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► To cite this version:

Nicolas Honvault, David Houben, Manhattan Lebrun, Charlotte Vedere, Cécile Nobile, et al.. Positive or neutral effects of biochar-compost mixtures on earthworm communities in a temperate cropping system. *Applied Soil Ecology*, 2023, 182, pp.104684. <10.1016/j.apsoil.2022.104684>. <hal-03810055>

HAL Id: hal-03810055

<https://normandie-univ.hal.science/hal-03810055v1>

Submitted on 5 Sep 2025

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1 **Positive or neutral effects of biochar-compost**
2 **mixtures on earthworm communities in a temperate**
3 **cropping system**

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19 **Abstract**

20 Biochar is increasingly considered an efficient
21 amendment to improve soil fertility and store carbon in
22 soils for climate change mitigation. However, few field
23 studies have evaluated biochar impacts on soil fauna and
24 especially earthworms in temperate areas despite their
25 importance for soil structure and laboratory-based

26 evidence of potential adverse effects. We investigated the
27 effects of three biochars (from rapeseed, miscanthus and
28 compost reject) in mixture with a green waste compost on
29 the abundance and composition of earthworm
30 communities during a three-year field experiment in
31 northern France. Compost alone or biochar-compost
32 mixtures were applied at 11.7 t dry matter ha⁻¹ and
33 compared to equivalent mineral fertilization. Biochar-
34 compost mixtures did not affect total earthworm density
35 and biomass, while contrasting effects concerning
36 specific ecological groups were observed. Compost
37 addition increased juvenile endogeic earthworm density
38 roughly one month after amendment while no adverse
39 short-term effects of biochar-compost mixtures were
40 observed. Two years after amendment application,
41 anecic earthworm density was higher for the mixture with
42 compost and biochar from compost rejects, which also
43 resulted in the highest soil organic carbon concentrations.
44 We conclude that biochar-compost mixtures at low but
45 more economically viable biochar application rates could
46 be used as an increasingly understood beneficial soil
47 amendment under temperate climate conditions without
48 adverse effects on earthworm communities.

49 **Keywords**

50 Earthworms, Biochar, Compost, Anecic, Endogeic,
51 Agroecology

52 **1. Introduction**

53 Biochar, the solid carbon-rich pyrolysis residue, can have
54 many beneficial effects on soil functioning, fertility and
55 carbon sequestration (Dai et al., 2020; Ding et al. 2016;
56 Majumder et al. 2019; Jenkins et al. 2017; Joseph et al.,
57 2021), contributing toward planning for future large scale
58 deployment (Joseph et al., 2021). One way to implement
59 biochar application at scale may be its addition to soil
60 organic amendments such as compost. In particular,
61 biochar may be produced at large scale composting
62 facilities from non-compostable material. However, the
63 effects of such materials on soil fauna and associated
64 ecosystem services have received little attention, with
65 most studies focusing on microbial communities
66 (Güereña et al., 2013; Lehmann et al., 2011).
67 Earthworms are of great importance for soil functioning
68 (Lavelle et al., 1997). They are often presented as “soil
69 engineers” owing to their effects on soil structure (Lavelle,
70 1988) and nutrient availability (Blouin et al., 2013) and
71 more generally biogeochemical cycling (Angst et al.,
72 2019). Despite recent efforts to decipher biochar effects
73 on earthworm communities (Ameloot et al., 2013;
74 Brtnicky et al., 2021; Domene, 2016), the impacts of

75 organic amendments including biochar and biochar co-
76 application with organic or mineral fertilizer on earthworm
77 communities remain poorly predictable (Whalen et al.,
78 2021). Further comprehensive research is thus required
79 before such strategies can be implemented at scale
80 (Malev et al., 2016; Oleszczuk et al., 2013; Sohi et al.,
81 2009).

82 While biochar effects on earthworm communities were
83 found to be very inconsistent (Lehmann et al., 2011;
84 Weyers and Spokas, 2011), many studies reported a
85 negative short-term (<6 months) effect of biochar on
86 earthworm density and activity (Li et al., 2011; Weyers
87 and Spokas, 2011). Moreover, the presence of biochar
88 reduced microbial activity during organic matter
89 decomposition in the presence of earthworms (Barthod et
90 al., 2016). This deleterious effect can be attributed to the
91 presence of contaminants in biochar, such as polycyclic
92 aromatic hydrocarbons (PAH) (Malev et al. 2016; Ren et
93 al. 2018). PAH can be issued from direct carbonization
94 and aromatization at temperature below 500 °C or free
95 radical pathways at temperature above 500 °C, with
96 widely differing concentrations between biochar (Wang et
97 al. 2017). Decreased soil matric potential following
98 biochar addition (Tammeorg et al., 2014), or biochar
99 liming effects (Domene, 2016) can also contribute to

100 deleterious effects. Other authors have suggested that
101 physical protection of the microbial communities
102 inhabiting biochar particles would result in shifts in
103 microbial grazers including earthworms (Briones et al.,
104 2020). Studies on longer term effects (periods superior to
105 six months to one year) are scarce and inconsistent as
106 negative (Briones et al., 2020), neutral (Whalen et al.,
107 2021) or positive (Husk and Major, 2010) effects have
108 been reported. Earthworm diverse behaviours per
109 ecological groups, such as deep burrowing and mixing of
110 mineral and organic soil layers for endogeic earthworms,
111 limited shallow soil mixing for epigeic earthworms or
112 vertical burrowing and litter burying for anecic
113 earthworms (Bouché 1977; Bohlen et al. 2004) may in
114 part explain these inconsistencies. Nonetheless,
115 detrimental effects of biochar on earthworms have been
116 predominantly reported after biochar application at high
117 rates, ranging from 25 to 68 t ha⁻¹ (Briones et al., 2020;
118 Liesch et al., 2010; Malev et al., 2016), relative to the
119 recommended rates ranging from 5 to 20 t ha⁻¹ (Joseph et
120 al., 2021). In addition, most studies have focused on
121 tropical climates while temperate areas have been much
122 less investigated (Tammeorg et al., 2014), and the wide
123 heterogeneity of biochar properties (Dai et al., 2020;
124 Xiang et al., 2017) was often omitted by approaches

125 which used only one biochar type. Given the considerable
126 effects of organic matter on earthworm communities
127 (Leroy et al., 2008, 2007; Sizmur et al., 2017) and
128 especially the strong response of endogeic earthworms to
129 soil available C (Tiunov and Scheu, 2004), biochar co-
130 application with organic fertilization such as compost
131 could importantly impact its effects (Tammeorg et al.,
132 2014). Mixture with compost may also enhance other
133 benefits of biochar (Jeffery et al., 2017; Ye et al., 2020).
134 The large majority of lab-based evidence of biochar
135 impact on earthworm communities underlines the need
136 for more field-based experiments to confirm tendencies
137 observed ex-situ and their evolution over longer time
138 periods (Briones et al., 2020; Whalen et al., 2021).

139 In order to investigate the effects of different types of
140 biochar co-applied with organic fertilization on earthworm
141 communities we performed a field experiment with three
142 contrasting biochar-compost mixtures as well as compost
143 alone and a mineral control in northern France. Biochar-
144 compost mixtures were applied at a rate of 11.7 t ha⁻¹
145 with a 30:70 biochar compost ratio in order to investigate
146 realistic and economically viable practices. We aimed to
147 evaluate if contrasting biochar properties alter the
148 response of earthworm communities to biochar-compost
149 mixture addition. To this end, we monitored earthworm

150 density and biomass during three years. We examined
151 how earthworm diverse behaviour and especially feeding
152 behaviour influenced their response to amendment
153 addition by identification of earthworm species and their
154 classification into ecological group (i.e. anecic, endogeic
155 and epigeic earthworms) (Bouché, 1977). We
156 hypothesised contrasting effects of the different biochar-
157 compost mixtures on earthworm communities because of
158 their diverse properties induced by the different biochar
159 types.

160 **2. Material and methods**

161 2.1 Organic amendments

162 The compost used in the experiment was a green waste
163 compost issued mostly from grass, poplar and conifers
164 branches provided by Fertivert (Seine-Maritime, France).
165 Three compost-biochar mixtures were examined,
166 containing biochar produced with diverse temperature
167 conditions and from different feedstocks. Three
168 feedstocks for biochar production were examined
169 miscanthus straw, rapeseed straw and green waste
170 compost reject, issued from reject material
171 undecomposed after composting. Biochars were
172 produced using an industrial pyrolysis reactor (Biogreen®
173 Technology, ETIA, Oise, France) with pyrolysis

174 temperatures of 550 °C for miscanthus biochar, 650 °C
175 for rapeseed straws biochar and 450 °C for green wastes
176 compost rejects biochar and a 10 minutes pyrolysis time
177 for all biochars. Biochars were selected based on their
178 contrasting properties and effects on plant growth (higher
179 biomass after miscanthus biochar addition than rapeseed
180 straws biochar and intermediate for green wastes
181 compost rejects biochar) during a preliminary experiment
182 (Honvault et al., 2022). Compost-biochar mixtures and
183 compost characteristics are presented in Table 1 and
184 biochar properties are presented in Table 2

185 2.2 Field experiment

186 The field experiment was carried out in Beauvais
187 (UniLaSalle farm), northern France (49°25'49"N,
188 2°04'51"E). The soil was classified as an Haplic Luvisol
189 (IUSS Working Group WRB, 2015), with 21 % clay, 69%
190 silt and 10% sand (Table 3). Soil properties were
191 determined following protocols published by the French
192 Association for Normalization (AFNOR, 1996), such as
193 dry combustion on decarbonated soil for organic carbon
194 (NF ISO 1423) (Table 3). The climate was oceanic with
195 minimum and maximum temperatures averaging at 6.5
196 °C and 14.9 °C and the average annual precipitation was
197 655 mm year⁻¹. The experiment started in spring 2019

198 and lasted three cropping cycles until autumn 2021. The
199 crop rotation consisted of maize in 2019, wheat in 2020
200 and barley in 2021. The experimental design consisted in
201 five treatments applied in four replicates for a total of 20
202 randomly distributed plots of 32 m² (3.2 m x 10 m).
203 Treatments consisted of a green waste compost either
204 alone (“Compost”) or mixed with biochar issued from rape
205 straws (“Compost/BiocharRap”), miscanthus straw
206 (“Compost/BiocharMis”) or green waste compost rejects
207 biomass (“Compost/BiocharRej”). Compost alone was
208 applied at a rate of 19.6 t fresh matter ha⁻¹ corresponding
209 to approximately 7.8 t dry matter, while compost-biochar
210 mixtures were applied at a rate of 24.5 t fresh matter ha⁻¹
211 consisting of 7.8 t dry matter ha⁻¹ compost and 3.9 t dry
212 matter ha⁻¹ biochar. A mineral control (“Control”) adding
213 the same quantity of phosphorus and potassium as in the
214 Compost treatment as triple superphosphate and KCl
215 respectively was also part of the experiment. Nitrogen
216 was not included as standard recommended nitrogen
217 fertilization was applied (in several doses for a total of 60
218 kg ha⁻¹ in 2019, 164 kg ha⁻¹ in 2020 and 180 kg ha⁻¹ in
219 2021) to ensure optimum nitrogen nutrition. No additional
220 mineral fertilisation or organic fertilisation was applied
221 during the whole duration of the experiment. The
222 amendments were applied manually to the soil once in

223 2019 before mechanical incorporation in the first 5 cm
224 using rotary harrow. Control plots were prepared as the
225 other plots, i.e. by mechanically mixing inorganic fertilizer
226 within the first 5 cm. The tillage was then very reduced
227 and superficial throughout the experiment.

228 2.3 Earthworm sampling

229 Earthworms were sampled twice every year in spring and
230 autumn, except in autumn 2019 and in spring 2020
231 (Table 4). Earthworms were extracted with a
232 formaldehyde solution (10 mL 37 % in 10 L water) on
233 three 0.5 m² (0.7*0.7m) areas per plot (ISO 2018). Each
234 0.5 m² area was watered 3 times, each time followed by a
235 10 minute-collection period. Harvested areas were
236 identified with a plastic picket and progressively shifted
237 within the plots to avoid previously harvested areas. Due
238 to the long-term character of the still ongoing experiment,
239 earthworms were collected on the soil surface without soil
240 excavation. Earthworms were stored in 70% ethanol until
241 determination shortly after collection. Total earthworm
242 biomass was measured for spring samplings, as well as
243 earthworm biomass per ecological group in 2021. They
244 were classified by species and ecological group (i.e.
245 epigeic, endogeic, anecic) (Bouché, 1977).

246 2.4 Statistical analysis

247 All tests were performed with R v 3.6.0 and the package
248 Rcmdr 2.5-3 with a significance level of 0.05 (R Core
249 Team, 2013). The effects of year, season and treatments
250 were first examined via generalised linear models (GLM)
251 (quasi-poisson distribution, log link) with specified
252 interactions between year, season and treatment to
253 understand the overall effects of biochar-compost
254 mixtures. GLM with quasi-poisson distribution were
255 selected due to the skewed and slightly over dispersed
256 density (count) data overall. The proportion of juvenile to
257 adult earthworms was calculated and compared for each
258 ecological group as well as the proportion of each
259 ecological group to the total density. In order to examine
260 plausible effects on earthworm diversity the Shannon
261 diversity index was calculated as presented below
262 (Shannon, 1948).

263
$$H = - \sum [(p_i) \times \ln(p_i)]$$

264 With p_i proportion of each individual species in the
265 community.

266 As data per year and season was normally distributed,
267 differences among earthworm density, biomass, species
268 richness and diversity were examined with one-way
269 ANOVAs followed if necessary by Tukey post hoc tests.

270 **3. Results and discussion**

271 3.1 Earthworm total density and biomass

272 Earthworm total density at the beginning of the
273 experiment was very low between 7 and 14 individual m^{-2}
274 and more than doubled two years later with densities
275 between 24 and 44 individuals m^{-2} (Fig. 1 c and f). This
276 was also reflected in earthworm biomass which increased
277 from 5.7 to 11.9 $g m^{-2}$ on average in spring 2019 to 16.0
278 to 35.2 $g m^{-2}$ on average in spring 2021 (Supplementary
279 material S1). During the three cropping cycles, the
280 different organic amendments did not show any effect on
281 total earthworm abundance, richness or diversity (Table
282 5). Strong seasonal effects were underlined with
283 earthworm density, species richness and diversity being
284 higher in spring than autumn, likely due to dry summers
285 months reducing earthworm densities (Holmstrup, 2001;
286 Whalen, 2004). Strong temporal effects were also
287 underlined with earthworm density, species richness and
288 diversity increasing with time since mechanical
289 incorporation of the amendments (Table 5). Despite
290 significant interactions between treatment, year and
291 season (Table 5) no significant differences were
292 observed in earthworm species richness and diversity
293 (Supplementary material S2). Increasing earthworm
294 density in the first years after the establishment of the

295 field experiment is consistent with results of Denier et al.
296 (2022), who observed a similar two fold increase in
297 earthworm density after three years without tillage in a
298 field experiment adjacent to ours. This increase may thus
299 be related to initial mechanical disturbances and reduced
300 mechanical disturbances of soil during the experiment,
301 which is known to strongly affect earthworm communities
302 in agricultural fields (Karaca, 2011). Our results showed
303 that mechanical disturbances were the predominant
304 factor driving earthworm density over time irrespective of
305 the treatment. We thus overall highlight neutral effects of
306 biochar-compost mixtures on earthworm total densities
307 compared to equivalent fertilisation, in agreement with
308 recent observations (Whalen et al., 2021) but in contrast
309 with numerous studies showing adverse short term (<6
310 months) effects of biochar application on earthworm
311 density and activity (Li et al., 2011; Weyers and Spokas,
312 2011). We indeed observed no short-term negative
313 effects of biochar roughly one month after addition (Fig. 1
314 a, b and c) and up till three years after application.

315 3.2 Earthworm community composition

316 The earthworm communities from this cropped soil were
317 composed of two to five species and dominated by two
318 species, *Lumbricus terrestris* and *Allolobophora*
319 *chlorotica* (see Supplementary material S3 for a list of

320 species observed). Despite overall neutral effects of
321 organic amendments on total earthworm density,
322 significant differences between treatments were noted
323 per ecological group within the community, i.e. for anecic
324 earthworm density. Significant interactions between
325 treatment, year and season were also observed for
326 endogeic earthworm density (Table 6). Further
327 investigations highlighted significant differences in
328 juvenile endogeic earthworm density for organic
329 amendments compared to the mineral control and anecic
330 earthworm density between organic amendments in
331 spring 2019 and 2021 respectively, while no significant
332 differences were observed in autumn, likely due to lower
333 density (Supplementary material S4). Significantly higher
334 juvenile endogeic earthworm density was observed for
335 the compost treatment compared to the mineral control in
336 spring 2019 (Fig. 1 b), despite very low densities. All
337 organic amendments in turn tended to have higher, albeit
338 not significantly, juvenile endogeic earthworm density
339 than the mineral control in spring 2019 (Fig. 1 b). In
340 spring 2021, anecic earthworm density was higher for the
341 Compost/BiocharRej treatment (23 ± 3 individuals m^{-2})
342 compared to the Compost treatment (12 ± 2 individuals
343 m^{-2}) and to the other organic treatments (Fig. 1 d) but not
344 to the mineral control. Anecic earthworm biomass was

345 also significantly higher for the Compost/BiocharRej with
346 32 g m⁻² as compared to 14 g m⁻² for the
347 Compost/BiocharRap treatment.

348 Increased endogeic earthworm in spring 2019 may be
349 explained by boosting effects of organic carbon additions
350 on earthworm communities (Leroy et al., 2008, 2007),
351 which are expected to particularly affect endogeic
352 earthworms. Indeed endogeic earthworms feed on more
353 humidified substrates and are mostly limited by available
354 C in soil (Tiunov and Scheu, 2004), which can be directly
355 enriched by the carbon contained in organic fertilizers
356 (Briones et al., 2020). Moreover, endogeic earthworm
357 communities have been observed to decline less or even
358 increase after tillage (Chan, 2001; Ernst and Emmerling,
359 2009). Since the soil was slightly tilled to allow
360 amendment incorporation roughly one month before the
361 first sampling in spring 2019, the weaker sensitivity to
362 tillage of endogeic earthworms might explain why only
363 endogeic earthworms responded quickly positively to
364 compost addition. By contrast, tillage may have overrode
365 the very short-term amendment effect on the other
366 ecological groups which are more sensitive to mechanical
367 disturbances (i.e. anecic earthworms) (Denier et al., 2022;
368 Simonsen et al., 2010). Anecic earthworm indeed
369 represented a lower proportion of the total community at

370 the beginning of the experiment (Fig. 1 a, c, d, f).
371 However, no significant differences in total earthworm
372 density and anecic earthworm density were observed
373 compared to the mineral control, highlighting low short-
374 term impacts of organic inputs in our system.

375 Intermediate juvenile endogeic earthworm densities were
376 observed in Spring 2019 for compost-biochar mixtures
377 compared to compost alone and to the mineral control
378 (Fig. 1 b). The lack of adverse short-term effects of
379 biochar may be related to the low biochar application rate
380 which was well below the threshold rates reported for
381 negative effects such as 16 t ha⁻¹ in Malev et al. (2016) or
382 68 t ha⁻¹ in Liesch et al. (2010). Moreover, simultaneous
383 co-application with organic matter appears to largely
384 compensate short-term negative effects of biochar
385 (Weyers and Spokas, 2011), suggesting that co-
386 application of compost could have contributed to alleviate
387 possible negative effects of biochar. Slightly lower
388 juvenile endogeic earthworm density for biochar-compost
389 mixtures compared to compost alone could then be
390 related to the higher nutritional value of compost alone
391 (Leroy et al., 2008). Earthworms have indeed been
392 shown to prefer more labile and less humidified sources
393 of carbon (Leroy et al., 2008) and biochar is considered
394 to be of low nutritional value for earthworms (Briones et

395 al., 2020). Lower average nutritional value for biochar-
396 compost mixtures as well as protection of labile carbon
397 sorbed on biochar particles may thus have contributed to
398 slightly lower juvenile endogeic densities for biochar-
399 compost mixtures compared to compost applied alone.
400 Biochar liming effect and PAH content may also have
401 contributed, although low biochar application rates would
402 suggest minor effects.

403 Longer term effects of organic amendments contrasted
404 with the earlier observations, showing significantly higher
405 anecic earthworm density for the Compost/BiocharRej
406 treatment compared to compost alone and no significant
407 differences in endogeic earthworm density between
408 organic amendments. Endogeic (and total) earthworm
409 density did not significantly differ for the organic
410 amendments compared to the mineral control, showing
411 progressively decreasing effects of organic inputs. The
412 approximately two fold increase in anaecic earthworm
413 density for treatments amended with Compost/BiocharRej
414 as compared to the compost treatment is consistent with
415 observations by Husk and Major (2010) of an
416 approximately two times increase in earthworm density
417 one year and half after biochar addition as well as
418 observations by Tammeorg et al. (2014) of an 1.8 times
419 increase in earthworm density four months after biochar

420 addition (even though not significantly different for the
421 later). Biochar application can lead to positive effects on
422 earthworm density after several months (Husk and Major,
423 2010; Tammeorg et al., 2014). However, these effects
424 are scarcely reported with neutral effects or negative
425 effects being reported more frequently (Briones et al.,
426 2020; Weyers and Spokas, 2011; Whalen et al., 2021).
427 Increased anecic earthworm density could hardly be
428 related to increased crop biomass productivity, soil fertility
429 or soil pH which did not show significant differences
430 among treatments (data not shown, in preparation). We,
431 however, observed increased organic carbon
432 concentration in soils amended with biochar-compost
433 mixtures (Fig. 2). Specifically, organic carbon
434 concentration was higher for all soils amended with
435 organic amendments compared to the mineral control in
436 2019 (Fig. 2 a), for the Compost/Biochar treatments
437 compared to the mineral control in 2020 (Fig. 2 b) and for
438 the Compost/BiocharRej treatment compared to the
439 mineral control in 2021 (Fig. 2 c). To our knowledge, this
440 is the first observation of a plausible long-term (i.e. more
441 than one year) change in earthworm communities due to
442 biochar application in mixture with compost, simultaneous
443 to an increase in soil organic carbon concentration.
444 However, no significant correlation was observed

445 between soil organic carbon concentration and anecic
446 earthworm density, and differences in anecic earthworm
447 density were observed between organic treatments rather
448 than with the mineral control. Increased anecic
449 earthworms density after two years seems to contrast
450 with the increased juvenile endogeic earthworm density
451 observed roughly one month after amendment
452 incorporation and with the higher preference of endogeic
453 earthworms for humidified C possibly increased by
454 organic amendments (Tiunov and Scheu, 2004). Recent
455 evidence nonetheless points toward higher assimilation of
456 biochar carbon by anecic earthworms compared to
457 endogeic earthworm (Briones et al., 2020). However,
458 *Lumbricus terrestris*, the dominant specie in our
459 communities, appear to poorly assimilate carbon
460 originating from biochar (Briones et al., 2020). Moreover,
461 anecic earthworm density significantly differed between
462 organic amendments but not compared to the mineral
463 control despite increases in organic carbon concentration,
464 prompting further investigations. We thus overall
465 underline a mostly neutral long term (i.e. more than one
466 year after addition) effect of biochar-compost mixtures on
467 earthworm total density, coherent with the low biochar
468 rate applied and fertile temperate soils (Weyers and
469 Spokas, 2011), while also highlighting scarcely reported

470 changes in earthworm community composition that
471 require further investigations.

472 **4. Conclusion**

473 This study intended to gain insight into the sparsely
474 investigated effect of biochar-compost mixtures on
475 earthworm community in an agricultural field under
476 temperate climate. Our experiment indicated overall
477 neutral effect of biochar-compost mixtures. We conclude
478 that biochar addition to compost at low application rates
479 (4 t dry matter ha⁻¹) has no adverse effects on the
480 abundance of earthworm communities both on the short-
481 and the long-term. However, we also observed a group-
482 specific influence of the biochar type on the amendment
483 effect, as anecic earthworm density and biomass were
484 greater for compost containing compost rejects biochar
485 as compared to the other organic amendments. This
486 mixture also induced a simultaneous lasting increase in
487 organic carbon concentration, while our results don't
488 support a direct relationship, prompting further
489 investigations. To our knowledge, our results thus
490 constitute one of the very few evidences of a multiannual
491 impact of biochar compost mixtures on earthworm
492 communities in a temperate setting. We conclude that
493 biochar type could influence the effect of such mixtures.
494 However, as biochar interaction with soil environments

495 and its effect on soil biological response tend to be very
496 variable depending on biochar and soil properties, further
497 investigations are required in order to generalize our
498 observations.

499 **Acknowledgements**

500 The authors thank Bpifrance and the Région Hauts-de-
501 France for funding the project (FUI Biochar 2021). CR
502 acknowledges funding from ANR under the U2 worm
503 project (ANR-20-CE01-0015-01). All members of the FUI
504 biochar are acknowledged for their contribution to the
505 project. The authors also thank Vincent Hervé for his
506 technical assistance. We thank ETIA and Agriculteurs
507 Composteurs de France for providing compost and
508 biochars. We thank the “ASET 160-161-162” students for
509 technical assistance.

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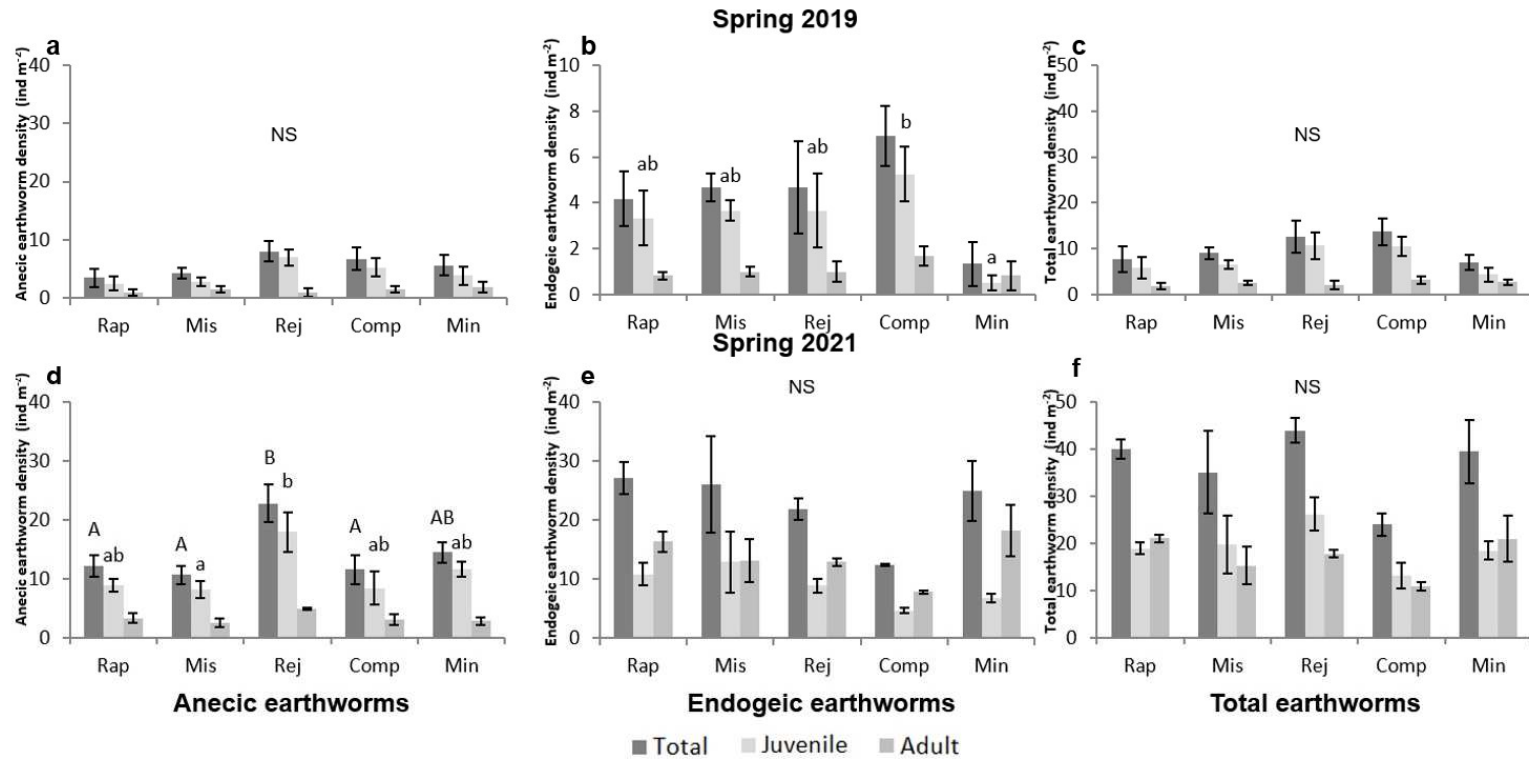


Fig. 1

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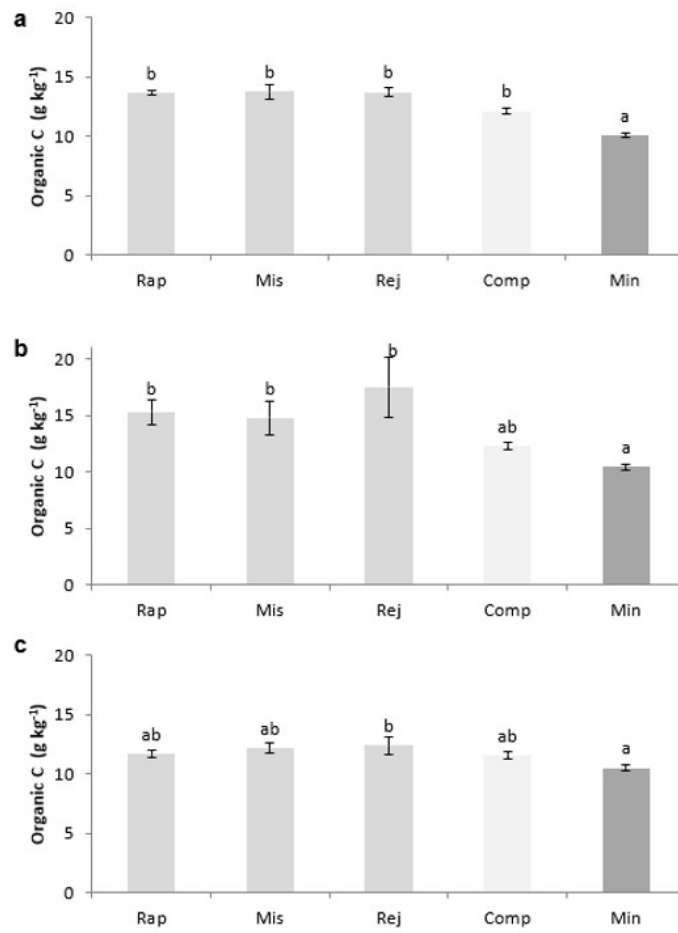


Fig. 2

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796 **Fig. captions**

797 **Fig. 1:** Mean total earthworm density and density per
798 ecological group (\pm SE) in spring 2019 and 2021.

799 Rap: mixture of green waste compost and biochar issued
800 from rape biomass; Mis: mixture of green waste compost
801 and biochar issued from miscanthus biomass; Rej:
802 mixture of green waste compost and biochar issued from
803 green wastes compost rejects biomass; Comp: green
804 waste compost; Min: Mineral control. Different letters
805 above the bars indicate statistically significant
806 differences. a Anecic earthworm density in spring 2019, b
807 Endogeic earthworm density in spring 2019, c Total
808 earthworm density in spring 2019; d Anecic earthworm
809 density in spring 2021, e Endogeic earthworm density in
810 spring 2021, f Total earthworm density in spring 2021.

811 **Fig. 2:** Mean soil organic carbon concentration (\pm SE) in
812 2019, 2020 and 2021.

813 a: Organic carbon concentration in 2019; b: Organic
814 carbon concentration in 2020; c: Organic carbon
815 concentration in 2021. Different letters above the bars
816 indicate statistically significant differences. Rap: mixture
817 of green waste compost and biochar issued from rape
818 biomass; Mis: mixture of green waste compost and
819 biochar issued from miscanthus biomass; Rej: mixture of

- 820 green waste compost and biochar issued from green
- 821 wastes compost rejects biomass; Comp: green waste
- 822 compost; Min: Mineral control.

Table 1

| Amendment | Organic C [†] (g kg ⁻¹) | Total N [‡] (g kg ⁻¹) | C:N | P [§] (g kg ⁻¹) | K [§] (g kg ⁻¹) | Ca [§] (g kg ⁻¹) | Mg [§] (g kg ⁻¹) |
|--------------------|---|---|-----|---|---|--|--|
| Compost | 292 | 27 | 10 | 4.2 | 17 | 29 | 2.8 |
| Compost/BiocharMis | 337 | 18 | 18 | 3.4 | 16 | 24 | 2.3 |
| Compost/BiocharRej | 344 | 22 | 15 | 5.3 | 19 | 31 | 2.9 |
| Compost/BiocharRap | 429 | 21 | 20 | 3.5 | 20 | 32 | 2.2 |

Table 1: Organic amendment properties

[†]Dry combustion; [‡]Dumas method; [§] Total, chemical digestion followed by ICP measurement (NF EN 13650, ICP MS NF EN ISO 17294) (EN 13650, 2002). NF and NF ISO determined following protocols published by the French Association for Normalization (AFNOR, 1996). Compost/BiocharMis : mixture of green waste compost and biochar issued from miscanthus biomass; Compost/BiocharRej: mixture of green waste compost and biochar issued from green wastes compost rejects biomass; Mixture Compost/BiocharRap: mixture of green waste compost and biochar from rape biomass; Compost : green waste compost.

Table 2

| | Unit (Dry Mass) | Miscanthus Rapeseed Compost | | |
|---------------------------|------------------------------------|-----------------------------|------|------|
| | | BC | BC | BC |
| Porosity [†] | % | 92.5 | 95.9 | 86.3 |
| Density [†] | g L ⁻¹ | 112 | 78 | 291 |
| Conductivity [‡] | dS m ⁻¹ | 1.30 | 1.22 | 3.54 |
| CEC [§] | cmol _c kg ⁻¹ | 31.0 | 2.9 | 8.5 |
| pH [#] | | 10.4 | 11.2 | 11.4 |
| Ash 550°C ⁺⁺ | % | 18.6 | 26.4 | 33.7 |
| C ⁺⁺ | % | 74.0 | 62.3 | 55.5 |
| Total N ^{§§} | % | 0.53 | 0.81 | 0.88 |
| C/N | | 141 | 77 | 63 |
| P ^{##} | g kg ⁻¹ | 0.31 | 0.38 | 0.37 |
| K ^{##} | g kg ⁻¹ | 2.52 | 1.82 | 1.78 |
| Ca ^{##} | g kg ⁻¹ | 0.21 | 6.57 | 3.38 |
| Mg ^{##} | g kg ⁻¹ | 0.06 | 0.41 | 0.50 |

Table 2 : Chemical and physical properties of the biochars used.

[†] (EN 13041, 2006) ; [‡] (EN 13038, 1999) ; [§] (NF X31-130, 1999) ; [#] (EN 13037, 1999); ⁺⁺ (ISO, 2016) ; ^f (EN 13039, 2011) ; ^{§§} (EN 13654-1, 2002) ; ^{##} total extracted with aqua regia, (EN 13650, 2002).

Table 3

| Soil depth (cm) | Clay (%) | Silt (%) | Sand (%) | pH [†] H ₂ O | Carbonates [‡] (%) | OM [§] (g kg ⁻¹) | CEC [#] (cmolc kg ⁻¹) | Total N ^{††} (g kg ⁻¹) | P ^{††} (g kg ⁻¹) | K ^{§§} (g kg ⁻¹) | Mg ^{§§} (g kg ⁻¹) | Ca ^{§§} (g kg ⁻¹) |
|-----------------|----------|----------|----------|-------------------------------------|--------------------------------|--|---|--|--|--|---|---|
| 0-15 | 21 ± 0.5 | 69 ± 0.8 | 10 ± 0.7 | 7.93 ± 0.03 | 0.8 ± 0.11 | 17.67 ± 0.2 | 109.17 ± 2.18 | 1.13 ± 0.01 | 0.07 ± 0 | 0.12 ± 0.01 | 0.07 ± 0 | 3.95 ± 0.36 |
| 15-30 | 21 ± 0.5 | 69 ± 0.8 | 10 ± 1.1 | 7.92 ± 0.03 | 0.83 ± 0.08 | 17.6 ± 0.26 | 109.83 ± 2.65 | 1.12 ± 0.01 | 0.07 ± 0 | 0.13 ± 0.01 | 0.07 ± 0 | 4.15 ± 0.38 |

Table 3: Soil properties

[†]1:5 soil: water ratio NF ISO 10 390; [‡] Total carbonates NF ISO 10693; [§] dry combustion on decarbonated soil NF ISO 14235; [#] CEC Metson NFX 31-130; ^{††} Dumas method; ^{†††} Available P, Joret Hebert NFX 31-161; ^{§§} Exchangeable cations NFX 31-108. NF and NF ISO determined following protocols published by the French Association for Normalization (AFNOR, 1996).

Table 4

| | Date | Days since amendment |
|----------------------|------------|----------------------|
| Soil amendment | 23/04/2019 | 0 |
| Spring sampling 2019 | 20/05/2019 | 27 |
| Autumn sampling 2019 | 19/09/2019 | 149 |
| Autumn sampling 2020 | 22/09/2020 | 518 |
| Spring sampling 2021 | 19/04/2021 | 727 |
| Autumn sampling 2021 | 22/09/2021 | 883 |

Table 4: Earthworm sampling dates

Table 5

| Variable | Source of variability | p value |
|----------|-----------------------|-----------------|
| Total | Year | <0.01 |
| | Treatment | 0.06 |
| | Season | <0.01 |
| | Year*Treatment | 0.09 |
| | Treatment*Season | 0.58 |
| Richness | Year | <0.01 |
| | Treatment | 0.57 |
| | Season | <0.01 |
| | Year*Treatment | <0.05 |
| | Treatment*Season | 0.35 |
| Shannon | Year | <0.01 |
| | Treatment | 0.99 |
| | Season | <0.01 |
| | Year*Treatment | <0.01 |
| | Treatment*Season | <0.01 |

Table 5: Result of GLMs for the effect of year, season, treatment and their interaction on total earthworm density, species richness and diversity.

GLM quasipoisson with log link. Significant ($\alpha < 5\%$) p value in bold. Total : Total earthworm density; Richness : Species richness; Shannon : Shannon diversity index.

Table 6

| Variable | Source of variability | p value |
|----------|-----------------------|-----------------|
| Anecic | Year | <0.01 |
| | Treatment | 0.01 |
| | Season | 0.40 |
| | Year*Treatment | 0.77 |
| | Treatment*Season | 0.73 |
| Endogeic | Year | <0.01 |
| | Treatment | 0.24 |
| | Season | <0.01 |
| | Year*Treatment | 0.03 |
| | Treatment*Season | 0.91 |

Table 6: Result of GLMs for the effect of year, season, treatment and their interaction on total earthworm density, species richness and diversity.

GLM quasi-poisson with log link. Significant ($\alpha < 5\%$) p value in bold. Anecic: Anecic earthworm density; Endogeic: Endogeic earthworm density.