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Spatial organization of earthworm assemblages in pastures of northwestern France

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ABSTRACT

Studying spatial patterns of soil engineers has become an important issue that may contribute to a better understanding of soil functioning. We investigated the spatial patterns of earthworm species assemblages in a recently settled and an old temperate pasture. Earthworms were sampled following a spatially explicit sampling design. Data were analysed using Spatial Analysis by Distance IndicEs methods to describe the main characteristics of earthworm spatial patterns: aggregation index, patch and/or gap number and size, and the frequencies of species association or dissociation. Ten lumbricid species composed the earthworm assemblages in both pastures, some of them displaying a spatial distribution characterized by clusters with areas of patches and gaps. By comparing aggregation indices between the pastures, an increase of the spatial organization level in the community was observed in the old pasture. We also observed differences between the pastures in the number of aggregated species, cluster characteristics, ratio between spatial association/dissociation and spatial overlap. We also found some significant relationships between species pairs that were already described in the literature, enabling us to discuss the possible nature of interactions. Our results suggest that earthworm spatial distribution and community assembly are likely driven by interspecific interactions at the local scale.

1. Introduction

During the last two decades, the study of spatial patterns in soil fauna distribution has been a major subject of research for soil ecologists. Many studies have shown that soil organisms are not randomly distributed, presenting structured patterns at multiple spatial scales and at different levels of organization from populations to communities. This was described e.g. with microorganisms [1,2], nematodes [3], arthropods [4] and isopods [5]. Earthworm communities have also been described in this aspect in many ecosystems. Distribution of earthworm populations is usually spatially structured at multiple scales, from a few tens to a few hundreds of metres [6–10], with patterns usually consisting in an alternation of patches with high and low population density. In tropical ecosystems, Rossi [11] and Jimenez et al. [12] also reported a short-range spatial variability of earthworm populations at a scale of 2–15 m. In temperate systems, earthworm communities often exhibit spatial patterns over a range of 20–80 m [13–15].

Understanding the driving factors of these spatial patterns is a central issue in soil ecology, because spatial organization of earthworm populations and communities is hypothesized to significantly impact soil functioning. Earthworms are considered as one of the main groups of soil ecosystem engineers (sensu Jones et al. [16]). They participate in essential soil ecological processes (dynamics of soil structure and organic matter) [17], significantly affect life conditions for the whole soil biota [18,19] and play a key role in the provision of major ecosystem services [20]. The distribution of their populations or communities is expected to spatially affect the rate of ecosystem processes such as soil aggregate formation or organic matter mineralization, ending in the creation or maintenance of soil heterogeneity at different scales of space and time with important consequences on ecosystem functioning. Spatial pattern in earthworm communities has been related to (1) environmental factors such as local physico-chemical soil

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properties [21–23] or vegetation structure that drives both the nature of soil organic matter inputs and soil microclimate [14]; and (2) the auto-organization of earthworm populations through their own dynamics, that depends on basic features of population dynamics (e.g. survival, fecundity, dispersion, etc) and on interspecific processes such as facilitation and competition [24–27].

These factors are proposed in a more general context as the main drivers of assembly rules for ecological communities, which refer to the general rules that explain how communities are constituted in response to their environment [28]. Keddy and Weiher [28] distinguish two main types of constraints (i.e. environmental factors and species interactions) that act as environmental filters by deleting from the regional species pool those species that do not present the necessary traits to adapt to local conditions. Moreover, as with any complex biological system [29], communities also present an internal dynamics with an expected trajectory driving the system towards a «self-organized» state through a succession of non-equilibrium stages [20,30,31].

Our knowledge of assembly rules of soil faunal communities remains relatively poor compared with what is known for above-ground biota [24], but for a few taxa such as ants [32,33], terrestrial molluscs [34] and beetles [35]. Some recent studies have, however, provided new insights into the understanding of earthworm community dynamics [7,26,27,36–39]. They highlighted that community organization is mainly driven by habitat constraints at large scales (i.e. region scale or environmental gradient), and by environmental heterogeneity (i.e. soil and vegetation patchiness) and biotic constraints (i.e. spatial segregation between species exhibiting a high niche overlap) at fine scales. At a local scale, earthworm species assemble to form complex communities whose spatial organization may also result from the existence of several structuring constraints acting at different spatio-temporal scales.

In this study, we aimed at testing the relevance of the organization concept of Kolasa and Pickett [29] in the context of soil communities. According to this concept, organized communities are likely to present a higher degree of spatial structuring due to the combined effect of competitive exclusion, niche partitioning and environmental heterogeneity [30]. We compared earthworm community structure in two temperate pastures of different ages using density and diversity measures and spatial metrics, and expected to find a higher degree of organisation in the older pasture compared to the younger. We finally discuss how our results highlight the relative importance of the physical environment and of interspecific competition in the spatial structuring of these communities.

2. Materials and methods

2.1. Study site description

The study was carried out at the “Lycée Agricole d’Yvetot” (Seine Maritime, France), on a loamy plateau located 200 km northwest of Paris (49°37′04.00″N, 0°45′18.76″E). Climate is temperate oceanic; average yearly rainfall and temperature are 800 mm and 10 °C, respectively. The almost entire surface of the surrounding landscape is covered by a thick layer of loess material (>80 cm). Soils are classified as NEOLUVISOL-LUVISOL (French Classification; INRA 1999; clays = 15%, silts = 66% and sands = 19%, mean pH = 6.1).

The site is mainly dedicated to agricultural production with a significant proportion of permanent pastures or pasture/crop rotations. We selected two pastures with contrasting ages: a 5-year-old pasture (P1) and a more than 42-year-old pasture (P2). Both pastures were located on the same topographic situation with some significant differences in soil properties between them (Table 1; Mathieu et al., unpublished data). Pasture establishment was conducted in a similar way for both plots: soil was deeply ploughed and an input of 50 tons ha^{-1} of cattle manure was incorporated superficially. Seeds of *Lolium* sp., *Trifolium repens* L., *Festuca elator* L., *Phleum pratense* L. were sown in autumn at a rate of 21 kg ha^{-1}. Afterward, an annual fertilization of 180 kg of N ha^{-1} was applied. Pastures were grazed by cattle for milk production from mid-March to mid-September with a stocking rate of 2–5 animal units ha^{-1} depending on the season.

2.2. Earthworm sampling

Earthworms were collected in March–April 2009. In each pasture, sampling was done on a 10-m mesh grid of 120 × 70-m, and a total of 104 points were sampled. In P2, the number of samples was reduced to 87 for logistical reasons. In both pastures however, the total number of points was higher than the minimal number recommended for relevant spatial analyses ($n = 60$ [40]). At each point, earthworms were sampled using a combination of formaldehyde extraction and hand-sorting. First, 10 l of 4‰ formaldehyde were applied on a 1 m^2 surface, and earthworms expelled at the soil surface were collected during a 15 min period. Then, a soil volume of 25 × 25 × 25 cm and 30 cm depth was dug out in the centre of the square meter and hand sorted in the field. Earthworms collected by the two different methods were stored separately. This allowed for the estimation of the density of individuals that were not successfully collected by formaldehyde extraction and readjusting density data when necessary. Specimens were fixed in pure alcohol and categorized as juveniles (aclitellates) and adults (clitellates). Adults were identified to species level according to Sims and Gerard [41]. Densities per species, per ecological categories and per age stages were expressed in adult individuals m^{-2}. Juveniles were therefore not taken into account for the estimation of species density but only when calculating total earthworm density.

2.3. Statistical analysis

The first step of data analysis consisted of comparing mean densities and diversity indices. As density data did not match the basic assumptions of normality and homoscedasticity required for parametric statistics (Wilk–Shapiro test at significant level of $p = 0.05$), we used Wilcoxon Rank-Sum test to compare these data between the two pastures. Diversity indices were calculated for each pasture: SR, the mean species richness (i.e. the number of species observed per sampling point or per m^2); and J', the mean Shannon Evenness index, a structural index which reflects the distance of the sample from equirepartition. Mean diversity indices were compared using Wilcoxon Rank-Sum test. Statistics were

Table 1
Main characteristics of the two sampled pastures. Standard error in parenthesis; different letters indicate significant difference at $p < 0.05$ (Wilcoxon Rank-Sum test).

Pasture type	Age (years)	pH	Organic C (g kg^{-1})	Total N (g kg^{-1})	C-to-N ratio	Organic matter (g kg^{-1})	CEC (cmol kg^{-1})
P1 (temporary)	5	5.79 (0.05) a	15.20 (0.29) a	1.54 (0.03) a	9.82 (0.05) a	26.29 (0.50) a	7.91 (0.05) a
P2 (permanent)	≤42	5.60 (0.04) a	27.76 (0.43) b	2.53 (0.03) b	10.95 (0.06) b	48.03 (0.74) b	10.25 (0.10) b

performed using the R software [42] and diversity indices using the PAST software [43].

In a second step, we analysed earthworm counts (individuals per sampling point) with the Spatial Analysis using Distance IndicEs (SADIE) [44], a method which allows an accurate description of count distribution and a delimitation of spatial clusters. It was successfully used in a few previous studies addressing earthworm spatial distribution [11,12]. SADIE refers to the term "cluster" as a region of either relatively high or low density, which are further referred to as "patches" or "gaps", respectively. It consists of three different phases:

1) For each species and each ecological category, we computed using count data an index of aggregation (I*a*) that allows detecting random (I*a* = 1), aggregated (I*a* >1) or regular (I*a* <1) distributions. Additionally, a local clustering index was calculated for each sample point. This index measures the degree of clustering of the data into patches or gaps, respectively positive (vi) and negative values (vj). Fixed values of 1.5 and −1.5 were used as heuristic thresholds for vi and vj index values respectively. Sampling units associated with index values >1.5 indicated patches, whereas sampling units associated with index values <−1.5 revealed the presence of gaps. These indices were tested with 5967 permutations (the maximum number of permutations supported by the SADIE software) following a Monte Carlo procedure under the null hypothesis of a random spatial pattern, and the number of significant I*a* indices was then compared between the two pastures.
2) We further mapped the values taken by the clustering index (vi and vj) to produce contour maps that described the characteristics of the clusters. Once clusters are identified, they are described as follows:
    a. the number of cluster (NC) of a given type (patches and gaps) observed in a given site;
    b. the % of the site area covered by a given cluster type;
    c. the mean cluster size in m^2 for each cluster type.
3) The interaction between spatial patterns of two species can be described using an SADIE association index [45]. This was used to test the spatial association or dissociation between all earthworm species pairs (45 comparisons). Each value taken by the index was further tested against the null hypothesis of complete spatial independence of species counts, allowing the identification of significant spatial association (positive values) or dissociation (negative values). The number of significant spatial relationships was then counted and expressed as a percentage of the total number of comparisons in the two pastures.

SADIE statistics were computed with the SADIE software (http://www.rothamsted.bbsrc.ac.uk/pie/sadie/). For detailed description on SADIEs procedures, see [44,46,47]. Contour maps were produced with the software SURFER (Golden software, Colorado, U.S.A.).

Finally, we used abundance data to calculate mean spatial overlap for each pasture. We built a matrix where each row represented a sampling point and each column represented a species. The spatial overlap was calculated for each pair of species using Pianka's index of niche overlap: $O_{12} = O_{21} = \sum_{i-1}^{n} p_{2i}p_{1i} / \sqrt{\sum_{i-1}^{n}(p_{2i}^2)(p_{1i}^2)}$ where O_{12} is the overlap between species 1 and species 2; and p_{2i} and p_{1i} are the proportion of resource i (space—proportion of species in the sample) used by species 1 and 2. Pianka's index is a symmetric index that ranges from 0 (absence of overlap) to 1 (complete overlap). We computed the mean spatial overlap at the scale of each pasture by averaging the values calculated for all possible pairs of species present in the corresponding assemblages. Niche overlap calculations were performed using the "spaa" package for the R software [42] and mean spatial overlaps were compared between pastures using Wilcoxon Rank-Sum test.

3. Results

3.1. Density and diversity patterns

A total of 10 earthworm species were found in each pasture (Table 2), including representatives of the three ecological categories defined by Bouché [48]. Endogeics (i.e. species living in the soil matrix and feeding on soil organic matter) were numerically dominant and were represented by four species: *Aporrectodea caliginosa* (Savigny 1826), *Aporrectodea icterica* (Savigny 1826), *Aporrectodea rosea* (Savigny 1826), and the green morph of *Allolobophora chlorotica* (Savigny 1826). Anecics (i.e. species living in the soil and feeding mainly on surface litter) were represented by *Lumbricus terrestris* (Linné 1758), *Lumbricus festivus* (Savigny 1826), *Aporrectodea giardi* (Ribaucourt 1901) and *Aporrectodea longa* (Ude 1885). Epigeics (i.e. surface dwelling earthworms that feed on surface litter) were also present in the assemblage with *Lumbricus castaneus* (Savigny 1826) and *Satchellius mammalis* (Savigny 1826).

Species richness per point or per sample ranged from 2 to 9 species m^{-2} in both pastures with no significant difference between the means. Means of Shannon Evenness were high (>0.69) in both pastures, although slightly lower in P2 than in P1 (Table 2).

Densities calculated for age classes and ecological categories varied between pastures, except for total anecics, adults and immatures. Total anecic and adult densities were significantly higher in P1, while immature density was higher in P2. Several species differed significantly in their density between pastures. *A. icterica*, *A. chlorotica*, *A. giardi*, and *S. mammalis* were more abundant in P1, while *A. rosea* and *L. castaneus* presented an opposite pattern (Table 2).

3.2. Spatial aggregation patterns

The values taken by the index of aggregation I*a* were contrasted among species, and were also different among pastures (Table 3).

Table 2
Mean earthworm density (ind m^{-2}) in the two pastures. Standard error in parenthesis; different letters indicate significant between pasture differences at $p < 0.05$ (Wilcoxon Rank-Sum test); samples number was of 104 and 87 in P1 and P2, respectively.

Species/groups	Pasture 1 (P1)	Pasture 2 (P2)
A. caliginosa	43.10 (3.51) a	53.21 (5.11) a
A. icterica	19.94 (2.10) a	12.11 (1.66) b
A. chlorotica	22.80 (3.42) a	2.74 (0.69) b
A. rosea	4.25 (1.01) b	7.26 (1.29) a
Total endogeics	90.09 (6.76) a	75.32 (6.15) a
A. giardi	26.67 (2.27) a	16.55 (2.06) b
L. festivus	5.70 (1.01) a	4.80 (0.88) a
A. longa	3.49 (0.94) a	2.05 (0.60) a
L. terrestris	0.45 (0.23) a	0.15 (0.06) a
Total anecics	36.32 (2.82) a	23.55 (2.32) b
L. castaneus	11.43 (1.58) b	18.55 (2.92) a
S. mammalis	9.38 (1.79) a	2.98 (0.81) b
Total epigeics	20.81 (2.57) a	21.53 (3.15) a
Adults	147.21 (9.16) a	120.40 (8.01) b
Immatures	172.44 (10.55) a	209.91 (10.74) b
Total	319.65 (16.74) a	330.31 (16.82) a
Richness min	2	2
Mean of richness	5.77 (0.16) a	5.31 (0.15) a
Richness max	9	9
Shannon evenness	0.76 (0.01) a	0.69 (0.02) b

Table 3
SADIE aggregation indices and associated probability levels for earthworm species and ecological groups in the two sampled pastures. P1 = pasture 1; P2 = pasture 2; I*a* = global index of aggregation; mean vj and vi = mean negative and positive index values indicating gaps and patches, respectively; probability levels of significant indices are indicated as follows: * $p < 0.05$; ** $p < 0.01$ (tested with 5967 permutations, Monte Carlo procedure under the null hypothesis of a random spatial pattern).

Species/Groups	P1			P2		
	I*a*	Mean vj	Mean vi	I*a*	Mean vj	Mean vi
A. caliginosa	1.321	−1.263	1.205	1.611 **	−1.759 **	1.506 *
A. icterica	0.987	−1.056	0.969	0.923	−0.904	1.017
A. chlorotica	1.639 **	−1.590 **	1.549 **	0.976	−0.951	0.970
A. rosea	1.178	−1.196	1.196	1.468 *	−1.433	1.421
A. giardi	0.998	−0.977	0.993	1.424 *	−1.445 *	1.419 *
L. festivus	1.263	−1.274	1.219	1.020	−1.031	1.014
A. longa	0.882	−0.868	0.843	1.051	−1.004	1.022
L. terrestris	1.323 *	−1.314	1.443 *	1.748 **	−1.815 **	1.710 **
L. castaneus	0.972	−0.981	0.909	1.034	−1.071	0.974
S. mammalis	1.085	−1.083	1.083	1.107	−1.106	1.119
Anecics	1.007	−1.007	0.984	1.665 *	−1.645 *	1.569 *
Endogeics	1.478 *	−1.440 *	1.330 *	1.581 *	−1.643 *	1.491 *
Epigeics	1.100	−1.083	1.133	1.145	−1.207	1.063
Adults	1.318 *	−1.299 *	1.136	1.380 *	−1.460 *	1.199
Immatures	1.673 *	−1.630 *	1.537 *	1.898 *	−1.888 *	1.689 *
Total	1.712 *	−1.640 *	1.576 *	1.782 *	−1.856 *	1.681 *

The spatial distribution was well structured in both pastures for most of the groups tested (i.e. ecological categories, demographic groups and earthworm total), except for anecics in P1 and epigeics in both plots (Fig. 1 and Supplementary data). Species distribution patterns were less consistent. Random spatial distribution was observed for most of species, except for a few ones: *L. terrestris* presented significant patches and gaps associated with a high I*a* index in both pastures, as did *A. chlorotica* in P1 and *A. caliginosa*, *A. rosea* and *A. giardi* in P2. Moreover, the total number of species displaying significant I*a* index was higher in the old than in the young pasture, with 4 and 2 species respectively.

3.3. Cluster characteristics

Table 4 shows the main cluster characteristics for each species observed in the two pastures. We found significant between species and between pasture differences in the number of patches and gaps and their relative spatial covering. The relative surface covered by clusters (patches and/or gaps) was generally low, meaning that species distribution was random in most of the plot surface. However, the mean cluster surface was higher in P2 than in P1. The numbers of patches and gaps were quite variable in the two pastures. In P1, the number of gaps ranged from 1 to 8 with a mean of 4.2 ± 0.6, while in P2 it was of 1–8 with a mean of 3 ± 0.7. The number of patches was lower than for gaps in both pastures, and it was higher in P1 where it ranged from 0 to 7 with a mean of 2.7 ± 0.7 than in P2 where it varied between 0 and 6 with a mean of 2.3 ± 0.6.

3.4. Species spatial association and spatial overlap

Table 5 presents all significant pair-wise indices of species spatial association. Spatial association among earthworm species varied across pastures. In P1, we found 6 pairs of species presenting a significant spatial association. In P2, we found 14 significant index values, 8 and 6 corresponding to spatial association and dissociation, respectively. As a consequence, the percentage of total significant indices was higher in P2 than P1 (31.1% and 13.3% respectively), and the ratio between spatial association/dissociation was more balanced in P2. The mean spatial overlap of the whole earthworm communities tended to be lower in P2 than in P1, but did not differ significantly between them (Table 6).

4. Discussion

4.1. Density and diversity patterns

The density and diversity values found in our study are in the range of results published in previous studies of similar agro-ecosystems [9,37,49]. The composition of the local species assemblages described herein is similar to what Decaëns et al. [36] call the habitat species pool of temperate pasture in their synthetic study of earthworm assembly rules at the scale of the Haute-Normandie region. Similarly, we found that species assemblages are dominated by endogeic species, which is a classical feature of temperate pastures that can be related to a low shoot–root ratio in the vegetation and the presence of a deep A-layer that provides

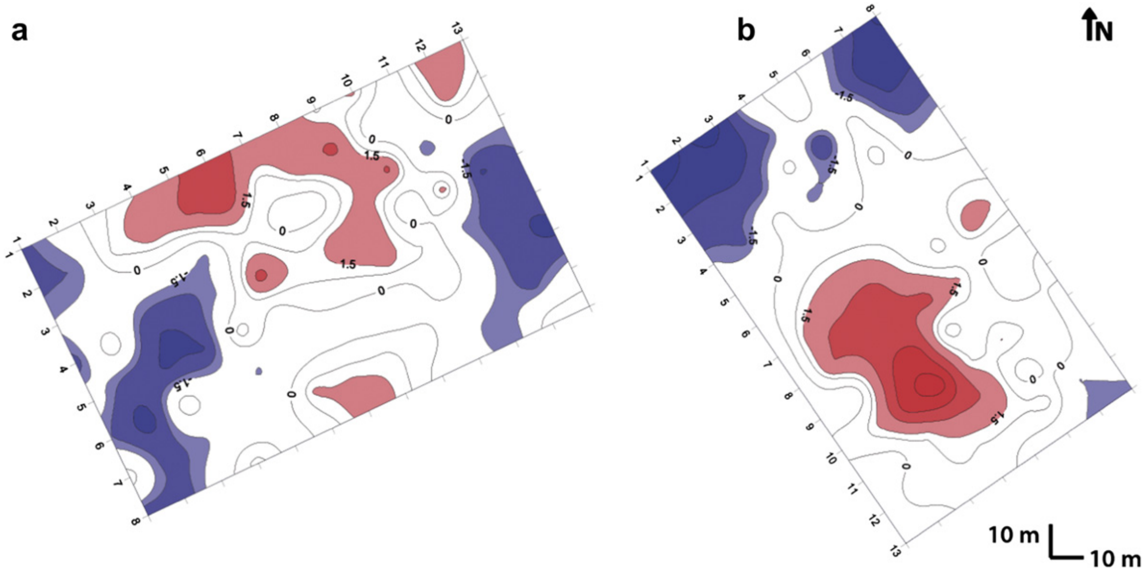

Fig. 1. Contour maps of clustering indices for counts of total earthworms in the two pastures: (a) Pasture 1, (b) Pasture 2. Red shading indicates significant patches (index values >1.5); blue shading indicates significant gaps (index values <−1.5). Lines represent iso − index values interpolated.

Table 4
Cluster characteristics for each species observed in the two pastures P1 and P2. P1 = pasture 1; P2 = pasture 2; NC = number of clusters of a given type (patch or gap); % site area = percentage of the site area covered by a given cluster type; SEM = standard error of the mean cluster area calculated for each species (in column) or of the means of the different indices calculated for the whole community (lower rows).

Species	P1							P2						
	Type	NC	% site area	Mean cluster area (m^2)	SEM	Min cluster area (m^2)	Max cluster area (m^2)	Type	NC	% site area	Mean cluster area (m^2)	SEM	Min cluster area (m^2)	Max cluster area (m^2)
A. caliginosa	Gap	8	10	104	76	6	628	Gap	5	18.6	312	185	3	769
	Patch	5	4.5	74	37	3	211	Patch	4	12.5	262	225	3	934
A. icterica	Gap	5	6.2	103	64	1	330	Gap	1	0.1	1	–	1	1
	Patch	2	0.2	4	3	1	7	Patch	3	4	112	24	67	151
A. chlorotica	Gap	4	28.8	605	465	3	1990	Gap	3	5.7	158	77	56	308
	Patch	7	9.9	118	47	1	330	Patch	0	0	0	–	0	0
A. rosea	Gap	4	19.7	412	409	1	1637	Gap	4	14.5	303	159	9	746
	Patch	4	1.6	32	30	1	121	Patch	4	5.3	111	44	4	205
A. giardi	Gap	3	3.2	89	55	7	192	Gap	8	13.1	137	103	1	847
	Patch	1	0.6	46	–	46	46	Patch	6	6.9	97	47	3	279
L. festivus	Gap	3	20.4	569	378	17	1291	Gap	2	12.5	523	104	419	626
	Patch	2	1.8	76	73	3	148	Patch	1	0.2	10	–	10	10
A. longa	Gap	3	6.8	188	90	43	353	Gap	3	14.4	401	346	4	1089
	Patch	0	0	0	–	0	0	Patch	0	0	0	–	0	0
L. terrestris	Gap	4	27.4	575	565	7	2271	Gap	1	41.7	3501	–	3501	3501
	Patch	2	0.5	20	3	17	23	Patch	2	4.5	187	142	46	329
L. castaneus	Gap	2	4.1	171	168	3	339	Gap	1	11.4	957	–	957	957
	Patch	1	0.2	10	–	10	10	Patch	1	0.1	7	–	7	7
S. mammalis	Gap	6	12.6	175	120	7	762	Gap	2	15.6	655	627	29	1281
	Patch	3	1.2	32	25	1	83	Patch	2	0.4	14	0	14	14
Mean	Gap	4.2	13.9	299.1		9.5	979.3		3	14.8	694.8		498	1012.5
	Patch	2.7	2.1	41.2		8.3	97.9		2.3	3.4	80		15.4	192.9
SEM	Gap	0.6	3	68.3		4	241.2		0.7	3.4	323.9		347.6	300
	Patch	0.7	1	12		4.5	34		0.6	1.3	28.7		7.2	91.4

abundant trophic resources (soil organic matter and dead roots) for this ecological category [36,48,50].

4.2. Species distribution patterns

We found that 5 species out of 10 had non-random spatial distribution and cluster (patches and gaps) characteristics varied among species in both number and size. This result is congruent with the findings of other studies in which earthworm spatial patterns were found to be clumped, whatever the ecosystem studied, in patches of varying size. At fine scales, patches are usually in the range of a few metres (i.e. less than 15 m [11,12]) to a few tens of metres [9], while at the largest scales species are usually aggregated at scales less than one hundred metres [15,21]. The scale at which *L. terrestris* was aggregated in P2 (15 m) is in accordance with the results reported by Valckx et al. [9] and Cannavacciuolo [13] who described a patch size ranging from 10 to 45 m for this species. Patch sizes found for *A. caliginosa* (26–60 m), *A. rosea* (18–53 m) and *A. longa* (40 m) are also in the same order of magnitude as those found in other studies [9,21,51]. Otherwise, for other species our results represent the first description of their spatial attributes. The patchiness observed in the distribution of the

Table 5
Significant SADIE associations index between species. Only SADIE indices with significant *p*-values are represented. P1 = pasture 1; P2 = pasture 2; $^{*}p < 0.025$ (associations); † $p > 0.975$ (dissociations).

Interactions groups	P1		P2	
	Species	Index of association	Species	Index of association
Anecics ∩ Anecics	–		*L. festivus*/*A. longa*	−0.363 †
	–		*L. terrestris*/*A. giardi*	0.323 *
	–		*A. longa*/*A. giardi*	0.207 *
Epigeics ∩ Epigeics	–		–	
Endogeics ∩ Endogeics	*A. chlorotica*/*A. caliginosa*	0.261 *	*A. rosea*/*A. icterica*	0.346 *
	A. rosea/*A. caliginosa*	0.261 *	*A. rosea*/*A. caliginosa*	0.441 *
Anecics ∩ Epigeics	–		*L. terrestris*/*L. castaneus*	−0.360 †
	–		*L. terrestris*/*S. mammalis*	−0.273 †
	–		*L. festivus*/*S. mammalis*	0.238 *
			A. longa/*S. mammalis*	−0.291 †
Anecics ∩ Endogeics	*L. terrestris*/*A. icterica*	0.249 *	*L. terrestris*/*A. caliginosa*	−0.345 †
			L. terrestris/*A. rosea*	−0.380 †
			A. longa/*A. chlorotica*	0.268 *
Epigeics ∩ Endogeics	*L. castaneus*/*A. chlorotica*	0.275 *	*S. mammalis*/*A. rosea*	0.346 *
	S. mammalis/*A. rosea*	0.235 *	*S. mammalis*/*A. caliginosa*	0.238 *
	S. mammalis/*A. icterica*	0.236 *		
% Total association		13.3		17.8
% Total dissociation		0.0		13.3
Mean spatial overlap		0.33 ± 0.05		0.26 ± 0.05
Min spatial overlap		0.01		0.00
Max spatial overlap		0.66		0.63

Table 6
Spatial overlap index between all species pairs ($n = 45$). P1 = pasture 1; P2 = pasture 2; SEM = standard error; Min = minimum value; Max = maximum value.

Spatial overlap	P1	P2
Mean ± SEM	0.33 ± 0.05	0.26 ± 0.05
Min	0.01	0.00
Max	0.66	0.63

dominant species further determined the patchiness in the distribution of ecological categories considered as a whole (Fig. 1, Supplementary data). Accordingly, the mean aggregation level differed significantly between these ecological groups, with endogeics and anecics being more aggregated than epigeics.

Clumped spatial distribution of earthworm species can be explained by the action of at least one or more driving factors such as biotic interactions or heterogeneity in soil characteristics. Many studies have demonstrated that earthworm spatial distribution can be correlated with soil physico-chemical properties such as organic carbon [51] or soil hydromorphy [13,15]. However, in other cases a lack of relationships was also reported between environmental conditions and species spatial distributions [11,23]. As proposed by Rossi [52], the presence of a high proportion of random clusters suggests that environmental factors are weakly involved as drivers of species spatial patterns. Obviously, a formal test of this idea would require a description of the spatial heterogeneity in soil properties at a plot scale in both pastures. Despite such soil data are not available in our study, the hypothesis of a weak influence of physical environment is at least partly supported by the rather homogeneous soil and vegetation conditions that prevailed at the plot scale, which seems unlikely to drive species spatial patterning. Although not formally tested in our study, we could find no a priori relationship between earthworm spatial features and topography, tractor tracks or the presence of drinking troughs that would have caused increased cattle trampling in some areas of the study plots.

In the absence of environmental heterogeneity, the formation of clusters in earthworms may for instance result from the associated influence of demographic processes, dispersal behaviour and interspecific interactions [24,53]. Rossi and Lavelle [54] and Jiménez et al. [55] have reported that aggregation may depend greatly on the demographic and morphological features of earthworms and this relationship was well established for species from tropical systems. Epigeic species are often reported to be strongly aggregated due to their small size, their low population density at the plot scale, their high reproductive rates and short life cycle (annual), and their demographic strategy [12,54]. Our study contrasts with these previous results, and the absence of aggregation in epigeics in our results may be attributed to low population density or to an excessive sample surface and inter-sampling distance to detect small-scale clumped distribution. However, as mentioned by Jiménez et al. [55], cautious conclusions must be drawn to establish the patterns that define the aggregation of earthworm species. These authors observed that species of medium to large size, such as endogeics and anecics, might be more aggregated than epigeics. Population patchiness could also result from particular behaviour such as aggregative behaviour [56], sedentary behaviour [57], or dispersal behaviour [53,58]. The low vagility of endogeic species makes it difficult to determine whether spatial aggregation of species belonging to this group is the result of demographic process [59,60].

Interspecific competition is thought to play a major role in the spatial structuring and general organization of earthworm communities [25,61]. Decaëns et al. [36] found consistent patterns suggesting a high importance of competition in the assembly of earthworm communities in temperate ecosystems of northern France. In a meta-analysis, Uvarov [62] also showed that most interspecific interactions are negative in the laboratory, suggesting a predominance of competition in the field conditions. Evidence of competition between pairs of species have already been provided in experimental studies, including cases of direct competition with consumption of cocoons [63,64] or indirect effects of anecic species such as *L. terrestris* that affect epigeic populations by reducing their food resources through their "litter-burying activities" [65]. However, the importance of interspecific competition in shaping earthworm community structure is difficult to assess *in situ*. Competition may be occasional and the apparent absence of competition does not represent clear evidence that no competition has influenced population dynamics and community assembly in the past (see the "*ghost of competition past*" [66]).

Recent studies that used factorial statistics and null-model analyses from spatially explicit sampling protocols have provided evidence that competition represents a key factor in the spatial structuring of earthworm assemblages in tropical systems [7,25]. In temperate systems, although no attempt has been made to date to analyse spatially explicit data using null-models, evidence of competition have yet been provided at both a regional scale and at the scale of a succession of ageing pastures [36,37]. In our study, the results of spatial dissociation between some species pairs in P2 and the weak spatial overlap support the hypothesis of the driving role of competition in an earthworm community. Further research is however needed to complement our findings on competitive interactions at the patch scale and assess how these may interact with soil physico-chemical properties to generate spatial patterning in earthworm assemblages.

Mechanisms of facilitation or earthworm engineering activities could also be proposed as factors generating the spatial structure of earthworm assemblages [24,53,58,62]. We found that the endogeic species *A. caliginosa* and *A. rosea* were spatially associated with each other, which supports previous observations by Valckx et al. [9] and Mathieu et al. [53]. Indeed, some soil-dwelling species are known to benefit from the presence of others in laboratory experiments [67]. For instance, Lowe and Butt [68] observed that endogeics could benefit from living in the neighbourhood of anecic species. It is tricky to define if this kind of relationship can account for the observed spatial interaction between anecics and endogeics, because we found as many associations as dissociations. Anecic species such as *L. terrestris* may also create a network of galleries that can represent favourable microsites for epigeic species [69,70], but in our results no anecic species was involved in significant positive relationship with epigeics. Conversely, we observed a high number of spatial dissociation between anecics and epigeics. This could be explained by the activities of anecics that reduced resource availability for epigeics by removing the litter layer from the soil surface [65].

4.3. *Community organization and dynamics*

The spatial and diversity patterns described herein constitute a relevant example to illustrate the organization and dynamics of local species assemblages [36,71]. Even if plots were not replicated, the differences in community structure between two pastures of contrasting ages can be confronted to the results of other studies to propose some hypotheses. Based on the theory of organization of ecological systems, the comparison of the two pasture systems suggests that earthworm communities were at two different stages of their assembly process [29]:

- In the young pasture, we observed high density and evenness values. Species aggregation and interspecific spatial dissociation were relatively low with an important spatial overlap between species. All these results suggest a state of non-equilibrium with interspecific interactions having little influence on assemblage structure. Indeed, this pasture system was relatively young (5 years) and may thus correspond to a post-colonisation stage following pasture settlement, in which species may co-occur in a transitory way after re-colonization mechanisms have begun.

- In the old pasture, a higher spatial organization of communities was observed, with more species presenting a significant spatial structure, lower species densities and evenness, more significant spatial interactions between species and lower spatial overlap observed between species pairs. These observations suggest a near equilibrium state in which species are spatially segregated as a result of past and/or present competitive exclusion, and environmental micro-heterogeneity [30,66].

5. Conclusion

In this study, we provided new insights in the understanding of earthworm assembly rules and how these result in spatial structuring of species assemblages at the local scale. By analysing diversity and spatial indices in two pastures of different ages, we provided evidence of the driving role of local factors on earthworm spatial distribution and community assembly. Moreover as initially hypothesized, we observed a higher strength, diversity and grain of spatial organization in the older pasture, which suggests a shift from non-equilibrium towards equilibrium states during pasture ageing. These results support the hypothesis of a structuring of earthworm communities by interspecific interactions. However further research is still needed to distinguish the nature of species interactions that shape earthworm assemblages and identify the age at which a pasture can be considered as exhibiting a stable interaction pattern. As used in a recent study [38], a combination of spatially explicit statistics and null-model analyses may represent a useful option to test whether communities are structured by competition or facilitation.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejsobi.2012.08.005.

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