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Interpretation of ecotoxicity tests using *Enchytraeus albidus:* Weight of soil characteristics in conditions of diffuse pollution

Andrea Chacon-Hurtado [a,1], Juliette Faburé [a,*], Laura Sereni [a,2], Sylvie Nélieu [a], Nathalie Cheviron [b], Ghislaine Delarue [a], Emmanuelle Baudry [c], Isabelle Lamy [a]

[a] University Paris-Saclay, INRAE, AgroParisTech, UMR Ecosys, Palaiseau 91120, France
[b] University Paris-Saclay, INRAE, AgroParisTech, UMR Ecosys, Biochem-Env Platform, Palaiseau 91120, France
[c] University Paris-Saclay, CNRS, AgroParisTech, Ecologie Systématique Evolution, Gif-sur-Yvette 91190, France

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ABSTRACT

One way to evaluate the impact of chemical substances released in the ecosystems is the use of terrestrial organisms in ecotoxicological bioassays. *Enchytraeus albidus* (Oligochaeta) is a species commonly used in such standardized bioassays aiming at identifying biological responses as life traits (survival and reproduction) using artificial spiked soils or in situ contaminated soils. However, in the latter case, it is known that other factors such as soil characteristics may affect the responses in *E. albidus*. We hypothesized that, in cases of low diffuse pollution, soil characteristics are major confounding factors that interfere with the interpretation of life trait responses in bioassays when their influence outweighs that of the diffuse pollution itself. To evaluate these confounding factors, 20 soils were collected from a low-contamination area encompassing diverse landscapes (urban, peri-urban, and rural) that exhibited gradients in pedological characteristics, biological parameters, and organic and inorganic contaminant levels, all within the range of classical values considered optimal for reproduction. *E. albidus* individuals were exposed to each soil sample, and survival and reproduction were monitored as life traits. Soil clustering and multifactorial analysis were employed to identify the confounding factors. Regardless of the life trait, low and sublethal contamination was rarely an explanatory factor for the responses of *E. albidus*. Survival was unaffected by soil characteristics or diffuse pollution. In contrast, reproduction was significantly and positively associated with soil pH and the levels of exchangeable sodium, calcium carbonate, molybdenum, PAHs, and insecticides, while it was negatively influenced by aluminum and exchangeable manganese. These findings suggest that studies conducted under conditions of diffuse, sublethal contamination should consider multiple natural soil characteristics in ecotoxicological bioassays to enable meaningful comparisons.

1. Introduction

Soil diffuse pollution is characterized by chronic inputs of a mixture of organic and inorganic contaminants at low doses. Multiple sources may be at the origin of this pollution such as industrial activities (fossil fuel combustion), urban activity (household products, waste incineration, traffic) or agriculture (fertilisers, soil amendments) (Grassi et al., 2022). Compared to contaminant hotspots with locally high concentrations in soil, the risks associated with diffuse soil pollution involving sublethal doses remain poorly documented.

To evaluate the ecotoxicological risks to ecosystems exposed to various chemical substances, several tools have been developed using standardized bioassays with model soil invertebrates (Bicho et al., 2015). These bioassays are traditionally conducted in standardized soils, such as artificial OECD soil (OECD 222, 2004) or natural soils as LUFA 2.2 soil (Lock and Janssen, 2002). Furthermore, these bioassays are conducted using model species of soil invertebrates, including oligochaetes such as earthworms (e.g., *Eisenia fetida* and *E. andrei*). Some

* Corresponding author.
E-mail address: juliette.fabure@agroparistech.fr (J. Faburé).

[1] Present address: Chemical and Behavioral Ecology, Gembloux Agro-Bio Tech, University of Liege, Avenue de la Faculté d'Agronomie 2B, Gembloux, 5030, Belgium.

[2] Present address: University Grenoble Alpes, CNRS, INRAE, IRD, Grenoble INP, IGE, Grenoble, France.

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bioassays have also been developed for enchytraeids (potworms, Enchytraeidae) (ISO 2004:16) due to their complementary role to earthworms in soil ecosystem functioning, as for example the degradation of leaf litter into smaller pieces, but also because of their presence in ecosystems unsuitable for earthworms. Enchytraeids are ubiquitous across various ecosystems and contribute significantly to soil dynamics, including soil structure, organic matter decomposition, bioturbation, and nutrient cycling (Bart et al., 2018; Didden and Römbke, 2001; Maraldo and Holmstrup, 2010). Like other soil-dwelling organisms, enchytraeids are exposed to diverse stress factors through soil solution, as well as solid and gaseous soil phases.

Moreover, their ease of use under both field and laboratory conditions facilitates comparative studies of different exposures, with responses that are readily measurable (Didden and Römbke, 2001). Consequently, enchytraeids have been widely employed as terrestrial biological models in bioassays to investigate the effects of various substances, including trace elements (e.g., copper, cadmium, zinc, and lead) and pesticides (e.g., dimethoate, atrazine, carbendazim) (Frampton et al., 2006; Gomes et al., 2015, 2011; Kobetičová et al., 2009; Lock et al., 2000; Novais et al., 2014, 2011)

These bioassays aim at evaluating a generic response to an induced stress on enchytraeids. However, Kuperman et al. (2006) argue that the physical and chemical properties of the standardized soils used in these bioassays fail to reflect the soil diversity present in real field conditions. Neglecting the vast diversity of in situ soil characteristics may result in inaccurate environmental risk assessments by misinterpreting biological assay responses as being solely attributable to contaminants. This raises concerns about using enchytraeids as biological models, as unfavorable soil conditions for their ecology could amplify stress levels beyond chemical stress, while particularly favorable conditions might mask the detrimental effects of contaminants. Some research has already demonstrated the influence of soil characteristics on enchytraeid responses. Luo et al. (2014) observed reduced survival and reproduction of *Enchytraeus crypticus* in acidic soils (pHCaCl$_2$ $\leq$3.8). In the absence of chemical pollution, Amorim et al. (2005) and Kuperman et al. (2006) assessed the influence of soil characteristics on the survival and reproduction responses of two enchytraeid species exposed to twenty-five soil samples, thirteen of which were collected *in situ*. They suggested that tests for *E. albidus* and *E. luxuriosus* should be conducted within the following ranges to avoid confounding factors on reproduction: pH > 5, organic matter (OM) content between 2.5 % and 8 %, clay content between 6 % and 26 %, and sand content between 4 % and 80 %.

Regarding the energy basal level and allocation as endpoints, Gomes et al. (2011) found that these factors were differently affected in *E. albidus* exposed to a pollutant when comparing two natural standardized soils: LUFA 2.2 and Hygum. Two main reasons related to changes in soil properties were identified: alterations in the bioavailability of pollutants and/or changes in soil properties that act as stressors. Energy intake and allocation are primarily dedicated to maintaining essential body functions such as locomotion, growth, and reproductive output. However, they can also be redirected under non-optimal soil conditions, beyond the presence of pollution. Similarly, Gomes et al. (2012) demonstrated that soil properties, particularly pH and organic matter (OM), influence the gene expression profile in *E. albidus*. Exposure to low pH (pHCaCl$_2$ $\leq$4.9) affected cellular structure, regulation, and stress responses. While these findings confirm the acidophobic characteristics of these enchytraeids *E. albidus* and highlight the role of soil OM and texture in their mobility and reproductive capacity, they also emphasize the limited understanding of the overall effects of soil properties when considered holistically.

It therefore seems necessary to expand the range of soil characteristics considered and to examine the state of soil contamination in greater detail to understand how life traits respond both to contaminants and to environmental conditions. This approach will help identify cases where the influence of soil characteristics outweighs that of contaminants. Given that bioassays have primarily been conducted under high contamination concentrations and with mostly standardized soils, there is a need to explore conditions of low, sublethal diffuse pollution using natural soils that vary in physico-chemical and biological characteristics. Indeed, references are needed to assess which soil characteristics might affect enchytraeid life traits in the same way as contaminants in bioassays, especially to determine the sensitivity of life traits in the case of diffuse soil pollution. To assess risks in the context of diffuse pollution, the key is to identify confounding factors and avoid misinterpretations when using bioassays with enchytraeids and field-contaminated soils.

In this study, we worked with enchytraeids and followed their responses after exposition in conditions of field sublethal diffuse pollution with a twofold objective: 1) to identify confounding factors as soil characteristics having a greater influence than that of contaminants when present in fairly low concentrations (i.e. diffuse pollution), and 2) to hierarchize the soil parameters exerting the highest influences on the life traits in enchytraeids. For that, we evaluated the respective influence of a set of physical, chemical and biological soil properties including diffuse pollution on two important life traits in ecotoxicological bioassays, survival and reproduction, using natural in situ no or low contaminated soils. We used *E. albidus* as model species and hypothesized that in the case of diffuse pollution, e.g., mixture of low to very low sublethal concentrations of trace elements and organic contaminants, life traits are more sensitive to soil characteristics than to the presence of contaminants. *E. albidus* were exposed to various soils sampled in different locations in a territory located south-west of Paris (France) which recently underwent one part of major urban development while retaining another part for agricultural vocation. This sampling allowed obtaining a gradient in soil diffuse pollution, originating from agriculture, roads, or proximity of towns. We ensured that conditions of bioassays for *E. albidus* were respected in terms of pH, contents in OM and soil texture while having a gradient of soil types chosen from soils in rural, in urban or in peri-urban locations. Data from survival and reproduction of *E. albidus* were combined with the full soil sample descriptors to highlight causality.

2. Materials and methods

2.1. Bioassay organisms

Enchytraeus albidus Henle, 1837 (white potworm; Enchytraeidae, Oligochaeta) has a short life cycle of approximately 40–50 days. *Enchytraeus albidus* is a complex of cryptic species, notable as one of the largest in its genus, measuring between 15 and 40 mm. This species is commonly found in surface soil layers, particularly in soils rich in organic matter (Bicho et al., 2015; Kovačević, 2021). The individuals used in this study originated from a private breeding, and their species were morphologically identified in the laboratory (personal communication, Dr Joël Amossé). However, no molecular identification has been made to confirm the species, so it's "*E. albidus* complex", which we'll refer to here as *E. albidus* for simplicity. Before launching the experiment, the individuals were acclimated in a soil (previously sterilised at -80°C for 48 h) used in our laboratory for enchytraeids breeding. Acclimation was made during 1 week at 75 % of the water holding capacity (WHC) of this soil, in an incubator at 20 ± 1 °C. The soil used is a Luvisol (FAO soil classification) sampled in a permanent grassland near Versailles (SW suburb of Paris, France). This grassland has not been treated with pesticides for more than 20 years (Bart et al., 2018) and can thus be considered as a control soil. Because enchytraeids live in close contact with the soil pore water fraction, the main route of exposure to the soil was dermal contact (Serbource et al., 2024). It is therefore essential to maintain soil moisture. Thus, moisture loss was twice per week checked by weight difference and replenished by adding water. The worms were fed with ground and sterilized oatmeal as recommended in ISO 16387 (ISO, 2023).

2.2. Soil samples

The selection of sampling locations is outlined in Renaud et al. (2022). In brief, soil samples were collected in 2017 from sites located approximately 20 km southwest of Paris, France. A portion of this area is undergoing significant urban development, encroaching on agricultural lands. The territory has been used in studies to assess the changes induced by this development on surrounding contamination and biodiversity (Nélieu et al., 2021). Sampling strategy was performed in order to cover a wide range of soil characteristics together with a gradient in diffuse pollution to evaluate the *E. albidus* responses to a large heterogeneity. The soil used for breeding the enchytraeids was also included (Bart et al., 2018).

Nineteen soil samples were collected from the top 0–20 cm layer, each 20 cm-high soil column was numbered from Soil 1 (S1) to Soil 19 (S19), while the breeding soil was named S20 for identification during the study (Table 1). After sampling, the main plant parts were discarded, and the soil was sieved in situ at 5 mm to be immediately used for determining enzymatic activities. A separate portion was kept at −20°C until the analysis of organic contaminants. The remaining samples were air-dried and sieved at 2 mm. One portion was used for soil analysis, while another was frozen at −80°C ± 1°C for 48 hours to remove soil invertebrates and potential enchytraeid cocoons for the microcosm devices. The soil was kept at a controlled temperature of 21°C ± 1 for one week before starting the tests.

2.3. Soil analysis

The main physical and chemical parameters were determined by the Laboratory of Soil Analysis INRAE following French (NF) or international (ISO) norms. The following physical and chemical parameters were determined: a) Granulometry five fractions without decarbonation (AFNOR (Association française de normalisation) NF X 31–107, (2003a)); b) Cationic exchange capacity cobaltihexamine (spectrocolorimetry), (ISO 23470, 2018); c) exchangeable Ca, Mg, Na, K, Fe, Mn, Al with cobaltihexamine (ISO 23470, 2018); d) pH_{water} (ISO 10390, 2021); e) Organic Carbon (C) and total nitrogen (N) ((ISO 10694, 1995) and (ISO 13878, 1998)); f) Total (HF mineralisation) major and trace elements: Al, Ca, Fe, K, Mg, Mn, Na, P_2O_5, Cr, Cu, Ni, Zn, Co, Pb, Cd, Tl, Mo, Bi, In, Sb, Sn (by ICP-AES or ICP-MS) (AFNOR NF X 31–147, 1996); ISO 17294-2, (2023); and g) extracted trace elements with EDTA, this extraction being assimilated to a pool of available metals (AFNOR NF X 31–120, 2003b): Cd, Cu, Ni, Pb, Zn, Cr (ICP-AES). The Limit Of Quantification (LOQ) in mg/kg were as follow: Co: 1, Cr: 2, Cu: 1, Ni: 1, Zn: 5, Pb: 0.1, Cd: 0.02, Tl: 0.01, Mo: 0.04, Mn: 10 and in g/100 g Al, Ca, Fe, K, Mg, Na: 0.02.

The polycyclic aromatic hydrocarbons (PAHs) were analyzed in our laboratory according to the European standard (AFNOR NF EN 17503, 2022), by pressurized liquid extraction and HPLC-fluorescence quantification. The determined PAHs (μg/kg) corresponded to 15 of the 16 most commonly searched: five PAHs with two or three cycles (Naphthalene, Acenaphthene, Fluorene, Phenanthrene, Anthracene), four PAHs with four cycles (Fluoranthene, Pyrene, Benz[*a*]anthracene, Chrysene), four PAHs with five cycles (Benzo[*b*]fluoranthene, Benzo[*k*] fluoranthene, Benzo*[a]*pyrene, Dibenz[*a,h*]anthracene) and two PAHs with six cycles (Benzo[*ghi*]perylene, Indeno[1,2,3-*cd*] pyrene). The LOQ were all below 0.3 μg/kg except 0.5 μg/kg for Indeno[1,2,3-*cd*] pyrene.

The pesticides determined in soil samples, including herbicides, fungicides and insecticides, were selected to cover the past and present uses in the studied area (Nélieu et al., 2021): cereals, maize, rapeseed, sunflower, orchard, vegetable crops. Ultrasonic extraction, purification and concentration of the extracts by solid-phase extraction were performed before analysis by ultra-high-performance liquid chromatography coupled to a triple quadrupole mass spectrometer (UHPLC-ESI-MS/MS), with detection in MRM mode.

The enzymatic activities of phosphatase (PHOS), βglucosidase (GLU), arylsulfatase (ARS), urease (URE) and arylamidase (ARN) involved in the biochemical cycles of P, Organic Carbon (Corga), S and N respectively were analyzed by the Biochem-Env platform on fresh soil, according to the norm ISO 20130 (2018) and (de Santiago-Martín et al., 2013), and derived from the respective protocols of: (Tabatabai and Bremner, 1969) (PHOS), (Eivazi and Tabatabai, 1988) (GLU), (Tabatabai and Bremner, 1970) (ARS), (Sinsabaugh et al., 2000) (URE), and (Acosta-Martinez and Tabatabai, 2000) (ARN).

2.4. Survival and reproduction bioassays

The microcosms consisted of plastic pots of 60 mL (Ø 55 mm, h = 38 mm) with perforated lids, into which 20 g of soil (dry weight equivalent) were placed. The soils were then moistened to reach 75 % of the water holding capacity (WHC).

The reproduction bioassay was performed according to the standard ISO 16387 (2023). Ten mature individuals with well-developed clitellum were introduced in each experimental microcosm; four replicates per soil sample were carried out. Soil moisture content was checked by weighting and adjusted twice a week with tap water. For

Table 1
Main soil samples properties: pH (water), Corga (organic carbon), C/N (Carbon/nitrogen), grain size distribution (clay, silt, sand, g/kg), CEC (cation exchange capacity, cobaltihexamine cmol+/kg) and WHC (water holding capacity).

Soil sample	type of landscape	pH	Corga (g/kg)	C/N	CEC (cmol+/kg)	Clay (%)	Silt (%)	WHC (%)
S1	Peri-urban	7.5	22.7	12	16.4	20.6	58	42.9
S2	Rural	8.5	29.2	11.8	18.7	20.9	64	43.4
S3	Rural	7.6	29.8	12	16.6	19.3	44.1	48.5
S4	Rural	7.7	22.1	12.6	14.7	17.3	54.2	40.0
S5	Rural	7.5	27.8	11.4	17.2	16.2	70.6	50.3
S6	Peri-urban	6.7	24.7	11.6	14.2	18.6	65.9	46.2
S7	Rural	7.7	47	12.2	19.7	18	46.9	49.9
S8	Rural	6.2	21	10.3	15.9	33.2	63	38.8
S9	Peri-urban	7.5	33.7	13.8	14.1	17.3	64.4	45.9
S10	Peri-urban	5.9	14.5	14.5	7.67	15.8	38	35.6
S11	Peri-urban	5.9	10.9	10.7	7.84	17.2	76.8	40.1
S12	Urban	7.7	24.7	12.1	17.6	20.6	67.3	47.6
S13	Peri-urban	5.7	13	13	3.16	6.2	9.9	30.6
S14	Urban	7.7	46.6	11.9	22.9	22.7	55.3	50.8
S15	Urban	8.3	28.3	34.5	9.93	10.3	24.9	30.4
S16	Urban	8.1	23	10.3	22	28.4	62.5	44.0
S17	Urban	8.4	10.1	13.8	6.97	10.5	12.6	28.3
S18	Peri-urban	7.7	24.2	14.1	11.8	11.1	11.7	37.1
S19	Urban	8.2	5.7	15	11.1	18.5	11.6	33.2
S20	Peri-urban	7.0	20.8	13.8	15.3	18.5	52	40.7

food supply, 50 mg of finely ground oatmeal were mixed with the soil samples at the beginning of the experiment and 25 mg was then added on days seven and 14. The microcosms were stored in an incubator (20 ± 1 °C, 24 h darkness) for a period of 21 days. On the 21st day, the adults were carefully extracted using tweezers while delicately removing the surrounding soil, and their survival was recorded. The microcosms were incubated for further three more weeks (until 42nd day) under the same experimental conditions. Feeding was added once a week until the day 35 (25 mg of ground oatmeal per container). After 42 days of incubation, the juveniles were counted. To facilitate counting, the substrate was fixed with ethanol (99.8 % v/v) and coloured with 300 µL of Bengal red (1 % solution in ethanol). The following day, the substrate was washed through a 100-µm sieve, and the juveniles were counted using a stereoscope to assess the reproduction rate.

2.5. Statistical analysis

For each soil sample, the analysis of contamination ended with measures of 15 PAHs, 26 herbicides, seven fungicides, two insecticides, and 11 trace elements. We combined the PAHs data into four groups as follows: one sum of the five 2–3-cycles PAHs, one of the four 4-cycles PAHs, one of the four 5-cycles PAHs and one of the two 6-cycles PAHs. The pesticides were combined as the sum of the 26 herbicides, as the sum of the seven fungicides, and as the sum of the two insecticides analyzed. When a pesticide was detected at a concentration below LOQ, the value corresponding to half of its LOQ was considered (Farnham et al., 2002).

Statistical analysis was performed to estimate the relative importance of soil variables in the life traits on *E. albidus*. We conducted two complementary analyses, one without *a priori* for soil clustering based on all the quantitative data describing the soil characteristics, and the second one based on *a priori* qualitative pool of data (e.g. chemical data, biological data…) for assessing the most determinant ones. The first clustering consisted in a hierarchical clustering on principal components (HCPC) taking into account the quantitative soil data, while for the second type of analysis we used multiple factor analysis (MFA) to highlight the relative importance between all the types of variables in the soil's variability.

Clustering or data gathering reduced the number of bio-physico-chemical variables in order to construct analysis emphasising the dominant variables in a co-correlated panel, and allowed estimating the dominant variables to explain soil variabilities. Adult's survival numbers of *E. albidus* and reproduction rate data were analyzed after clustering. The HCPC analysis was performed both after standardization of soil description variables to a common scale, so that their respective units will not influence the results, and after consolidation of groups based on k-means. Clusters were then constructed by minimising the variance into each cluster in order to obtain a hierarchization of genealogic type between clusters. Each cluster consisted in few variables being lower/upper in the group than in the whole pool of soils. Optimal number of clusters was determined by maximising the loss of inertia between clusters (e.g max(Inertia for n + 1 cluster/Inertia for n clusters)). Significance of differences in survival and reproduction rate between clusters was determined with Kruskal-Wallis and post hoc Dunnet tests with Benjamini-Hochberg correction for multiple testing and significant level at 0.05.

The MFA analysis is a weighted PCA that allows the simultaneous comparison of qualitative variables of different types, avoiding that one group with a large number of highly correlated variables mostly influences the first axis. Each variable of each group is weighted so that these weights are identical for the variables of a group (but vary from one group to another). The maximum axial inertia of one group is equal to one. In our case, we choose to define five groups according to their consistency in describing soil sample characteristics: soil chemistry, soil biology, soil physic, and mineral (i.e trace elements) and organic contamination data. The groups of variables were as follow:

(1) Soil chemical data included pH, total major elements as Al, Ca, Fe, K, Mg, Mn, Na, P_2O_5 and $CaCO_3$, cation exchange capacity (CEC), and exchangeable major elements,
(2) Soil biological data included total organic carbon (C) and nitrogen (N), and the enzymatic activities phosphatase, βglucosidase, arylsulfatase, urease and arylamidase,
(3) Soil physical data included % in clay and silt,
(4) Soil trace element data included total concentrations of Cu, Zn, Cr, Co, Mo, Pb, Cd, Ni, Bi, Sn, Sb measured after HF mineralization and contents in EDTA extractions of Cu, Cd, Zn as a surrogate of their bioavailability,
(5) Soil organic contaminant data included the sum of PAHs per number of cycles, and the contents in herbicides, fungicides and insecticides.

These five groups were initially used to construct the MFA, i.e., to determine the dimensions of variation. Subsequently, data on *E. albidus* survival and reproduction rates were added as passive supplementary variables to the constructed MFA (e.g., illustrative variables for Enchytraeidae).

All analyses were performed by R Core Team 4.1.1 (2021) using the statistical packages factoextra (v. 1.0.7) (Kassambara and Mundt, 2020) and FactoMineR (Lê et al., 2008).

3. Results

3.1. Soil sample characteristics

The main physicochemical characteristics of the soil samples tested are summarized in Table 1. The entire set of data can be found in the dataverse (https://doi.org/10.57745/4XC0SH). The gradients of the soil sample variables covered a large span depending on the variable in question.

Chemical data exhibited a broad range, spanning approximately four pH units (4.6–8.5), from acidic to alkaline soil samples. Total calcium content and calcium carbonate ($CaCO_3$) varied significantly, by approximately 50-fold, from non-calcareous to calcareous soils (0.09–4.90 g/100 g and 2–99 g/kg, respectively). Similarly, total magnesium content showed a 60-fold variation (0.06–3.80 mg/kg), while exchangeable potassium and manganese varied by 20-fold (0.15–3.50 cmol+/kg and 0.01–0.24 cmol+/kg, respectively). Exchangeable sodium also exhibited a large variation, spanning 40-fold (0.01–0.41 cmol+/kg).

Physical data exhibited a fivefold variation in clay content, ranging from 6.2 % to 33.2 %, and an eightfold variation in silt content, ranging from 9.9 % to 76.8 %. These values correspond to soil textures ranging from sandy loam to silty clay loam, according to the FAO classification.

Soil biology data showed a threefold variation in organic matter content, ranging from 36 to 113 g/kg, with a C/N ratio varying between 10.3 and 34.5. These values indicate soil samples with either a relatively high or low rate of organic matter mineralization. The enzymatic activity data reflect the range of values typically observed in soils, influenced by factors such as land use and soil characteristics (Bandick and Dick, 1999). In the absence of established references, interpreting these biochemical data remains challenging (Deng et al., 2017). However, for any given soil sample, the values align with the primary known drivers of soil enzyme activity, such as pH, organic matter content, and soil texture.

The gradient span of trace elements was around 25 times for total contents in Cd (0.09–2.26 mg/kg), 5 times for Pb (19.8104 mg/kg), 16 times for Zn (22.4, 351 mg/kg), 11 times for Cu (4.58, 51 mg/kg), and ten times for Sb (0.44–4.5 mg/kg), an indicator of road traffic. To interpret the trace element data using these median values as thresholds, 35 % of the soil samples can be considered as slightly contaminated in Cd by diffuse pollution. In the same manner 65 % of the soil samples can be considered as slightly contaminated in Zn and while 80 % in Cu and

90 % in Pb.

Concerning the organic contamination, the contents in PAHs (sum of 15 PAHs) were very different, from 215 to 52 371 µg/kg. Among the 26 herbicides of interest, diflufenican, (S)-metolachlor, chlorotoluron, isoproturon, diuron and its metabolite dichlorophenylmethylurea (DCPMU), were the most detected, but very often at the limit of quantification. The only one being quantified in more than 50 % of the soil samples at rather high contents was diflufenican. Among the seven fungicides of interest, only two were not quantified, while epoxiconazole, hexaconazole, metconazole, tebuconazole and boscalid were detected. Tebuconazole was the most frequently observed (70 % of the soil samples) and epoxiconazole presented the highest concentration (up to 17.1 µg/kg). Between the two insecticides of interest, imidacloprid was more frequently observed (40 % of the soil samples) while pirimicarb presented the highest concentration (5.4 µg/kg).

3.2. Survival and reproduction bioassays

The survival and reproduction data for *E. albidus* exposed to the 20 soil samples are presented in Table 2. Mortality was minimal throughout the experiment, with an average survival rate of 95 % (± 4.5 %), and all observed survival rates complied with the requirements of ISO 16387 (2023) (i.e., >80 %). The lowest survival rates (86–88 %) were recorded for samples S4, S5, and S16, while the highest survival rates (99–100 %) were observed for samples S8, S9, S14, and S20.

Reproduction was calculated as the ratio of juveniles found after the second incubation period to the number of surviving adult enchytraeids counted after the first incubation period. Table 2 indicates substantial variability in reproduction among the soil samples, with a median of 11.8 (± 16, median absolute deviation) juveniles per adult and a mean of 13 (± 12) juveniles per adult. No reproduction was observed in three soil samples (S6, S10, and S11), while low reproduction rates (i.e., ratio < 2.5) were recorded in four samples (S1, S8, S13, and S16). Conversely, eight soil samples (S2, S3, S7, S9, S14, S15, S17, and S18) exhibited high reproduction rates compared to the sample (S20).

3.3. Soil clustering after HCPC analysis

All the data except reproduction and survival rate were taken into account for soil clustering. HCPC analysis resulted in 4 clusters as determined from inertia optimization. The dendrogram (Fig. 1) shows the hierarchical tree of soils and the four clusters identifying the soils with closest physicochemical and biological characteristics as being on the same side of each node. The first cluster (C1, in Fig. 1) is composed of four soil samples, S13, S17, S18, and S19. The second cluster (C2, in Fig. 1) includes the highest number in soil samples (eight): S1, S4, S6, S8, S10, S11, S16 and S20. The third cluster (C3 in Fig. 1) includes seven soil samples: S2, S3, S5, S7, S9, S14 and S12. The fourth cluster (C4 in Fig. 1) is composed of only one soil sample, S15. The significant soil physico-chemical and biological characteristics of each cluster are presented in Table 3.

C1 is characterized by the lowest mean values of several soil physico-chemical and biological parameters. Focusing on the six most important variables in the characterization of each cluster (e.g. the 6 variables with the largest mean differences considering the respective cluster and the rest of the soils) C1 includes the soil samples which combine the lowest values compared to other soils of some major and trace elements, and of some physical parameters as Silt, Na (total), Mn (total), Cr (total), P_2O_5 and Ti (total).. To lesser extent enzyme activities as phosphatase, urease or arylase and soil organic parameters (Corga, Nitrogen) are also lower than in other soils. C2, cluster corresponding to soils in which no reproduction was observed (soils S6, S10, and S11), is characterized by mineral and organic contaminant concentrations with both the highest Mn (exchangeable) content and the lowest contents in Zn (EDTA), PAH (2–3 cycles), pH, Pb (EDTA), and PAH (four cycles) compared to the other soils. Concentration in Cu (EDTA) and five and six cycles PAH are also smaller in this cluster than in the others. C4 is characterized by the highest contents in Cu (EDTA and total), N, Corga, and Sn (total) combined with glucosidase values, compared to the other soils. But in this cluster, urease and arylamidase, and soil major and trace elements are in smaller concentrations than in the other soils. C4 is characterized by the highest combined total values of some major and trace elements: Mg, Co, Zn, Ni, Mo, and Fe compared to the other soils but also to a lesser extent highest values of K, Mn, Al, Zn, Ca or Cr.

Interestingly, some variables as pesticide's concentrations, $CaCO_3$, Al exchangeable, Sb (total) or Pb (total) were not found as variables of interest to characterize soil clusters. The other data such as CEC or enzymatic activities were not put forward first in this clustering exercise.

3.4. Life traits of E. albidus versus soil clustering

Survival and reproduction rate data were tested against the four clusters by the HCPC analysis. Results show a significant effect of soil clusters in the reproduction rate (Kruskal Wallis test, p.v = 3.10^{-9}) but not in the survival rate (Kruskal Wallis test, p.v. = 0.08). Fig. 2 shows the reproduction rate (median, 1st, 3rd quartile) in each soil cluster and the results of the post hoc Dunn test. Cluster 3 has the smallest reproduction rate with 3.5 ± 5.1 (mean, sd) and 0.6 ± 1 (median, mad) juveniles/ adult. Cluster 4 has the higher reproduction rate with 22.6 ± 10.9 (mean, sd) and 26.8 ± 8.5 (median, mad) juveniles/adult but without significant differences with cluster 2 (22.1 ± 0.6 mean, sd and 21.8 ± 0.1 median, mad juveniles/adult). However, if soil clustering was found a good way to explain reproduction data in *E. albidus* among a soil series, it seems that the clusters are not discriminating enough. Indeed, we observed in our study that *E. albidus* showed no reproduction in soil samples that belong to different clusters: S6, S8, S10 and S11, from cluster 3, but also S13, from cluster 1. This suggests that to hierarchize the factors affecting the life traits, other characteristics than those assessed in the actual soil clusters have to be taken into account.

3.5. Data multi factorial analysis

The representation of the 20 soils in the MFA is given in Suppl. Fig. 1a for the dimensions 1–2 and Suppl. Fig. 1b for the dimensions 3–4. The contribution of S15 to dim. 3 largely overwhelmed the contributions of the other soils. Indeed, S15 had a coordinate around 5 while all the other soil samples have coordinates around 1 in dim. 3. The specific

Table 2
Data of survival and reproduction as expressed by the ratio of juvenile/adults for *E. albidus* exposed to the 20 soil samples. Ratio juveniles per adults are calculated based on the surviving adults.

Soil type	Survivals %	Ratio Juveniles/adults
S1	95 (± 5.7)	2.5 (± 1.3)
S2	97 (± 5)	26.7 (± 1.2)
S3	95 (± 10)	34.0 (± 4.4)
S4	90 (± 0)	8.8 (± 1.5)
S5	88 (± 15)	3.8 (± 1.4)
S6	98 (± 5)	0 (± 0)
S7	100 (± 0)	29.5 (± 5 .6)
S8	100 (± 0)	0.1 (± 0.2)
S9	100 (± 0)	22.3 (± 6.9)
S10	93 (± 5)	0 (± 0)
S11	100 (± 0)	0 (± 0.1)
S12	95 (± 10)	12.3 (± 4.8)
S13	100 (± 0)	0.1 (± 0.3)
S14	100 (± 0)	29.5 (± 1.7)
S15	100 (± 0)	22.1 (± 0.6)
S16	93 (± 5)	2.0 (± 0.7)
S17	100 (± 0)	22.9 (± 3.7)
S18	93 (± 10)	16.2 (± 4.3)
S19	100 (± 0)	12.0 (± 4.0)
S20	98 (± 5)	14.2 (± 2.1)

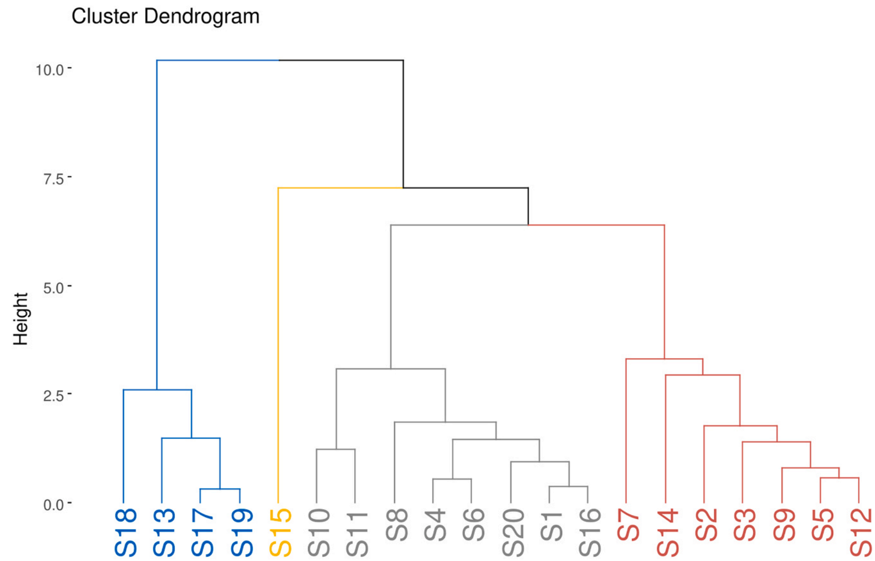

Fig. 1. Dendogram of soil clusters after hierarchical clusterisation with HCPC analysis. Soils with closest branches shared similar bio-physico-chemical characteristics. The 4 clusters defined for optimal inertia are represented with colours: C1 in blue, C2 in grey, C3 in red and C4 in yellow.

characteristics of soil S15 were already pointed out through HCPC analysis, where a cluster was designed by this unique soil sample. To assess the correlation between soil variables and enchytraeid life traits, the following section of results will thus consider a restricted MFA (further named MFAr) excluding sample S15 to avoid the weight of its specificities. Indeed, this sample with particularly high concentrations in soil trace elements and in some major ions (see Table 2, cluster 2) suggests that such parameters have an important effect but without robustness.

The representation of soils in the MFAr is given in Suppl. Fig. 2(a for dimensions 1–2 and b for dimensions 3–4), with all soils having comparable coordinates' magnitudes. Restricted multi factorial analysis explains 58.4 % of soil variances for the dim. 1–2 and 15 % for the dim. 3–4. Total variance explained by the dim. 1–4 is hence 73.4 %. The results of MFAr for groups are shown in Fig. 3a for dim.1–2 and in Fig. 3b for dim 3–4.

The dimensions 1–2 of MFAr are constructed by the similar groups of variables than MFA, with dimension 1 mostly constructed with the soil chemical, physical and biological data as well as the group of trace elements, and dimension 2 mostly constructed by organic contaminants (Fig. 3a. and Suppl. Fig. 2b.). However, compared to MFA, dimension 3 is mostly constructed by organic contaminants instead of trace elements, and dimension 4 by trace elements instead of organic contaminants and to a lesser extent by soil biology (Fig. 3b. and Suppl. Fig. 3d.). The contributions of the different variables to each dimension is presented in Suppl. Fig. 3. The contributions of the different variables summed over the 4th first dimensions are presented in Supplementary table 1.

3.6. Life traits of E. albidus versus data multifactorial analysis

The groups of variables relating to enchytraeid life traits, used here as illustrative variables, were found mostly correlated with the dim 2 and organic contaminants (Fig. 3). When looking for the variables mostly correlated with life traits as survival and reproduction rates, we noticed that enchytraeid survival rates were not well represented in the MFAr with respective $\cos^2$ of 0.01, 0.04, 9.10^{-4} and 0.01 for the dimensions 1–4 due to small variations between soils. Reproduction rates were better represented, especially in the dim.2 with respective $\cos^2$ of 0.03, 0.55, 0.03 and 0.02 for the dimensions 1–4. Thus, only the reproduction rate could be adequately analysed with MFAr. The better representation of reproduction rate being for dim 1–2 with total $\cos^2$ of 0.58, the correlations with variables will be analyzed in these dimensions and not in dimensions 3–4.

Fig. 4a represents the MFAr results for variables with acceptable quality of representation ($\cos^2 > 0.25$) for the dim. 1–2. The same results for dim. 3–4 are presented in Fig. 4b. In dim 1–2, reproduction rate is highly positively correlated with $CaCO_3$, exchangeable Na, pH, total Molybdenum and insecticides as these vectors are well grouped, and negatively correlated with exchangeable Mn and Al. To a lesser extent, reproduction rate is also correlated with PAH, glucosidase, Corga, nitrogen, urease, arylamidase that are in the 12 most important contributors of these dimensions (Supplementary table 1) and move in the same direction than reproduction rate (Fig. 4). Based on the dim 1–2 of MFAr, the reproduction rate is independent of total Mg, total Na, arylsulfatase, total Mn, total Ni, total Fe and silt that are well represented in these dimensions but orthogonal to reproduction rate (Fig. 4).

4. Discussion

4.1. Potential impact of diffuse contamination in E. albidus

Based on the trace element contents of the 20 soil samples, the contamination can be characterized as diffuse, ranging from very low to low, and sublethal. Despite the low concentrations of trace elements, some soil samples contained metal levels previously reported as toxic to *E. albidus* (Lock and Janssen, 2002; Novais et al., 2011). These toxic thresholds were Cu > 50 mg/kg, Pb > 55 mg/kg, and Zn > 180 mg/kg for survival, and Zn > 32 mg/kg for reproduction. Notably, copper exceeded the threshold in one sample (S5: 51 mg/kg), while lead exceeded the threshold in four samples (S3: 60.1 mg/kg; S11: 75.1 mg/kg; S12: 83.5 mg/kg; and S18: 104 mg/kg). Except for S13, all soil samples had zinc levels exceeding 32 mg/kg, ranging from 34.1 mg/kg (S10) to 351 mg/kg (S15). However, these trace element concentrations showed inconsistent associations with reproduction rates (Table 2), suggesting no direct relationship between diffuse trace element contamination and reproduction.

The PAH concentrations found in the present study are in the classical range found in peri-urban soils (Gaspéri et al., 2018; Vecchiato et al., 2021). Even if their bioavailability is low, PAHs were demonstrated in previous studies to exert a toxic effect on soil invertebrates as earthworms, springtails or enchytraeids, considering their survival or biomass, at thresholds (LC50, EC50) higher than 39 mg/kg (Kobetičová et al., 2011; Sverdrup et al., 2002). But in the studied soils none of the PAHs reached more than 12 mg/kg, each one being specifically at least

Table 3
Soils cluster characteristics. Mean in cluster is the mean of each variable in the cluster, overall mean is the mean of each variable is the 4 clusters, sd in cluster is the standard deviation of each variable in the cluster, overall sd is the standard deviation over the 4 clusters, v.test is a measure of association between variables so that variables with higher mean in the cluster that in overall soils have positive v.test values and variables with lower mean in the cluster than in the overall soils have negative v.test value. Relative values for ranking of variables within and between cluster should be used rather than absolute v.test values. P. values correspond to the test for significant difference in variable mean in cluster compared to the overall soils. NA= Not Applicable (when the cluster has only one soil, so there is no SD for the variable in the cluster).

	Mean in cluster	Overall mean	sd in cluster	Overall sd	v.test	p.value
Cluster 1						
Ca EE	8.1	4.5	12.9	5.4	−1.97	0.05
Cu total	12.9	5.9	23.2	11.7	−1.98	0.05
Urease	7.0	3.5	12.6	6.1	−2.03	0.04
Arylase	3.8	2.0	7.9	4.4	−2.09	0.04
Sn total	1.9	0.6	3.3	1.5	−2.10	0.04
Phosphatase	25.3	11.8	44.1	19.3	−2.17	0.03
Nitrogen	1.0	0.6	1.9	0.9	−2.23	0.03
Corga	13.3	7.9	24.0	10.7	−2.25	0.02
Ni total	8.6	3.8	17.6	8.5	−2.35	0.02
Co total	3.3	1.2	7.3	3.8	−2.36	0.02
Clay	115.8	51.1	180.6	60.6	−2.39	0.02
K total	0.9	0.4	1.3	0.4	−2.41	0.02
CEC	8.3	4.0	14.2	5.2	−2.55	0.01
Fe total	1.0	0.3	1.9	0.8	−2.57	0.01
Ni EDTA	0.6	0.3	1.3	0.6	−2.63	0.01
Bi total	0.1	0.0	0.2	0.1	−2.68	0.01
Mg EE	0.4	0.1	0.9	0.4	−2.95	3.21E−03
Al total	2.2	0.8	3.6	1.1	−2.96	3.07E−03
Tl total	0.3	0.1	0.4	0.1	−2.97	2.97E−03
P2O5 total	0.1	0.0	0.2	0.1	−3.13	1.77E−03
Cr total	23.9	5.6	45.3	14.3	−3.35	7.98E−04
Mn total	163.8	13.7	415.6	163.4	−3.45	5.67E−04
Na total	0.2	0.0	0.5	0.2	−3.62	2.92E−04
Silt	114.5	11.3	476.9	220.0	−3.68	2.31E−04
Cluster 2						
Mn EE	0.1	0.1	0.0	0.1	2.19	0.03
6 PAH	445.2	710.0	1800.5	2523.2	−1.96	0.05
5 PAH	593.3	960.4	2690.0	3838.4	−1.99	0.05
Cu EDTA	6.3	3.2	9.5	5.9	−2.00	0.05
4 PAH	955.3	1513.7	5556.9	8212.4	−2.05	0.04
Pb EDTA	9.5	4.3	18.8	16.2	−2.10	0.04
pH	6.9	0.9	7.4	0.9	−2.10	0.04
2/3 PAH	137.0	204.1	977.3	1429.7	−2.15	0.03
Cluster 3						
Zn EDTA	5.0	2.7	17.6	18.0	−2.56	0.01
Cu EDTA	15.5	5.3	9.5	5.9	3.34	8.50E−04
Cu total	34.6	9.7	23.2	11.7	3.19	1.42E−03
Nitrogen	2.8	0.8	1.9	0.9	3.19	1.45E−03
Corga	34.1	9.1	24.0	10.7	3.11	1.86E−03
Sn total	4.7	1.4	3.3	1.5	3.02	2.56E−03
Glucosidase	36.4	15.4	23.7	14.2	2.92	3.45E−03
Urease	18.0	4.7	12.6	6.1	2.87	4.08E−03
Ca EE	17.0	3.3	12.9	5.4	2.53	0.01
CEC	18.1	2.7	14.2	5.2	2.48	0.01
Zn EDTA	31.1	14.4	17.6	18.0	2.46	0.01
Arylamidase	22.0	7.1	15.7	8.8	2.36	0.02
P2O5 total	0.2	0.1	0.2	0.1	2.36	0.02
Bi total	0.2	0.1	0.2	0.1	2.34	0.02
Na EE	0.2	0.1	0.1	0.1	2.17	0.03
Cd EDTA	0.5	0.5	0.3	0.3	2.08	0.04
K EE	1.3	1.1	0.8	0.7	2.05	0.04
Cd total	0.7	0.7	0.4	0.5	2.04	0.04
Cluster 4						
Mg total	3.8	NA	0.4	0.8	4.32	1.53E−05
Co total	20.9	NA	7.3	3.8	3.69	2.26E−04
Zn total	351.0	NA	92.8	74.5	3.55	3.81E−04
Ni total	46.5	NA	17.6	8.5	3.48	5.07E−04
Mo total	1.8	NA	0.7	0.3	3.35	8.01E−04
Fe total	4.5	NA	1.9	0.8	3.32	9.08E−04
K total	2.3	NA	1.3	0.4	2.82	4.80E−03
Cr EDTA	0.5	NA	0.2	0.1	2.76	5.79E−03
Mn total	812.0	NA	415.6	163.4	2.49	1.28E−02
Zn EDTA	61.1	NA	17.6	18.0	2.48	1.32E−02
Al total	6.2	NA	3.6	1.1	2.36	1.82E−02
Ca total	4.4	NA	1.3	1.4	2.31	2.10E−02
Cr total	75.7	NA	45.3	14.3	2.18	2.93E−02

6.5 folds below this threshold value. The absence of evidence of PAH effects on survival is consistent with such results. However, considering the 10 % reduction of reproduction (EC10), the thresholds determined by (Sverdrup et al., 2002) are lower. The comparison with the PAH concentrations in the studied soils allows to point out a same order of magnitude for pyrene (a 4 cycles PAH) at 10 mg/kg, with the observed concentrations in soils S18, S7, S9 and S3 (8.1, 6.4, 5.4 and 4.1 mg/kg respectively). However, all except S18 were included in the C3 cluster, the one where enchytraeids reproduction was the least impacted in the present study.

The main pesticides observed in this study are globally the most frequently found in European cultivated areas (Panico et al., 2022; Pelosi et al., 2021; Silva et al., 2019). In particular, the herbicides diflufenican and (S)-metolachlor, the insecticide imidacloprid and the fungicides epoxiconazole, tebuconazole and boscalid were, as herein, found among the major ones considering both their frequency and concentration (Panico et al., 2022; Pelosi et al., 2021). Notably, as a trace from previous usages, the DCPMU, a degradation product issuing from the banned diuron and linuron, also appeared as major in the present study. The azole fungicides and imidacloprid were previously pointed out in field mixtures as contributing to a risk for soil invertebrates (Panico et al., 2022). In laboratory, the no effect concentration (NOEC) of imidacloprid on *E. crypticus* reproduction was estimated as 1000 μg/kg (de Lima e Silva et al., 2017), far above the maximal concentration of 4.13 μg/kg found in the present study. In the same way, even if enchytraeids are known to be sensitive to fungicides (Frampton et al., 2006), the concentration of each individual compound, and even their mixture, were certainly too low to exert an effect.

4.2. Complementary insights of the two statistical approaches

Soil clustering using HCPC analysis was found a good way to differentiate pools of soils in function of the reproduction rate of *E. albidus*. Cluster 3 has the smallest reproduction rates (Figs. 1 and 2) while bringing together a pool of soils with the lowest contents in contaminants as Zn, Cu and Pb (available i.e. EDTA extractable) and PAHs. This suggests that the low reproduction rates were not linked with the contaminants. Here, the explaining parameters were the high contents in exchangeable Mn combined with the low pH values of this cluster (Table 3). In the same manner, the intermediate cluster C1 with low reproduction rates was also characterized by mean lowest values of several soil physico-chemical and biological parameters also excluding contaminants. The low values concern Corga, N, P and the three enzymatic activities: urease, arylamidase, and phosphatase linked to the biogeochemical cycles of N and P. Contrarily cluster 4 has the highest reproduction rate with cluster 2. C4 is characterized by the highest values in total trace elements (Zn, Cu, Bi and Sn as micronutrient and Cd as contaminant), in CEC and in exchangeable cations (Ca, Na and K) as well as in biological parameters total N and Corga and the enzymatic activities linked to their biogeochemical cycles urease, glucosidase, and arylamidase. C2 is also characterized by the highest combined total values of some major and trace micronutrients but without biological parameters. Compared to clusters 3 and 1, it can be roughly concluded that the higher the soil content in major and trace elements is within the limits of this study, the higher the reproduction rates will be. This

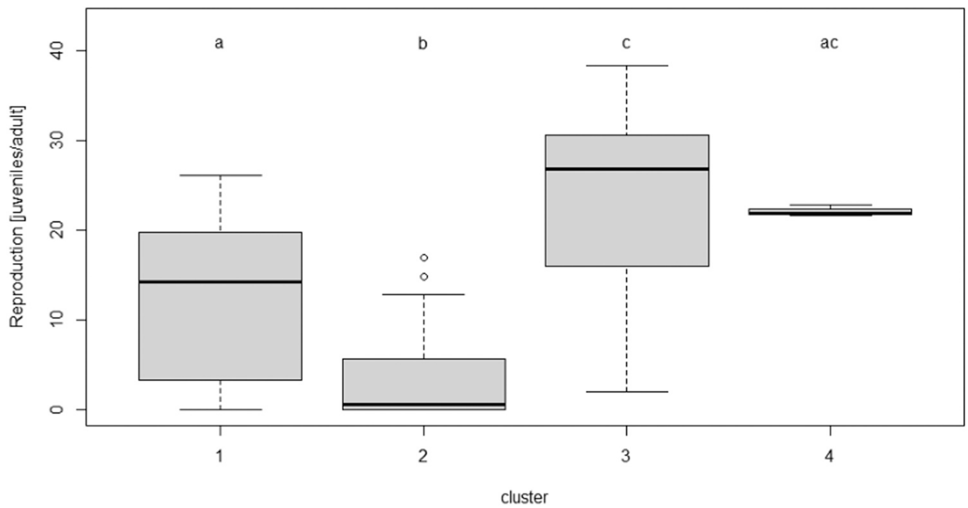

Fig. 2. Box plot of number of juveniles per adult per soil cluster as identified with the HCPC analysis. For each cluster the minimal, first quartile, median, third quartile and maximal number of juveniles/adult are represented. Letters on the top of the box plot refer to the results of post hoc Dunn test with significant threshold at 0.05. Clusters sharing a same letter have no significant differences in the juveniles/adult ratio.

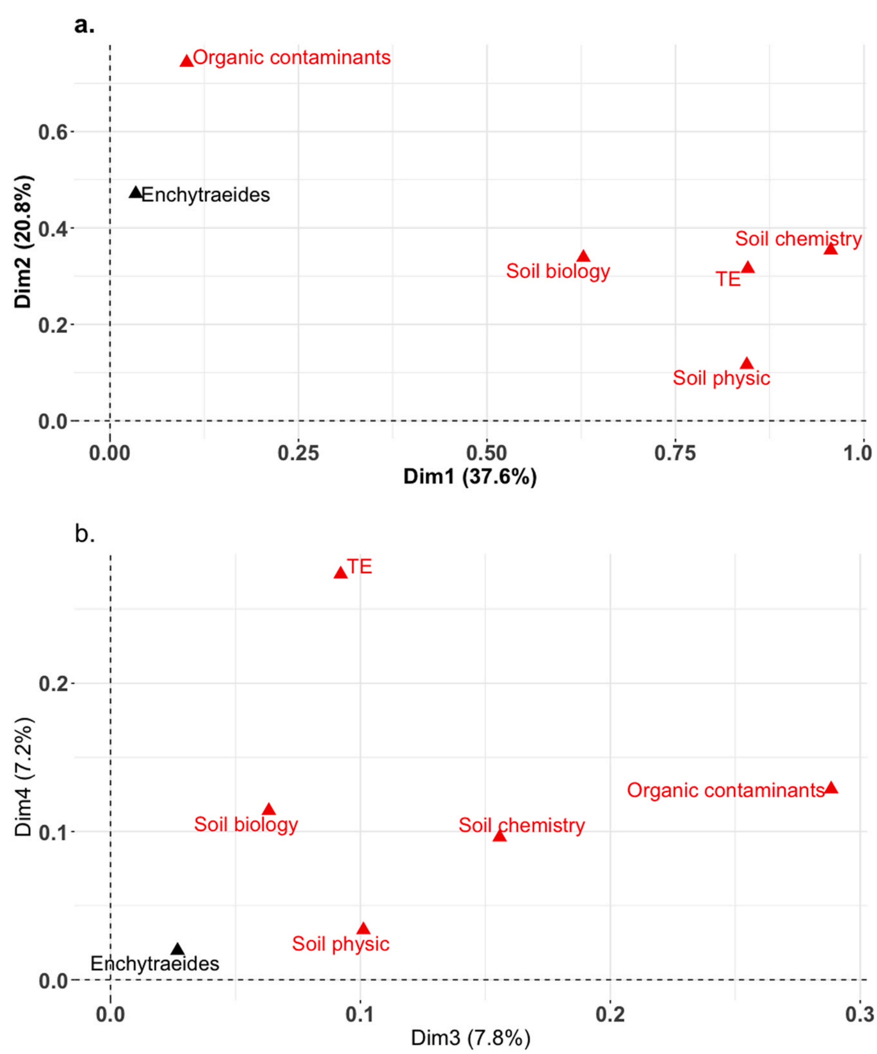

Fig. 3. MFA_r contribution of variables' families to dimension 1–2 (a) and dimension 3–4 (b). Families in red were actively used to construct MFA while families in black are illustrative.

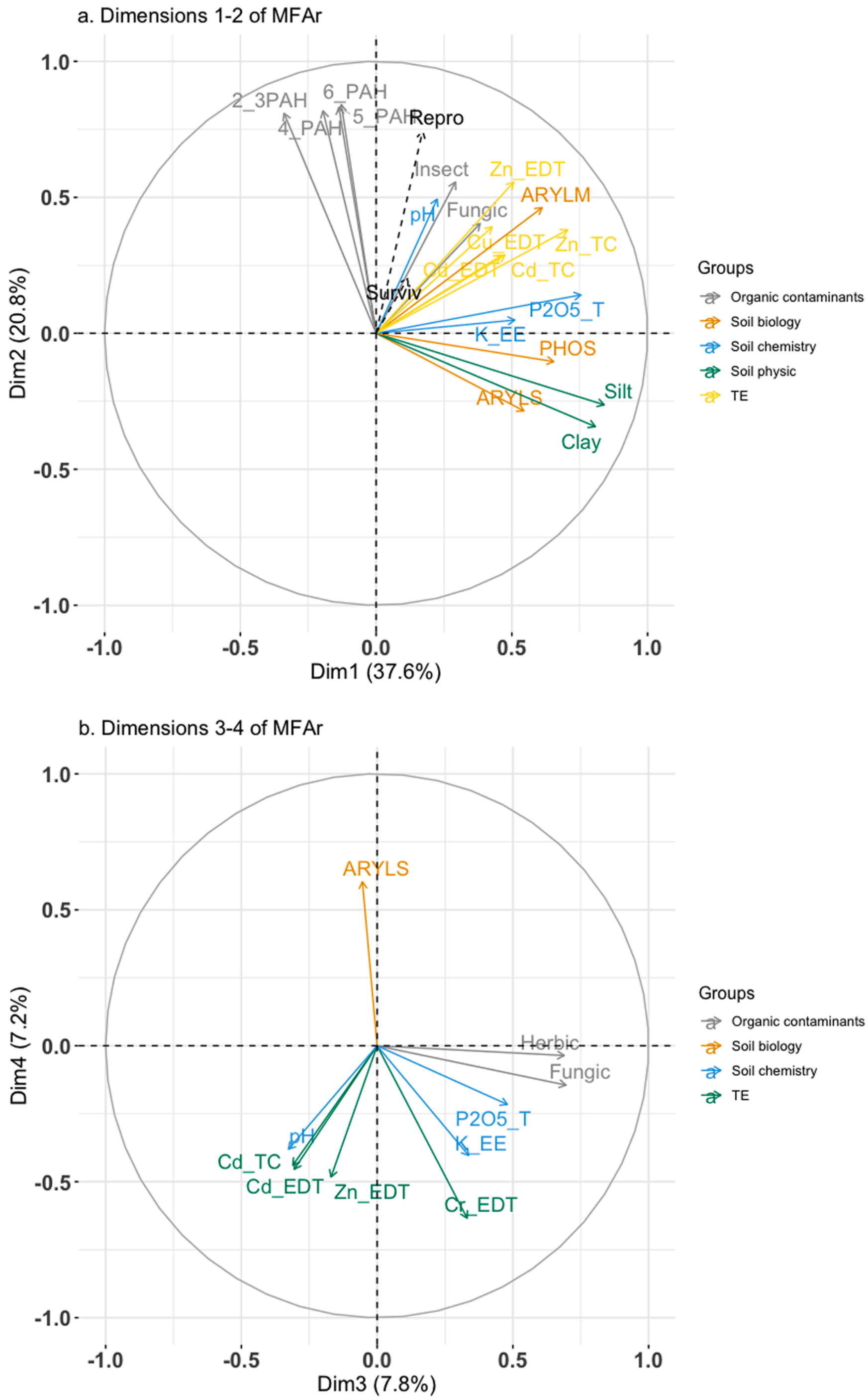

Fig. 4. Variables in the dimensions 1–2 and 3–4 of the MFA_r. Variables that contributed more than the equally distribute contributions to the dimensions and with $cos^2 > 0.25$ are represented. a. Representation of variables in dimension 1–2 b. Representation of variables in dimension 3–4. Colored variables were used for the construction of MFA with color according to their family of variable: dark blue is for biology, yellow for heavy metals, grey for organic contaminants, red for soil chemistry and light blue for soil physic. For *Enchytraeidae* end points (survival and reproduction rate), only reproduction rate has $cos^2 > 0.25$ and is represented in black. Nitrog is for soil nitrogen, URE is for urease, GLUC is for glucosidase, PHOS is for phosphatase, ARYLS is for arylase, ARYLM is for arylamidase, insec is for insecticides, fungic is for fungicides, herbic is for herbicides, 2/3, 4, 5, 6 PAH is for PAH of respectively 2/3, 4, 5 and 6 cycles, elements with suffixes EE are exchangeable, elements with suffixes EDT are with EDTA extraction and elements with suffixes TC are in total contents, Repro is for reproduction of *Enchytraeidae.*

includes the contents in contaminants when in conditions of diffuse contamination.

In a complementary way, soil data analysis with MFA was found as a good means to identify the pools of variables mostly correlated with reproduction rates of *E albidus*. The negative correlation with exchangeable Mn is consistent with the HCPC analysis, and the high correlation found with $CaCO_3$, pH, exchangeable Na, total Mo and insecticides pointed out on these variables. To a lesser extent reproduction rate was also correlated with PAHs, and biological data as Corga, nitrogen, and the associated enzyme activities. The positive correlation of reproduction rates with some contaminants at low doses could be due to hormesis, a process which had gained a renewed interest (Sebastiano et al., 2022). Interestingly, reproduction rates were found to be independent of the total content of certain elements but correlated with their exchangeable or bioavailable fractions. This finding aligns with the concept of bioavailability, which posits that only a portion of a substance's total content is accessible to organisms, referred to as its bioavailable fraction (Harmsen, 2007).

4.3. Hierarchization of soil parameters affecting life traits in E. albidus in case of diffuse pollution

Based on our soil data set and following literature recommendations, soil parameters such as pH, organic matter content, and texture (representing chemical, biological, and physical soil properties, respectively) were within the tolerance range for *E. albidus*. However, in addition to pH, we identified other chemical parameters influencing the reproduction of *E. albidus*. More generally, our findings suggest that the reproduction rate of *E. albidus* is better explained by a combination of factors rather than by a single parameter. Specifically, a positive relationship was observed with $CaCO_3$ and exchangeable sodium, while a negative relationship was found with exchangeable manganese and aluminum.

The pH value was not the primary parameter explaining our dataset, despite its established role in inducing stress responses in *E. albidus* under low soil pH conditions (Gomes et al., 2011). No reproduction was observed in three soil samples with pH values between 5.9 and 6.7, and very low reproduction rates were recorded in five soil samples with pH ranging from 5.7 to 8.1. These findings challenge the commonly accepted pH threshold of 5 for *E. albidus* reproduction, suggesting that other soil parameters, either independently or in combination with pH, may influence *E. albidus* stress responses. Interestingly, exchangeable sodium emerged as a key parameter influencing the reproduction rate of *E. albidus*. In our soil samples, exchangeable sodium concentrations ranged from 0.013 cmol+ /kg to 0.414 cmol+ /kg, with the highest concentration correlating with the highest reproduction rate. *E. albidus* is known to inhabit intertidal ecosystems, where it demonstrates high tolerance to saline conditions (Didden and Römbke, 2001). It is also found in colder regions, such as Arctic areas, where Silva et al. (2013) proposed that its resistance to low temperatures is partly mediated by internal salinity adjustments. Their study showed that individuals exposed to saline soil conditions exhibited greater resistance to low temperatures and that soil salinity up to 30 ‰ enhanced reproduction by approximately tenfold. Nevertheless, in soil sample S15, where the exchangeable sodium value was low (0.04 cmol+/kg) but the soil pH was 8.28, reproduction was still optimal. This suggests that favorable reproduction in *E. albidus* can occur under specific combinations of soil parameters, even when one key factor, such as exchangeable sodium, is low.

Limestone ($CaCO_3$), with values ranging broadly from < 1 g/kg to 99 g/kg, was positively correlated with reproduction rates. Soil samples with $CaCO_3$ content below 1 g/kg and pH < 7 did not show reproduction, while the soil sample S20, which had a $CaCO_3$ content < 1 g/kg but a pH = 7, exhibited no adverse impact on reproduction. These findings did not establish a direct relationship between $CaCO_3$ and reproduction rates but rather implied that multiple interacting factors influenced the outcomes. Molybdenum, a trace element, also displayed a positive correlation with reproduction rates. This element is naturally present in soils, with concentrations influenced by the pedogeochemical background, typically ranging from 0.2 mg/kg in sandstone-derived soils to 7 mg/kg in basaltic igneous soils (He et al., 2005). In the analyzed soil samples, molybdenum concentrations spanned from 0.3 mg/kg to 1.8 mg/kg, within the expected natural range for this trace element. Notably, higher reproduction rates were observed in soil samples with elevated molybdenum levels, particularly in cluster 4. The contribution of molybdenum may be attributed to its role as an essential element involved in various biochemical processes in microorganisms, plants, and animals (Williams and Fraústo da Silva, 2002), suggesting that soil organisms can derive benefits from it under favorable conditions.

Total manganese, a major soil element, did not show a direct relationship with reproduction rates; however, its exchangeable forms were negatively correlated. As soil pH decreases, the concentration of exchangeable manganese in the soil solution increases. In French soils, exchangeable manganese typically ranges from 0 to 2.11 cmol+ /kg (Antoni et al., 2013). In this study, exchangeable Mn concentrations were below 0.10 cmol+ /kg in all soil samples except for S10 (0.24 cmol+/kg) and S11 (0.13 cmol+/kg). These samples, both with a pH of 5.8, did not present reproduction. Kuperman et al. (2004) reported that increasing total Mn concentrations negatively impacted survival and reproduction rates in *E. crypticus*, a finding that contrasts with the current study. Here, manganese availability, rather than its total concentration, appeared to be a critical factor influencing reproduction. Although manganese toxicity, particularly in plants, is well documented, the physiological effects of excess manganese on soil fauna indicators, including enchytraeid life traits, remain underexplored. This is particularly relevant given the significant reserves of manganese in soils, which become more bioavailable as pH decreases. Similarly, total aluminum content did not impact reproduction, while exchangeable aluminum showed a notable trend. In soil samples with exchangeable Al concentrations exceeding 0.1 cmol+ /kg—specifically S10 (0.13 cmol+/kg), S11 (0.23 cmol+/kg), and S13 (0.17 cmol+/kg)—*E. albidus* failed to reproduce. This trend mirrored the behavior of manganese, where a pH < 6 increased metal availability, adversely affecting life traits. Soil acidification mobilizes toxic aluminum ions, which can disrupt numerous metabolic pathways in soil organisms (Barabasz and Albi, 2002).

5. Conclusion

Our study was designed to analyse the effects of a wide range of field soil characteristics including low diffuse contamination on *Enchytraeus albidus* life traits. The 20 soils used had values in the range of those known to be optimal for reproduction as soil pH and texture, and organic matter contents. Contrary to survival, the reproduction of *E. albidus* as end-point allowed observing an effect of soil characteristics. In our conditions, we found a previously unrecorded effect of the presence of major elements which were positively correlated with the reproduction rates of *E. albidus* as $CaCO_3$, exchangeable sodium and pH, but negatively correlated with exchangeable manganese and aluminium. The fact that exchangeable element contents but not total element contents were involved in this biological response, confirms the importance of element bioavailability in the process of exposure. Total contents in diffuse contaminants (mixture of contaminants at very low, sublethal doses) did not affect, even poorly, the reproduction rate. On the contrary, their positive correlation with the reproduction rate is consistent with the status of micronutrients of some trace elements and with the hormetic effect known at low concentrations. For future *E. albidus* bioassay studies, we recommend better describing the natural soil used with parameters that go beyond conventional characteristics. Depending on knowledge of soil history and objectives, these data could explain unexpected results as ours where, in certain soil exposures, the reproduction rate exceeded that indicated in the standard norm.

CRediT authorship contribution statement

Nélieu Sylvie: Writing – review & editing, Validation, Investigation, Formal analysis. **Cheviron Nathalie:** Validation, Investigation. **Chacon Hurtado Andrea:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation. **Faburé Juliette:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Sereni Laura:** Writing – review & editing, Investigation, Formal analysis. **Baudry Emmanuelle:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Lamy Isabelle:** Writing – review & editing, Funding acquisition. **Delarue Ghislaine:** Validation, Investigation, Formal analysis.

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.

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The authors dedicate this article to the memory of Isabelle Lamy, our late colleague.

Supplementary material

The supplementary material contains a table with the total contribution of variables to the dimensions 1–4 of MFAr. Three supplementary figures present the 20 soils in the MFA (suppl. Fig. 1), the 19 soils in the MFAr (suppl. Fig. 2) and the contribution of variables to dimensions 1–4 of MFAr (suppl. Fig. 3)

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2025.117986.

Data availability

Data will be made available on request.

References

Acosta-Martinez, V., Tabatabai, M.A., 2000. Arylamidase activity of soils. Soil Sci. Soc. Am. J. 64, 215–221. https://doi.org/10.2136/sssaj2000.641215x.

AFNOR (Association française de normalisation), 1996. NF X31-147. Qualité des sols - Sols, sédiments - Mise en solution totale par attaque acide.

AFNOR (Association française de normalisation), 2003a. NF X31-107. Qualité du sol - Détermination de la distribution granulométrique des particules du sol - Méthode à la pipette.

AFNOR (Association française de normalisation), 2003b. NF X31-120. Afnor Ed. URL ⟨https://www.boutique.afnor.org/fr-fr/norme/nf-x31120/qualite-des-sols-determination-du-cuivre-du-fer-du-manganese-et-du-zinc-ext/fa123335/21412⟩ (accessed 3.25.24).

AFNOR (Association française de normalisation), 2022. NF EN 17503. Soil, sludge, treated biowaste and waste - Determination of polycyclic aromatic hydrocarbons (PAH) by gas chromatography (GC) and high performance liquid chromatography (HPLC).

Amorim, M.J.B., Römbke, J., Soares, A.M.V.M., 2005. Avoidance behaviour of *Enchytraeus albidus*: effects of benomyl, carbendazim, phenmedipham and different soil types. Chemosphere 59, 501–510. https://doi.org/10.1016/j.chemosphere.2005.01.057.

Antoni, V., Arrouays, D., Bispo, A., Brossard, M., Le Bas, C., Stengel, P., Villanneau, E., Baize, D., Barriuso, E., Blanca, Y., Boulonne, L., Briand, O., Cabidoche, Y.-M., Caria, G., Chéry, P., Cluzeau, D., Cousin, I., Couturier, A., Decaëns, T., Walter, C., 2013. The state of the soils in France in 2011 - A synthesis.

Bandick, A.K., Dick, R.P., 1999. Field management effects on soil enzyme activities. Soil Biol. Biochem. 31, 1471–1479. https://doi.org/10.1016/S0038-0717(99)00051-6.

Barabasz, W., Albi, D., 2002. Ecotoxicology of aluminium. Pol. J. Environ. Stud. 11, 199–203.

Bart, S., Roudine, S., Amossé, J., Mougin, C., Péry, A.R.R., Pelosi, C., 2018. How to assess the feeding activity in ecotoxicological laboratory tests using enchytraeids. Environ. Sci. Pollut. Res. 25, 33844–33848. https://doi.org/10.1007/s11356-018-1701-3.

Bicho, R.C., Santos, F.C.F., Gonçalves, M.F.M., Soares, A.M.V.M., Amorim, M.J.B., 2015. Enchytraeid Reproduction TestPLUS: hatching, growth and full life cycle test—an optional multi-endpoint test with Enchytraeus crypticus. Ecotoxicology 24, 1053–1063. https://doi.org/10.1007/s10646-015-1445-5.

Deng, S., Dick, R., Freeman, C., Kandeler, E., Weintraub, M.N., 2017. Comparison and standardization of soil enzyme assay for meaningful data interpretation. J. Microbiol. Methods 133, 32–34. https://doi.org/10.1016/j.mimet.2016.12.013.

Didden, W., Römbke, J., 2001. Enchytraeids as indicator organisms for chemical stress in terrestrial ecosystems. Ecotoxicol. Environ. Saf. 50, 25–43. https://doi.org/10.1006/eesa.2001.2075.

Eivazi, F., Tabatabai, M.A., 1988. Glucosidases and galactosidases in soils. Soil Biol. Biochem. 20, 601–606. https://doi.org/10.1016/0038-0717(88)90141-1.

Farnham, I.M., Singh, A.K., Stetzenbach, K.J., Johannesson, K.H., 2002. Treatment of nondetects in multivariate analysis of groundwater geochemistry data. Chemom. Intell. Lab. Syst. 60, 265–281. https://doi.org/10.1016/S0169-7439(01)00201-5.

Frampton, G.K., Jänsch, S., Scott-Fordsmand, J.J., Römbke, J., van den Brink, P.J., 2006. Effects of pesticides on soil invertebrates in laboratory studies: a review and analysis using species sensitivity distributions. Environ. Toxicol. Chem. 25, 2480–2489. https://doi.org/10.1897/05-438R.1.

Gaspéri, J., Ayrault, S., Moreau-Guigon, E., Alliot, F., Labadie, P., Budzinski, H., Blanchard, M., Muresan, B., Caupos, E., Cladière, M., Gateuille, D., Tassin, B., Bordier, L., Teil, M.-J., Bourges, C., Desportes, A., Chevreuil, M., Moilleron, R., 2018. Contamination of soils by metals and organic micropollutants: case study of the Parisian conurbation. Environ. Sci. Pollut. Res. 25, 23559–23573. https://doi.org/10.1007/s11356-016-8005-2.

Gomes, S.I.L., Novais, S.C., Scott-Fordsmand, J.J., De Coen, W., Soares, A.M.V.M., Amorim, M.J.B., 2012. Effect of Cu-nanoparticles versus Cu-salt in Enchytraeus albidus (Oligochaeta): differential gene expression through microarray analysis. Comp. Biochem. Physiol. Toxicol. Pharmacol. CBP 155, 219–227. https://doi.org/10.1016/j.cbpc.2011.08.008.

Gomes, S.I.L., Novais, S.C., Soares, A.M.V.M., Amorim, M.J.B., 2011. Effects of soil properties and time of exposure on gene expression of *Enchytraeus albidus* (Oligochaeta). Soil Biol. Biochem. 43, 2078–2084. https://doi.org/10.1016/j.soilbio.2011.06.006.

Gomes, S.I.L., Scott-Fordsmand, J.J., Amorim, M.J.B., 2015. Cellular energy allocation to assess the impact of nanomaterials on soil invertebrates (enchytraeids): the effect of Cu and Ag. Int. J. Environ. Res. Public. Health 12, 6858–6878. https://doi.org/10.3390/ijerph120606858.

Grassi, G., Lamy, I., Pucheux, N., Ferrari, B.J.D., Faburé, J., 2022. State of the art of Triad-based ecological risk assessment: current limitations and needed implementations in the case of soil diffuse contamination. Front. Environ. Sci. 10, 878238. https://doi.org/10.3389/fenvs.2022.878238.

Harmsen, J., 2007. Measuring bioavailability: from a scientific approach to standard methods. J. Environ. Qual. 36, 1420–1428. https://doi.org/10.2134/jeq2006.0492.

He, Z.L., Yang, X.E., Stoffella, P.J., 2005. Trace elements in agroecosystems and impacts on the environment. J. Trace Elem. Med. Biol. 19, 125–140. https://doi.org/10.1016/j.jtemb.2005.02.010.

ISO (International Organization for Standardization), 1995. ISO 10694. Soil quality — Determination of organic and total carbon after dry combustion (elementary analysis).

ISO (International Organization for Standardization), 1998. ISO 13878. Soil quality — Determination of total nitrogen content by dry combustion ("elemental analysis").

ISO (International Organization for Standardization)., 2018. ISO 23470. Soil quality – Determination of effective cation exchange capacity (CEC) and exchangeable cations using a hexamminecobalt(III)chloride solution.

ISO (International Organization for Standardization), 2018. ISO 20130. Soil quality – Measurement of enzyme activity patterns in soil samples using colorimetric substrates in micro-well plates.

ISO (International Organization for Standardization), 2021. ISO 10390. Soil, treated biowaste and sludge – Determination of pH.

ISO (International Organization for Standardization)., 2023. ISO 16387. Soil quality — Effects of contaminants on Enchytraeidae (Enchytraeus sp.) — Determination of effects on reproduction.

ISO (International Organization for Standardization), 2023. ISO 16387. Soil quality — Effects of contaminants on Enchytraeidae (Enchytraeus sp.) — Determination of effects on reproduction.

ISO (International Organization for Standardization), 2023. ISO 17294-2. Water quality — Application of inductively coupled plasma mass spectrometry (ICP-MS).

Kassambara, A., Mundt, F., 2020. Factoextra: extract and visualize the results of multivariate data analyses. R. Package Version 1 (0), 7.

Kobetičová, K., Hofman, J., Holoubek, I., 2009. Avoidance response of *Enchytraeus albidus* in relation to carbendazim ageing. Environ. Pollut. 157, 704–706. https://doi.org/10.1016/j.envpol.2008.09.032.

Kobetičová, K., Šimek, Z., Brezovský, J., Hofman, J., 2011. Toxic effects of nine polycyclic aromatic compounds on *Enchytraeus crypticus* in artificial soil in relation to their properties. Ecotoxicol. Environ. Saf. 74, 1727–1733. https://doi.org/10.1016/j.ecoenv.2011.04.013.

Kovačević, M., Hackenberger, D.K., Lončarić, Ž., Hackenberger, B.K., 2021. Measurement of multixenobiotic resistance activity in enchytraeids as a tool in soil ecotoxicology. Chemosphere 279, 130549. https://doi.org/10.1016/j.pestbp.2022.105198.

Kuperman, R.G., Amorim, M.J.B., Römbke, J., Lanno, R., Checkai, R.T., Dodard, S.G., Sunahara, G.I., Scheffczyk, A., 2006. Adaptation of the enchytraeid toxicity test for use with natural soil types. Eur. J. Soil Biol., ICSZ 42, S234–S243. https://doi.org/10.1016/j.ejsobi.2006.07.028.

Kuperman, R.G., Checkai, R.T., Simini, M., Phillips, C.T., 2004. Manganese toxicity in soil for *Eisenia fetida*, *Enchytraeus crypticus* (Oligochaeta), and *Folsomia candida* (Collembola). Ecotoxicol. Environ. Saf. Ecol., Physiol. Physiochemical Factors Earthworm Ecotoxicol. Third Int. Workshop Earthworm Ecotoxicol. 57, 48–53. https://doi.org/10.1016/j.ecoenv.2003.08.010.

Lê, S., Josse, J., Husson, F., 2008. FactoMineR: an R package for multivariate analysis. J. Stat. Softw. 25, 1–18. https://doi.org/10.18637/jss.v025.i01.

de Lima e Silva, C., Brennan, N., Brouwer, J.M., Commandeur, D., Verweij, R.A., van Gestel, C.A.M., 2017. Comparative toxicity of imidacloprid and thiacloprid to different species of soil invertebrates. Ecotoxicology 26, 555–564. https://doi.org/10.1007/s10646-017-1790-7.

Lock, K., Janssen, C.R., 2002. Multi-generation toxicity of zinc, cadmium, copper and lead to the potworm *Enchytraeus albidus*. Environ. Pollut. 117, 89–92. https://doi.org/10.1016/S0269-7491(01)00156-7.

Lock, K., Janssen, C.R., De Coen, W.M., 2000. Multivariate test designs to assess the influence of zinc and cadmium bioavailability in soils on the toxicity to Enchytraeus albidus. Environ. Toxicol. Chem. 19 (11), 2666–2671. https://doi.org/10.1002/etc.5620191108.

Luo, W., Verweij, R.A., van Gestel, C.A.M., 2014. Contribution of soil properties of shooting fields to lead biovailability and toxicity to *Enchytraeus crypticus*. Soil Biol. Biochem. 76, 235–241. https://doi.org/10.1016/j.soilbio.2014.05.023.

Maraldo, K., Holmstrup, M., 2010. Enchytraeids in a changing climate: a mini-review. Pedobiologia 53, 161–167. https://doi.org/10.1016/j.pedobi.2009.10.003.

Nélieu, S., Lamy, I., Karolak, S., Delarue, G., Crouzet, O., Barraud, C., Bimbot, M., Allaoui, F., Hanot, C., Delorme, A., Lévi, Y., Hulot, F.D., Baudry, E., 2021. Impact of peri-urban landscape on the organic and mineral contamination of pond waters and related risk assessment. Environ. Sci. Pollut. Res. 28, 59256–59267. https://doi.org/10.1007/s11356-020-10355-5.

Novais, S.C., Gomes, S.I.L., Gravato, C., Guilhermino, L., De Coen, W., Soares, A.M.V.M., Amorim, M.J.B., 2011. Reproduction and biochemical responses in Enchytraeus albidus (Oligochaeta) to zinc or cadmium exposures. Environ. Pollut. Barking Essex 1987 159, 1836–1843. https://doi.org/10.1016/j.envpol.2011.03.031.

Novais, S.C., Gomes, N.C., Soares, A.M.V.M., Amorim, M.J.B., 2014. Antioxidant and neurotoxicity markers in the model organism Enchytraeus albidus (Oligochaeta): mechanisms of response to atrazine, dimethoate and carbendazim. Ecotoxicology 23, 1220–1233. https://doi.org/10.1007/s10646-014-1265-z.

OCDE, 2004. Test No. 222: Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei). Éditions OCDE, Paris. https://doi.org/10.1787/9789264070325-en.

Panico, S.C., van Gestel, C.A.M., Verweij, R.A., Rault, M., Bertrand, C., Menacho Barriga, C.A., Coeurdassier, M., Fritsch, C., Gimbert, F., Pelosi, C., 2022. Field mixtures of currently used pesticides in agricultural soil pose a risk to soil invertebrates. Environ. Pollut. 305, 119290. https://doi.org/10.1016/j.envpol.2022.119290.

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, 107167. https://doi.org/10.1016/j.agee.2020.107167.

Renaud, E., Heraudet, V., Deparis, M., Basquin, H., Bessa-Gomes, C., Baudry, E., 2022. Non-linear effects of landscape on pollination service and plant species richness in a peri-urban territory with urban and agricultural land use. Urban For. Urban Green. 68, 127454. https://doi.org/10.1016/j.ufug.2021.127454.

de Santiago-Martín, A., Cheviron, N., Quintana, J.R., González, C., Lafuente, A.L., Mougin, C., 2013. Metal contamination disturbs biochemical and microbial properties of calcareous agricultural soils of the Mediterranean Area. Arch. Environ. Contam. Toxicol. 64, 388–398. https://doi.org/10.1007/s00244-012-9842-8.

Sebastiano, M., Messina, S., Marasco, V., Costantini, D., 2022. Hormesis in ecotoxicological studies: a critical evolutionary perspective. Curr. Opin. Toxicol. 29, 25–30. https://doi.org/10.1016/j.cotox.2022.01.002.

Serbource, C., Petit-Dit-Grezeriat, L., Pelosi, C., 2024. A meta-analysis to compare the sensitivities of earthworms and enchytraeids to different stressors. Eur. J. Soil Biol. 122, 103656. https://doi.org/10.1016/j.ejsobi.2024.103656.

Silva, A.L.P., Holmstrup, M., Kostal, V., Amorim, M.J.B., 2013. Soil salinity increases survival of freezing in the enchytraeid Enchytraeus albidus. J. Exp. Biol. 216, 2732–2740. https://doi.org/10.1242/jeb.083238.

Silva, V., Mol, H.G.J., Zomer, P., Tienstra, M., Ritsema, C.J., Geissen, V., 2019. Pesticide residues in European agricultural soils - a hidden reality unfolded. Sci. Total Environ. 653, 1532–1545. https://doi.org/10.1016/j.scitotenv.2018.10.441.

Sinsabaugh, R.L., Reynolds, H., Long, T.M., 2000. Rapid assay for amidohydrolase (urease) activity in environmental samples. Soil Biol. Biochem. 32, 2095–2097. https://doi.org/10.1016/S0038-0717(00)00102-4.

Sverdrup, L.E., Jensen, J., Kelley, A.E., Krogh, P.H., Stenersen, J., 2002. Effects of eight polycyclic aromatic compounds on the survival and reproduction of Enchytraeus crypticus (Oligochaeta, Clitellata). Environ. Toxicol. Chem. 21, 109–114. https://doi.org/10.1002/etc.5620210116.

Tabatabai, M.A., Bremner, J.M., 1969. Use of *p*-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1, 301–307. https://doi.org/10.1016/0038-0717(69)90012-1.

Tabatabai, M.A., Bremner, J.M., 1970. Arylsulfatase activity of soils. Soil Sci. Soc. Am. J. 34, 225–229. https://doi.org/10.2136/sssaj1970.03615995003400020016x.

Vecchiato, M., Bonato, T., Barbante, C., Gambaro, A., Piazza, R., 2021. Organic pollutants in protected plain areas: the occurrence of pahs, musks, UV-filters, flame retardants and hydrocarbons in woodland soils. Sci. Total Environ. 796, 149003. https://doi.org/10.1016/j.scitotenv.2021.149003.

Williams, R.J.P., Fraústo da Silva, J.J.R., 2002. The involvement of molybdenum in life. Biochem. Biophys. Res. Commun. 292, 293–299. https://doi.org/10.1006/bbrc.2002.6518.