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**Earthworm abundance response to conservation agriculture practices in organic arable farming under Mediterranean climate**

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**Abstract:**

Earthworms are one of the most important soil macrofaunal groups, and they play a major role in agricultural ecosystems. Agricultural practices, such as reduced tillage, the use of green manures and organic fertilization, can be beneficial for earthworm populations in agricultural systems. However, under a Mediterranean climate, not much is known regarding their response to agricultural management. The aim of this study was to analyse the effects of tillage type, organic fertilization, and green manures on the density and biomass of earthworms in organic arable dryland. The trial was conducted in a four-year crop rotation with a complete factorial design that combined tillage system (mouldboard ploughing vs. chisel), fertilization (composted farmyard manure

25 vs. no fertilizer) and green manures (green manures vs. no green manures). Earthworms  
26 were assessed in each plot by the extraction of all individuals in soil areas of 33 cm × 33  
27 cm that were excavated to a depth of 25 cm. Only five earthworm species were found in  
28 this trial, and the earthworm community was dominated by such endogeic ecotypes as  
29 *Aporrectodea rosea* and *Allolobophora georgii*, and the anecic ecotype *Aporrectodea*  
30 *trapezoides*. Endogeic species can benefit from soil inversion because of the  
31 incorporation of organic matter, but the anecic ones can be negatively affected by it.  
32 The results show that plots with farmyard manure had higher density and biomass of  
33 earthworms. We observed that the type of tillage significantly affected earthworm  
34 populations: plots that had been ploughed with mouldboard ploughing (soil inversion)  
35 the year prior to sampling presented more juveniles. The biomass of earthworms was  
36 significant lower in plots with green manures and chiselling. Our results indicated that  
37 the combination of chiselling and green manures was not optimal for earthworm  
38 populations, but organic fertilization played a considerably more important role and  
39 enhanced their abundances.

40

41 **Keywords:** organic farming; Mediterranean soils; green manures; earthworms; chisel

42

## 43 **1 Introduction**

44 Earthworms play a major role in ecosystem functioning because their burrowing and  
45 feeding activities modify the soil structure and several soil properties. In particular,  
46 earthworms increase soil macroporosity, relocate nutrients along the soil profile and  
47 form stable aggregates (Crittenden et al., 2014; Ernst and Emmerling, 2009; Metzke et  
48 al., 2007). The significant role of earthworms has been revealed by experiments in  
49 which they were eliminated in grass swards causing soil bulk density to increase, while

50 organic matter, soil moisture and infiltration rate greatly decreased (Riley et al., 2008).  
51 Conversely, earthworm populations are influenced by soil moisture, organic matter,  
52 texture, pH and soil management (Crittenden et al., 2014). Soil tillage can modify the  
53 relative abundance of earthworm species and their community structure (Riley et al.,  
54 2008). Some studies concerning the impact of inversion tillage on the abundance of  
55 earthworm populations have found that the largest and most fragile earthworms (those  
56 with soft epitheliums) are most affected by intensive tillage, and species inhabiting the  
57 topsoil are at risk of being negatively affected by ploughing (Pelosi et al., 2014). The  
58 variability in burrowing and feeding behaviours can be important in determining the  
59 effects that tillage type can have on earthworms (Capowiez et al., 2009). According to  
60 Bouché (1972), earthworms can be divided into the following ecological groups based  
61 on soil habitats and feeding habits: (1) Epigeic species live and feed in the organic  
62 layers above the mineral soil surface. (2) Anecic species live in vertical burrows in  
63 mineral soil layers, but come to the surface to feed on leaf litter that they drag into their  
64 burrows (0–200 cm depth). (3) Endogeic species live in mineral soil layers and feed on  
65 soil organic matter. They make horizontal burrows through the soil that they sometimes  
66 reuse to feed and move around. Capowiez et al. (2009) and Ernst and Emmerling (2009)  
67 showed that soil layer inversion by mouldboard ploughing negatively affected the  
68 density of anecic earthworm species, while the density of endogeic species was  
69 enhanced.

70 The influences of other farming practices, such as crop rotation, crop residue  
71 management and fertilization, are also important for earthworm populations (Riley et  
72 al., 2008). Eriksen-Hamel et al. (2009) reported that the addition of crop residues to  
73 tilled soils could alleviate some of the negative impacts of tillage on earthworms, thus  
74 improving their growth and maintaining more stable populations. While many studies

75 demonstrate the role of cover crops in decreasing soil erosion and improving weed  
76 control and soil fertility (Ward et al., 2012), there are few that investigate the effect of  
77 cover crops on earthworms. Farmyard manure is an organic amendment alternative to  
78 mineral fertilizers that can be beneficial for earthworm populations in arable fields  
79 (Andersen, 1979). Brown et al. (2004) reported that organic manures benefit  
80 earthworms both directly and indirectly by providing additional food resources and  
81 shelter (through the mulching effect), and stimulating plant growth and litter return.

82 Diversified crop rotation and green manures are used to manage weeds and pests, and  
83 the use of less intensive soil tillage (such as reduced tillage with no soil inversion) can  
84 reduce soil erosion, thus ensuring the sustainability of farming systems (Pelosi et al.,  
85 2014). Due to the potential beneficial effect of reduced tillage, green manures and  
86 organic fertilization on earthworms, a sensible hypothesis could be that the integration  
87 of conservation agriculture techniques into organic farming systems should increase  
88 their populations and diversity. For instance, Henneron et al. (2014) indicated that  
89 conservation agriculture and organic farming increased the density and biomass of all  
90 soil organisms. Several studies have found higher biodiversity in organically managed  
91 systems than in conventional systems (Scullion et al., 2002), Padmavathy and  
92 Poyyamoli (2013) reported higher earthworm populations in organically managed  
93 fields. Organic farming is fundamentally different than conventional systems due to the  
94 exclusion of synthetic pesticides and fertilizers. However, notably few studies provide  
95 results confirming that earthworm populations and diversity increase in arable cropping  
96 systems with a combination of conservation agriculture techniques and organic farming.

97 The aim of this study is to analyse the individual and collective effects of tillage type,  
98 organic fertilizer and green manures on the density and biomass of earthworms in  
99 organic arable cropping systems in the Mediterranean region. Indeed, there is a lack of

100 studies of earthworm populations in Mediterranean agricultural areas. Monitoring  
101 earthworms in these areas can be challenging because environmental conditions  
102 strongly limit earthworm distribution. Frequently, earthworms are distributed in small  
103 patches because many species have narrow ecological requirements that are determined  
104 by the high spatial variability of soil and soil water regimes in many Mediterranean  
105 landscapes (Gutiérrez-López et al., 2016).

106 The hypotheses of this study are that (1) the application of farmyard manure as fertilizer  
107 will increase earthworm density and biomass; 2) mouldboard ploughing will decrease  
108 earthworm populations; 3) the incorporation of cover crops into the soil as green  
109 manures can increase earthworm density and biomass; and 4) the integration of  
110 conservation agriculture techniques into organic farming systems could help increase  
111 the abundance of earthworms in arable fields under a Mediterranean climate.

112 To answer these questions, we took advantage of a trial designed to evaluate the effects  
113 of tillage, fertilization and green manures on a Mediterranean rainfed crop rotation and  
114 measured the abundance of earthworm populations in relation to these factors.

115

## 116 **2 Materials and Methods**

### 117 2.1 Experimental site and design

118 In November of 2011, a midterm field experiment was established in Gallecs, a rural  
119 area of Catalonia, Spain. This location is a peri-urban agricultural area of 753 ha  
120 situated in the region of Vallès Oriental, 15 km North of Barcelona (41°33'31.9"N  
121 2°11'59.5"E). It has a Mediterranean climate; the mean annual temperature and  
122 precipitation are 14.9 °C and 647 mm, respectively. At the beginning of the experiment,  
123 soil properties of the field were evaluated. On average, the mineral fraction consisted of

124 43.3 ± 6.9 % sand, 26.9 ± 4.7 % loam and 29.7 ± 3.7 % clay; the texture was classified  
125 as loamy-clay (Soil Survey Staff, 1998); and the soil type was a Haplic Cambisol (IUSS  
126 Working Group WRB, 2015). At the beginning of the experiment the average soil  
127 organic matter was 1.5 ± 0.1 % (Walkley-Black) and the pH (H<sub>2</sub>O) was 8.1 ± 0.1.

128 The trial consisted of a four-year crop rotation in a split-block design of three factors:  
129 tillage system (mouldboard ploughing (P) vs. chisel (C)) fertilization (composted  
130 farmyard (+F) vs. no fertilizer (-F)) and green manures (with green manures (+G) vs. no  
131 green manures (-G)). In total 32 plots measuring 13 m × 12 m were established,  
132 comprising four replicates of each treatment. The field had been under organic  
133 management for five years prior to the trial, with a typical dryland Mediterranean crop  
134 rotation that alternated cereals and legumes for human consumption. The crop rotation  
135 of this trial consisted of spelt (*Triticum spelta* L., 2011–2012), chickpeas (*Cicer  
136 arietinum* L., 2013) winter wheat (*Triticum aestivum* L., 2013-2014) and lentils (*Lens  
137 culinaris* Medik., 2015).

138 Two tillage systems were used: a mouldboard plough (P) (soil inversion at 25 cm depth;  
139 EG 85-240-8, Kverneland) plus a rotary harrow (5 cm depth; HR3003D, Kuhn); and a  
140 chisel plough (C) (no soil inversion at 25 cm depth; KCCC 1187 - A00, Kverneland)  
141 plus a rotary harrow (same as for plough). The fertilization (+F) treatment utilized six-  
142 month-long composted cow farmyard manure sourced near the field. The farmyard  
143 manure was applied every year before sowing the main crop. The total amount of  
144 manure applied differed per the nutritional demands of each crop. The year before  
145 sampling, 40 Ton ha<sup>-1</sup> (138.28 kg ha<sup>-1</sup> N<sub>tot</sub>) of farmyard manure was applied before  
146 winter wheat was sown. The organic fertilizers were mixed in the soil by means of a  
147 chisel or mouldboard plough in accordance to the tillage treatment. In September 2012  
148 and 2014, green manure (+G) was sown in the corresponding 16 plots. It consisted of a

149 mixture of oat (*Avena sativa* L.), white mustard (*Sinapis alba* L.), bitter vetch (*Vicia*  
150 *ervilia* (L.) Willd.) and common vetch (*Vicia sativa* L.). At the end of March of the  
151 following year, green manure was incorporated into the soil by disc harrowing.

## 152 2.2 Earthworm sampling

153 In February 2015, after three years of crop rotation, earthworms were assessed during  
154 green manure or stubble (depending on the type of treatment). Three square sampling  
155 frames of 33 cm × 33 cm were placed 2 m from the edge of each plot, with two on the  
156 mid-line and one on the centre of a randomly chosen side and were manually excavated  
157 to a depth of 25 cm. All earthworm and cocoons were extracted by hand sorting and  
158 were preserved alive with moist soil at 4 °C until their fixation. In the laboratory, the  
159 earthworms were fixed using formalin (4 % formaldehyde) and preserved in alcohol  
160 (90 %) (Kuntz et al., 2013; Peigné et al., 2009). They were counted and sorted by adults  
161 (with a clitellum), juveniles (without a clitellum and tubercula pubertatis) and cocoons.  
162 Adults and juveniles were identified following Bouché (1972) and weighed (conserved  
163 weight in alcohol with gut contents).

## 164 2.3 Statistical analysis

165 The individual and combined effects of the type of tillage (P vs. C), fertilization (+F vs.  
166 -F) and green manure (+G vs. -G) on adult and juvenile earthworm density and biomass  
167 was evaluated using linear mixed-effects models. Tillage, fertilization and green  
168 manures were used as fixed factors and the block was introduced as a random factor  
169 (block for the fertilization treatment and block for the tillage treatment). The normality  
170 of data was verified by the Shapiro-Wilk test and homoscedasticity was assessed using  
171 the Barlett test. To meet the normality and homoscedasticity requirements we used  
172 logarithmic transformation on data when necessary. The differences in mean density



173 and biomass among the treatments resulting from the combination of all three factors  
174 were verified using Tukey's HSD test. The same statistical procedure was followed  
175 while analysing the effect of tillage, fertilization and green manures on the densities of  
176 the main earthworm species. All the analyses were performed in R version 3.2.2 (R  
177 Core Team, 2015) with the packages lme4 (Bates et al., 2011) for linear mixed effects  
178 model fitting and multcomp (Hothorn et al., 2008) for post hoc multiple comparisons.

### 179 **3 Results**

#### 180 3.1 Overview of earthworm diversity

181 Overall, five earthworm species were found: *Aporrectodea trapezoides* (Duges, 1828),  
182 *Aporrectodea rosea* (Savigny, 1826), *Allolobophora georgii* (Michaelsen, 1890),  
183 *Octodrilus complanatus* (Duges 1828) and one unidentifiable specimen belonging to the  
184 family Hormogastridae (Michelsen, 1900). In this study, we have focused on the three  
185 most abundant species: *A. rosea*, *A. georgii* and *A. trapezoides*. The first two are  
186 endogeic ecotypes (Bouché, 1972), but the latter is quite variable in its behaviour. Some  
187 authors have considered it an endogeic ecotype that sometimes feeds on the surface  
188 (Lee, 1985) but is primarily considered to be anecic (Gutiérrez-López et al., 2016). Only  
189 one specimen of *Octodrilus complanatus* (anecic) and one unidentified Hormogastridae  
190 (endogeic) were observed. The most abundant species was *A. rosea* (mean  $53 \pm 12$  ind  
191  $m^{-2}$ ) followed by *A. georgii* (mean  $30 \pm 6$  ind  $m^{-2}$ ), and *A. trapezoides* (mean  $24 \pm 8$  ind  
192  $m^{-2}$ ). However, the highest earthworm biomass was of *A. trapezoides*, ( $14.80$   
193  $\pm 1.94$  g  $m^{-2}$ ), followed by *A. rosea* ( $7.56 \pm 15.57$  g  $m^{-2}$ ) and *A. georgii* ( $5.59 \pm$   
194  $1.93$  g  $m^{-2}$ ).

195 3.2 Effect of organic fertilization, tillage and green manures on the density and  
196 biomass of earthworms

197 Fertilization was the main factor that influenced earthworm populations (Table 1). The  
198 density and biomass of earthworms were significantly enhanced by farmyard manure  
199 (Figure 1(I) and 1(II)). The densities of juveniles, adults and total earthworms were  
200 significantly higher in plots with farmyard manure (mean  $\pm$  standard error of total  
201 density: +F= 585 ind  $\pm$  94 m<sup>-2</sup>; -F= 273  $\pm$  47 ind m<sup>-2</sup>) (Table 2). This pattern is related to  
202 *A. rosea* and *A. georgii*, but not *A. trapezoides* (Table 1 and Table 2). Similarly,  
203 biomass was significantly enhanced by farmyard manure (mean  $\pm$  standard error of total  
204 biomass: +F= 158.75  $\pm$  36.78 g m<sup>-2</sup>; -F= 64.87  $\pm$  17.81 g m<sup>-2</sup>). In contrast, total and  
205 adult densities were not affected by the type of tillage, but the density of juveniles was  
206 significantly higher in plots with mouldboard ploughing (Table 1). Total biomass of  
207 earthworms was not affected by the type of tillage. The presence of green manures did  
208 not affect the density of earthworms; however, significant differences were found for  
209 total and adult biomass (Table 1). The highest biomass was found in plots without green  
210 manures and chiselling (49.10  $\pm$  10.01 g m<sup>-2</sup>). Furthermore, there was a significant  
211 interaction between tillage and green manure factors due to the significant decrease of  
212 total earthworm biomass in plots with chiselling and without green manures when no  
213 farmyard manure was incorporated (Table 1).

214 3.3 Effects of the combination of conservation agriculture practices and organic  
215 fertilization on the biomass and abundance of earthworms

216 Contrasting results were found regarding the combined effects of the factors on density  
217 and biomass of earthworms. The combination of the two techniques of conservation  
218 agriculture (C and +G) was not the best combination for earthworm populations,  
219 regardless of fertilization (Figure 1(I)). Total biomasses were significantly lower in

220 plots with green manure and chiselling in comparison to those managed without green  
221 manure; the incorporation of green manures seems to negatively affect the total biomass  
222 of earthworms in unfertilized plots managed with chisel (Figure 1(II)).

## 223 4 DISCUSSION

### 224 4.1 Overview of earthworm diversity

225 Our results show a low diversity of earthworms, with only four species identified in the  
226 study area. Studies in temperate climates had shown higher species richness (9-13  
227 species) compared to our study in the Mediterranean region (Kuntz et al., 2013; Peigné  
228 et al., 2009). Furthermore, the earthworm community is dominated by only three  
229 species: two endogeic (*Aporrectodea rosea* and *Allolobophora georgii*) and one anecic  
230 ecotype (*Aporrectodea trapezoides*).

231 Arable cropping systems with annual rotation schemes, high rates of soil disturbance  
232 and habitat simplification likely contribute to low species richness. Smith et al. (2008)  
233 reported low species richness in a study in Michigan, US, dominated by the genus  
234 *Aporrectodea*. These researchers relate the findings to the fact that this genus is  
235 relatively tolerant to agricultural activities, as it is able to persist deeper in the subsoil  
236 than other species.

237 Boström (1995) suggested that the large amount of organic matter ploughed under the  
238 soil, which served as food for the earthworms, together with the supply of cocoons  
239 allowed the endogeic earthworms to make a fast recovery. Capowiez et al. (2009)  
240 reported that *Aporrectodea caliginosa* increased with mouldboard ploughing due to an  
241 increase in food resources (buried organic matter) and a decrease in competition for  
242 food with anecic species. Anecic species, on the other hand, could be the most  
243 negatively affected by intensive and repeated soil disturbance because of direct physical

244 damage and an indirect effect on food resources (burial of surface organic matter) and  
245 their habitat (destruction of burrows) (Capowiez et al., 2009).  
246 Pelosi et al. (2014) suggested that anecic species are less abundant, or even absent, in  
247 ploughed fields. Though several studies have shown that ploughing reduced the number  
248 of the large-bodied anecic species (Chan, 2001; Ernst and Emmerling, 2009; Pelosi et  
249 al., 2014), our results suggested *A. trapezoides* were not significantly affected by the  
250 type of tillage. Kuntz et al. (2013) reported that the implementation of reduced tillage in  
251 an organically managed clay soil over a six-year crop rotation enhanced the density and  
252 biomass of earthworms while also influencing their community structure. In this four-  
253 year experiment, we did not find that the type of tillage significantly affected earthworm  
254 communities, perhaps indicating that the response of earthworm populations occurs  
255 over a longer time period.

#### 256 4.2 Effect of organic fertilization, tillage and green manures on the density and 257 biomass of earthworms

258 It is known that fertilizers have long-term benefits for earthworm populations through  
259 increased input of nutrients, organic matter and enhanced production of litter material,  
260 which ultimately result in more food for earthworms. Furthermore, farmyard manure is  
261 considered most suitable for earthworm population growth (Brown et al., 2004; Curry,  
262 2004, 1976). Our study supports the claim that farmyard manure enhances earthworm  
263 growth and reproduction.

264 The effect of fertilization is significant for both density and biomass of adults and  
265 juveniles, while the effect of tillage is only significant for the density of juveniles. The  
266 high number of juveniles in plots using mouldboard ploughing can be explained by the  
267 enhancement of reproduction of earthworms in response to tillage disturbance. Several  
268 studies have shown that tillage with soil inversion can reduce earthworms by 70% both

269 in numbers and biomass, but populations generally recover within a year if the  
270 disturbance is not repeated (Boström, 1995). Eriksen-Hamel et al. (2009) reported that  
271 ploughing reduced the earthworm population by 73-77%, but one year later, there were  
272 five times as many earthworms, and the biomass was similar to the pre-tillage level.  
273 This could be explained because disturbance creates a pressure which reduces  
274 earthworm populations, but right after ploughing reproduction is increased. In this  
275 experiment, earthworm sampling was carried out six months after the soil was  
276 disturbed, probably leaving enough time for their recovery.

277 In this study, the highest biomass was found in plots without green manures, which is  
278 contrary to our initial hypothesis that the incorporation of organic matter from green  
279 manure should favour earthworms. However, few studies are available which analyse  
280 the effects of green manures and their interaction with tillage systems on earthworm  
281 populations, and thus our results are difficult to frame within existing evidence. Stroud  
282 et al. (2016) reported that *Lumbricus terrestris* L. was not enhanced by oilseed radish as  
283 cover crop in a long-term rotation. Valckx et al. (2011) also studied *Lumbricus*  
284 *terrestris* food and habitat preferences for cover crops and have found that rye grass was  
285 the preferred food resource, but no preference or repulsion was found for mustard  
286 (*Sinapis alba*). However, they found an increasing trend for repulsion against oats  
287 (*Avena sativa*) over time, suggesting that the allelopathic effect of oats may affect  
288 earthworms indirectly by changing habitat and food preference. Since we used oats and  
289 white mustard for the green manure mix, this could explain our results, although we  
290 cannot confirm that there is a repellent effect of green manures on earthworms.  
291 Furthermore, no studies were found regarding the food and habitat preferences of *A.*  
292 *rosea*, *A. georgii* or *A. trapezoides*. There is a need to study in more detail whether

293 there are some allelopathic effects of oats or mustard as cover crops that may affect  
294 earthworms.

#### 295 4.3 Effects of the combination of conservation agriculture practices and organic 296 fertilization on the biomass and abundance of earthworms

297 Density and biomass of earthworms respond differently to the combination of  
298 experimental factors, but in no case conservation agriculture techniques were the best  
299 combination for earthworm populations. Nevertheless, it is important to note that  
300 similar density and biomass of earthworms resulted in plots with mouldboard ploughing  
301 and fertilization compared to plots with chiselling and no fertilization. Several studies  
302 have indicated that the use of conservation agriculture techniques increases populations  
303 of earthworms (Scullion et al., 2002). Our results showed that earthworms can have  
304 similar abundances with both tillage systems. Perhaps in the Mediterranean region,  
305 where extreme climate conditions -particularly summer drought- play an important role  
306 on agroecosystems functioning, the use of all conservation agriculture techniques does  
307 not contribute to a more sustainable cropping system. Organic fertilization seems to  
308 play an important role in these systems and could be a crucial factor to maintain and  
309 benefit earthworm populations in Mediterranean agricultural systems.

310 The highest density of earthworms was found in plots with mouldboard ploughing,  
311 fertilization and the presence of green manures. This can be explained due to a species-  
312 specific response. *Aporrectodea rosea*, which was the most abundant species, avoids  
313 compaction and reduced tillage seemed to create more compacted zones. Some authors  
314 have shown that minimum tillage and no-tillage result in more compacted soil than  
315 ploughing (Capowiez et al., 2009; Peigné et al., 2009). Compacted zones are created by  
316 wheel tracks, and in tilled plots those zones are fragmented into clods by the plough; but  
317 in reduced tillage plots only parts of the compacted zones are fragmented (Capowiez et

318 al., 2009). In consequence, a soil structure with more macropores is obtained with  
319 mouldboard ploughing and, in the short term, earthworms are not able to improve soil  
320 macroporosity (Peigné et al., 2009). Furthermore, *A. rosea* lives mainly in the upper  
321 layers of the soil, eating soil organic matter. Therefore, the species could take advantage  
322 of increased availability of crop residues incorporated by inversion tillage (Chan, 2001).  
323 The highest total biomass was found in plots tilled with chisel and fertilization, but  
324 without green manures. *A. trapezoides* had the highest biomass since it is the largest  
325 species and it was the only anecic ecotype found in this experiment. This makes it more  
326 likely that they are affected by soil inversion tillage since they go to the surface for food  
327 (Gutiérrez-López et al., 2016). Therefore, reduced soil tillage with non-inversion could  
328 benefit this species.

329

## 330 5 CONCLUSIONS

331 Farmyard manure demonstrated the strongest effects on the growth and reproduction of  
332 earthworm populations. The two tillage systems did not show significant differences in  
333 total density and biomass of earthworms, but they did show an important effect  
334 depending of the ecotype of earthworm. Endogeic species can benefit from soil  
335 inversion because of the incorporation of organic matter, but anecic species can be  
336 negatively affected by it. It is essential to study the effects of cover crops on earthworm  
337 populations and whether there are allelopathic effects of specific cover crops such as  
338 oats and mustard on earthworms. Furthermore, there is a need to study in more detail  
339 the biology of *Aporrectodea trapezoides*, *A. rosea* and *Allolobophora georgii*, the  
340 species dominant in our experiment, which are abundant in Mediterranean systems.

341 Our study did not show any positive effects of the combination of conservation  
342 agriculture techniques on earthworms. In our trial the combination of reduced tillage by  
343 chiselling and green manure did not enhance earthworm populations. It is important to  
344 understand how these different factors interact when designing a sustainable organic  
345 system under certain climate conditions, soil properties, and crop rotations.

346 This study shows the importance of analysing the combination of such conservation  
347 agriculture techniques as reduced tillage and green manures in organic arable crop  
348 rotations, and it adds relevant information for the Mediterranean region. However, long-  
349 term trials in a Mediterranean climate are needed to fully understand the ecology of  
350 different earthworm species, their interactions and relation with conservation agriculture  
351 techniques, as well as their potential roles in promoting more sustainable farming  
352 systems.

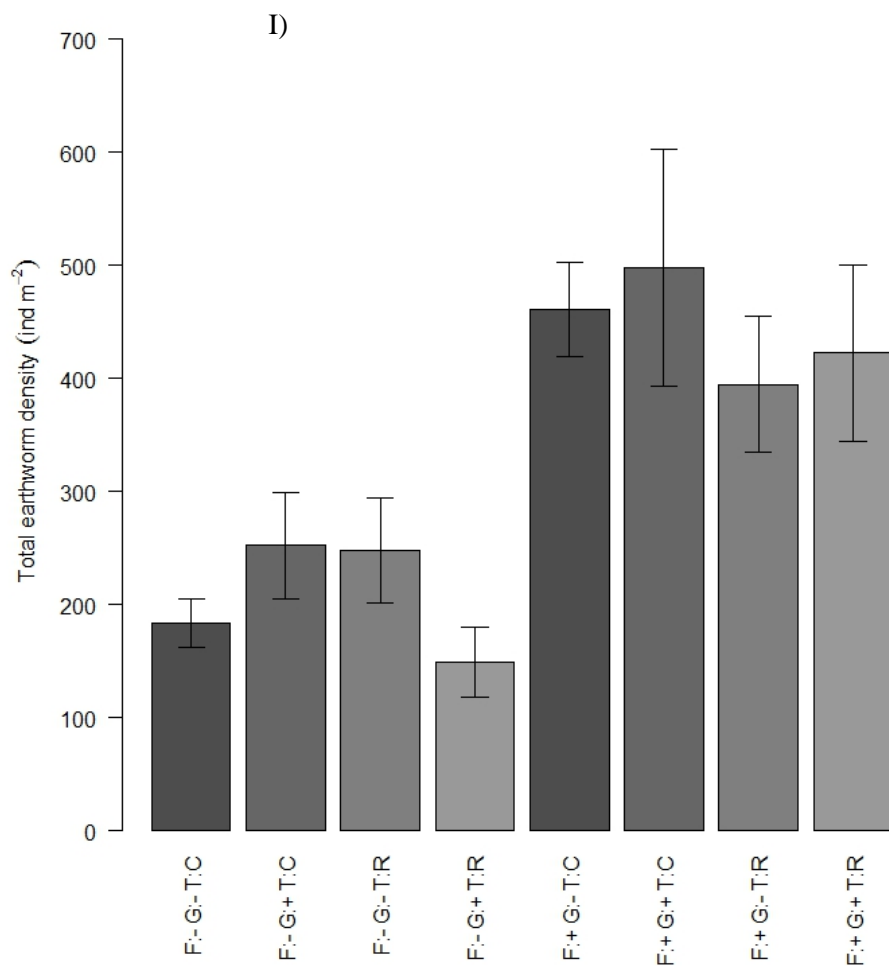


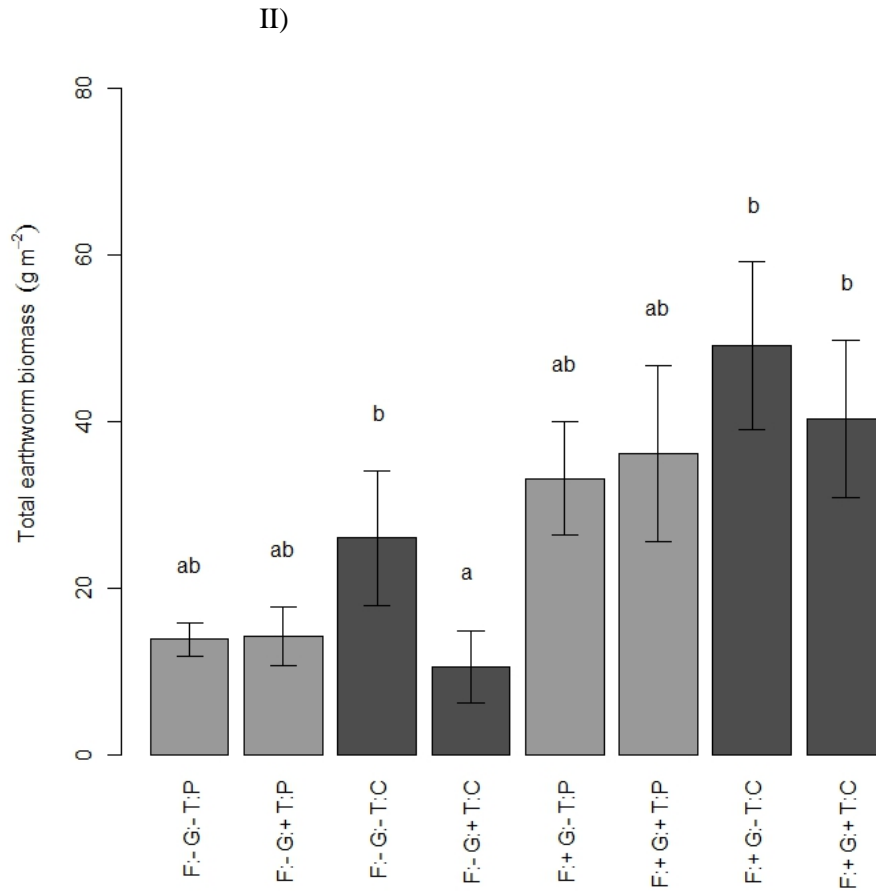
353 Table 1. Coefficients (estimate  $\pm$  standard error) from the linear mixed models testing the effect of fertilization (F: + fertilization with farmyard manure, F: - not fertilized;  
 354 T: tillage system (P: mouldboard ploughing, C: chisel ploughing); G: + with green manure, G: - no green manure), and the interaction between factors (F, T and G) on the  
 355 different variables measured.

	F: + vs -	T: P vs C	G: + vs -	FxT	FxG	TxG
Total density	0.390**	0.076 <sup>NS</sup>	-0.021 <sup>NS</sup>	0.006 <sup>NS</sup>	0.034 <sup>NS</sup>	0.091 <sup>NS</sup>
Density adults	0.480***	-0.045 <sup>NS</sup>	-0.081 <sup>NS</sup>	0.028 <sup>NS</sup>	0.017 <sup>NS</sup>	0.046 <sup>NS</sup>
Density juveniles	0.305*	0.203**	0.042 <sup>NS</sup>	0.042 <sup>NS</sup>	0.103 <sup>NS</sup>	0.100 <sup>NS</sup>
<i>A. rosea</i> total density	19.984*	8.046 <sup>NS</sup>	3.890 <sup>NS</sup>	8.621 <sup>NS</sup>	5.965 <sup>NS</sup>	8.053 <sup>NS</sup>
<i>A. georgii</i> total density	0.0323*	0.009 <sup>NS</sup>	-0.050 <sup>NS</sup>	-0.019 <sup>NS</sup>	-0.097 <sup>NS</sup>	-0.074 <sup>NS</sup>
<i>A. trapezoides</i> total density	9.656 <sup>NS</sup>	-1.137 <sup>NS</sup>	-0.381 <sup>NS</sup>	-3.031 <sup>NS</sup>	0.762 <sup>NS</sup>	3.218 <sup>NS</sup>
Total biomass	0.489*	-0.091 <sup>NS</sup>	-0.151*	-0.068 <sup>NS</sup>	0.060 <sup>NS</sup>	0.160*
Biomass adults	0.582*	-0.166 <sup>NS</sup>	-0.232*	-0.028 <sup>NS</sup>	0.112 <sup>NS</sup>	0.150 <sup>NS</sup>
Biomass juveniles	1.209*	0.521 <sup>NS</sup>	0.121 <sup>NS</sup>	-0.378 <sup>NS</sup>	0.234 <sup>NS</sup>	0.334 <sup>NS</sup>
<i>A. rosea</i> total biomass	3.121*	0.215 <sup>NS</sup>	-0.221 <sup>NS</sup>	0.509 <sup>NS</sup>	-0.090 <sup>NS</sup>	0.903 <sup>NS</sup>
<i>A. georgii</i> total biomass	0.408*	-0.086 <sup>NS</sup>	-0.133 <sup>NS</sup>	0.006 <sup>NS</sup>	-0.049 <sup>NS</sup>	0.026 <sup>NS</sup>
<i>A. trapezoides</i> total biomass	7.134 <sup>NS</sup>	-2.834 <sup>NS</sup>	-1.222 <sup>NS</sup>	-2.234 <sup>NS</sup>	1.091 <sup>NS</sup>	2.347 <sup>NS</sup>

356 Total density and biomass refers to the sum of adults and juveniles. §: Square root transformation was applied to accomplish homoscedasticity and normality of residuals.

357 Significance levels according to the following codes: \*\*\*  $p < 0.001$ . \*\*  $p < 0.01$ . \*  $p < 0.05$ . <sup>NS</sup> not significant).





359

360 Figure 1. Mean ( $\pm$  SE standard error of differences) total number of individuals (I) and total  
 361 biomass (II) in each treatment: F: - not fertilized, F: + fertilization with farmyard manure; G: -  
 362 no green manure, G: + sowed with green manure; T: tillage system (P: mouldboard ploughing,  
 363 C: chisel ploughing). Bars with no letters in common are significantly different (Tukey HSD  
 364 test,  $P < 0.05$ ).

365 Table 2. Mean ( $\pm$  SE standard error of the differences) number of total individuals of *Aporrectodea rosea*, *Allolobophora georgii* and *Aporrectodea*  
 366 *trapezoides*, and their sum per square meter: +F: fertilization with farmyard manure, -F: not fertilized, P: mouldboard ploughing, C: chisel, G+: with green  
 367 manure and G-: no green manure.

Species	F+				F-			
	P		C		P		C	
	G+	G-	G+	G-	G+	G-	G+	G-
<i>A. rosea</i>	108.35 $\pm$ 33.13	71.95 $\pm$ 10.09	58.32 $\pm$ 11.24	55.30 $\pm$ 11.55	38.62 $\pm$ 4.17	27.25 $\pm$ 4.11	24.25 $\pm$ 9.51	43.92 $\pm$ 14.66
<i>A. georgii</i>	24.22 $\pm$ 1.24	53.05 $\pm$ 7.97	44.70 $\pm$ 14.77	34.85 $\pm$ 6.37	24.27 $\pm$ 7.21	19.70 $\pm$ 5.16	18.17 $\pm$ 2.76	19.70 $\pm$ 3.81
<i>A. trapezoides</i>	31.82 $\pm$ 8.97	27.27 $\pm$ 8.65	36.37 $\pm$ 13.67	39.40 $\pm$ 10.71	19.70 $\pm$ 6.84	12.90 $\pm$ 3.12	6.82 $\pm$ 4.35	18.20 $\pm$ 7.42
Total_abundance	164.40 $\pm$ 34.64	152.27 $\pm$ 13.71	139.40 $\pm$ 25.81	129.50 $\pm$ 20.71	82.57 $\pm$ 14.98	59.87 $\pm$ 6.82	49.22 $\pm$ 10.08	81.82 $\pm$ 15.50

368

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381 **REFERENCES**

- 382 Andersen, C., 1979. The influence of farmyard manure and slurry on the earthworm  
383 population (Lumbricidae) in arable soil, in: Dindal, D.L. (Ed.), *Soil Biology as*  
384 *Related to Land Use Practices*. Environmental Protection Agency, Washington  
385 DC, pp. 325–335.
- 386 Bates, D., Maechler, M., Bolker, B., 2011. lme4: Linear mixed-effects models using S4  
387 classes.
- 388 Boström, U., 1995. Earthworm populations (Lumbricidae) in ploughed and undisturbed  
389 leys. *Soil Tillage Res.* 35, 125–133. doi:10.1016/0167-1987(95)00489-0
- 390 Bouché, M.B., 1972. *Lombriciens de France. Ecologie et systématique*. INRA, Paris.
- 391 Brown, G.G., Edwards, C.A., Brussaard, L., 2004. How earthworms affect plant  
392 growth: burrowing into the mechanisms, in: Edwards, C.A. (Ed.), *Earthworm*  
393 *Ecology*. CRC Press, Boca Raton, FL, USA. doi:doi:10.1201/9781420039719.ch2
- 394 Capowiez, Y., Cadoux, S., Bouchant, P., Ruy, S., Roger-Estrade, J., Richard, G.,  
395 Boizard, H., 2009. The effect of tillage type and cropping system on earthworm  
396 communities, macroporosity and water infiltration. *Soil Tillage Res.* 105, 209–216.  
397 doi:10.1016/j.still.2009.09.002
- 398 Chan, K.Y., 2001. An overview of some tillage impacts on earthworm population  
399 abundance and diversity - implications for functioning in soils. *Soil Tillage Res.*  
400 57, 179–191.
- 401 Crittenden, S.J.J., Eswaramurthy, T., de Goede, R.G.. G.M., Brussaard, L., Pulleman,  
402 M.M.M., 2014. Effect of tillage on earthworms over short- and medium-term in  
403 conventional and organic farming. *Appl. Soil Ecol.* 83, 140–148.  
404 doi:10.1016/j.apsoil.2014.03.001
- 405 Curry, J.P., 2004. Factors affecting the abundance of earthworms in soils, in: Edwards,

406 C.A. (Ed.), *Earthworm Ecology*. CRC Press Boca Raton, Fla, USA, pp. 91–113.

407 Curry, J.P., 1976. Some effects of animal manures on earthworms in grassland.

408 *Pedobiologia* (Jena). 16, 425–438.

409 Eriksen-Hamel, N.S., Speratti, A.B., Whalen, J.K., Légère, A., Madramootoo, C.A.,

410 2009. Earthworm populations and growth rates related to long-term crop residue

411 and tillage management. *Soil Tillage Res.* 104, 311–316.

412 doi:10.1016/j.still.2009.04.006

413 Ernst, G., Emmerling, C., 2009. Impact of five different tillage systems on soil organic

414 carbon content and the density, biomass, and community composition of

415 earthworms after a ten year period. *Eur. J. Soil Biol.* 45, 247–251.

416 doi:10.1016/j.ejsobi.2009.02.002

417 Gutiérrez-López, M., Moreno, G., Trigo, D., Juárez, E., Jesús, J.B., Díaz Cosín, D.J.,

418 2016. The efficiency of earthworm extraction methods is determined by species

419 and soil properties in the Mediterranean communities of Central-Western Spain.

420 *Eur. J. Soil Biol.* 73, 59–68. doi:10.1016/j.ejsobi.2016.01.005

421 Henneron, L., Bernard, L., Hedde, M., Bertrand, M., Girardin, C., Blanchart, E., Pelosi,

422 C., Villenave, C., Chenu, C., Bertrand, M., Girardin, C., Blanchart, E., 2014.

423 Fourteen years of evidence for positive effects of conservation agriculture and

424 organic farming on soil life. *Agron. Sustain. Dev.* doi:10.1007/s13593-014-0215-8

425 Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric

426 models. *Biometrical J.* 50, 346–363.

427 IUSS Working Group WRB, 2015. World reference base for soil resources 2014, update

428 2015. International soil classification system for naming soils and creating legends

429 for soil maps, World Soil Resources Reports No. 106 FAO, Rome.

430 doi:10.1017/S0014479706394902

431 Kuntz, M., Berner, A., Gattinger, A., Scholberg, J.M., Mäder, P., Pfiffner, L., 2013.  
432 Influence of reduced tillage on earthworm and microbial communities under  
433 organic arable farming. *Pedobiologia (Jena)*. 56, 251–260.  
434 doi:10.1016/j.pedobi.2013.08.005

435 Lee, K.E., 1985. *Earthworms: their ecology and relationships with soils and land use*.  
436 Academic Press.

437 Metzke, M., Potthoff, M., Quintern, M., Heß, J., Joergensen, R.G., 2007. Effect of  
438 reduced tillage systems on earthworm communities in a 6-year organic rotation.  
439 *Eur. J. Soil Biol.* 43, S209–S215. doi:10.1016/j.ejsobi.2007.08.056

440 Padmavathy, A., Poyyamoli, G., 2013. Biodiversity comparison between paired organic  
441 and conventional fields in Puducherry, India. *Pakistan J. Biol. Sci.* 16, 1675–1686.

442 Peigné, J., Cannavaciolo, M., Gautronneau, Y., Aveline, A., Giteau, J.L., Cluzeau, D.,  
443 2009. Earthworm populations under different tillage systems in organic farming.  
444 *Soil Tillage Res.* 104, 207–214. doi:10.1016/j.still.2009.02.011

445 Pelosi, C., Pey, B., Hedde, M., Caro, G., Capowiez, Y., Guernion, M., Peigné, J., Piron,  
446 D., Bertrand, M., Cluzeau, D., 2014. Reducing tillage in cultivated fields increases  
447 earthworm functional diversity. *Appl. Soil Ecol.* 83, 79–87.

448 R Core Team, 2015. *R: A Language and Environment for Statistical Computing*.

449 Riley, H., Pommeresche, R., Eltun, R., Hansen, S., Korsæth, A., 2008. Soil structure,  
450 organic matter and earthworm activity in a comparison of cropping systems with  
451 contrasting tillage, rotations, fertilizer levels and manure use. *Agric. Ecosyst.*  
452 *Environ.* 124, 275–284. doi:10.1016/j.agee.2007.11.002

453 Scullion, J., Neale, S., Philipps, L., 2002. Comparisons of earthworm populations and  
454 cast properties in conventional and organic arable rotations. *Soil Use Manag.* 18,  
455 293–300. doi:10.1079/SUM2002132



456 Smith, R.G., Mcswiney, C.P., Grandy, A.S., Suwanwaree, P., Snider, R.M., Robertson,  
457 G.P., 2008. Diversity and abundance of earthworms across an agricultural land-use  
458 intensity gradient. *Soil Tillage Res.* 100, 83–88. doi:10.1016/j.still.2008.04.009  
459 Soil Survey Staff, 1998. Keys to soil taxonomy, Soil Conservation Service, United  
460 States Department of Agriculture.  
461 Stroud, J.L., Irons, D.E., Watts, C.W., White, R.P., Mcgrath, S.P., Whitmore, A.P.,  
462 2016. Population collapse of *Lumbricus terrestris* in conventional arable  
463 cultivations and response to straw applications. *Appl. Soil Ecol.* 108, 72–75.  
464 doi:10.1016/j.apsoil.2016.08.002  
465 Valckx, J., Cordón, A., Govers, G., Hermy, M., Muys, B., 2011. Food and habitat  
466 preferences of the earthworm *Lumbricus terrestris* L. for cover crops. *Pedobiologia*  
467 (Jena). 54, S139–S144. doi:10.1016/j.pedobi.2011.07.004