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

**Authors:** Jeanne Maréchal<sup>1,2</sup>, Kevin Hoeffner<sup>1</sup>, Xavier Marié<sup>2</sup>, Daniel Cluzeau<sup>1</sup>

**Addresses:**

**1.** University of Rennes, CNRS, ECOBIO [(Ecosystèmes, biodiversité, évolution)] - UMR 6553, Rennes, France

**2.** Sol Paysage company, 8 bis boulevard Dubreuil, F-91400 Orsay, France

**Corresponding author:**

Jeanne Maréchal, [jeanne.marechal@univ-rennes1.fr](mailto:jeanne.marechal@univ-rennes1.fr)

## Abstract

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

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

## **1. Introduction**

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

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

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

Several studies have been undertaken at a large scale to evaluate the impact of landscape diversity on earthworm communities (Joschko et al., 2006; Frazão et al., 2017; Hoeffner et al., 2021). For example, Vanbergen et al. (2007) highlighted a positive correlation between earthworm diversity and habitat richness at landscape-scale (1 km<sup>2</sup>); between earthworm abundance and plant species richness at a local scale (200 m<sup>2</sup>). In the urban context, Xie et al. (2018) observed that the ratio of asphalted surface and green space surface within different buffers from 200 m to 1 km was negatively correlated with an anecic species. However, the majority of the previous studies did not report the impact of landscape on earthworm communities at a very fine scale (Vanbergen et al., 2007; Lüscher et al., 2014; Frazao et al., 2017; Xie et al., 2018). In particular, the effect of asphalted surfaces such as roads or cycle paths surrounding roadside soils on earthworm communities remains unclear. Tiho and Josens (2000) highlighted similar earthworm communities between soils surrounded by two roads and soils of local urban parks, whereas Dupont et al. (2017) reported that for two endogeic species roads could constitute barriers to earthworm dispersal at a regional scale (~130 km<sup>2</sup>). Thus, earthworm communities of roadside soils and the effect of surrounding landscapes at a very fine scale are still to be explored.

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

## **2. Materials and methods**

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

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

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

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

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

## 2.2. Earthworm sampling and laboratory analyses

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



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

### 2.3. Soil sampling and physicochemical analyses

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

### 2.4. Statistical analyses

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

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

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

### **3. Results**

#### **3.1. Physicochemical characteristics of soils**

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

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

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

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

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

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

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

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

#### 4. Discussion

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

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

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

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

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

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

## **5. Conclusion**

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

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

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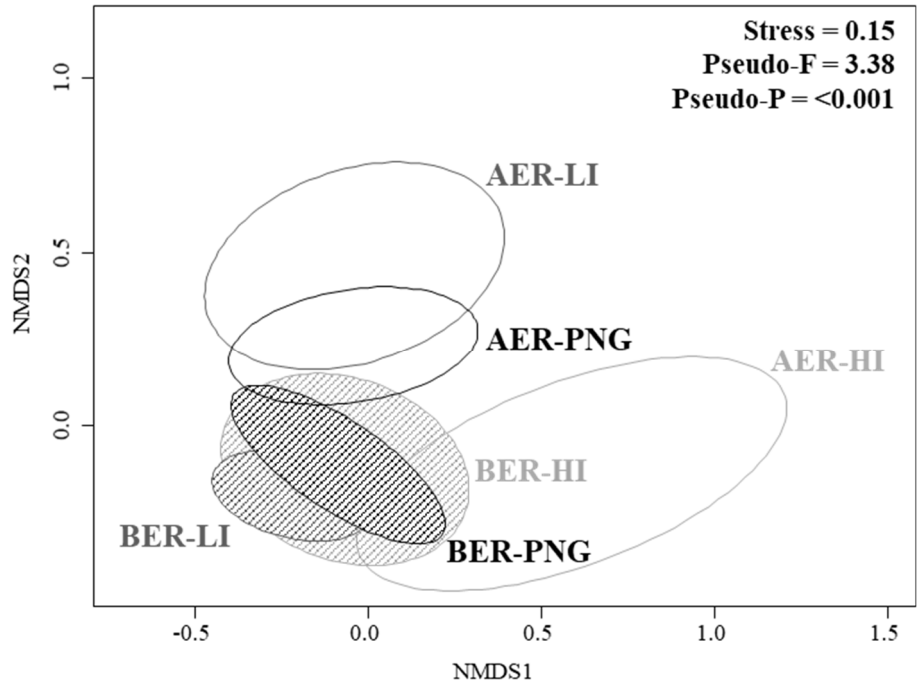
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# **FIGURES**

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



soils).

**TABLES**

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

	Basic engineered roadside soils (BER)		Advanced engineered roadside soils (AER)		Eng Anova   p-value		Iso Anova   p-value		Eng * Iso Anova   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)						
Total abundance (ind.m <sup>-2</sup> )	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

**Table 2** Effects of soil engineering and soil isolation on earthworm abundance per ecological categories. Values are mean  $\pm$  standard deviation. K-values and P-values from Kruskal-Wallis test were given for epigeic, epi-anecic, strict-anecic, and endogeic abundance. 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 <sup>-2</sup> )	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 <sup>-2</sup> )	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 <sup>-2</sup> )	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 <sup>-2</sup> )	280 $\pm$ 96 a	252 $\pm$ 122 ab	422 $\pm$ 157 a	151 $\pm$ 92 b	K = 11.6	0.009