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1 Combined effects of annual crop agricultural practices on earthworm communities

2

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13

14 **Abstract**

15 Earthworm communities are subjected to a great variety of agricultural practices in cropping  
16 systems. Most studies that focus on the impacts of agricultural practices on earthworm  
17 communities have been carried out on a small combination of practices (e.g., tillage, pesticides,  
18 fertilization...) while their simultaneous effects in the field remain little known. In this context,  
19 the aim of the present study was to jointly evaluate the effects of (i) tillage (ploughing vs  
20 reduced tillage), (ii) fertilization (mineral and organic) and (iii) pesticide applications  
21 (herbicides and a combination of insecticides, fungicides and molluscicides) on earthworm  
22 communities. Abundance, biomass and richness of earthworm communities were monitored for  
23 four consecutive years (2017 to 2020) on a network of 26 fields where annual crops are  
24 cultivated, in Brittany (France). The results of this medium-term multifactorial study  
25 highlighted that tillage was the most influential agricultural practice on earthworms, followed  
26 respectively by fertilization and pesticides application. Our study demonstrated that ploughing  
27 was more detrimental to earthworm biomass and richness than to earthworm abundance.  
28 Compared to reduced tillage, plough decreased earthworm richness by 1.2-fold, total earthworm  
29 biomass by 1.6-fold, *Aporrectodea* anecic biomass and abundance by respectively 2.7-fold and  
30 2.8-fold. Increased mineral fertilization increased epigeic abundance and biomass as well as  
31 *Lumbricus* anecic biomass. In contrast, organic fertilization did not affect any earthworm  
32 community parameters. Increase use of insecticides, fungicides and molluscicides decreased  
33 the biomass of *Aporrectodea* anecic earthworms. Finally, endogeic earthworms have not been  
34 impacted by any agricultural practices. To move towards sustainable agriculture, it is necessary  
35 to adapt crop management in order to preserve the ecosystem services provided by soil  
36 biodiversity, in particular earthworms.

37 **Keywords:** Soil biodiversity; tillage; fertilization; pesticide; crop management

## 38 1. Introduction

39 In order to cope with crop needs, pedo-climatic contexts and an increasing demand for  
40 food, fodder, fiber, and bioenergy of a growing human population, very diverse practices have  
41 been developed in annual crops systems (Filson, 2005; Bruinsma, 2015; Shrestha et al., 2021;  
42 Abudulai et al., 2022). However, the intensification of agricultural practices has led to soil  
43 degradation (Lal et al., 2020; Kraamwinkel et al., 2021) caused by, among others, soil  
44 compaction, nutrient leaching and pesticide application (Parris, 2011; Gaupp-Berghausen et al.,  
45 2015; Socorro et al., 2016; Rumschlag et al., 2020), reducing the capacity of agricultural lands  
46 to provide ecosystem services and contributing to biodiversity loss (Gomiero et al., 2008;  
47 Gabriel et al., 2013; Bai et al., 2018; Giannakis et al., 2019).

48 Earthworms are widespread in temperate climates and play a role in a wide range of soil  
49 ecosystem services (Blouin et al., 2013) particularly valuable in annual crops systems (Bertrand  
50 et al., 2015). For instance, they contribute to the improvement of soil structure (Capowiez et  
51 al., 2012; Sharma et al., 2017; Schon et al., 2017), to water infiltration (Bouché and Al-Addan,  
52 1997; Capowiez et al., 2014; Cunha et al., 2016), to nutrient mineralization (Van Groenigen et  
53 al., 2019), leading to an increase in primary production (Scheu, 2003; Brown et al., 2004). A  
54 meta-analysis by Van Groenigen et al. (2014) concluded that, on average, the presence of  
55 earthworms increased aboveground biomass by 34 % for barley, 28 % for maize and 29 % for  
56 wheat. However, if intensive land management practices are carried out on an annual crop, it  
57 can negatively influence earthworm communities and therefore their contribution to ecosystem  
58 services (Capowiez et al., 2009; Pelosi et al., 2016; Nuutinen and Butt, 2019).

59 The effects of agricultural practices on earthworm communities have been extensively  
60 studied and reviewed (Pelosi et al., 2009; Datta et al., 2016; Briones and Schmidt, 2017) with  
61 special attention to soil tillage (Emmerling, 2001; Crittenden et al., 2014; Pelosi et al., 2014b),

62 pesticides inputs (Krogh et al., 2007; Pelosi et al., 2014a; Maggi and Tang, 2021), plus organic  
63 and mineral fertilization (Leroy et al., 2008; Niswati et al., 2022). It is well known that the  
64 negative effects of some agricultural practices on earthworm communities may be compensated  
65 by others that are more favorable (Nuutinen, 1992; Overstreet et al., 2010; Torppa and Taylor,  
66 2022). Simonsen et al., (2010) examined the combined effect of tillage, manure inputs, pesticide  
67 use, and crop diversity in a long term observatory and concluded that organic fertilization and  
68 tillage had most impact on total earthworm abundance. However, only a few studies have been  
69 carried out to determine the combined effects of a range of agricultural practices on earthworm  
70 communities: thus, it remains generally difficult to rank which practices have the most or least  
71 effects on earthworm communities (Pelosi et al., 2009; Ashworth et al., 2017). Besides, the  
72 effects of these agricultural practices on earthworm communities are usually studied in  
73 experimental sites focusing on specific agricultural practices (Capowiez et al., 2009; Crittenden  
74 et al., 2014; Rodríguez et al., 2020). Therefore, it is necessary to evaluate the combined effects  
75 of agricultural practices on earthworm communities through diversified practices implemented  
76 by farmers currently in the field.

77         The aim of this study was to evaluate the combined effects of (i) tillage (either ploughing  
78 or reduced tillage), (ii) mineral and organic nitrogen (N) fertilization and (iii) pesticides  
79 applications (herbicides and insecticides-fungicides and molluscicides) on earthworm  
80 community parameters. We hypothesized that the simultaneous intensification of tillage and  
81 pesticide application would have a negative effect on those parameters, while fertilization  
82 would have a positive effect. The study was conducted in Brittany (France), on 26 annual crops,  
83 sampled each spring from 2017 to 2020. Several parameters describing earthworm communities  
84 were evaluated, that included earthworm total abundance, biomass and species diversity as well

85 as the abundance and biomass within each earthworm ecological category (i.e., epigeic,  
86 *Lumbricus anecic*, *Aporrectodea anecic* and endogeic).

87

## 88 **2. Materials and methods**

### 89 **2.1. Study site and field network**

90 The study site is in the departments of Côtes d'Armor and Ille-et-Vilaine near the city  
91 of Rennes, in Brittany, northwestern France (48°2'N, 2°3'W). Agriculture in the study area is  
92 mainly dedicated to dairy farming and cereal crops (wheat, maize and rapeseed), temporary  
93 grassland and annual forage. The surface area of the study site was approximately 2000 km<sup>2</sup>  
94 and the climate of the area is oceanic with a mean annual rainfall of 733.0 mm, a mean annual  
95 temperature of 11.7 °C, a monthly minimum of 2.3 °C and a monthly maximum of 22.3 °C  
96 (data from Météo France 2007–2020). The main soil types encountered are humic silty-textured  
97 cambisols developed on aeolian silts (GIP Bretagne Environnement, 2005; FAO, 2014).

98 We used the data from DEPHY (Demonstration, Experimentation and Reference  
99 Production of pHYtosanitary Products Efficient Systems) farm network which was created in  
100 2010 as part of the national ECOPHYTO plan, set up in 2008 by the French government  
101 (Barzman and Dachbrodt-Saaydeh, 2011). The DEPHY farm network is managed at national  
102 scale by the French Ministry of Agriculture (Ministère de l'Agriculture et de la Souveraineté  
103 alimentaire) with the aim of promoting and evaluating the implementation of practices to reduce  
104 the use of phytosanitary products (Fouillet et al., 2022). We selected 26 fields with annual crops  
105 in conventional agriculture and where applied management practices were representative of  
106 those used in the region (tillage, fertilization and pesticide). Wheat was cultivated on all fields

107 at the beginning of the study in 2017, which allowed for a start of earthworm monitoring on the  
108 same crop.

109 In 2018,  $5 \times 100$  g soil cores (0-25 cm depth) were collected near the earthworm  
110 sampling points from each plot, pooled and homogenized to analyze a composite soil sample.  
111 The analytical laboratory AUREA AGROSCIENCE (Brittany, France) analyzed particle-size  
112 distribution (NF X 31.107), organic matter content (NF ISO 14235), pH (NF ISO 10390) and  
113 cation exchange capacity (CEC; NF X 31.130) of the composite soils. The 0-25 cm soil layer  
114 was characterized by a silty texture and, on average, an organic matter content of  $2.5 \pm 0.5$  %,   
115 a pH of  $6.8 \pm 0.4$ , and a CEC of  $10.01 \pm 1.35$  meq/100 g (mean  $\pm$  standard deviation;  
116 Supplementary Table S1).

117

## 118 **2.2. Agricultural practices**

119 Five agricultural practices reflecting the management of annual crops were selected:  
120 tillage, fertilization (mineral and organic) and pesticide application (herbicides only and  
121 grouping insecticides, fungicides and molluscicides; Supplementary Table S2). An annual  
122 farmers survey allowed information to be obtained about agricultural practices up to 12 months  
123 prior to earthworm sampling. It is important to note that farming practices within each plot,  
124 such as fertilizer and pesticide use, can vary from year to year due to farmers' adaptation to the  
125 crops.

126 Tillage modalities were either ploughing i.e., opening and turning over the soil (with a  
127 plough or a spade at a depth of 20 cm) or reduced tillage i.e., loosening the soil to less than 15  
128 cm of depth without turning it (unploughed). Over the entire study, 63 plots were ploughed and  
129 41 were under reduced tillage.

130 Fertilizers were organic and/or mineral, the organic fertilization derived mostly from  
131 livestock waste application (pig and cattle manure). The farmers that we followed for this study  
132 were supervised by members from COMIFER (French Committee for the Study and  
133 Development of Reasoned Fertilization) to help them know the quantities of organic matter to  
134 spread according to the crops. Thus, to convert organic fertilizers into units of nitrogen per  
135 hectare (N units ha<sup>-1</sup>), the formula used was (Agasse et al., 2013) :

136 
$$\text{N units ha}^{-1} = \text{N content of organic matter} \times \text{total amount of organic matter applied} \times$$
  
137 
$$\text{coefficient of equivalence of efficient mineral N fertilizer}$$

138 On all plots in our study, the mean organic fertilization was 96 (± 71 standard deviation (sd))  
139 N units ha<sup>-1</sup> ranging from 0 to 320 N units ha<sup>-1</sup>. Mineral fertilization mainly consisted in N,  
140 phosphorus and potassium application. Over the entire study, the average mineral fertilization  
141 was 60 (± 51 sd) N units ha<sup>-1</sup>, ranging from 0 to 180 N units ha<sup>-1</sup>.

142 The Treatment Frequency Index (TFI) was calculated (i) for herbicides only, and (ii) by  
143 grouping insecticides, fungicides and molluscicides together. The grouping of insecticides,  
144 fungicides and molluscicides was done firstly because they are used in small quantities and this  
145 avoided overparameterization of the models and secondly because some broad-spectrum  
146 insecticide, fungicide and molluscicide molecules may impact non-target organisms (Reinecke  
147 and Reinecke, 2007; Pereira et al., 2009; Sánchez-Bayo, 2011; Jayaraj et al., 2016). The TFI of  
148 Herbicides (TFI\_H) and the combination of Insecticides, Fungicides and Molluscides  
149 (TFI\_IFM) were calculated using the following formula described by Barzman and Dachbrodt-  
150 Saaydeh, (2011).

151 
$$TFI = \frac{\text{active substance dose applied} \times \text{area treated}}{\text{active substance dose recorded} \times \text{total plot area}}$$



152 Over the entire study, the average TFI\_H was 1.62 ( $\pm$  0.88 sd) ranging from 0 to 4 and the  
153 average TFI\_IFM was 0.52 ( $\pm$  0.63 sd) ranging from 0 to 3.25.

154

### 155 **2.3. Earthworm sampling and laboratory analysis**

156 Earthworms were sampled from the 26 plots each spring (2017 to 2020) with a modified  
157 protocol (ISO 23611-1:2018) that consisted of extracting six blocks of soil, two meters from  
158 each other, with a size of 20 cm  $\times$  20 cm, at a depth of 25 cm, by using a spade and hand-sorting  
159 to collect the earthworms. Sampling was undertaken 10 meters from any field edge (e.g., road,  
160 hedge, slope). Each year, samples were staggered by five meters to avoid the exact location  
161 used before. Collected earthworms were stored in 96 % ethanol before laboratory analysis.

162 In the laboratory, earthworms were counted, weighed, assigned to a developmental  
163 stage (juvenile, sub-adult, and adult), identified to subspecies level, and assigned to an  
164 ecological category: epigeic, *Lumbricus anecic*, *Aporrectodea anecic*, and endogeic (Bouché,  
165 1972, 1977; Jégou et al., 1998; Hoeffner et al., 2019). Juvenile earthworms that could not be  
166 determined at the species level were assigned to species based on the proportion of adults and  
167 sub-adults identified in the plot. Earthworm community parameters derived from the sampling  
168 data were: total abundance and biomass, abundance and biomass of ecological categories,  
169 richness and evenness index which was calculated with the following formula: Evenness index  
170 =  $H' / H_{max}$  where  $H'$  is the Shannon diversity index. All were expressed at the scale of one  
171 square meter by multiplying by 25 the figures obtained from the six blocks.

172

### 173 **2.4. Statistical analysis**

174 Statistical analyses were performed with R software version 4.1.3 (R Core Team, 2022).  
175 A significant effect always corresponded to a P-value less than 0.05. We used generalized linear  
176 mixed models (GLMM) to test jointly the effects of tillage, fertilizations and pesticides  
177 applications on all the mentioned parameters describing earthworm communities. Two random  
178 factors were included in the models: plot identification and year of sampling due to the cross-  
179 over experimental design (Harrison et al., 2018). The variance inflation factor (VIF) of each  
180 variable entered into the model was evaluated, to test for multicollinearity between agricultural  
181 practices. Agricultural variables with a VIF higher than 5 were removed from the model. The  
182 conditions of homoscedasticity, normality and overdispersion of the residuals were checked.  
183 Since the overdispersion was not respected, we replaced the Poisson distribution by the negative  
184 binomial distribution. The best model was selected based on the second-order Akaike  
185 information criterion (AICc).

186

### 187 **3. Results**

188 In the 26 annual crop plots sampled during the four years of observation, average  
189 earthworm species richness, abundance, and biomass reached respectively  $5.4 \pm 1$  species,  $233$   
190  $\pm 166$  individuals  $m^{-2}$ , and  $61 \pm 40$  grams  $m^{-2}$ . On average, earthworm total abundance was,  
191 179, 268, 187 and 300 individuals  $m^{-2}$  for 2017, 2018, 2019, 2020 respectively. In addition,  
192 epigeic amounted to 7% of earthworm abundance while *Lumbricus anecic* accounted for 9%,  
193 *Aporrectodea anecic* accounted for 7% and endogeic to 77% (Supplementary Table S3).  
194 Average biomass was composed of 5% of epigeic, 19% of *Lumbricus anecic*, 20% of  
195 *Aporrectodea anecic*, and 56% of endogeic (Supplementary Table S3). The most common  
196 species were *Lumbricus castaneus* (Savigny, 1826) for epigeic, *Lumbricus terrestris* (Linnaeus,  
197 1758) for *Lumbricus anecic*, *Aporrectodea nocturna* (Evans, 1946) for *Aporrectodea anecic*

9

198 and *Aporrectodea calignosa calignosa* (Savigny, 1826) and *Allolobophora chlorotica*  
199 (Savigny, 1826) for endogeic. In total, 18 earthworm species were recorded during this study.  
200 The list of all earthworm species found in the 26 annual crop plots monitored during the four  
201 years of sampling is presented in Supplementary Table S4.

202         Regarding the type of tillage, total earthworm biomass was 1.6 times lower in ploughed  
203 plots than under reduced tillage (Figure 1a; Table 1), which is probably due to the significant  
204 decrease of *Aporrectodea anecic* biomass (Figure 1c; Table 1) as well as a trend of both  
205 *Lumbricus anecic* and endogeic biomass to decline (Table 1). *Aporrectodea anecic* abundance  
206 was 2.8 times lower in plots with ploughing than in plots with reduced tillage (Figure 1d; Table  
207 1). Indeed, there were twice as many *Aporrectodea nocturna* in plots managed with reduced  
208 tillage. In addition, there was a decreasing trend in the abundance of *Lumbricus anecic* (Table  
209 1), which may have also contributed to this decrease in total biomass. The decrease of  
210 *Lumbricus anecic* was related to the decrease of *Lumbricus rubellus rubellus* and *Lumbricus*  
211 *centralis* in the ploughed plots. The other ecological categories were balanced between the two  
212 types of tillage. Thus, the species richness was 1.2 times lower in plots with ploughing than  
213 under reduced tillage (Figure 1b; Table 1). The type of tillage did not significantly impact total  
214 earthworm abundance, evenness index, epigeic abundance and biomass, nor endogeic abundance  
215 (Table 1).

216         Regarding fertilization, an increase in mineral fertilization significantly increased  
217 epigeic abundance and biomass (Figures 2a and 2b; Table 1), as well as *Lumbricus anecic*  
218 biomass (Figure 2c; Table 1). However, no significant influence of organic fertilization was  
219 observed on all the earthworm parameters studied. Furthermore, mineral fertilization did not  
220 affect total earthworm abundance and biomass, evenness index, *Lumbricus anecic* abundance,  
221 *Aporrectodea anecic* biomass, species richness, or endogeic abundance and biomass (Table 1).

222 An increase in TFI\_IFM resulted in a decrease in biomass of *Aporrectodea anecic*  
223 (Figure 3a; Table 1), whereas an increase in TFI\_H tended to reduce the abundance and biomass  
224 of *Lumbricus anecic* (Figures 3b and 3c; Table 1). However, the increased TFI caused no  
225 significant decrease in any particular species. In addition, the evenness index, *Aporrectodea*  
226 *anecic* abundance, epigeic, endogeic and the total earthworm were not affected by TFI\_IFM  
227 and TFI\_H (Table 1).

228

## 229 4. Discussion

230 In the present study, average earthworm species richness and abundance respectively  
231 reached  $5.4 \pm 1$  species and  $233 \pm 166$  individuals  $m^{-2}$ , which are similar range to those found  
232 in previous studies conducted on crop plots under temperate climate. For instance, in 52 annual  
233 crops in western France Cluzeau et al. (2012) observed 215 individuals  $m^{-2}$  and 7.4 species  $m^{-2}$ ,  
234 while in 58 annual crops in Estonia Ivask et al. (2007) observed 81.4 individuals  $m^{-2}$  and 3.7  
235 species  $m^{-2}$  on average. Moreover, in the present study, eighteen earthworm species were found,  
236 which is close with the twenty three earthworm species recorded by Cluzeau et al. (2012) in the  
237 same region.

238

### 239 4.1. Ploughing was the most detrimental agricultural practice for earthworms

240 Total earthworm biomass was 160% higher in plots under reduced tillage than in  
241 ploughed plots. This finding is consistent with the meta-analysis of Briones and Schmidt  
242 (2017), that found +101% of earthworm biomass in conservation agriculture plots and +196 %  
243 in no-till plots than in ploughed plots. In this study, the higher earthworm biomass in plots under  
244 reduced tillage was particularly due to an increase in anecic biomass (significant for

245 *Aporrectodea* anecic and as a trend for *Lumbricus* anecic). In addition, earthworm richness also  
246 increased in plots under reduced tillage compared to those that were ploughed. These results  
247 are consistent with previous studies that highlighted a negative effect of ploughing on  
248 earthworms, especially on the anecic ecological group (Chan, 2001; Ernst and Emmerling,  
249 2009; Crittenden et al., 2014; Briones and Schmidt, 2017). Our study highlighted that ploughing  
250 has a greater impact on biomass and richness than on earthworm abundance. Indeed, by turning  
251 the soil, ploughing injures and kills earthworms directly and exposes them to harsh  
252 environmental conditions and predators. Anecic earthworms in particular face the destruction  
253 of their vertical burrows while their food sources are buried (Chan, 2001; Pelosi et al., 2014b;  
254 Briones and Schmidt, 2017). However, in the present study, the increase in the abundance of  
255 *Aporrectodea* anecic in the reduced tillage plots did not affect the total earthworm abundance  
256 but rather the total biomass. This result could be explained by the higher individual weight of  
257 anecic earthworms compared to endogeic and epigeic earthworms (Bouché, 1972; Sims and  
258 Gerard, 1999). Because they accounted for 16% of total earthworm abundance and for 39% of  
259 total earthworm biomass in the study, the reduction of anecic earthworms under ploughing  
260 systems notably impacted total earthworm biomass and, to a lesser extent, total earthworm  
261 abundance compared to reduced tillage. Epigeic and endogeic earthworms were not affected by  
262 ploughing which could be explained by their relatively small size compared to large anecic  
263 (Schmid et al., 2003; Postma-Blaauw et al., 2010). Therefore, by reducing anecic abundance  
264 and biomass, ploughing limits their contribution to ecosystem services such as the development  
265 of soil structure and porosity (Bastardie et al., 2003; Capowiez et al., 2015; Frazão et al., 2019),  
266 the creation of habitat for soil biodiversity (Eisenhauer, 2010; Andriuzzi et al., 2015; Hoeffner  
267 et al., 2018) or plant growth (Scheu, 2003; Van Groenigen et al., 2014). Finally, it is important  
268 to consider that the present study did not investigate differences between tillage depths, tools

269 and methods diversity (e.g. chisel, rotatory harrow, cultivators...) nor tillage frequency, which  
270 are all variables likely to influence earthworm communities (Capowiez et al., 2009; Briones  
271 and Schmidt, 2017).

272

#### 273 **4.2. Mineral nitrogen fertilization has positive effects on epigeic and *Lumbricus anecic*** 274 **earthworms**

275 We observed a positive effect of mineral fertilization on epigeic abundance and biomass as  
276 well as on *Lumbricus anecic* biomass, however *Aporrectodea anecic* as well as endogeic  
277 abundance and biomass were not affected by an increase of mineral fertilization. In addition,  
278 the increase of organic fertilization did not affect any earthworm community parameters.  
279 Consistent with most of these results, several studies also found no effect of organic or mineral  
280 fertilization on earthworm abundance and biomass (Timmerman et al., 2006; Valdez et al.,  
281 2020). Other studies observed an increase in earthworm abundance and biomass with increasing  
282 organic or mineral fertilization (Iordache, 2012; Zhu and Zhu, 2015; Blanchet et al., 2016,  
283 Betancur-Corredor et al., 2023). Indeed, it is commonly accepted that increasing N fertilization  
284 increases primary production, which, for earthworms, increases food supply, and provides  
285 refuge from predator and extreme climatic events (Leroy et al., 2007, 2008; Iordache, 2012;  
286 Zhu and Zhu, 2015; Niswati et al., 2022). If mineral fertilization does not provide a direct source  
287 of food for earthworms, in our study it was generally associated with intercrops in the rotation,  
288 requiring additional fertilization that can be rapidly assimilated by the plants. Hence, intercrops  
289 could provide fresh plant-derived material, refuge against predator and extreme climatic events  
290 preferentially for epigeic and *Lumbricus anecic* earthworms living in close contact with the soil  
291 surface, compared to *Aporrectodea anecic* and endogeic (Edwards and Lofty, 1982). Indeed,

292 *Aporrectodea* anecic and endogeic earthworms preferentially feed on aged plant-derived  
293 materials (i.e., decomposed organic matter) while epigeic and *Lumbricus* anecic earthworms  
294 rather feed on fresh plant-derived materials on the soil surface (Bouché, 1977; Andriuzzi et al.,  
295 2015; Larsen et al., 2016, Hoeffner et al., 2022). As this remains hypothetical, further studies  
296 are needed to elucidate the effect of mineral and organic fertilizer on earthworm communities  
297 and especially on these two earthworm ecological categories.

298

### 299 **4.3. Pesticide application negatively impacts anecic earthworms**

300 In the present study, we observed a negative effect of pesticides on anecic earthworms  
301 (significant for *Aporrectodea* and as a trend for *Lumbricus*) but no effect on epigeic and  
302 endogeic ecological categories, nor on total earthworm abundance, biomass, and richness.  
303 Previous studies, investigating the effects of different types of pesticides (fungicides,  
304 insecticides, herbicides...) on earthworm communities in cropland, reported inconsistent  
305 results. While some studies found a negative relationship between pesticides application and  
306 earthworm abundance or biomass (Krogh et al., 2007; Pelosi et al., 2013; Treder et al., 2020;  
307 Maggi and Tang, 2021) others demonstrated no response (Tarrant et al., 1997; Simonsen et al.,  
308 2010; Treder et al., 2020). This is probably related to several confounding factors that may  
309 aggravate or limit the effects of pesticides on earthworms, such as their active ingredient target  
310 (e.g., inhibition of cell respiration, disruption of cell division, permeability of membranes...),  
311 their persistence in the soil, application frequencies and doses, as well as environmental  
312 conditions during application (Laitinen et al., 2006; Pelosi et al., 2013; Gaupp-Berghausen et  
313 al., 2015). In addition, very little research has been done on cocktail effects of pesticides on  
314 earthworm communities (Pelosi et al., 2013; Gunstone et al., 2021), even though they are likely  
315 to occur in the field. A recent meta-analysis by Beaumelle et al (2023) showed that pesticides

316 with the most negative effects on soil fauna diversity were those that targeted a wide range of  
317 organisms. This could occur either through the application of a single broad-spectrum  
318 substance, or through the combined application of multiple substances at the same time or along  
319 the crop season. In the present study, the different confounding factors were not considered in  
320 the calculation of the TFI, which allows comparison of the intensity of pesticide use only.  
321 Except for epigeic which was the least representative ecological category (the most affected by  
322 fertilization), pesticides impact on anecic earthworms is consistent with previous studies that  
323 highlighted a greater impact than on endogeic earthworms (Krogh et al., 2007; Pelosi et al.,  
324 2013; Gaupp-Berghausen et al., 2015). This could be explained by their behavior, as they are  
325 more frequently in contact with the soil surface and are more exposed to pesticides. Besides,  
326 since anecic earthworms represented less than 17% of total earthworm abundance in the present  
327 study, a decrease in their abundance consequently impacted the total earthworm abundance to  
328 a limited extent only. Interestingly we observed that the increase of TFI\_IFM significantly  
329 decreased the biomass of *Aporrectodea* anecic, while the increase of TFI\_H tended to lower  
330 *Lumbricus* anecic abundance and biomass. This greater negative effect of TFI\_IFM could be  
331 explained by the fact that TFI\_IFM harm a wider range of organisms by combining the direct  
332 and indirect effects of all three types of pesticide (insecticides, fungicides and molluscicides;  
333 Beaumelle et al., 2023). Given their functional role in soils, it would be useful to undertake  
334 more studies on the contrasting effects of pesticides on these two anecic earthworm groups.  
335 Endogeic earthworms were not affected by pesticides which could be explained by the fact that  
336 they are not directly exposed to pesticides sprayed on crop foliage or on the soil surface, and  
337 they do not feed on fresh surface organic matter that may contain pesticide residues (Pelosi et  
338 al., 2013; Edwards and Arancon, 2022) which could reduce their vulnerability to hazardous  
339 chemicals (Hedde et al., 2012; Pelosi et al., 2013;). Overall, our study highlighted, the harmful



340 effects of pesticides applied at typical doses in cropping systems on earthworms. Consequently,  
341 our results strengthen the challenge to pesticide approval protocols, which should be based on  
342 robust studies that fully understand the long-term effects of pesticides under typical field  
343 conditions (Beaumelle et al., 2023). It is crucial to develop transparent new approaches that  
344 take into account the combined effects of pesticides on earthworm communities under realistic  
345 field conditions (Panico et al., 2022; Beaumelle et al., 2023).

346

## 347 **5. Conclusions**

348 Our study highlighted the importance of considering multiple agricultural practices to  
349 assess the effects of agriculture on earthworm communities. The results of this medium-term  
350 multifactorial study clearly illustrate that tillage was the most influential agricultural practice  
351 on earthworms, followed respectively by fertilization and pesticides application. Furthermore,  
352 this study highlights that ploughing negatively impacted total biomass, species richness and  
353 *Aporrectodea* anecic earthworms. Increased mineral fertilization favored epigeic abundance  
354 and biomass as well as *Lumbricus* anecic biomass while organic fertilization did not affect any  
355 earthworm community parameters. A higher TFI\_IFM decreased *Aporrectodea* anecic biomass  
356 while a greater TFI\_H tended to lower *Lumbricus* anecic abundance and biomass as well as  
357 species richness. Since our study demonstrated that agricultural practices have both negative  
358 (tillage and pesticides application) and positive (mineral fertilization) effects on earthworms in  
359 the field, it is therefore necessary to reconsider all agricultural practices in order to preserve soil  
360 biodiversity, particularly earthworms.

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669

670 **Legends**

671 **Figure 1:** Box plot of mean earthworm biomass (a), species richness (b), biomass (c) and  
672 abundance (d) of *Aporrectodea anecic* by tillage type (ploughing and reduced tillage). The  
673 number of plots is indicated by the value of the letter n below each tillage type modality.  
674 Asterisks above the whiskers represent the degree of significance based on a Tukey-type post  
675 hoc analysis. Box plot legend: Top edge of box = 75th percentile; bottom edge of box = 25th  
676 percentile; horizontal line in box = median; the black square (■) = mean; top whiskers = 75th  
677 percentile at 75th percentile + 1.5 × interquartile range; bottom whiskers = 25th percentile to  
678 25th percentile – 1.5 × interquartile range and the black dot (•) = point outside the whiskers but  
679 inside the edge of the box ± 3 × interquartile range.

680

681 **Figure 2:** Relationship between epigeic biomass (a), epigeic abundance (b) and *Lumbricus*  
682 anecic biomass (c) and mineral fertilization applied in the plots. Above the scatterplots, the R  
683 values represent the correlation coefficient and P the p values obtained after the ANOVA of the  
684 GLMM models.

685

686 **Figure 3:** Relationship between *Aporrectodea anecic* biomass (a), biomass (b) and abundance  
687 (c) of *Lumbricus anecic* and the TFIs (TFI\_IFM and TFI\_H) applied in the plots. Above the  
688 scatterplots, the R values represent the correlation coefficient and P the p values obtained after  
689 the ANOVA of the GLMM models.

690

691

692



693 **Tables**

694 **Tables 1:** Results of generalized linear mixed models testing the effects of agricultural practices  
695 on earthworm abundance, total biomass, species richness, evenness index, biomass and  
696 abundance of the ecological categories. Significant P values are indicated in bold ( $P < 0.05$ ).  
697 SE = Standard error; Z = model z-value, P = model p-value and NS = variable not selected by  
698 the best model.

699

700

## FIGURES

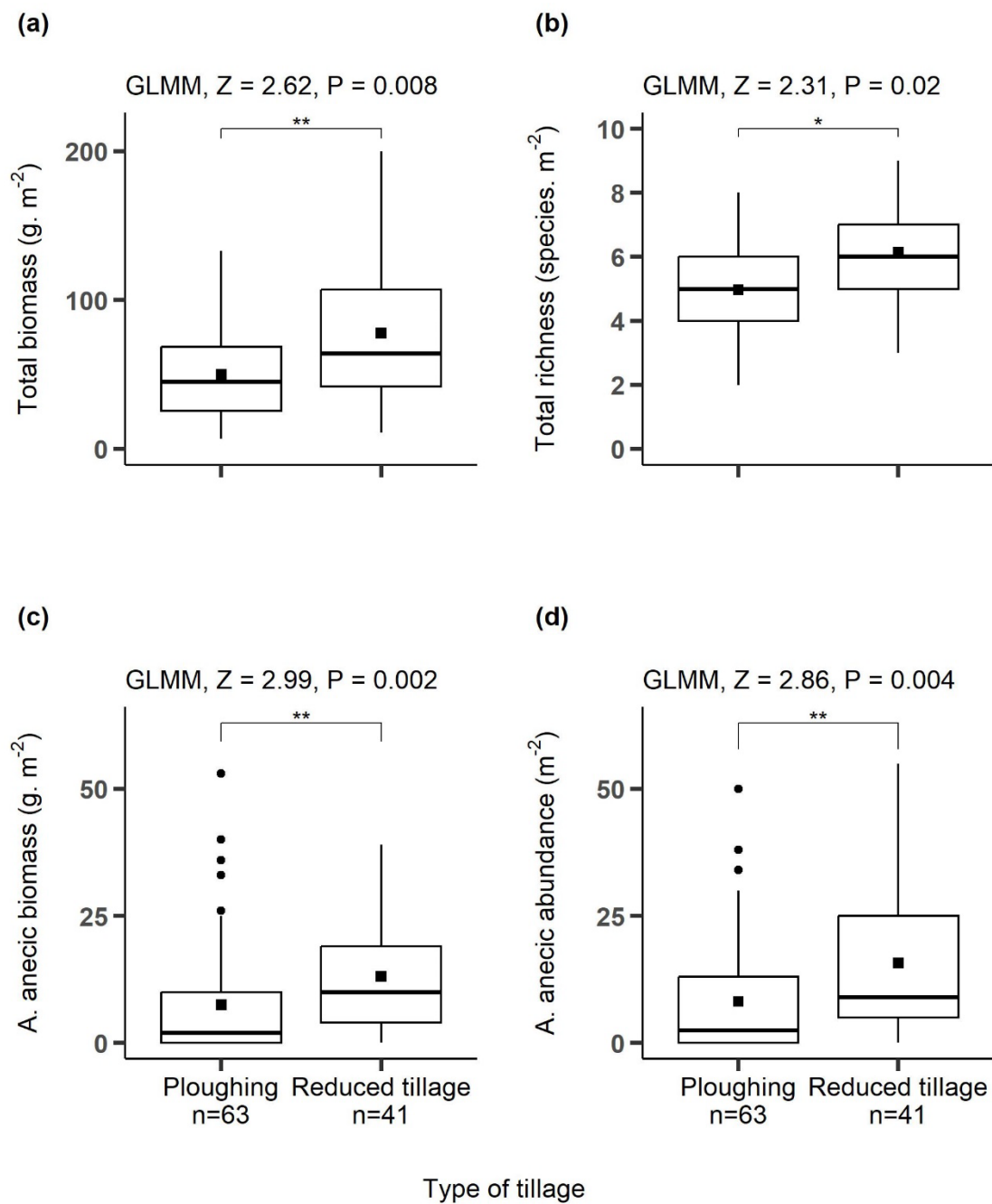
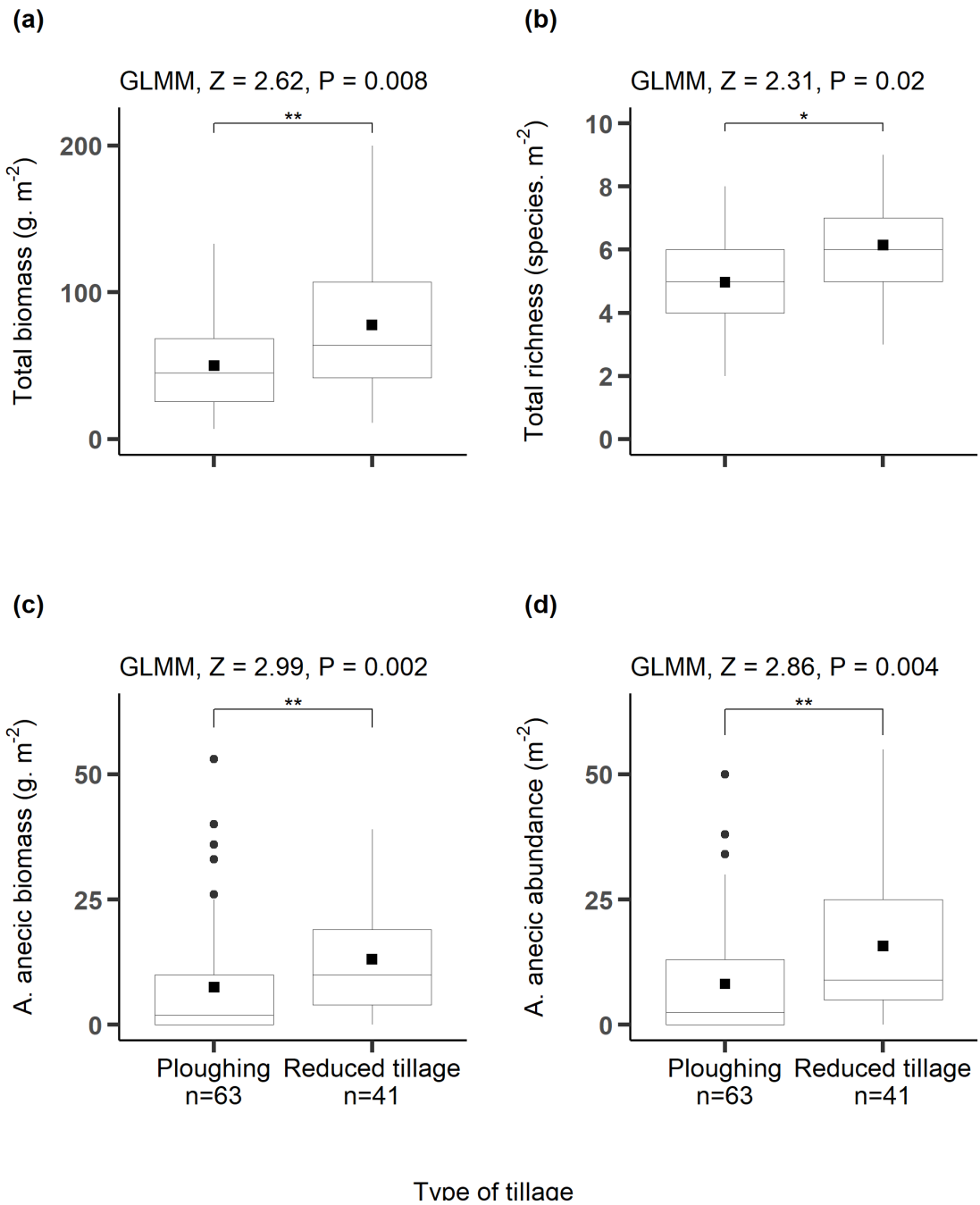
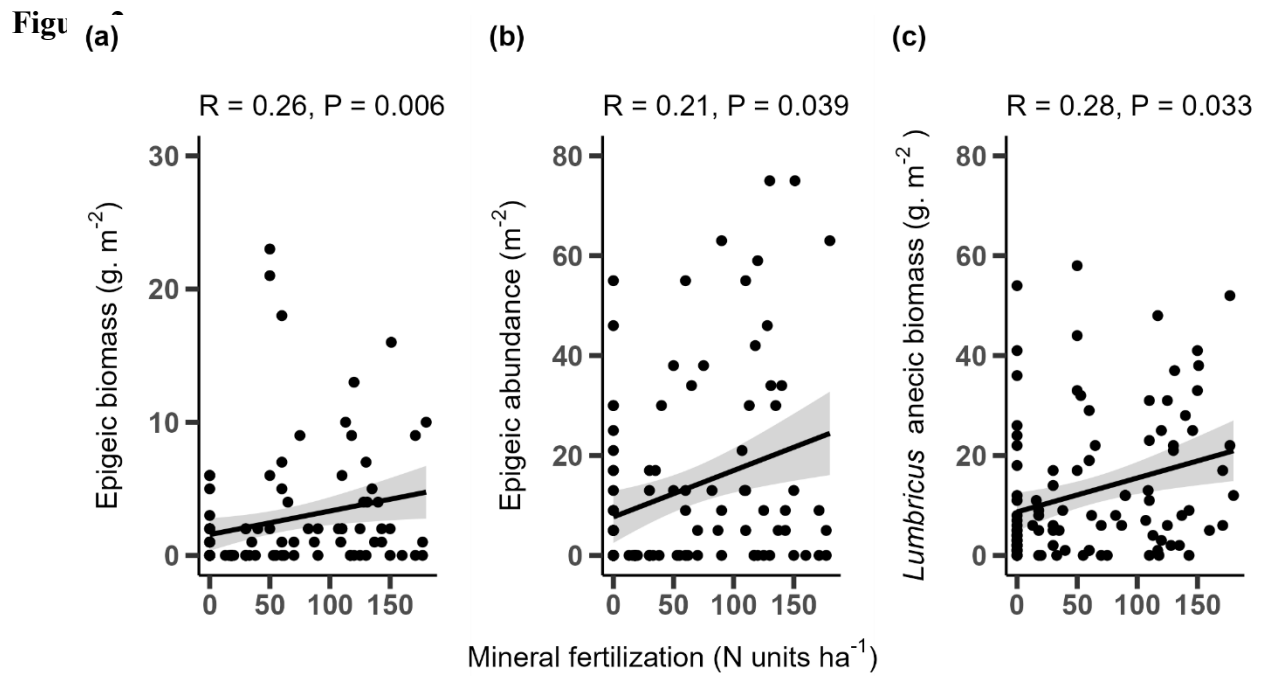
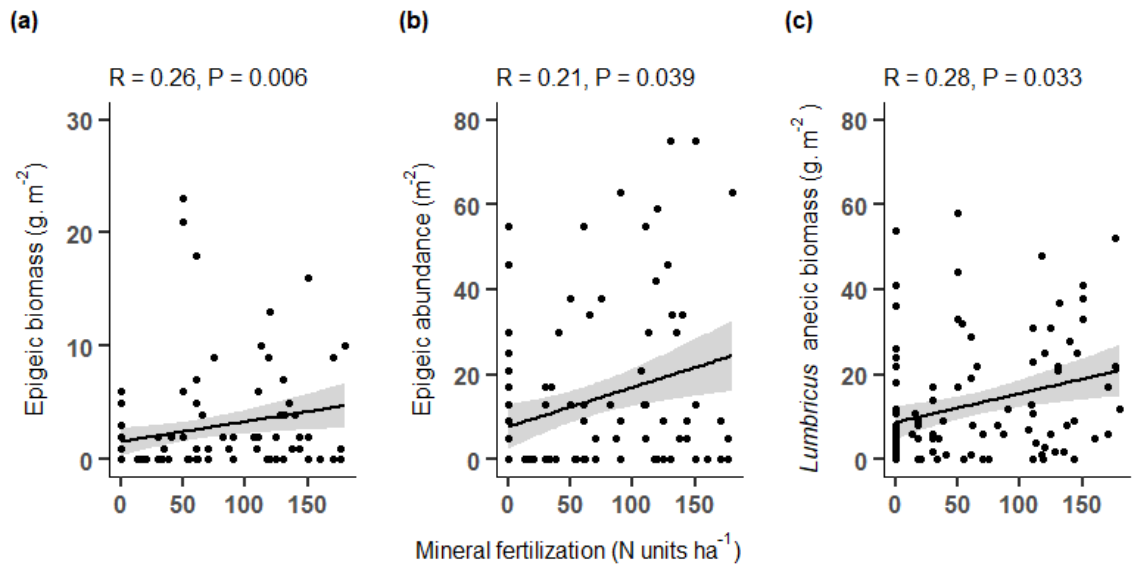


Figure 1





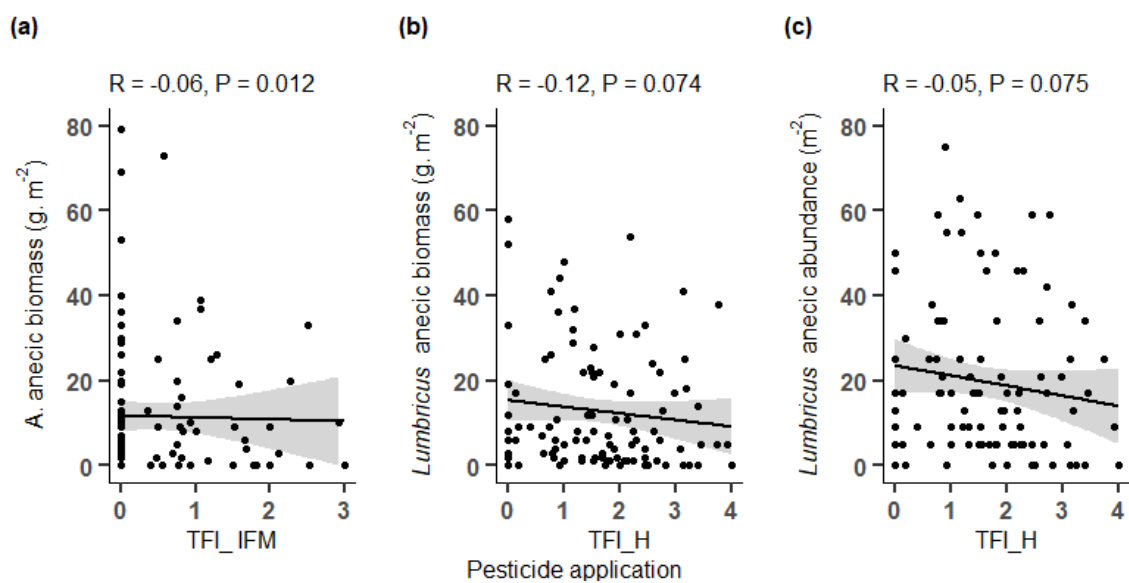


Figure 3

