Earthworm assemblages in different intensity of agricultural uses and their relation to edaphic variables

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Abstract

The objective of this study was to relate earthworm assemblage structure with three different soil use intensities, and to indentify the physical, chemical, and microbiological variables that are associated to the observed differences in earthworm assemblage structure between soils. Three soil uses were evaluated: 1- Fifty year old naturalized grasslands; 2- Cattle-grazing fields converted to feedlot within the two years before the start of this work, and 3- Fifty year old intensive agricultural fields. Three different sites for each soil use were evaluated from winter 2008 through summer 2011. Nine earthworm species were identified across all sampling sites. The sites shared five species: the native *Microscolex dubius*, and the introduced *Aporrectodea caliginosa*, *A. rosea, Octalasion cyaneum*, and *O. lacteum*, but they differed in their relative abundances according to the system. The results show that earthworm community structure is linked to and modulated by soil properties. Both, species abundance and diversity showed significant differences depending on soil use intensity. A PCA analysis showed that species composition is closely related to the environmental variability. The ratio of native to exotic species was significantly lower in the intensive agricultural system when compared to the other two, lower disturbance Systems. *Microscolex dubius* was shown to be related to the naturalized grasslands and it was associated to Ca, pH, Mechanical Resistance, and to respiration. *Aporrectodea caliginosa* was related to high K levels, low enzymatic activity, slightly low pH, and low Ca, and appeared related to the highly disturbed environment. *Eukerria stagnalis* and *Aporrectodea rosea*, commonly found un the cattle-grazing system, were related to high soil humidity, low pH, low Ca and low enzymatic activity. These results show that earthworm assamblages can be good descriptors of different soil use intensities. In particular, *Microscolex dubius*, *Aporrectodea caliginosa*, and *Aporrectodea rosea*, showed different temporal patterns and species associations, due to the changes in soil properties attributable tos oil use intensity.

Keywords: soil ecology, soil use intensity, soil biota

1. Introduction

The soil biota plays a crucial role in regulating processes like water infiltration and storage, decomposition and nutrient cycling, humus formation, nutrient transformation and transport; moreover, they stimulate the symbiotic activity in the soil, improve the organic matter storage, and prevent erosion (Lavelle et al., 2006; Coleman and Crossley, 1996).

Several of the ecosystem services of the soil depend on the community of soil invertebrates (Lavelle et al., 2006), being earthworms one of the most common component of edaphic communities. Earthworms are considered ecosystem engineers because they improve decomposition processes and nutrient cycling (Lavelle et al, 1997; Six et al, 2004) and have a strong effect on the soils´ hydraulic properties (Lee, 1985; Edwards and Bohlen, 1996; Lavelle and Spain, 2001; Lavelle et al., 2006; Johnson-Maynard, Umiker, and Guy, 2007; Jouquet et al, 2008).

The most important factors limiting earthworm populations are food supply, moisture, temperature, and the texture and soil chemical characteristics such as pH, organic matter and macronutrients content (Satchell, 1967; Lee, 1985; Curry, 2004). Earthworm populations are also affected by the direct and indirect effects related to the type and extension of the vegetation cover (Mather and Christensen, 1988; Falco and Momo, 1995). The use of soils in agriculture can modify the physical and chemical soil environment thus modulating changes in abundance and composition of earthworm communities (Curry, Byrne and Schmidt, 2002). In this regard, Dale and Polasky (2007) indicate that in agricultural systems, changes in land cover are the direct result of management practices. Therefore, when changes in soil use occur due to different agricultural practices, earthworms' assemblages rapidly respond to them (Lavelle et al, 1997; Johnson-Maynard, Umiker, and Guy, 2007).

Since earthworm abundance and distribution are strongly influenced by the environmental conditions and the ecological status of the system (Falco and Momo, 2010), earthworm community structure can be successfully used as biological indicators of soil conditions (Momo, Falco, Craig, 2003).

The use of bioindicators has the advantage of providing historical and functional information about soils. Earthworm community structure integrate this information on soil conditions both in space and time and provide signals of the soil ecological state.

In this context, the objectives of this study were: 1) to assess earthworm community composition under three different soil use intensities: intensive agriculture, cattle grazing, and naturalized grasslands. 2) To identify the physical, chemical, and microbiological variables related to the observed community structure. 3) To detect which earthworms species are typical of each set of soil conditions and of each use.

2. Materials and Methods

2.1 Sampling sites

This study was performed in the rolling pampas within the Argentine pampas, a wide plain with more than 52 million hectares (520.000 km^2) of land suitable for cattle rising and cropping (Viglizzo et al, 2004). It is one of the largest and most productive agricultural regions in the world.

Agricultural systems based on crop–crop and crop–pasture rotations under grazing conditions have been very common in the region for over a century until the 1980s. The adoption of conservative tillage and no-till practices has significantly increased during the 1980s and 1990s. Although pesticides were extensively used since the 1960s, crops and pasture fertilization increased noticeably only during the 1990s (Viglizzo et al, 2003). The expansion of the land area used for annual crops on different environments means that the pampean ecosystem is currently under an intense disturbance regime (Navarrete et al, 2009).

The selected study sites are located in central Argentina, on argiudol soils, (Mollisols, Typical argiudols (USDA, 2010)). The study sites were privately owned fields located in Navarro, Buenos Aires Province (34° 49’35” S, 59° 10´ 38” W), and Chivilcoy (35º 03’10” S; 59º 41’ 08” W) approximately 75 and 150 km west of Buenos Aires City, respectively.

Weather regime in this region is temperate humid, with an average annual rainfall around 1000 mm. The mean annual temperature is 17 ◦C. Phytogeographycally, it is within the neotropical region, oriental district of the Pampean Province, and the dominant vegetation is a gramineous steppe (Cabrera and Willink, 1973).

2.2 Land use intensity in the selected sites

The systems analyzed differed only in their use intensity. Samplings were carried out on three different type of soil uses (agroecosystems) which represent three different levels of disturbance of the same argiudol soil:

Agroecosystem 1: agricultural systems, sites with 50 years of continuous intensive agricultural practices, and under no-tillage during the last 16 years. Under a regular corn-wheat-soybean rotation, currently under no-tillage, chemical weed control is used. During the cropping season, heavy machinery is used and insecticides, herbicides, and fertilizers are applied several times a year.

Agroecosystem 2: Cattle-grazing systems, sites with 40 years under direct grazing, turned to a feedlot system within the two years before the start of this work. Originally managed under grazing with high instantaneous animal load per hectare, it moved to bale production (oat, maize, and sorghum) two years prior to the start of this study.

Agroecosystem 3: Naturalized grasslands, sites with no significant anthropic impact during the last 50 years.

Nine sampling fields (three replicates for each one of the three treatments) were evaluated. At each site, five samples were taken every three months covering a two year period.

Each sampling date, five random samples were taken 25 meters apart from each other per each replicate (3) and treatment (3). Thus, a total of 45 samples were taken per sampling date. The size of each sample was of 25 x 25 x 25 cm.

The measured environmental variables were bulk density (BD), mechanical resistance (MR), humidity (RH), electrical conductivity (EC), organic mater (OM), pH, N, P, Ca, Mg, K, and Na. To characterize the sites, microbiological activity was assessed through soil respiration and free nitrogen-fixing bacteria activity (Nitrogenase Acetylene Reduction Activity, ARA). Methods used for chemical and physical analyses are shown in Table 1.

Earthworm extraction from the soil samples was performed by handsorting. Earthworms were preserved with soil until identification in the laboratory, and later fixed and preserved in alcohol- formalin - glycerin following Righi (1979) and identified by external morphology using keys from Righi (1979) and Reynolds (Reynolds, 1996). Clitelated individuals were identified to species level and pre-clitelated ones to genus.

At each site, earthworm taxonomic composition and population density were measured. Earthworm communities were characterized at each soil use intensity by population density, species richness, both observed and estimated (Chao index), and diversity (Shannon).

2.3 Statistical analyses

Due the to non-normal distributions of the physical and chemical data, Kruskall-Wallis ANOVA tests were carried out to compare variables between treatments.

The relationship between environmental variables and earthworm species abundances was further analyzed at the genus level to include non-clitelated individuals, by means of a principal component analysis (PCA) using abundances. Prior to analysis, the species abundances data were transformed using the Hellinger method of Legendre & Gallagher (2001) such that the resulting PCA represents Hellinger distance between samples rather than Euclidean distance. Physical and chemical variables were then fitted into the ordination space described by the first two principal components of the earthworm data by projecting biplot vectors. The statistical significance of the environmental variables is based on random permutations of the data and P-values were adjusted by a sequential multiple test procedure Hommel (1988). The ordination analysis and vector fitting were produced using the R statistical language (R Core team, 2012) and the Vegan package (Oksanen et al, 2011).

3. Results

3.1 Physical and chemical soil parameters

Of all the physical - chemical and microbiological parameters evaluated, only four variables (Na, EC, MR, and respiration) showed significant statistical differences between each of the three systems and only OM presented no differences (Table 1),. From the four variables that separate the three systems, the naturalized grasslands showed the highest Na and EC values.

Microbiological activity and soil microfauna were assessed through soil respiration and nitrogen fixing bacteria activity, which separated the naturalized grasslands for their high value when compared to the other two agroecosystems.

3.2 Earthworm assemblage response to soil use intensity

Results show that each soil use presents a different species composition and abundance (Fig 1). The relative abundances of the earthworm species found in each system is shown in figure 2.

A total of 9 earthworm species were identified across all systems. Five species were common to all of them: the native *Microscolex dubius* and the exotic *Aporrectodea caliginosa*, *A. rosea*; *Octolasion cyaneum*, *O. lacteum*, but differed in their abundances. The differences in abundance explain the significant differences found for the Shannon index values (ANOVA test $p<0.05$). The richness estimate (Chao) and the observed richness only differed in the cattle grazing system (Table 2).

In the naturalized grasslands the species identified as being the dominant (44% of all the individuals collected) was the epigeic native *Microscolex dubius*, followed by the endogeic exotics *Aporrectodea caliginosa*, *A. rosea*, *Octolasion cyaneum,*and *O. lacteum*. The other endogeic species, *A. trapezoides*, and the native *M. phosphoreus* were less frequent (Fig. 2a). Forty seven percent of all the individuals collected belonged to native species, and the ratio natives / exotics was 1:2.5.

In the cattle-grazing system the endogeic native *Eukerria satgnalis* was dominant and the exotic *A. rosea* was also common. Other species that were present albeit with a low frequency were *A. caliginosa,* and *Microscolex dubius. M. phosphoreus; Octolasion cyaneum,* and *O. lacteum* appeared on either one or two sampling dates only. In this system, *E. stagnalis* represents 68% of all the individuals collected and *A. rosea* represents 22% (Fig. 2b).The ratio of native species / exotic was 1: 1.3.

In the agricultural system, the most common species were the endogeic exotics *Aporrectodea caliginosa*, *A. rosea*, and *A. trapezoides*. The other endogeic species *Octolasion cyaneum*, and the epigeic native *Microscolex dubius* were less frequent. *Octolasion tyrtaeum* was only detected in the first sampling date, and O. *lacteum* appears in two sampling dates with a single individual each. Here, the exotic species represent 95% of the individuals (Fig. 2c). The agricultural system also had the lowest ratio of native species /exotic (1:6).

The differences in the chemical and physical soil parameters, as well as the different temporal distribution and species requirements determined the species´ co-occurrences found in each system. We observed these associations involving both native and introduced species, and combining different ecological categories. In this way the associations most frequently found in naturalized grasslands were: *A. rosea* – *Microscolex dubius* (appearing together in 33% of the samples), *Octolasion cyaneum* – *O. lacteum* (10%), and *A. rosea* –*Octolasion cyaneum* (10%). In the cattle grazing sites *A. rosea* –*Eukerria stagnalis* (67%) and in the agricultural system the most common associations were *A. caliginosa* –*A. rosea* (12.5%), and *A. rosea* – *Microscolex dubius* (12,5%).

The relationship between the characteristics of the environment and earthworm presence was further analyzed at the genus level, assessing the sensitivity of the groups with the soil parameter values through a Mann-Whitney U-test (Table 3).

Aporrectodea, *Octolasion* and *Microscolex* were present in samplings with the same levels of Mg, K, and BD. *Octolasion* separated from *Aporrectodea* only for Ca levels, and its response to soil humidity, MR, and Respiration put it close to *Microscolex*. In turn, *Microscolex* differed from the other groups due to Na, pH, ARA, and high MR (RM 10 cm). On the other hand, *Eukerria* was related to places with low levels of Ca, K, pH, EC, ARA, BD, MR and high humidity.

In order to know how the species´ composition explain the environmental variability, an indirect ordination PCA analysis was used, followed by a vector fitting (Fig. 3). Interestingly, the analysis showed no relationship between species with fertility levels (N, P and OM), but it did with elements of low soil mobility.

The first two axes explain 57% of the variance. The environmental variables that were significantly related to the species ordination were: RH, K, ARA, Respiration, MR, Ca, and pH (adjusted $P < 0.05$).

As it can be seen in Fig. 3, the ordination method shows that *Microscolex dubius* appeared related to the levels of Ca, pH , MR and respiration. This species is well adapted to environments rich in Ca, neutral pH, high microbiological activity, and high mechanical resistance. The environment defined by *Microscolex dubius* was related to the characteristics of the Naturalized Grassland system, and this species can be considered as indicative of the conditions prevailing in this system. In the same way *Aporrectodea caliginosa* (Fig. 3) is related to high K levels, low enzymatic activity, low pH, and low Ca. These are characteristic of the Agricultural system, being this cosmopolite, invasive species a good indicator of high perturbation sites. Finally, *Eukerria stagnalis* and *Aporrectodea rosea*, were related to the second ordination factor, and they describe an environment with high soil humidity, low pH, low Ca levels, and low ARA. These characteristics describe the Naturalized Grassland system. In this way, these species are clearly good descriptors of the three studied use intensity regimes of the same soil.

4. Discussion

These results show that the structure of the earthworm assemblage changes in relation to differences in soil use intensity in terms of its composition, abundance, seasonal dynamics and species associations. The data presented here show that, on the same soil and the same regimen of temperature and precipitations, the earthworm assemblage composition and abundance varied across the different systems studied, thus reflecting the differences due to land use intensities and their associated management practices. Tillage, weed control, fertilization and soil cover are parameters that best characterize the different land use intensities (Decaëns et al, 2008; Viglizzo et al, 2004; Curry, 2004), modifying the physical (water and air movement) and chemical environment, thus changing habitat suitability.

In the AG system under highest use intensity, earthworm communities were affected directly by the changes caused by tillage practices or indirectly through changes in food supply. Several studies indicate that earthworm communities are more abundant and rich in species in undisturbed soils when compared to cropland (Feijoo et al, 2011; Felten and Emmerling, 2011; Emmerling 2001; Curry et al, 2008; Decaëns et al, 2008). In this study, however, this pattern was not observed. All three systems have the same richness value and the abundances are consistently higher in the AG system with highest use intensity. This system also showed the highest native species replacement by exotic ones (ratio 1:1.6). These results agree with those of Lee (1985), Paoletti (1999), and Smith et al. (2008), who found that annual croplands have higher earthworm abundance than older fields. The dominance of introduced species is another characteristic of highly disturbed sites, as pointed out by Fragoso et al. (1999), Winsome (2006), and Chan and Barchia (2007).

The results presented in this work indicate that earthworm assemblage response to the same soil subjected to different use intensities can be used as indicator of agroecosystem soil use intensity. This response can be explained in terms of quality and quantity of food (Bohlen et al, 1997), the long term use of inorganic fertilizers which have a positive effect on the total number of worms (Edwards and Bohlen, 1996; Curry, 2004), pH changes and the level of Ca in the soil (Lee, 1985; Paoletti, 1999; Smith et al, 2008).

In the agricultural and cattle grazing systems, microbiological activity was low (as assessed through respiration and ARA) when compared to the naturalized grasslands. This can be explained, as the result of a reduction in pH and Ca, as well as to the ecological categories of species present (Scheu et al, 2002). Indeed, Scheu (2003) indicates that the presence of endogean species significantly reduces bacterial biomass and the functioning of the microbial assemblage. In AG, 95% of the species present are exotic endogeans, while in the CG system the 97% are endogean (70 % native, 30% exotic).

These results show that the ecological categories of the earthworm assemblages are also related to the microbiological activity of each studied system, being another indication that earthworms are good descriptors of the functioning of the edaphic environment.

Soil use intensity is also indicated by the presence of a few species that closely related to environmental variability. The intensification of the agricultural activities in the Pampas determine up to a 50% reduction in the calcium level (Casas, 2005). The ordination analysis related *Microscolex dubius* with high Ca levels and thus, to less disturbed environments. On the other hand, *A. caliginosa* and *Eukerria stagnalis* are present in low Ca soils.

In this sense, Mele and Carter (Mele and Carter, 1999) point out that the distribution and number of native species are negatively correlated with P, K, and Mg levels, these species being adapted to lower nutrient levels. In our study the only species that is related to higher K levels is *A. caliginosa*, which is the most abundant in the agricultural system.

5. Conclusions

The richness, composition and abundance as well as the species associations found, reflected the physical, chemical, and biological changes, brought about as a result of the different intensities of the agricultural practices used on each tested system. The data gathered indicate that the different environments are well characterized by the levels of cations (Ca, K), pH, microbiological activity, and physical variables such as mechanical resistance and moisture. Earthworm species assemblage reflected the changes in these variables and are therefore good descriptors of the studied systems.

Microscolex dubius was associated to sites with high levels of calcium, microbiological activity and high mechanical resistance and describes the naturalized grassland. *Eukerria stagnalis* is primarily associated with high humidity as seen in the cattle grazing system in which it is the dominant species. *Aporrectodea caliginosa* is associated to highly disturbed environments, with high K levels, low CE and NA, and low microbiological activity, all typical of the Agricultural system. It is interesting to note that the earthworm species most related to the different systems, are not related to the variables most usually measured: OM, N, and P. Therefore, monitoring these species would provide indirect estimations of those scarcely measured variables, thus complementing the information provided by other more common soil analyses in agroecosystems.

Eukerria stagnalis is indicative the high humidity, increased soil acidity, and a reduction in the levels of calcium and potassium, which are conditions prevalent in the intermediate use intensity system.

Aporrectodea caliginosa is the species best adapted to the most disturbed environment. This implies that the population recovers quickly after a disturbance (Curry, 2004, Felten and Emmerling, 2011; Decaëns, 2011), it is not significantly affected by changes in litter quality (Curry and Schmidt, 2007).

The spatial and temporal patterns in the distribution and abundance of earthworm species observed in this work followed the differences in the physical and chemical variables measured on the different systems studied. These differences are, in turn, a reflection of the different management practices applied to the same argiudol soil. Therefore, these results show that the structure of the earthworm assemblages can be reliably used for monitoring different soil use intensity management practices.

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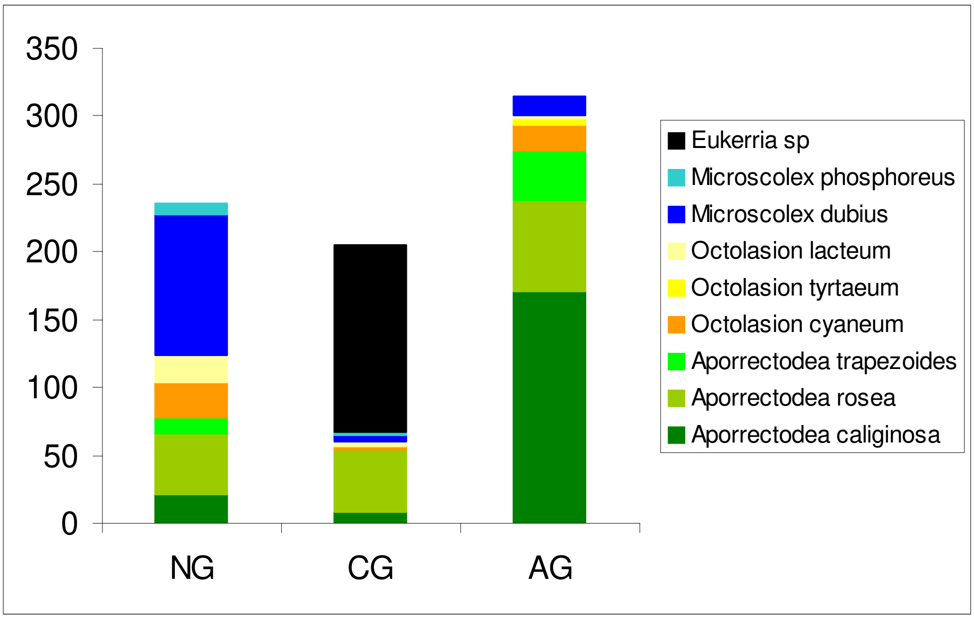

Figure 1: Abundance (N) of each earthworm species throughout the total sampling period for each system.

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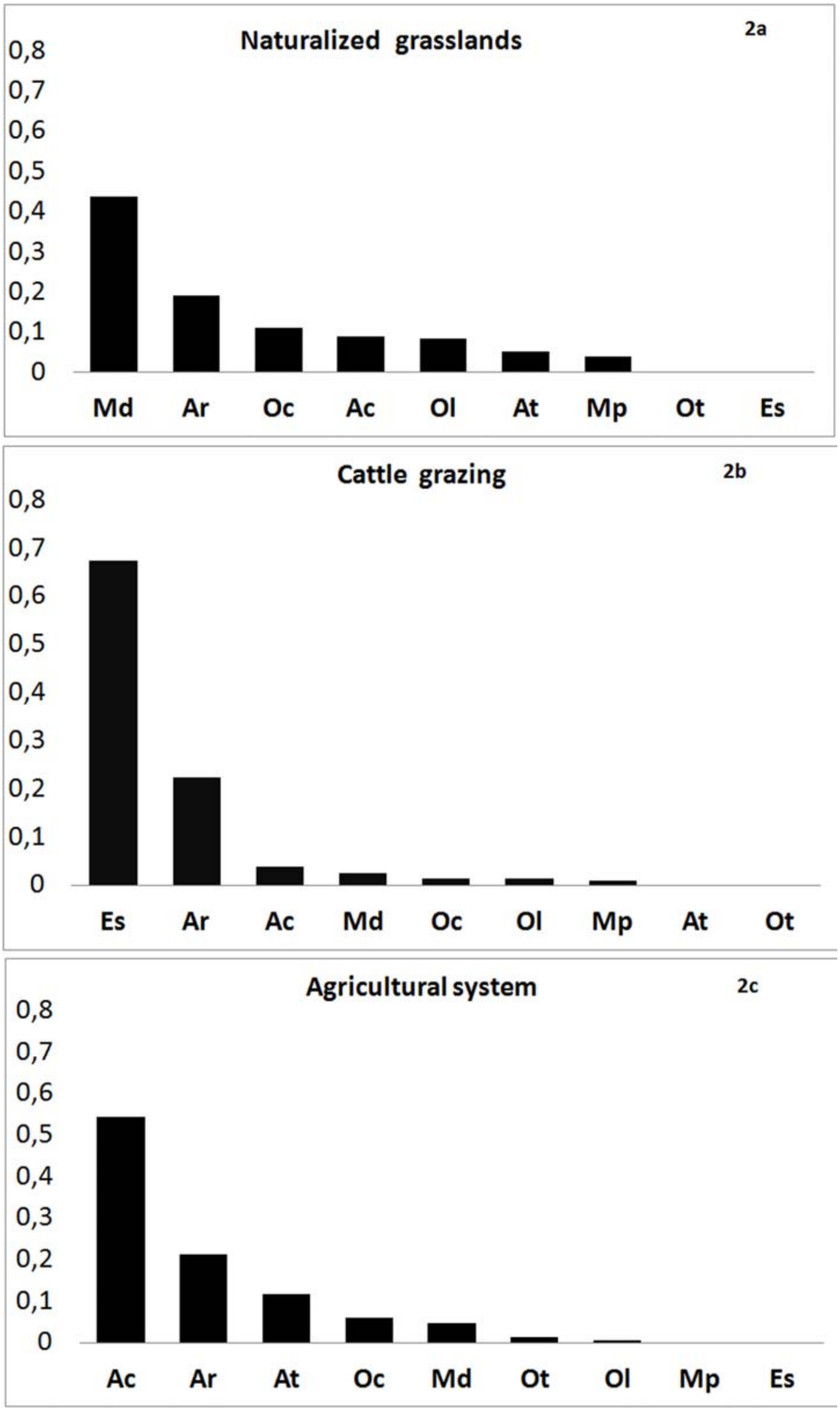

Figure 2: Relative abundance (ni/N) for each earthworm species identified in the three systems. 2a Naturalized grassland (NG), 2b Cattle grazing (CG), 2c Agricultural system (AG). Ac :*Aporrectodea caliginosa*, Ar: *Aporrectodea rosea*; At: *Aporrectodea trapezoides*; Ot: *Octolasion tyrtaeum*; Md: *Microscolex dubius*; Oc: *Octolasion cyaneum*; Ol: *Octolasion lactuem*; Es: *Eukerria stagnalis; Mp: Microscolex phosphoreus.*

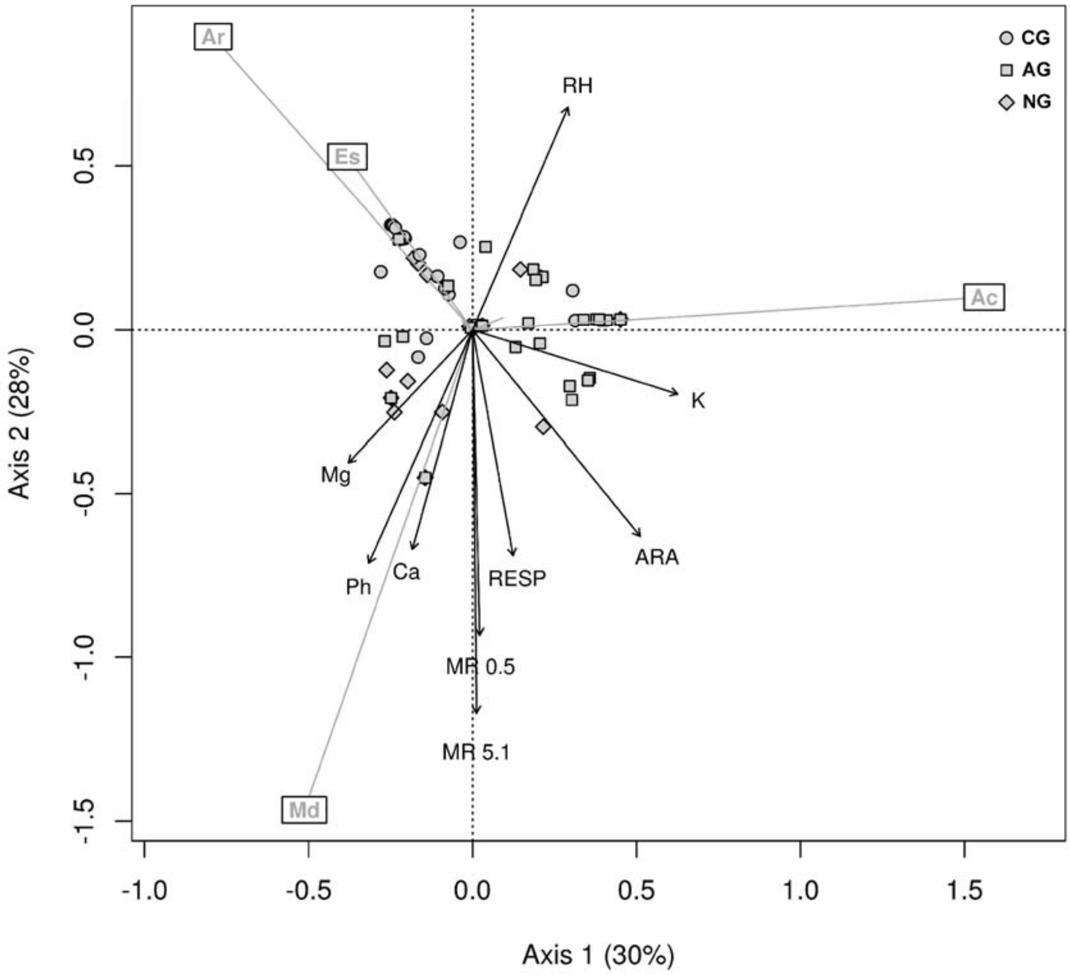

Figure 3: PCA biplot of Hellinger transformed earthworm species, only the four most abundant ones are shown. The arrows are significant environmental variables fitted into the ordination space. The percentage of explained variance is shown in each axis. Ac: *Aporrectodea caliginosa;* Ar: *Aporrectodea rosea;* Es: *Eukerria stagnalis*; Md: *Microscolex dubius.*

Table 1: Physicochemical and microbiological parameters measured (n=150 per system)

Parameters	Method	System		
		NG	CG	AG
P (ppm)	Kurtz y Bray	11+/- 8,5 b	15 +/- 12 a	14+/- 12 a
OM (%)	Walkey-Black	4 +/- 1,5 a	4 +/- 1,5 a	4 +/- 1,4 a
Ec ($dS*m^{-1}$)	conductivimeter	1,5+/- 1,3 a	0,8 +/- 0,5 b	0,7+/- 0,5 c
pH		7,5+/- 1 a	6 +/- 0,6 b	6 +/- 0,5 b
Bulk density ($gr*cm^{-3}$)	Porta	1,2+/-0,2 a	1,1 +/- 0,1 b	1,2+/- 0,1 a
Rh (%)	calculation	0,2+/- 0,1 a	0,3 +/- 0,1 b	0,2+/- 0,1 a
Ca ($cmol*Kg\ soil^{-1}$)	titration with EDTA	6,7 +/-1,3 a	5 +/- 0,5 b	6 +/- 0,7 a
Mg ($cmol*Kg\ soil^{-1}$)	titration with EDTA	1,8+/- 0,4 a	1,5+/- 0,7 b	1,6+/- 0,5 b
Na ($cmol*Kg\ soil^{-1}$)	flame photometry	1,3+/- 0,5 a	0,8 +/- 0,2 b	0,7 +/- 0,2 c
K ($cmol*Kg\ soil^{-1}$)	flame photometry	1,6 +/- 0,5 a	1,3+/-0,3 b	1,6+/- 0,5 a
N (%)	Kjeldahl	0,28 +/- 0,1 a	0,32+/- 0,1 b	0,29+/-0,05 b
Nitrogenase activity (nanolitres of ethylene* gr dry soil*incubation hour $^{-1}$)	ARA	0,3+/- 0,3 a	0,2+/-0,2 b	0,2+/-0,3 b
Respiration (mg de CO_2*gr dry soil day^{-1})	alkaline incubation	0,09+/-0,06 a	0,07+/-0,05 b	0,05+/-0,05 c
MR 0-5 ($Kg*cm^{-2}$)	cone	10+/- 6 a	2,5+/- 3 b	5,5 +/- 4 c
MR 5=10 ($Kg*cm^{-2}$)	cone	13+/-7 a	5 +/-5 b	8+/- 5 c

NG: naturalized grassland; CG: cattle grazing, AG; agricultural system. Different letters within a row indicate significant differences between systems, $P < 0.05$ Kruskall-Wallis ANOVA tests.

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Table 2: Observed and estimated species richness, mean density, and Shannon diversity index.

	Richness observed (Sob)	Richness estimate (Chao)	Density (ind/m^2)	Shannon index	
Naturalized grassland	7	7 +/- 0	46 +/- 19	0,53	a
Cattle - grazing	7	8,5 +/- 1,5	40 +/- 55	0,37	b
Agricultural system	7	7,25 +/- 0,4	76 +/- 56	0,57	a

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Table 3: Mean (range) values of each measured variable as they relate to earthworm genus presence (Non-clitelated specimens included)

Parameters	Genus			
	Aporrectodea	*Octolasion*	*Microscolex*	*Eukerria*
OM (%)	4,4 (3,7-5,3)	4,8 (4,4-6,1)	4,9 (3,2-5,9)	4,7 (4-5,9)
N (%)	0,29 (0,26-0,33)	0,29 (0,26-,33)	0,29 (0,27-0,34)	0,29 (0,25-0,34)
P (ppm)	8,7 (4,4-17,6)	7,7 (3,6-15,2)	9,3 (4,8-15)	6,8 (4,4-14,1)
Ca (cmol*Kg soil^{-1})	6,0 (5,5-6,4) a	6,6 (6,1-9) b	6,1 (5,8-7) c	5 (4,6-5,4) d
Mg (cmol*Kg soil^{-1})	1,7 (1,1-2) a	1,7 (1,1-1,9) a	1,6 (1,5-1,9) a	1,1 (1-1,6) b
Na (cmol*Kg soil^{-1})	0,8 (0,7-1) a	0,74 (0,4-0,9) a	0,9 (0,7-1,1) b	0,8 (0,7-1,1) a
K (cmol*Kg soil^{-1})	1,3 (1,1-1,7) a	1,5 (1,2-1,8) a	1,3 (1,1-1,8) a	1,1 (1-1,4) b
pH	6,2 (5,8-7) a	6,3 (6-6,8) a	6,8 (6,2-7,2) b	6 (5,6-6,5) c
Ec (dS*m^{-1})	0,6 (0,3-0,9) a	0,6 (0,3-0,9) a	0,7 (0,3-1,2) a	0,4 (0,2-0,7) b
Nitrogenase activity (nanolitres of ethylene* gr dry soil*incubation hour^{-1})	0,15 (0,07-0,3) a	0,26 (0,11-0,35) a	0,27 (0,14-0,37) b	0,15 (0,12-0,18) a
Respiration (mg de CO_2*gr dry soil day^{-1})	0,04 (0,03-0,09) a	0,04 (0,03-0,09) ab	0,07 (0,04-0,1) b	0,05 (0,02-0,07) a
Rh (%)	0,3 (0,2-0,3) a	0,25 (0,17-0,29) ab	0,2 (0,2-0,3) b	0,3 (0,3-0,4) c
Bulk density (gr*cm^{-3})	1,2 (1,1-1,3) a	1,21 (1,1-1,3) a	1,2 (1,1-1,3) a	1,1 (1-1,2) b
MR 0-5 (Kg/cm^2)	4,6 (2,25-8,2) a	4,9 (3-8) ab	7,8 (4,3-12,5) b	0,78 (0-3) c
MR 5=10 (Kg/cm^2)	6,5 (3,5-10,8) a	7,6 (4-11,5) a	10 (7-17) b	2,6 (0,8-5,5) c

Variables measured at the sampling points were each earthworm genus was recorded. Different letters within each row indicate significant differences between earthworm genus, $P < 0,05$, Mann–Whitney U-test pairwise comparisons.