between taxonomic groups (Van Straalen, 1993). Also, isopods and Collembola are highly efficient in biotransforming organic chemicals whereas earthworms seem less efficient. While this can provide better resistance to certain chemicals, it can also cause a possible consequence of this rapid biotransformation, because potentially toxic metabolites may be produced (van Gestel, 2012).

Concerning physiology, explanatory factors can be found in the capacity of organisms or species to adapt to a contaminated environment through physiological adaptations (Fitzgerald et al., 1996). Chronic exposure to pesticides for generations may allow adaptation by favoring individuals that face them (Givaudan et al., 2014). Physiological adaptation, which induces, for example, a better ability to detoxify chemicals, may lead to genetic adaptation if it implies hereditary mechanisms. Genetic adaptation could also pass on the selection of genotypes allowing a better tolerance to chemicals by avoiding exposure (e.g., digging deeper or maturating faster; Givaudan et al., 2014). Such adaptations in terrestrial invertebrates have been demonstrated for metals (e.g., Gudbrandsen et al., 2007; Posthuma & Van Straalen, 1993) and also for pesticides.

For example, Givaudan et al. (2014) have demonstrated the pesticide acclimation of an earthworm population from conventionally cropped fields. Compared with the responses measured of earthworms from organic farming, the exposure to the agricultural fungicide epoxiconazole induced an increase in burrowing behavior and a higher detoxification rate in earthworms from conventional farming. This means that the different explanatory factors are not independent and that other responses of organisms, such as cast production and burrowing, can be observed rather than lethality or reproduction responses and have consequences on the ecosystem functioning.

A functional trait approach, which has been extensively developed in ecology (Pey et al., 2014), particularly in Collembola (e.g., Joimel et al., 2018) and earthworms (Pelosi et al., 2014), could help to better highlight the explanatory factors. Moreover, these traits also take into account behavioral abilities, which were not included in our study. Tests could thus be carried out on other effects of pesticides to give a holistic view of the effects of pesticides on soil fauna, for example on the movement of organisms or on pesticide avoidance. This type of test remains rare (Ximenes et al., 2020) but see, for example, Niemeyer et al. (2018), who carried out several studies on behavior. It is also important to note that avoidance tests are already used in ecotoxicology.

Differences in sensitivity between taxonomic groups could also be explained by the different modes of action of molecules. Indeed, the neurotoxic molecules of neonicotinoids, for example, have an affinity for insects, to which Collembola are more related than earthworms (Akeju, 2014).

It has also been hypothesized that proteins within the chitinous exoskeleton of some arthropods may increase the potential for bioaccumulation (Prosser et al., 2016). Alternate sources of exposures may include a contamination of primary food sources for Collembola, fungi, which could absorbed pesticides within the soil environment (Princz et al., 2018).

Nevertheless, a high degree of heterogeneity in sensitivity is observed between studies. The variation in the experimental conditions is often put forward to explain data variability between different studies, but this does not seem to be the case in the studies reviewed.

Indeed, the experimental conditions of the different studies are very similar because they follow the OECD guidelines for testing chemicals (OECD, 2016a), which standardize the methodologies for ecotoxicological tests. The high variability could therefore be explained mainly by the multitude of molecules tested and the fact that the substances are mostly tested only once on several taxonomic groups.

The variability observed could also be explained by other factors, such as the type of soil and differences in its composition and structure. Indeed, the amount of organic matter in the soil, and its type, would influence the bioavailability of pesticides (Ogungbemi & van Gestel, 2018). Similarly, the soil texture (proportion clay/sand/silt) seems to influence the bioavailability of molecules (Bandeira et al., 2020) and the soils organisms' exposure to the substance. For example, EC50 values in soils with high clay and silt content (>90%) were twice as low as those found in soils with <20% of these elements, with the same organic matter content (Bandeira et al., 2020).

Soil risk assessment, remediation, and contaminant management strategies should represent the soil functional aspects (Princz et al., 2018). To meet part of this challenge, a soil toxicity testing approach has been developed on a diversity of species: earthworms, Collembola, Acari, and more recently enchytraeids, nematodes, and isopods. However, it is obvious that the current battery is not complete and also not well balanced (van Gestel, 2012). Earthworms and Collembola are the most used species for tests, as models of nontarget macro- (Pelosi et al., 2014) and mesofauna (Amorim et al., 2012; de Santo et al., 2018). It is therefore not surprising that Collembola are mostly compared with earthworms in our study. Collembola inhabit various organic substrates and use a wide range of food sources. They are also involved in the food chain basis for other species, acting as nutrient cycling catalyzers as well as changing the soil structure through litter comminution, casting, and other mechanisms (de Santo et al., 2018; Domene et al., 2011; Potapov et al., 2016). As for earthworms, they are responsible for a large fraction of the biomass in the soil, they play an important role in the functioning of the soil ecosystem (de Santo et al., 2018), and they are considered as ecosystem engineers due to their action on the soil structure and nutrient cycling (Lavelle & Spain, 2003). Earthworms and Collembola are considered to be key groups in the soil ecosystem, but this is also the case for other groups such as Acari or isopods, and many justifications for their use are also given in review (Castro-Ferreira et al., 2012; Hägerbäumer et al., 2015; Lebrun & van Straalen, 1995; van Gestel et al., 2018). Moreover, most ecotoxicological studies mainly focus on earthworms to assess the ecotoxicity of the compounds (Alves et al., 2013; Pelosi et al., 2014), but they may not be ideal indicators of the risks posed to terrestrial fauna by insecticides and other similar substances (Alves et al., 2013; Jänsch et al., 2006). In our study, the standard earthworm test species, *E. fetida sensu lato*, seems to be less sensitive than Collembola when considering the reproduction data and in Frampton et al. (2006) it is the least sensitive species to insecticides based on acute mortality, whereas the standard Collembola test species, *F. candida* (Willem, 1902), is among the most sensitive species to a broad range of toxic modes of action (biocide, fungicide, herbicide, and insecticide). However, although *F. candida* is faced with little criticism, it is different for *E. fetida*, whose use in ecotoxicological tests is often decried and would benefit from a replacement by *Apporectodea caliginosa*, a more sensitive and common species in agricultural land use (e.g., Pelosi et al., 2013).

To conserve soil biodiversity, some regulations still use lethality to assess substance toxicity in regulation tests (see, for example, Brazil and Argentina where mostly lethality tests are used on earthworms [Camargo Carniel et al., 2019] which is not the best parameter to provide accurate information [Cortet et al., 1999]). This parameter does not respond strongly to substance toxicity with few differences between taxonomic groups compared with the response of soil organisms in terms of reproduction. Within the ecotoxicological tests, sublethal endpoints (i.e., burrowing activity, acute toxicity, cast production, avoidance, biomarkers, survival, and reproduction) are more relevant to assess the toxicity of chemical products (de Lima e Silva et al., 2020). Specifically, reproduction is a more relevant endpoint when translating effects to the population level (van Gestel, 2012). Nevertheless, the development of subindividual biomarkers could be even more interesting for the early detection of the sensitivity of springtails to relatively low pesticide concentrations. For instance, Saha and Joy (2016) showed a strong biochemical impact of agriculturally recommended doses of insect growth regulator on tissue nutrient levels and digestive enzyme activities in *Cyphoderus javanus* within 7 days of exposure in microcosms. Although subindividual biomarkers are not widely used in regulatory environmental risk assessment, the precocity of their response makes them relevant tools for assessing the potential ecotoxicity of pesticides.

Some studies have demonstrated that in each taxonomic group there are differences in sensitivity, which could be explained by many factors (e.g., in earthworms Pelosi et al., 2013), including reproduction mode and strategy. Collembola have been used since the beginning of the 1960s as a model organism for assessing the toxicity of chemicals in soils (de Lima e Silva et al., 2021). Species models are reduced to the species *F. candida*, which is the most studied, whereas other species are recommended notably by OECD standardized tests (as *F. fimetaria*) or the Canadian Ministry for Environment and Climate Change *(Proisotoma minuta*). However, studies about the indicating value of this parthenogenetic species (*F. candida*) for species of Collembola which reproduce sexually are very recent (de Lima e Silva et al., 2021). Reproduction strategy (*r* or *k* strategy, for example) could also play a role in the differences in responses to pesticides in reproduction tests for different soil organisms. Organisms that focus on a reproductive strategy, especially when confronted with stress, may show less effect in terms of reproduction than a species focusing on growth. Also, the differences observed may be explained by differences in stress responses, rather than differences in sensitivity to pesticides. Moreover, Frampton & van den Brink (2007) show differences in sensitivity to insecticides between springtails species and explain that the in situ assessment approach based on total community abundance does not identify taxon-specific effects. They therefore recommend targeted monitoring of representative and sensitive species, such as *Entomobrya multifasciata*.

Finally, since the introduction of the term ecotoxicology, the question of "putting more eco into ecotoxicology" has been raised (van Gestel, 2012). This notion has triggered the focus on more ecologically relevant test designs, integrated approaches including responses at different levels of biological organizations, and taking into account the normal operating range of parameters describing the structure and functioning of soil ecosystems (van Gestel, 2012). The aim is to validate the laboratory studies under more realistic conditions. To meet this challenge, more complex issues have been highlighted, including ecological vulnerability, trait-based analysis, and effects on functional endpoints (so-called *ecosystem services*; van Gestel, 2012). This is why it is crucial to carry out soil toxicity tests on a diversity of endpoints and species/groups. In addition, biotic interactions will definitely need more attention in the future and so will long-term effects involving several generations (Filser et al., 2014). Current environmental guidelines in Europe use single-species data in environmental protection tests. Today, it is of high importance to develop a method that can easily derive a community effect. Renaud et al. (2021) suggested incorporating community data into the assessment of the heavy metal effect on the environment. They used community similarity dose–response curves to measure community effects and demonstrate their potential for inclusion in risk assessment schemes as a measure of community response (Renaud et al., 2021). In the same way, the intracommunity variability should be considered to assess the pesticide effects on Collembola. Thus, Fountain et al. (2007) showed that the addition of the insecticide chlorpyrifos to the soil decreased the diversity and species richness of springtails, but the total abundance of springtails increased. The springtail *Ceratophysella denticulata* was found to dominate the overall community. In addition, Frampton & van den Brink (2007) showed that the sensitivity of Collembola prey species could differ from that of its predator species due to indirect effects of pesticides. This is the reason why the pesticide regulation process should take into account the community responses to chemicals. The goal would be to improve knowledge of trophic interactions to better understand the direct and indirect effects on organisms and consequences on the scale of communities and ecological functions. This is required to provide adequate environmental protection.

CONCLUSION

Soil risk assessment, remediation, and contaminant management strategies should represent soil functional aspects. To meet this challenge, our results promote the diversification of soil organism models for pesticides ecotoxic effects. Indeed, in using the case of Collembola, we demonstrated that different soil organism models have different responses to pesticides. Collembola are among the most sensitive soil fauna groups to a variety of pesticides, notably with effects on reproduction. Because Collembola are closer to insects than earthworms, they could be a good indicator of insecticide effects.

Because there are several modes of exposure, and cascading effects that are possible, there is a challenge to move from a single-species study to food chains integrating different taxonomic groups. Their differences in sensitivity or response could have effects on soil communities but also on soil functions. New soil organism models need to be tested to complement the test batteries that still rely too heavily on earthworms.

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