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Using the ecosystem engineer concept to test the functional effects of a decrease in earthworm abundance due to an historic metal pollution gradient

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Abstract

In a companion study, carried out in a fallow meadow close to a lead recycling factory, we showed that earthworms were absent in the first 20 m and then gradually increased in abundance from 30 to 110 m from the factory. Here we assessed in the same meadow whether these differences in earthworm abundance were associated with the loss of physical soil properties. Soil cores were sampled and infiltration measured in situ at five distances from the factory (10, 30, 50, 80 and 110 m). X-ray tomography was used to characterize the earthworm burrow systems within cores. The burrow systems were minimal at the first two distances, with the only macropores observed probably produced by insects such as ants. Typical earthworm burrows were seen at 50 m but most of their characteristics (volume, diameter, continuity) were similar to those observed at 10 or 30 m. Dense and well-developed burrow systems were observed at 80 and 110 m from the factory with significantly larger volume and continuity. Burrow diameter at 80 m was significantly higher than at closer distances but it significantly decreased at 110 m associated with the higher abundance of endogeics earthworm species. Water infiltration followed the same trend with significantly lower rates at the first two distances compared to those further from the factory, where rates increased from 70 to 250%. This study emphasizes the need to develop a more functional approach to study the spatial effects of contamination on soil ecological processes and fertility, beyond modifications in earthworm communities.

Keywords: Burrow ; Macroporosity ; Water infiltration ; Lead

1 Introduction

Earthworms are generally considered soil « ecosystem engineers » (sensu Jones et al., 1994). This means that, through their different activities (organic matter burial, burrow creation, cast deposition), these organisms create favourable habitats and modified conditions of resource availability for many other soil inhabitants, from micro-organisms to plant roots (Jouquet et al., 2014; Liu et al., 2019). For example, as recently reviewed by Medina-Sauza et al. (2019) micro-organisms are more diverse and active close to the burrow wall or fresh casts. Moreover, plant roots are often observed within earthworm burrows presumably to save energy (Passioura, 2002). However, the concept of ecosystem engineer is now called upon in soil biology as an easy way to justify the relevance of studies about earthworm communities. Yet quantitative empirical evidence of the importance of earthworms for selected soil functions remain incredibly scarce, especially under field conditions. For example, Blouin et al. (2013) reviewed the impacts of earthworms on soil functions and ecosystem services but only cited two quite old studies which suggested that earthworm abundance in some field studies was correlated with increased water infiltration (Ehlers et al., 1975; Bouché and Al-Addan, 1997).

Earthworms are often chosen as model organisms for studying the biological effects of soil pollution by metals or pesticides (Pelosi et al., 2014). However, in most studies, these effects were only assessed at the community level without addressing the consequences on soil functioning. There are however a few exceptions carried out under field conditions (Creamer et al., 2008; van Gestel et al., 2009; Naveed et al., 2014) or in laboratory experiments (Zorn et al., 2005; Capowiez and Bérard, 2006; Prado et al., 2016; Mombo et al., 2018; Wang et al., 2019). In these experiments, pollutants were reported to negatively impact earthworm communities and/or their main functions in soil ecosystems (soil macroporosity or organic matter burial). Among them, studies that focused on soil macroporosity showed a relatively good agreement between macrofauna abundance and soil macroporosity. However, in the absence of a strong disturbance such as contamination, this relationship was not always observed in other ecological studies that tried to correlate earthworm abundance to the density of burrows in croplands (Pérès et al., 1998; Lamandé et al., 2011; van Schaik et al., 2014; Pelosi et al., 2017), at the colonization front (Capowiez et al., 2000) or at the landscape scale (Schneider et al., 2018).

Indeed, soil porosity is important since soil structure greatly influences or even controls most soil functions as reviewed by Rabot et al. (2018). According to these authors, macroporosity is one the most relevant indicators of soil structure. Although macroporosity does not always have the same definition in soil physics and soil biology (Bottinelli et al., 2015), the macropores that earthworms or other soil inhabitants create strongly influence water infiltration through preferential flow (Badorreck et al., 2012; van Schaik et al., 2014), which in turn is also thought to increase water storage in soil and limit soil erosion. Different methods are available to directly or indirectly assess soil structure (Rabot et al., 2018) and with regards to earthworm burrow systems, X-ray tomography using medical scanners is a common approach due to its accuracy and availability in hospitals or research institutes (Pierret et al., 2002; Amossé et al., 2015; Bottinelli et al., 2017). Overall, experiments carried out under laboratory conditions (Capowiez et al., 2015; Bottinelli et al., 2017), semi-field (Capowiez et al., 2009a; Andriuzzi et al., 2015; Schon et al., 2017) or field conditions (Capowiez et al., 2012) demonstrated that when earthworm abundance increased, water infiltration did the same, presumably associated with macroporosity. The role of burrows made by anecic earthworms, which are vertical and continuous, is relatively clear but there is less evidence for endogeic burrows (Capowiez et al., 2014b). Among the characteristics of the burrow system that are thought to be important for water infiltration (e.g. burrow length, continuity or connectivity), no consensus has yet emerged mainly due to the scarcity of datasets where all these parameters were accurately and simultaneously measured.

In the present study, we benefited from a previous study (Lévêque et al., 2015) focusing on earthworm abundance and diversity along a sharp gradient of pluri-metallic persistent contamination in a fallow land close to an old lead-recycling factory. We previously showed that earthworm abundance increased from zero close to the factory wall to 150 individuals m^{-2} at 140 m from this wall. We decided to use this gradient to test, under field conditions, the relationships between earthworm communities, soil macroporosity and water infiltration in this meadow. For that, we sampled soil cores and carried out in situ water infiltration tests at four distances from the wall (10, 30, 50, 80 and 110 m). We hypothesized that (i) the volume and length of the earthworm burrow system will gradually increase with earthworm abundance and that (ii) the characteristics of the burrow system will depend on the proportions of anecic and endogeic earthworms in the community.

2. Material and Methods

2.1. The study site

The study site is located in Bazoches-les-Gallérandes (N 48.164554, E 2.042537), 80 km south of Paris, France. The study was carried out in a fallow metallophyte meadow, on a calcic cambisol (clay: 22.8% ; silt 54.6% ; sand : 22.6% ; A horizon 0-25 cm depth), adjoining a lead recycling factory: the Société de Traitements Chimiques des Métaux (STCM) which has been in operation since 1967. This plant treats lead batteries and the recycling process is carried out in several stages (crushing, fusion, reduction and refining), each generating, in addition to Pb, undesirable by-products such as Cu, Zn, As and Sb. Metal and metalloids are emitted from the factory through a chimney (furnace emissions) and diffuse emissions are also produced due to the recycling processes. The prevailing winds blow from the South-West. Thus, the soil of the meadow (located in the North of the factory) was contaminated with a cocktail of metal and metalloids due to atmospheric fallout with, for example, soil lead concentrations ranging from 29600 at 10 m to 480 mg kg^{-1} at 140 m from the factory wall. The French "ICPE" regulation was recently reinforced to not only avoid environmental emissions near habitations, but also to manage persistent historic pollution in order to reduce its impacts on health and the environment.

In spring 2013, we selected five distances (10, 30, 50, 80 and 110 m) based on the findings of the previous study (Lévêque et al., 2015): there were no worms at 10 m; the earthworm abundance increased between 30 and 50 m but remained very low (less than 20 individuals m^{-2}); from 50 to 80 and 110 m the abundance greatly increased up to about 125 individuals m^{-2}. The mean abundance of the anecic and endogeic earthworms at these distances is summarized in Table 1. Key soil parameters (pH, $C_{organic}$, N_{total}) along the gradient can be found in Lévêque et al., 2015.

2.2. Assessment of the burrow system

In spring 2013, for each of the five chosen distances (10, 30, 50, 80 and 110 m from the factory wall), eight soil cores were sampled across the transects of the previous study. Soil cores were manually taken using a PVC tube (16 cm in diameter and 20 cm in length). An undisturbed soil zone was selected and the PVC tube was placed vertically on the soil. The soil around the tube was carefully excavated up to a width of 15 cm and up to a depth of a few cm using a small hand-spade. Then the soil just and only below the PVC tube was removed using a knife vertically cm by cm. After each cm, the tube was gently inserted in the soil using a hammer on top of the PVC tube. The process continued until the depth of the soil core within the PVC was 15 cm. It took at least 30 min to sample one soil core. The soil below the core was horizontally cut with a knife and the core was put in a plastic bag. The macrofauna inside each core was killed to prevent further burrowing by pouring 3 mL of chloroform on top of each core and closing the bags.

The macroporosity within the soil cores was analysed by X-ray tomography using a medical scanner (BrightSpeed Exel 4, General Electric) at the INRAE Nancy research centre. The scanner settings were 50 kV and 130 mA and 1.25 mm between images. The final image resolution was 0.38 mm per pixel. The images were first transformed into 8-bit images (using the following settings −1000 and 2000 HU as minimum and maximum greylevel values, respectively) and then binarized using a fixed threshold (185) since the separation between peaks (void and soil matrix) in the greylevel histograms was obvious.

The macroporosity was characterised using the following parameters: the volume as the sum of the volumes of the burrows (excluding those smaller than 0.5 cm^3); the number of burrows; the percentage of macroporosity in four soil layers (0-5, 5-10, 10-15 and 15-20 cm depth); the estimated diameter computed as the equivalent circular diameter for only the most circular 2D pores (i.e. whose circularity, computed in ImageJ, was higher than 0.8); the vertical continuity assessed by counting the number of burrows whose vertical extension was larger than 30% of the length of the soil cores (i.e. 6 cm).

2.3. Water infiltration

Water infiltration was assessed in situ in the fallow meadow at 10 locations (separated by at least 5 m from each other) for each distance to the factory wall. These locations were positioned on the same line (i.e. distance from the factory wall) but not at the same location where soil cores were sampled. For each point, a 30 cm (diameter) PVC ring was inserted into the soil for 1 or 2 cm. The vegetation was cut within the ring. Then known volumes of water (corresponding to a height of 1 cm) were poured within the ring and the time before complete absorption into the soil was noted. As soon as the water disappeared a new volume was added until steady state conditions were reached (i.e. the same time between volumes which occurred after about 8 to 10 volumes under our conditions). The resulting water infiltration rates were computed as the coefficient of linear regression for the last 6 to 5 points (when steady state was reached ; see Fig.1 in Capowiez et al., 2015). Since the cores were not sampled at the same location where water infiltration was assessed, we did not try to correlate water infiltration rate to burrow system characteristics. These correlations with only five points (i.e. distances) would have been of limited interest.

2.4. Statistical analysis

To compare the different parameters (relative to macroporosity or infiltration) between distances to the factory wall, we used one-way ANOVA followed by a Tukey HSD post-hoc test. First, normality or homoscedasticity were checked using Levene and Bartlett tests respectively and data were log-transformed when necessary (for example for diameter). Continuity, as count data with a lot of zero values, was compared using zero-inflated regression (using 'pscl' package). All computations were carried out using R (R core team, 2019).

3. Results

3.1. Burrow system

In the images provided by the medical scanner, the mean greylevels corresponding to the soil matrix were not significantly different between the core samples taken at varying distances to the factory wall suggesting that there was no significant variation in the soil bulk density.

Visual assessment of the burrow systems showed clear differences in terms of density, continuity, global design and size, between the two first distances (10 and 30 m) and the others (Fig. 1). The burrow systems at 10 and 30 m were less developed in depth and showed relatively intense burrowing in the first cm with burrows that appeared to be mostly horizontally orientated. No obvious differences could be visually observed for the soil cores sampled at the three last distances.

Macroporosity volume increased with distance to the factory wall albeit non-linearly. The volume was low (about 20 cm^3) for 10 and 30 m (Fig. 2). Then it increased a bit but was very variable at 50 m and thus was not significantly different from the two first distances. However, at 80 and 110 m it increased significantly to an average of 50 cm^3 (i.e. an increase of about 250% compared to the volume observed at 10 and 30 m).

The estimated burrow diameter first increased from 10 to 80 m with mean values increasing from 3.3 to 4.4 mm and then decreased at 110 m with a mean value of 3.7 mm (Fig. 3). Only the burrow diameter at 80 m was significantly different from those observed at all the other distances.

The continuity of the burrow systems (i.e. the number of burrows whose vertical extension is larger than 6 cm) was significantly lower at 10, 30 and 50 m (with on average 1.6 burrows) than at 80 and 110 m (with on average 6.3 burrows) as reported in Fig. 4.

Regarding the distribution of burrow volume with depth, the volume of the burrow system was always highest in the first layer. Moreover, the proportion of the burrow system in the four layers became more homogeneous the further the cores were sampled from the factory, with almost equality in the four layers at 110 m (Table 2).

The number of burrows ranged from 80 at 50 m to 280 at 110 m and was significantly higher furthest from the factory (Table 2).

3. 2. Water infiltration

The water infiltration rate was very low at 10 and 30 m from the factory wall, with on average 1 L mn^{-1} or less (Fig. 5). It then significantly increased at 50 m and at 80 m to 1.7 and 2.6 L mn^{-1} respectively, these two values being significantly different from each other. At 110 m the water infiltration rate decreased slightly but was still significantly different from the first two distances but not from 50 and 80 m.

4. Discussion

4.1. Relationships between earthworm communities and soil functions are not straightforward

In a previous study carried out in the spring of 2012 in this meadow, earthworm communities were assessed along five linear transects from 0 to 140 m from the factory wall (Lévêque et al., 2015): no earthworms were found from 0 to 20 m and then the abundance increased to reach about 240 individuals m^{-2} at 140 m from the factory walls. In the present study, the first striking result is that despite an absence of earthworms in the first 20 m from the factory wall, some macropores were found in soil cores sampled at 10 m. This suggest that either other arthropods were active in the soil (e.g. insects) or that these macropores were « ghost burrows » or both. Indeed, due to the relatively high earthworm abundance in most agricultural lands, the role of other burrowing soil macrofauna is often neglected. Is it then not surprising that their role is more often revealed in highly perturbed soils such as metal contaminated soils (Nahmani et al., 1995), at the initial soil development stages (Badorreck et al., 2012) or during the restoration of degraded lands (Yang et al., 2020). Generally, these soil-dwelling insects produce burrows that are open to the surface and not very deep (about a few cm). The intense and superficial burrowing in the soil cores at 10 and 30 m could be due to such insects (Fig. 1). The other important soil ecosystem engineer in temperate climates is the ants which are known to make nests and burrows within the soil. On some 3D reconstructions of soil cores sampled at 10 m form the factory wall, we observed round-shaped structures connected by small vertical burrows (see Fig. 1 first image in the second row). These looked like structures resulting from ant activities (Li et al., 2019). Ghost burrows would mean that earthworms, which are no longer present or active due to soil pollution, made these burrows in the past. Indeed, there is limited information on the rate of burrow disintegration in a meadow where no tillage is applied and, as observed by Potvin and Lilleskov (2017) some burrows can last for more than seven years. Although in that specific case the burrows were inhabited and thus maintained by earthworms.

At 30 and 50 m, the earthworm abundance remained very low with about five and 10 individuals m^{-2} respectively, and almost only anecic adult earthworms were sampled there (Lévêque et al., 2015). However, the earthworm burrow systems observed at 10, 30 and 50 m were very similar. This simply means that with such low abundance and eight soil cores of 0.02 m^2 each, the probability of observing a burrow was thus very low. We only noticed that the variability and the mean values, increased, albeit non-significantly, at 50 compared to 10 or 30 m for all parameters computed on the burrow systems. At 80 m, pronounced and significant changes in the burrow systems were observed. The burrow systems were now well-developed with burrow volume, diameter and continuity. Consequently, water infiltration was also much higher (+190%). The increase in burrow diameter also suggests than the burrows observed at 10 and 30 m were either made by small insects and possibly ants or were degraded and thus possibly old (Le Mer et al., 2021). At 110 m the burrow system was also well-developed with high burrow volume and continuity. However, the burrow diameter decreased and this was linked to the fact that at 110 m, earthworm communities comprised a much greater proportion of endogeic earthworms (their percentage increased from 10 to 45% between 90 and 120 m; Lévêque et al., 2015). The body diameter of endogeic species is generally lower than those of anecics (Bouché, 1972; Lee and Foster, 1991) and this is particularly true in this meadow where the only endogeic species found were *A. caliginosa*, *A. rosea* and *A. chlorotica*. The changes in burrow continuity between 80 and 110 m (a non-significant decrease) could perhaps be due to the greater percentage of endogeics at 110 m since it is known that endogeics refilled their burrows by casts (Capowiez et al., 2014a; Bottinelli et al., 2017).

At a first glance, there should be a direct relationship between earthworm communities (abundance and diversity) and the resulting tubular macroporosity within the soil especially in croplands under temperate climate (in other lands or climates, ants and other insects may play a larger role). The diameter range for macroporosity that can be assessed using a medical scanner normally excludes macropores created by (dead) roots except for some plants with large roots such as alfalfa or other taproots (Koestel and Schlüter, 2019), which were not observed in this meadow. However, the relationship between macropores and earthworm abundance is not actually straightforward mainly due to the following reasons: (i) not all earthworm species burrow continuously and (ii) the rate of burrow disintegration which is influenced by soil texture, soil climate and land use is still poorly known (Ligthart, 1997; Capowiez et al., 2014a). Regarding rate of burrow creation, it is well known that, for example, most if not all endogeic species tend to burrow continuously as soon as the conditions (soil water content and temperature) are within optimal ranges (Roeben et al., 2020). In contrast, epianecic species such as *L. terrestris* first build a main quasi-vertical burrow, a shelter, and then reuse it intensively to forage at the soil surface during the night and to hide at depth during the day (Joyner and Harmon, 1961; Nuutinen and Butt, 2005). The two kinds of resulting burrows are also very different regarding stability in time: as *L. terrestris* intensively re-uses its burrow and crushes some casts along the burrow wall, these burrows are long-lasting especially when inhabited (Potvin and Lilleskov, 2017). In contrast, endogeic burrows are rarely reused and often refilled by casts (Capowiez et al., 2014a) and thought to be more fragile (Lee and Foster, 1991).

When analyzing earthworm burrow systems , even an a priori separation between endogeic and anecic species based on the burrow diameter is complicated since young anecic earthworms, which are often more abundant than adults, may have the same diameter range as older endogeic earthworms (Pérès et al., 1998). Moreover, the separation into three exclusive ecological categories leads to some confusion for species that are in between the three poles defined by Bouché (1977). For example, *A. trapezoides* is sometimes classified as anecic, *L. terrestris is* classified as anecic or epi-anecic depending on the authors and *A. chlorotica is* classified as an endogeic whereas Bouché unambiguously stated that this species was ‘intermediate’, i.e. in between the three poles (Bottinelli et al., 2020). This further adds to our difficulties to really distinguish the species or ecological categories involved in each burrow system. However, despite these complications, some authors reported significant correlations between earthworm communities and macropore quantity under field conditions even though the percentage of variance explained remained low and below 10% (Schneider et al., 2018).

There are also similar difficulties to establish a clear and consistent relationship between soil macroporosity and soil water infiltration. Although the importance of preferential flow is largely recognized (Jarvis et al., 2016), the macropore characteristics which have the most influence and the conditions for which this phenomenon is dominant remains an open question (Nimmo, 2012). Specifically, regarding earthworm burrows and water infiltration, the main problem is that not all macropores contribute equally to water infiltration. This was clearly demonstrated in field experiments using blue tracers (Capowiez et al., 2009b; van Schaik et al., 2014), but also more recently using ‘time-lapse’ X-ray tomography and simulated rain applied to a natural soil core (Sammartino et al., 2015). This last study showed that some macropores contributed a lot (water was detected in them for most of the scanning event, i.e. every 3 min) whereas others contributed far less (water was not often or rarely detected in them).

4.2. Towards a more functional approach of investigating soil contamination effects

Several very recent publications in the area of ecotoxicology described new tools for detecting toxic effects on earthworms using locomotion or burrowing activities as endpoints (Lee et al., 2019; Djerdj et al., 2020; Xu et al., 2020) with the authors suggesting that earthworm activities are crucial for predicting the impacts on soil functioning. This leads to a paradoxical situation because the consequences of the observed effects on earthworms are rarely studied. Thus, ecotoxicology and risk assessment procedures may not really address the “things that matter” in protecting the environment (Forbes and Calow, 2012), i.e., ecosystems services provided by ecosystem engineers. Therefore, we believe it is crucial for field studies to go beyond the simple characterization of earthworm communities and to also measure changes to soil functions traditionally associated with earthworm activities such as soil structure or organic matter burial. Examples of such complete assessments are, however, rare, especially under field conditions. Naveed et al. (2014) described the effects of field Cu contamination on several soil organisms and a set of chemical and physical soil characteristics. They observed that soil porosity (assessed using X-ray tomography) and gas diffusivity (related to soil aeration) decreased with increasing Cu concentration. Other rare examples of functional effects can be found in field studies with emphasis on soil porosity (Eijsackers et al., 2005), cast production (Lal et al., 2001; Norgrove, 2007), and litter decomposition (Creamer et al., 2008; van Gestel et al., 2009). In addition, some studies under laboratory conditions investigated macroporosity or burrows (Nahmani et al., 2005; Dittbrenner et al., 2011; Prado et al., 2016), cast production (Capowiez et al., 2010), rate of litter burial (Hobbelen et al., 2006), gas diffusion (Capowiez and Bérard, 2006) and water infiltration (Zaller et al., 2014). A last example, applicable to forest soils only, is the use of an ‘humus index’ as an indicator of the macrofauna activity in response to industrial pollution (Korkina and Vorobeichik, 2018).

These examples remain scarce, but one important point to keep in mind is that nowadays the necessary tools are readily available and do not require highly specialised skills. To assess the soil macroporosity resulting from earthworm activities, X-ray tomography is a useful tool but image processing and structure quantification remains tricky. Otherwise, simplified estimates are available for field studies and are based on visual assessment and counting or the use of pictures of vertical openings of macropores on vertical planes under field conditions (Capowiez et al., 2009a; Lamandé et al., 2011; van Schaik et al., 2014). For lab experiments using soil cores, the quantification of the burrows along the core walls is also feasible (Capowiez et al., 2014a). The assessment of associated soil functions under field conditions could rely on widely used classical field methods such a litterbags (Lucisine et al., 2015) or the Beerkan method (Bagarello et al., 2017). All these technics are to be used if we want to demonstrate quantitatively the role of earthworms in soil functioning and thus provide further evidence on the ecosystem engineer concept.

Conclusions

If we want to be able to evaluate the impacts of contaminants in soils at the ecosystem level, it is crucial to measure soil functions under field conditions. To achieve this aim, earthworms are an interesting target group as these soil organisms can directly or indirectly influence a wide range of soil processes. This, however, requires better knowledge of the relationships between earthworm activities (burrow creation, cast production and litter decomposition) and soil functions (aeration, water infiltration and biogeochemical cycles). To date, only qualitative relationships are available (Keith and Anderson, 2012). Moreover, these relationships were always based on the use of earthworm ecological categories (i.e epigeics, endogeics, and anecics) as functional groups. However, there is more and more evidence that variation within these ecological categories can sometimes be very high (as observed by Bastardie et al. (2005) for burrowing behaviour) or unexpectedly low or null (as recently observed by van Groenigen et al. (2019) for the fertility of earthworm casts). The findings of the present study suggest that ecotoxicology, by providing atypical situations and sharp gradients, can lead to a better understanding of the functioning of the soil ecosystem. Further, various kinds of polluted soils could be studied in order to highlight the precise ecological and biogeochemical mechanisms involved.

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Captions for Figures

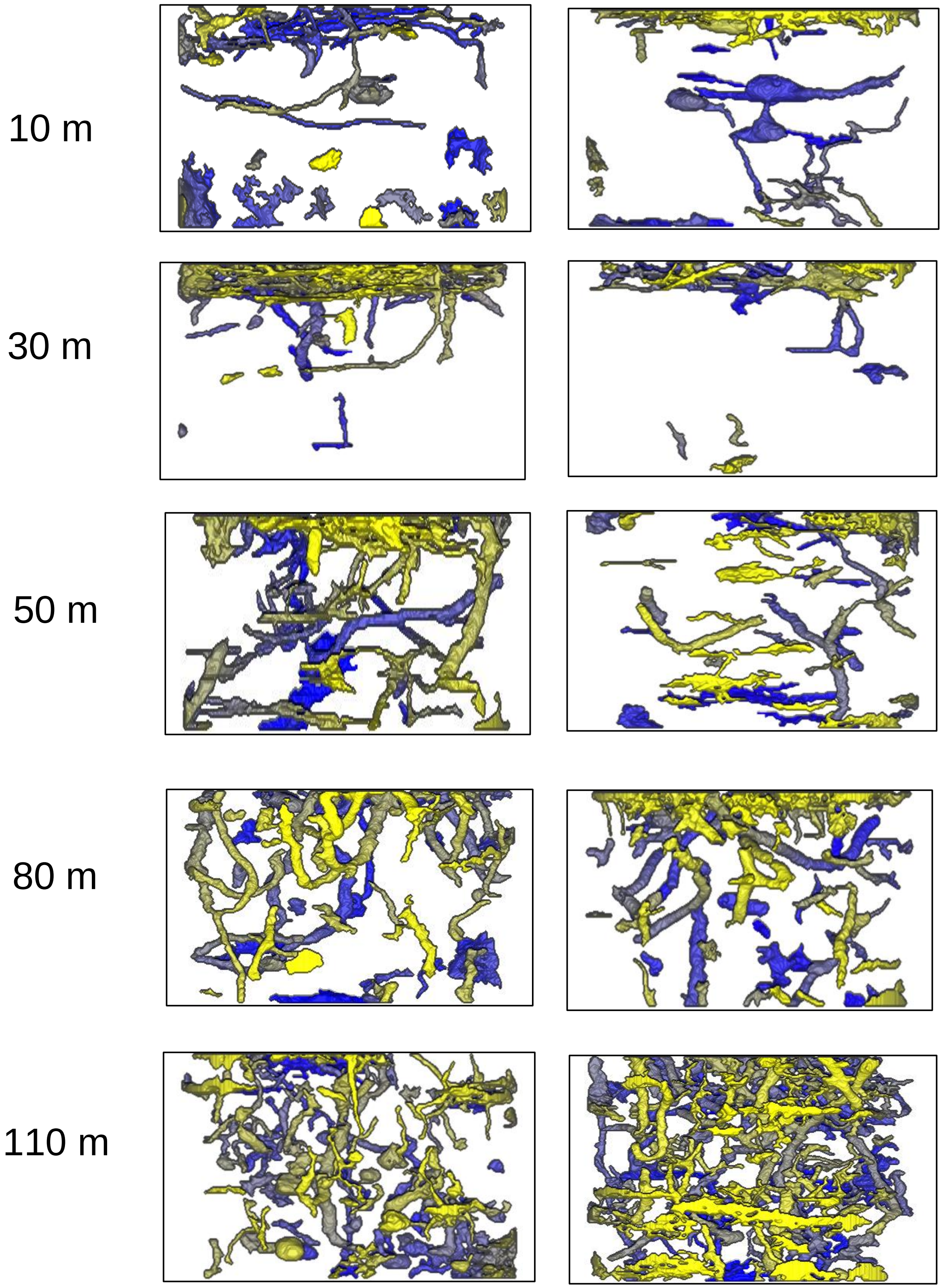
Fig. 1. Examples of 3D burrow systems observed at different distances from the wall of a lead recycling factory.

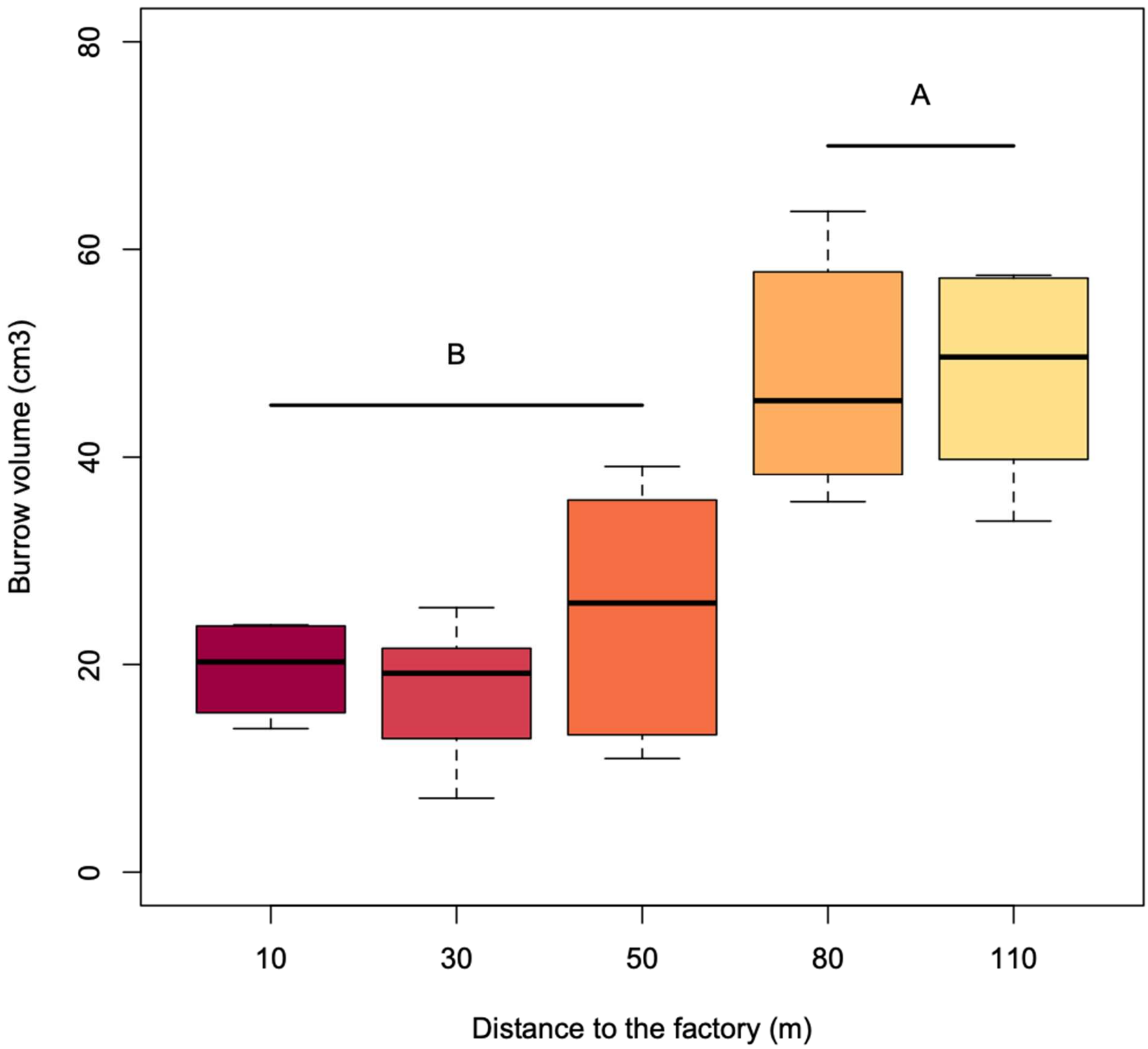
Fig. 2. Box and whisker plot of the volume of the burrow systems found in the soil cores sampled at different distances from the wall of a lead recycling factory. Boxes bearing different letters are different at the 5% threshold level (ANOVA).

Fig. 3. Box and whisker plot of the estimated diameter of the burrows found in the soil cores sampled at different distances from the wall of a lead recycling factory. Boxes bearing different letters are different at the 5% threshold level (ANOVA).

Fig. 4. Box and whisker plot of the continuity of the burrow system (assessed by the number of burrows whose vertical length is larger than 6 cm) found in the soil cores sampled at different distances from the wall of a lead recycling factory. Boxes bearing different letters are different at the 5% threshold level (zero-inflated regression).

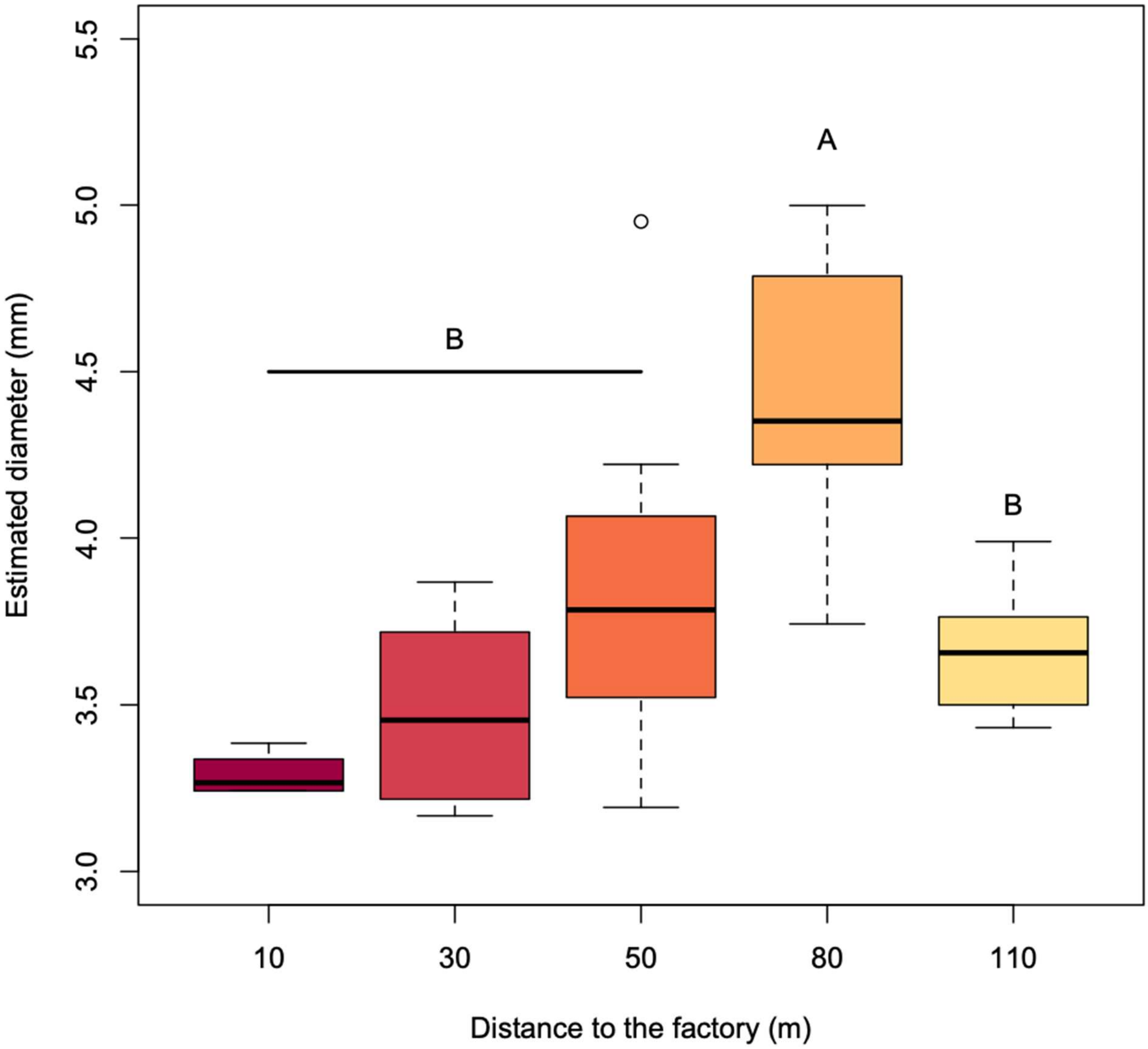
Fig. 5. Mean (and SE) infiltration rate, assessed in situ using the single ring method, measured in soil cores sampled at different distances from the wall of a lead recycling factory. Bars bearing different letters are different at the 5% threshold level (ANOVA).


10 m
30 m
50 m
80 m
110 m



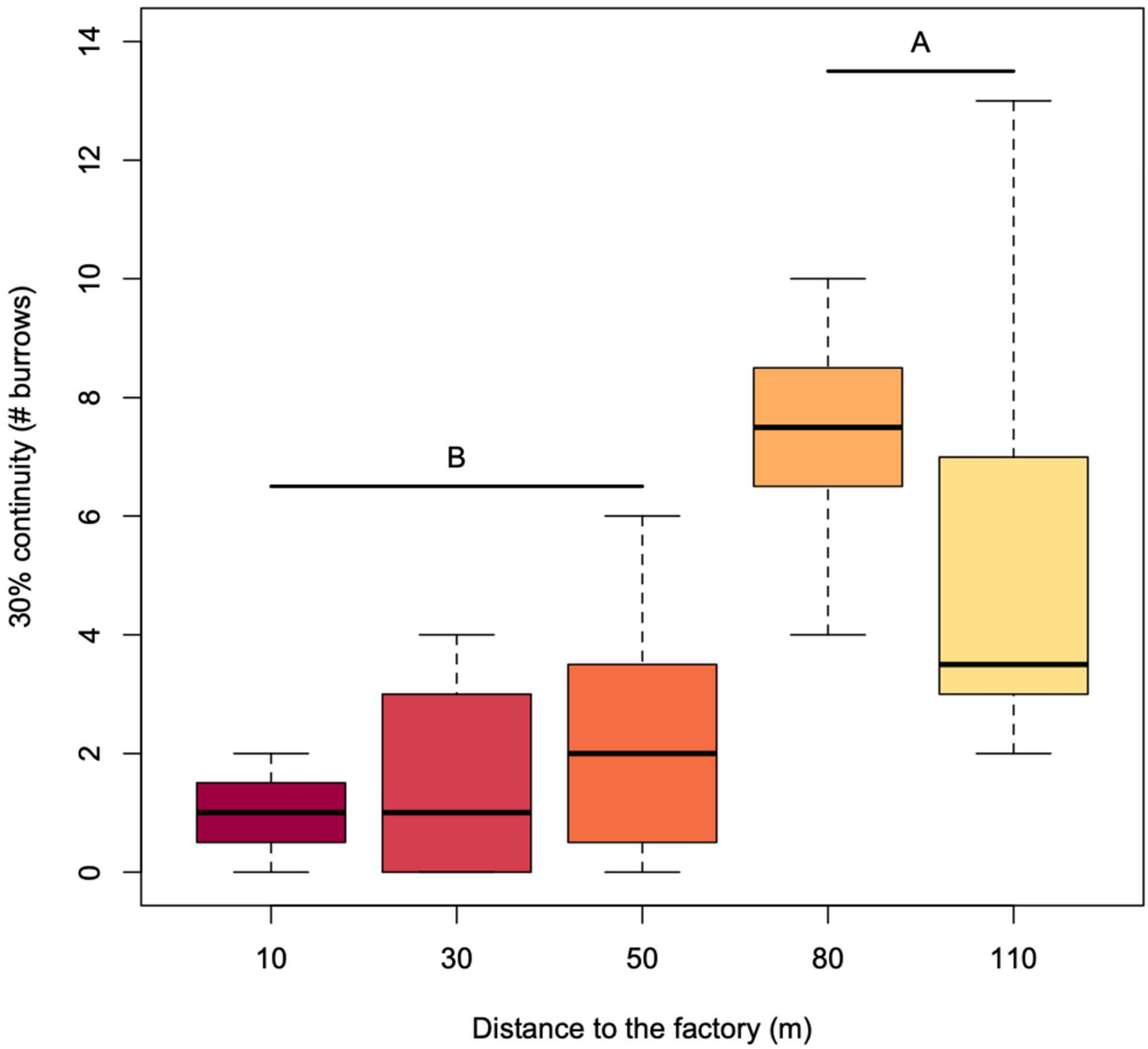
Burrow volume (cm3)
0
20
40
60
80
B
A
10
30
50
80
110
Distance to the factory (m)

Fig. 2



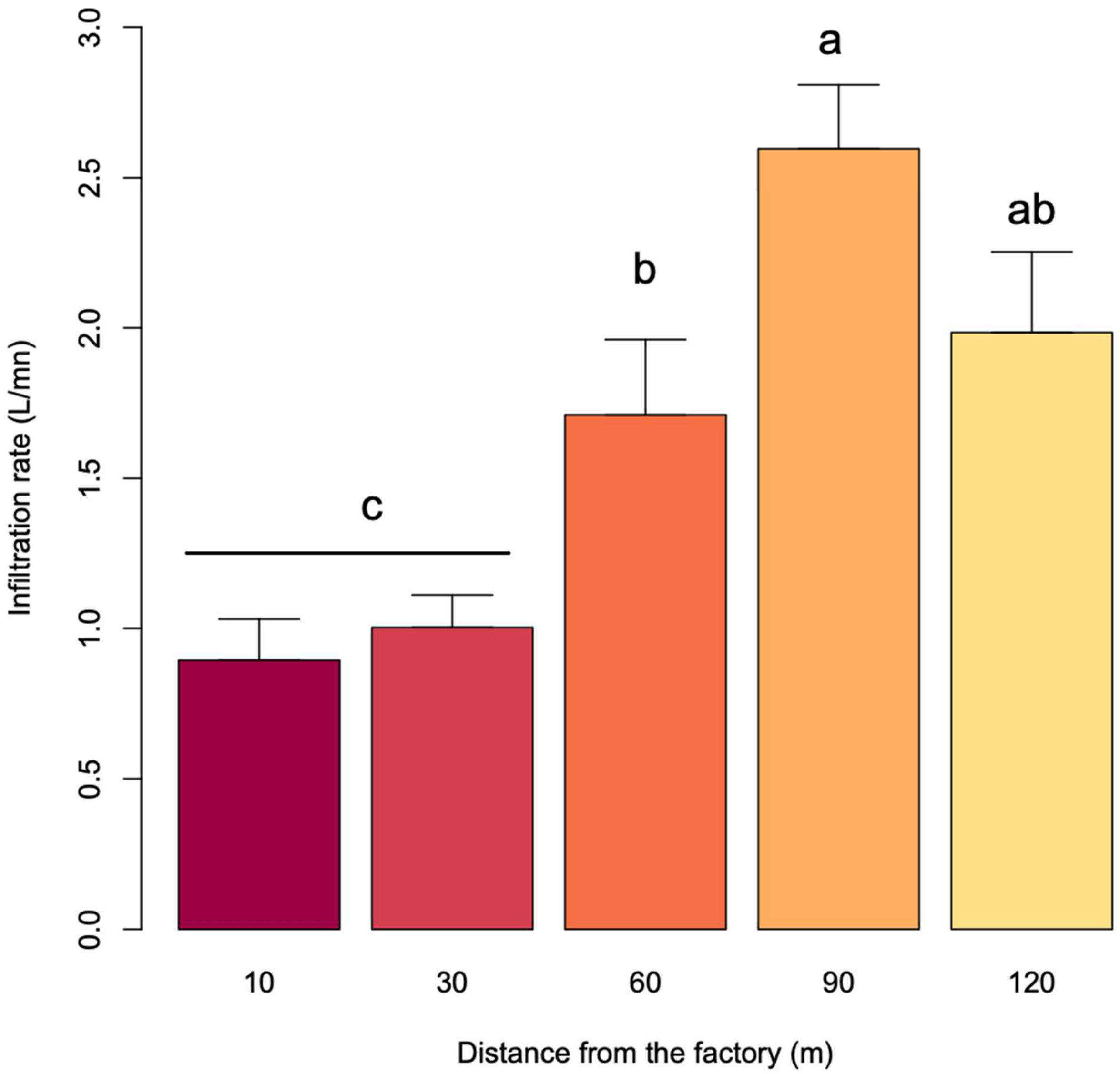
Estimated diameter (mm)
5.5
5.0
4.5
4.0
3.5
3.0
A
B
B
10
30
50
80
110
Distance to the factory (m)

Figure 3



A
B
14
12
10
8
6
4
2
0
30% continuity (# burrows)
10
30
50
80
110
Distance to the factory (m)

Figure 4



Infiltration rate (L/mn)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
a
ab
b
c
10
30
60
90
120
Distance from the factory (m)

Figure 5

Table 1. Abundance and biomass (means and SE) of anecic and endogeic earthworms and soil lead concentration and other key soil parameters in the fallow meadow in function of the distance to the factory wall (data taken from Lévêque et al. 2015).

	Distance to the factory (m)				
	10	30	50	80	110
ANECIC					
Abundance (m^{-2})	0	4.19 (0.54)	11.43 (0.71)	40.53 (1.11)	72.78 (1.02)
Biomass (g m^{-2})	0	10.42 (1.36)	23.15 (1.54)	71.18 (2.43)	126.34 (2.48)
ENDOGEIC					
Abundance (m^{-2})	0	0	0.29 (0.07)	2.79 (0.24)	41.33 (2.61)
Biomass (g m^{-2})	0	0	0.21 (0.03)	1.38 (0.54)	17.91 (1.15)
TOTAL					
Abundance (m^{-2})	0	4.19 (0.54)	11.72 (0.74)	43.32 (1.26)	114.11 (2.94)
Biomass (g m^{-2})	0	10.42 (1.36)	23.36 (1.54)	72.56 (2.44)	144.25 (2.57)
Percentage of anecic (% of total abundance)	-	100	98	94	64

Soil Pb concentration (mg kg^{-1})	29 600	3517	2350	1166	893
pH_{water}	7.9	8.3	8.3	8.3	8.2
$C_{organic}$ (g kg^{-1})	37.9	24.0	25.4	21.3	22.5
N_{total} (g kg^{-1})	2.9	1.8	2.1	1.8	1.9

Table 2 Volumes (and percentage) of the burrow systems in 4 soil layers and total burrow number in function of the distance to the factory wall. Values bearing different letters are significantly different at the 5% threshold level (only burrow number was tested).

	Distance to the factory (m)				
Volumes (cm^3) in the	10	30	50	80	110
[0-3.75 cm] layer (and %)	9.44 (45.2%)	13.63 (61.0%)	9.95 (34.6%)	20.84 (40.8%)	16.55 (29.7%)
[3.25-7.5 cm] layer (and %)	4.86 (23.3%)	3.23 (14.5%)	6.50 (22.6%)	6.52 (22.1%)	12.76 (22.9%)
[7.5-11.25 cm] layer (and %)	2.63 (12.6%)	2.07 (9.2%)	6.51 (22.7%)	8.34 (16.3%)	13.48 (24.2%)
[11.25-15 cm] layer (and %)	3.96 (18.9%)	3.42 (15.3%)	5.76 (20.1%)	10.67 (20.9%)	12.98 (23.2%)
Number of burrows	127.8 ^{bc}	99.4 ^{bc}	82.0 ^{c}	143.3 ^{b}	281.8 ^{c}