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Earthworms, microbial biomass and enzymatic activities as useful bioindicators of agroecosystem sustainability in protected and deteriorated savannas

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Abstract

We studied soil parameters and the earthworm biomass and density in a protected savanna (PS) from fire and cattle raising for more than fifty years located at Estación Biológica de los Llanos, Venezuela (EBLL), and an adjacent natural savanna (NS) under frequent burning and agricultural activities. Earthworm sampling was carried out at the end of the dry season (April), and at the peak of the wet season (July-August. The main physical, chemical, microbial (biomass) and enzymatic activities of soils in both systems were estimated. The establishment of tree canopies and the presence of litter layer at PS have increased the soil water retention capacity. In the PS also have increased the organic-C, C-microbial biomass, dehydrogenase activity and fertility levels respect NS. The presence of earthworms, in turn, affects the physical properties of the soil through the construction of channels and galleries.The average density of earthworms in the PS ranged between 25.6 and 85 individuals m-2, and average biomass varied between 6.92 and 23.23 g m-2. In the NS earthworms only were found in the wet season, with a mean density of 22.40 individuals m-2 and a mean biomass of 5.17 g m-2. Results suggest that, agricultural management in savannas can decrease their earthworm communities and microbial and enzymatic activities. However, earthworm populations and soil quality indicators can be restored after long-term protection. Therefore, we can emphasise the potential use of the density and biomass of earthworms as bioindicators of soil fertility and as active actors in the regeneration of deteriorated savanna's soil.

Key Words: macrofauna, Glossoscolecidae, microbial biomass, dehydrogenase, fire

Introduction

The Trachypogon dry savannas in Venezuela and Colombia occupy a very important part of the territory known as Orinoco's Llanos and have been recorded as one of the less productive terrestrial ecosystems of the world [1,2]. Those savannas are currently used to cattle raising and agricultural activities which include fire as the main management tool, after such agro practices are long used, a drastic decrease in pedofauna community (particularly earthworm populations) and, in microbial and enzymatic activities generally occur [3,4,5]. Therefore, when a savanna is protected for an extended period of fire and agricultural activities, an increasing in the woody component occur [6,7], as well as an improvement of the physical-chemical and biological properties of the soil including the reestablishment of the earthworm communities [3,4,5].

The Estación Biológica de los Llanos (EBLL), located in Calabozo, Guárico State, Venezuela has been protected from fire, grazing and cultivation since 1960 [6], However, in recent years, in some places within EBLL, despite management efforts, occasional fires have occurred. The extended period of protection has resulted in an increased woody component [6,8] and improved physical-chemical and biological properties of the soil [3,4]. All those factors may have a strong influence in the reestablishment of the earthworm communities in the protected savanna.

It is a well-known fact that soil macrofauna modify the soil and litter environment indirectly by the accumulation of their biogenic structures (casts, pellets, galleries, etc.). Thus, in savanna earthworms have a dominant role in shaping the soil environment due to both their abundance and effects of their biological activities; moreover, earthworms influence soil fertility and plant growth through the production of casts which impact the availability and nature of the spatial and trophic resources in soil [9,10]. The beneficial effects of the earthworm activities include: improvement of soil physical properties [11], and chemical and biological properties [12,13,14,15]. All these processes significantly impact the soil fertility through the activation of enzymatic activities and the release of available forms of nitrogen (N) and phosphorus (P) once the soil passes through the gut of the earthworm species [16,17,18,19,20,21,22].

Earthworm activities may also stabilise soil organic matter (SOM) through its incorporation and protection in their casts [13,23]. After cast deposition, compounds of microbial origin produced as result of the earthworm activities, in addition to earthworm mucilage, bind soil particles and contribute to the formation of highly stable aggregates [13,23]. This enhanced microbial activity decreases with ageing when the casts dry. Thus C mineralisation rate decreases and mineralisation of SOM from casts may be blocked for several months [13,23].

This contribution examines information on earthworm communities and other soil quality indices in a Trachypogon savanna long-term protected (PS) from fire and cattle raising for more than five decades at Estación Biológica de los Llanos in Central Llanos, Venezuela, and the adjacent non-protected savanna (NS) under cattle raising and low input agriculture practices.

Therefore, the specific objectives of this study were to: i) analyse the effect of a long period of protection from fire and agricultural activities has had on physical and chemical soil properties in a nutrient-poor savanna which generate conditions leaving to the establishment of Oligochaeta communities, ii) analyse the effect of a long period of protection has on microbial and biochemical soil properties in a nutrient-poor savanna iii) compare the biomass and density of earthworms existing in EBLL and the adjacent agro-managed savanna, iii) iv) compare the physico-chemical and biochemical properties of earthworm casts and adjacent soil in the chosen

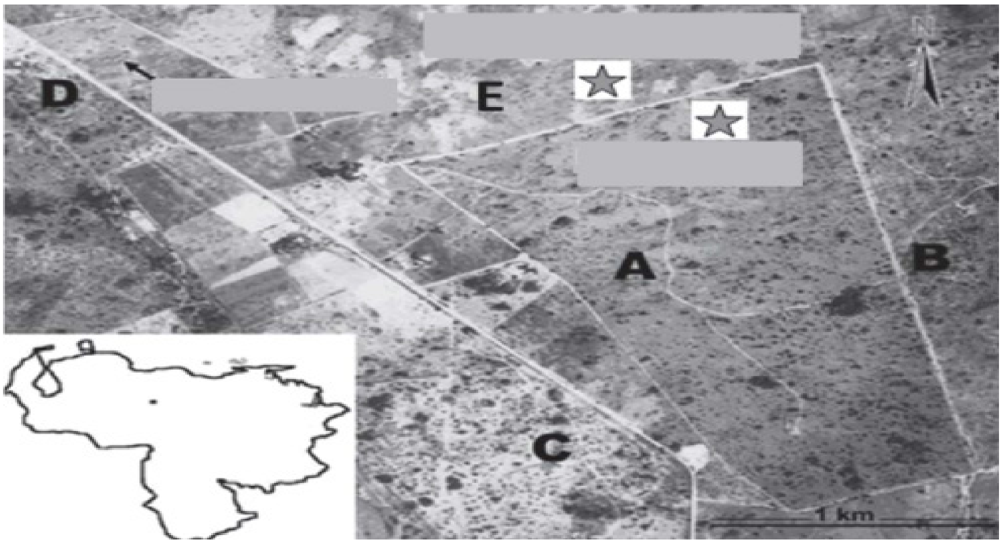

Fig. 1 Location of Estación Biológica de los Llanos in Venezuela (EBLL). (**A**) EBLL protected 500 from fire and cattle raising (PS). (**E**) Fundo "La Matica", NS.

savanna´s sites, and v) assess seasonal variations in earthworm cast production in both savannas.

Site Description

The experimental sites were located about 12 km southeast of the city of Calabozo (8° 56'N, 67°25' W), State of Guárico, Venezuela. The study included six experimental plots in one protected savanna (PS) located in the EBLL and six plots in an adjacent natural savanna (NS) located in Fundo "La Matica" (Fig. 1). PS have been under protection from annual fires and grazing and the adjacent natural savanna (NS) has been grazed at a rate of approximately 0.16 head/ha; and in addition has experienced recurring fires and agriculture practices.

Both sites are located less than 1 km apart, in the same soil type (Oxisols) with similar texture and relief. EBLL have been the place for extensive ecological studies since early sixties [3,8,24].

The climate of the zone is megathermic (Aw) with a rainy period which begins in the month of May and ends in November. Annual mean precipitation and temperature are 1364 mm and 26.7° C, respectively. The soil is characterised by the presence of a lateritic cuirass near the surface that influences the distribution of vegetation [8]. This soil has been characterised as a Haplustox, kaolinitic, flat, well-drained, and moderately acidic and has low natural fertility [25]. Soil texture is between loamy-sandy and sandy-clayey. The herbaceous component of the plant community is constituted mainly of grasses belonging to the genus Trachypogon sp. while the tree component is mostly composed of Curatella americana, Bowdichia virgilioides and Byrsonima crassifolia.

Earthworms, Casts And Soil Sampling

All collected earthworms belong to the Glossoscolecidae family, originally the species were classified in the genus Aicodrilus [4]; however the earthworms collected in both savannas have been recently classified as Rinhodrilus pinangoi [27]. Details about earthworms, soil and casts sampling, and, as well analytical methods are presented in previous publications [4,5,27].

Statistical Analysis

An analysis of variance (ANOVA) was performed to assess the differences between the two systems, with a HSD-Tukey post-hoc test of significance. Analyses were performed using the statistical package SPSS 13.0 with a level of significance $p < 0.05$.

Changes in Soil Physical, Chemical and Biological Parameters in the Protected and Non-Protected Savannas

As a result of the protection of the savanna (PS) in the EBLL some physical parameters were modified in relation to NS (Table 1). The PS soils had greater soil moisture with significant differences between sites ($p < 0.05$, Table 1). Field capacity in the PS (16.4%±0.6) was significantly greater than that in the NS (14.1%±0.6). The PS soils had lower soil temperature (0-10 cm) at the top of the rainy season (July). The apparent bulk density for the surface layer of soil (0-10 cm) was significantly lower in the PS (1.37 g/ml ±0.06) than in the NS (1.84 g/ml ±0.38) reflecting a lower soil compaction in the PS. The predominant textural class within the profile was the loamy-sand type (LS), except the surface layer (0-10 cm) in the NS which was sandy-loam (SL), possibly associated with the tillage management.

Thus, in EBLL the tree encroachment has influenced some environmental parameters; particularly, soil-moisture contents have increased (Table 1). The lower bulk density of the PS compared with the NS; without doubt is a consequence of the higher compaction and lower organic content that occurs in the man-affected NS due to tillage management and cattle raising activities. Hernández-Valencia [25] and López-Hernández et al. [3] in comparative studies of soil physical parameters in the same protected and man-managed savannas have presented similar results. It seems possible that the management implemented in the NS in recent years (extensive cattle raising and conventional tillage) has been responsible for the presence of a sandy-loam textural class found in the first layer of soil (0-10 cm) that changed to loamy-sand at deeper depths.

Parameter	NS	PS
Temperature˚C	27.0 b	25.3 a
Soil water content (%)	10.0 a	14.4 b
Field capacity (%)	14.1 a	16.4 b
Bulk density(%)	1.84 b	1.37 a

Table-1 Soil physical characteristics in protected (PS) and non-protected (NS) savannas. Different letters indicate significant differences ($p<0.05$).

This may influence, directly or indirectly, the lower presence of earthworms at that depth because of the abrasive sand effect and/or alteration in the drainage of the soil.

Changes in Soil Chemical Properties in the Protected and Non-Protected Savannas

Soil chemical parameters (pH, organic matter, nitrogen and available phosphorus) in each locality are presented in Table 2. As expected, there was an important increment in soil fertility parameters due to the protection of the savanna. The total contents of organic carbon (OC) and nitrogen in the first 10 cm tend to be significantly higher in the PS (9900 mg OC kg^{-1} and 234 mg N kg^{-1}, respectively) than the NS (7300 mg OC kg^{-1} and 186 mg N kg^{-1}, respectively) (Table 2), whereas the natural acidity of the superficial soil (pH 4.1) was slightly neutralized (4.8) under protection (PS).

The establishment of tree canopies and the presence of an abundant litter layer above the soil have resulted in higher organic-C (Table 2) and soil parameters associated with fertility level (e.g. increase in total N and in a lesser extent available P contents). Preliminary results showed that protection has also improved soil fertility [3], because of a reduction of compaction, an increase in the water holding capacity, organic matter, pH, exchangeable bases, organic phosphorus and total phosphorus and nitrogen.

Changes In Microbial Biomass And Enzymatic Activities In Protected And Non-Protected Savanna

The most active form of C corresponding to the microbial biomass fraction increased significantly in the PS soil compared with the NS soil (Table 3), whereas in the case of microbial N-biomass there was no difference between the two sites. Values of microbial P were higher in NS than in PS; however there was no significant difference between means. All the enzymatic activities have increased under protection (PS) respect NS; however differences between means were not reached (Table 4).

Microbial C-biomass for the EBLL native savanna were higher than the values presented by Gómez et al. [30] and López-Hernández et al. [31] in Trachypogon savannas of Eastern Llanos and Venezuelan Amazonia, respectively. On the contrary, the dehydrogenase values although greater in PS respect NS were lower than the information reported by López-Gutiérrez et al. [32] and Gómez and Paolini [33] in savannas of Eastern Llanos, Venezuela. The combination of both factors: higher fertility and better microclimatic conditions create an environment more adequate for soil fauna; therefore, it has allowed the establishment and the dominance of earthworms, as well other components of the pedofauna populations. Pedofauna components, particularly earthworms, in turn, affect the soil physical properties of the soil through the construction of channels and galleries which ameliorate soil structure and the soil water retention.

Earthworm Biomass Abundance And Density In The Protected And Non-Protected Savannas

Soil properties		Soil (0– 10cm)	Casts
pH	PS	4.80aB	5.30bA
	NS	4.10aA	5.20bA
OC ($mgCkg^{-1}$)	PS	9900aB	11400bB
	NS	7300aA	10900bA
Total N ($mgNkg^{-1}$)	PS	234aB	265bA
	NS	186aA	339bB
Avail P ($mgP\ kg^{-1}$)	PS	0.7aB	2.2bB
	NS	0.3aA	1.8bA

Table-2 Chemical characteristics of earthworm casts and adjacent soil in protected (PS) and non-protected (NS) savannas in a protected savanna (PS). Different small and capital letters indicate significant differences ($p<0.05$) between soil and casts, and between PS and NS, respectively. OC= Organic carbon; Avail P= Available.

The improvement of the physical, chemical and biochemical conditions under protection (PS) induces better microclimatic and nutritional conditions for the proliferation of earthworms, which was reflected in a higher density and biomass of earthworms compared with NS (Table 5). Araujo and López-Hernández [29] have also recorded a drastic increase in earthworm density and biomass after the long-term organic amendment of the sandy savannas of Puerto Ayacucho, Venezuelan Amazonia. Also similar results were reported in the Eastern Llanos of Carimagua, Colombia [34], where it was found in the natural savanna a yearly average of both earthworm density and biomass, of 57.8 individuals (ind) m^{-2} and 5.00 g m^{-2}, respectively. Those values significantly increased when the natural savanna was replaced by pasture species corresponding to an association between an exotic African grass, and a tropical forage legume species [34].

This association produces a high belowground production resulting in an increased availability of superficial litter residues and concomitant soil organic matter.

The number and biomass of earthworms found in the non-protected (NS) well-drained Trachypogon savanna were low compared with other tropical sites. However, in the case of the PS, the biomass and density of earthworms exceeded the values reported by Jiménez et al. [34] and Araujo and López-Hernández [29] in neotropical savannas. Lavelle [35] also registered greater values of density and biomass of earthworms than the values presented here for a sandy soil in a tropical savanna located in Lamto, Ivory Coast.

Chemical Characteristics Of The Soil And Surface Casts

Soil chemical parameters (pH, organic carbon, nitrogen and available phosphorus) in the soil and associated casts are presented in Table 2. As expected, there was a small increment in soil fertility parameters (total C and N) in the casts with respect to the savanna soils (NS and PS). Also available P was higher in casts with respect to the adjacent soil. These results are similar to previous studies [21,36,37,38,39,40].

A little higher pH value in fresh earthworm cast compared to adjacent soil was also observed in the present study (Table 2). The higher pH of cast may be due to the ammonia secreted in the worm's gut, which may act as a neutralising factor [41] or the production of calcium carbonate in calciferous glands and its release into the oesophagus [42], especially for species of genus Rhinodrilus which has three pairs of tubular calciferous glands. Casts in PS presented higher values of OC and available P respect NS, on the contrary total N in casts was lower in PS than in NS.

In general, the cast:soil ratios for C and N contents in different ecosystems surpass 2.0 [9,38],

	PS		NS	
Microbial biomass	Soil	Casts	Soil	Casts
Microbial C (mgC kg^{-1})	217aB	232aA	124aA	152aB
Microbial N (mgN kg^{-1})	4.6aA	9.5bB	4.3 aA	6.9 bA
Microbial P (mgP kg^{-1})	1.0	0.0	5.3	0.0

Table-3 Microbial biomass in the soil and the earthworm casts in protected (PS) and non-protected (NS) savannas. Different small and capital letters indicate significant differences (p<0.05) between soil and casts, and between PS and NS, respectively.

	PS		NS	
Enzymatic activity	Soil	Casts	Soil	Casts
PA (mgP-NP $kg^{-1}h^{-1}$)	3.0aB	4.7aA	1.1aA	1.1aA
UA (mg N-NH4 $kg^{-1}h^{-1}$)	3.3bB	5.1aA	0.9aA	1.9aA
DA (mg$kg^{-1}h^{-1}$)	14aB	88bA	5aA	133bB

Table-4 Enzymatic activities in the soil) and the earthworm casts in protected (PS) and non-protected (NS) savannas. Different small and capital letters indicate significant differences (p<0.05) between soil and casts, and between PS and NS, respectively. PA. UA and DHA correspond to phosphatase, urease and dehydrogenase activities, respectively.

a much higher value than the values here presented for C and N, respectively (1.13-1.82), no doubt the harsh environmental conditions of EBLL with the extended lateritic layer, low fertility and low primary productivity contribute to the local establishment of an earthworm population with a low biomass [4] that also generates casts non well enriched in soil nutrients as have been presented for casts of earthworm species of other savanna ecosystems [9].

Microbial Biomass And Enzymatic Activities Of The Soil And Surface Casts

Microbial activities and mineralisation are known to be greatly enhanced during the transit of soil through the gut and in deposited fresh casts; however after cast ageing there is a tendency to decrease C and N contents. It has been emphasised that over longer periods of time, the preliminary enhanced microbial activity decreases when the casts dry, and aggregation physically protect SOM against mineralisation [23]. Thus C mineralisation rate decreases and mineralisation of SOM from casts may be blocked for several months [13,23,43].

The most active forms of C and N corresponding to the microbial biomass fractions were higher in the casts of both protected and non-protected savannas compared with the adjacent soils; however significance was not reached in the case of C-microbial biomass (Table 3). When comparing the savannas (PS and NS), microbial C and N in casts were higher in PS than in NS.

In the protected savanna the phosphatase (PA) and urease (UA) activities (Table 4) were higher in the casts with respect to the surface soil, however significance was not reached. While the dehydrogenase activity (DHA) was significantly higher in the casts compared with the soil. A similar information was found in the case of the natural savanna.

Parameter	Locality	Depth (cm)			
		0-10	10-20	20-30	0-30
Density	PS	38.4	25.6	0.0	64.0
	NS	9.6	9.6	3.2	22.4
Biomass	PS	14.8	8.1	0.0	22.9
	NS	2.6	1.1	1.4	5.1

Table-5 Earthworm density (N/m^2) and biomass (g/m^2) at different depths in a protected savanna (PS) and the corresponding adjacent natural savanna (NS).

Month	Mean density of casts (Indiv./m^2)	
	PS	NS
April	0.00 (±0.00)	.00 (±0.00)
July	16.80 (±9.00) bB	1.15 (±3.30) aA
August	21.13 (±1.93) bB	1.44 (±0.51) aA

Table-6 Mean monthly density of casts (n/m^2) and standard deviations at Estación Biológica de 490 los Llanos. Venezuela. Different small and capital letters indicate significant differences 491 ($p<0.05$) between PS and NS, and between sampling months respectively.

In synthesis, C and N biomass and enzymatic activities, were higher in the cast as compared to the soil from which the cast was mostly derived. Those results are in accordance with a high C and N contents in the cast respect the adjacent soil [36], similar results have been presented for several soil enzymes [44].

Cast Production In The Protected And Non-Protected Savannas

From the comparison of earthworm abundance in the dry and the rainy seasons, it was determined that the activity of earthworms was mainly limited to the rainy season, as noted in other studies [45]. Therefore, earthworm casts were found during the sampling dates of the wet season of the year (July–August), no cast production was registered during the sampling date of the dry season (Table 6). The dominant cast structures were of globular forms whereas in a minor proportion thread-like casts were also found. The stability of this earthworm cast usually depends on the geometrical form being the globular structure more stable than the other ones because they are less susceptible to rain impact. The higher weight of earthworm casts, in comparison with individual earthworm individuals´ biomass, indicates that earthworms remain in the same region of cast deposition for at least for three or four dejections.

As expected according with the biomass production, there was a drastic decreased in the density of cast production in the non-protected savanna as compared with the protected savanna (Table 6). The superficial casts generated during the wet season correspond to a global annual production of 7.7 and 0.2 Mg of dry casts per ha in the protected and non-protected savanna, respectively (approximate calculation based on individual cast mass weight data of the sampling during the months of July and August and on the assumption that earthworm activity period is of 6 months per year). Our estimations of cast production in both savannas are lower to what is usually found in tropical ecosystems, e.g. 21.8– 27.8 Mg ha^{-1} $year^{-1}$ in savannas of Côte d'Ivoire [35], and 14 Mg ha^{-1} $year^{-1}$ in Colombian savannas located in Carimagua [9]. The low result for cast production in the protected savanna is not at all surprising if it is considered: i) that the studied site (EBLL) is a dry savanna when compared with the moist savanna of Carimagua, Colombia, ii) the soil is an Oxisols with the presence of endured laterite which makes a non-convenient environment for earthworm development, iii) moreover the year of the casts sampling was affected by a prolonged dry season with precipitation below 800 mm [4] which is far from the mean average of the zone (1364 mm). The very low production value of the deteriorated savanna (NS) correspond well with the also low earthworm biomass of the site (Table 5).

Conclusions

The protection of the savanna in the EBLL promotes an improvement in the physical, chemical and biological (microbial biomass and enzymatic activities) properties of the oxisols studied. This may allow for an increase in the density and biomass of earthworms in the PS compared with the adjacent NS (less than 1 km apart) that is subjected to recurrent burning and grazing (Fig. 1).

Mean cast density was significantly higher in the protected savanna than the values reported in an adjacent savanna located on the same Oxisols but under traditional management with fire, grazing and agricultural activities. Consequently, intensive agriculture activities have dramatic effects on earthworm and other components of the soil macrofauna. The cast production in the protected savanna corresponds to a global annual production of 7.7 Mg of dry casts per ha with a very drastic reduction (0.2 Mg of dry casts per ha) in the agricultural managed savanna. Therefore, we can emphasise the potential use of the density and biomass of earthworms as bioindicators of soil fertility and as active actors in the regeneration of the soil used as have previously reported.

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