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# Soil properties, grassland management, and landscape diversity drive the assembly of earthworm communities in temperate grasslands

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## Abstract

Earthworms are widespread soil organisms that contribute to a wide range of ecosystem services. As such, it is important to improve our knowledge, still scanty, of the factors that drive the assembly of earthworm communities. The aim of the present study was to conjointly evaluate the effects on the assembly of earthworm communities of i) soil properties (texture, organic matter content, and pH), ii) grassland management (grassland age, livestock unit, and type of fertilization), iii) landscape diversity (richness, diversity of surrounding habitats, and grassland plant diversity), and iv) presence of hedgerows. The study was conducted in temperate grasslands of Brittany, France. Earthworms were sampled in 24 grasslands and, in three of these grasslands, they were sampled near a hedgerow or near a ditch (control without a hedgerow). Soil properties explained the larger portion of the variation in the earthworm community parameters compared to grassland management or landscape diversity. The increase in soil organic matter content and pH were the most favorable factors for earthworm abundance and biomass, in particular for endogeic species. Regarding grassland management, the increase in the livestock unit was the most damaging factor for earthworm communities, in particular for the anecic earthworm biomass and endogeic species richness. Surprisingly, landscape diversity negatively affected the total earthworm abundance and epigeic earthworm biomass, but it was related to an increase in the epi-anecic species. At a finer scale, we also demonstrated that the presence of hedgerows surrounding grasslands enhanced earthworm species richness, especially within the epigeic and anecic ecological categories. This study highlights that the earthworm ecological categories respond specifically to environmental filters; further studies need to be conducted to elucidate the factors that drive the assembly of earthworm communities at this ecological category level. We recommend that policymakers should act on landscape management to favor earthworm diversity in order to improve the ecosystem services they drive.

## 49 INTRODUCTION

50

51 Earthworms are widespread soil organisms constituting the most important terrestrial biomass in  
52 temperate climate zones (Hole, 1981; Bar-On et al., 2018). They are usually classified into three main  
53 ecological categories depending on their physiology, morphology and behaviour: epigeic, anecic and  
54 endogeic species (Bouché, 1972, 1977). Briefly, epigeic earthworms live in and consume surface organic  
55 matter, anecic earthworms burrow vertical galleries to feed on a mixture of surface and soil organic matter,  
56 and endogeic earthworms burrow horizontal galleries to feed on soil organic matter (Bouché and  
57 Kretzschmar, 1974; Bouché, 1977; Jégou et al., 1998). Additionally, within the anecic earthworms, epi-  
58 anecic species feed preferentially on fresh surface organic matter (i.e. leaf litter) and are thereby  
59 distinguished from strict-anecic species that feed preferentially on humified organic matter already  
60 incorporated into the soil (Jégou et al., 1998; Larsen et al., 2016; Hoeffner et al., 2019). Depending on their  
61 ecological categories and associated feeding and burrowing behaviour, earthworms contribute to important  
62 ecosystem services provided by the soil such as nutrient cycling, water and climate regulation and primary  
63 production (Blouin et al., 2013; Bertrand et al., 2015). For example, van Groenigen et al. (2014) reported in  
64 a meta-analysis that an increase in crop production was observed in presence of earthworms, this increase  
65 ranging from 18% in presence of epigeic species up to 32% in presence of anecic species.

66 Earthworm communities are governed by different environmental filters, including biogeographical  
67 history, soil properties, land use and management as well as species interactions within the community (e.g.  
68 competition or facilitation; Lavelle, 1983; Curry, 2004; Decaëns et al., 2008). Previous studies focusing on  
69 the impact of soil properties on earthworm communities highlighted the key role played by soil pH, soil  
70 organic matter content and soil texture (Joschko et al., 2006; Lee, 1985; Decaëns et al., 2008). Other studies  
71 focused on the impact of land use on these earthworm communities (Boag et al., 1997; Decaëns et al., 2003,  
72 2008; Cluzeau et al., 2012). For example, Ponge et al. (2013) reported that grasslands exhibited higher  
73 anecic earthworm abundance than croplands. In addition, Zaller and Arnone (1999) observed a positive  
74 correlation between the density and the biomass of earthworm communities and the plant species richness  
75 of grasslands, and in particular for endogeic species. Concerning land management, previous studies  
76 reported that ploughing (Chan, 2001; Briones and Schmidt, 2017), pesticide application (Pelosi et al., 2014)  
77 and low permanent cover (Vršic, 2011) negatively impact earthworm communities with a response intensity  
78 depending on the ecological category considered.

79 Other studies have been undertaken at a greater scale to evaluate the impact of landscape diversity  
80 on earthworm communities within croplands (Vanbergen et al., 2007; Lüscher et al., 2014; Frazão et al.,  
81 2017). For example, Flohre et al. (2011) observed that the earthworm species richness in croplands

82 decreased with the percentage of surrounding agricultural fields. Regulska and Kolaczowska (2015) also  
83 reported that a cropland surrounded by a diverse landscape supported a higher earthworm diversity, density  
84 and biomass than the same type of cropland surrounded by a simpler landscape. However, the majority of  
85 the previous studies did not report effect of landscape diversity on earthworm communities of croplands  
86 and vineyards (Kovács-Hostyánszki et al., 2013; Frazão et al., 2017; Buchholz et al., 2017). Moreover, field  
87 margins of croplands were reported to exhibit higher abundance and diversity of earthworms than in the  
88 croplands itself but, surprisingly, these field margins were not reported to favor earthworm populations of  
89 these croplands (Smith et al., 2008; Roarty and Schmidt, 2013; Crittenden et al., 2015). Whether and how  
90 earthworms disperse within agricultural landscapes hence remains an unresolved issue.

91 A strong research effort has been done in the past decades to study the earthworm communities of  
92 croplands. Grasslands are the largest terrestrial ecosystem in the globe and produce many key ecosystem  
93 services, such as carbon storage, soil erosion mitigation or support for pollinators (Costanza et al., 1997;  
94 Conant and Paustian, 2002; Werling et al., 2014). The main objective of the present study was to conjointly  
95 evaluate the effects of soil properties, grassland management and landscape diversity on the assembly of  
96 grassland earthworm communities. Specifically, we hypothesized that the intensity of grassland  
97 management would negatively affect earthworm community parameters while the landscape diversity  
98 surrounding the grasslands would increase earthworm community parameters. The second objective was to  
99 evaluate the effect of hedgerows on these earthworm communities. By increasing the number of available  
100 niches, we hypothesized that the presence of a hedgerow in the grassland edge would increase earthworm  
101 community parameters (Tews et al., 2004). We conducted the study in an agricultural landscape of Brittany,  
102 France. Earthworms were sampled in 24 grasslands and, within three of them, they were oversampled near  
103 a hedgerow and near a ditch (control without hedgerow). Several parameters of the earthworm communities  
104 were evaluated including (i) the total abundance, total biomass, species richness and species diversity and  
105 (ii) the abundance, biomass and richness within each earthworm ecological category.

106

## 107 MATERIALS AND METHODS

### 108 *Study site*

109 The study site covers 10 km<sup>2</sup> and is a part of the Long Term Ecological Research (LTER) ‘‘Zone  
110 Atelier Armorique’’, located in Brittany, France (48°50’ N, -1°58’ W). The climate of the area is oceanic  
111 with a mean annual temperature of 11.7 °C, a mean annual rainfall of 815.0 mm and a mean annual relative  
112 humidity of 80.9 % (mean values over the period 2010-2016, data from Météo France). The main soil types  
113 encountered are Cambisols (IUSS Working Group, 2015) with high bedrock heterogeneity (granite, soft  
114 schist and aeolian loam). Moreover, the study area presents a substantial micro-topography, mainly due to

115 a high variability of landscape structures (e.g. hedges and ditches as field margins) with a hedge density  
116 ranging from 50 to 100 m.ha<sup>-1</sup> (Baudry et al., 2000; Thomas et al., 2016). Land use comprises mainly annual  
117 crops (corn, wheat, barley) and temporary or permanent grasslands, forest and unmanaged areas.

118 We used ground-truth aerial photos, which were taken every year since 1990, to construct a detailed  
119 land-use history for all grasslands, allowing us to precisely determine the age of each grassland. Based on  
120 this land-use history and after verification with grassland owners, we selected 24 grasslands ranging from 1  
121 to 25 years since the last crop. Among them, three grasslands with an age gradient of 1-, 2- and 7-year-old  
122 were selected and oversampled from a hedgerow and a ditch at their surroundings to take into account a  
123 specific effect of hedgerow on soil properties (Marshall and Moonen, 2002; Walter et al., 2003).

124

### 125 *Earthworm sampling and laboratory analyses*

126 Earthworms were sampled in 2016 within the 24 grasslands at a 30 m distance from any grassland  
127 edge, and then in the 3 selected grasslands near a ditch and near a hedgerow. For the 3 selected grasslands,  
128 we standardized the sampling with 3 sampling points in order to consider 3 replicates with hedgerow (at 1,  
129 5 and 10 m from the hedgerow) and 3 replicates without hedgerow (at 1, 5 and 10 m from the ditch).

130 Earthworms sampling followed the normalized protocol ISO 23 611-1, that was modified and  
131 validated during the RMQS BioDiv program (Cluzeau et al., 2012) combining chemical and physical  
132 extractions. Briefly, each earthworm sampling was characterised by a mean of three sub-sampling spaced  
133 of 10 m in line. Earthworm sub-sampling consisted of three waterings of 10 L with a gradient concentration  
134 of formaldehyde (0.25, 0.25 and 0.4%) on one square meter. After each watering, earthworms were collected  
135 for 15 min. Afterwards, a block of soil (25 × 25 × 20 cm, length × width × depth) was excavated within  
136 each sub-sampling area and earthworms were hand-sorted. The number of hand-sorted earthworms (HS)  
137 was multiplied by 16 to obtain an estimation per square meter. This number was then added to the number  
138 of earthworms counted with the formaldehyde extraction (F) to obtain the total number of earthworms per  
139 square meter (FHS):  $FHS = F + (16 \times HS)$ . Earthworms were fixed and preserved in formaldehyde solution  
140 (4%).

141 In the laboratory, each earthworm individual was counted, weighed, assigned to a stage of  
142 development (juvenile, sub-adult and adult), identified at the sub-species level and assigned to its ecological  
143 category: epigeic, anecic or endogeic (Bouché, 1972, 1977). Additionally, we distinguished within anecic  
144 earthworms, the epi-anecic (genus *Lumbricus*) from the strict-anecic earthworms (genus *Aporrectodea*)  
145 (Ferrière, 1980; Jégou et al., 1998). For juvenile individuals, identification was first limited to the genus and  
146 thereafter they were attributed a species name according to the proportions of sub-adults and adults present

147 of the same genus on each square meter. Earthworm diversity was analysed through three levels: total  
148 species richness, Shannon diversity index and species evenness index.

149

### 150 *Environmental filters*

151 We selected three environmental filters to explain earthworm community parameters: soil  
152 properties, grassland management and landscape diversity.

153 Soil properties were characterized by the soil texture, organic matter content and pH (water). Ten  
154 soil samples were randomly collected at 3 m around the earthworm sub-samplings using a cylindrical soil  
155 corer (5 cm diameter  $\times$  20 cm depth) in each grassland. These 10 soil samples were pooled and homogenized  
156 in order to consider one composite soil sample per grassland and sent to the analytical laboratory of  
157 LABOCEA (Combourg, France). Briefly, clay content ranged from 9.5% to 19.7%, sand content from  
158 13.3% to 68.9%, organic matter content from 1.8% to 5.2% and soil pH from 5.5 to 6.7 (Supplementary  
159 Table S1).

160 Grassland management was assessed from interviews with farmers (Supplementary Table S1) and  
161 from ground-truth aerial photos. The grassland age ranged from 1 to 25 years since the last row-cropping  
162 using quite similar species sown (*Lolium perenne* and *Trifolium repens* or *pratensis*). In addition, livestock  
163 unit per hectare varied from 0 to 4.3. Fertilisation rate was declarative so we used only the distinction  
164 between organic and mineral input.

165 Landscape structure within 100 m radius around the sampled fields was classified into 9 habitats  
166 based on aerial photos (forest, grassland, crop, hedge, water, building, garden, asphalt area, road). The radius  
167 of 100 m was chosen to reflect the overall low mobility of earthworms (Bardgett et al., 2005; Eijsackers,  
168 2010, 2011). Landscape diversity was characterized by two indexes: total richness of habitats within the  
169 radius and Shannon Diversity Index of habitats (hereafter called SHDI). Mapping and analysis were done  
170 using the softwares QGIS 2.8.1 and FRAGSTATS 4.296. In addition, we characterized the plant community  
171 of the 24 grasslands in spring 2015 using 10 quadrats (1  $\times$  1 m) evenly distributed in each grassland,  
172 characterizing for each plant species its covering percentage. Among the 24 grasslands selected, landscape  
173 richness varied from 1 to 7 habitats (maximum number of habitats has never been observed), SHDI from  
174 0.1 to 1.6 and plant Shannon index (hereafter called Plant diversity) within grasslands from 1.2 to 3.2  
175 (Supplementary Table S1).

176

### 177 *Statistical analysis*

178 We used multiple linear regression models to test the effects of soil properties (decomposed in clay,  
179 sand, organic matter contents and pH), grassland management (decomposed in grassland age, livestock unit

180 and fertilisation), and landscape diversity (decomposed in landscape richness, SHDI and plant diversity) on  
181 all earthworm community parameters (i.e. total abundance and biomass, total diversity indexes, ecological  
182 categories abundance and biomass). We constructed a full model comprising all environmental filters, and  
183 then we selected the significant environmental filters using a backward stepwise selection procedure that  
184 selects the best model using the AIC criterion (Crawley, 2012; stepAIC function of the “MASS” package).  
185 We also evaluated the variance inflation factor (VIF) of each variable selected by the previous procedure to  
186 test for multicollinearity among environmental filters. We removed all environmental filters that showed a  
187  $VIF > 5$ , even if significant from the model. Data met the conditions of normality and homoscedasticity.

188 Second, within each of the three selected grasslands (i.e. 1-, 2- and 7-year-old), we compared  
189 earthworm communities with and without hedgerow (ditch) using the three sampling points per plot as  
190 replicates. We used separated *t*-tests within the three selected grasslands to assess the differences in  
191 earthworm abundance, earthworm biomass, and species richness according to the presence or absence of a  
192 hedgerow.

193 Statistical analyses were performed with the R software 3.2.3 (R. Core Team, 2017). Significance  
194 was evaluated in all cases at  $P < 0.05$ .

195  
196 **RESULTS**  
197 *Impact of soil properties, grassland management and landscape diversity on earthworm*  
198 *communities*

199 Over the 24 grasslands sampled, the average earthworm abundance and biomass were  $517.0 \pm 57$   
200 individual.m<sup>-2</sup> and  $219.4 \pm 20$  g.m<sup>-2</sup>, respectively. The mean earthworm species richness was  $10.8 \pm 0.3$ .  
201 Eighteen species belonging to the three ecological categories were identified (Supplementary Table S2).  
202 *Allolobophora chlorotica* and *Aporrectodea caliginosa* were the most abundant species whereas *Eisenia*  
203 *tetraedra*, *Dendrobaena rubida* and *Octolasion lacteum* were present in one grassland only (Supplementary  
204 Table S2).

205 Higher soil organic matter content increased the total earthworm abundance ( $F = 5.3$ ,  $P = 0.033$ ,  
206 Table 1), the endogeic species abundance ( $F = 5.7$ ,  $P = 0.028$ , Supplementary Table S3) and the endogeic  
207 species richness ( $F = 5.4$ ,  $P = 0.031$ , Supplementary Table S4), while the endogeic species abundance was  
208 negatively correlated to the sand content ( $F = 6.9$ ,  $P = 0.017$ , Supplementary Table S3). In addition the total  
209 earthworm abundance and biomass increased when soil pH was more alkaline ( $F = 5.0$  and  $6.8$ ,  $P < 0.05$ ,  
210 Fig. 1, Table 1) but no category-specific impact was observed with respect to pH variation.

211 The increase in livestock unit decreased total earthworm biomass ( $F = 5.7$ ,  $P = 0.028$ , Table 1), and  
212 in particular the biomass of anecic species ( $F = 9.6$ ,  $P = 0.005$ , Fig. 2a, Supplementary Table S5). However,

213 this negative effect was only confirmed for the biomass of epi-anecic species ( $F = 4.4$ ,  $P = 0.049$ , Fig. 2b,  
214 Supplementary Table S5). The increase in livestock unit also decreased the earthworm species richness, the  
215 Shannon diversity index and the species evenness ( $F = 2.8$  to  $9.6$ ,  $P < 0.05$ , Fig. 2c, Table 1), and in particular  
216 the endogeic species richness ( $F = 9.5$ ,  $P = 0.006$ , Supplementary Table S4). Mineral fertilisation enhanced  
217 the epigeic species abundance and biomass compared to organic fertilisation ( $F = 6.6$  and  $8.6$ ,  $P < 0.02$ ,  
218 Supplementary Tables S3 and S5).

219 Landscape richness decreased the biomass of epigeic species ( $F = 4.9$ ,  $P = 0.041$ , Supplementary  
220 Table S4) but enhanced the epi-anecic species richness ( $F = 6.6$ ,  $P = 0.019$ , Supplementary Table S4). The  
221 increase of SHDI decreased the total earthworm abundance ( $F = 4.6$ ,  $P = 0.047$ , Table 1). In addition, the  
222 increase in plant diversity was positively correlated to Shannon diversity index and species evenness ( $F =$   
223  $5.0$  and  $4.8$ ,  $P < 0.04$ , Table 1).

224 Interestingly, the abundance of strict-anecic species, their biomass and richness were not affected  
225 by any of the environmental filters measured (Supplementary Tables S3, S4 and S5).

### 227 *Impact of hedgerow presence on earthworm communities*

228 Over the 3 grasslands oversampled, earthworm abundance was higher in the 2-year-old grassland  
229 ( $834 \pm 76$  individuals.m<sup>-2</sup>) compared to the 1-year-old ( $306 \pm 32$  individuals.m<sup>-2</sup>) and 7-year-old grasslands  
230 ( $385 \pm 32$  individuals.m<sup>-2</sup>). Earthworm species richness was higher in the 2- and 7-year-old grasslands ( $11.0$   
231  $\pm 0.4$  and  $10.2 \pm 0.3$ , respectively) compared to the 1-year-old grassland ( $7.9 \pm 0.4$ ). Earthworm species  
232 composition was also strongly different between these three grasslands. For example, the presence of  
233 *Eisenia tetraedra* occurred only in the 2-year-old grassland and the presence of *Aporrectodea caliginosa*  
234 *meridionalis* occurred only in the 7-year-old grassland.

235 Earthworm species richness was 21.0% and 23.2 % higher with the presence of a hedgerow,  
236 compared to the presence of a ditch, in the grasslands of 1- and 2-year-old ( $t = 5.8$  and  $13.9$ ,  $P < 0.03$ , Fig.  
237 3a and b). It was however not affected in the 7-year-old grassland ( $t = 0.0$ ,  $P = 0.85$ , Fig. 3c). The abundance  
238 of earthworms was not affected by the presence of hedgerows in the three selected grasslands ( $t = 0.0$  to  
239  $0.03$ ,  $P > 0.865$ ).

240 Overall, except *Allolobophora icterica* and *Aporrectodea nocturna* that were more abundant with  
241 the presence of a hedgerow, the strict-anecic and endogeic species were evenly distributed between the plots  
242 with and without a hedgerow. The distribution of epi-anecic earthworm species was heterogeneous, but  
243 *Lumbricus rubellus rubellus* and *Lumbricus terrestris* were more often observed in presence of a hedgerow.  
244 The distribution of epigeic earthworm was species dependent: *Dendrobaena mammalis* occurrence was  
245 higher in presence of a hedgerow and *Eisenia tetraedra* was observed in presence of a hedgerow in the 2-



246 year-old grassland only. *Lumbricus castaneus* and *Lumbricus rubellus castanoïdes* occurrences were overall  
247 similar between the plots, independent from the presence of a hedgerow.

248

## 249 DISCUSSION

250 In the present study, we clearly demonstrated that soil properties, grassland management and  
251 landscape diversity conjointly affected the selected parameters of the earthworm communities. Our findings  
252 hence contrast with those of Frazão et al. (2017) who reported that earthworm communities of the croplands  
253 were impacted by agricultural practices only but neither by soil properties nor landscape diversity.

254 Contrary to previous studies that observed an effect of soil properties at the regional scale (Decaëns  
255 et al., 2003; Vanbergen et al., 2007; Decaëns et al., 2008), here, by taking the earthworm ecological category  
256 into account, we evidenced that soil properties impact on a finer scale (i.e. 10 km<sup>2</sup>), the abundance, biomass  
257 and richness of earthworm ecological categories. This result might be due to the strong spatial heterogeneity  
258 of the soil properties in the studied region (Jamagne, 2011). In agreement with previous studies, we observed  
259 that higher soil sand content decreased the total abundance of earthworms (Hendrix et al., 1992; Lapied et  
260 al., 2009), which could be due to the low capacity of sandy soils to hold water, leading to an unfavorable  
261 habitat for earthworms (Lee, 1985). In addition, the increase in soil pH was positively correlated to both  
262 earthworm species richness (Joschko et al., 2006) and total abundance (Ma et al., 1990; McCallum et al.,  
263 2016). Nonetheless, several reviews observed that earthworm preference to soil pH was species-dependent  
264 due to their synecology (Bouché, 1972; Edwards and Lofty, 1977; Lee, 1985) but the underlying  
265 mechanisms for pH preference are not fully understood yet. In line with their feeding behaviour that consists  
266 in consuming mainly humified organic matter, endogeic earthworm communities were more abundant and  
267 diversified in grasslands presenting high contents of soil organic matter (Bouché, 1977; Pearce, 1978;  
268 Ferrière, 1980).

269 Regarding grassland management, increasing livestock unit was the most damaging factor for  
270 earthworm communities as it decreased the total biomass, species richness, the Shannon diversity index and  
271 the species evenness. This strong negative effect could be associated to the trampling at high stocking levels  
272 that damages soil structure and thus adversely affect earthworm communities and burrows (Cluzeau et al.,  
273 1992; Pietola et al., 2005; Chan and Barchia, 2007). Interestingly, earthworms' response to livestock unit  
274 was almost entirely confined to the largest epi-anecic and endogeic species and only the earthworm biomass  
275 was affected, contrary to their abundance, suggesting a decrease in the mean body size rather than in  
276 individuals' number. Surprisingly, mineral fertilisation enhanced the abundance and biomass of epigeic  
277 species, but this finding is nonetheless in line with some previous studies that reported an increase in  
278 earthworm abundance in relation to N mineral fertilisation (Muldowney et al., 2003; King and Hutchinson,

279 2007; Curry et al., 2008). Mineral fertilisation would probably allow a better primary production leading to  
280 higher leaf litter inputs that constitute a source of refuge and food for earthworms. Further studies are  
281 needed, in grassland, to elaborate the different impacts of manure versus mineral fertilisation on  
282 earthworms. Overall, we observed that within grasslands, grazing pressure led to smaller and less-diversified  
283 earthworm communities.

284 We observed a negative effect of increasing landscape diversity (richness and Shannon Index) on  
285 the total abundance of earthworms and, to our knowledge, for the first time, the biomass of epigeic  
286 earthworms in grasslands. A negative correlation between the total abundance of earthworms and landscape  
287 diversity was also observed by Flohre et al., (2011) in croplands, and the authors hypothesized that landscape  
288 diversity increases the number of earthworm predators. Indeed, several studies highlighted that landscape  
289 diversity enhance the abundances of invertebrates, mammals and birds (Marshall and Moonen, 2002;  
290 Maudsley et al., 2002; Vickery et al., 2009) that are potential predators for earthworms (Granval and Aliaga,  
291 1988; O'Brien et al., 2016). We can also hypothesize that the capacity of epigeic species to disperse is  
292 hindered by physical barriers (i.e. hedge or ditch) and different soil properties (shelter and litter availability)  
293 in neighboring habitats that nonetheless constitute landscape diversity. In contrast, the species richness of  
294 epi-aneic earthworm was enhanced by the landscape diversity. As epi-aneic earthworm species have a  
295 great mobility varying from 1.5 to 14 m. year<sup>-1</sup> (Hoogerkamp et al., 1983; Eijsackers, 2011; Nuutinen et al.,  
296 2014) and the ability to burrow into the soil to protect themselves, higher landscape diversity around  
297 grasslands could enhance their areas of emigration. Endogeic earthworm species were not impacted by  
298 landscape diversity and were highly abundant in each grassland as previously reported (Lavelle, 1983;  
299 Decaëns et al., 2008). Overall, it is possible that low agricultural practices in grasslands, compared to  
300 croplands or vineyard, could increase the effect of the surrounding landscape diversity on earthworm  
301 communities (Roarty and Schmidt, 2013; Buchholz et al., 2017; Frazão et al., 2017).

302 In addition to the effect of landscape diversity, we highlighted the importance of hedgerows  
303 surrounding grasslands. Hedgerows especially acted in young grasslands (i.e. 1- and 2-year-old grassland),  
304 which is probably due to the increase earthworm species aggregation with the age of the grasslands (Richard  
305 et al., 2012). It is well known that hedgerows locally modify soil properties (i.e. soil moisture, temperature  
306 or organic matter content; Marshall and Moonen, 2002), and especially the amount and type of litter  
307 deposited at the soil surface (Walter et al., 2003). This litter input is a key factor for the development of  
308 earthworm communities (Lee, 1985; Edwards, 2004), and in particular for epigeic and epi-aneic species  
309 that have a diet mainly composed of fresh leaf litter (Bouché and Kretzschmar, 1974; Pearce, 1978; Ferrière,  
310 1980). In field, earthworm communities living in grasslands surrounded by a hedgerow were richer in  
311 earthworm species compared to earthworm communities in grasslands surrounded by a ditch, especially for

312 epigeic and epi-aneic earthworm species. Thus, hedgerows presence could promote earthworm diversity  
313 in grasslands. Increasing epi-aneic earthworm diversity in grasslands landscape could have consequences  
314 on ecosystem services provided by these species. Hoeffner et al. (2018) observed that burrows' fungal  
315 communities were regulated by epi-aneic species identity, which could increase the diversity of the  
316 drilospheric microbiota and improve soil functioning. Besides, as it is difficult to monitor the earthworm  
317 diversity response to global change drivers, earthworm databases often concern surveys carried out at  
318 regional or national scales (Rutgers et al., 2009; Cluzeau et al., 2012; Cameron et al., 2016). A first  
319 predictive model on the abundance and diversity of earthworms was created by Rutgers et al. (2016) taking  
320 into account soil occupation and properties. Future predictive models could therefore take into account the  
321 landscape as an additional factor regulating these earthworm communities.

322

## 323 CONCLUSION

324 Our study clearly illustrated that earthworm communities in grasslands were affected by the three  
325 environmental filters considered: soil properties, grassland management and landscape diversity. Soil  
326 properties was the main environmental filter controlling earthworm communities. However, we also  
327 highlighted important effects of grassland management, for instance a strong decrease in abundance of  
328 earthworms with increasing livestock unit. We observed various effects of landscape diversity, such as a  
329 surprising overall decrease of earthworm abundance or a higher epi-aneic richness in diverse landscapes.  
330 Therefore, our findings demonstrated conjoint effects of various environmental filters as drivers of  
331 earthworm communities. Taken together, our results suggest a strong context dependency in the assembly  
332 rules of earthworm communities, despite the fact that these communities are well known to be ubiquitous  
333 and resilient.

334

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342

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546

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Revised manuscript

548 TABLES

549

550 **Table 1** ANOVA results of multiple linear models testing for the effects of soil properties, grassland management and landscape diversity  
 551 on total earthworm abundance, total biomass, species richness and Shannon diversity index and evenness index (when VIF > 5). *F*-  
 552 values and associated *P*-values are indicated. Significant *P*-values are indicated in bold ( $P < 0.05$ ). df = degrees of freedom, %SS =  
 553 percentage of sum of square.

	Total abundance				Total biomass				Total richness				Shannon				Equitability			
	df	%SS	<i>F</i>	<i>P</i>	df	%SS	<i>F</i>	<i>P</i>	df	%SS	<i>F</i>	<i>P</i>	df	%SS	<i>F</i>	<i>P</i>	df	%SS	<i>F</i>	<i>P</i>
<b>Soil properties</b>																				
Clay content	1	5.9	2.2	0.157																
Sand content	1	5.2	1.9	0.182	1	0.4	0.1	0.711	1	0.1	0.0	0.841								
Organic matter content	1	14.4	5.3	<b>0.033</b>	1	6.0	2.1	0.164												
pH	1	13.4	5.0	<b>0.039</b>	1	19.4	6.8	<b>0.018</b>												
<b>Grassland management</b>																				
Grassland age													1	7.5	9.9	0.107	1	7.3	2.4	0.141
Livestock unit					1	16.3	5.7	<b>0.028</b>	1	31.3	9.6	<b>0.005</b>	1	26.2	2.8	<b>0.005</b>	1	15.1	4.8	<b>0.040</b>
Fertilisation																				
<b>Landscape diversity</b>																				
Landscape Richness																				
SHDI	1	12.3	4.6	<b>0.047</b>	1	6.4	2.2	0.153												
Plant diversity													1	13.2	5.0	<b>0.037</b>	1	15.1	4.8	<b>0.040</b>
Residuals	18	48.7			18	51.5			21	68.6			20	53.1			20	62.5		

554

555 FIGURE LEGENDS

556

557 **Fig. 1.** Relationship between total earthworm biomass and soil pH.  $R^2$  and associated  $P$ -value of  
558 the linear regression are indicated.

559

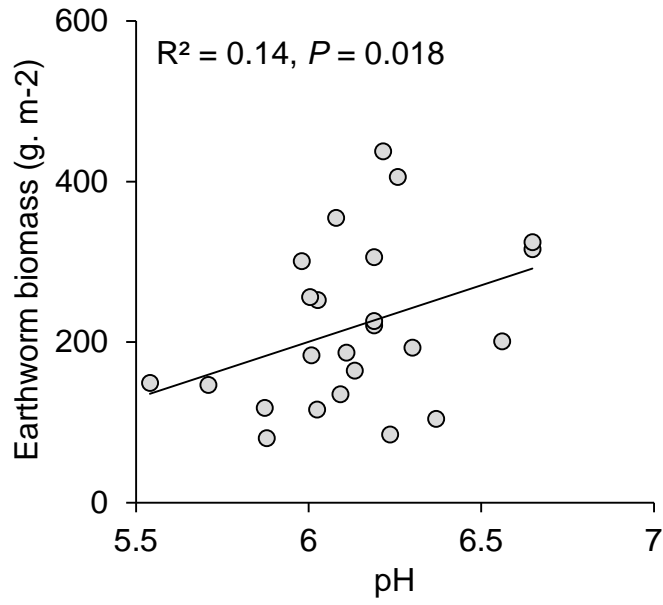
560 **Fig. 2.** Relationships between livestock unit and (a) anecic earthworm abundance, (b) epi-anecic  
561 earthworm abundance and (c) Shannon index.  $R^2$  and associated  $P$ -values of linear regressions are  
562 indicated.

563

564 **Fig. 3.** Earthworm species richness in plots with a hedgerow or with a ditch (i.e. control plot without  
565 hedgerow) for grassland of (a) 1-year-old, (b) 2-year-old and (c) 7-year-old. Values are means  $\pm$   
566 SD;  $n = 3$ . Different letters denote significant differences between the two plots with  $a > b$  (post  
567 hoc Tukey test results).

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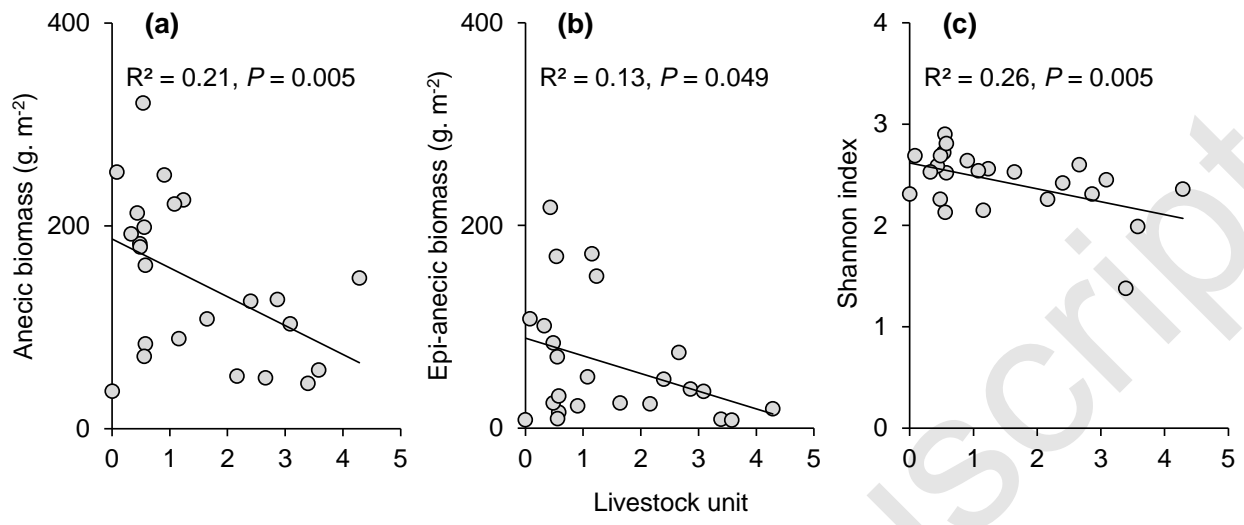
569 **Fig. 1.**



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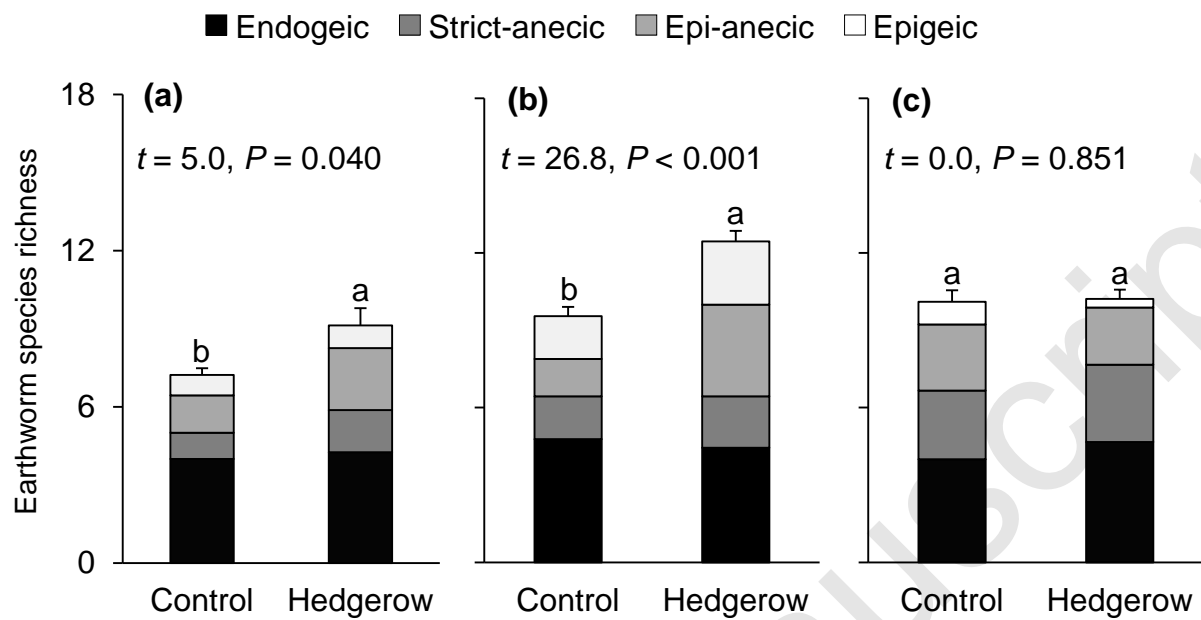
Revised manuscript

573 **Fig. 2.**  
574



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577 **Fig. 3.**



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