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A meta-analysis to compare the sensitivities of earthworms and enchytraeids to different stressors

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► **To cite this version:**

Cécile Serbource, Lucas Petit-Dit-Grezeriat, Céline Pelosi. A meta-analysis to compare the sensitivities of earthworms and enchytraeids to different stressors. *European Journal of Soil Biology*, 2024, 122, pp.103656. <10.1016/j.ejsobi.2024.103656>. <hal-04703594>

HAL Id: hal-04703594

<https://hal.science/hal-04703594v1>

Submitted on 23 Sep 2024

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HAL Authorization

European Journal of Soil Biology

A meta-analysis to compare the sensitivities of earthworms and enchytraeids to different stressors --Manuscript Draft--

Manuscript Number:	EJSOBI-D-24-00241R3
Article Type:	Research Paper
Keywords:	Ecotoxicological endpoints; Multiple stressors; Soil engineers; Indicators; LC50.
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Abstract:	<p>Earthworms and enchytraeids are soil organisms involved in key soil functions, such as organic matter turnover and soil structure, at different scales. In natural soils, these organisms are exposed and sensitive to different abiotic factors (e.g., climate, land use and management) and are often used as bioindicators of human disturbances, particularly chemical stress. However, the sensitivity of these two groups of Oligochaeta (Annelida) to different stressors has never been compared. Using data from 49 publications and 330 observations, we performed a meta-analysis to compare the sensitivities of earthworms and enchytraeids to all kinds of stressors under similar test conditions. Earthworms and enchytraeids were found to be equally sensitive to chemical stressors (mean effect size -0.61 [-2.53; 1.30]) regardless of the studied endpoint (mortality or reproduction). Most of the observations dealt with the effects of pesticides (42%) and heavy metals (40%) on both organisms. No difference in sensitivity was revealed when these two stressors were considered separately. Regarding the two most studied species of enchytraeids and earthworms, the mean effect sizes of all the possible combinations of <i>Eisenia fetida</i> (41% of the studies) or <i>Eisenia andrei</i> (48%) or <i>Enchytraeus crypticus</i> (73% of the studies) or <i>Enchytraeus albidus</i> (27%) did not reveal any differences in sensitivity. This study also highlights the lack of studies on environmentally relevant (i.e., representative of natural soils) enchytraeid and earthworm species. We also revealed that mostly ecotoxicologists have compared the sensitivities of these two key soil organisms when they are exposed to and threatened by other important factors, such as agricultural practices and climate change.</p>
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84914 Avignon Cedex 9

Avignon, July 27th 2024

Dear Editor,

Our research article entitled “A meta-analysis to compare the sensitivities of earthworms and enchytraeids to different stressors” has undergone the last requested revisions. We are again very grateful for that feedback that helped improving the quality of our manuscript.

We sincerely hope that our manuscript meets the publication standards of European Journal of Soil Biology.

Sincerely yours,

Cécile Serbourg, on behalf of co-authors

A handwritten signature in blue ink, appearing to read 'Serbourg', with a large, stylized flourish extending to the left.

Answers to comments, in blue

Dear Dr Serbource,

Apologies for asking for one final change but there is one phrase in your new conclusion that just does not seem quite right. Apologies also for the complexity of the English language! Where you say "loosen organic materials" in line 372, could you change it to " organic rich habitats" or something similar. Then we'll get it published.

Thanks again,

Bryan Griffiths

Dear Dr Griffiths,

I would like to thank you for your meticulous efforts in improving my work and its clarity.

L 372 "organic rich habitats" is indeed more understandable. I changed it.

Thank you,

Cécile Serbource

1 Abstract

2 Earthworms and enchytraeids are soil organisms involved in key soil functions, such as organic matter
3 turnover and soil structure, at different scales. In natural soils, these organisms are exposed and
4 sensitive to different abiotic factors (e.g., climate, land use and management) and are often used as
5 bioindicators of human disturbances, particularly chemical stress. However, the sensitivity of these
6 two groups of Oligochaeta (Annelida) to different stressors has never been compared. Using data from
7 49 publications and 330 observations, we performed a meta-analysis to compare the sensitivities of
8 earthworms and enchytraeids to all kinds of stressors under similar test conditions. Earthworms and
9 enchytraeids were found to be equally sensitive to chemical stressors (mean effect size -0.61 [-2.53;
10 1.30]) regardless of the studied endpoint (mortality or reproduction). Most of the observations dealt
11 with the effects of pesticides (42%) and heavy metals (40%) on both organisms. No difference in
12 sensitivity was revealed when these two stressors were considered separately. Regarding the two most
13 studied species of enchytraeids and earthworms, the mean effect sizes of all the possible combinations
14 of *Eisenia fetida* (41% of the studies) or *Eisenia andrei* (48%) or *Enchytraeus crypticus* (73% of the
15 studies) or *Enchytraeus albidus* (27%) did not reveal any differences in sensitivity. This study also
16 highlights the lack of studies on environmentally relevant (i.e., representative of natural soils)
17 enchytraeid and earthworm species. We also revealed that mostly ecotoxicologists have compared the
18 sensitivities of these two key soil organisms when they are exposed to and threatened by other
19 important factors, such as agricultural practices and climate change.

20

1. Introduction

Earthworms and enchytraeids are macro- and meso-organisms, respectively, that perform several key functions in soils. At different spatial and temporal scales, both taxa are involved in litter fragmentation and organic matter decomposition (Marinissen & Didden, 1997, Brown et al., 2000), soil nutrient dynamics (Lavelle et al., 1998; Cole et al., 2002), soil formation (Lavelle et al., 1988, Topoliantz et al., 2000), soil structure dynamics via bioporosity (van Vliet et al., 1993, Edwards & Bohlen 1996), and regulation of microbial biomass (Hedlund & Augustsson, 1995, Brown et al., 2000). Because they play key roles in the functioning of agroecosystems and are abundant in many ecosystems, earthworms and enchytraeids are both used as bioindicators of land use, agricultural practices and chemical stress (OECD, 1984, Paoletti, 1999, Didden & Römbke, 2001, Pelosi & Römbke, 2017).

The ecological niches and behaviours of these two groups do not necessarily overlap (Didden et al., 1997): For example, the earthworm *Lumbricus terrestris* creates extensive burrows that improve water infiltration. In contrast, enchytraeids, such as *Enchytraeus albidus*, play a crucial role in nutrient cycling by decomposing finer organic materials in the soil, their activities are more concentrated in the upper soil layers and leaf litter. Therefore, they can provide complementary functions in soils and complementary information on soil quality (Postma-Blaauw et al., 2012). Under natural conditions, both earthworms and enchytraeids face stressors of different origins. In agricultural soils, tillage or chemical inputs such as pesticides may harm these soil organisms (van Capelle et al., 2012, Beketov et al., 2013). Moreover, climate change has increased the vulnerability of soils and terrestrial biodiversity (IPCC 2022). Phillips et al. (2019) showed that climate variables were more important than soil properties or habitat cover in shaping earthworm communities. Drought periods and intense precipitation events now occur more frequently and cause dramatic disruptions to soil biological, physical and chemical parameters (Bellard et al., 2012). Thus, the extreme variations in soil moisture as well as the exposure to soil tillage and high quantities of chemicals—directly applied to the soil

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26 surface—can disturb these organisms, their metabolism and the interactions between them (Mäder et
27 al., 2002, Bengtsson et al., 2005).

28 Earthworms and enchytraeids are both soft-bodied soil invertebrates, and enchytraeids are considered
29 the closest relatives to earthworms according to Erséus and Källersjö (2004). Enchytraeids have been
30 compared to other soil meso-organisms, such as springtails (Urarov & Karaban, 2015, Hlavkova et al.,
31 2020) or nematodes (Hagner et al., 2010), more often than to earthworms. Moreover, in some studies,
32 different endpoints have been assessed for enchytraeids and earthworms to obtain additional data on
33 the effects of stressors (Niva et al., 2021); therefore, the sensitivities of these two organismal groups
34 cannot be compared. Among the studies that have properly compared earthworms and enchytraeids,
35 some have shown that enchytraeid species exhibit a lower sensitivity than earthworms to certain
36 chemicals and climatic parameters. In Bart et al. (2017), earthworms experienced a decrease in
37 biomass, and they avoided soil contaminated with a copper fungicide at a lower concentration than
38 enchytraeids. Holmstrup et al. (2022) noted that, in Arctic areas, the enchytraeid *Enchytraeus albidus*
39 individuals had superior freezing tolerance than earthworms. Moreover, Sverdrup et al. (2002)
40 reported that *Enchytraeus crypticus* exhibited a lower sensitivity to polycyclic aromatic compound
41 exposure than the earthworm *Dendrobaena veneta*; however, their respective sensitivities can vary
42 according to the compound itself. In contrast, enchytraeids reportedly exhibit a greater sensitivity to
43 pesticides (e.g., Sechi et al., 2014 – insecticide alpha-cypermethrin) and pig manure (Segat et al.,
44 2020) than earthworms. Finally, Römbke and Moser (2002), based on the results of an international
45 ring test involving 29 institutions from 15 European countries, noted that there was no clear answer as
46 to the sensitivity of the species tested (*Eisenia fetida*/*Eisenia andrei* or *E. albidus*) to two chemicals
47 (the fungicide carbendazim and the industrial chemical 4-nitrophenol). The authors concluded that
48 there was no "most sensitive species". Other studies comparing earthworms to enchytraeids have
49 shown similar sensitivities between both soil organisms through a wide range of stress factors, such as
50 uranium (Caetano et al., 2014), electrical conductivity (Gainer et al., 2019), polycyclic aromatic
51 compounds (Kobeticova et al., 2008) and lindane, an insecticide (Lock et al., 2002). A report
52 published in 2009 by Jarratt and Thompson showed mixed conclusions on the sensitivities of

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enchytraeid and earthworm species to chemicals, although they suggested that the mortality test results indicate that Enchytraeidae species may be less sensitive than earthworms. They reported a lower sensitivity for enchytraeids in some cases but noted that the current data do not show consistent differences in sensitivity between Lumbricidae and Enchytraeidae in the laboratory or under semifield/field conditions. To summarize, the message is not clear, perhaps due to the non-quantitative nature of all these assessments.

The aim of this study was to quantify the relative sensitivities of earthworms and enchytraeids to all kinds of stressors (i.e., any factor likely to negatively affect earthworms and enchytraeids at any level of biological organization). For that purpose, we performed a meta-analysis based on an exhaustive bibliographical review that included all the studies using both earthworms and enchytraeids under the same test conditions (e.g., same soil, tested stressor). We determined the difference in sensitivity to specific stressors according to the following assumptions: **(H1)** enchytraeids are generally less sensitive than earthworms to different stressors, **(H2)** differences in sensitivity are mainly observed when mortality is assessed compared to reproduction or avoidance, and **(H3)** the relative sensitivities of both groups of organisms vary according to the tested stressor and the considered species. Our findings highlight the specific gaps in the literature regarding the sensitivity of these organisms to different stressors, emphasizing the need for future research to address these gaps.

2. Methods

2.1. Literature search and inclusion/exclusion criteria

A systematic literature review was conducted in January 2023 to find publications dealing with earthworms and enchytraeids exposed to different stressors. The literature search was carried out on the basis of keywords in the ISI Web of Knowledge, using the “All Databases” option, with the following scheme: ‘(enchytr* OR potworm* OR achaet* OR bryodril* OR buchlol* OR cernosvitoviel* OR cognat* OR frideric* OR guaranidril* OR lumbricil* OR hemienchytr* OR oconnorel* OR hemifrideric* OR henl* OR marionin* OR mesenchytr*) and (earthworm* OR lumbric* OR aporrect* OR eisen* OR dendrob* OR alloloboph* OR octalas* OR dendrodril* OR

79 diporodril* OR eophil* OR helodril* OR kritodril* OR octodril* OR prosellodril* OR scherothec*
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2 80 OR satchell* OR proctodril* orodril* OR postandril* OR perelia* OR andrei* OR fetid* OR foetid*)’
3
4 81 in Topics. From a total of 967 references, a first selection was made using titles and abstracts. The full
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6 82 texts were examined when the information was considered consistent for the meta-analysis. To
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8 83 complete the search, bibliographic reviews that included both annelids were selected, and references
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10 84 were carefully examined to extract any new articles that could be relevant for the meta-analysis.
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14 85 We considered only publications providing data on terrestrial earthworms and enchytraeids, all species
15
16 86 included. For instance, papers dealing with aquatic and bioluminescent worm species were excluded
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18 87 (Rodionova et al., 2017). The data on earthworms and enchytraeids had to be from the same study to
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20 88 compare the sensitivity of these groups under the same conditions (e.g., active substance, type of
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22 89 substrate). Moreover, we did not consider studies dealing with chemical bioaccumulation in animals,
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24 90 as bioaccumulation cannot be easily correlated with certain factors (Tosza et al., 2010; Santorufo et al.,
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26 91 2012). We selected only the studies where the tested factors were supposed to be harmful to soil
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28 92 organisms, specifically those detrimental to their life cycle parameters (e.g., survival, growth,
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30 93 fecundity, juvenile survival). We did not consider studies on biochar addition (possibly beneficial to
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32 94 them, e.g., Briones et al., 2020) or on tillage (Engell et al., 2021) because these factors can be
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34 95 beneficial to enchytraeid populations (e.g., Pelosi & Römbke, 2016). Regarding organic and inorganic
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36 96 waste, one paper was kept because it focused on contaminated incineration ash (Kobetičová et al.,
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38 97 2010). The final set was composed of 49 publications (i.e., 48 papers and one PhD thesis), which
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40 98 included 330 lines in the database (Table S1).
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46 99 2.2. Data extraction

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49 100 For each of the 49 studies, the following data were recorded (Table S1):
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- 51 101 - author, date of publication,
- 52 102 - stressor type (i.e., pesticides, metals, wastes, others);
- 53
54 103 - stressor subcategory (i.e., for pesticides: fungicide, herbicide and insecticide; for metals:
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56 104 antimony, barium, beryllium, cadmium, chromium, copper, lanthanum, manganese, mercury,
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- 105 molybdenum, nickel, lead, silver, uranium and zinc; for wastes: inorganic waste, organic waste,
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2 106 vinasse, pig manure waste, mineral byproducts, and dredged sediment; for others: pesticide
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4 107 additive, boric acid, electrical conductivity, linear alkylbenzene sulfonate, chlorinated paraffins
5
6 108 and polycyclic aromatic compounds);
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9 109 - earthworm species (i.e., *Aporrectodea caliginosa*, *Aporrectodea longa*, *Dendrobaena veneta*,
10
11 110 *Eisenia andrei* and *Eisenia fetida*) and enchytraeid species (i.e., *Enchytraeus albidus*,
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13 111 *Enchytraeus crypticus* and *Enchytraeus luxuriosus*);
14
15 112 - the observed variable (i.e., survival, reproduction, avoidance, growth, population abundance)
16
17 113 and its measured endpoint (LC_x: lethal concentration; EC_x: effect concentration; NOEC: no
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19 114 observed effect concentration; NEC: no effect concentration; and LOEC: lowest observed
20
21 115 effect concentration);
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23
24 116 - the mean value of the endpoints with the standard error, standard deviation, or the respective
25
26 117 95% confidence intervals;
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29 118 - and the number of replicates.
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32 119 As the choice was made to select studies that provided ecotoxicological endpoint values to ease the
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34 120 comparison between earthworms and enchytraeids, all the selected studies were laboratory
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36 121 experiments.
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39 122 First, the data were collected from available tables; otherwise, the data were collected from the text.
40

41 123 Searching for data in the figures (graphs) was not ruled out, but none of the data matched our search
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43 124 criteria. Because of compliance with ISO standards (ISO, 1998, OECD, 2015), some methodological
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45 125 differences were tolerated when comparing data for earthworms and enchytraeids. For example, in
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47 126 reproduction tests, cocoons or juveniles were counted for earthworms, while the number of juveniles
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49 127 was counted for enchytraeids. The duration of the experiment was also not the same for standard tests
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51 128 of earthworms and enchytraeids, i.e., 21 and 42 days, respectively.
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55 129 For both earthworms and enchytraeids, when the endpoint value was not given as a precise number but
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57 130 was bounded by > or <, the data were adjusted as follows: in the case of LC₅₀ > x, the data were not
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131 considered to indicate that the sensitivity of the organism would be overestimated by considering x as
1 the LC₅₀ value. In the case of LC₅₀ < x, the x value was used, and it is conservative to consider x as the
2 132 the LC₅₀ value. This latter case represented 20 of the 330 observations in the database (i.e., 6% of the
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4 133 data).
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9 135 Four comparisons involving boric acid were found in two studies (Becker et al., 2011, Niemeyer et al.,
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11 136 2018). Becker et al. (2011) characterized boric acid as a fertilizer and fungicide, but Niemeyer et al.
12
13 137 (2018) did not mention any role or expected effect. Boric acid can be used as an insecticide, acaricide,
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15 138 herbicide, or fungicide (Büyükgüzel et al., 2013). Since it can be placed in different subcategories
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17 139 (insecticide, fungicide, and fertilizer), it was placed into the "others" category.
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21 140 2.3. Effect size calculation

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24 141 When the standard deviation (SD) was not given directly from a table, it was calculated with the t
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26 142 distribution for small samples (n<60) using the following formula (Higgins & Green, 2011):
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$$28 \quad SD = \sqrt{n} * \frac{\text{upper limit CI} - \text{lower limit CI}}{t_{\alpha,df} * 2}$$

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34 144 With $t_{\alpha,df}$, the T.INV.2T functions in syntax with two associated arguments: probability, associated
35
36 145 with Student's t-distribution (here, the alpha=0.05 to respect the 95% CI from extracted data), and
37
38 146 degrees of freedom (equal to the group sample size - 1).
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41 147 When neither the 95% CI nor the SD were given, we used the worst-case scenario, i.e., the highest SD
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43 148 calculated for the same endpoint in the database (LC₁₀, LC₅₀, EC₁₀, EC₂₀, EC₅₀). NOEC and LOEC
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45 149 values have no variance, so their standard deviation (SD) was set to zero. When the number of
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47 150 replicates was not the same between the treatments and the control, the lower number was considered
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49 151 to be conservative (i.e., less weight was given to the study). When the number of replicates was not
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51 152 given and the tests referred to ISO standards, we considered the number of replicates advised in
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53 153 official procedures (e.g., ISO, 1998, OECD, 2015). The confidence intervals (CIs) for each estimate of
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55 154 effect size were calculated using bias-corrected bootstrapping methods, which corrects for
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57 155 nonnormality of the data and nonconstant standard errors (Efron & Hastie, 2016).
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156 Hedge's d is a standardized mean difference that accounts for the potential bias in estimating
157 population effect size (Hedges & Olkin, 1985). The magnitude of the difference between earthworm
158 and enchytraeid sensitivities was calculated with the Hedge metric using the natural logarithm of the
159 response ratio (LRR) as follows (van Groenigen et al., 2014):

$$LRR = \ln\left(\frac{x_i}{x_u}\right)$$

161 with x_i and x_u being the values of the enchytraeid and earthworm endpoints, respectively. Therefore,
162 when the LRR equals zero, the two organisms have the same sensitivity.

163 The LRR variance (V) was calculated as follows (van Groenigen et al., 2014):

$$V = \frac{1}{n_i} * \left(\frac{SD_i}{x_i}\right)^2 + \frac{1}{n_u} * \left(\frac{SD_u}{x_u}\right)^2$$

165 where x_i and x_u are the mean values of the endpoints for earthworms and enchytraeids, respectively;
166 SD_i and SD_u are the standard deviations of the endpoint values for enchytraeid and earthworm,
167 respectively; and n_i and n_u are the sample sizes in the enchytraeid and earthworm tests, respectively.
168 Finally, effect sizes were weighted by the inverse of their variance in all the models, giving more
169 weight to well-replicated studies (Koricheva et al., 2013).

170 3. Meta-analysis

171 3.1. General modelling approach

172 Every model incorporated a random effect associated with the study's identity to accommodate
173 correlated data arising from the same study (Viechtbauer, 2010). The models were selected using the
174 Akaike information criterion (AIC). This criterion represents a compromise between bias decreasing
175 with the number of free parameters and parsimony, the desire to describe the data with as few
176 parameters as possible. Lower AIC values indicate a better-fit model; therefore, for each calculation,
177 the smallest AIC was considered for the LRR calculation. Random effect models were tested for the
178 lines (to allow the real effect to vary from one dataset to another), the authors (to allow the actual

179 effect to vary from one article to another), and a combination of the two (Borenstein et al., 2010). A
180 combination of the two modalities was associated with a lower AIC for every case, and these models
181 were systematically used. For each model, we used the restricted maximum likelihood (REML)
182 method, which aims to provide unbiased estimates of variance components by accounting for fixed
183 effects in the model. All analyses were performed with R Software (R Development Core Team, 2014)
184 using the metafor package (Viechtbauer, 2010).

185 3.2. Analysis

186 First, a meta-analysis was carried out using estimated overall effect size in a multivariate linear model,
187 calculated using the `rma.mv` function from the metafor package in R (Viechtbauer, 2010). In the
188 context of a meta-analysis, the effect size (with its 95% confidence interval) is a standardized measure
189 that quantifies the magnitude of the treatment effect or association across all included studies. Then,
190 the logarithm of the response ratio (LRR) was calculated to explore, through earthworm and
191 enchytraeid endpoints, the results of this model on all the data, all the factors and all the species
192 combined. This main model enabled us to test our first assumption (H1).

193 We also compared earthworm and enchytraeid data by splitting them according to the studied
194 endpoints (i.e., reproduction or survival rate) to test the second assumption (H2). We further
195 investigated the effects of the different stressors (H3) on the response ratio (LRR) by stressor category,
196 a moderator with 4 levels: pesticides, metals, wastes, and others. We finally investigated the potential
197 "species effect" of the most represented earthworm and enchytraeid species (H3), using the response
198 ratio of the earthworm species categorical moderator at 2 levels (i.e., *E. andrei* and *E. fetida*, which
199 accounted for 48% and 41%, respectively; Table S1), coupled with the enchytraeid species categorical
200 moderator at 2 levels (i.e., *E. albidus* and *E. crypticus*, which accounted for 27% and 73%,
201 respectively; Table S1).

202 Publication bias was assessed by Egger's regression test for funnel plot asymmetry between the
203 standard normal deviation of the effect sizes and precision (i.e., inverse of their standard errors). We
204 retained the multilevel linear model `rma.mv` by adding a standard error moderator. This test is

205 powerful given the fairly large number of studies. The findings demonstrated the absence of
1
2 206 publication bias: we detected no significant asymmetry (z -value = -0.62; p value = 0.53) in the funnel
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4 207 plot (Fig. S1), as none of the intercepts from Egger's regression exhibited a significant deviation from
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6 208 zero.

209 4. Results

210 4.1. Data description

211 **Location Fig. 1**

212 The 49 selected studies addressed a total of 30 different stressors (Figure 1) classified into four
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20 213 different categories. “Metals” accounted for 39.7% of the observations, with the most represented
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22 214 being molybdenum (2 studies, 36 observations) and zinc (2 studies, 19 observations). “Pesticides”
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24 215 represented 25.5%, with subcategories including insecticides (13 studies, 52 observations), fungicides
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26 216 (6 studies, 31 observations) and herbicides (1 study, 1 observation). Among all the observations,
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28 217 14.2% were classified into the “Wastes” category, which is widely represented by vinasse (1 study, 26
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30 218 observations). Finally, “Others” accounted for 20.6% of the observations, with polycyclic aromatic
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32 219 compounds (2 studies, 30 observations) and pesticide additives (1 study, 22 observations) being the
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34 220 most represented. In total, 98.8% of the data concerned contaminant tests (all the stressors studied
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36 221 except for the 4 observations for the electrical conductivity), even though no restrictions were imposed
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38 222 during the literature search.

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43 223 The 49 selected studies reported reproduction endpoints in 71.2% of the cases (43 studies, 235
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45 224 observations), survival in 20.9% (20 studies, 69 observations), avoidance in 4.54% (3 studies, 15
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47 225 observations), growth in 2.4% (1 study, 8 observations), and population abundance in 1% (1 study, 3
48
49 226 observations), with EC₅₀ (106 observations) and NOEC (68 observations) calculations dominating
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51 227 (Table 1).

228 **Location Tab.1**

229 In total, five earthworm species and three enchytraeid species were used in the comparisons (Table 2).
230 Together, *E. andrei* and *E. fetida* accounted for 89% of the total number of observations. *E. crypticus*
231 was the most represented enchytraeid species among the observations.

232 **Location Tab.2**

233 4.2. Meta-analysis results

234 When considering all the stressors together, earthworms and enchytraeids were found to be equally
235 sensitive, as the mean effect size was -0.61 [-2.53, 1.31] (Fig. 2a). The results were considered non-
236 significant because the confidence interval crosses zero, indicating no statistically significant effect.
237 Similarly, exploration of the data across the four stressor types yielded nonsignificant results (Fig. 2b),
238 with each confidence interval intersecting zero.

239 **Location Fig.2**

240 Similarly, when the data on mortality (i.e., LC₁₀, LC₅₀, and some NOEC and LOEC data) and those on
241 reproduction (i.e., some EC₁₀, EC₂₀, EC₂₅, EC₅₀, LOEC, NEC and NOEC) were considered separately
242 (Fig. 3), no difference in sensitivity was found between earthworms and enchytraeids.

243 **Location Fig3**

244 The sensitivities of the different species were assessed when the dataset was statistically sufficient for
245 comparison. Therefore, *E. andrei* (earthworm) data were compared with *E. crypticus* (enchytraeid)
246 data, *E. andrei* with *E. albidus* (enchytraeid), *E. fetida* (earthworm) with *E. albidus* and *E. fetida* with
247 *E. crypticus*. The sensitivities of these species were similar (Fig. S2).

248 5. Discussion

249 This study allowed us to quantitatively compare the relative sensitivities of earthworms and
250 enchytraeids to different types of stressors. On the basis of 49 published studies and 330 observations,
251 we revealed similar sensitivities to stressors in these two soil organisms (H1). The conclusion was the
252 same when considering the different stressors, species or endpoints separately (H2 and H3). Although

253 the differences were not significant, enchytraeids tended to be slightly more sensitive to metals,
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2 254 pesticide additives and electrical conductivity. In contrast, earthworms tended to be slightly more
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4 255 sensitive to pesticides regardless of their mode of action (fungicide or insecticide), but the differences
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6 256 were still nonsignificant. Large confidence intervals were found for all mean effect sizes. This
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8 257 variability was probably due to the variety of test conditions used for the observations; in particular,
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10 258 the tested substances revealed alternating greater sensitivities of enchytraeids or earthworms.
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12 259 Moreover, the high number of studies with no information on data uncertainty (e.g., standard
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14 260 deviation) (i.e., 58 observations) could explain the absence of significant differences since when
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16 261 neither the 95% CI nor the SD was given, we used a conservative approach by giving the highest SD
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18 262 calculated for the same endpoint in the database.
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22 263 These results are not in accordance with our assumptions that enchytraeids are less sensitive than
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24 264 earthworms to different stressors (H1), that differences in sensitivity are mainly observed when
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26 265 mortality is assessed (H2), and that the relative sensitivity of both groups of organisms varies
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28 266 according to the tested stressor and the considered species (H3). Enchytraeids, which are smaller than
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30 267 earthworms and 80% microbivorous and 20% saprovorous (Didden, 1990, 1993), ingest less material
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32 268 and smaller particles than earthworms in soils and thus could be less exposed to chemicals (Tourinho
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34 269 et al., 2021, Coleman et al., 2004). However, the relationship between size and sensitivity is debatable,
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36 270 as earthworms are much larger than springtails but are less sensitive to all kinds of pesticides, such as
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38 271 formulations or active substances (Joimel et al., 2022). Moreover, in the same study (Joimel et al.,
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40 272 2022), differences in sensitivity were reported between springtails and enchytraeids, but both of these
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42 273 taxonomic groups belong to the same size class (i.e., soil mesofauna). Small animals have a greater
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44 274 surface area-to-volume ratio than larger organisms and are thus potentially more exposed to external
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46 275 stressors. Regarding chemicals, the size class would be of particular importance if the main route of
47
48 276 exposure was dermal contact. Enchytraeids live in close contact with the soil porewater fraction, and
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50 277 their routes of exposure are dermal, intestinal and respiratory and earthworms one is dermal but also
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52 278 dietary exposure (Lock & Janssen, 2003, Römbke, 2003, Hirano & Tamae, 2011). Earthworms, which
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54 279 might be thought to be more sensitive to chemicals at first glance due in particular to their larger size,
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280 can ultimately be less exposed than small enchytraeids in soils. Both earthworms and enchytraeids
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2 281 must maintain a moist body surface to facilitate oxygen absorption through their tegument, which is
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4 282 highly permeable to water. Pesticide characteristics (e.g., polarity, solubility in water) influence the
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6 283 available concentrations in water and in soils, leading to varying sensitivities among taxonomic
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9 284 groups. In accordance with our results, Römbke and Moser (2002) concluded that the overall range of
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11 285 sensitivities of oligochaetes to chemicals was usually quite similar. These authors linked the lack of
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13 286 differentiation between the sensitivities of *E. albidus* and *E. fetida* exposed to carbendazim to the high
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15 287 toxicity of this persistent compound. More research should be dedicated to quantifying the different
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17 288 routes of exposure to chemicals for soil organisms to better understand their respective sensitivities in
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19 289 different situations. Moreover, both earthworms and enchytraeids can be considered in risk
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22 290 assessments, and while earthworms are most commonly used, there are many reasons to support the
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24 291 use of enchytraeids in testing. Enchytraeids can thrive in soils where earthworms are scarce (Jarratt &
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26 292 Thompson, 2009). Moreover, Römbke et al. (1994) encouraged the use of enchytraeids when
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28 293 comparing laboratory results with those obtained under semifield and field conditions, as the same
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30 294 species (or closely related species) can be used at all test levels. Other practical and ecological points
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32 295 (ease of handling and rearing, short generation time, reduced soil requirements, cost-effectiveness)
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34 296 make them good organisms for inclusion in laboratory experiments (Römbke & Moser, 2002).
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38 297 Among the tested species, the earthworm *Eisenia* sp. represented 89% of all species included in the
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40 298 selected studies. *E. fetida* and *E. andrei* are composting worms that are not representative of mineral
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42 299 soils and are less sensitive to pesticides than species found in cultivated fields (Pelosi et al., 2013).
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45 300 However, these species are recommended as standard test organisms in various guidelines, such as
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47 301 those of the OECD (OECD, 1984) and ISO (ISO, 1993, 1998, 2019), and have been widely used in
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49 302 published ecotoxicological studies, mainly because they are easy to rear. A few studies have used
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51 303 species that are found in agricultural soils. In Bart et al. (2017), the LC₅₀ and EC₅₀ values were
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53 304 systematically lower for *A. caliginosa* (earthworm) than for *E. albidus* (enchytraeid) (Table S1). In
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55 305 Holmstrup and Krogh (2001), the opposite occurred, with systematically higher LC₅₀ and EC₅₀ values
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57 306 for *A. caliginosa* and *A. longa* (earthworm) than for *E. albidus* (Table S1). Thus, the results should be
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1 307 contrasted according to the tested stressor, and additional data are needed to assess the sensitivity of
2 308 earthworms and enchytraeid species commonly found in the field.

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5 309 The choice was made here to select studies that provided ecotoxicological endpoint values to facilitate
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7 310 comparisons between earthworms and enchytraeids. Potentially, interesting studies have been
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9 311 excluded that compared both organisms. For instance, Amossé et al. (2018) assessed the short-term
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11 312 effects of two fungicides (Cuprafor Micro® made of copper oxychloride and Swing Gold® made of
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13 313 epoxiconazole and dimoxystrobin) on enchytraeid and earthworm communities under field conditions.
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15 314 They found greater negative effects on the diversity and community structure of earthworms than on
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17 315 those of enchytraeids. Similarly, Pereira et al. (2018) studied the degree to which seawater intrusion
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19 316 and irrigation threaten biodiversity via a terrestrial model ecosystem (TME) approach. They found that
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21 317 the abundance of only enchytraeids significantly decreased, although earthworms generally suffered.
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23 318 These field or semifield studies allowed us to investigate the sensitivity of several species found in
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25 319 natural soils under realistic conditions, which offers more comprehensive insight into the ecological
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27 320 dynamics of the systems (Wall & Moore, 1999). TMEs, which were proposed in the 1990 s, are useful
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29 321 experimental systems for assessing the impact of factors such as pollution or climate change on
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31 322 terrestrial ecosystems.

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37 323 Although the literature search was performed to include all kinds of stressors in this study, the great
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39 324 majority of the articles in our final selection concerned chemicals. Very few data are available on the
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41 325 effects of climatic parameters on soil organisms (Classen et al., 2015), and data comparing their
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43 326 responses to these parameters remain scarce (Otomo, 2011). However, earthworms and enchytraeids,
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45 327 in addition to all other soil organisms, are increasingly having to cope with climate change (Pritchard,
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47 328 2011). Warming, changes in precipitation regimes, and changes in the quantity and quality of soil
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49 329 carbon inputs can disrupt the habitat and food resources of soil-living organisms, affecting their
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51 330 survival and reproductive capabilities (Maraldo & Holmstrup, 2009). Some species, such as the
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53 331 enchytraeid *Cognettia sphagnetorum*, which has strong potential to recover from severe drought stress
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55 332 events, appear to have adaptive potential to climate parameters (Maraldo & Holmstrup, 2009). They
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57 333 can vertically migrate as a strategy for evading dry surface layers and high temperatures (Springett et

334 al., 1970). However, these organisms were found to have no genetic adaptation to drought, as drought-
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2 335 exposed populations of enchytraeids had the same high sensitivity as those from unexposed control
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4 336 plots (Maraldo, 2009). *E. albidus* has a high osmotic pressure on its body fluids and can live in
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6 337 environments with strong fluctuations in moisture (Maraldo, 2009), which also enables *Fridericia*
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8 338 *galba* to survive in soil with a water retention capacity of less than 20% (corresponding to values
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10 339 below -9.8 bar) for more than 49 days (Dózsa-Farkas 1973). However, additional research is needed to
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12 340 understand the physiological responses of enchytraeids to desiccation. Earthworms are known to be
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14 341 sensitive to drought and high temperatures (Bayley et al., 2010; Lima et al., 2015; González-Alcaraz
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16 342 & van Gestel, 2016), but they have adaptive strategies such as diapause or other physiological
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18 343 mechanisms, such as an increase in the free amino acid alanine, to survive long periods of drought
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20 344 (Holmstrup et al., 2016; Jiménez & Decaëns, 2004). Thus, in the actual context of climatic
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22 345 disturbances and knowing the potential effects of combined stressors on soil organisms (e.g.,
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24 346 Bindsbøl et al., 2005; Jensen et al., 2009; Singh et al., 2019), the equilibrium between species and
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26 347 taxa could be modified in natural ecosystems in the future. Maraldo et al. (2006) noted that the
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28 348 synergistic effects of climate change factors and environmental contamination on enchytraeids have
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30 349 been insufficiently explored. Further studies should assess the effects of climatic parameters on
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32 350 functionally useful soil organisms and their relative sensitivity to desiccation or heat in realistic
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34 351 contexts.

352 We also know that enchytraeids are tolerant of soil acidity (Römbke, 1991), whereas earthworm
353 abundance and diversity are generally low in acidic soils (Chan & Mead, 2003, Moore et al., 2013, Wu
354 and al., 2020). Enchytraeids exhibit high tolerance to other soil properties (clay and organic matter
355 contents) (Kuperman et al., 2006; van Gestel et al., 2011, Castro-Ferreira et al., 2012) and temperature
356 (Holmstrup et al., 2022). However, once again, there is no real comparison to date. The combined
357 effects of stressors related to soil properties and climate were examined by Yli-Olli and Huhta (2000),
358 who assessed the co-occurrence of soil pH, moisture and resource addition on *Dendrobaena octaedra*
359 (Lumbricidae) and *Cognettia sphagnetorum* (Enchytraeidae). They found that *D. octaedra* exhibited

360 the highest biomass in spruce stand humus at higher pH values, while *C. sphagnetorum* thrived best at
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2 361 higher moisture levels.
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5 362 6. Conclusion 6

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8 363 For the first time, this exhaustive synthesis provides a quantitative comparison of the sensitivity of
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10 364 earthworms versus enchytraeids to extrinsic factors (such as metals and pesticides) potentially
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12 365 affecting soil organisms. Under laboratory conditions, comparable sensitivities were found for
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14 366 earthworms and enchytraeids regardless of the studied species, or endpoint. Therefore, to test the
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16 367 effects of metals or pesticides, we could recommend testing on one of these groups in order to limit
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18 368 costs and labour. However, for other stressors such as climate parameters and for a thorough
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20 369 understanding of soil functioning, it remains crucial to consider the physiological responses of both
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22 370 groups due to their distinct ecological roles and potential sensitivity difference. Moreover, to fully
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24 371 capture the complexity of natural habitats, it would be relevant to use species inhabiting mineral soils
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26 372 instead of model species commonly found in organic rich habitats. For decades, researchers have been
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28 373 deepening their understanding of the impacts of natural and anthropic stressors on soil fauna and their
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30 374 related functions, which has allowed to reveal the importance of maintaining the presence and function
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32 375 of biodiversity in our soils. Let's keep digging in that direction.
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38 376 Acknowledgements 39 40

41 377 The authors thank the PACA region and INRAE AgroEcoSystem Department for PhD funding. The
42
43 378 authors also thank David Makowski and Guillaume Cinkus for useful advice with the R scripts.
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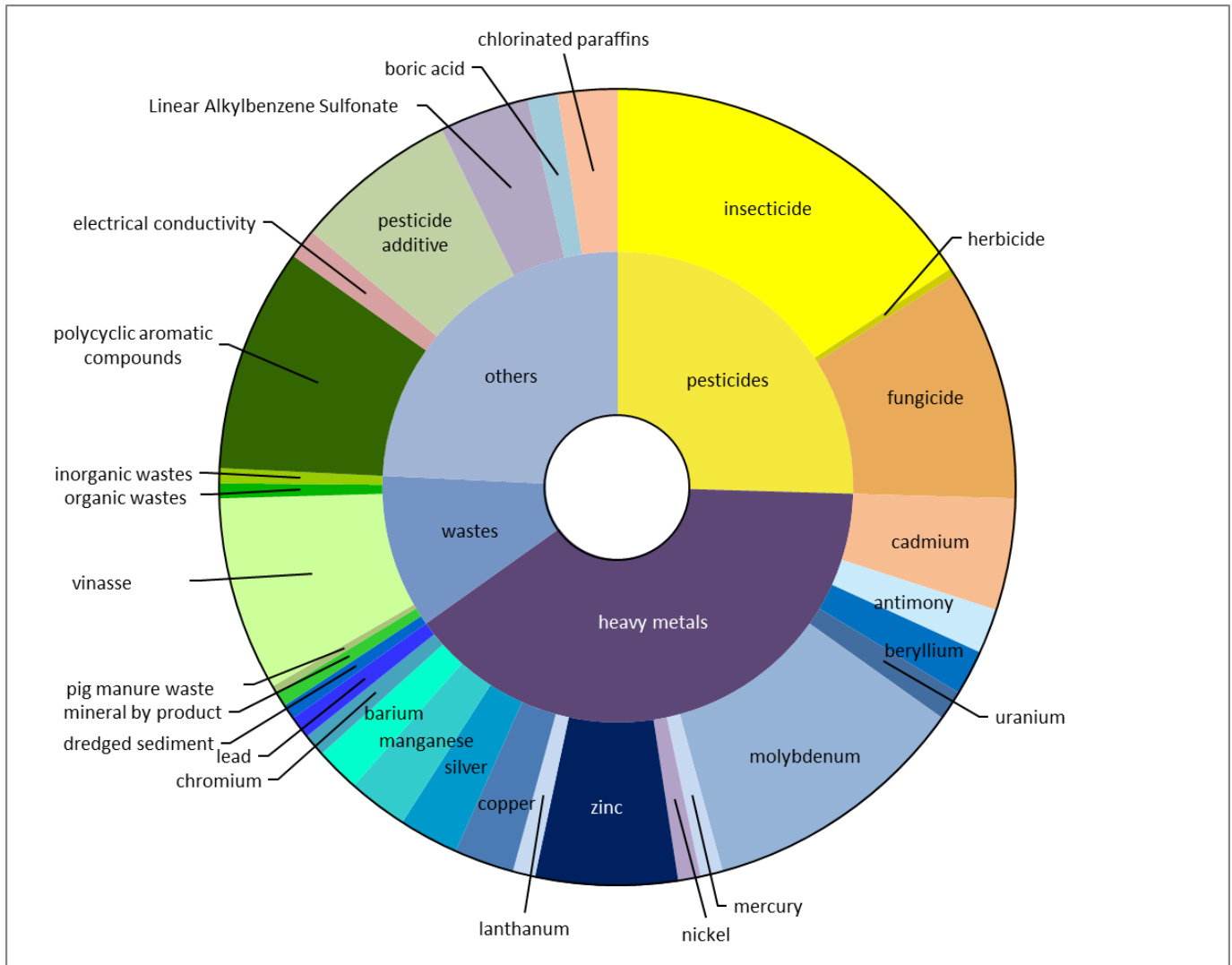
1 **Figure captions – EVERY FIGURES IN COLOR PLEASE**

2 Figure 1. Relative coverage of different stressors tested for the earthworms and enchytraeids
3 sensitivity comparisons, in 330 observations from 49 studies. Sunburst diagram representing
4 proportions of observations for each category.

5
6 Figure 2. Log ratio of the mean effect size (in bold) and its 95% confidence interval (in square
7 brackets) for (a) all stressors, and (b) the different stressor types separately: pesticides, metals, wastes
8 and others stressors. The number of observations and papers are into round brackets. Error bars
9 correspond to 95% confidence intervals computed either from the standard deviations reported by the
10 authors when available (292 data for earthworm; 293 data for enchytraeids) or from the standard
11 deviation using the worst-case scenario (38 data for earthworms; 37 data for enchytraeids). On the left
12 of the 0: higher sensitivity of enchytraeids; on the right: higher sensitivity of earthworms.

13
14 Figure 3. Log ratio of the mean effect size (in bold) and its 95% confidence interval (in square
15 brackets) for (a) survival endpoints, and (b) reproduction endpoints. The number of observations and
16 papers are into round brackets. Error bars correspond to 95% confidence intervals computed either
17 from the standard deviations reported by the authors when available (314 data for earthworm; 309 data
18 for enchytraeids concerning reproduction data; 316 data for earthworm; 318 data for enchytraeids
19 concerning survival data) or from the standard deviation using the worst-case scenario (16 data for
20 earthworms; 21 data for enchytraeids concerning reproduction data; 14 data for earthworms; 14 data
21 for enchytraeids concerning survival data). On the left of the 0: higher sensitivity of enchytraeids; on
22 the right: higher sensitivity of earthworms.

23 Figure 1



24

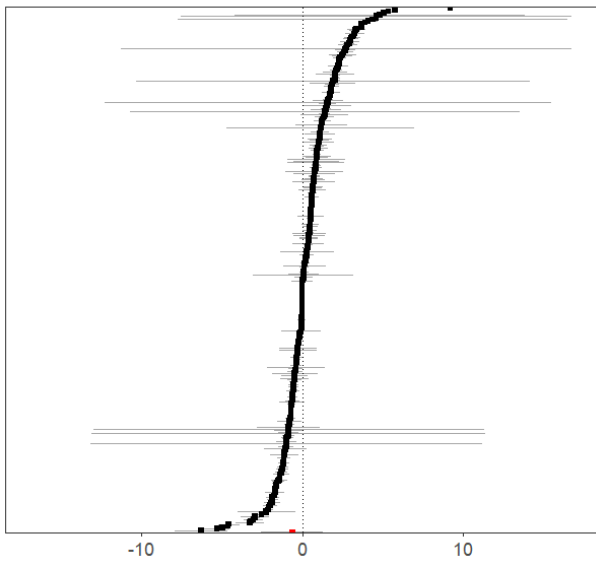
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26 Figure 2

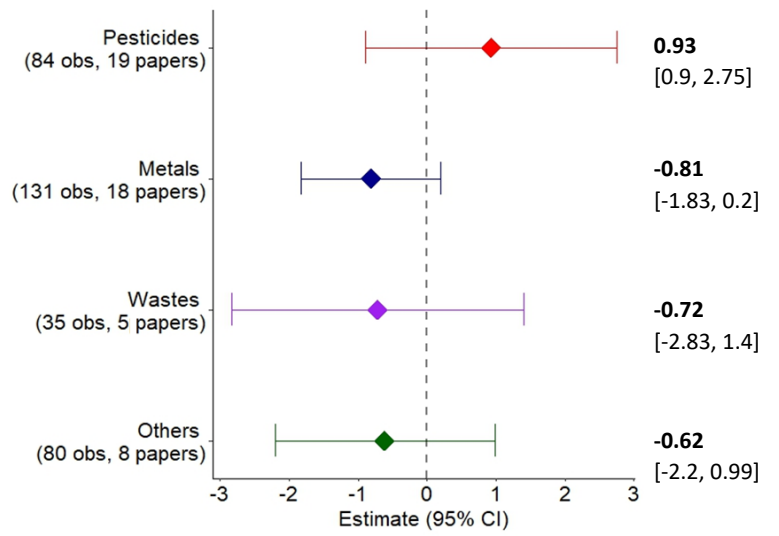
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(a)

All data (330 obs, 49 papers) | **-0.61** [-2.53, 1.31]

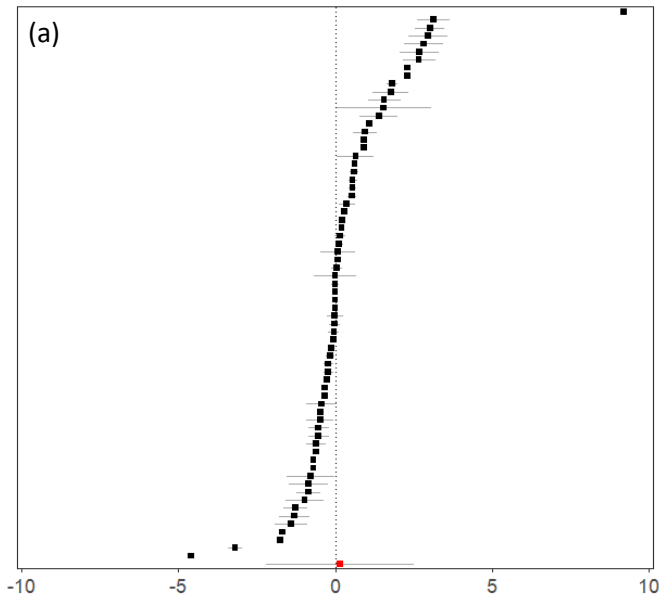


(b)

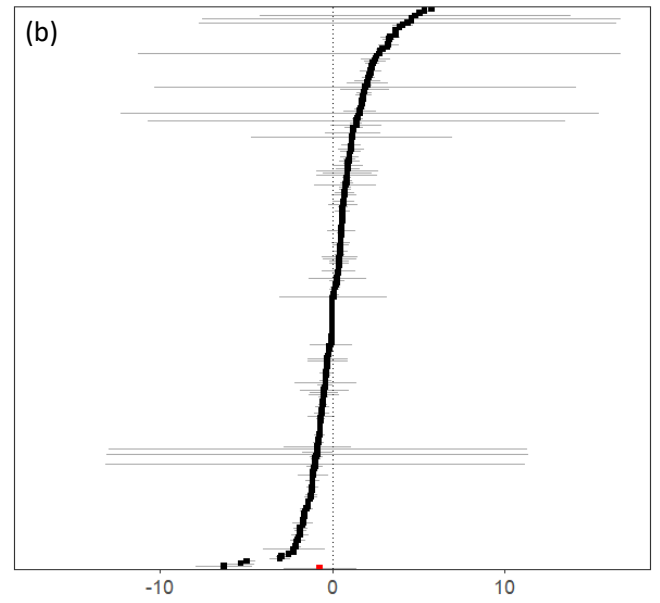


29 Figure 3

Survival data (69 obs, 20 papers) | **0.16** [-2.19, 2.51]



Reproduction data (235 obs, 43 papers) | **-0.72** [-2.83, 1.4]



30

31

1 TABLES

2 Tab.1. Summary table of the number of observations relating to the different endpoints used for the
 3 comparisons of the sensitivity of earthworms and enchytraeids in 330 observations from 49 studies.
 4 EC_x Effect Concentration; LC_x Lethal Concentration; NOEC No Observed Effect Concentration;
 5 LOEC Lowest Observed Effect and NEC No Effect Concentration.

EC _x	Number of observations	LC _x	Number of observations	Other	Number of observations
EC ₅₀	106	LC ₅₀	37	NOEC	68
EC ₁₀	44	LC ₁₀	10	LOEC	36
EC ₂₀	23			NEC	2
EC ₂₅	4				

6

7

8 Tab.2. Summary table of the repartition of the species (in percent) used in for the comparisons of the
 9 sensitivity of earthworms and enchytraeids in 330 observations from 49 studies.

Earthworm species	%	Enchytraeid species	%
<i>Eisenia andrei</i>	48	<i>Enchytraeus crypticus</i>	73
<i>Eisenia fetida</i>	41	<i>Enchytraeus albidus</i>	27
<i>Dendrobaena veneta</i>	5	<i>Enchytraeus luxuriosus</i>	<1
<i>Aporrectodea caliginosa</i>	4		
<i>Aporrectodea longa</i>	2		

10

Declaration of interests

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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:



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1. Introduction

Earthworms and enchytraeids are macro- and meso-organisms, respectively, that perform several key functions in soils. At different spatial and temporal scales, both taxa are involved in litter fragmentation and organic matter decomposition (Marinissen & Didden, 1997, Brown et al., 2000), soil nutrient dynamics (Lavelle et al., 1998; Cole et al., 2002), soil formation (Lavelle et al., 1988, Topoliantz et al., 2000), soil structure dynamics via bioporosity (van Vliet et al., 1993, Edwards & Bohlen 1996), and regulation of microbial biomass (Hedlund & Augustsson, 1995, Brown et al., 2000). Because they play key roles in the functioning of agroecosystems and are abundant in many ecosystems, earthworms and enchytraeids are both used as bioindicators of land use, agricultural practices and chemical stress (OECD, 1984, Paoletti, 1999, Didden & Römbke, 2001, Pelosi & Römbke, 2017).

The ecological niches and behaviours of these two groups do not necessarily overlap (Didden et al., 1997): For example, the earthworm *Lumbricus terrestris* creates extensive burrows that improve water infiltration. In contrast, enchytraeids, such as *Enchytraeus albidus*, play a crucial role in nutrient cycling by decomposing finer organic materials in the soil, their activities are more concentrated in the upper soil layers and leaf litter. Therefore, they can provide complementary functions in soils and complementary information on soil quality (Postma-Blaauw et al., 2012). Under natural conditions, both earthworms and enchytraeids face stressors of different origins. In agricultural soils, tillage or chemical inputs such as pesticides may harm these soil organisms (van Capelle et al., 2012, Beketov et al., 2013). Moreover, climate change has increased the vulnerability of soils and terrestrial biodiversity (IPCC 2022). Phillips et al. (2019) showed that climate variables were more important than soil properties or habitat cover in shaping earthworm communities. Drought periods and intense precipitation events now occur more frequently and cause dramatic disruptions to soil biological, physical and chemical parameters (Bellard et al., 2012). Thus, the extreme variations in soil moisture as well as the exposure to soil tillage and high quantities of chemicals—directly applied to the soil

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7 26 surface—can disturb these organisms, their metabolism and the interactions between them (Mäder et
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9 27 al., 2002, Bengtsson et al., 2005).

10
11 28 Earthworms and enchytraeids are both soft-bodied soil invertebrates, and enchytraeids are considered
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13 29 the closest relatives to earthworms according to Erséus and Källersjö (2004). Enchytraeids have been
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15 30 compared to other soil meso-organisms, such as springtails (Urarov & Karaban, 2015, Hlavkova et al.,
16 31 2020) or nematodes (Hagner et al., 2010), more often than to earthworms. Moreover, in some studies,
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18 32 different endpoints have been assessed for enchytraeids and earthworms to obtain additional data on
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20 33 the effects of stressors (Niva et al., 2021); therefore, the sensitivities of these two organismal groups
21 34 cannot be compared. Among the studies that have properly compared earthworms and enchytraeids,
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23 35 some have shown that enchytraeid species exhibit a lower sensitivity than earthworms to certain
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25 36 chemicals and climatic parameters. In Bart et al. (2017), earthworms experienced a decrease in
26 37 biomass, and they avoided soil contaminated with a copper fungicide at a lower concentration than
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28 38 enchytraeids. Holmstrup et al. (2022) noted that, in Arctic areas, the enchytraeid *Enchytraeus albidus*
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30 39 individuals had superior freezing tolerance than earthworms. Moreover, Sverdrup et al. (2002)
31 40 reported that *Enchytraeus crypticus* exhibited a lower sensitivity to polycyclic aromatic compound
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33 41 exposure than the earthworm *Dendrobaena veneta*; however, their respective sensitivities can vary
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35 42 according to the compound itself. In contrast, enchytraeids reportedly exhibit a greater sensitivity to
36 43 pesticides (e.g., Sechi et al., 2014 – insecticide alpha-cypermethrin) and pig manure (Segat et al.,
37
38 44 2020) than earthworms. Finally, Römbke and Moser (2002), based on the results of an international
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40 45 ring test involving 29 institutions from 15 European countries, noted that there was no clear answer as
41 46 to the sensitivity of the species tested (*Eisenia fetida*/*Eisenia andrei* or *E. albidus*) to two chemicals
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43 47 (the fungicide carbendazim and the industrial chemical 4-nitrophenol). The authors concluded that
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45 48 there was no "most sensitive species". Other studies comparing earthworms to enchytraeids have
46 49 shown similar sensitivities between both soil organisms through a wide range of stress factors, such as
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48 50 uranium (Caetano et al., 2014), electrical conductivity (Gainer et al., 2019), polycyclic aromatic
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50 51 compounds (Kobeticova et al., 2008) and lindane, an insecticide (Lock et al., 2002). A report
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52 52 published in 2009 by Jarratt and Thompson showed mixed conclusions on the sensitivities of

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7 53 enchytraeid and earthworm species to chemicals, although they suggested that the mortality test results
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9 54 indicate that Enchytraeidae species may be less sensitive than earthworms. They reported a lower
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11 55 sensitivity for enchytraeids in some cases but noted that the current data do not show consistent
12 56 differences in sensitivity between Lumbricidae and Enchytraeidae in the laboratory or under
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14 57 semifield/field conditions. To summarize, the message is not clear, perhaps due to the non-quantitative
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16 58 nature of all these assessments.

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18 59 The aim of this study was to quantify the relative sensitivities of earthworms and enchytraeids to all
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20 60 kinds of stressors (i.e., any factor likely to negatively affect earthworms and enchytraeids at any level
21 61 of biological organization). For that purpose, we performed a meta-analysis based on an exhaustive
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23 62 bibliographical review that included all the studies using both earthworms and enchytraeids under the
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25 63 same test conditions (e.g., same soil, tested stressor). We determined the difference in sensitivity to
26 64 specific stressors according to the following assumptions: **(H1)** enchytraeids are generally less
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28 65 sensitive than earthworms to different stressors, **(H2)** differences in sensitivity are mainly observed
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30 66 when mortality is assessed compared to reproduction or avoidance, and **(H3)** the relative sensitivities
31 67 of both groups of organisms vary according to the tested stressor and the considered species. Our
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33 68 findings highlight the specific gaps in the literature regarding the sensitivity of these organisms to
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35 69 different stressors, emphasizing the need for future research to address these gaps.

36 37 70 2. Methods

38 39 71 2.1. Literature search and inclusion/exclusion criteria

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41 72 A systematic literature review was conducted in January 2023 to find publications dealing with
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43 73 earthworms and enchytraeids exposed to different stressors. The literature search was carried out on
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45 74 the basis of keywords in the ISI Web of Knowledge, using the ‘‘All Databases’’ option, with the
46 75 following scheme: ‘(enchytr* OR potworm* OR achaet* OR bryodril* OR buchlol* OR
47
48 76 cernosvitoviel* OR cognat* OR frideric* OR guaranidril* OR lumbricil* OR hemienchytr* OR
49
50 77 oconnorel* OR hemifrideric* OR henl* OR marionin* OR mesenchytr*) and (earthworm* OR
51 78 lumbric* OR aporrect* OR eisen* OR dendrob* OR alloloboph* OR octalas* OR dendrodri* OR

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7 79 diporodril* OR eophil* OR helodril* OR kritodril* OR octodril* OR prosellodril* OR scherothec*
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9 80 OR satchell* OR proctodril* orodril* OR postandril* OR perelia* OR andrei* OR fetid* OR foetid*)
10
11 81 in Topics. From a total of 967 references, a first selection was made using titles and abstracts. The full
12 82 texts were examined when the information was considered consistent for the meta-analysis. To
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14 83 complete the search, bibliographic reviews that included both annelids were selected, and references
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16 84 were carefully examined to extract any new articles that could be relevant for the meta-analysis.
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18 85 We considered only publications providing data on terrestrial earthworms and enchytraeids, all species
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20 86 included. For instance, papers dealing with aquatic and bioluminescent worm species were excluded
21 87 (Rodionova et al., 2017). The data on earthworms and enchytraeids had to be from the same study to
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23 88 compare the sensitivity of these groups under the same conditions (e.g., active substance, type of
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25 89 substrate). Moreover, we did not consider studies dealing with chemical bioaccumulation in animals,
26 90 as bioaccumulation cannot be easily correlated with certain factors (Tosza et al., 2010; Santorufo et al.,
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28 91 2012). We selected only the studies where the tested factors were supposed to be harmful to soil
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30 92 organisms, specifically those detrimental to their life cycle parameters (e.g., survival, growth,
31 93 fecundity, juvenile survival). We did not consider studies on biochar addition (possibly beneficial to
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33 94 them, e.g., Briones et al., 2020) or on tillage (Engell et al., 2021) because these factors can be
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35 95 beneficial to enchytraeid populations (e.g., Pelosi & Römbke, 2016). Regarding organic and inorganic
36 96 waste, one paper was kept because it focused on contaminated incineration ash (Kobetičová et al.,
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38 97 2010). The final set was composed of 49 publications (i.e., 48 papers and one PhD thesis), which
39
40 98 included 330 lines in the database (Table S1).

41 42 99 2.2. Data extraction 43

44 100 For each of the 49 studies, the following data were recorded (Table S1):
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- 46 101 - author, date of publication,
- 47
48 102 - stressor type (i.e., pesticides, metals, wastes, others);
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50 103 - stressor subcategory (i.e., for pesticides: fungicide, herbicide and insecticide; for metals:
51 104 antimony, barium, beryllium, cadmium, chromium, copper, lanthanum, manganese, mercury,
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105 molybdenum, nickel, lead, silver, uranium and zinc; for wastes: inorganic waste, organic waste,
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9 106 vinasse, pig manure waste, mineral byproducts, and dredged sediment; for others: pesticide
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11 107 additive, boric acid, electrical conductivity, linear alkylbenzene sulfonate, chlorinated paraffins
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13 108 and polycyclic aromatic compounds);
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15 109 - earthworm species (i.e., *Aporrectodea caliginosa*, *Aporrectodea longa*, *Dendrobaena veneta*,
16 110 *Eisenia andrei* and *Eisenia fetida*) and enchytraeid species (i.e., *Enchytraeus albidus*,
17 111 *Enchytraeus crypticus* and *Enchytraeus luxuriosus*);
18
19 112 - the observed variable (i.e., survival, reproduction, avoidance, growth, population abundance)
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21 113 and its measured endpoint (LC_x: lethal concentration; EC_x: effect concentration; NOEC: no
22 114 observed effect concentration; NEC: no effect concentration; and LOEC: lowest observed
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24 115 effect concentration);
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26 116 - the mean value of the endpoints with the standard error, standard deviation, or the respective
27 117 95% confidence intervals;
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29 118 - and the number of replicates.

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31 119 As the choice was made to select studies that provided ecotoxicological endpoint values to ease the
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33 120 comparison between earthworms and enchytraeids, all the selected studies were laboratory
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35 121 experiments.

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37 122 First, the data were collected from available tables; otherwise, the data were collected from the text.
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39 123 Searching for data in the figures (graphs) was not ruled out, but none of the data matched our search
40 124 criteria. Because of compliance with ISO standards (ISO, 1998, OECD, 2015), some methodological
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42 125 differences were tolerated when comparing data for earthworms and enchytraeids. For example, in
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44 126 reproduction tests, cocoons or juveniles were counted for earthworms, while the number of juveniles
45 127 was counted for enchytraeids. The duration of the experiment was also not the same for standard tests
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47 128 of earthworms and enchytraeids, i.e., 21 and 42 days, respectively.

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49 129 For both earthworms and enchytraeids, when the endpoint value was not given as a precise number but
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51 130 was bounded by > or <, the data were adjusted as follows: in the case of LC₅₀ > x, the data were not

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7 131 considered to indicate that the sensitivity of the organism would be overestimated by considering x as
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9 132 the LC₅₀ value. In the case of LC₅₀ < x, the x value was used, and it is conservative to consider x as the
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11 133 LC₅₀ value. This latter case represented 20 of the 330 observations in the database (i.e., 6% of the
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13 134 data).

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15 135 Four comparisons involving boric acid were found in two studies (Becker et al., 2011, Niemeyer et al.,
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17 136 2018). Becker et al. (2011) characterized boric acid as a fertilizer and fungicide, but Niemeyer et al.
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19 137 (2018) did not mention any role or expected effect. Boric acid can be used as an insecticide, acaricide,
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21 138 herbicide, or fungicide (Büyükgüzel et al., 2013). Since it can be placed in different subcategories
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23 139 (insecticide, fungicide, and fertilizer), it was placed into the "others" category.

24 140 2.3. Effect size calculation

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26 141 When the standard deviation (SD) was not given directly from a table, it was calculated with the t
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28 142 distribution for small samples (n<60) using the following formula (Higgins & Green, 2011):

$$29 \quad SD = \sqrt{n} * \frac{\text{upper limit CI} - \text{lower limit CI}}{t_{\alpha,df} * 2}$$

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33 144 With $t_{\alpha,df}$, the T.INV.2T functions in syntax with two associated arguments: probability, associated
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35 145 with Student's t-distribution (here, the alpha=0.05 to respect the 95% CI from extracted data), and
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37 146 degrees of freedom (equal to the group sample size - 1).

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39 147 When neither the 95% CI nor the SD were given, we used the worst-case scenario, i.e., the highest SD
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41 148 calculated for the same endpoint in the database (LC₁₀, LC₅₀, EC₁₀, EC₂₀, EC₅₀). NOEC and LOEC
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43 149 values have no variance, so their standard deviation (SD) was set to zero. When the number of
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45 150 replicates was not the same between the treatments and the control, the lower number was considered
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47 151 to be conservative (i.e., less weight was given to the study). When the number of replicates was not
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49 152 given and the tests referred to ISO standards, we considered the number of replicates advised in
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51 153 official procedures (e.g., ISO, 1998, OECD, 2015). The confidence intervals (CIs) for each estimate of
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53 154 effect size were calculated using bias-corrected bootstrapping methods, which corrects for
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55 155 nonnormality of the data and nonconstant standard errors (Efron & Hastie, 2016).

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7 Hedge's d is a standardized mean difference that accounts for the potential bias in estimating
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9 population effect size (Hedges & Olkin, 1985). The magnitude of the difference between earthworm
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11 and enchytraeid sensitivities was calculated with the Hedge metric using the natural logarithm of the
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13 response ratio (LRR) as follows (van Groenigen et al., 2014):

$$14 \quad LRR = \ln\left(\frac{x_i}{x_u}\right)$$

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17 with x_i and x_u being the values of the enchytraeid and earthworm endpoints, respectively. Therefore,
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19 when the LRR equals zero, the two organisms have the same sensitivity.

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21 The LRR variance (V) was calculated as follows (van Groenigen et al., 2014):

$$22 \quad V = \frac{1}{n_i} * \left(\frac{SD_i}{x_i}\right)^2 + \frac{1}{n_u} * \left(\frac{SD_u}{x_u}\right)^2$$

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24 where x_i and x_u are the mean values of the endpoints for earthworms and enchytraeids, respectively;
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3. Meta-analysis

3.1. General modelling approach

Every model incorporated a random effect associated with the study's identity to accommodate correlated data arising from the same study (Viechtbauer, 2010). The models were selected using the Akaike information criterion (AIC). This criterion represents a compromise between bias decreasing with the number of free parameters and parsimony, the desire to describe the data with as few parameters as possible. Lower AIC values indicate a better-fit model; therefore, for each calculation, the smallest AIC was considered for the LRR calculation. Random effect models were tested for the lines (to allow the real effect to vary from one dataset to another), the authors (to allow the actual

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7 179 effect to vary from one article to another), and a combination of the two (Borenstein et al., 2010). A
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9 180 combination of the two modalities was associated with a lower AIC for every case, and these models
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11 181 were systematically used. For each model, we used the restricted maximum likelihood (REML)
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13 182 method, which aims to provide unbiased estimates of variance components by accounting for fixed
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15 183 effects in the model. All analyses were performed with R Software (R Development Core Team, 2014)
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17 184 using the metafor package (Viechtbauer, 2010).

18 185 3.2. Analysis

19
20 186 First, a meta-analysis was carried out using estimated overall effect size in a multivariate linear model,
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22 187 calculated using the `rma.mv` function from the metafor package in R (Viechtbauer, 2010). In the
23
24 188 context of a meta-analysis, the effect size (with its 95% confidence interval) is a standardized measure
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26 189 that quantifies the magnitude of the treatment effect or association across all included studies. Then,
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28 190 the logarithm of the response ratio (LRR) was calculated to explore, through earthworm and
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30 191 enchytraeid endpoints, the results of this model on all the data, all the factors and all the species
31
32 192 combined. This main model enabled us to test our first assumption (H1).

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34 193 We also compared earthworm and enchytraeid data by splitting them according to the studied
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36 194 endpoints (i.e., reproduction or survival rate) to test the second assumption (H2). We further
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38 195 investigated the effects of the different stressors (H3) on the response ratio (LRR) by stressor category,
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40 196 a moderator with 4 levels: pesticides, metals, wastes, and others. We finally investigated the potential
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42 197 "species effect" of the most represented earthworm and enchytraeid species (H3), using the response
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44 198 ratio of the earthworm species categorical moderator at 2 levels (i.e., *E. andrei* and *E. fetida*, which
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46 199 accounted for 48% and 41%, respectively; Table S1), coupled with the enchytraeid species categorical
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48 200 moderator at 2 levels (i.e., *E. albidus* and *E. crypticus*, which accounted for 27% and 73%,
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50 201 respectively; Table S1).

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52 202 Publication bias was assessed by Egger's regression test for funnel plot asymmetry between the
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54 203 standard normal deviation of the effect sizes and precision (i.e., inverse of their standard errors). We
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56 204 retained the multilevel linear model `rma.mv` by adding a standard error moderator. This test is

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7 205 powerful given the fairly large number of studies. The findings demonstrated the absence of
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9 206 publication bias: we detected no significant asymmetry (z -value = -0.62; p value = 0.53) in the funnel
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11 207 plot (Fig. S1), as none of the intercepts from Egger's regression exhibited a significant deviation from
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13 208 zero.

14 15 209 4. Results

16 17 210 4.1. Data description

18 19 211 Location Fig. 1

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21 212 The 49 selected studies addressed a total of 30 different stressors (Figure 1) classified into four
22
23 213 different categories. "Metals" accounted for 39.7% of the observations, with the most represented
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25 214 being molybdenum (2 studies, 36 observations) and zinc (2 studies, 19 observations). "Pesticides"
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27 215 represented 25.5%, with subcategories including insecticides (13 studies, 52 observations), fungicides
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29 216 (6 studies, 31 observations) and herbicides (1 study, 1 observation). Among all the observations,
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31 217 14.2% were classified into the "Wastes" category, which is widely represented by vinasse (1 study, 26
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33 218 observations). Finally, "Others" accounted for 20.6% of the observations, with polycyclic aromatic
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35 219 compounds (2 studies, 30 observations) and pesticide additives (1 study, 22 observations) being the
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37 220 most represented. In total, 98.8% of the data concerned contaminant tests (all the stressors studied
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39 221 except for the 4 observations for the electrical conductivity), even though no restrictions were imposed
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41 222 during the literature search.

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43 223 The 49 selected studies reported reproduction endpoints in 71.2% of the cases (43 studies, 235
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45 224 observations), survival in 20.9% (20 studies, 69 observations), avoidance in 4.54% (3 studies, 15
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47 225 observations), growth in 2.4% (1 study, 8 observations), and population abundance in 1% (1 study, 3
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49 226 observations), with EC₅₀ (106 observations) and NOEC (68 observations) calculations dominating
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51 227 (Table 1).

52 53 228 Location Tab.1

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In total, five earthworm species and three enchytraeid species were used in the comparisons (Table 2). Together, *E. andrei* and *E. fetida* accounted for 89% of the total number of observations. *E. crypticus* was the most represented enchytraeid species among the observations.

Location Tab.2

4.2. Meta-analysis results

When considering all the stressors together, earthworms and enchytraeids were found to be equally sensitive, as the mean effect size was -0.61 [-2.53, 1.31] (Fig. 2a). The results were considered non-significant because the confidence interval crosses zero, indicating no statistically significant effect. Similarly, exploration of the data across the four stressor types yielded nonsignificant results (Fig. 2b), with each confidence interval intersecting zero.

Location Fig.2

Similarly, when the data on mortality (i.e., LC₁₀, LC₅₀, and some NOEC and LOEC data) and those on reproduction (i.e., some EC₁₀, EC₂₀, EC₂₅, EC₅₀, LOEC, NEC and NOEC) were considered separately (Fig. 3), no difference in sensitivity was found between earthworms and enchytraeids.

Location Fig3

The sensitivities of the different species were assessed when the dataset was statistically sufficient for comparison. Therefore, *E. andrei* (earthworm) data were compared with *E. crypticus* (enchytraeid) data, *E. andrei* with *E. albidus* (enchytraeid), *E. fetida* (earthworm) with *E. albidus* and *E. fetida* with *E. crypticus*. The sensitivities of these species were similar (Fig. S2).

5. Discussion

This study allowed us to quantitatively compare the relative sensitivities of earthworms and enchytraeids to different types of stressors. On the basis of 49 published studies and 330 observations, we revealed similar sensitivities to stressors in these two soil organisms (H1). The conclusion was the same when considering the different stressors, species or endpoints separately (H2 and H3). Although

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7 the differences were not significant, enchytraeids tended to be slightly more sensitive to metals,
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9 pesticide additives and electrical conductivity. In contrast, earthworms tended to be slightly more
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11 sensitive to pesticides regardless of their mode of action (fungicide or insecticide), but the differences
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13 were still nonsignificant. Large confidence intervals were found for all mean effect sizes. This
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15 variability was probably due to the variety of test conditions used for the observations; in particular,
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17 the tested substances revealed alternating greater sensitivities of enchytraeids or earthworms.
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19 Moreover, the high number of studies with no information on data uncertainty (e.g., standard
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21 deviation) (i.e., 58 observations) could explain the absence of significant differences since when
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23 neither the 95% CI nor the SD was given, we used a conservative approach by giving the highest SD
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25 calculated for the same endpoint in the database.

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27 These results are not in accordance with our assumptions that enchytraeids are less sensitive than
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29 earthworms to different stressors (H1), that differences in sensitivity are mainly observed when
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31 mortality is assessed (H2), and that the relative sensitivity of both groups of organisms varies
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33 according to the tested stressor and the considered species (H3). Enchytraeids, which are smaller than
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35 earthworms and 80% microbivorous and 20% saprovorous (Didden, 1990, 1993), ingest less material
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37 and smaller particles than earthworms in soils and thus could be less exposed to chemicals (Tourinho
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39 et al., 2021, Coleman et al., 2004). However, the relationship between size and sensitivity is debatable,
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41 as earthworms are much larger than springtails but are less sensitive to all kinds of pesticides, such as
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43 formulations or active substances (Joimel et al., 2022). Moreover, in the same study (Joimel et al.,
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45 2022), differences in sensitivity were reported between springtails and enchytraeids, but both of these
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47 taxonomic groups belong to the same size class (i.e., soil mesofauna). Small animals have a greater
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49 surface area-to-volume ratio than larger organisms and are thus potentially more exposed to external
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51 stressors. Regarding chemicals, the size class would be of particular importance if the main route of
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53 exposure was dermal contact. Enchytraeids live in close contact with the soil porewater fraction, and
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55 their routes of exposure are dermal, intestinal and respiratory and earthworms one is dermal but also
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57 dietary exposure (Lock & Janssen, 2003, Römbke, 2003, Hirano & Tamae, 2011). Earthworms, which
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59 might be thought to be more sensitive to chemicals at first glance due in particular to their larger size,
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7 280 can ultimately be less exposed than small enchytraeids in soils. Both earthworms and enchytraeids
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9 281 must maintain a moist body surface to facilitate oxygen absorption through their tegument, which is
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11 282 highly permeable to water. Pesticide characteristics (e.g., polarity, solubility in water) influence the
12 283 available concentrations in water and in soils, leading to varying sensitivities among taxonomic
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14 284 groups. In accordance with our results, Römbke and Moser (2002) concluded that the overall range of
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16 285 sensitivities of oligochaetes to chemicals was usually quite similar. These authors linked the lack of
17 286 differentiation between the sensitivities of *E. albidus* and *E. fetida* exposed to carbendazim to the high
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19 287 toxicity of this persistent compound. More research should be dedicated to quantifying the different
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21 288 routes of exposure to chemicals for soil organisms to better understand their respective sensitivities in
22 289 different situations. Moreover, both earthworms and enchytraeids can be considered in risk
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24 290 assessments, and while earthworms are most commonly used, there are many reasons to support the
25
26 291 use of enchytraeids in testing. Enchytraeids can thrive in soils where earthworms are scarce (Jarratt &
27 292 Thompson, 2009). Moreover, Römbke et al. (1994) encouraged the use of enchytraeids when
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29 293 comparing laboratory results with those obtained under semifield and field conditions, as the same
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31 294 species (or closely related species) can be used at all test levels. Other practical and ecological points
32 295 (ease of handling and rearing, short generation time, reduced soil requirements, cost-effectiveness)
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34 296 make them good organisms for inclusion in laboratory experiments (Römbke & Moser, 2002).
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36 297 Among the tested species, the earthworm *Eisenia* sp. represented 89% of all species included in the
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38 298 selected studies. *E. fetida* and *E. andrei* are composting worms that are not representative of mineral
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40 299 soils and are less sensitive to pesticides than species found in cultivated fields (Pelosi et al., 2013).
41 300 However, these species are recommended as standard test organisms in various guidelines, such as
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43 301 those of the OECD (OECD, 1984) and ISO (ISO, 1993, 1998, 2019), and have been widely used in
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45 302 published ecotoxicological studies, mainly because they are easy to rear. A few studies have used
46 303 species that are found in agricultural soils. In Bart et al. (2017), the LC₅₀ and EC₅₀ values were
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48 304 systematically lower for *A. caliginosa* (earthworm) than for *E. albidus* (enchytraeid) (Table S1). In
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50 305 Holmstrup and Krogh (2001), the opposite occurred, with systematically higher LC₅₀ and EC₅₀ values
51 306 for *A. caliginosa* and *A. longa* (earthworm) than for *E. albidus* (Table S1). Thus, the results should be

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7 307 contrasted according to the tested stressor, and additional data are needed to assess the sensitivity of
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9 308 earthworms and enchytraeid species commonly found in the field.

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11 309 The choice was made here to select studies that provided ecotoxicological endpoint values to facilitate
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13 310 comparisons between earthworms and enchytraeids. Potentially, interesting studies have been
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15 311 excluded that compared both organisms. For instance, Amossé et al. (2018) assessed the short-term
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17 312 effects of two fungicides (Cuprafor Micro® made of copper oxychloride and Swing Gold® made of
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19 313 epoxiconazole and dimoxystrobin) on enchytraeid and earthworm communities under field conditions.
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21 314 They found greater negative effects on the diversity and community structure of earthworms than on
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23 315 those of enchytraeids. Similarly, Pereira et al. (2018) studied the degree to which seawater intrusion
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25 316 and irrigation threaten biodiversity via a terrestrial model ecosystem (TME) approach. They found that
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27 317 the abundance of only enchytraeids significantly decreased, although earthworms generally suffered.
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29 318 These field or semifield studies allowed us to investigate the sensitivity of several species found in
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31 319 natural soils under realistic conditions, which offers more comprehensive insight into the ecological
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33 320 dynamics of the systems (Wall & Moore, 1999). TMEs, which were proposed in the 1990 s, are useful
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35 321 experimental systems for assessing the impact of factors such as pollution or climate change on
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37 322 terrestrial ecosystems.

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39 323 Although the literature search was performed to include all kinds of stressors in this study, the great
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41 324 majority of the articles in our final selection concerned chemicals. Very few data are available on the
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43 325 effects of climatic parameters on soil organisms (Classen et al., 2015), and data comparing their
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45 326 responses to these parameters remain scarce (Otomo, 2011). However, earthworms and enchytraeids,
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47 327 in addition to all other soil organisms, are increasingly having to cope with climate change (Pritchard,
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49 328 2011). Warming, changes in precipitation regimes, and changes in the quantity and quality of soil
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51 329 carbon inputs can disrupt the habitat and food resources of soil-living organisms, affecting their
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53 330 survival and reproductive capabilities (Maraldo & Holmstrup, 2009). Some species, such as the
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55 331 enchytraeid *Cognettia sphagnetorum*, which has strong potential to recover from severe drought stress
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57 332 events, appear to have adaptive potential to climate parameters (Maraldo & Holmstrup, 2009). They
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59 333 can vertically migrate as a strategy for evading dry surface layers and high temperatures (Springett et

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7 334 al., 1970). However, these organisms were found to have no genetic adaptation to drought, as drought-
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9 335 exposed populations of enchytraeids had the same high sensitivity as those from unexposed control
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11 336 plots (Maraldo, 2009). *E. albidus* has a high osmotic pressure on its body fluids and can live in
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13 337 environments with strong fluctuations in moisture (Maraldo, 2009), which also enables *Fridericia*
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15 338 *galba* to survive in soil with a water retention capacity of less than 20% (corresponding to values
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17 339 below -9.8 bar) for more than 49 days (Dózsa-Farkas 1973). However, additional research is needed to
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19 340 understand the physiological responses of enchytraeids to desiccation. Earthworms are known to be
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21 341 sensitive to drought and high temperatures (Bayley et al., 2010; Lima et al., 2015; González-Alcaraz
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23 342 & van Gestel, 2016), but they have adaptive strategies such as diapause or other physiological
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25 343 mechanisms, such as an increase in the free amino acid alanine, to survive long periods of drought
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27 344 (Holmstrup et al., 2016; Jiménez & Decaëns, 2004). Thus, in the actual context of climatic
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29 345 disturbances and knowing the potential effects of combined stressors on soil organisms (e.g.,
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31 346 Bindesbøl et al., 2005; Jensen et al., 2009; Singh et al., 2019), the equilibrium between species and
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33 347 taxa could be modified in natural ecosystems in the future. Maraldo et al. (2006) noted that the
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35 348 synergistic effects of climate change factors and environmental contamination on enchytraeids have
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37 349 been insufficiently explored. Further studies should assess the effects of climatic parameters on
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39 350 functionally useful soil organisms and their relative sensitivity to desiccation or heat in realistic
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41 351 contexts.

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38 352 We also know that enchytraeids are tolerant of soil acidity (Römbke, 1991), whereas earthworm
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40 353 abundance and diversity are generally low in acidic soils (Chan & Mead, 2003, Moore et al., 2013, Wu
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42 354 and al., 2020). Enchytraeids exhibit high tolerance to other soil properties (clay and organic matter
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44 355 contents) (Kuperman et al., 2006; van Gestel et al., 2011, Castro-Ferreira et al., 2012) and temperature
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46 356 (Holmstrup et al., 2022). However, once again, there is no real comparison to date. The combined
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48 357 effects of stressors related to soil properties and climate were examined by Yli-Olli and Huhta (2000),
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50 358 who assessed the co-occurrence of soil pH, moisture and resource addition on *Dendrobaena octaedra*
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52 359 (*Lumbricidae*) and *Cognettia sphagnetorum* (*Enchytraeidae*). They found that *D. octaedra* exhibited

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7 the highest biomass in spruce stand humus at higher pH values, while *C. sphagnetorum* thrived best at
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9 higher moisture levels.

10 11 6. Conclusion 12

13 For the first time, this exhaustive synthesis provides a quantitative comparison of the sensitivity of
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15 earthworms versus enchytraeids to extrinsic factors (such as metals and pesticides) potentially
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17 affecting soil organisms. Under laboratory conditions, comparable sensitivities were found for
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19 earthworms and enchytraeids regardless of the studied species, or endpoint. Therefore, to test the
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21 effects of metals or pesticides, we could recommend testing on one of these groups in order to limit
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23 costs and labour. However, for other stressors such as climate parameters and for a thorough
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25 understanding of soil functioning, it remains crucial to consider the physiological responses of both
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27 groups due to their distinct ecological roles and potential sensitivity difference. Moreover, to fully
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29 capture the complexity of natural habitats, it would be relevant to use species inhabiting mineral soils
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31 instead of model species commonly found in ~~organic rich habitats~~ loosen organic materials. For
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33 decades, researchers have been deepening their understanding of the impacts of natural and anthropic
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35 stressors on soil fauna and their related functions, which has allowed to reveal the importance of
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37 maintaining the presence and function of biodiversity in our soils. Let's keep digging in that direction.

38 Acknowledgements

39 The authors thank the PACA region and INRAE AgroEcoSystem Department for PhD funding. The
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41 authors also thank David Makowski and Guillaume Cinkus for useful advice with the R scripts.

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