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SOIL-EARTHWORM RELATIONSHIP REFLECTED IN LUMBRICIDAE DYNAMICS

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INTRODUCTION

Soil is a natural component of terrestrial ecosystems, in a long-term balance with climate and vegetation. Biota is also a very important part of the soil. Soil invertebrates are particularly good indicators of soil conditions due to their specific ecological requirements (Oehlmann & Schulte-Oehlmann, 2003; Gurcio-Ruiz et al., 2009). Together with other macroinvertebrates in the soil, earthworms were defined "ecosystem engineers" because of their role in the formation of soil structure by creating macropores (Blanchart et al., 2004; Edwards & Shipitalo, 1998), stable macroaggregates (Blanchart et al., 2004; and complex organic minerals. Considering the various means by which earthworms can alter soil structure, it is obvious that both earthworms and the factors that regulate their populations can have an important impact on soil organic matter dynamics (Brown et al., 2000). Brown et al. (2004) identified several factors involved in the impact of earthworms on primary production. Improving knowledge about how these factors interact to influence the dynamics of organic matter in soil is therefore relevant to agricultural systems management accountability. A high density and abundance of earthworms in soil indicate that dietary supplements, usually biocides, are not used for various crops since soil structure and water stability are maintained at an optimum level (Kladivko-Eileen, 2001; Miura et al., 2008). The changes in the structure and function of lumbricidae, biomass and ecological groups of species also reflect the influence of anthropogenic factors (Bauchhenb, 2006) and soil characteristics on lumbricidae populations. The soil ingested by earthworms undergoes chemical and microbial changes when passing through their gut. Organic matter is digested and both pH and gut microbial activity increase. (Edwards et al., 1996; Lukkari et al, 2006). Earthworms accelerate mineralization, but the effect depends on the species and their interaction with physical and chemical characteristics of the soil, organic matter and soil biota (Butenschoen et al., 2009, Sierra et al. 2014). In their dynamics and functions, earthworms are influenced by physical and chemical characteristics of the soil. Recently, it has been shown that earthworms increase organic matter stabilization in the soil only when debris is applied (Fonte & Six, 2010).

Earthworms fall into three ecological groups depending on their morphological and behavioural features (Bouche', 1977; Lavelle, 1983; Sims & Gerard, 1999): epigeic, endogeic and anecic. Each group is distinguished by way of making burrows, food preference and their contribution to the biosystem.

1. *Epigeic earthworms* live in litter, consume considerable amounts of crude organic matter and have a wide range of enzymatic capacities, mainly from ingested microflora (Curry et al., 2007). Their primary role is fragmentation, grinding, decomposition of organic matter (Sampedro et al., 2008) and changes in fungi composition (McLean et al. 2000). This group is represented by some pigmented earthworms like *Dendrobaena octahedral, Lumbricus rubellus.*

2. *Endogeic earthworms* live in the soil. They feed mainly on soil organic matter and dead roots. Living roots are rarely eaten by endogeic earthworms (Lavelle, 1983; Lavelle et al, 1989). Endogeic species are further divided into oligo-, meso- and polihumic according to the type of soil ingested: (i) deep layer soil, with a low organic content; (ii) bulk soil in the upper layers 10 to 25cm; and (iii) upper layer soil rich in organic matter or ingestion of fine particles with high organic content rather than coarse sand particles. Endogeic earthworms are active "excavators" that can greatly increase soil aggregation. These mainly include humivorous earthworms.

3. *Anecic earthworms* feed on plant debris, but live in underground burrows. Their main role is to transport organic matter from the soil surface in the deeper layers where it is decomposed by changing soil physical characteristics (e.g., water infiltration and soil aeration by digging burrows). This category includes *Lumbricus terrestris.* These are not defining classifications because the behavior of many species is intermediate and can vary depending on the environmental conditions (Edwards & Bohlen, 1996).

The study aims to highlight the feed-back relationship between soil and lumbricidae in a maize-cultivated agrosystem (*Zea mays*). The objectives are to analyze the soil profile, to characterize the soil physical chemical structure, to analyze lumbricidae dynamics in terms of: density (ind./m^2), frequency (%), relative abundance (%) and the correlations between density, soil texture, relative abundance and soil pH.

MATERIALS AND METHODS

Study area - The agroecosystem in which soil samples were collected is located in Arges county, Piedmont Cândeşti (Romania). The geographic coordinates are: $44^0$55'06''N, $25^0$06'28''E.

Pedogenetic conditions – Glacis relief on the right side of Cârcinov River, slightly inclined surface (10°), Eastern exhibition and absolute altitude 327m. Parental material: groundwater, 5-10m in depth, good drainage. Climate (Pitesti Weather Station) - 307m abs. alt., T_m=9.8^{0}C; $T_{(I)}$=-2.4^{0}C; $T_{(VII)}$=20.8^{0}C; P_m=700mm; ET=662mm; I_{ar}=34. Natural vegetation (underneath the oak forests), mixed
with beech, acacia, hornbeam. Use: arable (maize).

Soil morphological characterization – The soil profile is necessary to characterize its morphological structure. The soil samples were collected in labeled plastic bags from genetic horizons and subhorizons using a spatula, on a 10-15cm thick layer.

Physical analyses - consisted of a granulometric analysis on fine soil (<2mm), by pipetting the fraction, 0.02mm in diameter or even smaller, and sieving the fraction, over 0.02mm in diameter. Pretreatment and dispersion of the soil sample preceded soil content in fractions: 2-0.2mm (sand), 0.2-0.002mm (dust) <0.002mm (clay). The sum of granulometric fractions was always 100. The soil granulometric composition was necessary to establish classes (coarse, medium, fine) and subclasses (sand, loamy sand, sandy loam, clay loam, clay) of soil texture.

Chemical analyses were based on ICPA analysis methodology (1981, 1986, 1987), and STAS, ASRO standards in line with European standards in the field of soil analysis.

Sampling and determination of lumbricidae. Lumbricidae have been sampled manually (Raw, 1960) and randomly on 50/50 m surface, on soil levels, 30 cm in depth. Ten units of monthly samples were extracted during June-August 2014. The sample unit was 25/25 cm. After sampling, the earthworms were immersed in 8% formalin. In order to identify species, earthworms were transported to the laboratory. They were measured up to species level using the stereomicroscope, the Identification Manual "*Lumbricidae in Romania*" (Pop, 1949) and "*A guide to the valid names of Lumbricidae* (*Oligochaeta) (1983).*

Statistical analysis. Statistical interpretation of the results was performed using SPSS 16 for Windows, Microsoft Office Excel 2007. The parameters applied LSD analysis of variance for the significance threshold p<0.05. The trend line was drawn and Pearson simple correlation coefficient was calculated.

Numerical density expressed in ind/m^2 was necessary to calculate other structural indicators: standard error of the mean (S') with a confidence level of 95%, the coefficient of variation (C_V), variance (S^2), variance / mean ratio ($S^2/\bar{x}$).

The coefficient of variation (C_V) was used for the quantitative expression of the standard error/mean ratio. The calculation of this indicator was performed using the formula:

$$C_v = 100 \times \frac{s^2}{\bar{x}},$$

where: S^2 = standard error; $\bar{x}$ = arithmetic mean

Relative abundance was calculated using the formula:

$$A_r = \frac{n}{N} \times 100,$$

where : n = number of individuals for species A (from the samples); N total number of individuals (for all species)

Frequency was determined using the frequency index "F" according to the relationship:

$$F = \frac{p}{P} \times 100,$$

where p = the number of sample units for "A" species ; P = total number of sample units collected.

RESULTS AND DISCUSSION

Morphological and physicochemical characterization of soils

Five pedogenetic horizons were identified in the soil profile: Apcol (0-28cm), AC (28-49cm), C1 (49-73cm), C2 (73-90cm), C3 (90-113cm). The soil profile was coluvic-eutric aluvisol on coluvial-proluvial deposits, sandy loam.

Apcol horizon (0-28cm) showed a moist grayish-brown medium loam (10YR 5/3 with 5/6) and 10YR 6/3, dry, small angular polyhedral, slightly developed, moist, friable, slightly plastic, medium sticky, where earthworms were identified. There were plant remains (cobs), undecomposed in the first 8cm, rare thin and medium roots, gradual passages, AC line (28-49 cm), which was brown medium sandy loam (10 YR 4/4) with rusty stains 10 YR 4/6, moist and 10 YR 5/6 dry, medium angular polyhedral, moderately developed, damp, slightly compact, slightly plastic, slightly sticky; there were worms, very rare thin roots, rare medium roots, clear passage, C1 line (49-73cm) - this horizon was a yellowish-brown medium sandy loam (10 YR 5/3) dry, dry angular polyhedral, small angular polyhedral, slightly developed, damp, slightly compact, slightly plastic, slightly sticky, with rare roots, gradual passage, C2 line (73-90cm), characterized by a brown medium sandy loam with a slight blue tinge (10 YR 5/4 with 2.5 Z 6/2), moist and 10 YR 5/2 dry, slightly sticky, slightly medium compact, damp, unstructured, rare medium roots, clear passage, C3 line (90-113cm) – brown-gray medium sandy loam (10 YR 5/4 with spots 10 YR 5/8), moist and 10 YR 5/3 dry, damp, non-sticky, medium.

Physical analyses showed a granulometric composition which allowed determination of soil classes shown in Table 1.

The results of soil physical and chemical analyses showed a highly acid reaction in Apcol and AC horizons and moderately acid in the others. Humus content correlated with soil texture was low and very low in Ap-Ac-C horizons and very low in the other horizons. Nitrogen index (H x V_{Ah})/100 was low throughout the soil profile, mobile phosphorus content (extractable in ethyl lactate) was low in Ap horizon and very low in the other horizons. Mobile potassium content (extractable in ethyl lactate) was medium in Ap horizon, low in C2 horizon and very low in the other horizons. The total capacity of cation exchange (T_{sh}) was low in Ap, C1, C2, C3 horizons and slightly higher to medium in C4 horizon.

The sum of exchangeable bases (SB) was low throughout the soil profile; hydrolytic acidity (A_h) was high in Ap horizon, medium in AC horizon and low in the other horizons. The degree of base saturation (V_{SH}) indicated an *oligomesobasic* soil in Ap horizon and *mesobasic* in the other horizons (on base saturation scale from highly oligobasic to base saturated) (Table 2). Soil texture was medium sandy loam throughout the soil profile, with small humus reserve.

Table 1. Soil physical analyses

Horizont	Depth	Granulometric fractions (mm) în %						Texture
		Sample depth (cm)	2.0-0.2 (coarse sand)	0.2-0.02 (fine sand)	0.02-0.01 (dust I)	0.01-0.002 (dust II)	Below 0.002 (loam)	
Apcol	0-20	10-20	11.6	51.1	6.6	10.2	20.5	Medium sandy loam
AC	28-49	33-43	27.8	49.4	2.0	3.0	17.8	Medium sandy loam
C1	49-73	57-67	26.1	50.8	3.8	3.7	15.6	Medium sandy loam
C2	73-90	76-86	30.8	42.3	3.3	7.8	15.8	Medium sandy loam
C3	90-113	95-105	28.8	44.1	6.7	5.5	14.8	Medium sandy loam

Table 2. Soil chemical analyses

Horizons	Apcol	AC	C1	C2	C3
Sample depth	10-20	33-43	57-67	76-86	95-105
pH in H_2O	4.68	4.85	5.21	5.09	5.01
Carbonates (%)	-	-	-	-	-
Organic carbon (%);	1.136	0.663	0.588	0.158	0.492
Humus (Corg x 1.724) (%);	1.958	1.140	1.014	0.272	0.849
Mobile phosphorus P ppm	10.0	7.0	5.0	3.0	3.0
Mobile potassium K ppm	156	56	52	102	60
Exchange bases (SB) me/100g soil	8.8	9.8	11.4	10.6	15.0
Hydrolitic acidity (Ah) me/100g soil	6.51	4.32	2.73	2.57	3.92
Changeable hydrogen ($SH_{8.3}$)	7.26	5.72	6.76	6.27	6.60
Cation exchange capacity (T_{sh});	16.07	15.52	18.16	16.87	21.60
Cation exchange capacity (T_{AH});	15.31	14.20	14.13	13.17	18.92
Degree of base saturation (V_{Ah})	57.0	69.0	80.6	89.5	79.2
Degree of base saturation (V_{SH})	54.7	63.1	62.7	62.9	69.5

Soil - Lumbricidae relationship

Lumbricidae community in June and July 2014 comprised 9 species. Identified species belong to the three ecological groups (epigenic, endogeic, anecic), each with an important role in the soil (Table 3). The analysis shows that epigenic species have a higher share in this habitat. Similar results were also obtained by Bertrand et al., (2012) in their studies. Pop (1998) showed that *Octodrilus* species in the Romanian Carpathians affect clay mineralogy and formation of illite in the soil, a process that takes hundreds of thousands of years in the absence of soil biota. In the laboratory experiments, Carpenter et al., (2007) showed that *Eisenia veneta* epigeic species, accelerated the weathering of anorthite, biotite, smectite and kaolinite; smectite was transformed to illite and kaolinite reacted to produce a new mineral phase (Carpenter et al., 2007). Whether it is the earthworms, microorganisms stimulated in their gut (Brown, 1995) or a collective action of both organisms that are responsible for the mineral weathering effect is still open to debate.

The number may change over time depending on certain biotic or abiotic factors (annual, seasonal or accidental fluctuations influencing the spatial distribution of individuals, structure according to age, size or sex (Mustață & Mustață, 2003).

Variation coefficient values were higher in species with low density and high standard errors, and low in species with high density and low standard error. The higher the average standard error, the higher the degree of biotope heterogeneity. The more uniform the sample numerical representation, the lower the average standard error and a high degree of biotope homogeneity (Table 4). Dynamics of lumbricidae populations in the two months highlighted their numerical density evolution expressed by the average number of individuals / m^2 (Figure 1). In June 2014 lumbricidae density ranged between 0.6 ind / m^2 and 4.53 ind / m^2. *Lumbricus rubellus species* recorded the highest density (4.53 ind/m2), followed by *Octodrilus complanatus* (4.0ind/m^2), while *Octolasion lacteum,* common species in all soil types, recorded the lowest density (0.6ind/m^2). *Octolasion lacteum* recorded the highest density in July (8.26 ind/m^2) compared to the other species which recorded similar densities ranging between 2.4ind/m^2 and 4ind/m^2). The lumbricidae values within two months were pretty low thus confirming other studies in the specialized literature, according to which lumbricidae populations in agroecosystem soils recorded low density probably due to anthropogenic interventions, without excluding the influence of soil type. According to Curry et al. (2002), Hendrix & Edwards, (2004), earthworms populations in cultivated lands are generally lower than those in undisturbed habitats.

Table 3. Lumbricidae community in the soil analysed in June-July 2014

Species	Total number of species belonging to three ecological groups		
	Epigeic	Endogeic	Anecic
Octodrilus complanatus (Dugès 1828)	-	-	+
Dendrobaena octaedra (Savigny 1826)	+	-	-
Octolasion lacteum (Őrley 1885)	-	+	-
Lumbricus rubellus (Hoffmeister 1843)	+	-	-
Dendrodrilus rubidus rubidus (Savigny 1826)	+	-	-
Lumbricus castaneus (Savigny 1826)	+	-	-
Lumbricus terrestris (Savigny 1826)	-	-	+
Octolasion lissaense (Michaelsen, 1891)	-	+	-
Dendrobaena alpina (Rosa,1884)	+	-	-

Table 4. Statistical data of lumbricidae in June-July 2014

SPECIES	JUNE					
	D (ind/m^2)	STDEV (D ind/m^2)	F (%)	STDEV (F%)	A_r (%)	CV
Lumbricus rubellus	4.53	3.56	21.66	11.6	30.35	78.65
Octolasion lissaense	1.6	2.6	6.66	12.11	10.90	167.33
Octolasion lacteum	0.8	1.3	3.33	5.16	4.68	167.33
Dendrobaena octaedra	1.06	1.3	5.0	5.47	5.88	122.47
Dendrodrilus rubidus	2.93	2.1	16.66	10.32	13.92	72.4
Octodrilus complanatus	4	4.1	13.33	10.32	22.72	103.53
SPECIES	JULY					
Octolasion lacteum	8.26	4.34	35	16.43	45.58	54.93
Lumbricus castaneum	3.46	2.93	15	12.24	20.63	84.68
Dendrobaena alpina	2.4	1.6	11.66	7.52	18	69.92
Dendrobaena octaedra	4	3.61	20	17.88	36.58	90.33
Lumbricus terrestris	3.46	2.75	15	8.36	50	79.49
Octolasion lissaense	3.46	2.35	20	14.14	100	67.93

Abbreviations: D-density (ind/m^2) ; STDEV- standard deviation (D ind/m^2) ; F-frequency (%); A_r- relative abundance (%); CV-coefficient of variation

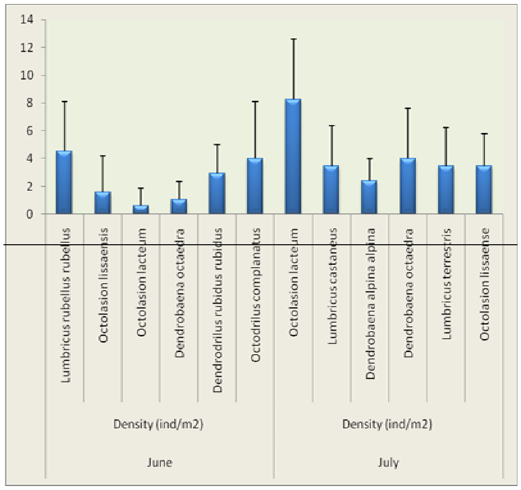

Figure.1. Lumbricidae Density (ind/m^2) in June-July 2014

Individuals' frequency contributes to the characterization of lumbricidae to highlight each species share in the populations' relationships. Average frequency of individuals was low in June, ranging between 3.33% and 21.66% (Figure 2).

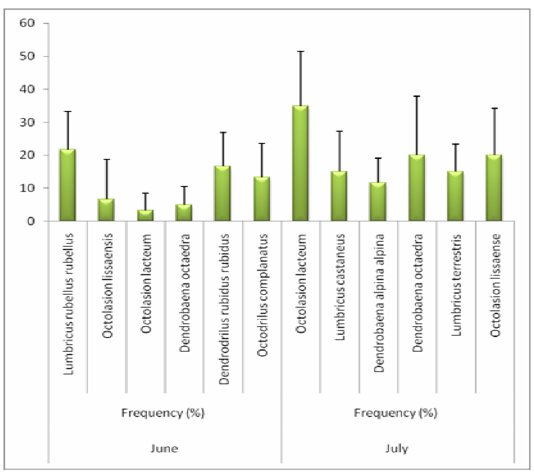

Figure 2. Lumbricidae frequency (%) in June-July 2014

The average frequency of individuals in July was slightly higher than in June, ranging between 15% and 35%.

The highest values of the samples' frequency were recorded by epigeic *Lumbricus rubellus* (21.66%) and *Dendrodrilus rubidus* (16.66%), in June, and *Octolasion lacteum* (35%), *Octolasion lissaense* (20%) in July. The latter species recorded a value equal in frequency to epigenic *Dendrobaena alpine* (20%). There is conformity between density, spatial distribution and frequency: (1) – high frequency for high numerical density and uniform spread species; (2) – very low frequency for very high density and random or clustered distribution species; (3) – very low frequency for low density and uniform distribution species.

Species relative abundance is not always directly linked to its importance in biocenosis, in the sense that the most abundant species are not always the most important and vice versa (Botnariuc & Vădineanu, 1982). The way in which lumbricidae participate in the bio-system structure and operation can be expressed by this index, very important in assessing the role of each species in biocoenosis activity.

Abundant lumbricidae had a great influence on soil characteristics. *Lumbricus rubellus* (30.35%) and *Octolasion complanatus* (22.72%) (figure 3) had a higher contribution to biocenosis in June. *Octolasion lissaense* (100%), *Lumbricus terrestris* (50%) and *Octolasion lacteum* (45.58%) (Figure 3) recorded the highest relative abundance values in July.

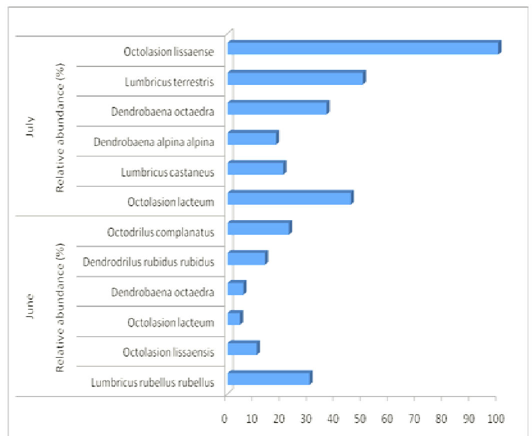

Figure 3. Lumbricidae relative abundance (%) in June-July 2014

Endogeic and epigeic species inhabiting the surface soil layers had a greater role in the ecosystem structure and function in June and July 2014. One of the most important roles of earthworms in the soil was to control humification rate by feeding, drifting, and interaction with microorganisms (Ponge, 1991).

This seems to be achieved mainly by controlling soil C inputs, burying litter, improving the rate of decomposition, and regulating microbial activity in the drilosphere (soil surrounding earthworms' burrows) as well as C protection in stable aggregates (Brown et al., 2000). Garden soil blackening is also a slow process involving chemical reactions and microbial activities. This process can be pushed by earthworms which prepare mixtures of soil and litter composed of fragmented, macerated leaves and soil fine particles for microbial activity. It is well known that humus can be produced from organic matter in a few months (Edwards et al., 2011).

Analysis of the correlation between relative abundance (%) and soil texture (mm) is not significant (R = 0.315, p = 0.058) (Figure 4). This correlation can suggest that species abundance is not associated with granulometry and there must be other local factors affecting the abundance of earthworms' population. Similar results were also found by Martin et al (2011) in their studies. Earthworms' abundance may be affected by pests such as aphids, possibly due to the quality of products used in increasing crops and plant communities (Laossi et al, 2011; Wurst et al, 2011).

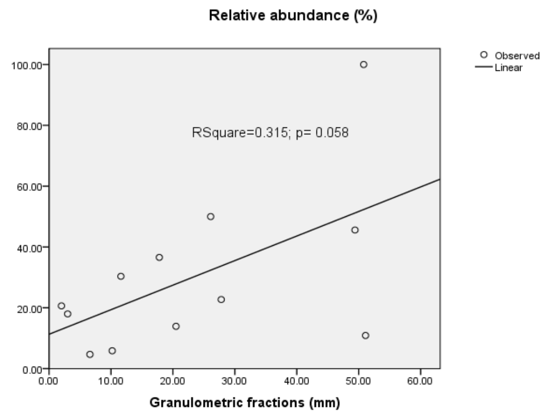

Figure 4. Correlation between lumbricidae relative abundance (%) and granulometric fractions (mm) in June-July 2014

In general, soil texture, the proportion of clay, sand, and humus in the soil layers correlate with humidity and affect the species abundance. Depending on the soil level where samples were collected, humidity was between 50-100% during the two months. Although earthworms prefer moist environments, humidity was too high due to the large amount of rainfall in this period. Species abundance was therefore affected by burrows' flooding and oxygen deprivation, particularly on 0-10cm and 10-20cm levels. Epigeic species in subalpine meadows are considered flooding bioindicators as they provide useful information regarding soil texture and organic matter quality (Bullinger-Weber et al., 2012).

Other studies show that coarse-textured sandy soils restrict species abundance due to reduced capacity for holding water or rapid water drainage. Fine-textured clay and humus have high water retention capacity and limit the activity of earthworms due to lack of oxygen to survive. Recent studies (Jänsch et al., 2013) have shown that *Octolasion* species is rarely found in sandy textures, while *Dendrobaena octaedra* is rarely found in clay textures.

Also, aggregate stability is a key factor to soil fertility and affects soil organic matter dynamics (Abiven et al., 2009), while the size, quantity, and soil aggregates' stability reflect a balance between factors such as soil fauna (Six et al., 2002).

The correlation between species density and soil pH showed that the higher the soil pH, reaching neutral values, the lower the species density The correlation for this study is justified by the higher number of epigeic species, which are generally acid-tolerant. The correlation between density and soil pH was significant (June - R^2=0.240; p<0.002; July – R^2=0.202; p <0.006)- (Figure 5).

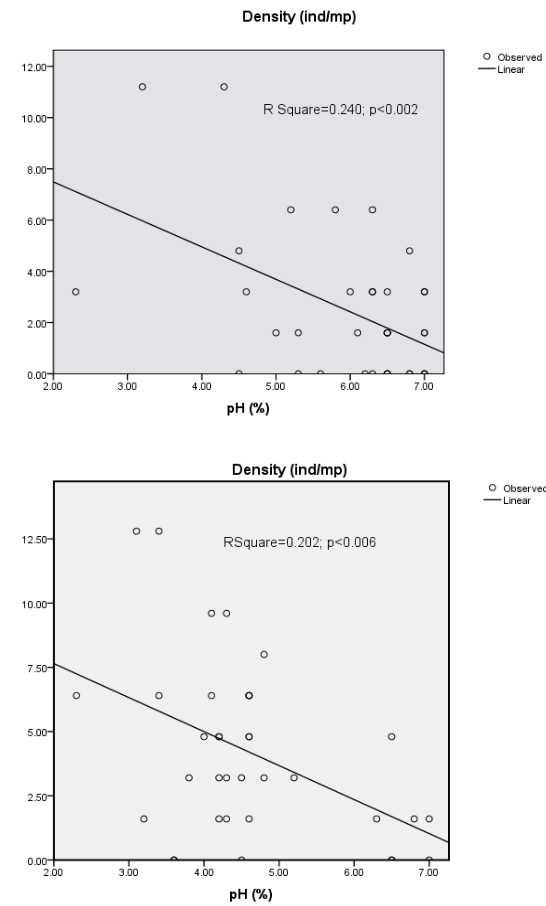

Figure 5. Significant correlations between species density (ind/m^2) (up) and soil pH (%) in June - July (down) 2014

Soil pH is one of the major limiting factors of lumbricidae populations. Most species prefer neutral or slightly acid soils. *Dendrobaena, Dendrodrilus* species are generally acid-tolerant, while *Octolasion, Octodrilus* prefer slightly acid environments with pH around 5.0%. According to Bouché (1972) *Octolasion* species was described as a limited taxon in organic, neutrophil and relatively acid-tolerant biotopes, while *Octodrilus* is a taxon present in agricultural soils at woodside and in moist substrates (Monroy et al., 2007). Jänsch et al., 2013 showed in their studies that epigeic species (except *Lumbricus castaneus*) are highly acid-tolerant and *Dendrobaena octaedra* species is rarely found in sites with a pH>5.5. Previous studies showed that anecic species such as *Lumbricus terrestris* are closely related to soil depth and tolerant to pH variations (Bouche, 1972; Guenat et al., 1999).

CONCLUSIONS

A further approach would be to study earthworms in soil plots on a long term, to evaluate their effects on soil structure dynamics and vice versa. Earthworms contribute to soil ecosystem functions significantly, and further research will lead to a better understanding of their role in these processes.

The functions' management of these organisms in the soil can be achieved by a better knowledge of their diversity and dynamics, as well as populations' disturbing factors.

Agroecosystems are, by definition, systems providing positive and negative feed-back, so it is difficult to make simple predictions about the consequences of changing size of lumbricidae populations. It should also be noted that soil is a complex environment in which the interaction of several factors, such as bedrock, climate, topography and vegetation, is not negligible. All these parameters can positively or negatively influence earthworm populations' dynamics considering the period under research.

ABSTRACT

Interactions between soil fauna and their habitat can be described in "positive" and "negative" terms according to the feed-back of the ecosystem balance. The soil cultivated with maize was analyzed in terms of morphological, physical and chemical structure in June and July 2014, in order to determine the specific species and to highlight the relationship between soil and earthworms. The prevalent soil was coluvic-eutric aluvisol on colluvial-proluvial deposits, sandy loam. Lumbricides community comprised nine species classified in three environmental categories (epigeic, endogeic and anecic). Statistics showed low density (ind/m^2) and species frequency (%) on this type of soil. Epigeic species recorded the highest abundance. The correlation between species density and soil texture was insignificant (R=0.315; p=0.058), while the correlation between soil pH and relative abundance was significant for two months: June (R=0.240, p<0.002) and July (R=0.202; p<(0.006).

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