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## Management of grassland: A necessary tool to maintain plant and earthworm diversity

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1 **Title: Management of grassland: a necessary tool to maintain plant and earthworm diversity**

2

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18

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20

21 **Abstract**

22 In temperate grassland, earthworms contribute to the major soil processes which determine most  
23 of the ecosystem services. The characteristics of plant communities in grassland are key factors in  
24 maintaining earthworm communities, however effects of different herbage management on  
25 earthworms remain largely unknown. In this context, the aim of the present study was to determine  
26 the long-term effects of herbage management on grassland plant and earthworm communities.  
27 Plants and earthworms were sampled in a 14-years-old experiment in upland grasslands (Massif  
28 central, France). Abandoned grasslands were compared with mowed grasslands and with pastures  
29 grazed by cattle (at low or high intensities) or grazed by sheep (at low intensity). Compared to  
30 abandoned grassland, herbage management by grazing or by mowing display higher leguminous  
31 plant, community-weighted mean Ellenberg light values as well as plant richness while they display  
32 lower percentage of plant litter and community-weighted mean Ellenberg nitrogen values. The  
33 differences in plant richness were associated with a significant change in plant community  
34 structure. Compared to the abandoned grassland, herbage management by grazing or mowing  
35 significantly display higher earthworm biomass and total richness. Except for pastures grazed by  
36 cattle at high intensity, earthworm abundance was at least twice that in the grassland at low grazing  
37 intensity or mowing compared to the abandoned grassland. Earthworm communities were  
38 significantly different between grazed and mown treatments notably due to changes within  
39 *Aporrectodea* anecic and endogeic earthworm species. Overall, herbage management by animals  
40 or by mechanical export is beneficial for plant and earthworm diversity although no clear  
41 differences between management practices for earthworm richness, total biomass or total  
42 abundance were observed. Our results highlight that abandonment does not preserve biodiversity

43 (plant, soil macrofauna) while management of grassland by grazing or mowing is a necessary tool

44 for biodiversity conservation and improvement.

45

## 46 **1. Introduction**

47 Grassland ecosystems are widespread around the world, comprising 67% of agricultural area  
48 [1]. Grasslands provide several important ecosystem services such as the provision of forage,  
49 biodiversity habitat, in addition to the regulation of carbon storage, although this is dependent on  
50 objectives [2–4]. It is well known that these ecosystem services rely on complex biotic interactions  
51 constituting ecosystem processes [5–7].

52 Herbage management of grassland, through mowing and grazing impact plant functional  
53 types and diversity. Reduced disturbance [8] enhanced plant types with later flowering date and  
54 higher plant size with higher leaf dry matter content [8], foliar trait being linked to slower tissues  
55 decomposition [9]. Reduced grazing intensity [10] favor stress tolerant grasses to the detriment of  
56 competitive grasses [10], these grass strategies contrasting with species long-lived tissues  
57 producing litter of lower quality than species with opposite traits [11]. Management, also alters the  
58 quantity of plant litter [10], and the quality through changes in root N concentration [12] or  
59 production of secondary compounds in response to herbivory [13]. These changes in the amount  
60 of litter and the litter traits strongly impact the soil decomposer communities [14,15], including  
61 earthworms [16–18], which contribute to a large range of ecosystem services [19]. For example,  
62 Eisenhauer et al. [17] observed that earthworm performance was driven by the presence of certain  
63 functional plant groups, such as legumes, that were linked to high quality inputs of plant residues.  
64 In contrast, abandoned grassland produced a lower litter quality, closer to that found in scrubland  
65 or forests environments [20] unfavourable for common earthworm species [18,21].

66 The effects of grazing animals on earthworm communities are balanced between the negative  
67 effects of soil compaction under heavy loading [22–24], and the positive effects of organic matter

68 return through animal faeces, leading to improved trophic resources [25,26]. These positive effects  
69 can be direct, notably via animal faeces which constitute a resource for decomposers, or indirect,  
70 as faeces contribute to soil fertility. However, these effects are strongly depending on the livestock  
71 level, as demonstrated by King and Hutchinson [25] who showed that 20 sheep ha<sup>-1</sup> were  
72 favourable to the development of earthworm communities while 30 sheep ha<sup>-1</sup> were unfavourable.  
73 While previous studies have investigated the influence of grassland management (abandoned,  
74 mowed, or grazed) on earthworm communities [24,27–31], we are unaware of any research  
75 comparing the simultaneous effect of both grazing or mowing with abandoned grasslands on these  
76 communities.

77 The objectives of this study were to assess the long-term effects of an increasing herbage  
78 management by grazing or mowing on plant and earthworm communities. We hypothesized that  
79 the intensity of herbage utilisation gradually modifies plant community characteristics and  
80 consequently improves earthworm community structure. At a long-term experimental site, after 14  
81 years of differentiated management practices, abandoned grasslands were compared with mown  
82 grasslands and with grasslands grazed by sheep or by cattle, at different herbage utilization  
83 intensity.

84

## 85 **2. Materials and methods**

### 86 2.1 Study site and experimental design

87 The study site consists of upland grassland plots and is part of the long-term observatory  
88 SOERE-ACBB (Systems of Observation and Experimentation in Environmental Research -  
89 Agroecosystems, Biochemical cycles and Biodiversity), managed by the French National Institute

90 of Agricultural Research and Environment (INRAE) and supported by AnaEE-F. The study site is  
91 located in Theix, central France (45°43'43''N, 03°1'21''E, 880 m a.s.l.). Historically, the site,  
92 which contains all the studied plots, was used for grazing and mowing. In the late 1960s, following  
93 localized clearing of undergrowth and overseeding, the site was managed as a mixed regime with  
94 cuts for silage or hay until 2003, with applications of mineral and organic fertilizers, followed by  
95 autumn grazing. In the two years prior to experimentation (2003-2004), three annual cuts were  
96 carried out without fertilizer. In 2005, five treatments, representing different management  
97 practices, were established to test for the effect of intensity of grassland utilisation: (i) mowing  
98 regime (MO), (ii), grazing by cattle at a high level of herbage utilization (CA+), (iii) grazing by cattle  
99 at a low level of herbage utilization (CA-), (iv) grazing by sheep at a low level of herbage utilization  
100 (SH-) and (v) abandonment (AB). None of the treatments involved fertilization. The AB and MO  
101 treatments were conducted in 350 m<sup>2</sup> plots. SH- was set in 1100 m<sup>2</sup> plots grazed by five ewes  
102 whereas CA- and CA+ were set in 2200 m<sup>2</sup> plots grazed by 2 and 4 heifers, respectively. The  
103 grazing activity consisted of five annual rotations occurring in April, May, July, September and  
104 November. The mean length of the grazing period for these rotations (periods 1 to 5) was 8.6, 9.6,  
105 9.1, 7.2 and 2.6 days, respectively, based on data from 2006-2019. MO plots were mowed three  
106 times per year (in May, July, October) with the cuts made at a height of 5.5 cm and plant material  
107 subsequently removed from the field. Each treatment was replicated in 4 blocks, in a randomized  
108 block design, resulting in a total of 20 plots, for further details see Louault et al. (2017).

109

110 2.2 Climate and soil properties of the study site

111 The climate is semi-continental with an annual rainfall of 754 mm and a mean annual  
112 temperature of 8.8°C with a monthly minimum of -3.7°C and a monthly maximum of 22.5°C (site  
113 station data, 2009-2019). The soil, which is at least 60 cm deep, is classified as a eutric cambisol  
114 for blocks one and two and colluvic cambisol for blocks three and four, both developed from a  
115 granite bedrock [32]. The soil is characterized by an overall texture of sandy clay loam. Compared  
116 with blocks 1 and 2, blocks 3 and 4 are richer in clay and poorer in nitrogen. Moreover, organic  
117 matter is higher in blocks 1 and 2 than blocks 3 and 4, while bulk density and pH are lower in  
118 blocks 1 and 2 than in blocks 3 and 4 (Table 1, data from 2012 campaign).

119

### 120 2.3 Sampling and analysis of plant and earthworm communities

121 In spring 2019, in each plot, three square meters (referred to as pseudo replication) spaced at  
122 least 5 m apart were selected to analyze plant communities and sample earthworm communities in  
123 the same location.

124 For plants, in each square meter, first, a list of plant species was established to quantify species  
125 richness and a visual assessment of plant cover was performed to quantify relative abundance per  
126 species and per groups in terms of leguminous, dicotyledon (not presented) and grass (not presented).  
127 Second, within each square meter, divided into 16 cells, the cover was visually estimated for plant  
128 litter including senescent plant tissue, bare soil (not presented) and living plant (not presented). Using  
129 species Ellenberg nitrogen value or Ellenberg light indicator values and species abundance in each  
130 meter square quadrat, we calculated community-weighted mean (CWM) for Ellenberg Nitrogen  
131 (CWM EN) and for Ellenberg Light (CWM EL) indicator values considering only the dominants  
132 species, i.e., species accounting for at least 85% of the cumulative species relative abundance.



133 Moreover, we calculated CWM values considering the dominant species for two functional traits:  
134 Leaf Dry Matter Content (LDMC; g dry mass. g water-saturated fresh mass<sup>-1</sup>), and Reproductive  
135 Plant Height (RPH, cm). LDMC reflects the biomechanical properties of the leaves [33] and  
136 account for community digestibility [34] and plant height is related to species competitive ability  
137 [35]. Plant trait data were extracted from a local trait database which provide trait's values  
138 measured per species per plot of the experimental study site, for more details see Louault et al.  
139 [32].

140 Earthworms were sampled according to the ISO 23611-1 protocol modified by Pérès et al.  
141 [36]. This protocol combines a chemical and a physical extraction. The chemical extraction  
142 consisted of three application of 10 L of formaldehyde solution on one square meter with increasing  
143 concentrations (0.25%, 0.25% and 0.4%), each followed by 15 minutes of earthworm collection.  
144 The physical extraction consisted of hand sorting a 25 cm × 25 cm × 25 cm block removed from  
145 each square meter. Collected earthworms were stored in bottles containing formaldehyde (4%). In  
146 the laboratory, the earthworms were identified to the lowest possible taxonomic rank (species or  
147 genus), individually weighted ( $\pm 1$  mg) and assigned to an ecological category : epigeic, anecic,  
148 and or endogeic [37–39] (within the anecic, we distinguished between species of the genus  
149 *Lumbricus* and *Aporrectodea*). The earthworm community parameters were total abundance and  
150 biomass, abundance and biomass of the ecological categories and species richness.

151

152 2.4 Statistical analyses

153 Statistical analyses were performed with the R software 4.2.2 [40]. Significance was  
154 evaluated in all cases at  $P < 0.05$ . When necessary, data were log or square root transformed to  
155 meet the conditions of normality and homoscedasticity.

156 First, we used separated two-way ANOVAs followed by Tukey HSD tests for post hoc  
157 pairwise comparisons, to test the effects of treatments (AB, SH-, CA-, CA+ and MO) and blocks  
158 (B1, B2, B3, B4) on each physico-chemical soil parameter (loam, silt, sand contents, bulk density,  
159 nitrogen, organic matter and pH).

160 Second, we used separated linear mixed-effects models (“nlme” package), followed by  
161 Tukey HSD tests for post hoc pairwise comparisons, to test the effects of treatments (AB, SH-,  
162 CA-, CA+ and MO) and blocks (B1, B2, B3, B4) on plant species richness, percentage of plant  
163 litter and leguminous, CWM-EL, CWM-EN, CWM-LDMC and CWM-RPH. To account for  
164 pseudo replication, the random part of the model indicated that each square meter was nested within  
165 plot. The model's syntax was:  $\sim$  Treatment + Block, random =  $\sim 1$  | Pseudo replication.

166 Third, we used separated linear mixed-effects models (“nlme” package), followed by Tukey  
167 HSD tests for post hoc pairwise comparisons, to test the effects of treatments (AB, SH-, CA-, CA+  
168 and MO) and blocks (B1, B2, B3, B4) on earthworm total abundance, biomass and ecological  
169 category (except epigeic biomass due to low values) and species richness. To account for pseudo  
170 replication, the random part of the model indicated that each square meter was nested within a plot.  
171 The model's syntax was:  $\sim$  Treatment + Block, random =  $\sim 1$  | Pseudo replication.

172 To compare the structure of plant and earthworm communities between the treatments (AB,  
173 SH-, CA-, CA+ and MO) we constructed a data matrix of pairwise comparisons using the Bray-  
174 Curtis distance index. Non-Metric multi-Dimensional Scaling (NMDS, “vegan” package) was used

175 to find the best low-dimensional representation of the distance matrix. Subsequently, we employed  
176 a Permutational Multivariate Analysis of Variance (PERMANOVA) to statistically evaluate the  
177 null hypothesis that there was no difference in the patterns of plant or earthworm community across  
178 treatments. For a comprehensive analysis, both the ‘Treatments’ and ‘Blocks’ were included as  
179 influencing factors in the PERMANOVA, run on the Bray-Curtis distance with 1000 permutations  
180 per analysis (PERMANOVA, “vegan” package). Lastly, to discern finer patterns and specific  
181 differences between treatments, we performed post-hoc pairwise comparisons and adjusted the p-  
182 values using the Bonferroni correction to account for multiple testing.

183

### 184 **3. Results**

#### 185 3.1 Plant communities

186 Herbage management by grazing (SH-, CA- and CA+) or mowing (MO) compared to  
187 abandoned grassland (AB) displayed a significantly lower percentage of plant litter, CWM-EN  
188 (community-weighted mean for Ellenberg Nitrogen values) and higher CWM-EL (community-  
189 weighted mean for Ellenberg light values; at least 1.7, 1.2 and 1.1 times respectively, Table 2).  
190 Compared to abandoned grassland (AB), herbage management by cattle with a high level of  
191 herbage utilization (CA+) or by mowing (MO) displayed a significantly higher percentage of  
192 leguminous plant (at least 15.3 times, Table 2). In addition, compared to abandoned grassland  
193 (AB), CWM-RPH (community-weighted mean for reproductive plant height values) was  
194 significantly lower in mowed grasslands (MO; 1.2 times, Table 2), CWM-LDMC (community-  
195 weighted mean for leaf dry matter content values) was significantly lower in grazed pasture by  
196 cattle with a high level of herbage utilization (CA+, 1.1 times) and plant richness was significantly

197 higher in mowed and grazed pastures by cattle with low and high levels of herbage utilization (MO,  
198 CA- and CA+; at least 1.5 times). Herbage management by grazing (SH-, CA- and CA+) or  
199 mowing (MO) significantly modified plant community structures compared to those of abandoned  
200 grassland (AB, Fig. 1).

201       Regarding the effect of animal presence, the CWM-EN was significantly higher in grazed  
202 pastures (SH-, CA- and CA+) than in mowed grassland (MO; at least 1.1 times, Table 2). The  
203 CWM-EL and plant richness were significantly lower in grazed plots at low level of utilization  
204 (CA- and SH-) while the percentage of plant litter and CWM-RPH were higher in grazed plots at  
205 low level of utilization (CA- and SH-) compared to MO. Similarly, compared to MO, pasture with  
206 sheep at low level of herbage utilization (SH-) significantly displayed a lower percentage of  
207 leguminous plants (4.7 times), meanwhile, pasture grazed by cattle at high level of herbage  
208 utilization (CA+) displayed significantly lower CWM-LDMC (1.1 times). Animal presence  
209 contributed to the modification of plant community structure, but these are linked to grazing  
210 intensity: pasture with sheep or cattle at a low level of herbage utilization (SH-, CA-) presented  
211 different plant community structure compared to mowed grassland (MO), while there was no  
212 difference between mowed grassland (MO) and pasture with cattle at a high level of herbage  
213 utilization (CA+; Fig. 1).

214       The increased herbage utilization by cattle (CA+ vs CA-) decreased the percentage of plant  
215 litter and CWM-LDMC (4.2 and 1.2 times, Table 2) and increased the percentage of leguminous  
216 plant and CWM-EL (4.9 and 1.1 times, Table 2). In addition, this increase of herbage utilization  
217 by cattle significantly modified plant community structures (Fig. 1) while plant richness, CWM-  
218 RPH and CWM-EN were not significantly affected (Table 2).

219 With respect to effects of grazing animal species (SH- vs CA-), no significant changes were  
220 observed in plant richness, percentage of plant litter and leguminous plant, CWM-EL, CWM-EN,  
221 CWM-LDMC, CWM-RPH and plant community structures, between grazed plots by sheep or by  
222 cattle at a low level of herbage utilization (Table 2 and Fig. 1).

223

### 224 3.2 Earthworm communities

225 Whatever the treatment, each earthworm ecological group was dominated by one or two  
226 species: *Lumbricus castaneus* and *Dendrobaena mammalis* for epigeic, *Lumbricus terrestris*,  
227 *Aporrectodea giardi* for anecic, and *Aporrectodea caliginosa caliginosa* and *Allolobophora rosea*  
228 *rosea* for endogeic earthworms (Supplementary Table S1).

229 Herbage management by grazing (SH-, CA- and CA+) and mowing (MO) compared to  
230 abandoned grassland (AB) displayed a significantly higher earthworm biomass (at least 1.9 times,  
231 Table 3) in addition to *Aporrectodea* anecic abundance and biomass (at least 3.1 and 2.7 times,  
232 Table 3). Quite similarly, herbage management by grazing with a low level of herbage utilization  
233 (SH-, CA-) or mowing (MO) compared to abandoned grassland (AB) displayed a significantly  
234 higher total earthworm abundance (at least 2.0 times, Table 3). In addition, herbage management  
235 by grazing (SH-, CA- and CA+) and mowing (MO) compared to abandoned grassland (AB)  
236 displayed a significantly higher earthworm richness (at least 1.3 times, Table 3) mainly due to  
237 *Lumbricus rubellus rubellus* and *Lumbricus centralis* (both anecic earthworms) and to a lesser  
238 extent due to *Allolobophora chlorotica chlorotica* and *A. r. rosea* (both endogeic earthworms,  
239 Supplementary Table S1). In contrast, Herbage management did not affect abundance of epigeics,  
240 endogeics and *Lumbricus* anecic biomass (Table 3). Herbage management modified earthworm

241 community structures, as those in abandoned grassland (AB) were different from those in grazed  
242 pasture (SH-, CA-, CA+) but showed no significant difference with mowed grassland (MO; Fig. 2)  
243 mainly due to the decreased of *A. giardi* abundance in abandoned grassland (AB, Supplementary  
244 Table S1).

245         Regarding the effect of animal presence, total earthworm abundance, biomass and species  
246 richness in mowed grassland (MO) were not different from those grazed by sheep or cattle (SH-,  
247 CA-, CA+, Table 3). Nevertheless, animal presence modified the earthworm community structure  
248 as epigeic abundance was at least 6.8 times higher in pastures grazed at a low level of herbage  
249 utilization (SH-, CA-) than in the mowed grassland (MO, Table 3). Similarly, *Lumbricus anecic*  
250 abundance was significantly higher in pastures grazed by cattle at a low level of herbage utilization  
251 (CA-) than in the mowed grassland (MO; 3.2 times, Table 3). In addition, animal presence modified  
252 earthworm community structure, as those in mowed grassland (MO) were different from those in  
253 grazed pastures (SH-, CA- and CA+, Fig. 2) mainly due to the increase of *Octolasion cyaneum*  
254 abundance by at least 3.2 times and the decrease by at least 6.6 times of *A. rosea* abundance in  
255 mowed grassland (MO, Supplementary Table S1).

256         The increase of herbage utilization by cattle (CA- vs CA+) did not modify total earthworm  
257 abundance, biomass, diversity indexes (Table 3) but modified the earthworm community structure  
258 as abundance of *Lumbricus anecic* was significantly higher in grazed pastures by cattle with a low  
259 herbage utilization (CA-) than in the high herbage utilization (CA+; 3.0 times, Table 3). Similarly,  
260 abundance of endogeic earthworms tended to be lower in grazed pastures by cattle with a low  
261 herbage utilization (CA-) than in the high herbage utilization (CA+; 1.8 times, Table 3).

262 Grazing animal species (SH-, CA-) did not modify total earthworm abundance, biomass,  
263 diversity indexes and no effect was observed on the functional structure (Table 3). Moreover,  
264 earthworm community structure was not modified between grazed pastures by sheep or cattle (SH-  
265 vs CA-, Fig. 2). Interestingly, earthworm community structure was significantly different between  
266 grazed pasture by sheep at a low level of herbage utilization (SH-) and by cattle at a high level of  
267 herbage utilization (CA+, Fig. 2) mainly because of the decrease, by at least 6.7 times, of *A. rosea*  
268 abundance in grazed pasture by cattle at a high level of herbage utilization (CA+, Supplementary  
269 Table S1).

270

#### 271 **4. Discussion**

272 Our study is based on observations of plant and earthworm communities after 14 year of different  
273 applications of grassland practices. The absence of initial (T0) earthworm distribution data could  
274 be considered as a limitation of our study; however, consistent management of the entire site for  
275 30 years prior to the experiment and the randomized plot arrangement within the four blocks help  
276 mitigate this by isolating long-term grassland management effects from other variabilities,  
277 including the original earthworm distribution.

278

##### 279 4.1 Earthworm abundance in comparison to other lowland grasslands

280 In the present study, mean earthworm abundance and biomass were  $214.1 \pm 18$  ind.  $m^{-2}$  in  
281 managed grassland,  $107.8 \pm 22$  ind.  $m^{-2}$  in abandoned grassland, much less than those observed in  
282 previous lowland studies [41–43]. For example, Didden et al. [44] observed a mean earthworm  
283 abundance of 384 ind.  $m^{-2}$  among 20 Dutch lowland grasslands. Similarly, Cluzeau et al. [21]

284 observed a mean earthworm abundance of 350 ind. m<sup>-2</sup> among 47 French lowland grasslands. The  
285 low abundance found in our study could be related to unfavourable soil habitat properties for  
286 earthworms [45,46]. The high percentage of stones in the soil (13.5 %) may have hindered  
287 movement or food sources in the soil for earthworms. Moreover the sand dominance in the soil  
288 (51.9 %) and the relatively shallow soil depth (60 cm at some points) may have restricted the  
289 capacity of soils to hold water leading to an unfavourable habitat for earthworms [47,45,48,36].  
290 Also, a few studies reported that an increase of altitude led to decreased earthworm abundance [49–  
291 52] as a result of a decrease in productivity and harsh environmental conditions. Given this, the  
292 880 m a.s.l. elevation of the study site may also have hindered the development of earthworm  
293 communities, in particular epigeic earthworms, as surface species are more sensitive to climatic  
294 hazards. Nonetheless, we found 2 to 5 earthworm species for each ecological category and a mean  
295 earthworm richness of  $7.6 \pm 0.2$  m<sup>-2</sup> in managed grasslands and  $5.8 \pm 0.4$  m<sup>-2</sup> in abandoned  
296 grasslands, which is similar in magnitude to that found in previous studies of lowland grasslands  
297 [16,42]. For example, [44] observed a mean earthworm richness of 2.1 species m<sup>-2</sup> in Dutch  
298 lowland grasslands while [21] observed a mean earthworm richness of 9.6 species m<sup>-2</sup> in French  
299 lowland grasslands.

300

#### 301 4.2 Herbage management maintained plant and earthworm richness

302 After 14 years of different types of management, we demonstrated that plant richness  
303 decreased under abandonment compared to managed grasslands. In line with these findings, several  
304 studies observed such decreases after the abandonment of grasslands [53,54,32,55–57] and could  
305 be explained by higher competition for light which promotes tall plant species at the expense of



306 shorter ones, which disappear. Moreover, our study highlighted that plant litter cover was the  
307 highest in abandoned grasslands which is often associated with a thick layer of plant litter [57] that  
308 could inhibit seedling recruitment and thus reduce the number of plant species [57,58]. Among  
309 managed grasslands, plant species richness tends to increase with the level of herbage utilization  
310 under grazing and mowing, as such practices could lead to the opening of gaps for the establishment  
311 of many fast-growing and short plant species in the lower layers of the sward [59,60]. CWM-EL  
312 gradually increased with the intensity of herbage utilization which meant that more light was  
313 gradually available in managed grasslands, allowing many light-demanding species to coexist,  
314 resulting in greater species richness [61,62,8,63]. In addition, the highest species richness in mowed  
315 grassland could result not only from a regularly cut sward but from the lower fertile status derived  
316 from this management practice, as exportation of forage over 14 years was not compensated by  
317 nutrient inputs. Indeed, mown grasslands have the lowest average CWM-EN value and an even  
318 higher CWM-LDMC value, and numerous studies have highlighted the greater plant richness of  
319 poor grasslands compared to rich grasslands [64,8,65].

320 After 14 years of different type of herbage management by grazing or mowing, we  
321 demonstrated that earthworm richness was higher in all managed grasslands compared to  
322 abandoned grassland, mainly due to the presence of *L. rubellus rubellus* and *L. centralis* and in  
323 some extent to endogeic earthworms. Unlike other earthworm ecological categories, *Lumbricus*  
324 anecic earthworms feed mainly on fresh surface plant-derived materials that they bury into their  
325 permanent vertical galleries [66,39], and are the most sensitive to the physical and chemical  
326 properties of fresh plant-derived materials [67–69]. In line with our findings, Hoeffner et al. [18]  
327 observed, under controlled conditions, that among two grassland plant species, *L. rubellus rubellus*

328 and *L. centralis* lose mass in the presence of *Holcus lanatus* while they maintain their mass with  
329 *Lolium perenne*, probably due to the higher C/N ratio and phenolic compounds in litter of the  
330 former. Thus, the highest earthworm richness observed in managed grassland (grazed or mown)  
331 could be explained by changes in plant-derived materials' quality following modifications of the  
332 plant communities. The absence of significant difference of CWM-LDMC, which is negatively  
333 correlated to forage digestibility [34], among abandoned and managed grasslands (except in CA+),  
334 suggests that the digestibility of the forage does not influence earthworm. Different species of  
335 *Lumbricus* anecic earthworms could, in return, modify soil functioning, as for example, [18]  
336 observed that gallery fungal communities were specific to each *Lumbricus* anecic species, implying  
337 that these earthworms could specifically affect the dynamics of carbon or nutrient regulation. To a  
338 lesser extent, we also observed that herbage management increased the presence of *A. chlorotica*  
339 *chlorotica* and *A. rosea*, although current knowledge does not allow us to provide an explanation  
340 of any underlying reasons.

#### 341 342 4.3 Herbage management increased earthworm abundance, biomass and changed earthworm 343 community structure

344 After 14 years of different types of herbage management, including both grazing and mowing, our  
345 results intriguingly deviated from our original hypothesis. We observed lower value in  
346 abandonment only and higher earthworm biomass and, to some degree, earthworm abundance  
347 across all management regimes, without any significant differences between these regimes,  
348 contrary to what we had initially anticipated. In our study, higher total abundance and biomass  
349 observed in managed grasslands was mainly due to *Aporrectodea* anecic earthworms and to some

350 extent to endogeic earthworms. In other words, anecic *Lumbricus* anecic in addition to epigeic  
351 species did not significantly profit from any management, in terms of biomass and abundance, in  
352 comparison to abandonment. Mowing seems even unfavorable for both groups and also for single  
353 species. This result fits with both groups feeding on litter from the soil surface, which is strongly  
354 reduced by mowing, but also by other management practices. Contrary to others earthworm  
355 ecological categories, *Aporrectodea* anecic and endogeic earthworms feed preferentially on aged  
356 plant-derived materials within the soil with a large vertical or horizontal burrow network. Thus, the  
357 positive effects of herbage management on *Aporrectodea* anecic and endogeic earthworms measured  
358 in our study could be related to changes in the availability of plant-derived materials or their foraging  
359 capacity through changes in root structure. An increase of *Aporrectodea* anecic abundance is  
360 expected to have a strong impact on soil functioning, especially on water infiltration and circulation  
361 within the soil [74–76]. In relation to the morphology of their burrow networks, *Aporrectodea*  
362 anecic earthworms allow a lower water infiltration rate than *Lumbricus* anecic earthworms [77];  
363 however, under saturated conditions, *Aporrectodea* anecic earthworms are the most efficient for  
364 water flow within the soil, due to higher burrow volume and higher openings at soil surface [74,75].  
365 Moreover, our study demonstrated that compared to mowed grassland, animal presence increased  
366 epigeic and to some extent *Lumbricus* anecic abundance. These findings are consistent with  
367 previous studies and could be explained by the supply of food for earthworms through animal  
368 excreta, unlike mowed grassland where plant above ground biomass is exported [26,28] and leads,  
369 with time, to a decline of nutrient availability, that could explain low CWM-EN nitrogen values.  
370 Animal faeces return organic matter to the soil and become a source of food for epigeic and  
371 *Lumbricus* anecic earthworms [78,26,79], which are closely associated with the soil surface where

372 they feed on fresh plant-derived materials [80,18,81]. Nevertheless, the positive effects of grazing  
373 on earthworm communities reaches its limits when there is an excessive stocking rate, beyond  
374 which trampling becomes detrimental to earthworm communities [22]. In our study, this  
375 detrimental effect was in part observed for *Lumbricus anecic* species. Unlike other ecological  
376 categories, animal trampling can be detrimental to the permanent burrows of the *Lumbricus anecic*  
377 earthworms [82,83]. We also observed that among endogeic earthworms, the abundance of  
378 *Octolasion cyaneum* decreased sharply in grazing treatments. This species, because of its proximity  
379 to the soil surface [37], its weak musculature that does not allow it to resist soil compaction and its  
380 relatively large size [37] appears more vulnerable to trampling.

381 Finally, earthworm communities were not altered by the grazing of sheep or cattle, which  
382 means that the feeding behaviour of these two grazing animals and the quantity and quality of their  
383 faeces had no effect on earthworm communities. It is likely that the level of utilization management  
384 appears to be the main driver of the change in plant community structure and biomass, so that when  
385 compared, CA- and SH- at low level of utilization, the grazing species effect cannot be revealed.

386

## 387 **5. Conclusion**

388 Our study showed that the increase in grassland herbage management led to a gradually  
389 increase in plant diversity. Faced with this gradient in grassland herbage management, the  
390 characteristics of the environment also evolved towards an environment with less plant litter, small  
391 plant species with more leguminous plants, to nutrient-poor when mowing without inputs. As a result,  
392 grassland herbage management, regardless of intensity and type (mowed or grazed), modified the  
393 earthworm communities and maintained at similar higher value earthworm biomass and richness

394 compared to abandonment. Grassland management practices have also specifically impacted the  
395 structure of earthworm communities particularly within anecic, and to a lesser extent within  
396 endogeic, earthworms.

397

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411 **References**

- 412 [1] FAO, Conservation Agriculture, Food Agric. Organ. U. N. (2019).  
413 <http://www.fao.org/conservation-agriculture/en/> (accessed December 10, 2019).
- 414 [2] O.E. Sala, J.M. Paruelo, Ecosystem services in grasslands, in: G. Daily (Ed.), *Nat. Serv. Soc.*  
415 *Depend. Nat. Ecosyst.*, Island Press, Island Press, Washington, 1997: pp. 237–251.
- 416 [3] Y. Zhao, Z. Liu, J. Wu, Grassland ecosystem services: a systematic review of research  
417 advances and future directions, *Landsc. Ecol.* 35 (2020) 793–814.  
418 <https://doi.org/10.1007/s10980-020-00980-3>.
- 419 [4] X. Li, X. Lyu, H. Dou, D. Dang, S. Li, X. Li, M. Li, X. Xuan, Strengthening grazing pressure  
420 management to improve grassland ecosystem services, *Glob. Ecol. Conserv.* 31 (2021)  
421 e01782. <https://doi.org/10.1016/j.gecco.2021.e01782>.
- 422 [5] R.D. Bardgett, W.H. van der Putten, Belowground biodiversity and ecosystem functioning,  
423 *Nature.* 515 (2014) 505–511. <https://doi.org/10.1038/nature13855>.
- 424 [6] D.H. Wall, U.N. Nielsen, J. Six, Soil biodiversity and human health, *Nature.* 528 (2015) 69–  
425 76.
- 426 [7] M. Delgado-Baquerizo, P.B. Reich, C. Trivedi, D.J. Eldridge, S. Abades, F.D. Alfaro, F.  
427 Bastida, A.A. Berhe, N.A. Cutler, A. Gallardo, L. García-Velázquez, S.C. Hart, P.E. Hayes,  
428 J.-Z. He, Z.-Y. Hseu, H.-W. Hu, M. Kirchmair, S. Neuhauser, C.A. Pérez, S.C. Reed, F.  
429 Santos, B.W. Sullivan, P. Trivedi, J.-T. Wang, L. Weber-Grullon, M.A. Williams, B.K. Singh,  
430 Multiple elements of soil biodiversity drive ecosystem functions across biomes, *Nat. Ecol.*  
431 *Evol.* 4 (2020) 210–220. <https://doi.org/10.1038/s41559-019-1084-y>.
- 432 [8] F. Louault, V.D. Pillar, J. Aufrère, E. Garnier, J.-F. Soussana, Plant traits and functional types  
433 in response to reduced disturbance in a semi-natural grassland, *J. Veg. Sci.* 16 (2005) 151–  
434 160.
- 435 [9] G.T. Freschet, R. Aerts, J.H.C. Cornelissen, A plant economics spectrum of litter  
436 decomposability, *Funct. Ecol.* 26 (2012) 56–65. <https://doi.org/10.1111/j.1365-2435.2011.01913.x>.
- 438 [10] B. Dumont, A. Farruggia, J.-P. Garel, P. Bachelard, E. Boitier, M. Frain, How does grazing  
439 intensity influence the diversity of plants and insects in a species-rich upland grassland on  
440 basalt soils?, *Grass Forage Sci.* 64 (2009) 92–105. <https://doi.org/10.1111/j.1365-2494.2008.00674.x>.
- 442 [11] J.P. Grime, K. Thompson, R. Hunt, J.G. Hodgson, J.H.C. Cornelissen, I.H. Rorison, G.A.F.  
443 Hendry, T.W. Ashenden, A.P. Askew, S.R. Band, R.E. Booth, C.C. Bossard, B.D. Campbell,  
444 J.E.L. Cooper, A.W. Davison, P.L. Gupta, W. Hall, D.W. Hand, M.A. Hannah, S.H. Hillier,  
445 D.J. Hodgkinson, A. Jalili, Z. Liu, J.M.L. Mackey, N. Matthews, M.A. Mowforth, A.M. Neal,  
446 R.J. Reader, K. Reiling, W. Ross-Fraser, R.E. Spencer, F. Sutton, D.E. Tasker, P.C. Thorpe,  
447 J. Whitehouse, Integrated Screening Validates Primary Axes of Specialisation in Plants,  
448 *Oikos.* 79 (1997) 259–281. <https://doi.org/10.2307/3546011>.
- 449 [12] M. Bahn, M. Knapp, Z. Garajova, N. Pfahringer, A. Cernusca, Root respiration in temperate  
450 mountain grasslands differing in land use, *Glob. Change Biol.* 12 (2006) 995–1006.
- 451 [13] R.D. Bardgett, D.A. Wardle, G.W. Yeates, Linking above-ground and below-ground  
452 interactions: how plant responses to foliar herbivory influence soil organisms, *Soil Biol.*  
453 *Biochem.* 30 (1998) 1867–1878.

- 454 [14] A. Milcu, S. Partsch, R. Langel, S. Scheu, The response of decomposers (earthworms,  
455 springtails and microorganisms) to variations in species and functional group diversity of  
456 plants, *Oikos*. 112 (2006) 513–524.
- 457 [15] A.C.W. Sabais, S. Scheu, N. Eisenhauer, Plant species richness drives the density and  
458 diversity of Collembola in temperate grassland, *Acta Oecologica*. 37 (2011) 195–202.  
459 <https://doi.org/10.1016/j.actao.2011.02.002>.
- 460 [16] J.G. Zaller, J.A. Arnone, Earthworm responses to plant species' loss and elevated CO<sub>2</sub> in  
461 calcareous grassland, *Plant Soil*. 208 (1999) 1–8.
- 462 [17] N. Eisenhauer, A. Milcu, A.C.W. Sabais, H. Bessler, A. Weigelt, C. Engels, S. Scheu, Plant  
463 community impacts on the structure of earthworm communities depend on season and change  
464 with time, *Soil Biol. Biochem.* 41 (2009) 2430–2443.
- 465 [18] K. Hoeffner, C. Monard, M. Santonja, D. Cluzeau, Feeding behaviour of epi-aneic  
466 earthworm species and their impacts on soil microbial communities, *Soil Biol. Biochem.* 125  
467 (2018) 1–9.
- 468 [19] M. Blouin, M.E. Hodson, E.A. Delgado, G. Baker, L. Brussaard, K.R. Butt, J. Dai, L.  
469 Dendooven, G. Peres, J.E. Tondoh, D. Cluzeau, J.-J. Brun, A review of earthworm impact on  
470 soil function and ecosystem services: Earthworm impact on ecosystem services, *Eur. J. Soil  
471 Sci.* 64 (2013) 161–182.
- 472 [20] J. Seeber, G.U.H. Seeber, Effects of land-use changes on humus forms on alpine pastureland  
473 (Central Alps, Tyrol), *Geoderma*. 124 (2005) 215–222.
- 474 [21] D. Cluzeau, M. Guernion, R. Chaussod, F. Martin-Laurent, C. Villenave, J. Cortet, N. Ruiz-  
475 Camacho, C. Pernin, T. Mateille, L. Philippot, A. Bellido, L. Rougé, D. Arrouays, A. Bispo,  
476 G. Pérès, Integration of biodiversity in soil quality monitoring: Baselines for microbial and  
477 soil fauna parameters for different land-use types, *Eur. J. Soil Biol.* 49 (2012) 63–72.
- 478 [22] D. Cluzeau, F. Binet, F. Vertes, J.C. Simon, J.M. Riviere, P. Trehen, Effects of intensive cattle  
479 trampling on soil-plant-earthworms system in two grassland types, *Soil Biol. Biochem.* 24  
480 (1992) 1661–1665.
- 481 [23] J. Mikola, H. Setälä, P. Virkajärvi, K. Saarijärvi, K. Ilmarinen, W. Voigt, M. Vestberg,  
482 Defoliation and patchy nutrient return drive grazing effects on plant and soil properties in a  
483 dairy cow pasture, *Ecol. Monogr.* 79 (2009) 221–244.
- 484 [24] J. Muldowney, J.P. Curry, J. O'Keeffe, O. Schmidt, Relationships between earthworm  
485 populations, grassland management and badger densities in County Kilkenny, Ireland,  
486 *Pedobiologia*. 47 (2003) 913–919.
- 487 [25] K.L. King, K.J. Hutchinson, The effects of sheep grazing on invertebrate numbers and  
488 biomass in unfertilized natural pastures of the New England Tablelands (NSW), *Aust. J. Ecol.*  
489 8 (1983) 245–255. <https://doi.org/10.1111/j.1442-9993.1983.tb01322.x>.
- 490 [26] N.B. Hendriksen, Consumption and utilization of dung by detritivorous and geophagous  
491 earthworms in a Danish pasture, *Pedobiologia*. 35 (1991) 65–70.
- 492 [27] J. Schlaghamerský, A. Šídová, V. Pižl, From mowing to grazing: Does the change in grassland  
493 management affect soil annelid assemblages?, *Eur. J. Soil Biol.* 43 (2007) S72–S78.
- 494 [28] J.P. Curry, P. Doherty, G. Purvis, O. Schmidt, Relationships between earthworm populations  
495 and management intensity in cattle-grazed pastures in Ireland, *Appl. Soil Ecol.* 39 (2008) 58–  
496 64.

- 497 [29] N.L. Schon, S. Dennis, P.M. Fraser, T.A. White, T.L. Knight, Intensification of pastoral  
498 systems influences earthworm populations, *N. Z. J. Agric. Res.* 60 (2017) 423–436.
- 499 [30] N.S. Schon, A.D. Mackay, R.A. Gray, Changes in the abundance and diversity of earthworms  
500 in hill soils under different long-term fertiliser and sheep stocking regimes, *N. Z. J. Agric.*  
501 *Res.* 0 (2019) 1–16.
- 502 [31] J. Singh, E. Cameron, T. Reitz, M. Schädler, N. Eisenhauer, Grassland management effects  
503 on earthworm communities under ambient and future climatic conditions, *Eur. J. Soil Sci.* 72  
504 (2021) 343–355. <https://doi.org/10.1111/ejss.12942>.
- 505 [32] F. Louault, J. Pottier, P. Note, D. Vile, J.-F. Soussana, P. Carrère, Complex plant community  
506 responses to modifications of disturbance and nutrient availability in productive permanent  
507 grasslands, *J. Veg. Sci.* 28 (2017) 538–549.
- 508 [33] H. Deraison, I. Badenhausser, L. Börger, N. Gross, Herbivore effect traits and their impact on  
509 plant community biomass: an experimental test using grasshoppers, *Funct. Ecol.* 29 (2015)  
510 650–661.
- 511 [34] A. Gardarin, É. Garnier, P. Carrère, P. Cruz, D. Andueza, A. Bonis, M.-P. Colace, B. Dumont,  
512 M. Duru, A. Farruggia, S. Gaucherand, K. Grigulis, É. Kernéis, S. Lavorel, F. Louault, G.  
513 Loucougaray, F. Mesléard, N. Yavercovski, E. Kazakou, Plant trait-digestibility relationships  
514 across management and climate gradients in permanent grasslands, *J. Appl. Ecol.* 51 (2014)  
515 1207–1217.
- 516 [35] C.L. Gaudet, P.A. Keddy, A comparative approach to predicting competitive ability from  
517 plant traits, *Nature.* 334 (1988) 242–243.
- 518 [36] G. Pérès, A. Bellido, P. Curmi, P. Marmonier, D. Cluzeau, Relationships between earthworm  
519 communities and burrow numbers under different land use systems, *Pedobiologia.* 54 (2010)  
520 37–44.
- 521 [37] M.B. Bouché, *Lombriciens de France: écologie et systématique*, INRA-Annales de Zoologie  
522 *Ecologie Animale*, INRA, France, 1972.
- 523 [38] D. Jégou, D. Cluzeau, J. Balesdent, P. Tréhen, Effects of four ecological categories of  
524 earthworms on carbon transfer in soil, *Appl. Soil Ecol.* 9 (1998) 249–255.
- 525 [39] K. Hoeffner, K.R. Butt, C. Monard, J. Frazão, G. Pérès, D. Cluzeau, Two distinct ecological  
526 behaviours within anecic earthworm species in temperate climates, *Eur. J. Soil Biol.* 113  
527 (2022) 103446. <https://doi.org/10.1016/j.ejsobi.2022.103446>.
- 528 [40] R. Core Team, *R: A language and environment for statistical computing.*, (2022).  
529 <https://www.R-project.org/>.
- 530 [41] B. Boag, L.F. Palmer, R. Neilson, R. Legg, S.J. Chambers, Distribution, prevalence and  
531 intensity of earthworm populations in arable land and grassland in Scotland, *Ann. Appl. Biol.*  
532 130 (1997) 153–165.
- 533 [42] T. Decaëns, P. Margerie, M. Aubert, M. Hedde, F. Bureau, Assembly rules within earthworm  
534 communities in North-Western France - A regional analysis, *Appl. Soil Ecol.* 39 (2008) 321–  
535 335.
- 536 [43] M. Rutgers, A.J. Schouten, J. Bloem, N.V. Eekeren, R.G.M.D. Goede, G.A.J.M.J. Akkerhuis,  
537 A.V. der Wal, C. Mulder, L. Brussaard, A.M. Breure, Biological measurements in a  
538 nationwide soil monitoring network, *Eur. J. Soil Sci.* 60 (2009) 820–832.
- 539 [44] W.A.M. Didden, Earthworm communities in grasslands and horticultural soils, *Biol. Fertil.*  
540 *Soils.* 33 (2001) 111–117.



- 541 [45] C.A. Edwards, ed., *Earthworm ecology*, 2nd ed., CRC Press, Boca Raton, 2004.
- 542 [46] M. Rutgers, A. Orgiazzi, C. Gardi, J. Römcke, S. Jänsch, A.M. Keith, R. Neilson, B. Boag,  
543 O. Schmidt, A.K. Murchie, R.P. Blackshaw, G. Pérès, D. Cluzeau, M. Guernion, M.J.I.  
544 Briones, J. Rodeiro, R. Piñeiro, D.J.D. Cosín, J.P. Sousa, M. Suhadolc, I. Kos, P.-H. Krogh,  
545 J.H. Faber, C. Mulder, J.J. Bogte, H.J. van Wijnen, A.J. Schouten, D. de Zwart, Mapping  
546 earthworm communities in Europe, *Appl. Soil Ecol.* 97 (2016) 98–111.
- 547 [47] P.F. Hendrix, B.R. Mueller, R.R. Bruce, G.W. Langdale, R.W. Parmelee, Abundance and  
548 distribution of earthworms in relation to landscape factors on the Georgia Piedmont, U.S.A.,  
549 *Soil Biol. Biochem.* 24 (1992) 1357–1361.
- 550 [48] E. Lapiéd, J. Nahmani, G.X. Rousseau, Influence of texture and amendments on soil  
551 properties and earthworm communities, *Appl. Soil Ecol.* 43 (2009) 241–249.
- 552 [49] C. Salomé, C. Guenat, G. Bullinger-Weber, J.-M. Gobat, R.-C. Le Bayon, Earthworm  
553 communities in alluvial forests: Influence of altitude, vegetation stages and soil parameters,  
554 *Pedobiologia.* 54 (2011) S89–S98.
- 555 [50] A. Rozen, R.W. Maslajek, L. Sobczyk, Altitude versus vegetation as the factors influencing  
556 the diversity and abundance of earthworms and other soil macrofauna in montane habitat  
557 (Silesian Beskid Mts, Western Carpatians), *Pol. J. Ecol.* 1 (2013).  
558 [https://www.infona.pl/resource/bwmeta1.element.agro-9e3b047b-f08f-46c2-9042-](https://www.infona.pl/resource/bwmeta1.element.agro-9e3b047b-f08f-46c2-9042-5b1cac7676e6)  
559 [5b1cac7676e6](https://www.infona.pl/resource/bwmeta1.element.agro-9e3b047b-f08f-46c2-9042-5b1cac7676e6) (accessed April 10, 2020).
- 560 [51] M. Kanchilakshmi, A. Thaddeus, Earthworm: A potential and sustainable source for soil  
561 fertility—an altitude based biophysical study, *Int. J. Environ. Prot. Policy.* 4 (2016) 77.
- 562 [52] J. Karungi, S. Cherukut, A.R. Ijala, J.B. Tumuhairwe, J. Bonabana-Wabbi, E.A. Nuppenau,  
563 M. Hoehner, S. Domptail, A. Otte, Elevation and cropping system as drivers of microclimate  
564 and abundance of soil macrofauna in coffee farmlands in mountainous ecologies, *Appl. Soil*  
565 *Ecol.* 132 (2018) 126–134.
- 566 [53] F. Krahulec, H. Skálová, T. Herben, V. Hadincová, R. Wildová, S. Pecháčková, Vegetation  
567 changes following sheep grazing in abandoned mountain meadows, *Appl. Veg. Sci.* 4 (2001)  
568 97–102.
- 569 [54] B. Köhler, A. Gigon, P.J. Edwards, B. Krüsi, R. Langenauer, A. Lüscher, P. Ryser, Changes  
570 in the species composition and conservation value of limestone grasslands in Northern  
571 Switzerland after 22 years of contrasting managements, *Perspect. Plant Ecol. Evol. Syst.* 7  
572 (2005) 51–67.
- 573 [55] S. Wehn, S. Taugourdeau, L. Johansen, K.A. Hovstad, Effects of abandonment on plant  
574 diversity in semi-natural grasslands along soil and climate gradients, *J. Veg. Sci.* 28 (2017)  
575 838–847.
- 576 [56] A. Józefowska, T. Zaleski, J. Zarzycki, K. Frączek, Do mowing regimes affect plant and soil  
577 biological activity in the mountain meadows of Southern Poland?, *J. Mt. Sci.* 15 (2018) 2409–  
578 2421.
- 579 [57] I. Jernej, A. Bohner, R. Walcher, R.I. Hussain, A. Arnberger, J.G. Zaller, F. Thomas, Impact  
580 of land-use change in mountain semi-dry meadows on plants, litter decomposition and  
581 earthworms, *Web Ecol.* 19 (2019) 53–63.
- 582 [58] K. Jensen, C. Meyer, Effects of light competition and litter on the performance of *Viola*  
583 *palustris* and on species composition and diversity of an abandoned fen meadow, *Plant Ecol.*  
584 155 (2001) 169–181.

- 585 [59] V. Pavlů, M. Hejcman, L. Pavlů, J. Gaisler, Restoration of grazing management and its effect  
586 on vegetation in an upland grassland, *Appl. Veg. Sci.* 10 (2007) 375–382.
- 587 [60] G. Niedrist, E. Tasser, C. Lüth, J. Dalla Via, U. Tappeiner, Plant diversity declines with recent  
588 land use changes in European Alps, *Plant Ecol.* 202 (2008) 195.
- 589 [61] M.J.A. Werger, T. Hirose, H.J. During, G.W. Heil, K. Hikosaka, T. Ito, U.G. Nachinshonhor,  
590 D. Nagamatsu, K. Shibasaki, S. Takatsuki, J.W. van Rheenen, N.P.R. Anten, Light  
591 partitioning among species and species replacement in early successional grasslands, *J. Veg.*  
592 *Sci.* 13 (2002) 615–626.
- 593 [62] H. Jacquemyn, R. Brys, M. Hermy, Short-term effects of different management regimes on  
594 the response of calcareous grassland vegetation to increased nitrogen, *Biol. Conserv.* 111  
595 (2003) 137–147.
- 596 [63] V. Pavlů, M. Hejcman, L. Pavlů, J. Gaisler, P. Nežerková, M.G. Andaluz, Vegetation changes  
597 after cessation of grazing management in the Jizerské Mountains (Czech Republic), 42 (2005)  
598 7.
- 599 [64] W.J. Roem, F. Berendse, Soil acidity and nutrient supply ratio as possible factors determining  
600 changes in plant species diversity in grassland and heathland communities, *Biol. Conserv.* 92  
601 (2000) 151–161. [https://doi.org/10.1016/S0006-3207\(99\)00049-X](https://doi.org/10.1016/S0006-3207(99)00049-X).
- 602 [65] H. Lambers, M.C. Brundrett, J.A. Raven, S.D. Hopper, Plant mineral nutrition in ancient  
603 landscapes: high plant species diversity on infertile soils is linked to functional diversity for  
604 nutritional strategies, *Plant Soil.* 348 (2011) 7–27. [https://doi.org/10.1007/s11104-011-0977-](https://doi.org/10.1007/s11104-011-0977-6)  
605 6.
- 606 [66] D. Jégou, D. Cluzeau, J. Balesdent, P. Trehen, Effects of four ecological categories of  
607 earthworms on carbon transfer in soil, *Appl. Soil Ecol.* 9 (1998) 249–255.
- 608 [67] M.A. Wright, Factors governing ingestion by the earthworm *Lumbricus terrestris* (L.), with  
609 special reference to apple leaves, *Ann. Appl. Biol.* 70 (1972) 175–188.
- 610 [68] M.J. Shipitalo, R. Protz, A.D. Tomlin, Effect of diet on the feeding and casting activity of  
611 *Lumbricus terrestris* and *L. rubellus* in laboratory culture, *Soil Biol. Biochem.* 20 (1988) 233–  
612 237.
- 613 [69] R. Neilson, B. Boag, Feeding preferences of some earthworm species common to upland  
614 pastures in Scotland, *Pedobiologia.* 47 (2003) 1–8.
- 615 [70] E.M. Spehn, J. Joshi, B. Schmid, J. Alpehi, C. Körner, Plant diversity effects on soil  
616 heterotrophic activity in experimental grassland ecosystems, *Plant Soil.* 224 (2000) 217–230.
- 617 [71] A. Milcu, S. Partsch, C. Scherber, W.W. Weisser, S. Scheu, Earthworms and legumes control  
618 litter decomposition in a plant diversity gradient, *Ecology.* 89 (2008) 1872–1882.
- 619 [72] D.A. Wardle, K.I. Bonner, G.M. Barker, G.W. Yeates, K.S. Nicholson, R.D. Bardgett, R.N.  
620 Watson, A. Ghani, Plant removals in perennial grassland: Vegetation dynamics, decomposers,  
621 soil biodiversity, and ecosystem properties, *Ecol. Monogr.* 69 (1999) 535–568.
- 622 [73] A. Gastine, M. Scherer-Lorenzen, P.W. Leadley, No consistent effects of plant diversity on  
623 root biomass, soil biota and soil abiotic conditions in temperate grassland communities, *Appl.*  
624 *Soil Ecol.* 24 (2003) 101–111.
- 625 [74] F. Bastardie, Y. Capowiez, J.-R. de Dreuzy, D. Cluzeau, X-ray tomographic and hydraulic  
626 characterization of burrowing by three earthworm species in repacked soil cores, *Appl. Soil*  
627 *Ecol.* 24 (2003) 3–16.

- 628 [75] F. Bastardie, Y. Capowiez, P. Renault, D. Cluzeau, A radio-labelled study of earthworm  
629 behaviour in artificial soil cores in term of ecological types, *Biol. Fertil. Soils*. 41 (2005) 320–  
630 327.
- 631 [76] Y. Capowiez, N. Bottinelli, S. Sammartino, E. Michel, P. Jouquet, Morphological and  
632 functional characterisation of the burrow systems of six earthworm species (Lumbricidae),  
633 *Biol. Fertil. Soils*. 51 (2015) 869–877.
- 634 [77] G. Pérès, Identification et quantification in situ des interactions entre la diversité lombricienne  
635 et la macro-bioporosité dans le contexte polyculture breton. Influence sur le fonctionnement  
636 hydrique du sol, Université Rennes 1, 2003. [https://tel.archives-ouvertes.fr/tel-  
637 00007432/document](https://tel.archives-ouvertes.fr/tel-00007432/document) (accessed October 2, 2018).
- 638 [78] N.B. Hendriksen, The effects of earthworms on the disappearance of particles from cattle  
639 dung pats during decay, *Pedobiologia*. 35 (1991) 139–146.
- 640 [79] K.R. Butt, Reproduction and growth of three deep-burrowing earthworms (Lumbricidae) in  
641 laboratory culture in order to assess production for soil restoration, *Biol. Fertil. Soils*. 16  
642 (1993) 135–138.
- 643 [80] M.B. Bouché, Strategies lombriciennes, *Ecol. Bull.* 25 (1977) 122–132.
- 644 [81] K. Hoeffner, M. Santonja, D. Cluzeau, C. Monard, Epi-aneic rather than strict-aneic  
645 earthworms enhance soil enzymatic activities, *Soil Biol. Biochem.* 132 (2019) 93–100.
- 646 [82] D. Jégou, V. Hallaire, D. Cluzeau, P. Tréhen, Characterization of the burrow system of the  
647 earthworms *Lumbricus terrestris* and *Aporrectodea giardi* using X-ray computed tomography  
648 and image analysis, *Biol. Fertil. Soils*. 29 (1999) 314–318.
- 649 [83] D. Jégou, S. Schrader, H. Diestel, D. Cluzeau, Morphological, physical and biochemical  
650 characteristics of burrow walls formed by earthworms, *Appl. Soil Ecol.* 17 (2001) 165–174.  
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652 **TABLES**

653 **Table 1.** Mean  $\pm$  standard deviation of the characteristics measured in fine soil: silt (%), clay (%),  
 654 sand (%), nitrogen (g. kg<sup>-1</sup>), organic matter (%), pH (H<sub>2</sub>O), or in bulk soil: stones (%), bulk density  
 655 (g. kg<sup>-1</sup>), for the four experimental blocks (0 – 40 cm depth, from the 2012 campaign).

	Blocks						Treatments	
	B1	B2	B3	B4	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Silt	29.3 <sup>a</sup> $\pm$ 0.3	26.9 <sup>ab</sup> $\pm$ 1.0	24.9 <sup>b</sup> $\pm$ 1.0	24.3 <sup>b</sup> $\pm$ 1.1	6.94	0.006	1.55	0.249
Clay	18.7 <sup>b</sup> $\pm$ 0.5	19.4 <sup>b</sup> $\pm$ 0.7	26.6 <sup>a</sup> $\pm$ 1.0	22.3 <sup>b</sup> $\pm$ 1.5	14.87	<0.001	1.47	0.271
Sand	52.0 <sup>a</sup> $\pm$ 0.5	53.8 <sup>a</sup> $\pm$ 1.4	48.6 <sup>a</sup> $\pm$ 2.0	53.4 <sup>a</sup> $\pm$ 2.5	1.92	0.180	1.30	0.330
Nitrogen	3.4 <sup>a</sup> $\pm$ 0.1	3.1 <sup>ab</sup> $\pm$ 0.2	2.8 <sup>bc</sup> $\pm$ 0.0	2.6 <sup>c</sup> $\pm$ 0.1	13.09	<0.001	2.60	0.089
Organic matter	6.4 <sup>a</sup> $\pm$ 0.2	5.9 <sup>a</sup> $\pm$ 0.3	5.1 <sup>b</sup> $\pm$ 0.0	4.7 <sup>b</sup> $\pm$ 0.1	14.95	<0.001	1.38	0.300
pH	6.04 <sup>a</sup> $\pm$ 0.06	5.89 <sup>a</sup> $\pm$ 0.07	6.40 <sup>b</sup> $\pm$ 0.03	6.32 <sup>b</sup> $\pm$ 0.02	38.88	<0.001	3.96	0.028
Stones	12.9 <sup>a</sup> $\pm$ 0.8	13.1 <sup>a</sup> $\pm$ 1.5	11.8 <sup>a</sup> $\pm$ 1.1	16.6 <sup>a</sup> $\pm$ 1.2	0.174	0.948	2.37	0.121
Bulk density	1.15 <sup>c</sup> $\pm$ 0.03	1.23 <sup>bc</sup> $\pm$ 0.03	1.26 <sup>ab</sup> $\pm$ 0.02	1.35 <sup>a</sup> $\pm$ 0.02	10.99	<0.001	0.88	0.503

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659 **Table 2.** Mean  $\pm$  standard deviation of plant species richness (species m<sup>-2</sup>), percentage of plant  
660 litter cover (%), percentage of leguminous plant cover (%), CWM-LDMC (g dry mass g water-  
661 saturated fresh mass<sup>-1</sup>), CWM-RPH (cm), CWM-EL and CWM-EN indicator value under the five  
662 treatments (n = 12): Abandonment (AB), low grazing by sheep (SH-), low grazing by cattle (CA-  
663 ), high grazing by cattle (CA+) and mowing (MO). Different letters denote significant differences  
664 between treatments with a > b > c (Tukey post-hoc test). *F*-values and associated *P*-values are  
665 indicated.

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	Treatments					<i>F</i>		<i>P</i>	
	AB	SH-	CA-	CA+	MO	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Species richness	7.8 <sup>c</sup> $\pm$ 0.7	11.3 <sup>bc</sup> $\pm$ 0.7	11.9 <sup>b</sup> $\pm$ 1.3	14.0 <sup>ab</sup> $\pm$ 0.7	17.0 <sup>a</sup> $\pm$ 0.9	14.2	<0.001	0.4	0.737
Plant litter (%)	66.7 <sup>a</sup> $\pm$ 4.4	35.0 <sup>b</sup> $\pm$ 3.9	39.9 <sup>b</sup> $\pm$ 4.7	9.6 <sup>c</sup> $\pm$ 2.5	14.3 <sup>c</sup> $\pm$ 1.9	42.2	<0.001	1.8	0.156
Leguminous plant (%)	0.4 <sup>c</sup> $\pm$ 0.2	1.3 <sup>c</sup> $\pm$ 0.7	2.1 <sup>bc</sup> $\pm$ 0.8	10.3 <sup>a</sup> $\pm$ 2.5	6.1 <sup>ab</sup> $\pm$ 2.1	9.6	<0.001	0.7	0.569
CWM LDMC	0.26 <sup>a</sup> $\pm$ 0.01	0.28 <sup>a</sup> $\pm$ 0.01	0.27 <sup>a</sup> $\pm$ 0.01	0.23 <sup>b</sup> $\pm$ 0.01	0.26 <sup>a</sup> $\pm$ 0.01	11.1	<0.001	2.9	0.046
CWM RPH	89.1 <sup>ab</sup> $\pm$ 1.1	90.4 <sup>a</sup> $\pm$ 3.0	88.6 <sup>ab</sup> $\pm$ 3.2	79.3 <sup>bc</sup> $\pm$ 4.5	73.5 <sup>c</sup> $\pm$ 2.9	6.7	<0.001	4.6	0.006
CWM EL	6.4 <sup>c</sup> $\pm$ 0.1	6.8 <sup>b</sup> $\pm$ 0.1	6.8 <sup>b</sup> $\pm$ 0.1	7.3 <sup>a</sup> $\pm$ 0.0	7.1 <sup>a</sup> $\pm$ 0.1	20.4	<0.001	10.1	0.003
CWM EN	7.7 <sup>a</sup> $\pm$ 0.2	6.1 <sup>b</sup> $\pm$ 0.2	6.2 <sup>b</sup> $\pm$ 0.2	6.1 <sup>b</sup> $\pm$ 0.1	5.5 <sup>c</sup> $\pm$ 0.11	32.5	<0.001	1.4	0.306

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670 **Table 3.** Mean ( $\pm$  standard deviation) earthworm abundance (individuals m<sup>-2</sup>), fresh biomass (g m<sup>-2</sup>) and diversity (m<sup>-2</sup>) under the five  
671 treatments (n = 12): Abandonment (AB), low grazing by sheep (SH-), low grazing by cattle (CA-), high grazing by cattle (CA+) and  
672 mowing (MO). Different letters denote significant differences between treatments with a > b (Tukey post-hoc test). *F*-values and  
673 associated *P*-values are indicated.

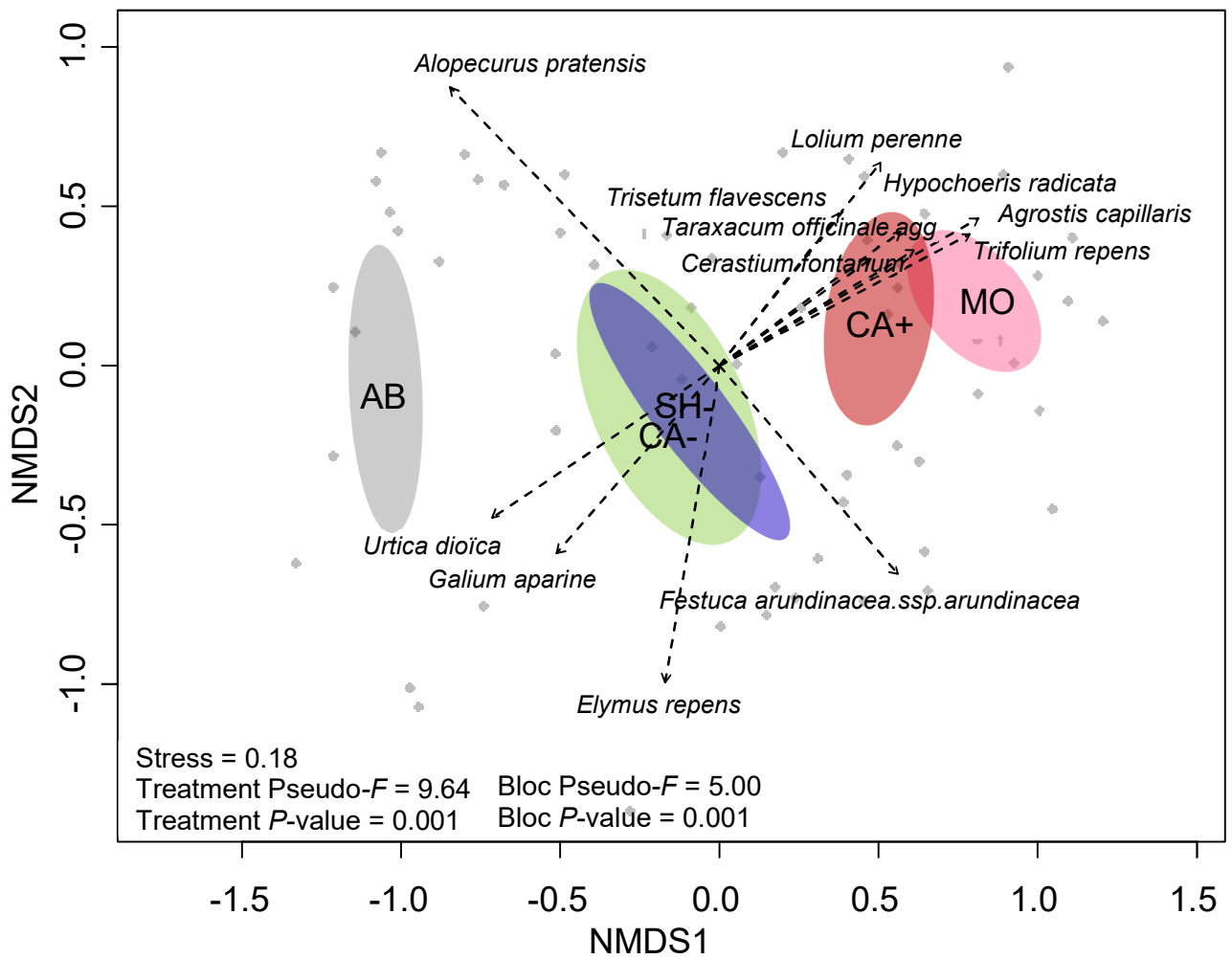
		Treatments							Blocks	
		AB	SH-	CA-	CA+	MO	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Abundance	Total	107.8 <sup>b</sup> ± 22	241.4 <sup>a</sup> ± 40	241.2 <sup>a</sup> ± 46	161.0 <sup>ab</sup> ± 23	212.8 <sup>a</sup> ± 25	4.2	0.005	3.3	0.027
	Epigeic	4.3 <sup>ab</sup> ± 2	8.1 <sup>a</sup> ± 2	10.7 <sup>a</sup> ± 4	6.9 <sup>ab</sup> ± 4	1.2 <sup>b</sup> ± 0.4	4.2	0.005	3.0	0.039
	<i>Lumbricus anecic</i>	14.2 <sup>ab</sup> ± 5	16.8 <sup>ab</sup> ± 4	25.0 <sup>a</sup> ± 4	8.2 <sup>b</sup> ± 3	7.8 <sup>b</sup> ± 2	3.0	0.028	2.0	0.135
	<i>Aporrectodea anecic</i>	15.1 <sup>b</sup> ± 5	47.4 <sup>a</sup> ± 10	39.3 <sup>a</sup> ± 5	51.3 <sup>a</sup> ± 6	49.5 <sup>a</sup> ± 6	9.6	<0.001	1.9	0.145
	Endogeic	74.3 <sup>a</sup> ± 19	169.2 <sup>a</sup> ± 33	166.2 <sup>a</sup> ± 44	94.7 <sup>a</sup> ± 18	154.3 <sup>a</sup> ± 23	7.41	0.050	4.63	0.201
Biomass	Total	60.8 <sup>b</sup> ± 15	118.7 <sup>a</sup> ± 10	132.4 <sup>a</sup> ± 17	115.0 <sup>a</sup> ± 10	125.5 <sup>a</sup> ± 15	6.6	<0.001	2.1	0.112
	Epigeic	0.4 ± 0.2	0.7 ± 0.2	1.2 ± 0.5	0.9 ± 0.5	0.1 ± 0.1	Na	Na	Na	Na
	<i>Lumbricus anecic</i>	13.7 <sup>a</sup> ± 7	15.6 <sup>a</sup> ± 4	19.8 <sup>a</sup> ± 5	6.4 <sup>a</sup> ± 1	10.1 <sup>a</sup> ± 2	1.2	0.304	1.3	0.278
	<i>Aporrectodea anecic</i>	23.1 <sup>b</sup> ± 9	62.9 <sup>a</sup> ± 5	67.0 <sup>a</sup> ± 9	84.1 <sup>a</sup> ± 8	69.4 <sup>a</sup> ± 13	10.1	<0.001	1.1	0.376
	Endogeic	23.7 <sup>a</sup> ± 6	39.5 <sup>a</sup> ± 7	44.4 <sup>a</sup> ± 11	23.6 <sup>a</sup> ± 4	45.8 <sup>a</sup> ± 7	2.4	0.060	1.6	0.239
Diversity	Species richness	5.8 <sup>b</sup> ± 0.4	7.6 <sup>a</sup> ± 0.4	7.8 <sup>a</sup> ± 0.4	7.6 <sup>a</sup> ± 0.5	7.4 <sup>a</sup> ± 0.4	4.5	0.004	3.0	0.420

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676 **FIGURES**

677 **Fig 1.** Non-metric multidimensional scaling ordinations showing similarities, based on Bray-Curtis  
678 distance index, of plant communities under five treatments (n = 12): Abandonment (AB), low  
679 grazing by sheep (SH-), low grazing by cattle (CA-), high grazing by cattle (CA+) and mowing  
680 (MO). Significant dissimilarities between modalities were assessed by PERMANOVA. Pseudo-*F*-  
681 values and associated *P*-values is indicated.

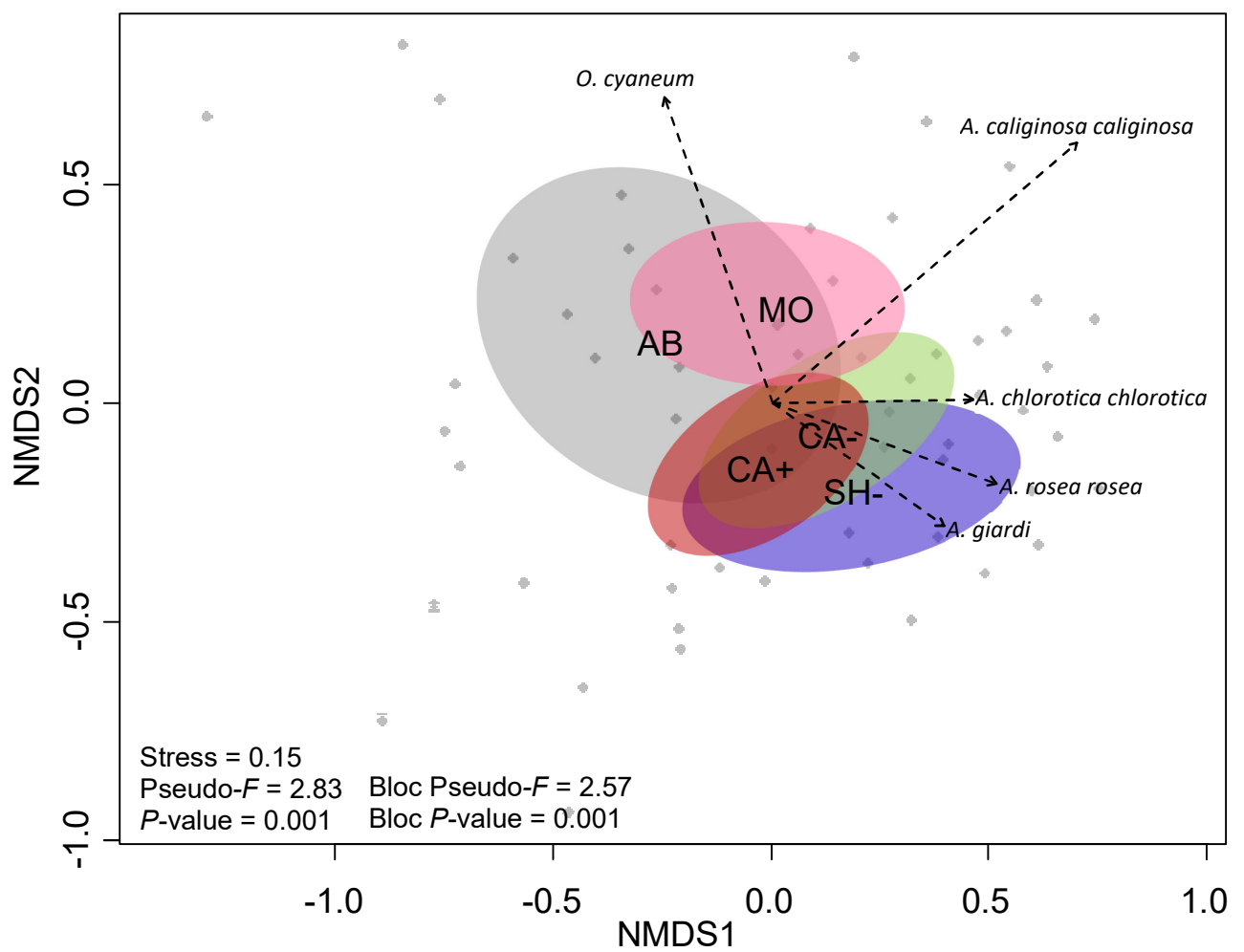


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685 **Fig 2.** Non-metric multidimensional scaling ordinations showing similarities, based on Bray-Curtis  
 686 distance index, of earthworm communities under five treatments (n = 12): Abandonment (AB), low  
 687 grazing by sheep (SH-), low grazing by cattle (CA-), high grazing by cattle (CA+) and mowing  
 688 (MO). Significant dissimilarities between modalities were assessed by PERMANOVA. Pseudo-*F*-  
 689 values and associated *P*-values is indicated.



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