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How good are epigeic earthworms at dispersing? An investigation to compare epigeic to endogeic and anecic groups

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ABSTRACT

Dispersal capacities can strongly determine an individual's ability to respond to changing environmental conditions, which would consequently influence the structure of natural communities. Nonetheless, we know little about the dispersal behaviour of soil organisms, despite some of these organisms, such as earthworms, have key roles in ecosystem functioning (e.g. organic matter decomposition). We expect that species exposed to frequent environmental changes would benefit from the capacity to escape from adverse environmental conditions and to disperse to settle in a more suitable habitat. In earthworms, we expect the epigeic group, which lives at or close to the soil surface, to have evolved higher dispersal capacities than the two other functional groups – anecic and endogeic, which live deeper in the soil. In this study, we investigated dispersal and diffusion behaviour of three species of epigeic earthworms (i.e. *Eisenia fetida*, *Eisenia andrei* and *Lumbricus rubellus*) and compared these behaviours with those of anecic and endogeic earthworms, whose behaviour has been previously measured through similar experiments. In accordance with our hypothesis, our study shows that dispersal behaviour of epigeic earthworms depends on habitat quality and population density, but that those responses vary among species and that it differs only to a limited extent from behaviour of anecic and endogeic earthworms.

Key words: density-dependent dispersal, departure, diffusion behaviour, ecosystem engineers, functional groups, habitat quality

1. Introduction

Dispersal is a central ecological process that allows colonization of new habitats and exploitation of spatially and temporally variable resources (Ronce, 2007). Active dispersal of animals (as opposed to passive dispersal, where individuals are transported by an external agent, and has not necessarily a cost for the disperser) is the result of three successive behavioural stages (following the definition given by Clobert et al., 2009, 2001). It involves the departure from a breeding site, crossing to a new place, and settlement (Clobert et al., 2009). It is thought to depend on the balance between the costs and benefits of dispersal (Bonte et al., 2012; Bowler and Benton, 2005), which are strongly determined by both environmental conditions (e.g. habitat quality, habitat fragmentation, patch size, density, predation; Bonte et al., 2006; Schtickzelle et al., 2006) and individual life traits (e.g. age, hormonal levels, movement abilities). Therefore, dispersal capacities are expected to strongly determine an individual's ability to respond to changing environmental conditions, which would consequently influence the dynamics and persistence of populations, the distribution and abundance of species, the structure of natural communities but also the functioning of ecosystems (Cuddington and Hastings, 2004). Nonetheless, we know little about the dispersal behaviour of soil organisms, even if some of these organisms, such as earthworms, play key roles in ecosystem functioning (Blouin et al., 2013).

Earthworms species are often classified into three functional groups based on their morphology, and their foraging behaviour (Bouché, 1977, 1972): endogeic earthworms live and feed in the soil, epigeic earthworms mainly live and feed on the leaf litter at the soil surface, anecic earthworms make vertical burrows in soil and feed on leaf litter which they drag into their burrows. Earthworms are of primary importance for ecosystem functioning because they modify the availability of resources for other organisms through physical and chemical changes in their surrounding soil environment (Jones et al., 2010, 1994; Rillig et al., 2016). As a consequence, they fulfil numerous soil-based ecosystem services (Blouin et al., 2013). While anecic earthworms, and to a lower extent endogeic and epigeic earthworms, are of particular importance for cropping systems (Bertrand et al., 2015; van Groenigen et al., 2014), epigeic earthworms play a key role in organic matter decomposition in deciduous forests because of the ingestion of poorly decomposed litter (Manna et al., 2003) and the interactions they established with decomposer microorganisms (Gómez-Brandón et al., 2012; Monroy et al., 2008), which explains their use in vermicomposting (e.g. Suthar et al., 2008). For these reasons, it is essential to identify the environmental factors that may influence earthworm prevalence and abundance in ecosystems (Curry, 1998; Palm et al., 2013), especially in the context of global changes. Indeed, anthropogenic activities, including urbanisation and agriculture, are responsible for considerable modifications of the natural environment through e.g. light, noise and chemical pollutions, temperature modifications and habitat fragmentation. These changes may have considerable impact at the individual level and in terms of population dynamics and functioning (e.g. Dupont et al., 2015; Johnston et al., 2015; Orwin et al., 2015). To cope with these natural (e.g. soil heterogeneity, daily and seasonal cycles, etc.) or human-induced environmental constraints, high sensory capacities associated with acclimatization or dispersal abilities may have been naturally selected in earthworm species (e.g. Fisker et al., 2011; Spurgeon and Hopkin, 2000). Amongst other things, we expect species exposed to frequent environmental changes to benefit from a large tolerance range, meaning from high flexibilities (e.g. earthworms exposed to changing concentrations of pollutants should benefit from high flexibility in the synthesis of detoxification proteins; Lukkari et al., 2004) or from the capacity to escape from these detrimental environmental conditions and to disperse to settle in a more suitable habitat.

The three functional groups of earthworms inhabit three different niches whose exposure to aboveground conditions increases from the endogeic group to the epigeic group. The aboveground environment is characterized by a high temporal and spatial heterogeneity. Therefore, epigeic earthworms have to face highly fluctuating environments (e.g. temperature and humidity changes) and are more directly exposed to soil inputs (e.g. pesticides, hydrocarbons, fertilizer, etc.), soil surface state (e.g. subsidence exerted by vehicles, ploughing or bioturbations) and predators. Consequently, we may expect epigeic earthworms to have evolved higher sensibility to surface conditions (i.e. quicker responses) and higher dispersal abilities (i.e. lower dispersal costs, associated with physiological and anatomical adaptations for low latency and high speed movements) than endogeic and anecic groups.

Habitat (i.e. soil and litter) structure (e.g. particle size distribution), composition (e.g. amount of organic carbon) and pH, both linked to bioavailability of chemicals in soils and earthworms' ability to move in the habitat, are expected to influence earthworm habitat preference (Lanno et al., 2004) and as a consequence dispersal behaviour. Moreover, the amount of food in the environment is often limited; therefore, food availability per individual is negatively correlated with population density (Curry, 1998). Alternatively, population density may affect soil physical and chemical properties (Jones et al., 2010, 1994; Rillig et al., 2016), which may lead to facilitating mechanisms (Caro et al., 2012; Mathieu et al., 2010). Nonetheless, to our knowledge, few studies have investigated the environmental factors that influence dispersal behaviour in earthworms. Caro et al. (2013, 2012) and Mathieu et al. (2010) showed that low soil quality increased dispersal rate of *Aporrectodea icterica*, *Allolobophora chlorotica*, *Aporrectodea caliginosa* (endogeic species) and of *Aporrectodea longa*, *Lumbricus terrestris* and *Aporrectodea giardi* (anecic species) (Caro et al., 2013; Mathieu et al., 2010). Moreover, the absence of litter increased dispersal rate in *Dendrobaena* *venata*, an epigeic species (Mathieu et al., 2010). High intraspecific density also increased dispersal rate in the three anecic species and in *A. icterica* but not in *A. chlorotica* and *A. caliginosa*. Finally, while dispersal speed was increased by conspecifics through the use of existing galleries in *Aporrectodea giardi* (Caro et al., 2012), dispersal rate was reduced by the pre-use of soil in *A. icterica* (Mathieu et al., 2010). Previous studies on earthworm dispersal were mostly carried out on endogeic and on anecic groups (Caro et al., 2013, 2012) or on a single epigeic species (Mathieu et al., 2010). Therefore, we still know little about the dispersal behaviour of the epigeic group and whether it is different from the two other groups.

To investigate diffusion and dispersal behaviour of epigeic earthworms, we performed three different experiments on three epigeic species (i.e. *Eisenia fetida*, *Eisenia andrei* and *Lumbricus rubellus*), following the same experimental protocol as a previous experiment (Caro et al. 2013). We investigated diffusion behaviour in a homogeneous environment (experiment 1), to measure earthworm propensity to explore, their distance of exploration and their tendency to follow their conspecifics. We measured dispersal rate in response to habitat quality (experiment 2) or in response to population density (experiment 3). Then, we compared diffusion and dispersal behaviours of epigeic earthworms with that of the two other functional groups: anecics and endogeics, whose behaviours have been previously measured through similar experiments (Caro et al., 2013). We expected high specificity in epigeic dispersal behaviours because of their specific relative selective pressures.

2. Methods

2.1 Subjects and housing

Free-living adult earthworms from three epigeic species – *Eisenia fetida*, *Eisenia andrei* and *Lumbricus rubellus* - were collected in November 2015 from several rural locations in Ile-de France, near Paris, France (between 48°69’N, 2°60’E and 48°74’N, 2°68’E). Earthworms were kept in acclimatizing mesocosms (12 cm x 10 cm x 8 cm) filled with suitable soil (see 2.2 Diffusion and dispersal mesocosms set-up) with a density of 10 earthworms per mesocosm, at a constant 17°C. Before the start of the trials (section 2.3) earthworms were acclimatized for a period of at least 3 weeks, depending on their capture date and the trial dates, to remove potential stress effects of capture. Acclimatizing mesocosms were humidified and enriched with homogenized horse dropping twice a month. All individuals were kept for at least three weeks before onset of experiments. The species names used herein conformed to the Fauna Europaea web site.

2.2 Diffusion and dispersal mesocosms set-up

First, we investigated diffusion in a homogeneous environment (experiment 1). Then, two different environmental factors were tested on epigeic earthworm dispersal: population density (experiment 2) and habitat quality (experiment 3). The experiments' setting followed the protocol used by Caro et al. (2013). Diffusion behaviour in a homogeneous environment was tested in mesocosms (300 cm x 20 cm x 20 cm) that consisted of a suitable habitat. The suitable habitat consisted of grassland soil collected from a brunisol at the IRD research centre (48°54’N, 2°29’E), which hosts large earthworm populations. Because epigeic earthworms mainly inhabit litter, lime leaves (*Tilia vulgaris*) were added on the surface of the suitable soil. This litter is generally well consumed by earthworms (Hendriksen, 1990). This first experiment tested the natural spread of individuals while removing the effect of heterogeneity. The mesocosms were divided in 13 regular sections (each 23 cm long) which were named according to their distance from the central section (see Fig. 1).

The influence of population density and habitat quality were investigated in dispersal mesocosms (100 cm x 20 cm x 20 cm; Mathieu et al., 2010), which were divided into three equal sections (see Fig. 1): the “inoculation” section (1) was a suitable habitat in population density trials and either a suitable or an unsuitable habitat in habitat quality trials; the “crossing” section (2) was always an unsuitable habitat; finally, the “arrival” section (3) was always a suitable habitat. The suitable habitat was similar to the one previously described (i.e. grassland soil + leaf litter). The unsuitable habitat consisted of a sandy soil collected from a luvisol in an area deprived of earthworms and litter in the forest of Fontainebleau (48°24’N, 2°44’E). Because of their niche, epigeic earthworms were expected to be strongly affected by litter quality. Therefore, the unsuitable soil was not topped with litter. We checked in a preliminary experience that the three species did prefer the suitable soil to the unsuitable one. Both soils were air dried, sieved to 2 mm and rewetted manually at 25% of humidity. Lime leaves were washed, air dried and soaked in tap water for 15 minutes. Soil (suitable or unsuitable) was filled to 5 cm high and 2 cm high of lime leaves were spread on the top of appropriate sections. This setup allowed the reproduction of the three stages of dispersal: departure, crossing and settlement in a suitable site (Clobert et al., 2009). The unsuitable habitat in the crossing section represented a physical barrier generating dispersal costs. It allowed us distinguishing between mechanisms of diffusion (random movements with potentially returns in the starting point) from active dispersal (Clobert et al., 2009). As expected, diffusion rate in a homogeneous high quality habitat was higher than dispersal rate from a high-quality habitat (see Table 1 and Fig. 2), verifying that our dispersal mesocosm set-up successfully induced a dispersal cost, and suggesting that dispersal rate rather than diffusion rate was measured in dispersal experiments.

2.3 Diffusion and dispersal trials

When investigating diffusion behaviour in a homogeneous environment (experiment 1), we simultaneously inoculated 10 earthworms of the same species in the central section of a three-meter long mesocosm (i.e. section 0). To avoid earthworms from escaping during inoculation, earthworms were placed in the centre of a rectangular plastic gate (12 cm x 10 cm x 6 cm) previously buried at 1.5 cm deep in the centre of the inoculation section, which was ever in the middle of the mesocosm (experiment 1) or at the left (experiments 2 and 3). We checked that all earthworms entered the soil or remained motionless before removing the gate and closure of the mesocosms. Each trial of the first experiment (experiment 1) lasted 24h; this time was short enough to prevent individuals from reaching the end of the mesocosms and going back towards the inoculation section. At the end of each trial, the 13 sections were separated and the number of earthworms per section was counted. We calculated diffusion rate as the proportion of individuals that left the central section. Despite the mesocosms being carefully closed, some earthworms escaped; the escape rate was low during the diffusion trials (mean ± se: 2 ± 7%, 0 ± 6% and 0 ± 0% in *E. fetida*, *E. andrei* and *L. rubellus*, respectively). In order to take into account these escapes, diffusion rates were calculated as the number of individuals that left the central section (i.e. section 0) compared to the number of individuals that were found in the inoculation section at the end of a trial. We also investigated the mean of the absolute distance crossed and the tendency to follow conspecifics as the absolute value of the difference in the diffusion rates to the right and to the left of a starting section; thus the tendency to follow their conspecifics varies from 0 (individuals equally distributed in each side) to 1 (all individuals in only one side).

When investigating the effect of habitat quality on dispersal rate (experiment 2), we simultaneously inoculated 10 earthworms of the same species in a one meter long mesocosm. When testing the effect of population density on dispersal rate (experiment 3), we inoculated earthworms in the mesocosm, at one of these four densities: 1, 10, 20 or 30 individuals of the same species. Each trial of experiments 2 and 3 lasted 7 days. At the end of each trial, the 3 sections were separated in order to prevent earthworms from switching between sections. Soil and lime leaves were carefully searched and earthworms were removed. Earthworms were then replaced in their resting mesocosms. The number of earthworms per section was counted; earthworms found in the arrival section were considered as disperser, while earthworms found in the inoculation and crossing section were considered as non-dispersers. Some of the earthworms also escaped these dispersal devices (mean ± se: 31 ± 5%, 27 ± 5% and 4 ± 5% in *E. fetida*, *E. andrei* and *L. rubellus*, respectively). The escape rate did not depend on the initial density ($\chi^2_{ddl=1}$=0.01, P=0.909). Nonetheless, dispersal rates were calculated as the number of individuals in the arrival section compared to the number of individuals found in the two other sections at the end of the trials, which allowed measuring dispersal behaviour rather than departure behaviour. Each experimental design (i.e. homogeneous environment, high quality, low quality, density 1, density 10, density 20 and density 30) was replicated 5 times per species under the same conditions of light (a daily photoperiod of 10h) with temperature fluctuating between 15°C and 17°C.

2.4 Comparison of diffusion and dispersal behaviour between functional groups

We compiled the data obtained in these experiments with those obtained in a previous similar study on anecic and endogeic earthworms (Caro et al., 2013) in order to compare dispersal behaviour between functional groups. Note that in order to take into account for the specific ecology of epigeics, the suitable and unsuitable habitats slightly differed in our study from the ones in Caro et al. (2013): we added a layer of leaf litter on top of the suitable soil, and soil depth was reduced to 5 cm high instead of 15 cm in the previous study. Both studies aimed at mimicking a suitable and an unsuitable habitat, which necessarily implied some minor habitat differences to adjust to the focus group.

2.5 Statistical analyses

2.5.1 Diffusion and dispersal behaviour in epigeic earthworms - To investigate whether the diffusion rate, the mean distance crossed and the tendency to follow conspecifics in a homogeneous environment varied between species (experiment 1), we performed a Generalized Linear Model (GLM) with a binomial distribution (model 1) and Linear Models (model 2 and 3), respectively: diffusion rate, diffusion direction or the mean distance crossed was the dependent variable and species identity was the explanatory variable. In order to test the effects of habitat quality (model 4) and population density (model 5) on dispersal rate, we used GLM with binomial distributions: dispersal rate was the dependent variable and habitat quality (i.e. high vs. low), species identity (i.e. *Eisenia fetida*, *Eisenia andrei*, *Lumbricus rubellus*) and their interactive effects (model 4) or population density (i.e. 1, 10, 20, 30), species identity and their interactive effect (model 5) were the explanatory variables. Because some of the earthworms escaped during the experiment and we have no means to know when it happened, we also tested the effect of final density on dispersal rate (model 5) by considering the number of earthworms that have been found at the end of the experiment as the explanatory variable instead of the number of earthworms inoculated.

2.5.2 Comparison of diffusion and dispersal behaviour between functional group – We compared the results from our three experiments on epigeic species to the ones obtained in a previous study with a similar experimental setup carried on three species of anecic (*A. longa*, *L. terrestris* and *A. giardi*) and three species of endogeic earthworms (*A. chlorotica*, *A. icterica* and *A. caliginosa*; Caro et al., 2013). We re-run our five previous models aggregating our results with the results from the previous study. In model 1 (Generalized Linear Mixed Models), 2 and 3 (Mixed-Effects Models), functional group was the explanatory variable and species was added as a random factor. In models 4 and 5 (Generalized Linear Mixed Models), the interactive effect between habitat quality or population density and functional group (i.e. epigeic, anecic, endogeic) was the explanatory variable and species identity was added as a random factor. Finally, we performed a Partial Least Squares – Discriminant Analysis (PLS-DA; mixOmics and RVAideMemoire packages in R) to compare dispersal behaviour between functional groups as a combination of the diffusion rate in homogeneous environment, the mean distance crossed in homogeneous environment, the tendency to follow conspecifics, the mean sensitivity to habitat quality (calculated as the difference between the mean dispersal rate from low quality habitat and from high quality habitat within a species), the sensitivity to population density (calculated as the slope of the regression between dispersal rate and population density), averaged for each species. PLS-DA is a supervised multivariate analysis that classifies groups of observations (i.e. functional groups) based on multivariate predictors (results from the different experiments; i.e. diffusion rate, mean distance crossed, tendency to follow conspecifics, sensitivity to habitat quality and sensitivity to population density), and which helps to identify which variables best discriminate the groups.

Statistical analyses were performed using R software (version 3.1.2). Full models included all factors and covariates and all their interactions. We retained final models based on their AIC.

3. Results

3.1 Diffusion and dispersal behaviour in epigeic earthworms

3.1.1 Homogeneous environment (experiment 1) – Diffusion behaviour in an homogenous environment was significantly different between species ($\chi^2_{df=2}$=14.97, P=0.001): diffusion rate was lower in *L. rubellus* than in *E. fetida* ($F_{1,8}$=6.57, P=0.010) and *E. andrei* ($F_{1,8}$=14.18, P<0.001; Table 1). The mean distance crossed was also significantly different between species ($F_{2,12}$=5.61, P=0.019): the mean distance crossed was shorter in *L. rubellus* than in *E.*

andrei ($F_{1,8}$=8.20, P=0.022, Table 1). The tendency to follow conspecifics was not significantly different between species ($F_{2,12}$=1.22, P=0.329; Table 1).

3.1.2 Habitat quality (experiment 2) –There was a significant interaction between habitat quality and species identity on the dispersal rate ($\chi^2_{df=2}$=10.49, P=0.005; Fig. 2): in all three species, dispersal rate was higher when earthworms were inoculated into a low quality habitat than into a high quality habitat ($\chi^2_{df=1}$=5.15, P=0.023; $\chi^2_{df=1}$=17.40, P<0.001 and $\chi^2_{df=1}$=45.09, P<0.001 for *E. fetida*, *E. andrei* and *L.rubellus*, respectively). However, dispersal rate from high quality habitat significantly differed between species ($\chi^2_{df=2}$=24.89, P<0.001): *L. rubellus* dispersed less from high habitat quality than the two other species ($\chi^2_{df=1}$=16.58, P<0.001 and $\chi^2_{df=1}$=21.48, P<0.001 for *E. fetida* and *E. andrei*, respectively); dispersal rate from low quality habitat did not differ between species ($\chi^2_{df=2}$=5.24, P=0.073).

3.1.3 Population density (experiment 3) – There was a significant interaction between population density (i.e. 1, 10, 20, 30) and species identity on the dispersal rate ($\chi^2_{df=6}$=27.38, P<0.001; Fig. 3): dispersal rate increased with increasing population density in *E. andrei* ($\chi^2_{df=1}$=7.85, P=0.005), while it decreased with increasing population density in *L. rubellus* ($\chi^2_{df=1}$=15.81, P=0.001); the correlation between dispersal rate and population density was not significant in *E. fetida* ($\chi^2_{df=1}$=3.00, P=0.083). The results were quite similar when considering the number of earthworms found at the end of the experiment as the explanatory variable; there was a significant interaction between the final density and species identity on the dispersal rate ($\chi^2_{df=2}$=24.44, P<0.001): dispersal rate increased with increasing the final density in *E. andrei* ($\chi^2_{df=1}$=12.95, P<0.001) while it decreased with increasing population density in *L. rubellus* ($\chi^2_{df=1}$=5.36, P=0.021) and *E. fetida* ($\chi^2_{df=1}$=5.19, P=0.023).

3.2 Comparison of diffusion and dispersal behaviour between functional groups

3.2.1 Homogeneous environment - There was no significant effect of functional group on diffusion rate in an homogenous soil ($\chi^2_{df=2}$=1.90, P=0.387; Table 2), on the mean distance crossed ($\chi^2_{df=2}$=0.91, P=0.634; Table 2) and on the tendency to follow conspecifics ($\chi^2_{df=2}$=0.23, P=0.890; Table 2).

3.2.2 Habitat quality - There was a significant interaction between habitat quality and functional group on the dispersal rate ($\chi^2_{ddl=2}$=16.59, P<0.001; Fig. 4): in all three functional groups, dispersal rate was higher when earthworms had been inoculated into a low quality habitat than into a high quality habitat ($\chi^2_{ddl=1}$=46.62, P<0.001; $\chi^2_{ddl=1}$=112.51, P<0.001 and $\chi^2_{ddl=1}$=67.05, P<0.001 for epigeic, anecic and endogeic earthworms respectively). However, dispersal rate from low quality habitat significantly differed between functional groups ($\chi^2_{ddl=2}$=7.34, P<0.026): it was lower in endogeic earthworms than in the two other functional groups ($\chi^2_{ddl=1}$=5.29, P=0.021 and $\chi^2_{ddl=1}$=4.43, P=0.035 when compared to anecic and epigeic earthworms, respectively); on the contrary, dispersal rate from high quality habitat did not significantly differed between functional groups ($\chi^2_{ddl=2}$=3.06, P=0.216).

3.2.3 Population density - There was no significant effect of population density ($\chi^2_{df=1}$=1.83, P=0.176), functional group ($\chi^2_{df=1}$=3.64, P=0.162) and their interactions ($\chi^2_{df=2}$=3.03, P=0.220) on dispersal rate (Fig. 5). The results were quite similar when considering the number of earthworms found at the end of the experiment as the explanatory variable: there was a significant interaction between the final density and species on the dispersal rate ($\chi^2_{ddl=2}$=15.66, P<0.001) but final density tended to influence dispersal rate in anecic earthworms only ($\chi^2_{ddl=1}$=0.30, P=0.584, $\chi^2_{ddl=1}$=2.81, P=0.094 and $\chi^2_{ddl=1}$=0.23, P=0.635 in epigeic, anecic and endogeic earthworms).

3.2.4 PLS-DA components analysis - Two components were retained in the PLS-DA: the first and the second component explained 67.20% and 12.58% of the among group variation respectively. The sensitivity to habitat quality, the sensitivity to population density and the dispersal rate in homogeneous environment were the most important variables in the projection (i.e. VIP index greater than 1). The first component was correlated with the sensitivity to habitat quality (t=-4.11, df=7, P=0.005), while the second component was correlated with the sensitivity to population density (t=-10.87, df=7, P<0.001), the dispersal rate in homogeneous environment (t=3.26, df=7, P=0.014) and the mean distance crossed in homogeneous environment (t=2.77, df=7, P=0.028).

4. Discussion

4.1 Diffusion and dispersal behaviour in epigeic earthworms

Our study demonstrated that dispersal behaviour of epigeic earthworms depends on habitat quality and population density, and that this response varies among epigeic species.

Firstly, results showed a higher dispersal rate of *Eisenia fetida* and *Eisenia andrei* than of *Lumbricus rubellus* from high quality habitat. This elevated basal dispersal rate may be related to the narrower ecological niche of *E. fetida* and *E. andrei*, characterized by a high proportion of organic matter (i.e. they inhabit compost and manure; Lee, 1985). Therefore, the so-called “high quality” habitat, composed of grassland soil topped with lime leaves may actually be moderately favourable for those species, consequently inducing high dispersal rates. Alternatively, both species might have high basal dispersal behaviour associated with high exploratory behaviour. This interpretation is supported by the results of our first experiment in a homogeneous environment. Indeed, diffusion rates in homogeneous environment were higher in *E. fetida* and *E. andrei* than in *L. rubellus*, and the mean distance crossed was also higher in *E. andrei* than in *L. rubellus*. It might also explain the higher escape rate measured in these two species than in *L. rubellus*. Moreover, our study clearly demonstrated an effect of habitat quality on dispersal rate, with earthworms exhibiting a higher dispersal rate in a low than in a high quality habitat. Therefore, as for anecic and endogeic earthworms (Caro et al., 2013), habitat quality should be a strong driver of epigeic earthworm spatial distribution in ecosystems. Future studies should identify more thoroughly which habitat characteristics count for epigeic earthworms' decision to disperse. In particular, we need to disentangle the role of leaf litter presence and characteristics (e.g. plant variety, decomposition state) from the role of soil characteristics (e.g. composition, humidity). *E. fetida* response to habitat quality was less pronounced comparing to *E. andrei* and *L. rubellus* but also more variable between individuals, suggesting that this species would either be less sensitive to habitat quality, or suffer high dispersal cost, which is doubtful regarding their high dispersal rate in high quality habitat.

Secondly, our study highlighted no clear pattern of response to population density in epigeic earthworms: dispersal rate increased with increasing population density in *E. andrei* but not in the two other species. Insufficient information is available regarding the biotic environmental factors that influence habitat choice in earthworms (but see Caro et al., 2013; Curry, 1998; Mathieu et al., 2010; Palm et al., 2013). For instance, the presence of conspecifics could be used as a clue of a high quality habitat (Lister, 2014). For instance, *E. fetida* lives at high density, arguably because they chose patches of abundant food. In accordance, attraction mechanisms were observed in this species (Zirbes et al., 2012, 2010). In contradiction, dispersal rate of *E. fetida* was not negatively correlated with population density in our study. Moreover, earthworms' motions and secretions (i.e. earthworm cast and mucus) induce strong modifications of the physical and chemical parameters of the soil (Jones et al., 2010; Rillig et al., 2016). Therefore, earthworm presence modifies the habitat in a way that may increase earthworm propensity to establish (Caro et al., 2014). In accordance with this hypothesis, dispersal rates of *Aporrectodea icterica* (endogeic) were lower when the soil has been pre-used by conspecifics in a former experiment (Mathieu et al., 2010). On the contrary, in the species *E. andrei*, population density would trigger dispersal. Positive density dependence in dispersal behaviour due to resource depletion is quite widespread in animals in general (Murray, 1967; Waser, 1985). Uvarov (2009) showed that more than 85% of the studies found negative effects of population density on earthworm demographic parameters, whatever their functional group. For instance, negative density dependence was found for growth rates and maturation in *E. fetida*, *E. andrei* and *L. rubellus*. The lack of a clear relationship between dispersal rate and population density in *E. fetida* and *L. rubellus* did not verify that density dependent dispersal is a key mechanism regulating population dynamics in the rapidly reproducing species such as *E. fetida* and most epigeic earthworms (Kammenga et al., 2003). It would suggest that, in these two species, either dispersal response to population density would not covary with fitness improvement, or dispersal costs were higher than its benefits (Ronce, 2007). We may also hypothesize that the density tested in our experiment (i.e. 1, 10, 20, 30) were under the threshold needed to induce the costs associated with crowding.

4.2 Comparison of dispersal and diffusion behaviour between functional groups

The comparison of dispersal behaviour of epigeic earthworms to the ones of anecics and endogeics, investigated in a previous study (Caro et al., 2013), suggests that dispersal rate of endogeic earthworms from low habitat quality was lower than the one of the epigeic and anecic earthworms (see Fig. 4). This would suggest that either endogeic earthworm have a broader ecological niche, a reduced sensitivity to habitat quality or poorer dispersal capacities (i.e. higher dispersal costs) compared to the two other functional groups. This last explanation would verify our hypothesis that earthworms experiencing high interactions with the aboveground environment would be more exposed to environmental stochasticity and therefore likely evolved higher dispersal capacities. Future studies should disentangle these hypotheses by investigating the effects of dispersal choice on fitness (i.e. survival and reproductive success) or health parameters (e.g. weight variation over time).

On the contrary, none of the three functional groups showed a clear response to population density (see Fig. 6) and the three groups exhibited similar behaviour in dispersal rate, in mean distance crossed and in the tendency to follow conspecifics in an homogeneous environment (see Table 2). Our study showed that there was significant variability in the dispersal behaviour of epigeic earthworm. Such variability had also been measured in anecic and endogeic groups (Caro et al., 2013). The high variability of dispersal behaviour among species of the same functional group may explain the lack of clear dispersal difference between earthworms' functional group. It suggests that functional group would not be the main factor explaining dispersal behaviour variability between species. It would be interesting to perform similar experiments on a greater number of species per functional group to lower the intragroup (i.e. within functional group) variation but also to extend the time and spatial scale of the diffusion experiment which may help measuring variations in diffusion behaviour dynamics along time in the longer term. Alternatively, functional groups may have to be redefined in view of species diffusion and dispersal behaviours; although these behaviours directly influence burrowing activities, which has known impact on soil processes, soil invertebrate species are mainly categorised according to their foraging behaviour and as a consequence to their position in foodwebs (Lavelle, 1996; Mariani et al., 2001). Indeed, several species, although belonging to a same functional group, inhabit different habitat. For instance, *E. fetida* and *E. andrei* habitat (i.e. mainly composts and manures) are very different from *L. rubellus* one (i.e. mainly leaf litter but also soil), despite they are all classified as epigeics. This may explain the low difference of dispersal behaviour between functional groups. Note also that the two comparative studies had slightly different experimental designs (i.e. presence of leaves and amount of soil). Because epigeic earthworms mainly live on the leaf litter at the soil surface, we assume that their dispersal behaviour only slightly depends on the amount of soil. However, the presence of leaf litter, by influencing food and shelter availability, likely affect dispersal behaviour of anecics, and to a lower extent of endogeic earthworms. Moreover, a deeper soil (> 15 cm depth) may have been more relevant in the previous study to mimic a high quality habitat for anecic earthworm. Consequently, we may think that in previous experiments, dispersal rate in high quality habitat might have been lower in these groups if the soil had been topped with leaf litter or had been thicker. However, dispersal rate of anecic and endogeic earthworms was already very low in high quality habitat (lower than 20%), suggesting that the amount of food and soil was sufficient for the experimental time and would not have significantly modulated dispersal behaviour.

In addition to identifying important factors influencing earthworms' dispersal choice, our study underlines the high variability in the responses to these factors among species and functional groups of earthworms. These complex patterns in dispersal behaviours contribute to earthworm community composition and as a consequence to ecosystem functioning. Efforts in maintaining ecosystem functioning and high ecosystem services, that often relies on species and functional diversity (Balvanera et al., 2006; Mace et al., 2012), should be optimized by taking into account such diversity in environmental factors sensitivity and consequently in dispersal behaviours.

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	E. fetida	*E. andrei*	*L. rubellus*
Diffusion rate	69 ± 11% (a)	81 ± 9% (a)	44 ± 14% (b)
Mean distance crossed (cm)	31 ± 6 (ab)	63 ± 1 (a)	18 ± 3 (b)
Tendency to follow conspecifics	0.18 ± 0.08 (a)	0.44 ± 0.19 (a)	0.37 ± 0.06 (a)

Table 1 Comparison of diffusion behaviour (mean ± se) among epigeic earthworms. Significant differences between species are indicated by different letters (a, b).

	Epigeic	**Anecic**	**Endogeic**
Diffusion rate	64 ± 6 %	83 ± 3 %	60 ± 10 %
Mean distance crossed (cm)	37 ± 7	60 ± 5	42 ± 13
Tendency to follow conspecifics	0.33 ± 0.07	0.43 ± 0.11	0.37 ± 0.13

Table 2 Comparison of diffusion behaviour (mean ± se) between epigeic, anecic and endogeic earthworms. The three functional groups did not exhibit significantly different behaviour in dispersal rate, in mean distance crossed and in the tendency to follow conspecifics.

Fig. 1 Schematic representation of the experimental designs. The soil disposition in the mesocosms is symbolized by rectangles whose colour represent the nature of the habitat (suitable vs. unsuitable). The arrows indicate the section where earthworms (e.) were inoculated at the beginning of each trial.

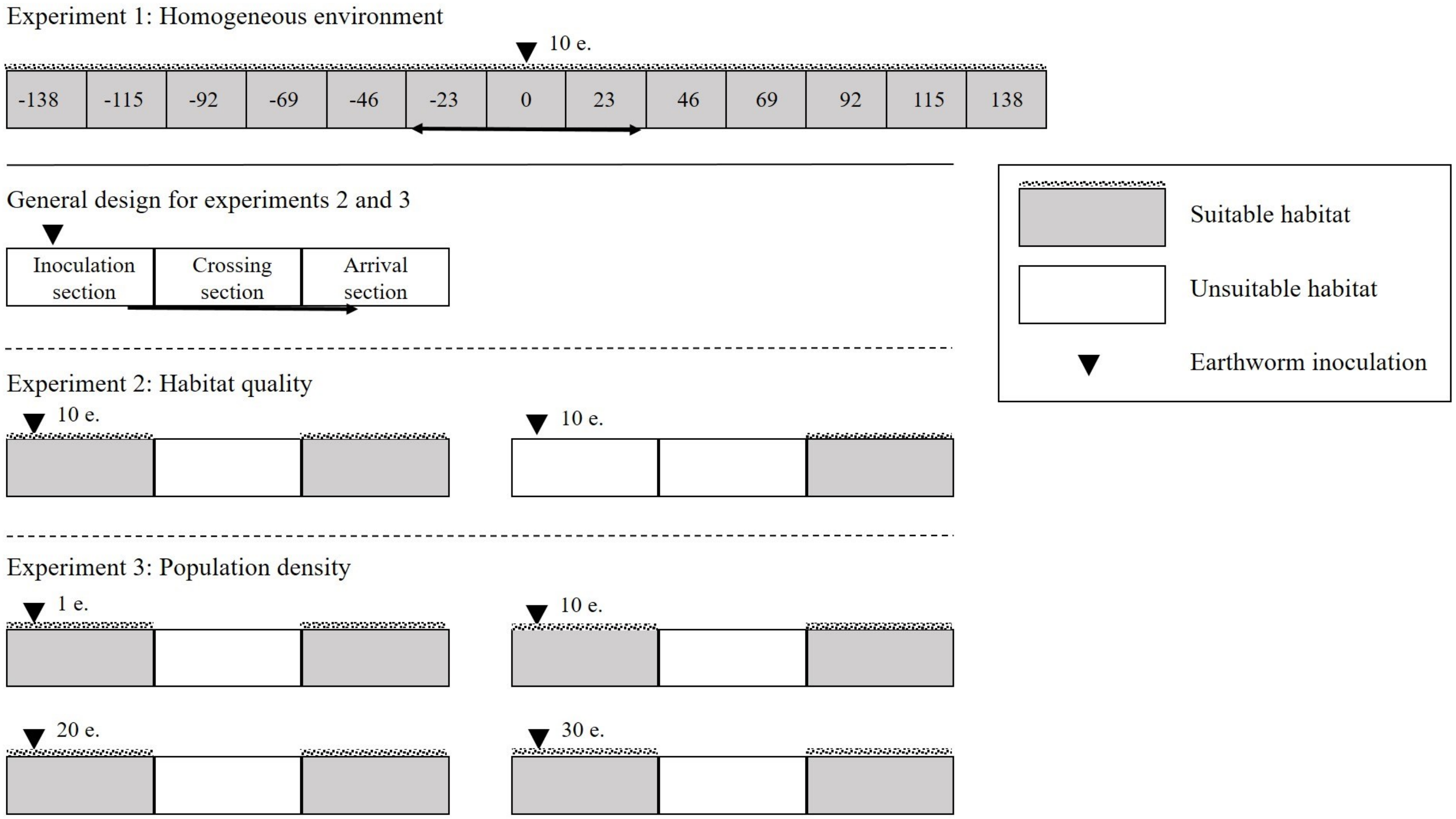

Fig. 2. Dispersal rate (mean ± se) of three epigeic earthworm species inoculated in low quality habitat (black symbols) or in high quality habitat (white symbols). Significant differences between and among species are indicated by different letters (a, b, c).

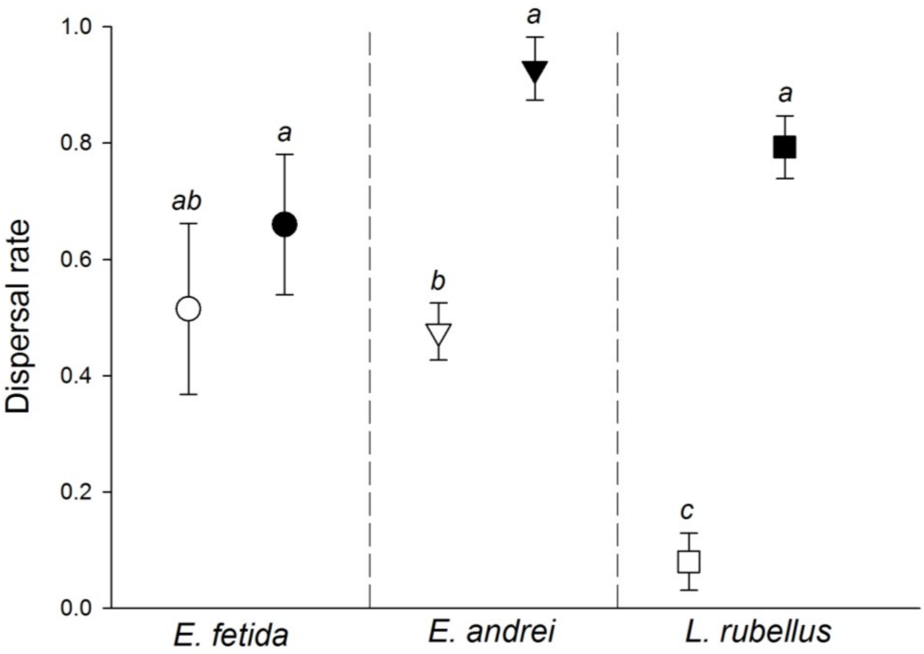

Fig. 3 Relationship between dispersal rate and population density (i.e. 1, 10, 20 or 30 earthworms) in three epigeic earthworm species. The points are slightly shifted around the correct densities to be clearly distinguishable one from another.

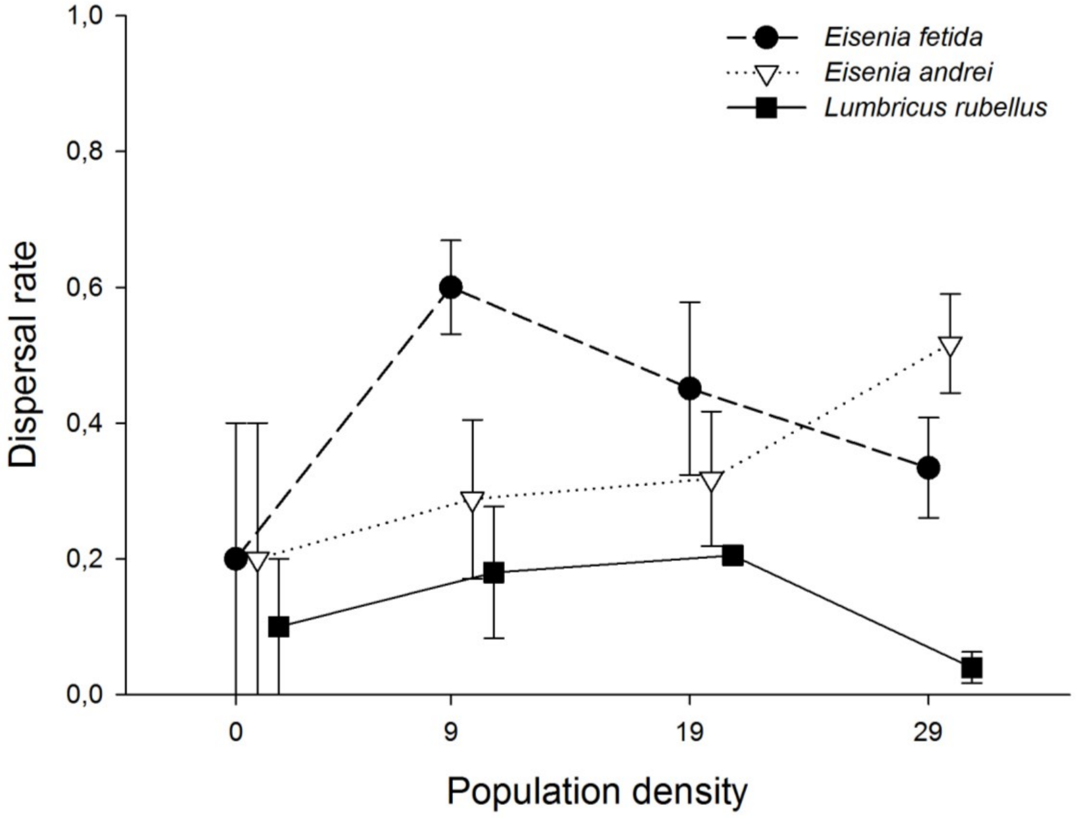

Fig. 4 Dispersal rate (mean ± se) of epigeic (circles), anecic (triangles) and endogeic (squares) earthworms when inoculated in low quality habitat (black symbols) or in high quality habitat (white symbols). Significant differences are indicated by different letters (a, b, c).

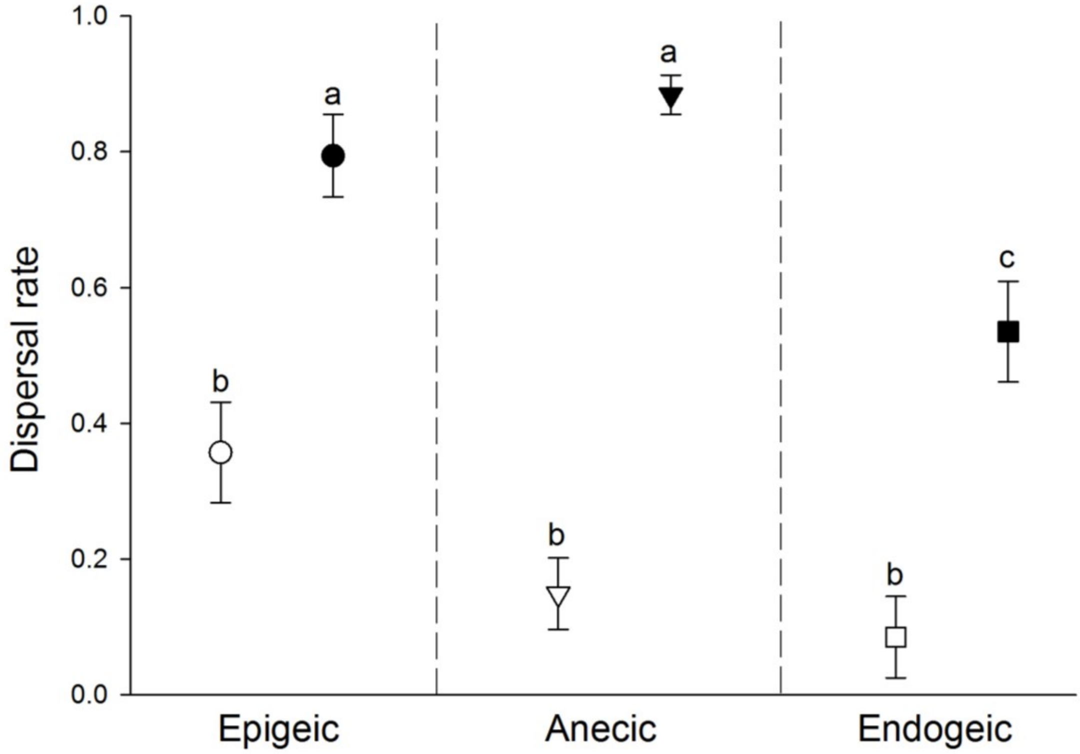

Fig. 5 Relationship between dispersal rate (calculated as the number of earthworms counted in the arrival section over the total number of earthworms) and population density (i.e. 1, 10, 20 or 30 earthworms) according to the functional group (i.e. epigeic, anecic, endogeic). The points are slightly shifted around the correct densities to be clearly distinguishable one from another.

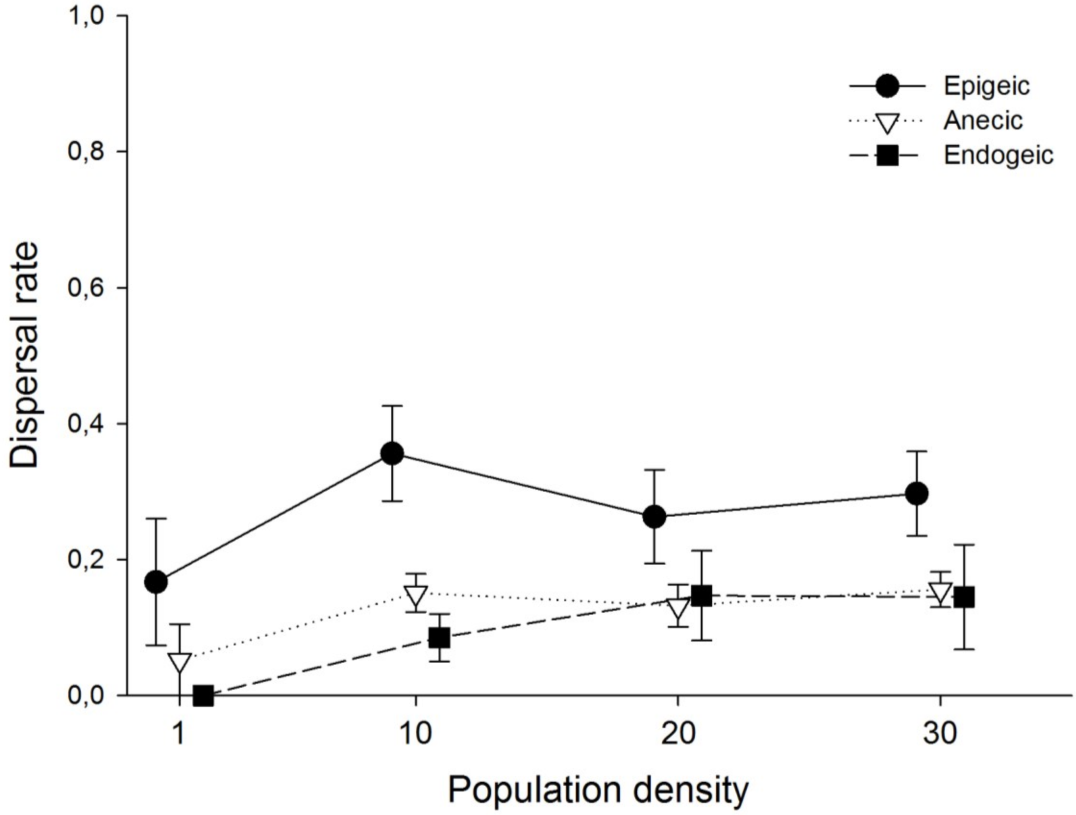

Fig. 6 Partial Least Squares - Discriminant Analysis (PLSDA function in R) on dispersal behaviour (i.e. density sensitivity, quality sensitivity, dispersal rate in homogeneous environment, mean distance crossed in homogeneous environment and tendency to follow conspecifics in homogeneous environment) between functional group (i.e. epigeic, anecic and endogeic).