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► To cite this version:

Jeanne Maréchal, Kevin Hoeffner, Xavier Marié, Daniel Cluzeau. Response of earthworm communities to soil engineering and soil isolation in urban landscapes. *Ecological Engineering*, 2021, 169, pp.106307. 10.1016/j.ecoleng.2021.106307 . hal-03283803

HAL Id: hal-03283803

<https://hal.science/hal-03283803>

Submitted on 2 Aug 2023

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1 **Title: Response of earthworm communities to soil engineering and soil isolation in urban**
2 **landscapes**

3

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13 **Abstract**

14 Engineered soils provide numerous ecosystem services in urban landscapes, such as water
15 regulation and plant growth. They are constructed to optimize soil physicochemical properties
16 but their biological properties are given little consideration. In particular, earthworm
17 communities may be highly impacted by soil engineering processes and soil isolation caused
18 by asphalted surfaces separating soils, and in particular roadside soils, from pseudo-natural
19 soils. In this context, this study aimed to evaluate (i) the effects of soil engineering processes
20 applied to construct roadside soils, and (ii) the effects of soil isolation from pseudo-natural
21 soils by asphalted surfaces on earthworm communities. The study was conducted in an urban
22 landscape in the suburbs of Paris, France. We sampled earthworms in roadside soils from two
23 distinct soil engineering processes: basic engineering (BER) associated with shallow stripping
24 depth and advanced engineering (AER) associated with deep stripping depth and organic
25 amendment. Within each soil engineering process, two levels of soil isolation were defined
26 depending on the asphalted surface separating roadside soils from pseudo-natural soils: light
27 isolation (LI) in case of a narrow cycle path, and high isolation (HI) in case of a wide road.
28 Soil engineering did not affect the total earthworm abundance whereas the total species
29 richness was negatively impacted in comparison to nearby pseudo-natural soils. High soil
30 isolation differently impacted earthworm communities depending on the engineering process.
31 Regarding AER, earthworm abundance and species richness were significantly lower in HI
32 than in LI while no differences were observed between BER-LI and BER-HI. Overall,
33 engineering processes define the soil's ability to host earthworms, and soil isolation defines
34 soil ability to be colonized from nearby environments. Considering the contribution of
35 earthworms to the provision of ecosystem services, both soil engineering and soil isolation
36 should be taken into account in urban projects to optimize their development in urban
37 landscapes.

38

39 **Keywords: urban soils, pedological engineering, connectivity, ecological continuity,**
40 **brown network**

41

42 **1. Introduction**

43 In response to global changes, urban landscapes integrate climate-resilient engineered
44 soils (e.g., parks, roundabouts, roadsides), especially to balance water flows and provide
45 thermal comfort through vegetation (Demuzere et al., 2014; Kim et al., 2016). Many
46 ecosystem services such as water regulation or support for plant growth are mainly provided
47 by urban soils depending on their physical, chemical, and biological properties (Dominati et
48 al., 2010). Engineered soil properties result from processes which include, among others, soil
49 stripping, transportation, and implementation in one or several layers (Marié and Rossignol,
50 1997; Vidal-Beaudet and Rossignol, 2018). Therefore, the construction of engineered soils in
51 the urban landscape is a key element to maintain functional soils and address urban issues
52 (Morel et al., 2015; Blanchart et al., 2018; Calzolari et al., 2020). If the physical and chemical
53 components of engineered soils have been studied (Damas and Coulon, 2016; Séré et al.,
54 2017), the biological component, despite being a key player in soil processes (Balsler and
55 Firestone, 2005; Ernst et al., 2009), remains largely unexplored.

56 Among soil biological actors, earthworms are widespread in temperate climates and are
57 involved in numerous ecosystem services (Lavelle et al., 2006; Blouin et al., 2013; Bertrand
58 et al., 2015), acting in particular on water regulation (Ernst et al., 2009; Pelíšek, 2018) and
59 plant growth (Scheu, 2003; van Groenigen et al., 2014). They are usually classified into four
60 main categories (Bouché, 1972, 1977; Jégou et al., 1998): epigeic earthworms live and feed
61 on organic matter of the soil surface, endogeic earthworms create horizontal burrows and feed
62 on soil organic matter, epi-anecic earthworms rather feed on fresh surface organic matter

63 whereas strict-anecic earthworms preferentially feed on aged organic matter already
64 incorporated into the soil (Bouché, 1977; Jégou et al., 1998). Earthworm communities of
65 urban soils are poorly described and the few studies available highlighted the effects of soil
66 physicochemical properties (Amossé et al., 2016; Audusseau et al., 2020), land-uses (Li et al.,
67 2020; Tóth et al., 2020), and land management (Smetak et al., 2007; Vergnes et al., 2017). For
68 example, Smetak et al. (2007) observed that additions of nitrogenous fertilizer in urban parks
69 were associated with an increase of an anecic species abundance. However, to our knowledge,
70 there is no study on the effect of engineering processes on earthworm communities in urban
71 landscapes.

72 Several studies have been undertaken at a large scale to evaluate the impact of landscape
73 diversity on earthworm communities (Joschko et al., 2006; Frazão et al., 2017; Hoeffner et al.,
74 2021). For example, Vanbergen et al. (2007) highlighted a positive correlation between
75 earthworm diversity and habitat richness at landscape-scale (1 km²); between earthworm
76 abundance and plant species richness at a local scale (200 m²). In the urban context, Xie et al.
77 (2018) observed that the ratio of asphalted surface and green space surface within different
78 buffers from 200 m to 1 km was negatively correlated with an anecic species. However, the
79 majority of the previous studies did not report the impact of landscape on earthworm
80 communities at a very fine scale (Vanbergen et al., 2007; Lüscher et al., 2014; Frazao et al.,
81 2017; Xie et al., 2018). In particular, the effect of asphalted surfaces such as roads or cycle
82 paths surrounding roadside soils on earthworm communities remains unclear. Tiho and Josens
83 (2000) highlighted similar earthworm communities between soils surrounded by two roads
84 and soils of local urban parks, whereas Dupont et al. (2017) reported that for two endogeic
85 species roads could constitute barriers to earthworm dispersal at a regional scale (~130 km²).
86 Thus, earthworm communities of roadside soils and the effect of surrounding landscapes at a
87 very fine scale are still to be explored.

88 The first objective of the present study was to evaluate the effects of soil engineering
89 processes applied to construct roadside soils on earthworm communities (total abundance,
90 total species richness, evenness index, and abundance within each earthworm ecological
91 category). We hypothesized that the more steps the engineering process includes, the more
92 degraded earthworm communities are likely to be. The second objective was to evaluate the
93 effects of soil isolation by asphalted surfaces on earthworm communities. We hypothesized
94 that earthworm communities in highly isolated roadside soils (i.e., separated from pseudo-
95 natural soils by a wide road) are likely to be more degraded than earthworm communities in
96 lightly isolated roadside soils (i.e., separated from pseudo-natural soils by a narrow cycle
97 path). We conducted a study in an urban landscape of Paris' suburbs, France. Earthworms
98 were sampled in pseudo-natural grassland soils (controls) and along linear roadside soils from
99 two different soil engineering processes: basic engineering involving shallow soil stripping
100 and the addition of a single unmodified soil layer; advanced engineering involving deep soil
101 stripping, the addition of two soil layers and an organic amendment in the surface soil layer.
102 Within each engineering process, we studied soil isolation by comparing lightly and highly
103 isolated roadside soils.

104

105 **2. Materials and methods**

106 **2.1. Study sites and sampling design**

107 The study area is located around the city of Paris (France), in the commune of
108 Guyancourt (48° 76' 18'' N; 2° 07' 23'' E) which belongs to the urban community of Saint-
109 Quentin-en-Yvelines, built as a "new city" in the 1970s to avoid urban concentration in Paris.
110 The city expands on large agricultural parcels where the substrate is characterized by fine
111 loess deposits more than 3 metres deep (Mégnyen, 1989). Guyancourt is structured by three
112 main avenues: two from north-east to south-west, and one from west to east. The climate is

113 temperate, with a mean annual precipitation of 563 mm and a mean annual temperature of
114 11.2°C, a monthly minimum of -2.3°C and a monthly maximum of 26.4 °C (data from Météo
115 France, 2008-2018 Trappes station).

116 Two linear roadside soils made by following distinct engineering processes were
117 selected: (i) basic soil engineering process (Léon Blum Avenue) which is commonly used in
118 the urban context and (ii) advanced soil engineering process (Europe Avenue) which is rarely
119 used in the urban context. These roadside soils were selected for the detailed historical
120 knowledge available (Table S1), their proximity (less than 2 km apart), their great age (30 and
121 20 years old respectively), and the presence of adjacent pseudo-natural grassland soils. Basic
122 engineered roadside soils (BER) were implemented at the end of the 1980s. The existing soil
123 was stripped to a depth of 30 cm to reach the desired road level. The topsoil was reconstituted
124 with an addition of 20 to 30 cm of organo-mineral material (silty topsoil). Advanced
125 engineered roadside soils (AER) were implemented at the end of the 1990s. The existing soil
126 was stripped to a depth of 150 cm. The soil was reconstituted from bottom to top with 125 cm
127 of organo-mineral material (silty topsoil), 25 cm of a mixture of organo-mineral material and
128 compost from green waste, and finally a thin layer of pine bark mulch (Table S1). In both
129 cases, the organo-mineral material came from the perimeter of the urban project and
130 corresponds to topsoil stripped down to 30 cm, stored in a windrow, and then transported to
131 the roadside location. Six soil profile descriptions per engineering process (BER and AER)
132 were performed in 110 cm deep pedological pits (NF EN ISO 25177; AFNOR, 2019). Soils
133 were identified according to the IUSS Working Group (WRB, 2015). In addition, for each
134 roadside soil, nearby pseudo-natural grasslands soils (BER-PNG and AER-PNG, respectively)
135 left untouched by urban engineering at least for 30 and 20 years old respectively were
136 selected.

137 Within each of the two types of engineered roadside soils, two levels of isolation from
138 adjacent pseudo-natural grassland soils were selected: (i) lightly isolated roadside soils (BER-
139 LI and AER-LI, respectively) between a 6 m wide asphalt road with a 20 cm high curb and a
140 3 m wide asphalt cycle path with no curb; (ii) highly isolated roadside soils (BER-HI and
141 AER-HI, respectively) between two asphalt roads, each 6 m wide with a 20 cm high curb
142 (Fig. S1). Details concerning land use and management of each group are shown in Table S1.
143 Earthworms and soil were sampled in order to cover all of the selected avenue portions. Each
144 replicate was separated from 60 m and the number of replicates for each group varied
145 depending on the avenue's layouts (i.e., intersections) and length. Thus, 6 groups were
146 studied: (i) BER-PNG (n=10), (ii) BER-LI (n=10), (iii) BER-HI (n=5), (iv) AER-PNG (n=7),
147 (v) AER-LI (n=7), and (vi) AER-HI (n=7).

148

149 **2.2. Earthworm sampling and laboratory analyses**

150 Each earthworm sampling consisted of extracting 6 blocks of soil (20 cm × 20 cm × 25
151 cm, length × width × depth) and hand-sorting to collect earthworms. Sampling of soil blocks
152 was undertaken at a distance of 2 m from a tree and equidistant from the edge of the tree
153 trench. Earthworms were stored in ethanol (96% solution). In the laboratory, earthworms were
154 counted, assigned to a stage of development (juvenile, sub-adult and adult), identified at the
155 sub-species level and assigned to its ecological categories: epigeic, epi-anecic (genus
156 *Lumbricus*), anecic-strict (genus *Aporrectodea*) and endogeic (Bouché, 1972, 1977; Jégou et
157 al., 1998; Hoeffner et al., 2019). For juveniles that could not be determined at the sub-species,
158 they were first assigned to a genus and an ecological category, then distributed in proportion
159 of sub-adults and adults identified at the sub-species level in each group. The number of hand-
160 sorted earthworms was multiplied by 25 to obtain an estimation per square metre. Earthworm
161 diversity was analysed by calculating the total species richness, and the evenness index. The

162 evenness index (J') was calculated as follows: $J' = \frac{H'}{H'_{max}}$, where H' is the Shannon diversity
163 index. Abundance was also analysed at the scale of ecological categories.

164

165 **2.3. Soil sampling and physicochemical analyses**

166 Soil samples were taken for analysis from the soil where earthworms had been
167 collected. Six sub-samples of soil (between 0 and 25 cm) were pooled and homogenized to
168 consider one composite sample per site. Composites were analysed by the analytical
169 laboratory AUREA AgroSciences (Orléans, France) for particle-size distribution (clay $<2 \mu\text{m}$;
170 silt $2 < \mu\text{m} < 50$; sand $50 < \mu\text{m} < 200$, %; NFX 31-107 without decarbonation; AFNOR, 2003),
171 soil organic matter (SOM; NF ISO 14235; AFNOR, 1998) and pH (water; NF ISO 10390;
172 AFNOR, 2005).

173

174 **2.4. Statistical analyses**

175 Wilcoxon tests were applied to compare total abundance, total species richness, evenness
176 index, and abundance of ecological categories between BER-PNG and BER, and between
177 AER-PNG and AER. Two-ways ANOVAs for parametric data were applied to test the factors
178 “soil engineering”, “soil isolation”, and their interaction on total abundance, total species
179 richness, and evenness index between BER-LI, BER-HI, AER-LI, and AER-HI. We used a
180 type III sum of squares ANOVA because the sampling design was unbalanced. In case of
181 significant interaction, one-way ANOVA, and pairwise multiple comparisons were performed
182 to highlight the significant differences between mean variables of BER-LI, BER-HI, AER-LI,
183 and AER-HI. Given the large contrasts of abundance between groups at the scale of
184 ecological categories, Kruskal-Wallis tests for non-parametric data were applied, followed by
185 Dunn tests for post-hoc pairwise comparisons to identify the significant differences between
186 BER-LI, BER-HI, AER-LI, and AER-HI.

187 The species composition of earthworm communities among BER-PNG, BER-LI, BER-HI,
188 AER-PNG, AER-LI, and AER-HI was compared thanks to a data matrix of pairwise
189 comparisons among samples calculated using the Bray-Curtis distance index. Non-Metric
190 multidimensional Scaling (NMDS) was then used to find the best low-dimensional
191 representation of the distance matrix. The null hypothesis of no difference in patterns of
192 earthworm community structure was tested with a permutational multivariate analysis of
193 variance (PERMANOVA). PERMANOVAs were run on the Bray-Curtis distance with 1000
194 permutations per analysis.

195 All statistical analyses were performed on R software (R Core Team, 2019). Significance
196 was evaluated in all cases at $P < 0.05$.

197

198 **3. Results**

199 **3.1. Physicochemical characteristics of soils**

200 BER-PNG and AER-PNG were identified as Haplic Luvisols, with a silt content of $63 \% \pm 3.5$ (\pm SD), a pH of 7.8 ± 0.3 and an organic matter content of $1.8 \% \pm 0.4$. BER and AER
201 were similar in pH (7.4 ± 0.6 and 7.2 ± 0.2 , respectively) and soil texture, with a dominant
202 percentage of silt ($71 \% \pm 4.0$ and $67 \% \pm 2.4$ respectively), while the percentage of organic
203 matter was higher AER in than in BER ($6.4 \% \pm 1.0$ and $1.8 \% \pm 0.3$, respectively) (Table
204 S2). BER were identified as Terric Luvisols (Transportic, Siltic) and AER were identified as
205 Horticultural Anthrosols (Organotransportic, Siltic) (Table S1).

206

207 **3.2. Earthworm communities of the study area**

208 Over the whole study area, the mean earthworm abundance and species richness were 369
209 ± 190 individuals m^{-2} and 5.6 ± 1.8 species, respectively (Table 1, Table S3). Fourteen species
210 were observed (Table S4): three epigeic (dominated by *Lumbricus castaneus*), two epi-anecic
211

212 (*Lumbricus rubellus rubellus* and *Lumbricus terrestris*), two strict-aneic (*Aporrectodea*
213 *giardi* and *Aporrectodea longa longa*), and seven endogeic earthworms (dominated by
214 *Aporrectodea icterica*).

215 Regarding both pseudo-natural grassland soils, earthworm abundance was 377 ± 150
216 individuals m^{-2} and 485 ± 163 individuals m^{-2} in BER-PNG and AER-PNG respectively, and
217 was not different to their respective engineered roadside soils ($W = 59.0$ and 29.0
218 respectively, $P > 0.05$; Table S3). Earthworm species richness was significantly higher in both
219 pseudo-natural grassland soils compared to their respective engineered roadside soils (at least
220 +34 %; $W = 6.0$ and 12.5 respectively, $P < 0.05$; Table S3), especially concerning epi-aneic
221 species in BER and strict-aneic species in AER. In addition, evenness index was
222 significantly higher in BER-PNG than in BER (+14 %, $W = 31.0$, $P = 0.016$; Table S3) while
223 no difference was observed between AER-PNG and AER ($W = 36.5$, $P > 0.05$; Table S3).
224 Among ecological categories, epi-aneic abundance was significantly higher in BER-PNG
225 than in BER (+93 %, $W = 35.0$, $P = 0.006$; Table S3), strict-aneic abundance was
226 significantly higher in AER-PNG than in AER (+72 %, $W = 15.5$, $P = 0.011$; Table S3) while
227 no differences were observed between grassland soils and their respective engineered roadside
228 soils regarding epigeic and endogeic abundance ($W < 39.0$, $P > 0.05$; Table S3).

229 Regarding lightly isolated roadside soils (LI), earthworm species richness and evenness
230 index were significantly lower in BER-LI than in AER-LI (at least +31 %, $F = 24.4$ and 10.3 ,
231 respectively, $P < 0.004$) while earthworm abundance was not affected by the engineering
232 process (Table 1). Among ecological categories, epigeic and epi-aneic abundances were
233 significantly lower in BER-LI than in AER-LI (at least +88 %, $K = 12.9$ and 15.8 ,
234 respectively, $P < 0.005$; Table 2) while strict-aneic and endogeic abundance were not
235 affected by the engineering process. In addition, earthworm communities were different in
236 BER-LI and AER-LI (Fig. 1). Regarding highly isolated roadside soils (HI), strict-aneic

237 abundance was significantly higher in BER-HI than in AER-HI (+100 %, $K = 15.1$, $P =$
238 0.002; Table 2) while earthworm abundance, species richness, evenness index, as well as
239 epigeic, epi-aneic and endogeic abundance were similar between BER-HI and AER-HI
240 (Table 1, Table 2).

241 In basic engineered roadside soils (BER) no differences were observed in both BER-LI or
242 BER-HI regarding earthworm abundance, species richness, evenness index, structure of
243 communities, as well as epigeic, epi-aneic, strict-aneic and endogeic abundance (Table 1,
244 Table 2, Fig.1). Regarding advanced engineered roadside soils (AER), earthworm abundance
245 and species richness were significantly higher in AER-LI than in AER-HI (at least +54 %, $F =$
246 10.8 and 24.4 respectively, $P < 0.003$) while evenness index was not affected by soil isolation
247 (Table 1). Among ecological categories, epi-aneic and endogeic abundance were
248 significantly higher in AER-LI than in AER-HI (+at least 64 %, $K = 11.6$ and 15.8 , $P <$
249 0.009). In addition, earthworm communities were different between AER-LI and AER-HI
250 (Fig. 1) in particular due to the absence of epigeic and strict-aneic earthworms in AER-HI
251 (Table 1).

252

253 **4. Discussion**

254 In the present study, mean earthworm abundance and species richness ranged from 153 to
255 538 individuals m^{-2} and from 3.1 to 7.7 species. The lowest mean earthworm abundance and
256 species richness observed are close to those observed in previous urban studies (Amossé et
257 al., 2016; Xie et al., 2018), for example Vergnes et al. (2017) found a mean of 93.4
258 individuals m^{-2} and 4.6 species in 20 urban parks of two districts of Paris. The highest mean
259 earthworm abundance observed in the present study is higher than those observed in
260 grasslands where earthworms are generally the most abundant in temperate climates (Decaëns
261 et al., 2008), while the highest mean earthworm species richness observed is lower than those

262 observed in grasslands. For examples, Didden (2001) found a mean of 384 individuals m⁻² and
263 10 species in 20 grasslands in the Netherlands, and Cluzeau et al. (2012) found a mean of 350
264 individuals m⁻² and 9.6 species among 47 grasslands in Brittany. These results underline the
265 high variability of earthworm communities within a single city as observed in previous studies
266 (Smetak et al., 2007; Xie et al., 2018). Indeed, urban landscapes are strongly influenced by
267 numerous anthropogenic factors (e.g., historical land-use, local soil management, urban
268 shapes) which differently affect earthworm communities at multiple spatial urban scales
269 (Swan et al., 2011; Aronson et al., 2016) such as city, district or neighbourhood. Thus,
270 earthworm communities of urban soils can be highly degraded as well as preserved,
271 modifying their level of contribution to ecosystem services (Lavelle et al., 2006; Blouin et al.,
272 2013).

273 Soil engineering did not affect the total earthworm abundance, as compared to their
274 respective pseudo-natural grassland soils, earthworm abundance was similar in basic and
275 advanced engineered roadsides soils. Thus, despite soil disturbances caused by engineering
276 processes (e.g., soil stripping, soil transport) and regardless of engineering intensity (e.g.,
277 stripping depth, soil addition), earthworm abundance reached comparable levels to pseudo-
278 natural soils after 20 and 30 years. This similar earthworm abundance between engineered
279 roadside soils and nearby pseudo-natural grassland soils can be explained by the absence of
280 soil disturbance in recent decades such as pedestrian activity or soil manipulation, which
281 favours earthworm development (Pižl and Schlaghamerský, 2007; Peigné et al., 2009).
282 Besides, the presence of a permanent cover in both engineered roadsides soils could also be a
283 favourable factor for earthworm development as it provides food resources and increases
284 protection against climatic hazards (Pélosi et al., 2009; Schreck et al., 2012). However, soil
285 engineering negatively affected total earthworm species richness which reinforces a previous
286 study that showed in urban landscapes that earthworm species richness declines due to soil

287 disturbance (i.e., soil engineering) and the loss of habitats due to urbanisation (Xie et al.,
288 2018). Given this erosion of earthworm diversity, engineering processes could include
289 population transfers from nearby pseudo-natural soils to engineered soils thanks to inoculation
290 techniques that would compensate for losses due to constructions (Butt et al., 1997; Butt,
291 1999).

292 Soil isolation differently impacted earthworm communities in both engineered roadside
293 soils. Regarding advanced engineered roadside soils, earthworm abundance was the highest in
294 lightly isolated roadsides among all the roadside soils considered. This result highlights a
295 particularly suitable environment for earthworm communities in advanced engineered
296 roadside soils. Although the entire soil profile has been reconstituted to a depth of 150 cm, the
297 use of topsoil and the organic amendment in the surface soil layer, highlighted by a high
298 percentage of organic matter, allowed a high level of earthworm development by providing
299 abundant trophic resources (Lapied et al., 2009; Ashwood et al., 2017). High levels of organic
300 matter in advanced engineered soils could explain the high abundance of the geophagous
301 endogeic earthworm *Aporrectodea caliginosa caliginosa*, which was almost two times more
302 abundant in lightly isolated soils than in nearby pseudo-natural soils (Bouché, 1972; Doube et
303 al., 1997). Earthworm abundance and species richness were in contrast the lowest in highly
304 isolated advanced roadside soils which shows, despite highly suitable habitats provided by the
305 advanced engineering process, that roads have a negative effect on earthworm communities.
306 This result could be explained by high mortality rates caused by cars and drought when
307 earthworms remain on asphalt surfaces, added to their probable difficulty to climb the 20 cm-
308 high curbs to access the soils. Besides, highly isolated advanced roadside soils are surrounded
309 by roads on both sides and are elevated from them which can limit water runoff and soil
310 moisture, considered as an essential factor to earthworm survival (Lee, 1985). Thus, resulting
311 isolated earthworm communities were almost exclusively composed of endogeic species that

312 are particularly tolerant to soil disturbances (Rosas-Medina et al., 2010; Briones and Schmidt,
313 2017). Overall, the absence of two ecological categories (epigeic and anecic earthworms) can
314 negatively affect the development of other earthworm species in terms of trophic relations, as
315 shown by Keith et al. (2018) who highlighted for example that the surface foraging activity of
316 anecics was beneficial for a soil-feeding endogeic species. More globally, the lack of several
317 earthworm ecological categories may be very damaging for the maintenance of soil functions
318 in which they are involved such as organic matter decomposition (especially epigeic species)
319 or water infiltration (especially anecic species) (Bastardie et al., 2003; Sharma et al., 2017).
320 Regarding soil from basic engineering processes, no differences were observed between
321 communities of lightly and highly isolated roadside soils, and both were very similar to
322 nearby pseudo-natural grassland soils. Similar observations were made in Brussels by Tiho
323 and Josens (2000), who observed similar earthworm communities between highly isolated
324 roadside soils of Roosevelt Avenue and 19 pseudo-natural soils of local urban parks.
325 Considering that basic roadside soils were stripped at shallow depth, it can be assumed that, as
326 suggested by Tiho and Josens (2000), earthworm communities could have developed from a
327 relict of the earthworm communities present before the road construction and have been
328 preserved under the modified layer of the soil. Thus, the issue of soil isolation should be taken
329 into account in urban projects, in particular if initial habitats and pool of species are likely to
330 be highly impacted along the construction process.

331

332 **5. Conclusion**

333 This study highlights the combined effects of soil engineering and soil isolation on
334 earthworm communities in urban landscapes. Soil engineering did not modify earthworm
335 abundance 20 or 30 years after roadside construction while earthworm species richness was
336 negatively impacted. Earthworm communities of highly isolated advanced roadside soils (i.e.,

337 surrounded by roads) were strongly negatively impacted while soil isolation did not impact
338 basic engineered soils. In the context of growing urbanisation and landscape fragmentation,
339 our results shows that urban soil biodiversity has to be taken into account all along the
340 different steps of urban projects. From the start, soil and biodiversity diagnostics could help to
341 define the quality of the habitat and identify the species already present on site; low impacting
342 engineering processes could limit the degradation of earthworm populations during the
343 project; the preservation of reservoir areas and the maintaining of connections between green
344 spaces could help the colonisation process; adapted soil management (e.g., ensuring sufficient
345 soil moisture) could maintain long-term high-quality habitats for soil biodiversity. To specify
346 and confirm technical recommendations, further studies should be conducted on other urban
347 shapes than more or less isolated roadsides, and different urban scales from the city to the
348 neighbourhood should be explored.

349

350 **6. Acknowledgments**

351 We thank AUREA Agrosiences for the soil physicochemical analysis and Météo France
352 for the meteorological data. We thank Véronique Keignaert from the environment and green
353 spaces department of Saint-Quentin-en-Yvelines for provision of access to Europe and Léon
354 Blum Avenues in Guyancourt. We sincerely thank Howaida Abbas, Nathan Colas, Mylène
355 Gauter, and Karina Gaspar, for their help and support during field sampling and lab work. We
356 also thank Rebecca Dingkuhn for helpful comments and proofreading in English. This
357 research was supported by the “Association Nationale de la Recherche et de la
358 Technologie” (ANRT) through an industrial CIFRE convention between the Sol Paysage
359 Company and the University of Rennes 1 (Number of convention N° 2018/0569,
360 <http://www.anrt.asso.fr/fr/cifre-7843>).

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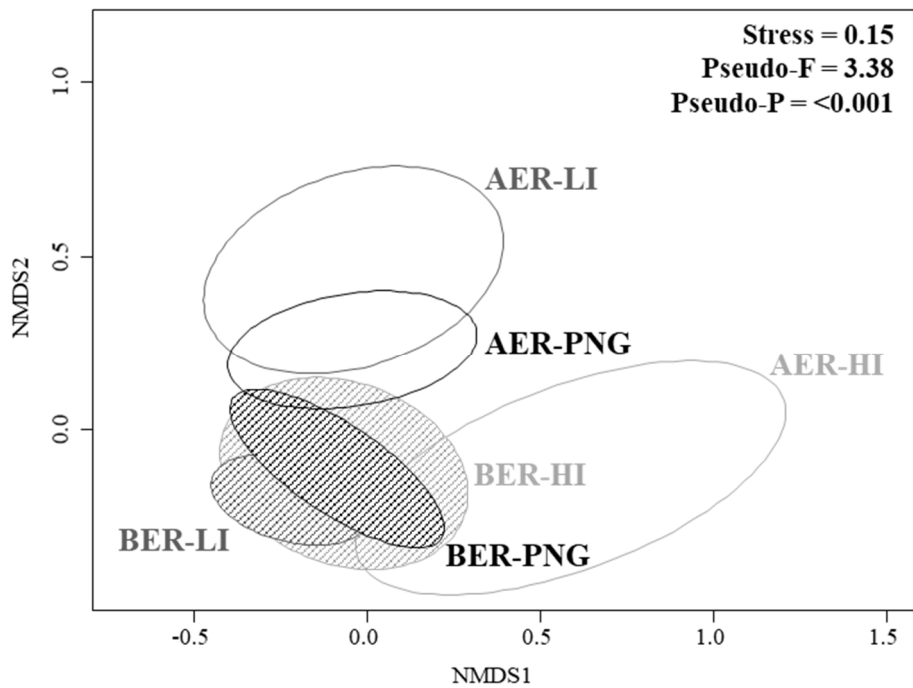
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550 **FIGURES**

551 **Fig. 1** Non-metric multidimensional scaling ordinations showing similarities, based on Bray-
552 Curtis distance index, of earthworm communities of each group. Significant dissimilarities
553 between groups were assessed by PERMANOVA. Pseudo-F values and associated P-values
554 are indicated. Ellipses correspond to groups (BER-PNG = pseudo-natural grassland soils
555 nearby basic engineered roadside soils; BER-LI = lightly isolated basic engineered roadside
556 soils; BER-HI = highly isolated basic engineered roadside soils; AER-PNG = pseudo-natural
557 grassland soils nearby advanced engineered roadside soils; AER-LI = lightly isolated
558 advanced engineered roadside soils; AER-HI = highly isolated advanced engineered roadside



559 soils).

560 **TABLES**

561 **Table 1** Effects of soil engineering and soil isolation on earthworm communities. Values are mean \pm standard deviation. F-values and
 562 P-values from Anova type III were given for total abundance, total species richness, and evenness index (Eng = Engineering; Iso =
 563 Isolation). Different letters denote significant differences regarding the interaction Eng*Iso among groups, with a > b > c (post-hoc
 564 Tukey test results).

	Basic engineered roadside soils (BER)		Advanced engineered roadside soils (AER)		Eng		Iso		Eng * Iso	
	Light isolation (BER-LI, n = 10)	High isolation (BER-HI, n = 5)	Light isolation (AER-LI, n = 7)	High isolation (AER-HI, n = 7)	Anova	p-value	Anova	p-value	Anova	p-value
Total abundance (ind.m ⁻²)	327 \pm 104 ab	340 \pm 181 ab	538 \pm 239 a	153 \pm 92 b	F = 0.0	0.848	F = 9.5	0.005	F = 10.8	0.003
Total species richness	4.6 \pm 0.5 b	4.4 \pm 1.1 bc	6.7 \pm 1.0 a	3.1 \pm 1.1 c	F = 1.6	0.221	F = 30.5	9.6e-06	F = 24.4	4.4e-06
Evenness index	0.5 \pm 0.1 b	0.7 \pm 0.1 ab	0.8 \pm 0.1 a	0.6 \pm 0.2 ab	F = 3.5	0.073	F = 0.3	0.570	F = 10.3	0.004

565

566 **Table 2** Effects of soil engineering and soil isolation on earthworm abundance per ecological categories. Values are mean \pm standard
 567 deviation. K-values and P-values from Kruskal-Wallis test were given for epigeic, epi-anecic, strict-anecic, and endogeic abundance.
 568 Different letters denote significant differences among groups with $a > b$ (post-hoc Dunn test results).

	Basic engineered roadside soils (BER)		Advanced engineered roadside soils (AER)		Kruskal-Wallis	p-value
	Light isolation (BER-LI, n = 10)	High isolation (BER-HI, n = 5)	Light isolation (AER-LI, n = 7)	High isolation (AER-HI, n = 7)		
Epigeic abundance (ind.m ⁻²)	6 \pm 5 b	28 \pm 31 ab	50 \pm 69 a	0 \pm 0 b	K = 12.9	0.005
Epi-anecic abundance (ind.m ⁻²)	1 \pm 3 b	0 \pm 0 b	28 \pm 40 a	2 \pm 2 b	K = 15.8	0.001
Strict-anecic abundance (ind.m ⁻²)	41 \pm 33 a	60 \pm 57 a	38 \pm 37 a	0 \pm 0 b	K = 15.1	0.002
Endogeic abundance (ind.m ⁻²)	280 \pm 96 a	252 \pm 122 ab	422 \pm 157 a	151 \pm 92 b	K = 11.6	0.009

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