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## REVIEW

# Pesticide effects on soil fauna communities—A meta-analysis

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**Handling Editor:** Margaret Stanley**Abstract**

1. Soil invertebrate communities represent a significant fraction of global biodiversity and play crucial roles in ecosystems. A number of human activities threaten soil communities, in particular intensive agricultural practices such as pesticide use. However, there is currently no quantitative synthesis of the impacts of pesticides on soil fauna communities.
2. Here, using a meta-analysis of 54 studies and 294 observations, we quantify pesticide effects on the abundance, biomass, richness and diversity of natural soil fauna communities across a wide range of environmental contexts. We also identify scenarios with the most detrimental effects on soil fauna communities by analysing the effects of different pesticides (herbicides, fungicides, insecticides, broad-spectrum substances and multiple substances), different application rates and temporal extents (short- or long-term), as well as the response of different functional groups of soil animals (body size categories, presence of exoskeleton).
3. Pesticides overall decreased the abundance and diversity of soil fauna communities across studies (Grand mean effect size (Hedge's  $g$ ) =  $-0.30 \pm 0.16$ ) and had stronger effects on soil fauna diversity than abundance. The most detrimental scenarios involved multiple substances, broad-spectrum substances and insecticides, which significantly decreased soil fauna diversity even at recommended rates. We found no evidence that pesticide effects dampen over time, as short-term and long-term studies exhibited similar mean effect sizes.
4. *Policy implications:* Our study highlights that pesticide use has significant detrimental non-target effects on soil biodiversity, eroding a substantial part of global biodiversity and threatening ecosystem health. This provides crucial evidence supporting recent policies, such as the European Green Deal, that aim to reduce pesticide use in agriculture to conserve biodiversity. The detrimental effects of multiple substances revealed here are particularly concerning because realistic

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pesticide use often combines several substances targeting different pests and diseases over the crop season. We suggest that future guidelines for pesticide registration, restrictions and banning should rely on data able to fully capture the long-term consequences of multiple substances for multiple non-target species in realistic conditions.

#### KEYWORDS

below-ground communities, biodiversity, data synthesis, invertebrates, multiple stressors, plant protection products, risk assessment

## 1 | INTRODUCTION

The wide use of pesticides across the globe to improve crop yield raises concerns regarding their impacts on biodiversity (Bernhardt et al., 2017; Pelosi et al., 2021; Wang et al., 2020). Despite recent initiatives to reduce the use of pesticides in many countries (e.g. European Commission, 2022), it continues to increase globally (Sharma et al., 2019), with a broad range of substances still being applied, and new substances continuously put on the market (Wang et al., 2020). It is, thus, crucial to synthesize the effects of pesticides on biodiversity and identify the most detrimental scenarios to gain a general understanding of their consequences for biodiversity. So far, most syntheses of the ecological consequences of pesticide use have focused on individual and population levels, on specific taxonomic groups such as pollinators and natural enemies (Desneux et al., 2007; Douglas & Tooker, 2016), or on specific pesticide types such as neonicotinoids (Pisa et al., 2015). To date, no quantitative synthesis has evaluated pesticide impacts on soil communities.

Soil organisms represent about a quarter of global biodiversity (Bardgett & van der Putten, 2014) and play important roles in multiple ecosystem services (Schuldt et al., 2018; Soliveres et al., 2016; Wagg et al., 2014). Soil biodiversity, quality and fertility are, thus, crucial for humanity (Wall et al., 2015). However, the response of soil communities to environmental change remains understudied compared to above-ground communities (FAO, ITPS, GSBI, SCBD, and EC, 2020; Phillips et al., 2017). This is particularly the case for soil invertebrates (such as earthworms or springtails) that not only constitute a significant fraction of global biodiversity (Eisenhauer et al., 2019; FAO, ITPS, GSBI, SCBD, and EC, 2020) but are also an essential part of terrestrial food webs, with many above-ground animals, including vertebrates, depending on this resource (Barnes et al., 2023; Scherber et al., 2010; Wardle, 2002).

Soil invertebrates are exposed to pesticides that often reach the soil through spraying, seed coating, fumigation, leaching and tillage practices (FAO, 2018). Several pesticides are persistent in soils (Hladik et al., 2018; Navarro et al., 2007), sometimes over the course of decades (chlordecone: Cabidoche et al., 2009). For instance, neonicotinoids can be found up to several years after application (Pisa et al., 2015; Wood & Goulson, 2017). Pesticides can induce direct toxic and sublethal effects on soil invertebrates, even at concentrations commonly found in the field (Pelosi et al., 2014; Pisa

et al., 2015), and many soil invertebrates can accumulate persistent chemicals such as pesticides in their tissues (Beaumelle et al., 2017; Pelosi et al., 2021; Römbke et al., 2017). Direct toxic effects include a range of responses from the individual to the population level. For example, pesticides can cause DNA damage, alter enzyme activities, growth rates, foraging activity and reproduction of soil invertebrates (Gunstone et al., 2021; Pelosi et al., 2014). On top of these direct effects, pesticides can further affect soil invertebrates through different indirect effects mediated by shifts in resources and biotic interactions with soil microbes, plants and above-ground arthropods (Chen et al., 2013; Clements & Rohr, 2009; Desneux et al., 2007; Morgado et al., 2018; Zaller et al., 2014).

A recent review summarized the results of 394 studies and found that a large proportion reports significant negative effects of pesticides on soil invertebrates (70.5% of the individual endpoints reported, Gunstone et al., 2021). Negative effects were far more frequent at the scale of individuals and populations, while soil invertebrate community responses were more variable. However, such qualitative, vote-counting approaches, cannot quantify the magnitude of pesticide effects. Quantitative synthesis is, thus, an important next step to generalize the consequences of pesticides for soil invertebrate communities across a range of environmental contexts (soil types, crops, geographic regions, etc.).

Although detrimental effects of pesticides are generally expected, several factors related to the type of exposure and organisms involved can alter the consequences of pesticides for soil invertebrate communities. As a result, it remains unclear whether pesticides generally decrease soil biodiversity and if certain scenarios are more detrimental than others. Indeed, soil invertebrates respond differently to different types of pesticides, according to their mode of action and chemical structure (Biondi et al., 2012; Frampton et al., 2006; Jänsch et al., 2006; Pelosi et al., 2014; Pisa et al., 2015). Pesticides that target invertebrates (insecticides) or other organisms (fungicides and herbicides), and broad-spectrum substances that simultaneously target several pests and diseases, could, thus, elicit contrasted effects on soil communities. Additionally, real-world scenarios often involve combinations of multiple substances with different targets throughout the crop season (Pelosi et al., 2021), and the impacts of pesticides can differ depending on whether they are applied alone or in conjunction with other substances (Wood & Goulson, 2017). Furthermore, exposure conditions in terms of dose and temporal extent (short vs. long-term exposures)

can greatly influence soil fauna response to pesticides, and detrimental effects on soil communities may increase with application rates and decrease over time (Amossé et al., 2018; Atwood et al., 2018).

Another important moderator of the exposure and response of soil fauna communities to pesticides relates to their functional traits. Indeed, different functional groups of soil animals can elicit contrasting responses to pesticides due to different sensitivities (De Silva et al., 2010; Douglas & Tooker, 2016; Jänsch et al., 2006; Pisa et al., 2015). For example, environmental change is generally expected to have stronger effects on larger organisms (Liess & von der Ohe, 2005; Yvon-Durocher et al., 2011). In addition, soil invertebrates that live in close contact with the soil surface have been found to be more sensitive to pesticides than litter-dwelling invertebrates (Pelosi et al., 2013; Römbke et al., 2004). Such group-specific sensitivities could lead to a wide range of responses at the scale of community diversity and abundance, including neutral responses due to shifts in community composition and indirect effects (Gunstone et al., 2021; Pelosi et al., 2014). Only by addressing these potential context-dependencies, we can generate a comprehensive understanding of pesticide impacts on soil biodiversity able to inform land managers and policymakers.

Here, we conducted the first meta-analysis of pesticide effects on natural soil fauna communities. We first asked how pesticides affect the diversity (richness or diversity indices) and abundance (densities or biomass) of soil fauna communities across 54 studies. We then identified scenarios with the strongest negative effects on soil fauna communities by testing if responses depended on the type of pesticides (herbicides, fungicides, insecticides, broad-spectrum substances and multiple substances), on the exposure conditions (application rate and temporal extent), and the functional traits of soil fauna (body size category and exoskeleton presence/absence). We tested the following hypotheses:

- **Overarching hypothesis (H1):** pesticides generally elicit negative effects on soil fauna diversity and abundance across different types of pesticides and studies, with:
  - (H1.1) stronger negative effects on diversity than abundance due to the removal of sensitive species in pesticide-treated plots (Pelosi et al., 2013); and
  - (H1.2) the magnitude of pesticide effects depends on the type of pesticide applied, with:
    - Stronger negative effects of insecticides than other types of pesticides due to direct toxic impacts on soil invertebrates (Jänsch et al., 2006; Pelosi et al., 2014); and
    - Stronger negative effects of multiple substances due to the combination of direct and indirect effects mediated by biotic interactions (plants, soil microbes; Chen et al., 2013; Desneux et al., 2007) and to potential additive or synergistic effects when multiple pesticides are used in combination (Rillig et al., 2019; Wood & Goulson, 2017).
- **Hypotheses related to exposure conditions (H2):** The dose and temporal extent of pesticide applications determine the responses of soil fauna, with:

- (H2.1) pesticides used at their recommended rates have no detectable negative impact on soil fauna communities;
- (H2.2) stronger negative effects occur shortly after application (days-weeks), while pesticide degradation in soils, as well as population recovery and adaptation over time, lead to lower negative effects in the long-term (several months-years; Amossé et al., 2020; Givaudan et al., 2014).

- **Hypotheses related to the sensitivity of different soil fauna groups (H3):** Functional traits influence the sensitivity of soil fauna communities to pesticides, with:

- (H3.1) macrofauna communities being more sensitive than meso- and microfauna due to longer generation time and lower population densities (Liess & von der Ohe, 2005);
- (H3.2) soil fauna without an exoskeleton (earthworms, enchytraeids and nematodes) being more sensitive than arthropods because they live in closer contact with the soil (Pelosi et al., 2013), while the exoskeleton of arthropods may act as a protective barrier against penetration of pesticides (Balabanidou et al., 2018; Jänsch et al., 2006).

## 2 | MATERIALS AND METHODS

### 2.1 | Literature search

We conducted a systematic literature search to collect studies on pesticide impacts on soil fauna communities. We used a previously compiled database of studies on global change effects on soil fauna communities (Beaumelle, Thouvenot, et al., 2021; Phillips et al., 2019). The search terms are fully reported in (Phillips et al., 2019). Specifically, they included keywords to retrieve pesticide studies (such as 'pesticide', 'fungicide', 'insecticide' and 'agrochemical') and soil fauna community studies (combination of keywords of the form 'soil' AND ('fauna' OR 'arthropod' OR 'invertebrate') AND ('community' OR 'biodiversity')). From the full systematic search, by screening the abstracts and full texts, we identified 104 studies focusing on pesticide impacts (Beaumelle, Thouvenot, et al., 2021). We further screened the full texts of those 104 studies against our inclusion criteria to identify our final set of 54 included studies (PRISMA plot: Figure S2). To verify the efficacy of the search, we checked that the initial literature search retrieved a set of preidentified papers relevant to the current meta-analysis scope (Burrows & Edwards, 2002; Fountain et al., 2007; Knacker et al., 2004; Scholz-Starke et al., 2011, 2013; Velcheva et al., 2012).

### 2.2 | Inclusion criteria

We included studies that met the following inclusion criteria:

- Test the effects of a gradient of pesticide use (application rates or soil concentrations, untreated versus treated comparisons, or low versus high application rates or concentrations) on soil fauna

communities in terms of abundance, biomass, diversity indices or taxa richness. We followed the authors' statements regarding pesticide categorization. For example, studies investigating the effect of copper on soil fauna communities were only included if they specified that their treatment aimed to reflect the effects of copper-based fungicides. Studies comparing organic and conventional farming systems were not included if they used different types of pesticides in organic and conventional fields and if they involved different types of farming practices on top of pesticide treatments.

- Focus on natural communities: We only included laboratory experiments that involved intact soil cores and excluded studies creating artificial communities by adding species to soil microcosms.
- Report pesticide use intensity (such as pesticide application rates or soil contamination levels) in both the reference and treatment cases.
- Sample soil fauna using soil and/or litter processing (pitfall trap data were excluded if not accompanied by soil samples), so that our analysis focuses on organisms living strictly below the ground.
- Report means and standard deviations of the community metrics (abundance, biomass, richness or diversity) in both the control and treatment cases. For the Shannon index, however, we collected data even if the standard deviation was not reported to maximize the final number of observations, and we estimated variances as described below.

### 2.3 | Data extraction

For each study, we collected soil fauna abundance, biomass, richness, and diversity indices along with several moderators. First, we defined the reference and treatment levels for each study based on the levels or intensities of pesticide use. For studies investigating multiple sites located along a gradient of pesticide intensities, the reference level was set as the condition with the lowest pesticide use intensity or concentration, and the treatment level was the treatment with the highest pesticide use intensity or concentration. We collected community data at the lowest taxonomic resolution reported by the study but higher than the family level (except for Enchytraeidae). When the study presented the response of several taxa or several pesticide treatments (different products, or different mixtures of pesticides), we collected them all and created separate observations for each taxon and pesticide treatment. If studies measured the same plot over multiple time points, we extracted control and treatment data from the latest time point to reflect long-term effects and control for temporal pseudoreplication (Ferlian et al., 2020; Hedges et al., 1999; Yue et al., 2018). We extracted means and variances for the reference and treatment levels from tables, text or figures (Rohatgi, 2018).

To test the effects of different pesticides (H1.2), we recorded pesticide identity (active ingredients) and assigned them to a pesticide type (Table S1). Pesticide types were herbicides, fungicides, insecticides, or broad-spectrum substances (i.e. a single substance

targeting several pests across taxonomic groups, e.g. fumigants). As a single study addressed the effect of a nematicide, we included that study in the insecticides category. When pesticide treatments involved multiple products combined into single, or several applications, we categorized the case study as 'multiple substances'. We acknowledge that this categorization does not allow us to address the effect of mixtures of pesticides in its strict sense. From a soil fauna community perspective, realistic pesticide exposure can involve tank mixtures (i.e. pesticides applied together, from the same sprayer), as well as the combination of several pesticides applied over the crop season. Both scenarios can lead to significant detrimental effects on soil biodiversity, and our approach enabled us to address them together in the present analysis.

We extracted pesticide application rates and the temporal extent of studies to test our hypotheses (H2.1) and (H2.2). We recorded if pesticides were applied at their recommended application rates or not based on information provided in the primary studies. When that information was missing, we compared the applied rates with the recommended application rates retrieved from the database ANSES E-Phy (ANSES, 2023) for similar crops and target pests. We also recorded if studies addressed the effects of a single pesticide application, or applied pesticides repeatedly (e.g. throughout the crop season). The temporal extent of the study reflected the time of the last sampling event after the start of the experiment (that is, the first pesticide application), with three categories: (1) 'short-term' studies measured soil fauna response up to 3 months after pesticide application; (2) 'intermediate-term' studies spanned 4 months to a year; and (3) 'long-term' studies from over a year to several years. It is important to note that our approach does not allow testing the temporal dynamics of pesticide effects on soil communities, as this was outside the scope of our study. Quantifying temporal effects would have required to use a different modelling framework to reduce temporal autocorrelation within each study. It would also further reduce the amount of available data, as fewer studies measure biodiversity changes over time. Instead, our approach, commonly used in other meta-analyses (Ferlian et al., 2020; Hedges et al., 1999; Yue et al., 2018), enables us to account for any variability in our mean effect sizes driven by different temporal extents in the primary studies.

To quantify whether functional traits moderated the response of soil fauna communities to pesticides (H3), we first assigned soil fauna taxa to taxonomic groups defined by the Global Soil Biodiversity Atlas (Orgiazzi et al., 2016) before assigning two functional traits. Taxonomic groups were categorized into (1) two functional groups based on the presence (arthropods) or absence (enchytraeids, nematodes and earthworms) of an exoskeleton and (2) three body size categories (microfauna, mesofauna and macrofauna, Table S2). Although soil animals operate along a continuum of body size, body size categories are often used in soil ecology, as they reflect different food web compartments (Decaëns, 2010; Potapov et al., 2021; Thakur et al., 2020) and are useful for comparing the response of different soil fauna groups to global change drivers in meta-analytical approaches (Phillips et al., 2019).

## 2.4 | Effect size calculation

We chose Hedge's  $g$  as the metric for our effect sizes because we were interested in the magnitude difference between control and pesticide treatments (Koricheva et al., 2013). Hedge's  $g$  represents a standardized mean difference (i.e. the raw mean difference is divided by the pooled standard deviation of the two groups) corrected for positive bias (Viechtbauer, 2010). Hedge's  $g$  is widely used in ecology, making our results comparable to other studies. Furthermore, this metric is more suitable than the log-response ratio to incorporate the zero or close to zero values in our dataset (such as very low abundance or richness values due to pesticide treatments; Lajeunesse, 2013).

For Shannon data, 11 observations from six studies lacked standard deviations (SDs) because studies did not report them. Based on our dataset that included 50 means and SDs of Shannon index responses (from nine other studies), we regressed the SD against the mean and used the slope to impute missing SDs (Lajeunesse, 2013). We note that a limitation of this approach was that the lack of data prevented the possibility of including the effects of moderators, such as pesticide type or community metric, on the relationship between means and SDs. For other diversity metrics, such as evenness, the number of studies was insufficient to use a similar approach, and observations that lacked SDs were not considered.

## 2.5 | Meta-analysis

### 2.5.1 | General modelling approach

We fitted meta-analytical models to test our hypotheses. All models included a random effect of the study identity, as data from the same study are not independent. In addition, an observation-level random effect was also used following Viechtbauer (2022), to ensure that the models did not assume identical effects for different observations within the same study. Effect sizes were weighted by the inverse of their variance in all models, giving more weight to well-replicated studies (Koricheva et al., 2013). We used Q-Q plots of the standardized deleted residuals and verified that they did not strongly depart from a normal error distribution (Viechtbauer, 2020). We also used Cook's distance plots to identify influential observations and checked that excluding them from the models yielded similar results. We tested interactive effects between moderators using likelihood ratio tests (LRTs). When interactions were not significant, we refitted the models without the interaction to test the main effect of each moderator using Wald-type Chi-square tests (QM). Main and interactive effects from the models were considered significant when  $p < 0.05$ . We identified the most detrimental scenarios based on the predicted mean effect sizes and their confidence intervals. A significant mean effect size is shown when the confidence interval does not overlap with zero. We used R software and the R package METAFOR (R Core Team, 2019; Viechtbauer, 2010).

### 2.5.2 | Main analysis

First, we fitted a main model of the form:  $Y \sim \text{PesticideType} \times \text{CommunityMetric}$ , with  $Y$  being the effect size,  $\text{PesticideType}$ , a categorical moderator with five levels (herbicides, fungicides, insecticides, broad-spectrum and multiple substances), and  $\text{CommunityMetric}$ , a categorical moderator with two levels: abundance (densities and biomass data) or diversity (richness and diversity indices) because there were too few studies to test separately abundance, biomass, diversity indices and richness. This main model allowed us to test our overarching hypothesis (H1) by quantifying the marginal grand mean effect size across all studies, along with the mean effect sizes of different types of pesticides and community metrics (H1.1). According to (H1.2) we expected stronger negative effects of multiple substances, broad spectrum substances targeting a wide range of organisms, and insecticides that have direct toxic effects on invertebrates. To formally test (H1.2), we used a post-hoc analysis and fitted a model similar to the main model, but with four categories of pesticides: (1) multiple substances, (2) broad spectrum substances, (3) insecticides and (4) fungicides and herbicides as a group of substances that do not target soil invertebrates (Table 1).

Publication bias was assessed based on the main model that included pesticide type and community metric as moderators. The funnel plot indicated no clear sign of publication bias (Figure S3). We further fitted Egger's regression between standard normal deviates of the effect sizes and precision (i.e. the inverse of their standard errors) with pesticide type and community metric as covariates interacting with precision (Koricheva et al., 2013). The results did not indicate publication bias, as none of the intercepts from Egger's regression differed significantly from zero showing no significant funnel plot asymmetry (Table S3).

### 2.5.3 | Second-level analyses

We further tested the influence of four moderators related to exposure conditions: 'recommended rates' (H2.1) and 'temporal extent' (H2.2), and to soil fauna functional traits: 'body size' (H3.1), and 'exoskeleton' (H3.2). Those subsequent models tested each of the four moderators in interaction with pesticide type because we expected that the influence of the moderators would depend on the type of pesticide (H1.2). Due to the lack of studies, we could not include interactions with the community metric (few diversity observations). The second-level models, therefore, addressed the combined responses of diversity and abundance. As the main model highlighted community metric as a significant moderator, we included a random effect of community metric in the second-level models to account for differences in diversity and abundance response across studies, in addition to the random effect of study identity. These second-level models were of the form:  $Y \sim \text{PesticideType} \times \text{Moderator}$ , with a random effect structure:  $(1|\text{CommunityMetric}) + (1|\text{StudyID}/\text{ObservationID})$ , and  $\text{Moderator}$  being either the temporal extent,

**TABLE 1** Description of the meta-analytical models used to test our hypotheses using different subsets of the data. We removed observations that did not discriminate between moderator levels (e.g. soil fauna responses encompass multiple body size levels), or if they belong to a moderator level combination having fewer than two studies and three observations (e.g. broad-spectrum substances in the models for temporal extent and body size).

Model name	Hypothesis	Main predictor	Moderator	N. Studies	N. Observations
Main model	H1.1	Pesticide type (five levels: herbicides; fungicides; insecticides; broad-spectrum substances; multiple substances)	Community metric (two levels: abundance and diversity)	54	294
Main model 2	H1.2	Pesticide type (four levels: herbicides and fungicides; insecticides; broad-spectrum; multiple substances)	Community metric (two levels: abundance and diversity)	54	294
Recommended rates	H2.1	Pesticides applied at recommended rates (five levels: herbicides; fungicides; insecticides; broad-spectrum substances; multiple substances)	—	31	158
Temporal extent	H2.2	Pesticide type (four levels: herbicides; fungicides; insecticides; multiple substances)	Temporal extent (three levels: short-, intermediate- and long term)	50	257
Body size	H3.1	Pesticide type (four levels: herbicides; fungicides; insecticides; multiple substances)	Body size (three levels: micro-, meso- and macro-fauna)	47	247
Exoskeleton	H3.2	Pesticide type (five levels: herbicides; fungicides; insecticides; broad-spectrum substances; multiple substances)	Presence of exoskeleton (two levels: with exoskeleton; without exoskeleton)	49	280

body size or exoskeleton. For recommended rates, we tested  $Y \sim \text{PesticideType}$  with the same random effect structure.

Each model was based on different subsets of the data suitable to test our hypotheses (Table 1). For recommended rates, the data subset included all studies and observations of pesticides used at their recommended rates ( $n=31$  studies; 158 observations), and all pesticide types were represented (although broad-spectrum and fungicides had few diversity observations). For temporal extent, we excluded broad-spectrum substances that did not have long-term observations (Table 1), which removed four studies and 37 observations for that analysis. For body size, three studies and 12 observations were removed. Primarily, this was due to the observations of soil invertebrates being at a taxonomic level that inherently spanned multiple body size categories (e.g. measuring all soil invertebrates). As a result of this subsetting, broad-spectrum pesticides were removed as only one study remained (addressing their impacts on macrofauna). Similarly, for the exoskeleton model, five studies and 14 observations were removed, as the observations were not at a taxonomic level that could be identified as with or without an exoskeleton (e.g. measuring all macrofauna). However, this model did include all five pesticide categories (broad spectrum, fungicides, herbicides, insecticides and multiple substances: Table 1).

Finally, we performed supplementary analyses focusing on the impact of multiple pesticides. We compared the mean effect sizes of multiple substances combining different types of pesticides versus multiple substances belonging to the same pesticide type. We also quantified the mean effect sizes of two well-represented pesticides in our dataset (glyphosate and neonicotinoids) and compared their effects when used

alone or in combination with other substances. Mean effect sizes were estimated with a similar modelling approach as described above (random effect structure, Wald-type Chi-square tests and data subsets of multiple substances, glyphosate or neonicotinoid studies).

## 3 | RESULTS

### 3.1 | Data description

Our study encompasses the results from 54 publications, with a total of 294 individual observations. Most were field experiments ( $n=51$  studies; 277 observations), covering a wide range of environmental contexts, although mostly located in the Northern Hemisphere (Figure S1). Studies addressed a total of 86 active substances (Table S1) belonging to different pesticide types (Figure 1): insecticides ( $n=19$ ; 116), herbicides ( $n=10$ ; 37), fungicides ( $n=8$ ; 42) and broad-spectrum pesticides (such as fumigants,  $n=7$ ; 37). Twenty studies addressed the impact of multiple substances, either applied together or as separate applications throughout the crop season. Multiple substances included combinations of several pesticide types ( $n=7$ ; 14), or several products belonging to the same pesticide type ( $n=14$ ; 48). Most studies applied pesticides at recommended application rates ( $n=31$ , 158), but their temporal extent varied greatly, with fauna sampled from 7 days up to 22 years after pesticide application.

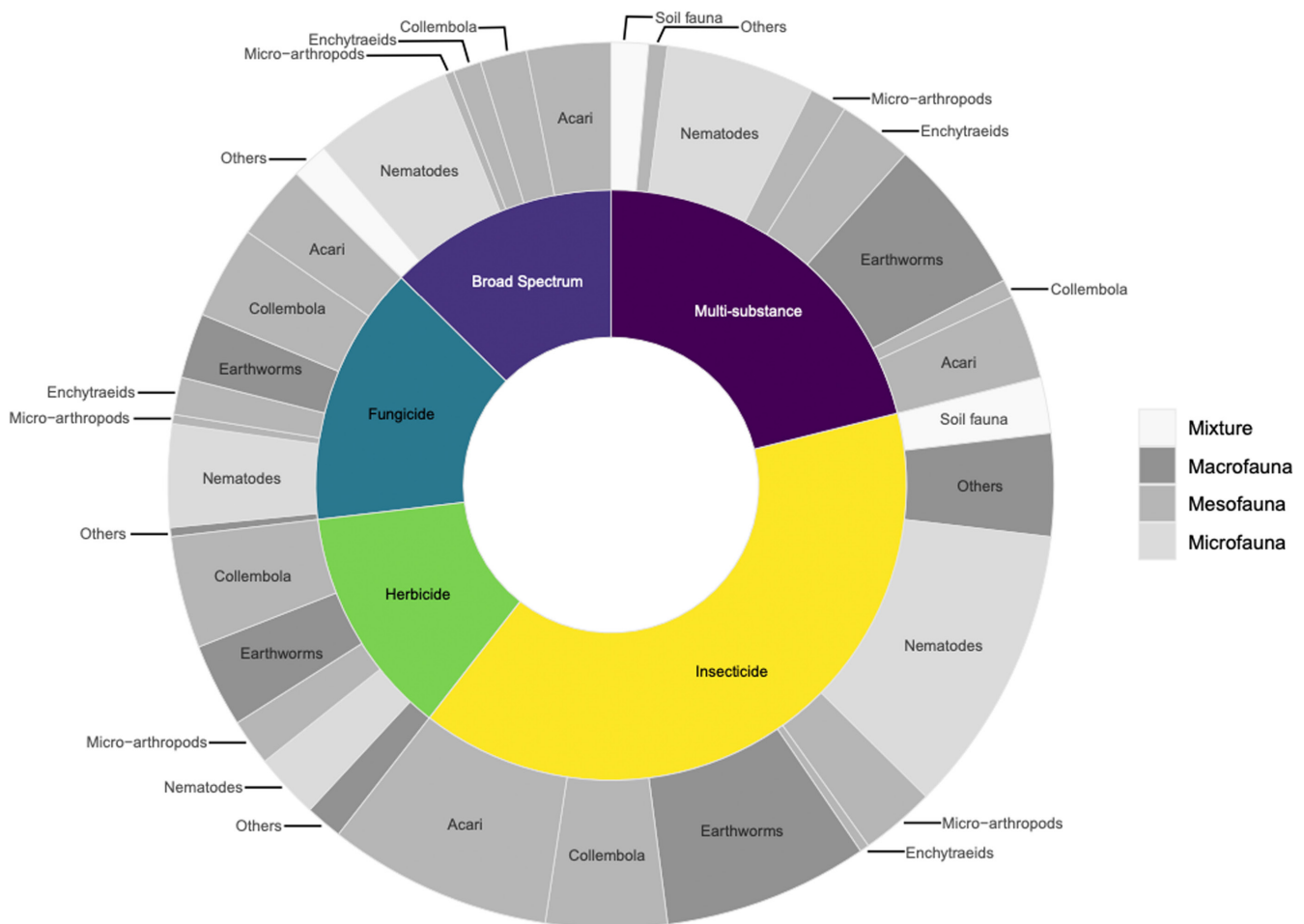
Studies reported soil fauna community abundance ( $n=47$ ; 190), diversity indices ( $n=16$ ; 41), richness ( $n=12$ ; 28), biomass ( $n=9$ ; 27)

and evenness indices ( $n=3$ ; 8). They covered 15 taxonomic groups (Figure 1; Table S2). Nematodes were the most represented ( $n=21$  studies; 80 observations), followed by Acari ( $n=13$ ; 50), Collembola ( $n=13$ ; 42) and earthworms ( $n=12$ ; 55). Several studies also reported the response of multiple taxa together (macro-arthropods, macrofauna, micro-arthropods or soil fauna). All pesticide types had a similar balance of observations among the most represented taxa (Figure 1). However, studies investigated insecticide effects on a much wider range of soil fauna groups (Figure 1, 16 groups) than the other pesticide types (9 for multiple substances, 7 for broad-spectrum and fungicides and 6 for herbicides).

### 3.2 | Contrasted effects of different types of pesticides

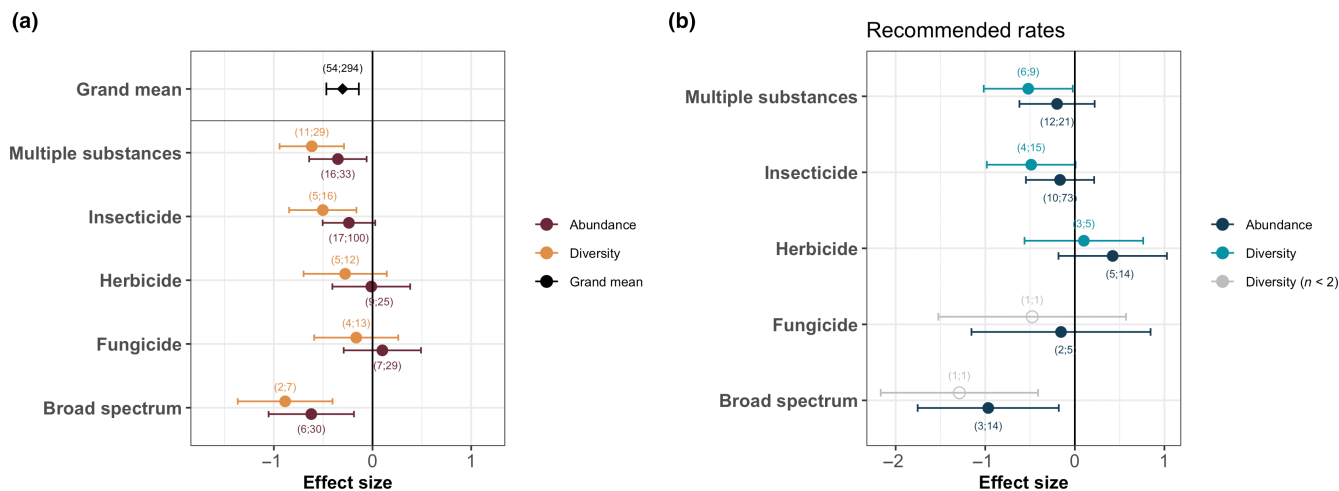
The results revealed a significant decrease in soil fauna abundance and diversity in response to pesticide use across studies. The grand mean effect size (Hedge's  $g$ ) was  $-0.30$  (CI:  $-0.47$ ;  $-0.14$ ; Figure 2a).

Richness and diversity indices were more negatively affected than abundance and biomass of soil fauna (significant effect of community metric:  $QM(df=1)=3.94$ ,  $p=0.0471$ ), an effect that was consistent across different pesticides (Figure 2a: non-significant pesticide type  $\times$  community metric interaction,  $LRT(df=4)=3.43$ ;  $p=0.4886$ ). Multiple substances and broad-spectrum substances significantly decreased soil fauna abundance and diversity, and insecticides significantly decreased soil fauna diversity (Figure 2a). Although the model indicated that all pesticides generally had a negative effect (non-significant effect of pesticide type  $QM(df=4)=8.35$ ,  $p=0.0797$ ), mean effect sizes of herbicides and fungicides had confidence intervals that overlapped with zero indicating non-significant (or neutral) effects when averaging across studies. A post-hoc analysis revealed that this was due to similarities between the effects of fungicides and herbicides. Grouping together these two types of pesticides, that do not target invertebrates, revealed a significant effect of pesticide type ( $QM(df=3)=8.20$ ,  $p=0.0420$ ), showing that broad-spectrum, multiple substances and insecticides elicited stronger negative effects compared with substances not targeting invertebrates (fungicides and herbicides).



**FIGURE 1** Relative coverage of different pesticide types and soil fauna taxa groups in 294 observations from 54 studies on the impacts of pesticide use on soil communities included in the meta-analysis. The sunburst diagram represents the proportions of observations for each category. Soil fauna groups have been categorized under different body size categories depicted in different shades of grey. Category 'Others' combines all taxa groups represented by fewer than 10 observations for a given pesticide type, and the body size category corresponds to the most common category across taxa groups (which differed depending on pesticide types).





**FIGURE 2** Effects of pesticides on soil fauna communities (a) and impact of recommended pesticide application rates (b). Grand mean and mean effect sizes (Hedge's *g*) and their 95% confidence intervals are predicted values from meta-analytic models of soil fauna abundance (densities and biomass) or diversity (richness or indices) response to different types of pesticides (multiple substances: several pesticides applied together or as separate applications throughout the crop season; broad-spectrum substances: single substances targeting a broad range of taxonomic groups). Significant mean effect sizes are those with confidence intervals not overlapping with zero, and the main model additionally tests whether effect sizes differ from each other. Numbers in parentheses indicate the sample size for each mean effect size as follows: (number of studies; number of observations).

Supplementary analyses suggested stronger negative effect sizes of multiple substances combining different types of pesticides (herbicides and fungicides, herbicides and insecticides, insecticides and fungicides) than of multiple substances combining several pesticides of the same type (broad-spectrum substances, herbicides, fungicides or insecticides; Figure S4a). Although that trend, based on a subset of the data (only for recommended application rates to avoid confounding effects of application rates and type of multiple substances) was not statistically significant ( $QM(df=1)=1.04$ ,  $p=0.3086$ ,  $n=15$ ; 30), the magnitude of the difference between mean effects was 0.43 (CI: -1.25; 0.39). We further compared the impacts of two types of widely used pesticides, glyphosate and neonicotinoids, when they were applied alone or combined with other pesticides. The results indicated a neutral mean effect size of these pesticides when applied alone (Figure S4). When glyphosate was combined with other pesticides, the mean effect size was also not significant (Figure S4b). However, we found a strong and significant negative effect of neonicotinoids applied in combination with other substances (Figure S4c).

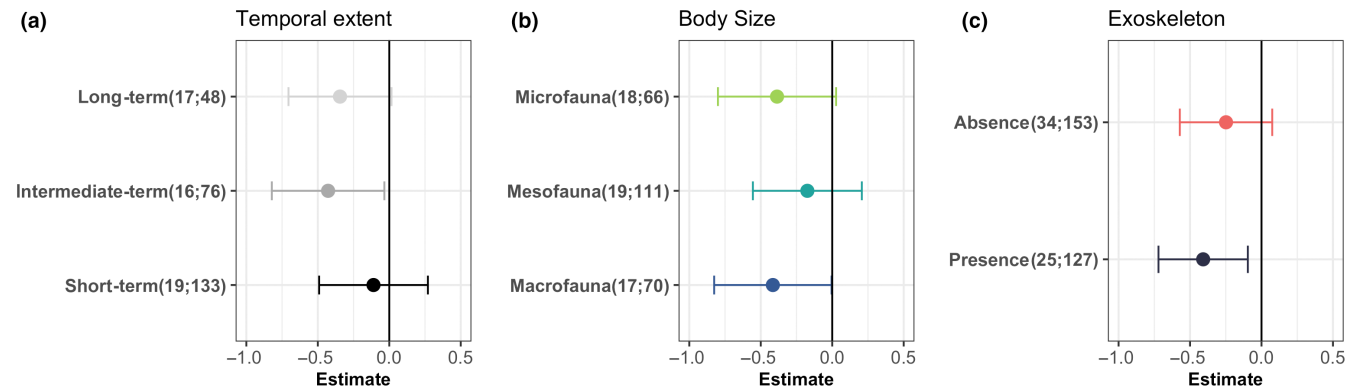
### 3.3 | Pesticide effects persisted at lower doses and longer temporal extents

When analysing studies that applied pesticides at their recommended rates (Figure 2b,  $n=31$ ; 158), the mean effect size on soil fauna abundance and diversity had a magnitude similar to that of the main analysis (-0.30) but with confidence intervals that overlapped zero (-0.66; 0.06). Responses did not differ according to pesticide types ( $QM(df=5)=9.65$ ,  $p=0.0858$ ), and only broad-spectrum substances caused significant declines when applied at recommended

rates (Table S4). However, in contrast with the main meta-analysis (Figure 2a), this model combined diversity and abundance responses due to the lack of diversity observations (Figure 2b). Focusing on pesticide types that had enough observations to quantify the separate mean effect for diversity and abundance (multiple substances, insecticides and herbicides), we found that recommended rates of multiple substances and insecticides had significant negative effects on diversity, and neutral mean effects on abundance, while herbicides had neutral effects on both diversity and abundance (Figure 2b).

The temporal extent of the study (the time between the start of the experiment and soil fauna sampling) did not significantly affect soil fauna communities (Figure 3a;  $QM(df=2)=1.26$ ,  $p=0.5320$ ,  $n=50$ ; 257). Across pesticide types, short- and long-term studies had the lowest mean effect sizes, but only the long-term mean effect size was significantly negative (Figure 3a). Long-term studies were dominated by insecticides ( $n=5$ ; 23) and multiple substances ( $n=9$ ; 18), while long-term effects of fungicides and herbicides were only addressed in  $n=2$ ; 4 and  $n=2$ ; 3 studies and observations, respectively. Furthermore, most long-term studies focused on repeated pesticide applications ( $n=14$  out of 17), while short- and intermediate-term studies mostly addressed the impacts of a single pesticide application ( $n=15$  out of 22, and 14 out of 19 studies, in short- and intermediate term, respectively).

Testing the effect of the temporal extent for single versus repeated applications separately across all pesticide types would have needed more data. For insecticides, we observed similar short-, intermediate- and long-term effects of single applications on the abundance of soil fauna ( $QM(df=2)=3.01$ ,  $p=0.2219$ ). Single insecticide applications had significant negative effects in the short term (-0.22; CI: -0.44; -0.01;  $n=7$ ; 63) and intermediate term (-0.45; CI: -0.85; -0.04;  $n=3$ ; 11). In the long-term category, we had only two studies



**FIGURE 3** Negative effects of pesticide use on soil fauna communities across temporal extents and functional groups. (a) Temporal extent reflects the time between the start of the experiment and fauna sampling (short term: 1–12 weeks; intermediate term: 4–12 months; long term: >1–22 years). (b) Body size categories covered different taxa groups listed in Table S2. (c) Communities of organisms with (arthropods) or without (earthworm, enchytraeid, and nematode) an exoskeleton. Values are mean effect sizes and 95% confidence intervals across pesticide types and community metrics (diversity and abundance) from meta-analytic models of different subsets of the data. Broad-spectrum pesticides lacked long-term and macrofauna studies and are not included in (a) and (b). Numbers in parentheses indicate sample size for each mean effect size as follows: (number of studies; number of observations).

addressing the effects of the same active ingredient (diflubenzuron) and the effect size was neutral (0.03; CI: -0.33; 0.39;  $n=2$ ; 15).

### 3.4 | Broad functional groups did not explain soil invertebrate communities' responses

There was no evidence that body size and the presence/absence of an exoskeleton significantly modulated the response of soil fauna communities to pesticides (Figure 3). In general, we found similar responses for the three body size categories ( $QM(df=2)=2.41$ ,  $p=0.2994$ ,  $n=47$ ; 247). Although the presence of an exoskeleton was associated with significant negative effects (effect size non-overlapping with zero, Figure 3), communities of organisms with an exoskeleton or a soft body had broadly overlapping effect sizes that were not significantly different from each other ( $QM(df=1)=1.11$ ,  $p=0.2917$ ,  $n=49$ ; 280). None of those traits interacted with pesticide types (Body size:  $LRT(df=6)=5.45$ ,  $p=0.4877$ ; Exoskeleton:  $LRT(df=4)=3.29$ ,  $p=0.5102$ ).

## 4 | DISCUSSION

Despite raising concerns about the ecological consequences of the widespread and intensive use of pesticides in agriculture, our study is the first meta-analysis to investigate their impacts on soil fauna communities. The results confirm the overarching hypothesis (H1) that pesticide use elicits negative effects on soil fauna communities across different environmental contexts. Our findings align with previous reviews (Jänsch et al., 2006; Pelosi et al., 2014) and vote-counting syntheses (Gunstone et al., 2021), but expand on them by quantifying the magnitude of pesticide use impacts on natural communities. Vote-counting approaches are sensitive to biases imposed by different sampling efforts in the original primary

studies (Koricheva et al., 2013). Quantitative synthesis is thus crucial to generalize the consequences of pesticides across a range of environmental contexts and further allows comparisons of their significance with respect to other global change drivers (Phillips et al., 2019). In one of the few existing meta-analyses on this topic, Pelosi et al. (2014) found neutral effects of pesticides on earthworm communities when averaged across studies, due to strong context dependencies. Our results provide new insights into the most detrimental scenarios for soil biodiversity, and the hierarchy of moderators influencing the general patterns of soil fauna communities' response to a range of ubiquitous pesticides. These findings highlight that realistic pesticide use represents a threat to soil fauna communities and have strong implications for future regulation and risk assessment of pesticides.

### 4.1 | Detrimental effects of pesticide use targeting a wide range of organisms

We found that pesticide scenarios with the most detrimental impacts were those that targeted a wide range of organisms, either through the application of a single broad-spectrum substance or through the combined application of multiple substances at the same time or along the crop season. Broad-spectrum substances were mostly fumigants, substances that target invertebrates as well as fungal crop diseases and weeds, while multiple substances often combined insecticides, fungicides and herbicides. This result probably reflects that broad-spectrum and multiple substances harm a wider range of organisms, and combine direct toxic and sublethal effects (Desneux et al., 2007; Pelosi et al., 2014) with indirect effects via soil microorganisms, plants, or above-ground biota (Chen et al., 2013; Douglas & Tooker, 2016; Rillig et al., 2019; Scherber et al., 2010).

Our findings further imply potential widespread additive or synergistic effects of multiple pesticides, as shown by the significant

negative effect of multiple substances across studies. Those results confirmed our hypothesis that multiple substances elicit stronger negative effects than single substances with a specific target (H1.2). For example, neonicotinoid applications resulted in significant declines only when combined with other pesticides. This is an alarming finding because pesticide mixtures are frequently found in agricultural soils (Pelosi et al., 2021). Panico et al. (2022) also found that 'natural' field mixtures of currently used pesticides in agricultural soil represent a risk for soil invertebrates. Although our result cannot indicate whether additive or synergistic effects dominate, several recent case studies have demonstrated synergistic toxic effects of combined pesticides on non-target organisms (Amossé et al., 2018; Niedobová et al., 2019; Tosi & Nieh, 2019; Wood & Goulson, 2017). At the community level, species interactions may be important drivers of such multiple stressors effects (Bruder et al., 2019; Orr et al., 2020). Multitrophic approaches combined with full-factorial studies will thus enable better predictions of the consequences of realistic pesticide use for soil biodiversity (Beaumelle, Thouvenot, et al., 2021). It would be particularly relevant to evaluate the relative magnitude of direct versus indirect effects by combining laboratory and field experiments (Clements & Rohr, 2009).

The present results suggest that direct effects can be important because insecticides had stronger negative effects than fungicides and herbicides. Soil fauna taxa groups are arthropods and invertebrates, thus more closely related to the main targets of insecticides than to those of fungicides and herbicides. The modes of action of insecticides can indeed operate on arthropods and invertebrates (e.g. neonicotinoids target neural pathways shared by insects and earthworms; Pisa et al., 2015). The stronger negative effects of insecticides reported here are thus probably due to their direct toxic and sublethal effects on soil fauna (Jänsch et al., 2006; Pelosi et al., 2014). In contrast, while non-target effects of herbicides and fungicides on soil fauna have been reported for several species (Bart et al., 2017; Zaller et al., 2014), our results indicate that they may not be generalizable across communities and pesticide ingredients. However, we caution against interpreting our results as evidence for the lack of effects of herbicides and fungicides on soil communities. Indeed, currently, too few studies are available to make strong generalizations (only 8 studies for herbicides and 10 studies for fungicides). These studies covered a small range of soil fauna taxa (Figure 1) and very few addressed long-term effects. Furthermore, a neutral mean effect size can arise from context-dependent responses ranging from negative to positive, and the lack of studies currently limits our ability to explain this variability. Finally, it is important to note that most studies were field experiments, which inherently present high variability, making it difficult to demonstrate significant effects of pesticides (Brulle et al., 2022; Gunstone et al., 2021).

## 4.2 | Pesticide use as a significant threat to soil biodiversity

We further revealed significant detrimental effects on soil fauna diversity across a range of functional groups, including when pesticides

were used at their recommended application rates. Together, these alarming results suggest that pesticide use represents a significant threat to soil biodiversity.

The stronger negative effects on diversity than abundance reported here probably reflect species-specific sensitivities to pesticides that lead to the removal or decline in the abundance of sensitive species from pesticide-treated plots (Amossé et al., 2018; Hedde et al., 2012; Pelosi et al., 2013). In contrast, abundance responses can range from negative (e.g. via direct toxic effects) to positive (e.g. via indirect competitive or predation release) depending on the functional or taxonomic group considered (Atwood et al., 2018). The lower mean effect of pesticides on the abundance of soil fauna could therefore arise from such variable responses and compensation processes between species (Pelosi et al., 2014). Future studies could address how different facets of diversity, such as richness and evenness, or abundance versus biomass, respond to pesticide use, as here it was not possible to separate their responses to different pesticide types.

Soil fauna diversity also declined in response to recommended rates of pesticides in several scenarios. In general, the effect sizes of recommended rates were lower than in the main analysis, in line with ecotoxicology theory where the dose is one of the primary factors determining the biological response to toxicants (Newman, 2009). Indeed, in the main analysis, most studies that did not apply pesticides at recommended rates used concentrations above those rates (13 studies, against only 2 studies below recommended rates). However, broad-spectrum substances, insecticides and multiple substances still showed clear negative effects at their recommended rates, implying significant detrimental non-target effects on soil communities also at lower doses. Pelosi et al. (2021) also reported a high risk of chronic toxicity to earthworms exposed to realistic concentrations of pesticide mixtures in agricultural soils. Our findings thus only partly confirmed hypothesis (H2.1) that low doses of pesticides have lower detrimental effects on soil invertebrate communities. One explanation is that recommended application rates are derived from simplified experiments that may not capture well the response of natural soil communities. For example, experiments do not necessarily consider the most sensitive species (Pelosi et al., 2013). We note that our meta-analysis of studies published from 1990 to 2018 includes pesticides now forbidden in several countries. Comparing the effects of forbidden and currently used pesticides was outside the scope of our global analysis, given that pesticide regulations vary widely across countries and over time within a country (e.g. neonicotinoids bans in the EU). As recent evidence points to considerable increases in the toxicity of currently used pesticides for several non-target organisms (Schulz et al., 2021), it is not clear if excluding forbidden substances from our meta-analysis would have changed our conclusions. Furthermore, we showed that pesticides had stronger negative effects on diversity than abundance, but we had few observations of diversity responses in this analysis. Including more data on the impact of recommended rates of pesticides on soil fauna diversity may thus lead to an even greater negative effect than reported here.

Our results further suggested persistent detrimental effects of pesticides on soil fauna communities over time. We expected lower negative effects on longer temporal extents due to pesticide degradation, population recovery and adaptation to pesticide exposure (Givaudan et al., 2014; Navarro et al., 2007). Due to the lack of data, we could not formally test this hypothesis because short-term studies mainly addressed the effects of a single pesticide application, while long-term studies were dominated by repeated pesticide applications that could lead to stronger negative effects due to the repeated disturbances for soil communities. In addition, the over-representation of insecticides and multiple substances that had the strongest negative effects in long-term studies could explain the similar negative effects found in short- and long-term studies. However, this result could also reflect the time it takes for soil fauna populations to recolonize after being affected by pesticides, given their low dispersal ability (Amossé et al., 2020; Pelosi et al., 2015). Indeed, we found that a single insecticide application could reduce soil fauna abundance with a magnitude similar to that several weeks or several months after application. Although our primary studies covered a wide range of temporal extents from a few days to several years, we acknowledge that we extracted the last point in time when studies reported temporal series. While this reduced temporal pseudo-replication in the modelling framework used here, we missed observations at shorter duration within each study and the temporal dynamics that they may show. Our results nevertheless suggest that realistic pesticide use with repeated applications has long-term detrimental effects on soil fauna communities that future studies, land managers and policymakers should take into account.

Lastly, different types of soil fauna communities were equally affected by pesticide use, highlighting that pesticides could similarly threaten a wide range of soil animal functional groups. Therefore, our results did not confirm the hypothesis that pesticides have stronger effects on larger organism groups (H3.1). Neither did our results support the hypothesis of different sensitivities of communities of arthropods versus non-arthropods (H3.2). It is conceivable that other functional groupings may better determine pesticide effects at this macroscopic scale. For example, endogeic earthworms have been found less sensitive to insecticides (chlorpyrifos) than epigeic ones (De Silva et al., 2010). Pesticides may thus affect communities of species living in close contact with the soil surface more than species dwelling on the surface (Hedde et al., 2012; Pelosi et al., 2013). Key traits such as dispersal ability, diet breadth and generation length may also shape soil fauna responses to pesticide use, as has been found in aquatic communities (Liess & von der Ohe, 2005). Furthermore, the effects of the functional traits studied here may depend both on the type of substance and on the community metric, but unfortunately, the lack of studies only enabled us to test interactions with broad categories of pesticides on combined diversity and abundance. These results suggest that the type of pesticide and application rate may influence soil fauna communities' response to pesticide use more than broad functional traits such as body size and the presence of an exoskeleton.

### 4.3 | Implications and future directions

The present study demonstrates that the use of pesticides has significant detrimental non-target effects on soil biodiversity, eroding a substantial part of global biodiversity and threatening ecosystem health. This provides crucial evidence to support recent policies that aim to reduce pesticide use. For example, the European Commission recently adopted a proposal for a new regulation of sustainable use of plant protection products that includes the aim to reduce by 50% the use and risk of chemical pesticides by 2030 (European Commission, 2022). Our results confirm the relevance of such regulation to conserve soil biodiversity.

However, we also reported significant knowledge gaps regarding the effects of low doses of pesticides on soil fauna communities, highlighting that policy initiatives would benefit from further research able to identify thresholds under which the detrimental effects of pesticides on soil biodiversity drop significantly. Linking pesticide use, animal exposures and potential side effects in agricultural landscapes could identify which substances should be managed in priority and reveal which degree of reduction is needed to safeguard biodiversity (Fritsch et al., 2022; Panico et al., 2022; Pelosi et al., 2021). Finding such thresholds will require multidisciplinary approaches that account for the consequences of pesticide reductions on crop productivity. Although our results imply cascading positive effects of pesticide reduction on the sustainability of agricultural production (by promoting a diversity of soil organisms involved in soil fertility, water infiltration or nutrient cycling) we cannot ignore potential short-term yield gaps that can result from pesticide reductions. Here, of the three studies that measured crop productivity, only one reported significant yield gain in response to pesticide use. Similarly, a large-scale study showed that reducing pesticide use generally does not result in losses in crop productivity and profitability (Lechenet et al., 2017). Additionally, integrated pest management strategies using natural enemies and building on and fostering nature-based solutions can further resolve apparent trade-offs between biodiversity conservation and agricultural production (Barnes et al., 2020; Beaumelle, Auriol, et al., 2021; Beaumelle, Giffard, et al., 2023; Dainese et al., 2019).

Our findings have strong implications for agriculture, as they suggest key actions to reduce the detrimental effects on soil fauna communities. First, limiting the number of pesticides should be considered (Rillig et al., 2019). Although reducing pesticide use is in line with recent policies, using a variety of substances is sometimes recommended to avoid the development of resistance to a particular pesticide (Barzman et al., 2015; van den Bosch et al., 2014). Thus, it would be interesting to explore potential trade-offs between biodiversity conservation and protection against pest resistance in the future. Second, since we found long-term detrimental effects of realistic pesticide use with repeated applications, we suggest that spraying patterns could be modified to ensure soil fauna population recovery between pesticide applications to meet the objectives of sustainable agricultural production (e.g. European Commission, 2022).

Finally, this study has important ramifications for future pesticide regulation. National guidelines for pesticide registration, restrictions and banning should rely on data able to fully capture the long-term consequences of multiple substances for multiple non-target species in realistic conditions. By showing detrimental effects at recommended rates, our results especially challenge the way pesticides are tested before their market authorization (Brühl & Zaller, 2019), as they should not cause detrimental non-target effects on soil communities. Recommended application rates vary depending on the crop, country, and manufacturer, and have recently been criticized because of the ways they are derived and their lack of transparency (Colin et al., 2020). The relevance of laboratory tests and risk assessment procedures to assess pesticide effects on biodiversity is increasingly being questioned (Brühl & Zaller, 2019). The development of new risk assessment approaches exposing multiple taxa to realistic mixtures of pesticides should, thus, be encouraged (Panico et al., 2022). Furthermore, by relying on long-term experiments lasting a maximum of 1 year, environmental risk assessment for soil organisms may currently miss the long-term detrimental effects over several years reported here.

## 5 | CONCLUSIONS

Our quantitative synthesis is an important step towards better predictions of the ecological consequences of pesticide use by focusing on natural soil fauna communities that are key components of global biodiversity and ecosystem functioning. Here, the negative effects of pesticides, especially in realistic scenarios with combinations of multiple substances and recommended rates, and largely based on field studies, confirm the general assumption and alarming conclusion that pesticides can represent a major threat to soil biodiversity. It is now recognized that increases in quantity, diversity and geographic distribution of synthetic chemicals have exceeded the rate of other important global change drivers, such as rising atmospheric CO<sub>2</sub>. This, along with our results, should reinforce current policies, such as the European Green Deal, aiming to reduce pesticide use to conserve biodiversity and achieve sustainable agriculture.

### AUTHOR CONTRIBUTIONS

Léa Beaumelle and Helen R. P. Phillips conceived the ideas and designed the methodology with inputs from Nico Eisenhauer, Jes Hines, Lise Thouvenot, and Céline Pelosi; Léa Beaumelle, Sandhya Malladi, Helen R. P. Phillips, Céline Pelosi and Léa Tison collected the data; Léa Beaumelle analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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### CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

### DATA AVAILABILITY STATEMENT

All data and R codes associated with this analysis are publicly available on the Zenodo archive <https://doi.org/10.5281/zenodo.7937860> (Beaumelle, Tison, et al., 2023), and can also be found on GitHub <https://github.com/leabeaumelle/MAPesti>.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Figure S1.** Geographic distribution of 54 studies on pesticide use impacts on soil fauna communities. Based on sampling or experimental locations of the primary studies.

**Figure S2.** PRISMA plot showing the steps to reach a final set of 54 studies for the present meta-analysis.

**Figure S3.** Assessment of publication bias. Funnel plot of the residuals from the main meta-analytic model of pesticide effects on soil fauna community against their standard errors. Model included the effects of pesticide type and community metric (diversity or abundance) as moderators.

**Figure S4.** Effects of multiple pesticides on soil fauna communities. (A) Differences between mean effect sizes of multiple pesticides of several types (herbicides and fungicides, herbicides and insecticides, insecticides and fungicides) and multiple pesticides of the same type (broad-spectrum substances, herbicides, fungicides, or insecticides) when applied at recommended rates (data subset to enable meaningful comparisons, because the category “several types” only applied pesticides at recommended rates in contrast to the category “same type”). Effects of neonicotinoids (B) and glyphosate (C) applied alone or combined with other pesticides. Values are mean effect sizes and 95% confidence intervals derived from meta-analytic models with a random effect structure accounting for non-independence within studies and community metrics.

**Table S1.** List of pesticides types and active ingredients covered in this meta-analysis, with the total number of studies (no.stu) and observations (no.obs) and CAS number for each. Active ingredient names from the Pesticide Properties DataBase (PPDB: <http://sitem.herts.ac.uk/aeru/ppdb/en/atoz.htm>).

**Table S2.** List of (taxonomic) groups and their body size category covered in this meta-analysis, with the total number of studies (no.stu) and observations (no.obs). Taxa groups as categorized from the Global Soil Biodiversity Atlas (GSBA), and as reported in the primary studies (from studies).

**Table S3.** Results from Eggers' regression indicating no publication bias.

**Table S4.** Results from meta-analytic models of pesticide effects on soil fauna communities.

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