

## Genetic variability of earthworm populations in soils with different perturbation levels

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### ABSTRACT

Density and genetic variability of both soil macro- and meso-fauna are disturbed by productive practices. This study aimed to analyze the genetic diversity and genetic structure of populations of the earthworm *Aporrectodea caliginosa caliginosa* (Briones, 1996) in two sites of the Argentine Pampa under different levels of disturbance: i) grassland, ii) livestock-raising plot, iii) agricultural-livestock raising plots and iv) agricultural plot (the most disturbed). The genetic diversity of the earthworm population was determined based on the allele number, polymorphism percentage and Similarity Index. Allele number and polymorphism percentage were lower in populations from one of the soils under agricultural-livestock practices, but differed significantly only from the values of the other agricultural-livestock raising plot and the livestock-raising plot. Population structure was low although significant. This study shows that allele number and polymorphism are very useful metrics to provide historical and functional information of soils. However, the genetic differences here recorded probably depend on multiple historical and recent causes. The stability of environmental conditions along with the degree of disturbance must be considered to understand their impact on population genetic structure.

### 1. Introduction

In Argentina, 72.8 % of the arable land is used for agriculture (FAO, 2014), 80 % of which is concentrated in the Pampean region (Paruelo et al., 2005; Satorre, 2005). Both agriculture and cattle raising disturb the soil ecosystem and impact on its physical and chemical variables. We have adopted the definition of disturbance as proposed by Borics et al. (2013). Disturbance signals include an increase in soil bulk density, a reduction in soil porosity, a decrease in organic matter content (Dexter, 1991; Sanzano et al., 2012), and the acidification of agricultural soils (Andreau et al., 2012).

The soil fauna is also affected by these perturbations. Different studies have concluded that agricultural and cattle-raising soils have a negative impact on the density and biomass of both the macrofauna (Duhour et al., 2009; Domínguez et al., 2010; Díaz Porres et al., 2014; Falco et al., 2015; Bedano et al., 2016; Domínguez and Bedano, 2016) and the mesofauna (Bedano et al., 2006a, 2006b, 2011, 2016; Arolfo et al., 2010; Menta et al., 2011; Sandler et al., 2018). Most studies have shown that these soils lead to a reduction in soil community abundance

and diversity. According to Domínguez et al. (2010), this reduction is associated with increased soil bulk density, a change in pH and a lower amount of soil organic matter, together with the increased application of agrochemicals. Similarly, Moço et al. (2010) highlighted the effect of pH on soil fauna richness, whereas Cabrera (2012) related the decreased richness of macrofauna of agricultural and livestock-raising soils to the absence of trees and lower contribution of organic matter to the soil. In addition, Díaz Porres et al. (2014) associated the absence of certain orders of macroarthropods with a reduced supply of available refuges and prey. The new stability achieved in disturbed systems results in a new reconfiguration of the community and its abundances, which is expected to be maintained until a new disturbance occurs.

The soil fauna participates in soil structure modification, nutrient cycling and organic matter decomposition (Jenny, 1983; Coleman and Whitman, 2005). In particular, earthworms play an important role in all of these processes (Lavelle et al., 1998; Edwards, 2004; Postma-Blaauw et al., 2006), and represent the largest living biomass in terrestrial ecosystems (Lavelle and Spain, 2001). The European earthworm *Aporrectodea caliginosa caliginosa* (Briones, 1996), which belongs to the

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family Lumbricidae, is within the group of peregrine species, showing a much wider distribution in Argentina than in its region of origin (Michaelsen, 1903). This distribution is associated with its high tolerance to environmental variations such as desiccation and extreme temperatures (Lee, 1985; Momo and Falco, 2009), which allows it to inhabit both agroecosystems (Paoletti, 1999) and little disturbed grasslands. This characteristic allows proposing it as an indicator organism (Römbke et al., 2007). Its sensitivity makes it potentially useful to be used as an environmental indicator (Nunes et al., 2007), as well as to analyze the effects of climate change on the soil biota in general and on the earthworm community in particular (Singh et al., 2019; Kaka et al., 2021). According to the classification of Lavelle (1983 and 1988), several authors (Räty, 2004; Falco and Momo, 2010; Rüdiger et al., 2021) consider *Aporrectodea caliginosa caliginosa* to be a mesohumic endogeic earthworm that lives permanently in the organic mineral substrate of the soil, feeds on dead roots and organic matter integrated into the substrate, and builds horizontal galleries.

Shekhovtsov et al. (2016) consider that the analysis of the genetic diversity of complex species such as *A. caliginosa* should include both the origin and the dispersion history of their populations, as well as the environmental filters to which they are exposed. Genetic variables are closely related to population size and keep a cumulative record of the expansions and reductions of the population size (Amos and Harwood, 1998), making them extremely valuable as components of a soil perturbation indicator. Little is known about the effects of perturbed soils on the genetic variability of soil populations. However, recent studies have shown a significant reduction in the genetic diversity of isopod populations of agricultural fields (Díaz Porres et al., 2018) and in invertebrate populations inhabiting soils with similar tillage systems (Kautenburger, 2006). It has been shown that a population can recover its abundance in a few generations, but this does not imply that it can recover its genetic variability. In environments that alternate different land uses, the effect of genetic drift due to bottlenecks that erode population genetic variability is increased. The duration, frequency, intensity and spatial extent of the disturbance pulse are aspects to be considered when studying its effects (Bengtsson, 2002). Several studies have also recorded a genetic structuring of *Allolobophora chlorotica* and *Aporrectodea icterica* populations in pastures with cattle (Dupont et al., 2015; Dupont et al., 2017) even at a very small scale. A population with little genetic variability is more vulnerable to environmental changes because of its reduced reaction capacity to such changes (Frankham et al., 2002). It is therefore important to quantify the genetic variability in populations from disturbed environments.

The objective of this study was to analyze the genetic diversity and genetic structure of *Aporrectodea caliginosa caliginosa* populations in soils of the Argentine Pampa, by comparing grassland with sites with different levels and durations of perturbation (agriculture, livestock raising, or agriculture-livestock raising), by analyzing the polymorphism generated by random amplification of sequences between repeated DNA regions (by the inter-simple sequence repeat technique or ISSR-PCR). We hypothesized that genetic diversity will be lower in earthworm populations inhabiting more intensified soils (agricultural soils) than in populations inhabiting non-agricultural intensified soils with a perturbation stable regimen (grassland). We also believe that this decrease in genetic diversity can lead to an increase in the sensitivity of earthworm populations to further disturbances, and thus affect the ecological functions they perform in that ecosystem. We expected to record some degree of genetic structuring in earthworm populations, related to the stability of management in the different plots and the reduced gene flow between populations.

## 2. Materials and methods

### 2.1. Study area and sampling

The study was conducted between 2009 and 2010 and between 2012

and 2014 in two sites of the Argentine Pampa, in the province of Buenos Aires: in an experimental field of the Luján University (34°36'S, 59°04'W) and in a private rural field of the town of Etchegoyen (34°26'S, 59°04'W). The distance between the two sites is about 17 km. We consider that, at this geographical scale of analysis, the *Aporrectodea caliginosa caliginosa* populations studied share the same migratory origin, despite having entered the continent in multiple human migratory events of similar origin (Shekhovtsov et al., 2016). The soil of both sites can be classified as Typic Argiudoll (Soil Survey Staff, 2010). The average annual precipitation ranges from 1000 to 1100 mm and the average air temperature is 22 °C in summer and 9 °C in winter (Servicio Meteorológico Nacional, San Miguel). The mean monthly rainfall in the four years sampled was 117 mm (Meteorological Station CIDEPA-U: 34°36' S, 59°04' W, 28 m a.s.l.).

Sampling was carried out in autumn, spring and summer, considering the following pre-established land-use gradient: two grassland plots, a livestock-raising plot, two agricultural-livestock-raising plots and an agricultural plot. We proposed an intensification gradient considering that a higher number of agrochemical applications, a higher concentration of pesticides, a short pulse of the disturbance (Lake, 2000), and a higher farm age are associated with higher soil disturbance. The soils of Luján University belong to the Mercedes Series, and are brown, loamy and moderately well drained, whereas those of Etchegoyen belong to the Portela Series, are dark, have a silty clay loam texture and are well drained (Panigatti, 2010). There is evidence of the introduction of lumbricids into the Pampean region with the human migrations from southern Europe since the end of the 19th century (Mischis and Herrera, 2006; Porco et al., 2013). The distance between the populations analyzed allows us to assume that they share the same origin. To distinguish the effect of founder drift on the genetic variability of populations of *Aporrectodea caliginosa caliginosa* that arrived in South America from genetic erosion that responds to land use, we compared genetic variables recorded in the reference soils with those recorded in soils under agricultural or livestock exploitation.

The two grassland plots (here called NU and NE) were the least disturbed soils and were considered as the closest to the pristine grasslands of the region. In these plots, no productive activities, pesticide application or land-use change has been recorded for >30 years (Table 1). NE was covered by grasses and some citrus trees: *Citrus x sinensis*, *Citrus x lemon*, *Ligustrum sinense*, *Bacharis* sp., *Gleditsia triacanthos*, *Eucalyptus camaldulensis*, *Elaeagnus* sp. and *Magnolia* sp., whereas NU was a pseudo-grass steppe combined with *Dipsacus fullonum*, *Sorghum halepense*, *Paspalum quadrifarium*, *Ligustrum sinense*, *Bacharis* sp., *Gleditsia triacanthos* and *Eucalyptus camaldulensis* as described by Duhour (2011). Next, the gradient was the livestock-raising Etchegoyen soil (which we called LE), which was covered by pasture without pesticide application or land-use change, used for sheep grazing for 7 years. This was followed in the gradient by the agricultural-livestock raising plots (which we called ALU<sub>2</sub> and ALU<sub>1</sub>), which corresponded to agricultural soils alternately used for livestock grazing for >30 years. ALU<sub>2</sub> was considered less disturbed than ALU<sub>1</sub> due to a longer pulse of disturbance (3 to 6 years) and, consequently with a lower number of insecticide applications (three times fewer applications than in ALU<sub>1</sub>). Finally, we studied an agricultural plot (which we called AE) at the extreme of greatest disturbance due to exclusively agricultural use, no land-use change, and a five-fold higher insecticide application than that proposed for ALU<sub>1</sub>, although its age of use (17 years) was shorter than that of ALU<sub>1</sub> and ALU<sub>2</sub>.

On each sampling time and in each plot (experimental unit), a transect was randomly drawn and three soil monoliths of 25 × 25 × 20 cm were extracted at 20, 30 and 40 m from the start. A total of 216 soil samples (6 plots × 3 monoliths × 12 samplings) were checked for the presence of earthworms in the plot being adult earthworms manually collected and carried alive to the laboratory. Earthworms were counted to estimate the relative ratio and identified to species level by using keys (Righi, 1979; Reynolds and Clapperton, 1996; Mischis and Moreno,

**Table 1**

Summary of the characteristics of the fields sampled: Grassland of UNLu (NU), Grassland of Etchegoyen (NE), Livestock-raising field of Etchegoyen (LE), Agricultural field of Etchegoyen (AE), Agricultural-livestock-raising field 1 of UNLu (ALU<sub>1</sub>), Agricultural-livestock-raising field 2 of UNLu (ALU<sub>2</sub>); Period 1999–2014.

	NU	NE	LE	ALU <sub>1</sub>	ALU <sub>2</sub>	AE
Surface (ha)	7	1	0.51	8.5	9	4
Years of use	–	–	7	31	30	>17
N° of herbicide applications (1999–2014) <sup>b</sup>	0	0	1	26	30	32
N° of fungicide applications (1999–2014) <sup>b</sup>	0	0	0	0	6	16
N° of insecticide applications (1999–2014) <sup>b</sup>	0	0	1	7	2	32
Conventional tillage (years) <sup>a</sup>	–	–	–	12	14	2
Reduced tillage (years) <sup>a</sup>	–	–	–	2	2	14
Zero tillage (years) <sup>a</sup>	–	–	–	13	13	–
Livestock (years)	–	–	6	13	18	–
Livestock type	–	–	Sheep	Cattle	Cattle	–
Total organic matter (% ± SD) <sup>f</sup>	5.02 ± 1.27	5.65 ± 1.26	5.52 ± 1.15	4.39 ± 0.54	4.79 ± 0.83	4.40 ± 1.19
Bulk Density(±SD) <sup>c</sup>	1.12 ± 0.10	1.12 ± 0.11	1.11 ± 0.12	1.23 ± 0.08	1.24 ± 0.14	1.23 ± 0.10
Gravimetric moisture(% ± SD) <sup>c</sup>	22.44 ± 5.06	21.99 ± 4.84	20.79 ± 6.36	21.93 ± 13.07	18.54 ± 4.94	18.35 ± 5.0
Total Nitrogen(% ± SD) <sup>f</sup>	0.29 ± 0.05	0.35 ± 0.06	0.30 ± 0.03	0.28 ± 0.04	0.33 ± 0.05	0.31 ± 0.09
pH (± SD) <sup>c</sup>	5.96 ± 0.43	6.22 ± 0.25	5.89 ± 0.34	5.78 ± 0.40	6.20 ± 0.84	5.89 ± 0.54

<sup>a</sup> The type of tillage was defined according to the FAO Integrated Management and Soil Conservation Practices Manual (Benítez and Friedrich, 2009).

<sup>b</sup> The numbers of herbicide, insecticide and fungicide applications were estimated according to the crop recorded. The concentrations follow the technical approaches of the Sarandon report (2013).

<sup>c</sup> Average of data recorded between 2009 and 2014 for the doctoral thesis of Rionda (2019).

1999) and preserved in 70 % ethanol. *Aporrectodea caliginosa caliginosa* specimens were used for genetic analysis.

## 2.2. DNA extraction and ISSR-PCR amplification

A total of 177 *Aporrectodea caliginosa caliginosa* earthworms were found evenly distributed in the six plots studied. DNA from all the earthworms was extracted using a HiYield™ Genomic DNA mini kit (Real Biotech Corporation) and amplified. The concentration of DNA extracted from 1 % agarose gel was quantified with reference to three known concentrations of phage Lambda DNA, using the Image J software. Samples with concentrations >5 ng/μl were selected.

To quantify genetic variability, we chose a dominant marker because it does not require prior information on the genomic sequence to be analyzed. As a preliminary step, 15 primers (Supplementary Table S1) were tested on a limited number of individuals. The four primers that provided the best result were used based on the size and number of amplified fragments, polymorphism, and the ability to distinguish between different individuals, calculated with the statistical package INFOGEN (Balzarini and Di Rienzo, 2014).

PCR amplification was performed in a 20-μl reaction volume containing: 25 ng of DNA (5 μl), 0.7 μM of the primer, 0.2 mM of each dNTP, 1 X of PCR buffer, 1.5 mM of Mg<sup>2+</sup>, 0.4 μg/μl of bovine serum albumin and 0.5 U of Taq DNA Polymerase HighWay from INBIO. Negative and positive controls (distilled water and bacterial DNA respectively) were included in each reaction. Then, 5 μl of PCR products was mixed with 3 μl of bromophenol blue, and electrophoresis was performed on a 1.5 % agarose gel using 1 × TBE buffer at 90 V for 2 h and stained using SYBR® Safe (Invitrogen). After this, 5 μl of 100-bp ladder plus (Dongsheng Biotech®) was run for each gel and photographed on an ultraviolet transilluminator.

## 2.3. Data analysis

A binary matrix of presence (1) and absence (0) was constructed from the reproducible band pattern of each individual. The genetic diversity of each population was measured with the allele number (A), percentage of polymorphism (P) and Sørensen-Dice Similarity Index with the InfoGen (Balzarini and Di Rienzo, 2014) and GenALEX (Peakall and Smouse, 2012) software. More details on the calculation and analysis of these variables can be found in the Supplementary Material.

To visualize the genetic relationship among individuals, a Neighbor-joining tree was constructed based on genetic distances, using the

NEIGHBOR and CONSENCE programs from the PHYLIP package version 3.66 (Felsenstein, 2004) and MEGA 5.2 (Tamura et al., 2011) with 1000 random subsamples (bootstraps). Genetic distances were computed as 1-S with the InfoGen package (Balzarini and Di Rienzo, 2014).

Genetic isolation by distance was analyzed using the Mantel test over the genetic distance matrix with the GenALEX package. To describe genetic structure and variability within and between populations, the non-parametric Analysis of Molecular Variance (AMOVA) was performed using the GenALEX (Peakall and Smouse, 2012) software. The components of genetic differentiation were estimated using the  $\phi_{PT}$ , with GenALEX software. The genetic structure of our dataset was derived with the STRUCTURE software (Pritchard et al., 2000) and the data were tested for fit to four different population models proposed in the HICKORY package (Holsinger et al., 2002). More details on the calculation and analysis of these parameters can be found in the Supplementary Material.

## 3. Results

### 3.1. ISSR-PCR profile

Four of the fifteen primers tested were selected based on the amplification percentage and the number and polymorphism of bands recorded (Supplementary Table S1). A total of 95 polymorphic loci (bands), ranging from 150 to 3000 bp, were studied for the 177 individuals of *Aporrectodea caliginosa caliginosa* evenly distributed in the plots analyzed ( $N_{AE} = 26$ ,  $N_{LE} = 30$ ,  $N_{NE} = 38$ ,  $N_{ALU1} = 25$ ,  $N_{ALU2} = 31$  and  $N_{NU} = 27$ ).

Each primer amplified between 3.28 and 4.20 bands/individual on average (Supplementary Table S2). More than 71 % of the bands were polymorphic. The most polymorphic primers were I-3 and I-13, the latter with the highest discriminatory capacity among loci (Polymorphic Information Content: PIC = 0.22 ± 0.02). The low probability of randomly sharing the same allele on the four primers indicates their high ability to distinguish individuals from one another.

### 3.2. Genetic diversity

A lower number of alleles (1.35) and a lower percentage of polymorphic bands (0.57 %) were observed in ALU<sub>1</sub> as compared with the other plots studied (Table 2). The diversity measured in AE, NE and NU was slightly higher than that in ALU<sub>1</sub>, although not significantly different. Only ALU<sub>2</sub> and LE populations showed significantly high

**Table 2**

Genetic diversity of six *Aporrectodea caliginosa caliginosa* populations with different levels of perturbation based on ISSR-PCR markers abundance values and proportion of *Aporrectodea caliginosa caliginosa* found, are also showed in each plot. Field codes are indicated in Table 1.

	Mean (±SE) of the number of alleles per locus	Polymorphism percentage (±SE)	Total bands	Earthworm density (ind/m <sup>2</sup> ± SD)	Proportion of <i>Aporrectodea caliginosa caliginosa</i> ± SD
NU	1.45 ± 0.09 ab	0.76 ± 0.01	84	31.11 ± 42.32 b	0.42 ± 0.45
NE	1.56 ± 0.09 ab	0.70 ± 0.05	90	20.44 ± 47.47 ab	0.13 ± 0.21
LE	1.71 ± 0.07 b	0.70 ± 0.05	90	18.67 ± 41.16 ab	0.41 ± 0.46
ALU <sub>1</sub>	1.35 ± 0.10 a	0.57 ± 0.05	84	8.89 ± 21.41 a	0.75 ± 0.43
ALU <sub>2</sub>	1.71 ± 0.07 b	0.71 ± 0.05	84	8.00 ± 20.72 a	0.75 ± 0.43
AE	1.58 ± 0.08 ab	0.67 ± 0.05	90	4.44 ± 10.71 a	0.33 ± 0.58
	$P_{KW} = 0.0178$			$P_{KW} = 0.0304$	$P_{KW} = 0.2231$

Different letters indicate significant differences tested by Kruskal Wallis test.

values of genetic diversity. It is interesting to consider these results together with the information on earthworm abundance found. Earthworm abundance was higher in both grasslands and in livestock-raising plot than in the other plots, although with a low to moderate proportion of *Aporrectodea caliginosa caliginosa* (i.e. the ratio of *Aporrectodea caliginosa caliginosa* to total earthworms). Similar values of earthworm abundance and proportion of *Aporrectodea caliginosa caliginosa* were recorded in ALU<sub>1</sub> and ALU<sub>2</sub>.

3.3. Genetic distances

The smallest Nei genetic distance was recorded between NU-ALU<sub>2</sub> and LE-AE individuals (Table 3), whereas the highest was recorded between NU-NE and NE-ALU<sub>2</sub> individuals. No significant correlation was found between genetic and geographic distances ( $r = 0.079$ ,  $N = 177$ ,  $p > 0.05$ ). The genetic distances allowed us to propose a relationship tree between individuals by using the Neighbor-joining algorithm (Fig. 1). The tree showed eight clades, three of them with a large predominance of NE individuals (clades 2, 4 and 8) and one of them grouping mostly ALU<sub>1</sub> individuals (clade 3). The algorithm grouped AE and LE individuals in the same clades (1 and 7), whereas it grouped ALU<sub>2</sub> and NU individuals in the clade 5. These results were reinforced by the AMOVA and STRUCTURE results. The AMOVA revealed a significantly lower genetic difference between AE and LE individuals (2.41 %,  $df = 1$ ,  $p = 0.0013$ ) and between ALU<sub>2</sub> and NU individuals (2.04 %,  $df = 1$ ,  $p =$

**Table 3**

Genetic distance and geographic distances (km, in brackets) for all pairwise comparisons between populations of *Aporrectodea caliginosa caliginosa*. Field codes are indicated Table 1.

	NU	NE	LE	ALU <sub>1</sub>	ALU <sub>2</sub>	AE
NU	–	0.76 (16.714)	0.65 (16.83)	0.66 (0.51)	0.48 (0.37)	0.55 (16.99)
NE		–	0.62 (0.48)	0.71 (16.36)	0.74 (16.22)	0.72 (0.31)
LE			–	0.63 (16.36)	0.64 (16.48)	0.43 (0.31)
ALU <sub>1</sub>				–	0.55 (0.20)	0.70 (16.52)
ALU <sub>2</sub>					–	0.61 (16.63)
AE						–

0.0011). Nevertheless, the genetic variability was higher within each land uses than between land uses.

3.4. Genetic differentiation

The population structure was low, although significant in both Etchegoyen and Luján University sites ( $\Phi_{PT-E} = 0.05$ ,  $\Phi_{PT-U} = 0.06$ ;  $p < 0.001$ ). The highest values of genetic differences (Table 4), were observed between individuals from ALU<sub>1</sub> and individuals from the other plots studied (except individuals from ALU<sub>2</sub>:  $\Phi_{PT} = 0.06$ ,  $p = 0.001$ ).

When we used the genetic membership of individuals estimated by the STRUCTURE software, individuals were separated into four clusters ( $K = 4$ ) (Fig. 2). NE individuals were almost entirely assigned to one cluster (white). The same happened with ALU<sub>1</sub> individuals, which were assigned to the light gray cluster. About half of the LE individuals shared the same origin with NE individuals. The predominant origin component in ALU<sub>1</sub> (light gray) was present in one third of ALU<sub>2</sub> and AE individuals, and in a smaller proportion in NU individuals. The dark gray component predominated in one third of AE and LE individuals and in a few ALU<sub>2</sub> individuals. The predominant origin component in NU (black) was present in one third of ALU<sub>2</sub> and AE individuals.

Bayesian analysis using the HICKORY software allowed to conclude that both Etchegoyen and Luján University populations are structured and without evidence of inbreeding. The values of deviance (Supplementary Table S3) were slightly different between the full model and the  $f = 0$  model. The genetic differentiation were higher in Luján University ( $\theta^H = 0.09$  or  $0.06$  according to the model) than in Etchegoyen ( $\theta^H = 0.0005$  or  $0.0003$ ). In Etchegoyen the  $\theta^H$  value was lower than  $\Phi_{PT}$  (Table 4). In Etchegoyen, we observed very low divergence values between agricultural and livestock-raising plots and low to moderate divergence between grassland and disturbed plots (AE or LE). In contrast, Luján University populations showed a low divergence between the agricultural-livestock ALU<sub>2</sub> system and the grassland system. These showed correspondence with the structuring  $\Phi_{PT}$  values shown in Table 4.

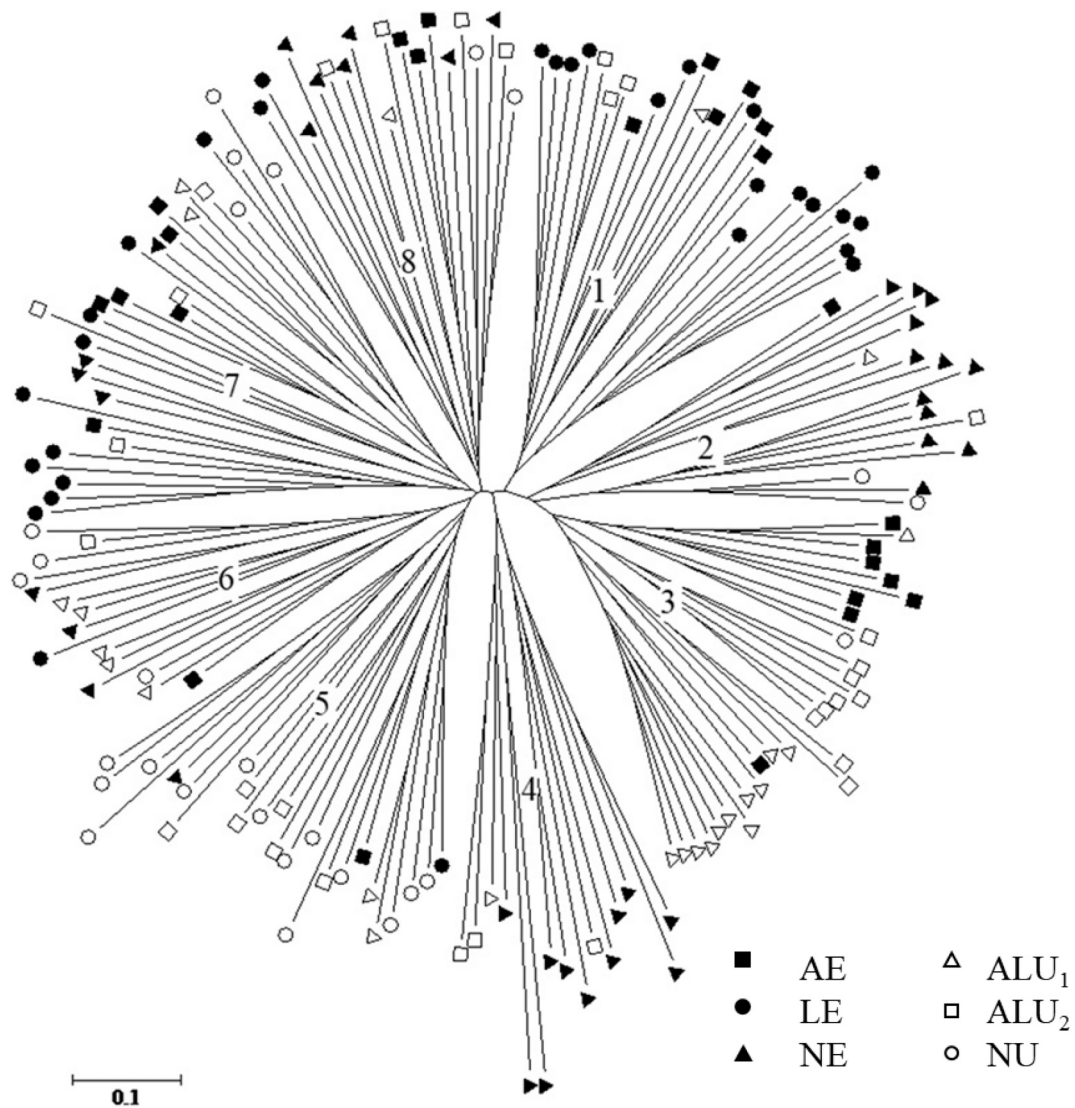
4. Discussion

4.1. Genetic diversity

The number of alleles recorded in this work was lower than that found by Torres-Leguizamon et al. (2014) for *Aporrectodea icterica* populations and by Dupont et al. (2019) for *Lumbricus castaneum* populations. These differences may be due to the methods used by the above-mentioned authors (microsatellites and mitochondrial DNA respectively), which allow detecting more genetic diversity than the ISSR-PCR technique. Previous studies of our group, in which we analyzed the genetic diversity of isopods with the ISSR-PCR technique (Díaz Porres, 2016; Díaz Porres et al., 2018), have revealed a similar number of alleles and lower values of genetic diversity in populations from ALU<sub>1</sub>, together with a greater polymorphism in the grassland population of NU. Rybak et al. (2020) also recorded polymorphism values similar to ours for populations of *Aporrectodea caliginosa*.

In the present study, we expected that the genetic diversity of the populations from more disturbed soils would be lower than that of the populations from less disturbed soils. The results obtained seem not to respond to a gradient of use, but rather to the disturbance regime. That is, the fact that the AE, LE, NE and NU soils had the same specific land use for a longer time could explain the similar genetic diversity values recorded in these disturbed soils.

At the beginning of this work we proposed that an unstable system should increase the bottleneck effect on earthworm populations, with repeated reductions and recoveries of population density and the consequent decrease in their genetic variability (Frankham et al., 2002). Under the same hypothesis, we think that the lower genetic diversity observed in ALU<sub>1</sub> as compared to ALU<sub>2</sub> could be due to differences in use



**Fig. 1.** Neighbor-joining tree based on Sørensen-Dice (1945) genetic distances between pairwise individuals of *Aporrectodea caliginosa caliginosa* in AE: agricultural plot of Etchegoyen, LE: livestock-raising plot of Etchegoyen, NE: grassland of Etchegoyen, ALU<sub>1</sub>: agricultural-livestock-raising plot 1 of UNLu, ALU<sub>2</sub>: agricultural-livestock-raising plot 2 of UNLu, NU: grassland of UNLu. Symbols represent individuals according to their perturbation degree of origin.

**Table 4**  
Genetic differentiation levels of *Aporrectodea caliginosa caliginosa* among soil perturbation levels within sites ( $\Phi_{PT}$ ; Weir & Cockerham). Significance levels in brackets. Field codes are indicated Table 1.

	NU	NE	LE	ALU <sub>1</sub>	ALU <sub>2</sub>	AE
NU	–	0.073 (0.001)	0.048 (0.001)	0.102 (0.001)	0.021 (0.05)	0.045 (0.001)
NE		–	0.054 (0.001)	0.093 (0.001)	0.045 (0.001)	0.065 (0.001)
LE			–	0.100 (0.001)	0.046 (0.001)	0.027 (0.001)
ALU <sub>1</sub>				–	0.064 (0.001)	0.100 (0.001)
ALU <sub>2</sub>					–	0.042 (0.001)
AE						–

Significance was estimated using GenALEx® with 999 permutations among 99 populations.

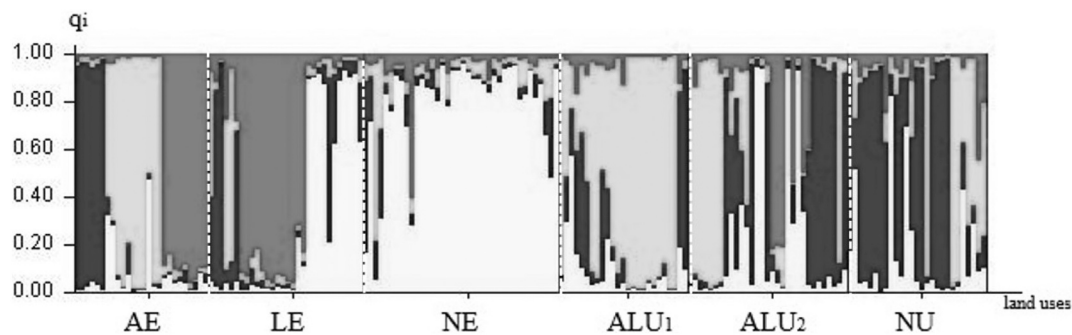
alternation: in ALU<sub>1</sub>, the alternation periods between cattle raising and agriculture were shorter (2 and 3 years), whereas, in ALU<sub>2</sub>, the alternation periods were larger (3 to 6 years). Also, the length of the rotation

cycles between pasture/forage and maize or soybean could also have contributed to these differences. However, we believe that it is necessary to extend the range of uses and periods analyzed to corroborate this hypothesis.

#### 4.2. Genetic distances

The Nei's genetic distance values found were higher than those recorded by Rybak et al. (2020) in *Aporrectodea caliginosa* populations from contaminated soils, as well as higher than those shown by Kautenburger (2006) for *Lumbricus terrestris* populations separated by 4 to 33 km. These differences could be explained by the type of genetic marker used and the relative low mobility of *Aporrectodea caliginosa* (Caro et al., 2013). The low correlation between genetic and geographic distances suggests a combination of factors in addition to geographic disposition, such as i) the migration rate between populations, ii) the effective population size and iii) the initial genetic diversity.

i) Populations sharing the same origin will show smaller genetic distances between individuals. This distance will be even smaller if a high active or passive migratory flow is maintained between



**Fig. 2.** Bayesian analysis of genetic differentiation in *Aporrectodea caliginosa caliginosa*. Each individual is represented by a single vertical line broken into four segments of length proportional to estimates of individual membership probability ( $q_i$ ) in each cluster. Dotted white line delimit natural populations. Plots codes are indicated Fig. 1.

populations. ii) At the same time, a stable population size will maintain this similarity between individuals by reducing the effect of genetic drift. This could explain the genetic similarity here recorded between NU and ALU<sub>2</sub> and between LE and AE. Several studies have pointed out the active role of humans in the dispersal of endogeic species such as *Allolobophora chlorotica* and *Aporrectodea icterica* (Dupont et al., 2015, 2017, 2019; Torres-Leguizamón et al., 2014). iii) Michaelsen (1903) described *Aporrectodea caliginosa caliginosa* as an opportunistic or peregrine species, capable of having a wider distribution range than in its place of origin and a high adaptation to disturbed environments. Directional selection would be expected to erode the genetic variability of populations in AE and LE in the same way, and although it does not directly affect the non-coding DNA regions analyzed in this work, it allowed us to observe a co-selection effect on these ISSR regions, which affects the diversity measured. At the other extreme, populations with different origins or isolated from each other, or with strong fluctuations in size, will show greater genetic distances between individuals. The populations of the Luján University and Etchegoyen grasslands and the latter with the ALU<sub>2</sub> population fit into this second scenario.

The results of the AMOVA are consistent with the genetic distance data presented above. In that sense, lower values of genetic distance collaborate with a lower genetic differentiation between populations. The percentages of genetic variation explained in the AMOVA coincide with those presented by Kautenburger (2006), who recorded less genetic variation between sampling points (equivalent to our plots) than within sampling points. The percentage of within plots genetic variation found in this work (94 %), however, was higher than that presented by Kautenburger (2006) (57.57 %) and similar to that reported between land uses (17.96 %). These differences can be partly explained by the greater geographical distances between the sampling points evaluated by this author (4–33 km), by the ecological (e.g. high vagility) and reproductive habits of the anecic earthworm studied (*Lumbricus terrestris*) and by differences in the used genetic markers (RAPDs).

#### 4.3. Genetic differentiation

Our results showed values of genetic differentiation (Table 4) similar to those published by Mezhzherin et al. (2021) for the same species in the Ukrainian forest steppe and lower among much more distant *Aporrectodea caliginosa* populations (Porco et al., 2013; Shekhovtsov et al., 2016). The low structuring may be explained by a recent separation between the populations and/or high gene flow between them. However, the limitations of the genetic marker used to detect levels of structuring should not be ruled out.

The highest (although low) structuring was recorded between ALU<sub>1</sub> and the other populations (except ALU<sub>2</sub>) and may indicate some barrier that reduces gene flow between these populations. The lack of fit to the isolation by distance model suggests that landscape fragmentation may be affecting or preventing gene flow between earthworm populations.

ALU<sub>1</sub> had the lowest genetic diversity and could be suffering from repeated bottlenecks, which erode its genetic diversity and differentiate it from other populations. Reduced gene flow may enhance this effect.

The evidence of polyploidy in *Aporrectodea caliginosa caliginosa* (Mezhzherin et al., 2008) makes us consider the possibility of polyploidy among the sampled individuals, which could explain the incipient structuring among the populations.

The analysis of the genetic components of origin of each population revealed a common history for the six populations studied. The exclusive predominance of one clade in NE and a different one in ALU<sub>1</sub> was observed, perhaps associated with particular events of high inbreeding and low gene flow with neighboring populations. This is supported by the reduced genetic diversity in these populations and the high genetic distance with other populations. We propose that divergence between ALU<sub>1</sub> and ALU<sub>2</sub> plots, which had similar land use, may be related to the pulse duration of the perturbation ecosystem inhabited by earthworms.

The presence of the same genetic components of origin was also recorded in populations that revealed smaller genetic distances (AE-LE and ALU<sub>2</sub>-NU), explained by gene flow between populations resulting from active or passive dispersal of earthworms. To explain the genetic distribution patterns in the invasive epigeic earthworm *Dendrobaena octaedra*, Cameron et al. (2008) proposed a “saltatory” dispersal, based on anthropogenic or animal transport. Although the probability of passive dispersal is much higher in this epigeic species than in *Aporrectodea caliginosa* (endogeic), a saltatory dispersal model could be also here proposed for those data that did not fit the isolation by distance model. This model could help us explain the presence of an origin component (light gray) in AE individuals, which was absent in the other Etchegoyen populations.

The management practices and perturbation history of the soils studied have led to a fragmentation pattern in the habitat of soil fauna (Dupont et al., 2017), reduced its population size (Bedano et al., 2016; Díaz Porres et al., 2014; Domínguez et al., 2010; Duhour et al., 2009; Falco et al., 2015; Menta et al., 2011), and affected its dispersion, reflecting the genetic structure of invertebrate populations. Kautenburger (2006) remarked that, in earthworm populations, these effects are related to agricultural practice and land-use change.

Finally, it is important to remark that frequent changes in land use, such as those observed in ALU<sub>1</sub>, are associated with lower records of genetic diversity and incipient population structuring values. These genetic characteristics may be explained by the reduction in population size added to the low rate of dispersion and the low initial number of alleles.

## 5. Conclusions

The ISSR-PCR molecular marker here used allows quantifying and comparing the population genetic diversity of soil fauna. This knowledge is useful to analyze the impact of soil perturbation on the

population genetic structure of species inhabiting the soil. Although the results of this study did not show less genetic variability in *Aporrectodea caliginosa caliginosa* populations inhabiting agricultural soils than in those inhabiting grasslands, they showed differences between populations inhabiting soils with different disturbance levels. These findings allow us to propose a hypothesis that considers both the disturbance levels and regime (land-use alternation periods) as variables that impact the genetic structure of the earthworm populations. Moreover, since the genetic variability of origin is also fundamental, further studies including the European populations that gave rise to the populations here studied and including the use of other molecular markers are needed to broaden the gradient of uses analyzed and to understand the multiple historical and recent causes of these differences. In particular, the low number of alleles and the reduced polymorphism recorded in earthworm populations inhabiting soils with short rotation cycles (like those of the agriculture-livestock plot ALU<sub>1</sub>, which changed its land use every 2 or 3 years) could indicate not only the degree of disturbance is the main cause, but also that the regime of disturbance could play a role in population genetic structure.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

Data will be made available on request.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.apsoil.2022.104760>.

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