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Journal of Hazardous Materials

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https://doi.org/10.1016/j.jhazmat.2024.135022

Journal of Hazardous Materials 476 (2024) 135022

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Effects of neonicotinoid residues on non-target soil animals: A case study of meta-analysis

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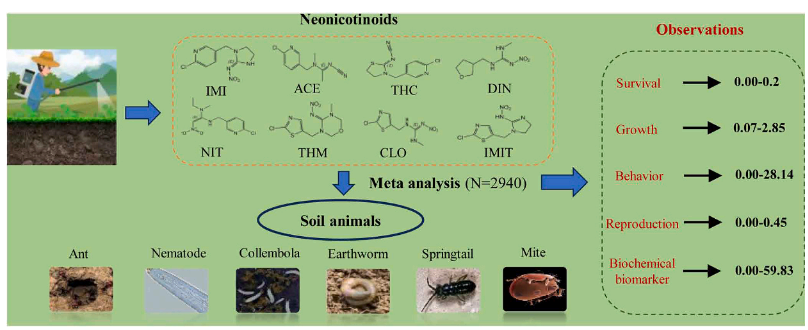
HIGHLIGHTS

- Impacts of NEOs on soil animals were quantified using a meta-analysis.
- Effects of NEOs on different species of soil animals were analyzed.
- Different soil animals exhibit varied responses to NEOs.
- NEOs inhabit survival, growth rate, behavior, reproduction of soil animals.
- NEOs can alter biochemical biomarkers of soil animals.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Neonicotinoid residues
Quantitative effect
Soil animals

ABSTRACT

Neonicotinoids (NEOs) are currently the fastest-growing and most widely used insecticide class worldwide. Increasing evidence suggests that long-term NEO residues in the environment have toxic effects on non-target soil animals. However, few studies have conducted surveys on the effects of NEOs on soil animals, and only few have

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https://doi.org/10.1016/j.jhazmat.2024.135022
Received 18 February 2024; Received in revised form 22 June 2024; Accepted 22 June 2024
Available online 24 June 2024

Soil ecosystem
Meta-analysis

focused on global systematic reviews or meta-analysis to quantify the effects of NEOs on soil animals. Here, we present a meta-analysis of 2940 observations from 113 field and laboratory studies that investigated the effects of NEOs (at concentrations of 0.001–78,600.000 mg/kg) on different soil animals across five indicators (i.e., survival, growth, behavior, reproduction, and biochemical biomarkers). Furthermore, we quantify the effects of NEOs on different species of soil animals. Results show that NEOs inhibit the survival, growth rate, behavior, and reproduction of soil animals, and alter biochemical biomarkers. Both the survival rate and longevity of individuals decreased by 100 % with NEO residues. The mean values of juvenile survival, cocoon number, and egg hatchability were reduced by 97 %, 100 %, and 84 %, respectively. Both individual and cocoon weights were reduced by 82 %, while the growth rate decreased by 88 % with NEO residues. Our meta-analysis confirms that NEOs pose significant negative impacts on soil animals.

1. Introduction

Neonicotinoids (NEOs) are a new class of broad-spectrum insecticides that act as agonists on nicotinic acetylcholine receptors (nAChRs) on the post-synaptic membrane, disrupting neural transmissions in the central nervous systems of insects [1]. NEOs have been on the market for more than 20 years since Bayer developed the first NEO, imidacloprid, in the mid-1980s Kollmeyer et al. [2]). By 2014, the NEO market accounted for more than 25 % of the total global insecticide sales [3,4]. However, the extensive use of NEOs in recent years has led to their frequent detection in different environmental media, such as soil, dust, surface water, sediment, and groundwater, globally [5]. Meanwhile, numerous studies have reported severe ecological risks of NEOs to non-target species such as bees [6,7], birds [8,9], earthworms [10,11], mammals [12], and aquatic organisms [13-16]. Without a doubt, NEO pollution has caused significant environmental concern globally.

Soils are complex and biodiverse ecosystems on the earth, that include organisms representing nearly a quarter of global biodiversity [17]. Soil animals play important roles in multiple ecosystem services for agricultural sustainability. According to reports, only a small percentage of NEOs is absorbed by crops after use, while more than 90 % of the active ingredients enter the soil [18,5,4]. Hou et al. [19] showed recently that the sum concentration of 13 NEOs collected from agricultural soils in China ranged from 0.04 to 702.00 μg/kg, while in Europe, the maximum sum concentration of NEOs in soils was observed with 138 μg/kg [20]. Surprisingly, NEOs can be found in soil even 1000 days after application, leading to their accumulation in the environment with repeated use [4,21,22]. Consequently, organisms in soil systems are likely to be exposed to high NEO concentrations [4,23]. Overall, the ecological and environmental problems in soil system caused by NEOs have aroused widespread concern globally [24].

Numerous studies have confirmed that NEOs are harmful to earthworms (a key indicator for soil health) and other non-target soil animals [25,26]. NEOs can accumulate in the tissues and/or digestive tracts of many soil animals [27-29]. NEOs can also destroy the physiological structures of soil animals, causing DNA damage, cell trauma, and oxidative stress [30,31]. The direct toxic effects of NEOs include reducing their growth, reproduction, and development [8,32]. In addition to these direct effects, NEOs can indirectly affect soil animals through resource changes and biotic interactions with soil microbes, plants, and ground animals [8,33]. A comprehensive assessment of the effects of NEOs on soil organisms is required because of their growing threat to soil organisms.

In recent years, numerous studies have investigated the effects of NEOs on non-target soil animals' performance indicators (i.e., survival, reproduction, behavior, reproduction, and biochemical biomarkers). However, there have been relatively few quantitative syntheses of this expanding literature. Meta-analysis is a statistical tool widely used to compare and integrate the results of multiple studies. Main et al. [34] conducted a meta-analysis to assess the effects of NEOs on non-target terrestrial arthropods. Douglas and Tooker [35] studied the negative effects of natural enemy abundance on arthropods using a meta-analysis. Beaumelle et al. [36] conducted the first meta-analysis of pesticide effects on natural soil fauna communities. However, the coverage of soil animals and related indicators in previous studies has been limited, and there is a lack of research focusing on NEOs. Therefore, conducting a comprehensive analysis of the effects of NEOs on soil animals is necessary.

In this study, we conducted the first meta-analysis of the effects of NEOs on soil animal communities and divided them into subgroups according to different species of soil animals. Biochemical biomarkers were included as indicators for the effects of NEOs on soil animals at the first time. Our goal is to answer the main question, "What are the effects of NEOs on non-target soil animals? Our main goal was to quantitatively assess repeatedly reviewed evidence to determine whether NEO treatments had any effect on the survival, growth, behavior, reproduction, and biochemical properties of non-target soil animals compared to untreated controls. Through this paper, we addressed the following questions: (1) Do NEOs affect five specific indicators (i.e., survival, growth, behavior, reproduction, and biochemical biomarkers) of soil animals? (2) Do various soil animal classes experience different effects from NEOs?

2. Materials and methods

2.1. Literature search

We used the literature search database of the Web of Science (WOS) cross-checked with PubMed to find studies evaluating NEO effects on non-target soil animals. The keywords used were the following: "neonicotinoid" or "imidacloprid" or "acetamiprid" or "nitenpyram" or "imidaclothiz" or "thiacloprid" or "thiamethoxam" or "clothianidin" or "dinotefuran" and "soil" or "terrestrial" and "animal" or "fauna" or "earthworm" or "enchytraeid" or "springtail" or "mite" to identify papers published from January 1, 1900 to June 24, 2022. The purpose of these search terms was to compile information that addresses our primary inquiries regarding the impact of NEOs on soil ecosystems. All the keywords associated with NEOs were linked by the Boolean operator "OR", and synonyms relevant to edaphic were connected with the operator "AND". In addition, the search terms of specific names of pesticides and animals are common NEOs and soil animals. By searching these keywords, we obtained 2439 scientific papers. After excluding duplicate papers and reviews, that did not include data, 2392 papers were retained. The details of the literature collection process are presented in Fig. S1 in Supporting Information (SI).

2.2. Criteria and relevance

For inclusion in this review, the following selection criteria were established [37,34,38]:

(1) The studied animal must be a soil-dwelling terrestrial species. Aquatic or terrestrial microbial organisms, such as bacteria and fungi, were not within the scope of our review.
(2) The study must include non-target soil animals, excluding the target animals or common agricultural pests (e.g., thrips, termites, or root-feeding beetle larvae).

(3) The study must evaluate the effects of NEOs on non-target animals based on observations.
(4) The pesticide studied must be a NEO.
(5) The study must compare experimental treatments against control treatments with two or more replicates.

Among the obtained scientific articles, the title and abstract of each identified paper were briefly scanned for relevance, and we identified 526 studies. Finally, after further reviewing all literature by applying these selection criteria, 113 laboratory and field studies with 2940 observations were selected in the present study for meta-analysis (Table S1). It needs to be noted that the proportion of field studies is small (i.e., 11 out of 113 articles), making it impossible to perform a separate sensitivity analysis.

2.2.1. Non-target soil animals

Soil animals were defined as organisms inhabiting the soil during part or all of their lifetimes. The soil animals from relevant studies that fit our criteria are organized by taxa in the Results section (Table 1). Additionally, arthropods, Thysanoptera, and ground-nesting bees, as well as various combinations of these taxa were included in our analysis. We also conducted a subgroup meta-analysis of soil animals to explore the effects of NEOs on different types of animals. However, aquatic and terrestrial microbial organisms, such as bacteria and fungi, were excluded from this study.

2.2.2. Observation

Observations were defined as unique combinations of the following variables: NEO, animal, and endpoints. Each observation measured a specific endpoint following the exposure of a specific animal to a specific NEO. Observations were identified and extracted from each study. We classified the observations into five major indicators: survival (523), growth (393), behavior (126), reproduction (362), and biochemical biomarkers (1536) (Table S2).

2.2.3. NEOs

We included all NEOs in our study: imidacloprid, acetamiprid, nitenpyram, imidaclothiz, thiacloprid, thiamethoxam, clothianidin, and dinotefuran. We included data not only on parent NEO molecules but also their metabolites (e.g., R-dinotefuran, S-dinotefuran, 1-methyl-3-(tetrahydro-3-furylmethyl) urea, and 1-methyl-3-(tetrahydro-3-furylmethyl) guanidium dihydrogen). However, studies with two or more NEO active ingredients applied together were categorized as mixtures and were not included in our analysis. We also did not include studies that focused on the behavior of NEOs, including sorption, degradation, transport, runoff, volatilization, dissipation, persistence, and leaching. Although these interactions in the soil are necessary to understand the environmental fate of pesticides, they go beyond the scope of our analysis.

Table 1
Soil animals included in the analysis and their functional groups.

Taxa	Animal	Functional group
Acari	Mites; Spider mites	Predator
Coleoptera	Beetles; Carabidae; Coccinellidae; Nitidulidae; Red palm weevil; Staphylinidae	Predator
Collembola	Springtails	Detritivore
Dermaptera	Earwig	Predator
Diapsida	Lizard	Predator
Diplura	Japygudae	Detritivore
Gastropoda	Snail	Mixed
Hemiptera	Bug	Predator
Hymenoptera	Ant; Araneae; Chilopada; Formicidae; Honeybee; Wild bee; Parasitoid wasp	Pollinator; Mixed; Parasitoid; Detritivore
Isopoda	Woodlice	Mixed
Isoptera	Termite	Mixed
Lepidoptera	Monarch butterfly; Pyralidae; Silkworm	Pollinator; Predator
Libellulidae	Odonatan	Predator
Nematoda	Roundworms	Detritivore
Neuroptera	Lacewing	Predator
Oligochaeta	Earthworms; Enchytraeid	Detritivore

2.3. Data extraction

After compiling all studies that met our established criteria, non-target soil animal performance measures were consistently evaluated in this literature. We extracted data for the five most common indicators: survival (523), growth (393), behavior (126), reproduction (362), and biochemical biomarkers (1536).

For each study included in the meta-analysis, six essential factors were extracted: number of replicates for the control (CK_n), number of replicates for the NEO treatment (T_n), mean of the control group (CK_{mean}), standard deviation for the control group (CK_{sd}), mean of the NEO treatment group (T_{mean}), and standard deviation for the NEO treatment group (T_{sd}). If the articles did not provide standard deviation (SD) or standard error (SE) values, the missing SDs were approximated using the average coefficient of variation of the dataset in which the SD was reported and multiplied by the reported mean (M_r) [30]. The equations used are as follows:

$$SD_i = m_r * M_i \tag{1}$$

$$m_r = \frac{\sum \frac{SD_r}{M_r}}{n_r} \tag{2}$$

where m_r refers to the average coefficient of variation, which is derived from the sum of the ratio of each known SD (SD_r) and the mean (M_r) divided by the number of known data points (n_r). SD_i is calculated from the data of articles that did not report SDs and is derived from the sum of m_r and the mean of the literature (M_i).

If SEs were given in a paper, SD was calculated as follows:

$$\mathrm{SD} = \mathrm{SE}\sqrt{\mathrm{N}} \tag{3}$$

where N is the sample size, and SD is the standard deviation of the treated or control group.

All data were extracted from in-text results, tables, or figures using GetData Graph Digitizer 2.26 (available online; http://getdata-graph-digitizer.com). The final database used for the meta-analysis contained 2940 observations from 113 studies (Table S1).

2.4. Statistical analyses

The analysis was conducted in R (version 4.2.2) using the *Metafor* package [39]. All data categories, including survival (523), growth (393), behavior (126), reproduction (362), and biochemical biomarkers (1536), were analyzed separately. The effects of NEO application on soil animal performance measures were examined using meta-analysis models.

The meta-analysis was based on the difference in effect size between NEO and control treatments. The effect value is the combined statistics in the quantitative meta-analysis, whose calculation method mainly depends on the acquisition of data from the original literature. For each pair of control treatment and NEO treatment observations extracted, we calculated Hedges'g as a measure of effect size [39]. Hedges' g is the calculated and bias-corrected standardized mean difference (SMD) or the estimate of the effect size between the treatment and control groups, an indication of how much one group may differ from another. Effect sizes were calculated by subtracting the mean of the control from the treatment mean and dividing it by the pooled and weighted standard deviation. We used the natural log-transformed response ratio (ln RR) as a metric of the effects of NEO residues on a response variable relative to the control, where no NEO was used. The effect size (g) was considered significant if the 95 % confidence intervals (CI) did not overlap with one.

$$\log RR = \ln\left(\frac{X_t}{X_c}\right) \tag{4}$$

where X_c denotes the mean of the control treatment, X_t denotes the mean of the NEO treatment. For our analysis, all models were random or mixed effects models fitted using the method of restricted maximum likelihood estimator (REML). Compared with other variance estimators, the REML provides a balance between unbiasedness and efficiency [40].

3. Results

A total of 113 laboratory and field studies fit our criteria, yielding 2940 observations. The concentrations of NEOs in the field experiments were 0.02–780.00 mg/kg based on 116 observations, while in the laboratory experiments they were 0.001–78,600.000 mg/kg based on 2824 observations. The detailed information of observation data is presented in an Excel file named Raw Data in SI. The meta-analysis results showed that NEO residues had different effects on the survival, growth, behavior, reproduction, and biochemical biomarker responses of soil animals (Table S2). For the same observations, soil animals were conducted as subgroups, which showed various levels of responses to NEOs. Soil animals were divided into 16 species (Table 1). Among them, Oligochaeta earthworms and Collembola springtails accounted for a large proportion.

3.1. Survival

As shown in Fig. 1, NEOs have significant negative effects on the survival of soil animals. Both the mean survival rate of individuals and longevity were reduced by 100 % in the pesticide treatment group compared to those in the control group ($p < 0.0001$).

Each index was divided into subgroups according to different soil animal categories, including Oligochaeta, Collembola, Acari, Coleoptera, Hymenoptera, Nematode, and Neuroptera. The mean survival rate decreased by 100 % (Oligochaeta), 100 % (Collembola), 100 % (Acari), 100 % (Coleoptera), 100 % (Hymenoptera), 98 % (Nematode), and 99.5 % (Neuropteran), with the response ratios of 0.00 [0.00, 0.00], 0.00 [0.00, 0.00], 0.00 [0.00, 0.00], 0.00 [0.00, 0.01], 0.00 [0.00, 0.00], 0.20 [0.10, 0.42], and 0.05 [0.01, 0.22], respectively ($p < 0.0001$). Neuroptera longevity decreased by 100 % with response ratios of 0.00 [0.00, 0.02] ($p < 0.05$). Overall, all types of animals' survival and longevity were greatly inhibited by NEOs.

3.2. Growth

As shown in Fig. 2, the growth rate of soil animals was reduced by 88 %, with stimulated reproductive organs response ratios of 0.12 [0.02, 0.62] ($p < 0.05$), indicating that NEOs significantly inhibit the soil animal growth. The growth inhibition rate was increased by 185 %, with

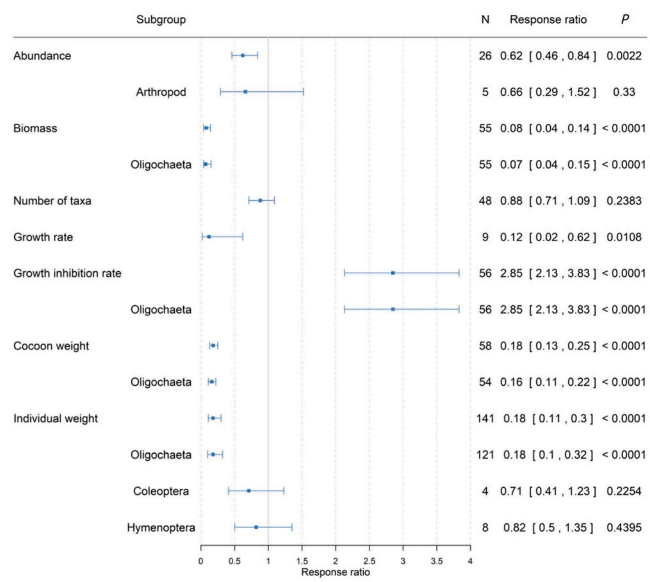

Fig. 2. Effects of NEO treatments on growth performance measures of non-target soil animals based on 393 observations. The blue square symbols show the average value of the response ratio for each type of NEO with error bars representing 95 % confidence interval (CI). A ratio > 1 indicates that the response from the treatment is higher compared to the control group. N refers to sample size and p means the p-value of the Q test. A significant effect of NEO use is indicated when CI does not overlap one and significance codes are as follows: * ** * $p < 0.0001$, * ** $p < 0.001$, * * $p < 0.01$, and * $p < 0.05$.

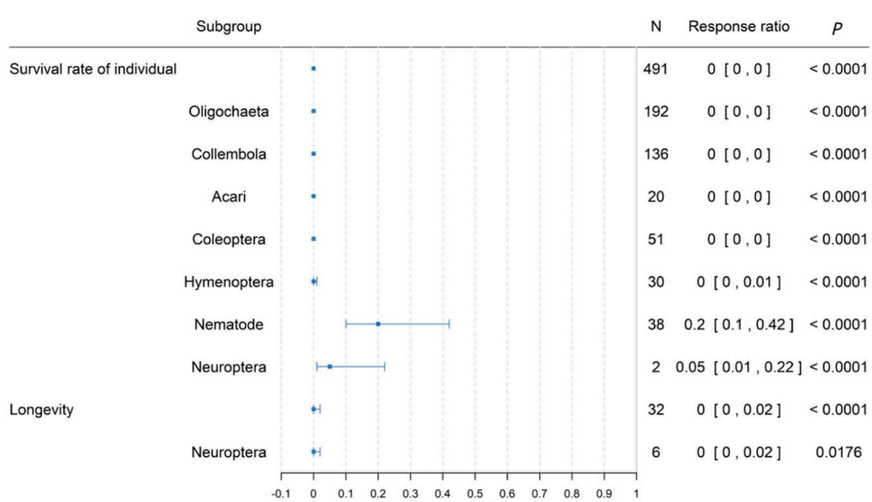

Fig. 1. Effects of NEO treatments on survival performance measures of non-target soil animals based on 523 observations. The blue square symbols show the average value of the response ratio for each type of NEO with error bars representing 95 % confidence interval (CI). A ratio > 1 indicates that the response from the treatment is higher compared to the control group. N refers to sample size and p means the p-value of the Q test. A significant effect of NEO use is indicated when CI does not overlap one and significance codes are as follows: * ** * $p < 0.0001$, * ** $p < 0.001$, * * $p < 0.01$, and * $p < 0.05$. Response ratio: the reason for the zeros is due to rounding.

response ratios of 2.85 [2.13, 3.83] ($p < 0.0001$). Abundance and biomass were reduced by 38 % and 92 %, with response ratios of 0.62 [0.46, 0.84] and 0.08 [0.04, 0.14] ($p < 0.01$). Moreover, individual weight and cocoon weight were both reduced by 82 %, with response ratios of 0.18 [0.13, 0.25] and 0.18 [0.11, 0.03], respectively ($p < 0.0001$).

The individual weight of Oligochaeta earthworms was reduced by 82 %, with response ratios of 0.18 [0.1, 0.32] ($p < 0.0001$), while Coleoptera and Hymenoptera had no distinct effect. The biomass and cocoon weight of Oligochaeta earthworms were reduced by 93 % and 84 %, with response ratios of 0.07 [0.04, 0.15] and 0.16 [0.11, 0.22], respectively ($p < 0.0001$). Similar to all soil animals, the growth inhibition rate of Oligochaeta earthworms were increased by 185 %.

3.3. Behavior

Soil animal behavior was also affected by NEO residues (Fig. 3). The avoidance response of soil animals was 27.14 times higher than the control group in all categories of Oligochaeta, with response ratios of 28.14 [11.8, 67.07] ($p < 0.0001$). Food consumption was reduced by 100 %, with response ratios of 0 [0, 0.12] ($p < 0.01$). Average burrow depth was 99 % lower in the treatment group compared to the control group, with response ratios of 0.01 [0, 0.05] ($p < 0.0001$), earthworms were reduced by 69 % ($p < 0.01$), and Isoptera termites were reduced by 100 % ($p < 0.0001$). Predation by soil animals was reduced by 69 %, with response ratios of 0.31 [0.2, 0.48] ($p < 0.0001$). However, there was no significant change in nest activity response to NEO exposure ($p = 0.1665$), although it was reduced by 71 % compared to that in the control group.

3.4. Reproduction

As shown in Fig. 4, NEOs have a significant negative effect on the reproduction of soil animals. The mean survival of juveniles, mean number of cocoons, mean cocoons/eggs/cells per animal, mean hatchlings per cocoon, and egg hatchability were reduced by 97 %, 100 %, 89 %, 96 %, and 84 %, with response ratios of 0.03 [0.02, 0.04], 0 [0, 0.04], 0.11 [0.07, 0.19], 0.04 [0.02, 0.1], and 0.16 [0.08, 0.3], respectively ($p < 0.0001$).

Each observation was divided into subgroups according to different soil animal categories. The mean survival of Oligochaeta and Collembola juveniles decreased by 95 % and 97 %, with response ratios of 0.05 [0.03, 0.09] and 0.03 [0.02, 0.05], respectively ($p < 0.0001$). The mean number of Oligochaeta cocoons decreased by 100 %, with response ratios of 0 [0, 0.04] ($p < 0.0001$). The mean cocoons/eggs/cells per Oligochaeta, Coleoptera, and Hemiptera decreased by 92 %, 55 %, and 100 %, with response ratios of 0.08 [0.04, 0.14], 0.45 [0.25, 0.81], and 0 [0, 0.24], respectively ($p < 0.05$). The egg hatchability of Oligochaeta and Coleoptera decreased by 83 % and 92 %, with response ratios of 0.17 [0.05, 0.53] and 0.08 [0.02, 0.4], respectively ($p < 0.005$).

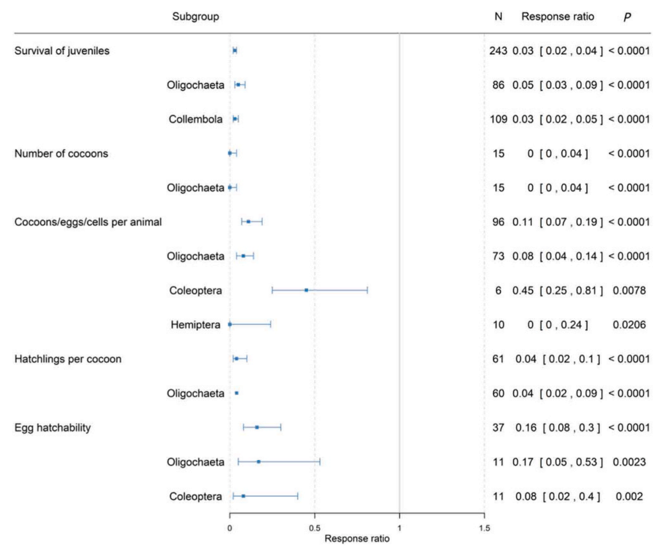

Fig. 4. Effects of NEO treatments on reproduction performance measures of non-target soil animals based on 362 observations. The blue square symbols show the average value of the response ratio for each type of NEO with error bars representing 95 % confidence interval (CI). A ratio > 1 indicates that the response from the treatment is higher compared to the control group. N refers to sample size and p means the p-value of the Q test. A significant effect of NEO use is indicated when CI does not overlap one and significance codes are as follows: * ** * $p < 0.0001$, * ** $p < 0.001$, * * $p < 0.01$, and * $p < 0.05$. Response ratio: the reason for the zeros is due to rounding.

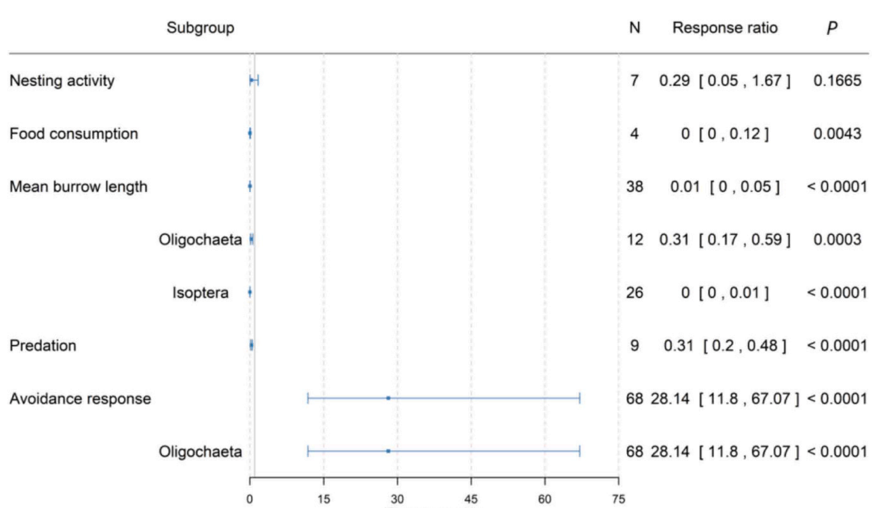

Fig. 3. Effects of NEO treatments on behavior performance measures of non-target soil animals based on 126 observations. The blue square symbols show the average value of the response ratio for each type of NEO with error bars representing 95 % confidence interval (CI). A ratio > 1 indicates that the response from the treatment is higher compared to the control group. N refers to sample size and p means the p-value of the Q test. A significant effect of NEO use is indicated when CI does not overlap one and significance codes are as follows: * ** * $p < 0.0001$, * ** $p < 0.001$, * * $p < 0.01$, and * $p < 0.05$. Response ratio: the reason for the zeros is due to rounding.

3.5. Biochemical biomarker

As shown in Fig. 5, NEOs have different effects on various physiological and biochemical characteristics of soil animals. The activities of superoxide dismutase activity (SOD), protein carbonyl activity (PCO), catalase activity (CAT), and glutathione reductase activity (GR) were increased by 2.18, 3.49, 1.87, and 2.25 times compared with control treatment, with response ratios of 3.18 [2.24, 4.51], 4.49 [2.98, 6.75], 2.87 [1.81, 4.55], and 3.25 [1.87, 5.66], respectively ($p < 0.0001$). Peroxidase activity (POD) increased by 1.05 times with response ratios of 2.05 [1.07, 3.95] ($p < 0.05$). Correspondingly, cellulase activity was inhibited by 67 %, with response ratios of 0.33 [0.19, 0.55] ($p < 0.0001$). However, the changes in the activities of acetylcholine esterase (AChE), glutathione S-transferase (GST), and carboxylesterases (CarE) were not significant ($p > 0.05$).

NEOs increased the contents of OH^{-}, 8-OHdG, reactive oxygen species (ROS), and malondialdehyde (MDA) by 6.76, 23.73, 12.95, and 2.66 times, with response ratios of 7.76 [3.87, 15.58], 24.73 [8.24, 74.16], 13.95 [8.36, 23.27], and 3.66 [2.54, 5.27], respectively ($p < 0.0001$). NEOs also induced DNA damage. Compared with the control treatment, olive tail moment (OTM), tail moment, and tail DNA in soil animals were increased by 58.83, 18.45, and 32.67 times, with response ratios of 59.83 [30.26, 118.3], 19.45 [2.17, 174.2], and 33.67 [12.17, 93.16], respectively ($p < 0.05$). Different expressions of genes caused different reactions: expression of the Hsp70 gene increased by 21.6 times ($p < 0.0001$), the expression of Ann gene decreased by 92 % ($p < 0.001$), while relative the expression of Tctp gene was not significantly affected ($p > 0.05$).

4. Discussion

This is the first meta-analysis to investigate the effect of NEOs on non-target soil animals. Among the five major indicators, survival, growth, behavior, and reproduction were significantly negatively affected, while observations of biochemical biomarkers showed different effects. Among the investigated response ratios, those with the

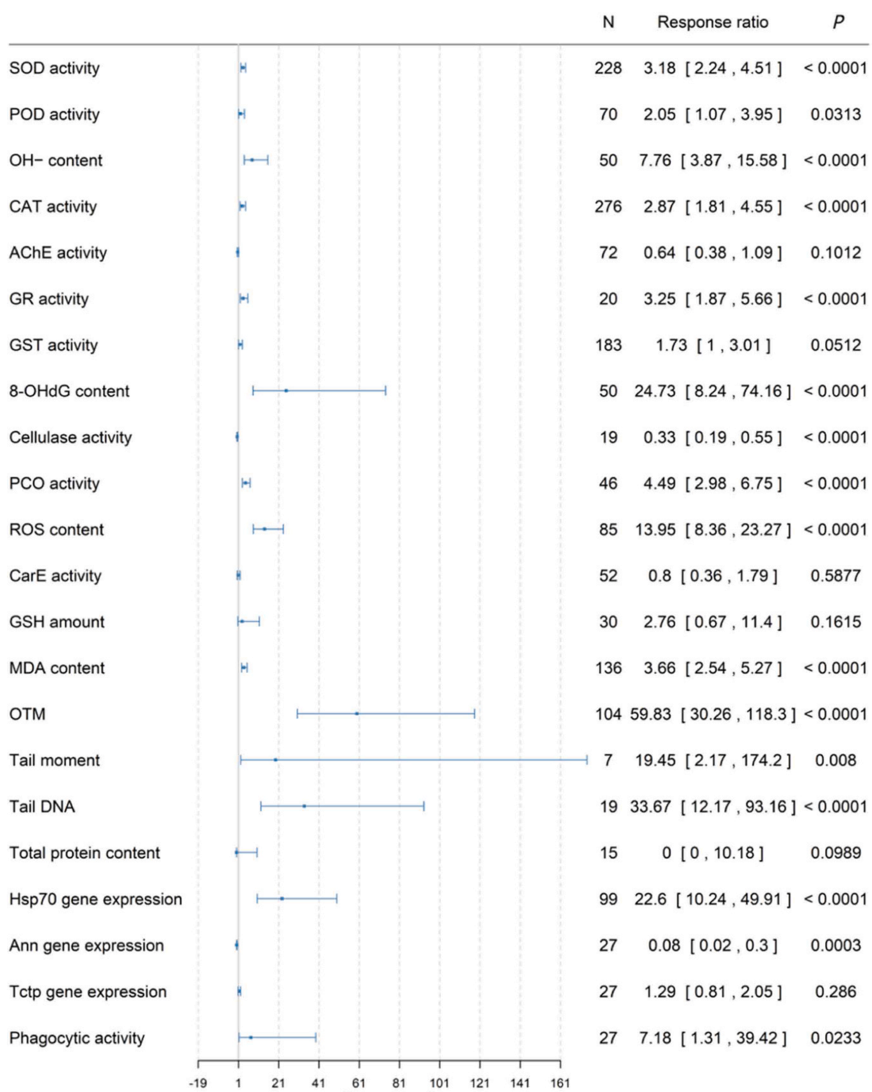

Fig. 5. Effects of NEO treatments on biochemical biomarker performance measures of non-target soil animals based on 1536 observations. The blue square symbols show the average value of the response ratio for each type of NEO with error bars representing 95 % confidence interval (CI). A ratio > 1 indicates that the response from the treatment is higher compared to the control group. N refers to sample size and p means the p-value of the Q test. A significant effect of NEO use is indicated when CI does not overlap one and significance codes are as follows: * ** * $p < 0.0001$, * ** $p < 0.001$, * * $p < 0.01$, and * $p < 0.05$. Full name of observations are as follows: SOD, superoxide dismutase activity; POD, peroxidase activity; CAT, catalase activity; AChE, acetylcholine esterase activity; GR, glutathione reductase activity; GST, glutathione S-transferase activity; PCO, protein carbonyl activity; ROS, reactive oxygen species content; CarE, carboxylesterase activity; GSH, glutathione peroxidase amount; MDA, malondialdehyde content; OTM, olive tail moments. Response ratio: the reason for the zeros is due to rounding.

greatest impact are the survival rate of individuals, longevity, and food consumption. Main et al. [34] assessed that NEOs significantly reduce terrestrial arthropods performance across broad ecological metrics, including abundance, behavior, condition, reproductive success, and survival, based on 44 studies with 372 observations. Compared with those in Main et al. [34], our study included more soil animals and more comprehensive test metrics, which also examined different effects of NEOs on different species of soil animals. According to subgroup analyses, we revealed different levels of sensitivity to NEO exposure at the species level. Soil animals belonging to different species have physiological, behavioral, and other characteristics that make them suitable for living in their respective environments, where they respond differently to pesticide pollution. This could explain the different responses of species to NEOs. However, these differences in our meta-analysis may help us distinguish between the various sensitivities of soil fauna and conduct more in-depth studies. Because the individual observations in each category had distinct interpretations, the five indicators were not combined and analyzed as a whole. In addition, NEO concentration in the field can be affected by various environmental factors (e.g., significant exposure, soil humidity, pH, climatic factors, and temperature), which can further impact soil animals [41]. Generally, sunlight induces NEO photodegradation. Using a photocatalyst under ultraviolet irradiation can promote the degradation of imidacloprid in soil [42]. The adsorption of pesticides in soil is negatively correlated with soil moisture content [43]. NEO concentrations increase with water temperature [41]. In this study, we examined the effects of NEOs on soil animals for each individual observation, which enabled us to understand their specific effects. Overall, the use of realistic NEOs poses harm to soil animal communities, as shown by these findings, which has significant implications for future pesticide regulation and risk assessment.

4.1. Survival

Among the investigated observations, the most important impacts on soil animals are survival rate and longevity. The effects of NEOs on soil animals can be best visualized through their survival. Since NEOs are designed to kill insects, it is not surprising that they can affect the survival of many non-target soil animals [21]. NEOs primarily act as agonists of nicotinic acetylcholine receptors (nAChRs) in the nervous system of insects [1]. By binding to these receptors, they disrupt the normal functioning of the nervous system, leading to the paralysis and death of target pests; however, they also hit the non-target animal as a side effect. As of 2011, 60 % of NEO use occurred through seed treatments and soil application [44], and 92 % of the increase in invertebrate toxicity loading was caused by NEOs [45]. In our result, although significant negative effects were observed for all species of soil animals, nematodes were less affected than other species of animals. That's because compared to arthropods, nematodes tend to be less sensitive to NEOs [46]. Literature indicates that arthropod survival is negatively affected by NEOs at all life history stages [34]. Meanwhile, early life stages and/or animals with more life stages are generally the most sensitive to pesticides [47]. Our results indicate that survival rate and longevity are negatively affected by NEOs. The effect on survival is severe for soil animals, which could potentially lead to greater reductions in ecosystem services [22].

4.2. Growth

Observations of abundance, biomass, growth rate, individual weight, and cocoon weight are markedly reduced, indicating NEO residues inhibit animal growth. Our meta-analysis confirms that NEOs have significant negative effects on the individual weight of soil animals, which is different from the conclusion of Main et al. [34], who found that NEOs have no significant effect on the weight of soil animals. Individual weight loss of soil animals is affected by NEOs, probably because NEOs inhibit the predation of soil animals [48]. We also found that the individual weight of the earthworms was significantly negatively affected. However, the individual weight of Coleoptera and Hymenoptera were not significantly affected. Coleoptera, represented by beetles, are not significantly affected by pesticides [37]. Previous studies have suggested that this is due to the different forms and rates of NEOs [49,50]. There is another assumption about the different toxicities of NEOs to insects. Insect nAChR subunits have different sensitivities to different NEOs; therefore, it is likely that different subunits may be activated with distinctive effects on their toxicity [51]. The mean cocoon and adult weight of the earthworms were affected remarkably in the presence of NEO residues. Earthworm (*Eisenia fetida*) cocoons were observed to be smaller and thinner with increased concentrations of the tested NEOs (imidacloprid, clothianidin, nitenpyram, acetamiprid, dinotefuran, and thiacloprid), which further verifies our conclusion [50].

Main et al. [34] verified significant negative effects on arthropod abundance, while our meta-analysis revealed no significant negative effects. This may be because the sample size (five observations) in this study is too small, which needs further verification to make a more solid assessment. Animal abundance responses may occur via direct toxic effects or predation release depending on the different functional groups [52]. The lower mean effect of NEOs on the abundance of soil animals could arise from the variable responses and compensation processes among species [53].

4.3. Behavior

NEOs impair the central nervous system function, leading to a variety of observable behavioral effects in soil animals [1], such as changes in nesting activity [54], predation [55], and burrow behavior [56]. The predation behavior of soil animals was greatly reduced when they were exposed to NEOs, which is consistent with the results of Main et al. [34]. NEOs may reduce the ability of soil animals to locate prey by interfering with their olfactory capacity, leading to decreased predation [57]. This inability to capture prey may explain the severe decline in predator survival rates. Our meta-analysis revealed NEOs had no significant effect on nesting activity, which may be related to NEO concentration and exposure time. A study of low-level (< 5 ppb) chronic dietary exposure revealed no effects on nesting activity of *Osmia bicornis* [58]. Conversely, the nesting activity of *Osmia lignaria* was reduced as a result of soil exposure to imidacloprid at a high level (> 50 ppb) [54]. Chronic dietary exposure to imidacloprid in bumble bees has also been associated with decreased nesting activity [59]. Among the behavioral effects on soil animals, burrowing and avoidance responses have been ranked as very sensitive parameters [21]. Changes in burrow depth may have an impact on the soil's ability to transmit gas and water, which might consequently have an impact on the ecosystem [60]. In this study, decreased burrow length and increased avoidance responses revealed behavior disturbances induced by NEOs. Imidacloprid has significant negative effects on the burrowing behavior of two earthworm species [56]. Most studied endpoints for soil animals provide clear evidence of damage, while avoidance informs us of an animal's response, which could indicate other negative impacts on the animal [61]. There can also be false negatives in avoidance tests. For example, dimethoate did not cause avoidance behavior in springtails but did cause stress or paralysis that prevented movement [62]. Furthermore, the effects of avoidance responses may alter depending on the soil type [63]. Alves et al. [64] suggested that avoidance responses vary across species. Compared with collembolans, earthworms may be more sensitive to detecting contaminants in avoidance tests.

4.4. Reproduction

Declines of the mean survival of juveniles, mean number of cocoons, mean cocoons/eggs/cells per animal, mean hatchlings per cocoon, and egg hatchability suggest that NEOs are harmful to animal reproduction.

NEOs can interfere with the development of nervous system of insects, which may affect their regeneration processes. In addition, NEOs may affect the ability of insects to regenerate by altering their endocrine systems [65]. The mean survival of juveniles is the most sensitive indicator of imidacloprid and clothianidin [50]. Both individual and juvenile survival rates are significantly affected by NEOs. The reproduction of *E. fetida*, which belongs to Oligochaeta earthworms, exposed to soil with low NEO concentrations was significantly inhibited [66]. According to the published research, imidacloprid can cause sperm abnormalities in earthworms and affect reproduction at doses as low as 0.5 mg/kg, leading to a serious decline in the number of earthworm cocoons and cocoons per earthworm [67]. The results of our study are different from the previous studies, in which cocoon weight is more sensitive than cocoon production and hatchability, and the hatchlings per cocoon is more sensitive than the number of cocoons [31,68]. The specific reasons for the slight difference require further investigation, though it is clear that NEOs have a large negative impact on these indicators. Earthworms showed significant negative effects in all reproduction observations, which was confirmed in our study. Compared with Oligochaeta and Hemiptera, the cocoons per animal of Coleoptera are less affected by NEOs.

4.5. Biochemical biomarker

NEO stimulation enhances the oxidative stress in animals [69]. Soil animals exposed to NEOs show higher levels of ROS, prompting cellular antioxidant defenses to become more active [69]. Antioxidant enzymes, including SOD, CAT, POD, and GST, play important roles in the removal of excess ROS from cells [70]. When ROS levels exceed the scavenging capacity of the antioxidant defense system, they can induce lipid peroxidation and cause oxidative damage to proteins and nucleic acids. This interferes with the regular function of cells, including reproductive cells [71]. When cells are subjected to oxidative stress, unsaturated fatty acids undergo oxidation under the action of ROS, leading to MDA formation [72]. MDA is the toxic end product of lipid peroxidation, and its elevated levels may lead to a decrease in the reproductive capacity of soil animals [29]. Therefore, studies on the effects of NEOs on animal reproduction should consider the responses of the entire reproductive system, including changes in the number and morphology of germ cells and disturbances in energy metabolism. Meanwhile, the increases in the content of antioxidant enzymes such as SOD, CAT, POD, and GR activity was shown to be used to eliminate oxidative damage because of their important role in scavenging excess ROS from cells [73,69,74]. CAT and POD activity in all treatments showed upward trends after exposure to clothianidin [32]. However, GST and AChE activity did not change significantly, which may be related to the NEO exposure time and concentration. GST can inactivate various xenobiotic compounds, including pesticides, and detoxify ROS in cells [75]. GST activity decreased after earthworms were exposed to clothianidin. For the 0.1 mg/kg treatment of clothianidin, the GST activity did not change significantly, while for the 0.5 and 1.0 mg/kg treatments of clothianidin, it decreased significantly [32]. NEOs cause DNA damage in soil animals, according to the increases in OTM, tail moment, and tail DNA. NEOs have been proven to increase ROS generation and damage the DNA of soil animals [31,32]. Furthermore, ROS accumulation can potentially damage cellular components, including DNA, proteins, and lipids [29,76]. In this study, NEOs had no significant effect on the protein content of soil animals. This is probably because different body parts have different protein contents. NEOs stimulate the reproductive organs, leading to increased protein production, while in the head region, they inhibited certain proteins, causing a decrease in their content [77]. The Hsp70 gene has the ability to protect cells from damage induced by stress conditions, and its relative expression levels changed obviously as NEO concentration and exposure time increased [78]. The overexpression of the Hsp70 gene indicated that NEOs had induced stress in soil animals. The Ann gene, an oxytocin-related neuropeptide hormone can control the egg-laying behavior of earthworms [79]. In Liu et al. [32], the downregulation of the Ann gene indicated that clothianidin may have affected the reproduction of earthworms. The Tctp gene is an important growth-related protein for cell growth and division that affects individual growth and reproduction. In our analysis, the Tctp gene had no significant effect on NEOs, which may also be due to the different NEO exposure times and concentrations.

4.6. Limitation

Our meta-analysis quantified the effects of NEOs on different soil animals across five indicators (i.e., survival, growth, behavior, reproduction, and biochemical biomarkers). However, our research mostly focused on laboratory studies of single pesticides on soil animals. In reality, multiple mixtures of NEOs always exist in realistic soils. Meanwhile, the NEO concentrations (0.001—78,600.000 mg/kg) in the laboratory studies are higher than those in the field soils (0.02—780.00 mg/kg), which could overestimate the effects of NEOs on soil animals in the real environment. In addition, the exposure time to NEOs was not assessed in our study because of the difficulty in integrating exposure times for different soil animals and the lack of sufficient data for certain soil animals, which requires further investigation.

5. Conclusion

Our quantitative synthesis is a significant step toward improving the predictions of the ecological effects of pesticide usage by concentrating on natural soil animal communities, which are essential elements of global biodiversity and ecosystem functioning. Our meta-analysis demonstrated significantly negative effects of NEOs on soil animal performance across five performance measures, including survival, growth, behavior, reproduction, and biochemical biomarkers, based on 2940 observations. NEOs changed two soil animal survival indicators with summary effect sizes of 0.00—0.20, seven soil animal growth indicators with summary effect sizes of 0.07—2.85, five soil animal behavior indicators with summary effect sizes of 0.00—28.14, five soil animal reproduction indicators with summary effect sizes of 0.00—0.45, and twenty-two soil animal biochemical biomarkers indicators with summary effect sizes of 0.00—59.83. Here, we validated the widely held belief and worrisome conclusion that NEOs can cause serious damage to soil biodiversity.

This review presents many pieces of evidence that NEOs pose a serious threat to soil animals as well as the basic ecosystem services they provide. With the growing use of NEOs, more NEOs may remain in soils as residues, posing risks to soil animals. To provide a more accurate picture of the effects of NEOs on soil ecosystems, we urge further long-term field experiments to be carried out by adding realistic concentrations of NEOs to soils in future studies. We also strongly support the inclusion of soil health analysis in the NEO risk assessment process to preserve biodiversity and achieve sustainable agriculture. These can advance our knowledge of the possible impacts of NEO pollution on ecosystem functioning in agricultural soils.

Environmental implication

Neonicotinoids (NEOs) have potential adverse effects on soil ecosystems. In this meta-analysis, we quantify the effects of NEOs on key indicators of soil animals (i.e., survival, growth, behavior, reproduction, and biochemical biomarkers) based on the 2940 observations from 113 studies. These quantitative results present many pieces of evidence that NEOs pose a serious threat to soil animals, thereby enhancing our comprehension of the impact of NEO residues on agricultural soil ecosystems.

CRediT authorship contribution statement

Huan Lu: Writing – review & editing. **Haoyuan Shi:** Resources. **Xin Li:** Resources. **Yuxuan Cao:** Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Jindong Li:** Writing – review & editing. **Siyang Ren:** Visualization. **Hongyu Mu:** Writing – review & editing. **Yiyi Li:** Writing – review & editing. **Wenting Zhao:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization. **Fanrong Zhao:** Writing – review & editing. **Jinrui Zhang:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Jiajun Han:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Kai Wang:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Daniel Figueiredo:** Writing – review & editing, Methodology. **Mingyu Zhao:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgements

The authors gratefully acknowledge the financial support from the National Key R&D Program of China (No. 2023YFD1702102, No. 2023YFD1900604 and No. 2023YFD1700700); the National Natural Science Foundation of China (No. 42207125 and No. 22306197); the Project of Cultivation for young top-notch Talents of Beijing Municipal Institutions (No. BPHR202203098), Beijing University Young Teachers' Innovation Teaching and Research Studio of Beijing Education Union; Professor station of China Agricultural University at Xinzhou Center for Disease Control and Prevention; the High-level Team Project of China Agricultural University.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.135022.

References

[1] Bass, C., Field, L.M., 2018. Neonicotinoids. Curr Biol 28 (14), R772–R773. https://doi.org/10.1016/j.cub.2018.05.061.
[2] Willy D. Kollmeyer, R.F.F, James P. Foster, James E. Powell, Mark E. Schroeder, & S. Barney Soloway 1999. Discovery of the Nitromethylene Heterocycle Insecticides. Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor. 71–89.
[3] Bass, C., Denholm, I., Williamson, M.S., Nauen, R., 2015. The global status of insect resistance to neonicotinoid insecticides. Pest Biochem Physiol 121, 78–87. https://doi.org/10.1016/j.pestbp.2015.04.004.
[4] Hladik, M.L., Main, A.R., Goulson, D., 2018. Environmental risks and challenges associated with neonicotinoid insecticides. Environ Sci Technol 52 (6), 3329–3335. https://doi.org/10.1021/acs.est.7b06388.
[5] Goulson, D., Kleijn, D., 2013. REVIEW: an overview of the environmental risks posed by neonicotinoid insecticides. J Appl Ecol 50 (4), 977–987. https://doi.org/10.1111/1365-2664.12111.
[6] Mitchell, E.A.D., Mulhauser, B., Mulot, M., Mutabazi, A., Glauser, G., Aebi, A., 2017. A worldwide survey of neonicotinoids in honey. Science 358 (6359), 109. https://doi.org/10.1126/science.aan3684.
[7] Woodcock, B.A., Bullock, J.M., Shore, R.F., Heard, M.S., Pereira, M.G., Redhead, J., Ridding, L., Dean, H., Sleep, D., Henrys, P., Peyton, J., Hulmes, S., Hulmes, L., Sárospataki, M., Saure, C., Edwards, M., Genersch, E., Knäbe, S., Pywell, R.F., 2017. Country-specific effects of neonicotinoid pesticides on honey bees and wild bees. Science 356 (6345), 1393. https://doi.org/10.1126/science.aaa1190.
[8] Gibbons, D., Morrissey, C., Mineau, P., 2015. A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. Environ Sci Pollut Res Int 22 (1), 103–118. https://doi.org/10.1007/s11356-014-3180-5.
[9] Lopez-Antia, A., Ortiz-Santaliestra, M.E., Mateo, R., 2014. Experimental approaches to test pesticide-treated seed avoidance by birds under a simulated diversification of food sources. Sci Total Environ 496, 179–187. https://doi.org/10.1016/j.scitotenv.2014.07.031.
[10] Chevillot, F., Convert, Y., Desrosiers, M., Cadoret, N., Veilleux, É., Cabana, H., Bellenger, J.-P., 2017. Selective bioaccumulation of neonicotinoids and sub-lethal effects in the earthworm Eisenia andrei exposed to environmental concentrations in an artificial soil. Chemosphere 186, 839–847. https://doi.org/10.1016/j.chemosphere.2017.08.046.
[11] Parveen Gill, D.D.S., Dommalapati Sudhakara Rao, Naresh Kumar, 2022. Assessment of Neonicotinoid Insecticide Acetamiprid LC 50 against Earthworm (Eisenia fetida L.).
[12] Costas-Ferreira, C., Faro, L.R.F., 2021. Neurotoxic effects of neonicotinoids on mammals: what is there beyond the activation of nicotinic acetylcholine receptors?-A systematic review. Int J Mol Sci 22 (16). https://doi.org/10.3390/ijms22168413.
[13] Amangeldi K. Apushev, B.Y., Nurdana N. Salybekova, Anvar Mamadaliev, 2023. Biomorphological Analysis of Tulip Varieties on Substrates in Covered Ground in Turkestan. https://doi.org/10.28991/HEF-2023–04-02–06.
[14] Jaroensutasinee, K., Jaroensutasinee, M., Boonsanong, P., 2023. Climatic factor differences and mangosteen fruit quality between on- and off-season productions. Emerg Sci J 7 (2), 578–588. https://doi.org/10.28991/esj-2023-07-02-020.
[15] Jensen, O.P., 2019. Pesticide impacts through aquatic food webs. Science 366 (6465), 566–567. https://doi.org/10.1126/science.aaz6436.
[16] Salerno, M., Berlino, M., Mangano, M.C., Sarà, G., 2021. Microplastics and the functional traits of fishes: a global meta-analysis. Glob Change Biol 27 (12), 2645–2655. https://doi.org/10.1111/gcb.15570.
[17] Ram, D.R.L., 2019. Curr Res Soil Fertil 107. https://doi.org/10.22271/ed.book.437.
[18] Alford, A., Krupke, C.H., 2017. Translocation of the neonicotinoid seed treatment clothianidin in maize. PLoS One 12 (3), e0173836. https://doi.org/10.1371/journal.pone.0173836.
[19] Hou, J., Chen, L., Han, B., Li, Y., Yu, L., Wang, L., Tao, S., Liu, W., 2023. Distribution characteristics and risk assessment of neonicotinoid insecticides in planting soils of mainland China. Sci Total Environ 902, 166000. https://doi.org/10.1016/j.scitotenv.2023.166000.
[20] Mamy, L., Pesce, S., Sanchez, W., Aviron, S., Bedos, C., Berny, P., Bertrand, C., Betoulle, S., Charles, S., Chaumot, A., Coeurdassier, M., Coutellec, M.-A., Crouzet, O., Faburé, J., Fritsch, C., Gonzalez, P., Hedde, M., Leboulanger, C., Margoum, C., Mougin, C., Munaron, D., Nélieu, S., Pelosi, C., Rault, M., Sucré, E., Thomas, M., Tournebize, J., Leenhardt, S., 2023. Impacts of neonicotinoids on biodiversity: a critical review. Environ Sci Pollut Res. https://doi.org/10.1007/s11356-023-31032-3.
[21] Pisa, L.W., Amaral-Rogers, V., Belzunces, L.P., Bonmatin, J.M., Downs, C.A., Goulson, D., Kreutzweiser, D.P., Krupke, C., Liess, M., McField, M., Morrissey, C.A., Noome, D.A., Settele, J., Simon-Delso, N., Stark, J.D., Van der Sluijs, J.P., Van Dyck, H., Wiemers, M., 2015. Effects of neonicotinoids and fipronil on non-target invertebrates. Environ Sci Pollut Res Int 22 (1), 68–102. https://doi.org/10.1007/s11356-014-3471-x.
[22] Wood, T.J., Goulson, D., 2017. The environmental risks of neonicotinoid pesticides: a review of the evidence post 2013. Environ Sci Pollut Res Int 24 (21), 17285–17325. https://doi.org/10.1007/s11356-017-9240-x.
[23] Saggioro, E.M., do Espirito Santo, D.G., Sales Junior, S.F., Hauser-Davis, R.A., Correia, F.V., 2019. Lethal and sublethal effects of acetamiprid on Eisenia andrei: behavior, reproduction, cytotoxicity and oxidative stress. Ecotoxicol Environ Saf 183, 109572. https://doi.org/10.1016/j.ecoenv.2019.109572.
[24] Cimino, A.M., Boyles, A.L., Thayer, K.A., Perry, M.J., 2017. Effects of neonicotinoid pesticide exposure on human health: a systematic review. Environ Health Perspect 125 (2), 155–162. https://doi.org/10.1289/EHP515.
[25] Bartlett, M.D., Briones, M.J.I., Neilson, R., Schmidt, O., Spurgeon, D., Creamer, R.E., 2010. A critical review of current methods in earthworm ecology: From individuals to populations. Eur J Soil Biol 46 (2), 67–73. https://doi.org/10.1016/j.ejsobi.2009.11.006.
[26] Burke, A.P., Niibori, Y., Terayama, H., Ito, M., Pidgeon, C., Arsenault, J., Camarero, P.R., Cummins, C.L., Mateo, R., Sakabe, K., Hampson, D.R., 2018. Mammalian susceptibility to a neonicotinoid insecticide after fetal and early postnatal exposure. Sci Rep 8 (1), 16639. https://doi.org/10.1038/s41598-018-35129-5.
[27] Pelosi, C., Bertrand, C., Daniele, G., Coeurdassier, M., Benoit, P., Nélieu, S., Lafay, F., Bretagnolle, V., Gaba, S., Vulliet, E., Fritsch, C., 2021. Residues of currently used pesticides in soils and earthworms: a silent threat? Agric, Ecosyst Environ 305. https://doi.org/10.1016/j.agee.2020.107167.
[28] Römbke, J., Schmelz, R.M., Pélosi, C., 2017. Effects of organic pesticides on Enchytraeids (Oligochaeta) in agroecosystems: laboratory and higher-tier tests. Front Environ Sci 5. https://doi.org/10.3389/fenvs.2017.00020.
[29] Wang, J., Wang, J., Wang, G., Zhu, L., Wang, J., 2016. DNA damage and oxidative stress induced by imidacloprid exposure in the earthworm Eisenia fetida. Chemosphere 144, 510–517. https://doi.org/10.1016/j.chemosphere.2015.09.004.
[30] Lee, D.K., In, J., Lee, S., 2015. Standard deviation and standard error of the mean. Korean J Anesth 68 (3), 220–223. https://doi.org/10.4097/kjae.2015.68.3.220.
[31] Wang, K., Qi, S., Mu, X., Chai, T., Yang, Y., Wang, D., Li, D., Che, W., Wang, C., 2015. Evaluation of the toxicity, AChE activity and DNA damage caused by

imidacloprid on earthworms, Eisenia fetida. Bull Environ Contam Toxicol 95 (4), 475–480. https://doi.org/10.1007/s00128-015-1629-y.
[32] Liu, T., Wang, X., You, X., Chen, D., Li, Y., Wang, F., 2017. Oxidative stress and gene expression of earthworm (Eisenia fetida) to clothianidin. Ecotoxicol Environ Saf 142, 489–496. https://doi.org/10.1016/j.ecoenv.2017.04.012.
[33] Clements, W.H., Rohr, J.R., 2009. Community responses to contaminants: using basic ecological principles to predict ecotoxicological effects. Environ Toxicol Chem 28 (9), 1789–1800. https://doi.org/10.1897/09-140.1.
[34] Main, A.R., Webb, E.B., Goyne, K.W., Mengel, D., 2018. Neonicotinoid insecticides negatively affect performance measures of non-target terrestrial arthropods: a meta-analysis. Ecol Appl 28 (5), 1232–1244. https://doi.org/10.1002/eap.1723.
[35] Douglas, M.R., Tooker, J.F., 2016. Meta-analysis reveals that seed-applied neonicotinoids and pyrethroids have similar negative effects on abundance of arthropod natural enemies. PeerJ 4, e2776. https://doi.org/10.7717/peerj.2776.
[36] Beaumelle, L., Tison, L., Eisenhauer, N., Hines, J., Malladi, S., Pelosi, C., Thouvenot, L., Phillips, H.R.P., 2023. Pesticide effects on soil fauna communities—a meta-analysis. J Appl Ecol 60 (7), 1239–1253. https://doi.org/10.1111/1365-2664.14437.
[37] Gunstone, T., Cornelisse, T., Klein, K., Dubey, A., Donley, N., 2021. Pesticides and soil invertebrates: a hazard assessment. Front Environ Sci 9. https://doi.org/10.3389/fenvs.2021.643847.
[38] Zhang, J., Ren, S., Xu, W., Liang, C., Li, J., Zhang, H., Li, Y., Liu, X., Jones, D.L., Chadwick, D.R., Zhang, F., Wang, K., 2022. Effects of plastic residues and microplastics on soil ecosystems: a global meta-analysis. J Hazard Mater 435, 129065. https://doi.org/10.1016/j.jhazmat.2022.129065.
[39] Viechtbauer, W., 2010. Conducting meta-analyses in R with the metafor package. J Stat Softw 36 (3), 1–48 https://doi.org/DOI 10.18637/jss.v036.i03.
[40] Viechtbauer, W., 2005. Bias and efficiency of meta-analytic variance estimators in the random-effects model. J Educ Behav Stat 30 (3), 261–293 https://doi.org/Doi10.3102/10769986030003261.
[41] Wang, J.Q., Yin, R., Liu, Y.L., Wang, B.J., Wang, N.N., Xiao, P.F., Xiao, T.F., Hirai, H., 2023. Meta-analysis of neonicotinoid insecticides in global surface waters. Environ Sci Pollut Res 30 (1), 1039–1047. https://doi.org/10.1007/s11356-022-22270-y.
[42] Sharma, T., Toor, A.P., Rajor, A., 2015. Photocatalytic degradation of imidacloprid in soil: application of response surface methodology for the optimization of parameters. RSC Adv 5 (32), 25059–25065. https://doi.org/10.1039/C5RA02224J.
[43] Zheng, T., Zhang, J., Tang, C., Zhang, Y., Duan, J., 2022. Persistence and vertical distribution of neonicotinoids in soils under different citrus orchards chrono sequences from southern China. Chemosphere 286. https://doi.org/10.1016/j.chemosphere.2021.131584.
[44] Jeschke, P., Nauen, R., Schindler, M., Elbert, A., 2011. Overview of the status and global strategy for neonicotinoids. J Agric Food Chem 59 (7), 2897–2908. https://doi.org/10.1021/jf101303g.
[45] DiBartolomeis, M., Kegley, S., Mineau, P., Radford, R., Klein, K., 2019. An assessment of acute insecticide toxicity loading (AITL) of chemical pesticides used on agricultural land in the United States. PLoS One 14 (8), e0220029. https://doi.org/10.1371/journal.pone.0220029.
[46] Freedman, J.H., Bradford, B.R., Whidden, E., Gervasio, E.D., Checchi, P.M., Raley-Susman, K.M., 2020. Neonicotinoid-containing insecticide disruption of growth, locomotion, and fertility in Caenorhabditis elegans. Plos One 15 (9). https://doi.org/10.1371/journal.pone.0238637.
[47] Stark, J.D., Banks, J.E., Acheampong, S., 2004. Estimating susceptibility of biological control agents to pesticides: influence of life history strategies and population structure. Biol Control 29 (3), 392–398. https://doi.org/10.1016/j.biocontrol.2003.07.003.
[48] Liu, T., Wang, X., Xu, J., You, X., Chen, D., Wang, F., Li, Y., 2017. Biochemical and genetic toxicity of dinotefuran on earthworms (Eisenia fetida). Chemosphere 176, 156–164. https://doi.org/10.1016/j.chemosphere.2017.02.113.
[49] Alptekin, S., Bass, C., Nicholls, C., Paine, M.J., Clark, S.J., Field, L., Moores, G.D., 2016. Induced thiacloprid insensitivity in honeybees (Apis mellifera L.) is associated with up-regulation of detoxification genes. Insect Mol Biol 25 (2), 171–180. https://doi.org/10.1111/imb.12211.
[50] Ge, J., Xiao, Y., Chai, Y., Yan, H., Wu, R., Xin, X., Wang, D., Yu, X., 2018. Sub-lethal effects of six neonicotinoids on avoidance behavior and reproduction of earthworms (Eisenia fetida). Ecotoxicol Environ Saf 162, 423–429. https://doi.org/10.1016/j.ecoenv.2018.06.064.
[51] Thany, S.H., Lenaers, G., Raymond-Delpech, V., Sattelle, D.B., Lapied, B., 2007. Exploring the pharmacological properties of insect nicotinic acetylcholine receptors. Trends Pharm Sci 28 (1), 14–22. https://doi.org/10.1016/j.tips.2006.11.006.
[52] Atwood, L.W., Mortensen, D.A., Koide, R.T., Smith, R.G., 2018. Evidence for multi-trophic effects of pesticide seed treatments on non-targeted soil fauna. Soil Biol Biochem 125, 144–155. https://doi.org/10.1016/j.soilbio.2018.07.007.
[53] Pelosi, C., Barot, S., Capowiez, Y., Hedde, M., Vandenbulcke, F., 2013. Pesticides and earthworms. A review. Agron Sustain Dev 34 (1), 199–228. https://doi.org/10.1007/s13593-013-0151-z.
[54] Fortuin, C.C., McCarty, E., Gandhi, K.J., 2021. Acute contact with imidacloprid in soil affects the nesting and survival success of a solitary wild bee, Osmia lignaria (Hymenoptera: Megachilidae). Chemosphere 264 (Pt 2)), 128572. https://doi.org/10.1016/j.chemosphere.2020.128572.
[55] Drobne, D., Blazic, M., Van Gestel, C.A., Leser, V., Zidar, P., Jemec, A., Trebse, P., 2008. Toxicity of imidacloprid to the terrestrial isopod Porcellio scaber (Isopoda, Crustacea). Chemosphere 71 (7), 1326–1334. https://doi.org/10.1016/j.chemosphere.2007.11.042.
[56] Dittbrenner, N., Moser, I., Triebskorn, R., Capowiez, Y., 2011. Assessment of short and long-term effects of imidacloprid on the burrowing behaviour of two earthworm species (Aporrectodea caliginosa and Lumbricus terrestris) by using 2D and 3D post-exposure techniques. Chemosphere 84 (10), 1349–1355. https://doi.org/10.1016/j.chemosphere.2011.05.011.
[57] Desneux, N., Decourtye, A., Delpuech, J.-M., 2007. The sublethal effects of pesticides on beneficial arthropods. Annu Rev Entomol 52 (1), 81–106. https://doi.org/10.1146/annurev.ento.52.110405.091440.
[58] Dietzsch, A.C., Kunz, N., Wirtz, I.P., Stähler, M., Heimbach, U., Pistorius, J., 2019. Does winter oilseed rape grown from clothianidin-coated seeds affect experimental populations of mason bees and bumblebees? A semi-field and field study. J Consum Prot Food Saf 14 (3), 223–238. https://doi.org/10.1007/s00003-019-01225-5.
[59] Leza, M., Watrous, K.M., Bratu, J., Woodard, S.H., 2018. Effects of neonicotinoid insecticide exposure and monofloral diet on nest-founding bumblebee queens. Proc Biol Sci 285 (1880). https://doi.org/10.1098/rspb.2018.0761.
[60] Capowiez, Y., Bastardie, F., Costagliola, G., 2006. Sublethal effects of imidacloprid on the burrowing behaviour of two earthworm species: modifications of the 3D burrow systems in artificial cores and consequences on gas diffusion in soil. Soil Biol Biochem 38 (2), 285–293. https://doi.org/10.1016/j.soilbio.2005.05.014.
[61] Niemeyer, J.C., de Santo, F.B., Guerra, N., Ricardo Filho, A.M., Pech, T.M., 2018. Do recommended doses of glyphosate-based herbicides affect soil invertebrates? Field and laboratory screening tests to risk assessment. Chemosphere 198, 154–160. https://doi.org/10.1016/j.chemosphere.2018.01.127.
[62] Pereira, C.M., Novais, S.C., Soares, A.M., Amorim, M.J., 2013. Dimethoate affects cholinesterases in Folsomia candida and their locomotion–false negative results of an avoidance behaviour test. Sci Total Environ 443, 821–827. https://doi.org/10.1016/j.scitotenv.2012.11.044.
[63] Bernardino, M.M., Alves, P.R.L., de Santo, F.B., Niemeyer, J.C., Leal, R.M.P., 2021. Ecotoxicity of imidacloprid to soil invertebrates in two tropical soils with contrasting texture. Environ Sci Pollut Res Int 28 (22), 27655–27665. https://doi.org/10.1007/s11356-021-12562-0.
[64] Alves, P.R.L., Bandeira, F.O., Giraldi, M., Presotto, R., Segat, J.C., Cardoso, E., Baretta, D., 2019. Ecotoxicological assessment of Fluazuron: effects on Folsomia candida and Eisenia andrei. Environ Sci Pollut Res Int 26 (6), 5842–5850. https://doi.org/10.1007/s11356-018-4022-7.
[65] James, K.L., Randall, N.P., Walters, K.F.A., Haddaway, N.R., Land, M., 2016. Evidence for the effects of neonicotinoids used in arable crop production on non-target organisms and concentrations of residues in relevant matrices: a systematic map protocol. Environ Evid 5 (1). https://doi.org/10.1186/s13750-016-0072-9.
[66] Wang, K., Pang, S., Mu, X., Qi, S., Li, D., Cui, F., Wang, C., 2015. Biological response of earthworm, Eisenia fetida, to five neonicotinoid insecticides. Chemosphere 132, 120–126. https://doi.org/10.1016/j.chemosphere.2015.03.002.
[67] Luo, Y., Zang, Y., Zhong, Y.A., Kong, Z.M., 1999. Toxicological study of two novel pesticides on earthworm Eisenia foetida. Chemosphere 39 (13), 2347–2356.
[68] Nyoka, N.W.-K., Ogbeide, O., Otomo, P.V., 2021. Reproduction and biomarker responses of Eisenia fetida after exposure to imidacloprid in biochar-amended soil. Biochar 3 (4), 615–624. https://doi.org/10.1007/s42773-021-00103-4.
[69] Li, B., Xia, X., Wang, J., Zhu, L., Wang, J., Wang, G., 2018. Evaluation of acetamiprid-induced genotoxic and oxidative responses in Eisenia fetida. Ecotoxicol Environ Saf 161, 610–615. https://doi.org/10.1016/j.ecoenv.2018.06.022.
[70] Xu, D., Li, C., Wen, Y., Liu, W., 2013. Antioxidant defense system responses and DNA damage of earthworms exposed to Perfluorooctane sulfonate (PFOS). Environ Pollut 174, 121–127. https://doi.org/10.1016/j.envpol.2012.10.030.
[71] Zhang, Q., Zhang, B., Wang, C., 2014. Ecotoxicological effects on the earthworm Eisenia fetida following exposure to soil contaminated with imidacloprid. Environ Sci Pollut Res Int 21 (21), 12345–12353. https://doi.org/10.1007/s11356-014-3178-z.
[72] Hajipour, M.J., Raheb, J., Akhavan, O., Arjmand, S., Mashinchian, O., Rahman, M., Abdolahad, M., Serpooshan, V., Laurent, S., Mahmoudi, M., 2015. Personalized disease-specific protein corona influences the therapeutic impact of graphene oxide. Nanoscale 7 (19), 8978–8994. https://doi.org/10.1039/c5nr00520e.
[73] Chowdhary, A.B., Singh, J., Quadar, J., Singh, S., Dutta, R., Angmo, D., Vig, A.P., 2022. Earthworm's show tolerance and avoidance response to pesticide clothianidin: effect on antioxidant enzymes. Int J Environ Sci Technol 20 (4), 4245–4254. https://doi.org/10.1007/s13762-022-04092-0.
[74] Van Gestel, C.A., Koolhaas, J.E., Hamers, T., van Hoppe, M., van Roovert, M., Korsman, C., Reinecke, S.A., 2009. Effects of metal pollution on earthworm communities in a contaminated floodplain area: linking biomarker, community and functional responses. Environ Pollut 157 (3), 895–903. https://doi.org/10.1016/j.envpol.2008.11.002.
[75] Hattab, S., Boughattas, I., Boussetta, H., Viarengo, A., Banni, M., Sforzini, S., 2015. Transcriptional expression levels and biochemical markers of oxidative stress in the earthworm Eisenia andrei after exposure to 2,4-dichlorophenoxyacetic acid (2,4-D). Ecotoxicol Environ Saf 122, 76–82. https://doi.org/10.1016/j.ecoenv.2015.07.014.
[76] Dizdaroglu, M., 2012. Oxidatively induced DNA damage: mechanisms, repair and disease. Cancer Lett 327 (1-2), 26–47. https://doi.org/10.1016/j.canlet.2012.01.016.

[77] Muhammad Faheem, M.F.K., 2010. Toxicity of imidacloprid (Nicotinoid) against earthworm, Pheretima posthuma with reference to its effects on protein. J Basic Appl Sci.
[78] Wang, J., Cao, X., Sun, J., Chai, L., Huang, Y., Tang, X., 2015. Transcriptional responses of earthworm (Eisenia fetida) exposed to naphthenic acids in soil. Environ Pollut 204, 264–270. https://doi.org/10.1016/j.envpol.2015.05.006.
[79] Zheng, S., Song, Y., Qiu, X., Sun, T., Leigh Ackland, M., Zhang, W., 2008. Annetocin and TCTP expressions in the earthworm Eisenia fetida exposed to PAHs in artificial soil. Ecotoxicol Environ Saf 71 (2), 566–573. https://doi.org/10.1016/j.ecoenv.2007.10.025.