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Geostatistical and multivariate analysis of the horizontal distribution of an earthworm community in El Molar (Madrid, Spain)

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Summary

The earthworm community in El Molar (Madrid) is studied, and its distribution patterns and relation with some soil factors are described by using geostatistic and multivariate tools. Six species were found, *Hormogaster elisae*, *Allolobophora rosea* and *Allolobophora caliginosa* being the three most abundant ones. These species exhibited a clumped distribution. The most dominant species, *H. elisae*, was distributed in patches of an average size of 45 m in spring and more than 100 m in autumn. *A. rosea* was aggregated in patches of an average size of 22 m and *A. caliginosa* formed patches of an average size of 38 m. There seemed to be a positive correlation between the abundance of *H. elisae* and the percentage of total and coarse sands, as well as a negative correlation with clay, nitrogen, carbon and coarse loams contents, opposite to what was observed for *A. rosea*. © 2007 Elsevier GmbH. All rights reserved.

Introduction

During the past decade many authors have analysed the spatial distribution of soil fauna, and have found that clumped or aggregated patterns are the most common in natural populations (Pielou

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1977), particularly for earthworms (Rossi and Lavelle 1998; Jiménez et al. 2001). Such a distribution may be caused, among other reasons, by spatial heterogeneity, as a response to abiotic factors, or by particular gregarious or reproductive behaviours. Some authors have used aggregation indices (Rossi and Lavelle 1998) to describe horizontal distribution patterns shown by earthworms, and have studied the factors that may influence this distribution by analysing the biological relationships that exist

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within earthworms and among them and other organisms (Judas 1989), as well as the relationship with soil factors (Rossi et al. 1997; Nuutinen et al. 1998). Other authors, such as Rossi (2003) used partial triadic analysis (PTA) to study spatiotemporal patterns shown by certain earthworm populations.

Geostatistics, developed by Matheron (1971), is another method used to determine the spatial structure of soil organisms, and has been applied in earthworm distribution studies by authors such as Cannavacciuolo et al. (1998), Jiménez et al. (2001) and Whalen and Costa (2003), among others. It is a more efficient and robust tool than dispersion indices, which only analyse distribution of organisms within sampling units.

Through the years, we have sampled numerous times in El Molar to carry out the study of different aspects of the biology of *Hormogaster elisae* (Valle et al. 1997, 1999; Garvín et al. 2002; Hernández et al. 2003). We have observed that this species appears alone in a part of the plot, while it coexists with other species elsewhere. This has led us to formulate hypotheses to explain this distribution, among which have been soil characteristics or competition among species.

In a previous contribution (Hernández et al. 2003), we initiated the study of the horizontal distribution of earthworms at El Molar. The results allowed us to confirm our initial expectations regarding earthworm distribution and to establish hypotheses concerning possible relationships between earthworm distribution patterns and soil factors. We have conducted two further sampling surveys (spring and autumn 2002) and obtained new data sets, which have been analysed using other techniques, such as Geostatistics, in order to complete the horizontal distribution of earthworm study at El Molar and characterize their spatial structure at El Molar.

Materials and methods

Study area

The sampling site is located on the outskirts of El Molar (Madrid, Spain) UTM. 30TVL524095. Climatic and edaphic characteristics of the site are described in Valle et al. (1997) and Garvín et al. (2002). A small stationary stream which only carries water a few days a year, after heavy rainfalls, flows at the far end of the plot. It is a cleared zone, quite steep, where remnants of old terraces prevail. Vegetation is typical of a transition zone, with annual plants and aromatic species such as *Lavandula stoechas* L. subsp. *pedunculata* (Miller) and *Thymus zygis* L. predominating.

Earthworms and soil sampling

We performed three sampling surveys. During the first one (spring 2001) we sampled a plot of $98 \text{ m} \times 70 \text{ m}$ divided by a grid into 28 subunits of $16 \text{ m} \times 14 \text{ m}$. Our previous field observations led us to increase the sampling area in order to include a specific zone located beyond the stream, where *H. elisae* was collected during exploratory sampling (Fig. 1). This resulted in a sampling plot size of $112 \text{ m} \times 84 \text{ m}$, where 42 subunits were sampled during spring and autumn 2002, respectively.

Sampling units were located in the corner of the subunits and separated by a minimal distance of 14 m; earthworms were sampled by the formol-manual separation method from a $100 \text{ cm} \times 50 \text{ cm} \times 25 \text{ cm}$ soil monolith cleared of vegetation. Specimens were fixed in a 1:1 mixture of 10% formalin and 96% ethyl alcohol solution. Earthworms were identified according to Álvarez (1971, 1977) and Bouché (1972). After sorted, they were kept in 10% formalin. Analyses of soil samples were done as indicated in Hernández et al. (2003).



Fig. 1. Representation of study zone. Shaded cells are the additional zone sampled at spring and autumn of 2002. Black line represents the stationary stream.

Statistical analyses

To detect earthworm species distribution patterns we used two dispersion indices: the Morisita index (I_{δ}) and Taylor's coefficient (b).

Taylor's Power Law is based on the relationship between mean (m) and variance (S^2) and is expressed as

 $S^2 = am^b$ or $\log S^2 = a + b \log(m)$,

where S^2 is the variance of the number of organisms in the samples, *m* is the mean, *a* is the intercept with the ordinate axis and *b* represents the slope of the regression curve between variance and the population's mean and indicates the level of aggregation.

In this equation, b takes the value 1 in a random distribution, 0 in a uniform distribution and is > 1 in an aggregated one.

We also calculated the percentage of combined dominance (PCD) (Jesús et al., 1981).

Geostatistics is also referred to as the Theory of Regionalized Variables, since each variable is associated with a position in space. To express the relationship between semivariance of a variable and separation among samples (lag), a semivariogram is used. Semivariance, $\gamma(h)$, is calculated as

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [Z(X_i) - Z(X_i + h)]^2,$$

where $Z(X_i)$ is the observed value of variable Z in X_i , $Z(X_i+h)$ the observed value of variable Z in X_i+h , h the separation between sampling spots and N(h)the number of comparisons between a given distance h. The shape of the semivariogram can be adjusted to various theoretical models and describes the degree of spatial dependence (autocorrelation) of the variable (Robertson 1987), which decreases as distance h increases (Moral 2004).

The semivariance value when $\gamma(0) > 0$ is known as nugget variance (Co) and is caused both by sampling errors and by the spatial variability occurring within the minimum distance interval. The part of the variance attributed to spatial correlation is the spatial variance (*C*). The sill (Co+*C*) is the asymptote of the model and the range (*A*) represents the larger distance that may occur between correlated samples and measures spatial dependence between them. The percentage of spatial dependence [*C*/(Co+*C*)] measures the proportion of the variance of a sample, which is explained by the spatial variance (*C*). If this proportion is close to 0, then the spatial dependence is low (Aubert et al. 2003).

Another of the tools used in Geostatistics is Kriging, which allows interpolation for non-sampled points (Wackernagel 2003); Kriging is based on the information provided by the semivariograms on the spatial dependence of a variable (Robertson 1987). We performed semivariogram and kriged maps using GS+ v.7 (Geostatistics for the Environmental Science) Gamma Design Software (www.gammadesign.com).

To detect possible relationships between the distribution of earthworms and edaphic factors, we performed a canonical correlation analysis between soil factors (independent variables) and earthworm species abundance (dependent variables) using Statistica v.6.

Results

Earthworms

Six earthworm species were found. H. elisae Álvarez, 1977, Allolobophora rosea bimastoides

Table 1.	Earthworm	species,	ecological	categories,	mean	abundance	$(N m^{-2}),$	standard	deviation	(St.D)	and
percentag	e of combine	d domina	nce (PCD) o	f earthworm	s durin	g three date	s at El Mo	olar			

Species	Ecological	Spring'01		Spring'02		Autumn'02			
	category	$Mean \pm St.D$	PCD	$Mean \pm St.D$	PCD	$Mean \pm St.D$	PCD	I_{δ}	b
Hormogaster elisae ^a	Endogeic	15.7±14.5	66.1	8.6±14.0	72.1	11.0±14.3	76.3	2.4	2.1
Allolobophora rosea bimastoides ^a	Endogeic	7.9 ± 13.9	22.1	2.1 <u>+</u> 6.9	14.6	0.8 ± 2.6	6.8	8.3	1.1
A. caliginosa trapezoides ^a	Anecic	1.9±5.0	9.6	1.1±4.0	7.3	1.6±5.9	11.8	11.6	1.2
Octodrilus complanatus	Anecic	0.07 ± 0.38	1.1	0.7 ± 4.6	3.8	0.8 ± 3.2	5.1	ND	ND
Microscolex phosphoreus	Endogeic	0.29±1.5	1.1	0.1±0.6	1.1	0	0.0	ND	ND
Microscolex dubius	Endogeic	0	0.0	0.1±0.6	1.1	0	0.0	ND	ND

ND: not determined.

Aggregation indices: Morisita's index (I_{δ}) and Taylor's index (b).

^aSpecies used as dependent variables in the canonical correlation analysis.

(Cognetti, 1901), Allolobophora caliginosa trapezoides (Dugès, 1828), Octodrilus complanatus (Dugès, 1828), Microscolex phosphoreus (Dugès, 1837) and Microscolex dubius (Fletcher, 1887). H. elisae, A. caliginosa and A. rosea were the most abundant species (Table 1).

The Taylor's index and the Morisita's index (I_{δ}) indicated a clumped distribution pattern (Table 1) for the three species.

H. elisae was the dominant species in the three sampling surveys (71.52% PCD), followed by *A. rosea* in the spring survey and *A. caliginosa* in the autumn one. These three species represented more than 90% of the PCD in the area, which indicates their great importance and the low relevance of the remaining other three species (Table 1).

 Table 2.
 Simple correlations between species' abundances and soil factors, and among species

Soil factors	He/m ²	Ac/m ²	Ar/m ²
%Carbon	-0.23 ^a	-0.06	0.05
%Nitrogen	-0.31 ^a	-0.07	0.11
C/N	0.25 ^a	0.03	-0.20 ^a
pH in H₂O	-0.17	0.09	0.05
%Moisture	-0.09	-0.03	0.15
%Porosity	0.05	0.12	0.08
%Aeration	0.10	0.12	-0.01
%Coarse sand	0.38 ^a	0.02	-0.36^{a}
%Fine sand	0.07	0.04	0.19 ^a
%Total sand	0.40 ^a	0.04	-0.28 ^a
%Coarse loam	-0.31^{a}	0.03	0.04
%Fine loam	-0.16	-0.07	-0.16
%Total loam	-0.26^{a}	-0.03	-0.09
%Clay	-0.35^{a}	-0.03	0.51 ^a
He/m ²	1.00	-0.15	-0.23 ^a
Ac/m ²	-0.15	1.00	0.15
Ar/m ²	-0.23^{a}	0.15	1.00

Hormogaster elisae (He); Allolobophora caliginosa (Ac); Allolobophora rosea (Ar).

^aMarked correlations are significant at p < 0.05.

There is a simple correlations between species' abundances and soil factors, and also among species (Table 2).

Table 3 shows the spatial parameters of the geostatistical analysis of the three dominant species in the study plot. They reveal the existence of a model, mainly spherical, for all species. The kriged maps (Fig. 2) show how these three species are distributed in patches of a variable size depending on the sampling survey. H. elisae is aggregated in patches of an average size of 45 m in spring (2001 and 2002 spring mean) and larger than 100 m in autumn. These patches were located mainly on the upper, right area of the plot, except in spring 2002, when they were in the central area. A. rosea appears in patches with an average size of 23 m located on the upper or central-left part of the plot, except during the 2001 survey, when this species was particularly abundant, forming patches larger than 100 m and spreading all over the left margin of the plot, bordering the stream. The aggregates formed by A. caliginosa had a mean size of 38 m and were always located on the stream's margin.

Soil characteristics

Soil characteristics are shown in Table 4. We have not found significant differences among the three surveys in soil characteristics, except for humidity (ANOVA; F = 24.780; df = 2; P < 0.01) and aeration (ANOVA; F = 6.824; df = 2; P < 0.01), which are very season-related variables.

The canonical correlation analysis extracted a statistically significant canonical variable, which shows a linear correlation (r = 0.76; P < 0.01) between dependent and independent variables, the extracted variance being 45.6% for all dependent variables together. The structural canonical coefficients show that the abundance of *H. elisae*

Date	Species	Model fitted	Со	Co+C	A (m)	SD
Spring'01	A. rosea	Gaussian	83.7	368.3	134.1	77.3
	H. elisae	Exponential	3.8	200.6	41.1	98.1
	A. caliginosa	Spherical	4.69	24.72	60.0	81.0
Spring '02	A. rosea	Spherical	1.30	35.86	18.4	96.4
	H. elisae	Spherical	72.2	291.7	50.0	75.2
	A. caliginosa	Spherical	1.02	16.19	30.1	93.7
Autumn'02	A. rosea	Spherical	1.06	7.89	26.2	86.6
	H. elisae	Gaussian	97.0	348.3	102.9	72.2
	A. caliginosa	Spherical	1.50	33.61	26.4	95.5

Table 3. Variogram model parameters for A. rosea. H. elisae and A. caliginosa

Nugget (Co). Sill (Co+C). Range (A) in metres. SD (spacial dependence (%) = [C/(Co+C)].



Fig. 2. Kriged maps of density (individuals/m²) of *A. rosea* (AR), *H. elisae* (HE) and *A. caliginosa* (AC) at three dates. Note the different parcels size (80 m in spring 2001 and 96 m in 2002).

Factor	$Mean \pm St.D$	Model	Co	Co+C	Α	SD
%Gravels (GRA) ^a	34.6±6.4	Spherical	63.4	189.1	138.9	66.5
%Total sand (SAND)	67.2 <u>+</u> 8.1	Gaussian	36.4	120.6	124.9	69.8
%Coarse sand (COA) ^a	53.0±7.9	Gaussian	26.9	104.8	130.1	74.3
%Fine sand (FIN) ^a	14.2±3.1	Spherical	2.3	5.3	74.7	56.2
%Total loam (LOA)	13.1±5.3	Exponential	0.0	20.9	48.3	100
%Coarse loam (CLO) ^a	3.6±2.5	Spherical	3.0	6.7	71.7	54.7
%Fine loam (FLO) ^a	9.5±5.4	Exponential	0.0	8.9	37.5	99.9
%Clay (CLA) ^a	19.7 <u>+</u> 5.4	Gaussian	12.5	40.5	104.8	69.2
%Porosity (POR) ^a	47.8 <u>+</u> 8.9	Spherical	0.1	106.5	17.9	99.9
pF 4.2	9.7 <u>+</u> 1.8	Gaussian	8.1	32.3	157.8	75.0
pF 2.7	15.9 <u>+</u> 2.7	Gaussian	19.4	69.8	121.9	72.2
%Carbon (C)	1.8±0.5	Exponential	0.0	0.2	43.8	99.9
%Nitrogen (N) ^a	0.2±0.1	Gaussian	0.0	0.0	81.8	52.5
C/N ratio $(C/N)^{a}$	11.8±1. 2	Exponential	0.0	1.6	26.7	99.9
pH in H ₂ O (PHH) ^a	6.6±0.5	Exponential	0.0	0.6	66.0	93.4
%Moisture-spring'01 (MOI) ^a	18.5 <u>+</u> 6.9	Lineal	52.0	52.0	48.0	0.0
%Aeration-spring'01 (AER) ^a	27.2 <u>+</u> 9.6	Spherical	30.8	100.8	47.5	69.4
%Moisture-spring'02 (MOI) ^a	17.3±4.9	Exponential	0.0	19.6	8.3	99.9
%Aeration-spring'02 (AER) ^a	31.8±11.7	Exponential	2.7	125.1	38.7	97.8
%Moisture-autumn'02 (MOI) ^a	12.3±3.0	Gaussian	3.6	12.3	101.3	70.4
%Aeration- autumn'02 (AER) ^a	35.7 <u>+</u> 6.4	Lineal	29.7	40.7	48.1	27.1

Table 4. Means, standard deviation (St.D) and variogram model parameters for soil factors

Nugget (Co). Sill (Co+C). Range (A) in metres. SD (spacial dependence (%) = [C/(Co+C)]). ^aVariables used in the canonical correlation analysis.

and sand percentage contribute negatively to this canonical variable, while the abundance of *A. rosea* and the percentages of nitrogen, carbon, coarse mud and clay do so positively. Hence, there is a positive correlation between *H. elisae*'s abundance and the percentage of total and coarse sand, and a negative one between the former and clay, nitrogen, carbon and coarse mud, which is exactly the opposite situation to what occurs with *A. rosea*.

Table 4 shows the parameters of the models to which the semivariograms of soil variables adjust. A spatial dependence of all soil factors under study is observed, except for humidity in the 2001 spring survey, where a big Nugget effect is clearly observed. The lack of a clear humidity spatial pattern might be due to the fact that the distance between samples might be too large to detect a spatial structure for this variable. Fig. 3 shows the kriged maps of some soil variables. Comparing species abundance maps and those of soil factors (Fig. 2 and 3), it can be observed that H. elisae appears in the sandiest areas, with the lowest carbon and nitrogen percentages, opposite to what happens with A. rosea, which is in accordance with the results obtained with the Canonical Correlation Analysis.

Discussion

The low organic matter percentages found in El Molar, in comparison with those observed by other authors in studies on earthworms in other parts of the Iberian Peninsula (Mariño et al. 1985), together with the extreme climatic conditions (cold winters and hot and dry summers) and the steep slope of the plot, make this place unsuited for most species of earthworms. This favours the predominance of endogeic species, as H. elisae, A. rosea, M. dubius and *M. phosphoreus*, which can protect themselves better from climatic and biological aggressions at deeper levels and are capable of feeding on poorer resources than epigeous ones (Lavelle 1981). The form of A. caliginosa that is present in El Molar is A. c. trapezoides, which is an anecic form, but that due to its parthenogenetic characteristics can show great variations within local populations.

Results from the canonical correlation analysis and the kriged maps show a relationship between *H. elisae* and *A. rosea* and soil factors similar to that obtained using principal component analysis and stepwise regression (Hernández et al. 2003). However, we have not detected a clear correlation between *A. caliginosa* and any of the factors under study, which might be due to the great plasticity of this species (which allows it to adapt to a wide



Fig. 3. Kriged maps for total sands (SAND), clay (CLA), coarse loam (CLO), carbon (C) and nitrogen (N).

range of environmental and soil factors; Álvarez 1971; Briones 1996), or to its low presence with respect to the total amount of sampled units.

The dominant species in the study area, *H. elisae*, *A. rosea* and *A. caliginosa*, showed clumped distribution patterns. This distribution model has been observed in numerous studies on earthworms in different parts of the world, such as the Colombian savannahs (Jiménez et al. 2001) or the tropical forests of Puerto Rico (González et al. 1999). Such aggregation may be partly due to the influence of edaphic factors, being the most important ones in the study plot, nitrogen and soil texture (mainly sand and clay content). The sole use of aggregation indices would not have allowed us to obtain information about the spatial distribution of these earthworm species in a bigger scale than the one of the sampling unit (Rossi et al. 1995). For this reason, geostatistical technigues have been used.

H. elisae is widely distributed all over the plot and tends to concentrate, especially during spring, in areas with a high percentage of coarse sand, particularly in the upper right area of the plot, which possibly due to its sandy character and steep slope is the one suffering from most nutrient and fine fraction losses as a consequence of runoff washing. The high abundance of *H. elisae* in El Molar suggests that it is adapted to environmental conditions that are unfavourable for most earthworms. This would be in agreement with the few data appearing in the literature which show that this species is often found in poor soils, with low carbon and nitrogen percentages (Moreno 1981). It is noteworthy that *H. elisae* showed a higher aggregation patch size in autumn (100 m) than in spring (45 m), probably due to the fact that during

this season it expands its distribution area and

appears from the central zone, covering the whole

right border, to the stream without going beyond it. On the other hand, we have observed that A. rosea is dominant and forms aggregation patches in those spots with a higher percentage of clay, nitrogen and carbon, which coincide with the upper left part of the plot, where there are some old abandoned terraces and the terrain shows a slighter slope and hence less losses in organic matter and fine fractions due to washing and runoff. This relationship between abundance of A. rosea and clay content has been observed before by other authors such as Briones et al. (1995) or Nordström and Rundgren (1974). In the spring of 2001, this species was very abundant and it spread largely in the plot, forming patches of up to 100 m. This could be due to the total precipitation in El Molar in that period, when it practically doubled the precipitation of 2002. That might have favoured the development of this species.

If we consider the high values of the concurrence index in El Molar (Hernández et al. 2003) and that *H. elisae* is mainly distributed as a monospecific community and forming opposite patches to those of *A. rosea* and *A. caliginosa*, one could think that this opposite distribution is a reflection of interspecific competition between species. This would be in accordance with the laboratory results of Garvín et al. (2002), who found evidence of the negative interactions between *H. elisae* and *A. caliginosa*, and a decrease in the production of cocoons of *A. rosea* and *H. elisae* in polyspecifics microcosms.

A comparison of the distribution maps of these three seasons showed temporal variations in the horizontal distribution of some species, indicating that this distribution is a dynamic process. This opens an interesting field of study aimed at understanding the distribution patterns of earthworms and their seasonal variations.

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