Scientific Paper

Relationship of edaphic biota with soil physical and chemical properties in five pasturelands of Granma province

Licet Chávez-Suárez[1] *https://orcid.org/0000-0002-7837-2168*, Idalmis Rodríguez-García[2] *https://orcid.org/0000-0001-5897-5431*, Verena Torres-Cárdenas[2] *https://orcid.org/0000-0002-7451-8748*, Diocles Benítez-Jiménez[1] *https://orcid.org/0000-0002-1046-1885* and Alexander Alvarez-Fonseca[1] *https://orcid.org/0000-0002-5218-445X*

[1]*Instituto de Investigaciones Agropecuarias Jorge Dimitrov. Carretera vía Manzanillo km 16 ½, Peralejo, Bayamo, Granma.* [2]*Instituto de Ciencia Animal, Apartado Postal 24, San José de las Lajas, Mayabeque, Cuba. E-mail: licet@dimitrov.cu, idalmisdoloresrodríguezgarcia@gmail.com, vtorres@ica.co.cu, diocles@dimitrov.cu, alexanderf@dimitrov.cu*

Abstract

Objective: To determine the relationship among some soil chemical, physical and biological properties and the plant component in pastureland agroecosystems of Granma province, Cuba.

Materials and Methods: The research was conducted in five pastureland agroecosystems of Granma province, Cuba, in the period 2014-2017. The granulometric composition, microstructure, structural stability, hygroscopic humidity, bulk density, real density, pH (H_2O), Na, K, Ca, Mg, P_2O_5 and electrical conductivity were determined in the laboratory of the National Institute of Agricultural Sciences. The edaphic macrofauna was collected according to the TSBF (Tropical Soil Biology and Fertility) and pitfall traps.

Results: There was significant canonical correlation between chemical and physical properties of soils and macrofauna communities. The orders Isopoda and Haplotaxida were the edaphic macrofauna variables that contributed most to the variance shared by both groups of variables. Meanwhile, bulk density, true density, clay content, hygroscopic moisture, pH, Ca, K and Mg, were the physical and chemical properties with the highest correlation.

Conclusions: The orders Haplotaxida and Isopoda are proposed as soil quality indicators, selected and validated from the analyses of canonical correlations with physical and chemical edaphic properties and with the plant component.

Keywords: soil organisms, soil properties, animal husbandry systems

Introduction

Edaphic biota comprises organisms that spend part or all of their life cycle within the soil or on its immediate surface. It includes arthropods, nematodes, and mollusks, among others. According to their body size, edaphic fauna is classified into microfauna, mesofauna and macrofauna (Mekonen, 2019).

The edaphic fauna performs multiple environmental services beneficial to human well-being and health: decomposition of organic matter, supply of plant nutrients, maintenance of soil structure, water movement and holding in the soil profile, biological control of pests and diseases, and carbon sequestration and release (Tanjung *et al*., 2020).

Macrofauna communities are often considered as bioindicators of soil quality because they are sensitive to environmental changes that can cause variation in their abundance and composition (Machado-Cuellar *et al*., 2020; Morel and Ortiz-Acosta, 2022). Soil quality depends on physical, chemical and biological properties. According to their spatial and temporal variability, sensitivity to changes in land use and ease of interpretation and execution, they can be used as quality indicators.

The study of the relationship between the physical and chemical properties of the soil, its intensity of use and the abundance and diversity of edaphic biota is used to select quality indicators, which is a current topic in soil ecology research (Díaz-Porres *et al*., 2014). In Cuba, several groups of edaphic macrofauna have been identified as indicators of soil quality. Nevertheless, extending the utilization of the already-generated indicators to different soils and ecosystems is recommended, in order to enhance the actions of research, validation and generalization of results in the country. (Cabrera-Dávila *et al*., 2022). The objective of this study was to determine the relationship between some chemical, physical and biological properties of the soil and the plant component in pastureland agroecosystems in Granma province, Cuba.

Materials and Methods

Location. The research was developed in five grassland agroecosystems in Granma province, located in the southwestern portion of the eastern

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region of Cuba, between coordinates 20°23′00″N and 76°39′09″W. Table 1 shows the main characteristics of the agroecosystems. Sampling was conducted twice a year, in the rainy season, (RS) and in the dry season (DS), from July, 2014, to March, 2017.

Sampling and identification of edaphic macrofauna and mesofauna. Two methods were used: the one recommended by the Tropical Soil Biology and Fertility program (Anderson and Ingram, 1993) and pitfall traps (Moreira *et al*., 2012). For the first method, the leaf litter was previously cleaned and all types of foreign bodies, such as stones and plant debris, were removed. On the diagonal of the sampling area, five monoliths per hectare, measuring 25 x 25 x 20 cm, were extracted at a distance of 20 m. Individuals of the macrofauna were collected *in situ* and counted by hand. The earthworms were preserved in 4 % formaldehyde and the remaining invertebrates in 70 % ethanol.

For the second sampling method, nine traps were placed in each study area, arranged in the two diagonals in the form of a cross, with a trap in the

Table 1. Main characteristics of agroecosystems.

Agroecosystem	Triángulo y Progreso	Cupeycito	Ojo de agua	Pasture station
Municipality	Bayamo	Jiguaní	Guisa	Bayamo
Affiliation	UBPC Francisco Suárez Soa	Animal Husbandry Enterprise Manuel Fajardo	Rafael Almaguer's farm, CCS Braulio Coroneaux	Jorge Dimitrov
Purpose	Milk production	Calf rearing	Bull fattening	Bull fattening
Soil type	Pellic vertisol	Carbonate loose brown	Carbonate loose brown	Fluvisol
Grazing method	Continuous	Rotational	Continuous	Rotational
Total grazing area, ha	T:18,5 P: 20,4	14,2	6,7	0,8
Sampling area, ha,+++ and percentage it represents of total area	T: 2 11 % P: 2 10 %	1,8 13 %	1,2 18 %	0,8 100 %
Prevailing pasture type	*Dichantium caricosum* L. A. Camus and *Cynodon nlemfuensis* Vanderyst.	*Megathyrsus maximus* (Jacq.)	*Dichantium caricosum* L. A. Camus)	Silvopastoral system of *M. maximus* grass and *Leucaena leucocephala* (Lam.)
Time of exploitation, years	20	10	7	10
Breed and stocking rate, LAU ha^{-1}	Siboney crossbred 1,5	Creole 1,7	Crossbred 2,2	Siboney crossbred 1
General conditions	Totally deforested grazing areas, without paddocks. It is flooded in the rainy season	Good shade level by trees and paddock establishment, high amount of stones. Tree species: *Cocos nucifera*); *Guazuma ulmifolia*); poplar *(Populus* sp.)	Good shade level by trees, without paddocks, relief with slope (10 %). Susceptibility to erosion. Tree species: *L. leucocephala*; *Samanea saman*); Cuban mahogany (*Swietenia mahagoni* (L.) Jacq.); Cuban cedar (*Cedrela odorata* L.)	Good shade level, zone of intense drought

T: El Triángulo P: El Progreso, UBPC: Basic unit of cooperative production, CCS: Cooperative of Credit and Service center. Plastic containers 8 cm in diameter and 10 cm deep were used, which were buried at ground level, with the least possible disturbance to the surrounding area. A 0,003 % aqueous detergent solution, prepared with LABIOFAM commercial liquid detergent, was then added and the containers were covered with dry leaves and plant debris from each agroecosystem. After seven days, the contents of the traps were collected in glass jars and transferred to the laboratory. Using a stereoscope, the individuals were extracted from the solution and counted and placed in vials with 70 % ethanol.

For the identification of the preserved specimens, the works by Hickman *et al*. (2008) and Brusca and Brusca (2003) were consulted. The entomological collection belonging to the Provincial Laboratory of Plant Health in Granma was also reviewed. The variables in the monoliths and in the pitfall traps were defined: number of individuals belonging to the orders Araneae, Hymenoptera, Hymenoptera-Formicidae, Coleoptera, Isopoda, Hemiptera and Haplotaxida.

The edaphic mesofauna was determined in the pitfall traps. After rinsing the individuals with 5 % sodium hydroxide and putting them through lactophenol with slight heating, they were finally mounted in FOR or Hoyer liquid for classification. Taxonomic identification was performed according to the work carried out by Brusca and Brusca (2003) and Díaz-Azpiazu *et al*. (2004). The variables number of mites and springtails were defined.

Microflora. Five samples composed of ten subsamples were taken at a depth of 0-20 cm, using a pickaxe. The method of serial dilutions and deep sowing in Petri dishes was used (Mayea *et al*., 1998). The number of colony forming units (CFU) of bacteria, fungi and actinomycetes were defined as variables.

The microflora isolation conditions are shown in table 2.

Determination of the chemical and physical properties of the soil. For the determination of chemical and physical indicators (table 3), five composite samples (ten subsamples taken in zigzag)

Table 2. Microflora isolation conditions.

Microbial group	Dilution	Culture medium	Temperature, ºC	Incubation time
Bacteria	106	Nutrient agar	30	24 hours
Fungi	104	Malt extract agar	30	5 to 7 days
Actinomycetes	105	Starch ammonium agar	30	5 to 7 days

Table 3. Physical and chemical properties of the soil. Methods for their determination.

Indicator	Methods
pH (H_2O) (1:2)	Potentiometry, NC ISO 13.080.10 :2015
OM, %	NC ISO-51, 1999
Na, K, Ca, Mg¥	Extraction with $AcNH_4$, By Maslova's method
Na, K, cmol kg^{-1}	Determination by flame photometry
Ca, Mg, cmol kg^{-1}	Determination by volumetry with EDTA
P_2O_5, ppm	Oniani (1964)
CE, dS m^{-1}	Conductometer NC 112:2001
Granulometric composition and microstructure, %	NRAG 408, 1981
Hygroscopic moisture, %	Gravimetric method NC 110, 2001
Bulk density, g cm^{-3}	NRAG 370, 1980
Real density, g cm^{-3}	NC 11 508, 2000
Dry sieving and structural stability, %	Savinov's method (Orellana *et al*., 1990)
Dry structure coefficient	Calculation
Total porosity	Pt = (1- Da/Dr)*100
Organic carbon, %	CO=MO*0,58

NC: Cuban standard, NRAG: Agricultural branch standard, ¥ Exchangeable cations.

were taken throughout the sampling area, at a depth of 0-20 cm, with the help of a helicoidal auger. These samples were air-dried and then ground and sieved (1 mm) for agrochemical analysis. For the determination of bulk density, undisturbed soil samples were taken.

Determination of botanical composition and biomass production. Botanical composition was determined in 80 randomly distributed frames ha^{-1} in each sampling area using the method of t'Mannetje and Haydock (1963). Botanical composition was estimated from the relative importance value, which depended on the frequency and relative dominance of grass, weeds and bare soil or depopulation, considered by the absence of vegetation.

Biomass availability was determined in each area, in 100 frames of 0,25 m^2, randomly taken according to the methodology proposed by Haydock and Shaw (1975). The grass sample patterns were cut at a height of approximately 10 cm. Four variables were defined: percentage of grasses, weeds and bare soil and biomass production (t ha^{-1}).

Statistical analysis. Canonical correlation analyses were performed among the groups of edaphic macrofauna variables in the monoliths and pitfall traps. Mesofauna and microflora were determined with the soil physical and chemical variables and plant component variables defined above. This analysis was performed for two groups of variables in each case. Two criteria for significance of canonical relationships were used, according to Badii *et al*. (2007): level of statistical significance of the functions ($p < 0,05$) and magnitude of the canonical correlation (Rcanonical > 0,75). The analysis was performed with the statistical package Statistica V 8.0 for Windows (Statsoft, 2008).

Results and Discussion

Canonical correlations of edaphic biota variables with soil physical and chemical properties. The canonical correlation analysis was significant between edaphic macrofauna and microflora variables and soil physical and chemical properties (table 4). When analyzing the structure of the factors obtained in the canonical correlation, it was observed that, in all cases, the order Isopoda was among the edaphic macrofauna variables that contributed most to the variance shared by both groups of variables. For the macrofauna determined by the monolith method, the order Haplotaxida, which was only observed by this method, was added. The above-explained fact allows to infer that in the studied agroecosystems these orders are the most related to the physical and chemical properties of these soils. There was no significant canonical correlation of the edaphic mesofauna variables with the physical and chemical properties of the soil.

There were differences in terms of the physical and chemical variables of higher contribution to variance when the analysis was performed with the macrofauna determined by the monolith method and by the pitfall traps. In the macrofauna determined by the trap method, there was a positive influence of Mg and Da, and by pH and Dr in a negative way. In the macrofauna determined by the monolith method, it was positively related to Ca, K and negatively to Hy and clay percentage. This could be due to the differences in the macrofauna collected in both methods, since the fauna with characteristics of greater mobility (diurnal or nocturnal activity) is more easily captured by the traps; while the monoliths concentrate their action on less mobile organisms, with diurnal activity fundamentally (Chávez, 2020).

Figures 1 and 2 show the most important simple correlations between edaphic biota organisms and soil physical and chemical variables. The order Isopoda was positively related to Dr, hygroscopic moisture and total porosity and negatively to Da, Ca and Mg. Organisms belonging to Haplotaxida

Table 4. Canonical correlations between edaphic biota variables and soil physical and chemical properties.

Group 1	Group 2	Canonical R	$\chi 2$	p	Variables with the highest contribution
Macrofauna-t	Physical	0,89	67,01	0,008	Isopoda, Dr (-), Da
Macrofauna-t	Chemical	0,92	77,83	0,004	Isopoda, Mg, pH (-)
Macrofauna-m	Physical	0,89	69,36	0,005	Isopoda, Haplotaxida, Hy (-), Clay (-)
Macrofauna-m	Chemical	0,91	72,95	0,012	Haplotaxida, Isopoda, Ca, K
Microflora	Physical	0,99	130,87	0,000	Bacteria, Hy (-)
Microflora	Chemical	0,99	137,86	0,000	Actinomycetes, Na, Ca

Macrofauna-t: macrofauna captured in the traps; Macrofauna-m: macrofauna captured in the monoliths

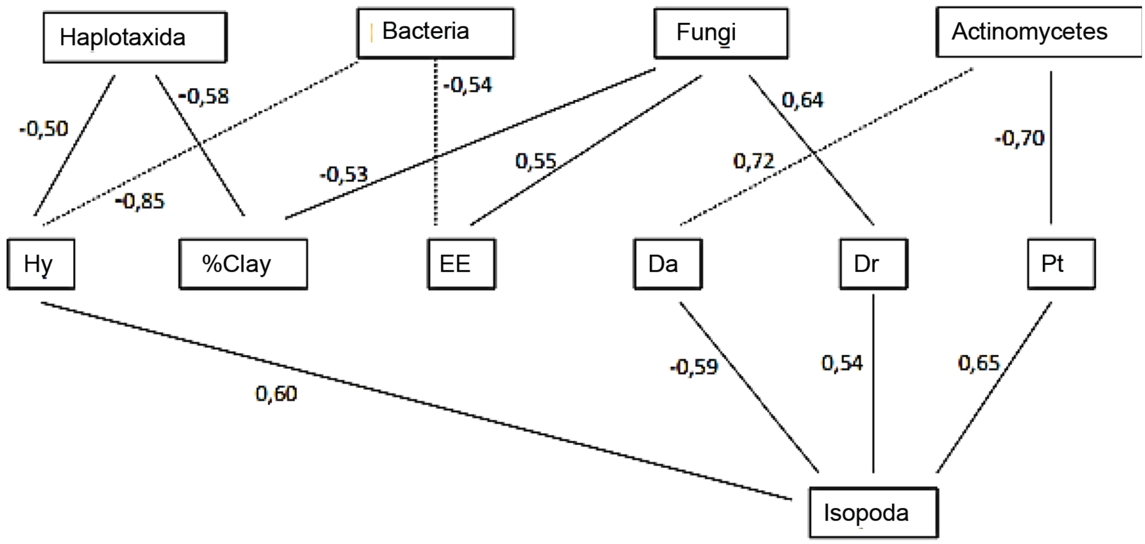

Fig. 1. Correlations between edaphic biota and soil physical variables (Hy: hygroscopic moisture, EE: structural stability, Da: bulk density, Dr: real density, Pt: total porosity).

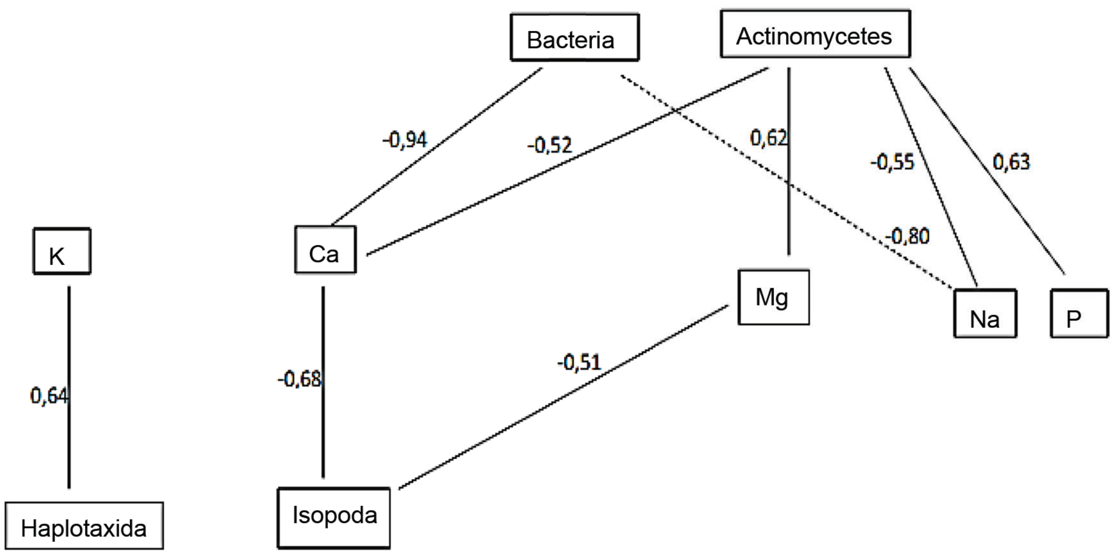

Fig. 2. Correlations between edaphic biota and soil chemical variables.

were positively related to hygroscopic moisture and K and negatively to clay percentage. All bacterial relationships were negative with regards to structural stability, clay percentage, hygroscopic moisture and Ca and Na.

Fungi were only positively related to physical variables (structural stability and Dr); while actinomycetes were only related to chemical variables (negatively to Ca and Na, and positively to Mg and P).

The distribution of soil macrofauna depends on several factors: soil type, nutrient content, organic matter, pH, texture and structure (Pollierer *et al.*, 2021). In addition, factors related to vegetation, climate, land use and developed anthropogenic management are very important (Tanjung *et al.*, 2020; Valkay-Halkova *et al.*, 2022). The correlations between edaphic biota and soil physical and chemical properties observed in this study have been reported by other authors. Díaz-Porres *et al.* (2014) found significant correlations between soil physical and chemical variables and the density of taxonomic groups of the edaphic macrofauna. These authors concluded that, when analyzing the structure of the factors obtained in the canonical correlation, the C/N ratio and the percentage of organic matter were the edaphic variables that best explained the distribution of macroarthropods. This differs from the results shown, since these factors were not the ones with the greatest contribution.

From the point of view of simple correlations, similar results to those reviewed in the literature were also recorded. Rosa *et al*. (2015) found that the soil chemical properties that correlated best with the edaphic macrofauna groups were organic matter, Ca, Mg, P and K, when they conducted a study that included pasturelands, in Santa Catarina, Brazil. Gholami *et al*. (2016) also reported a coincident result, finding a negative correlation of clay content and electrical conductivity, with some edaphic macrofauna indices (abundance, uniformity, richness and diversity) in a study conducted in a forest southwest of Irán. These indices were positively correlated with organic matter and silt content. Hani and Suhaendah (2019) noted that the factors that influenced soil macrofaunal diversity were soil pH, temperature, and soil moisture. Ge *et al*. (2021) distinguished organic carbon as the main factor among soil properties, which determines the composition of edaphic macrofauna.

Li *et al*. (2020) reported that the biota groups Bourletiella (Collembola), Symphyla and Armadillidae (Isopoda) were mainly influenced by the K content; while Staphylinidae (Coleoptera) larvae were mainly affected by soil temperature. Other groups, such as Muscidae (Diptera), Chironomid, Psychodidae, and Scydmaenidae (Coleoptera), showed minimal influence of soil factors.

Tulande *et al*. (2018) similarly found that macrofauna showed positive response to soil P, K, and Na content, in the Colombian Andes. Other authors reported that P and Na determine the presence of some groups of edaphic macrofauna, such as Araneae, Coleoptera, Isopoda and Haplotaxida (Ott *et al*., 2014). However, in rice fields in Indonesia, Tanjung *et al*. (2020) established that P availability did not influence the increase in diversity of edaphic macrofauna groups.

In a study conducted in natural, grazed savannas in the Venezuelan plains, Morales-Márquez *et al*. (2018) observed that edaphic macrofauna correlated positively with soil porosity and negatively with bulk density, as was the case for the order Isopoda in this research (fig. 1).

Several studies in tropical and subtropical regions have directly related, at local scale, the spatial distribution, density and biomass of earthworms and soil macrofauna communities to the content of organic matter, nitrogen and other nutrients, C/N ratio, pH, texture (sand and clay content), cation exchange capacity, water holding, aeration, porosity and soil structure (Rodríguez-Suárez *et al*., 2019; Sofo *et al*., 2020; Panklang *et al*., 2022).

The literature evidences the importance of soil nutrients for the development of edaphic macrofauna, such as calcium, which is involved in several mechanisms of osmotic regulation in invertebrates, as well as in growth processes (Rosa *et al*., 2015). Meanwhile, in oligochaetes, it is part of the spatial arrangement of hemoglobin polypeptide subunits (Moreira *et al*., 2011).

According to De-la-Cruz-Lozano (2005), potassium and phosphorus can be limiting factors in insect growth and magnesium is essential as an enzymatic cofactor.

If we analyze the large contribution of the orders Haplotaxida and Isopoda to the factors obtained in the canonical correlation together with Dr, Da, Hy, clay content, pH, Ca, K, Mg and Na, we also find elements that agree with literature. The dependence of earthworms on texture, true density, porosity and organic carbon manifests changes in composition and abundance on a short time scale (Zhukova and Mytiai, 2022). According to these authors, earthworms prevail in moist, non-compacted soil environments with high organic matter content.

Rodriguez (2020) in a study with various levels of diversification and intensification in crop rotation in Argentina, found that earthworm abundance and biomass were positively related, mainly with organic matter content. Similarly, Hoeffner *et al.* (2021), in 24 pasturelands in France, detected that increased organic matter was the most favorable factor for earthworm abundance and biomass. Nanganoa *et al.* (2019) recorded a strong and negative correlation of pH and earthworm numbers in five intensive land uses in the humid tropics of Cameroon.

It is also acknowledged that detritivorous organisms, such as those belonging to the order Isopoda, are very sensitive to physical and chemical changes in the soil, as well as to sudden changes in temperature and humidity in their habitats, so they can be used to indicate the state of disturbance in the edaphic environment. For these organisms, a diverse vegetation cover is indispensable, since it brings with it more heterogeneous litter and higher concentration of macronutrients in the soil. In this regard, Isopoda has been associated with higher vegetation cover, organic matter and soil moisture (Cabrera-Dávila, 2019). Other authors emphasized the importance of Isopoda in litter decomposition and interaction with soil microorganisms (Pey *et al*., 2019).

Canonical correlations among edaphic biota variables. Among the different groups of edaphic biota, significant canonical correlations were also established. This is the case of the macrofauna determined by the traps and the mesofauna (Rcanonical = 0,79; $\chi2$ = 29,12; p = 0,0038). Here, coleoptera and mites were the most negatively influencing variables. The microflora (bacteria, fungi, actinomycetes) was correlated with the macrofauna determined by the monoliths (canonical R=0,95; $\chi2$=29,12; p=0,0099). The number of bacteria and the order Isopoda were the variables that had the greatest negative contribution to variance.

Individual correlations showed that bacteria were positively related to the order Isopoda, Hemiptera, Haplotaxida and to actinomycetes, which were positively related to fungi (fig. 3).

Several biota groups were related to each other: mites and Coleoptera, spiders and Hymenoptera, ants and Coleoptera, presumably because of their similar mobility. Interestingly, all the relationships established among the components of the edaphic biota were positive. Díaz-Porres *et al*. (2014) also found this type of correlation between Araneae and Coleoptera and other groups of the edaphic macrofauna: Isopoda with Hemiptera and Lepidoptera with Chilopoda.

Canonical correlations between edaphic biota variables and plant component variables.

The edaphic biota variables that showed significant canonical correlations with plant component variables were macrofauna determined by monoliths (Rcanonical = 0,89; $\chi2$ = 54,39; p = 0,00038) and microflora (Rcanonical = 0,93; $\chi2$ = 59,71; p = 0,0000). Of the former, the variables that contributed most to the variance were depopulation percentage and order Haplotaxida. Of the latter, the percentage of weeds and fungi showed the highest contribution. Bacteria and individuals belonging to Isopoda were positively related to biomass production and pasture percentage (fig. 4). Actinomycetes and bacteria were negatively related to the percentage of weeds. Meanwhile, fungi were positively related, and it seems that soils with higher cover of weeds were those that provided better conditions for these microorganisms and, in turn, were negatively related to the percentage of grasses.

It has been widely reported in literature that vegetation diversity, as well as soil cover, are associated with the diversity and abundance of edaphic fauna (Lo-Sardo and Silva, 2019; Sabatté *et al*., 2021; Panklang *et al*., 2022). The above supports the results that showed the relationship between plant component variables and macrofauna, determined by monoliths and microflora.

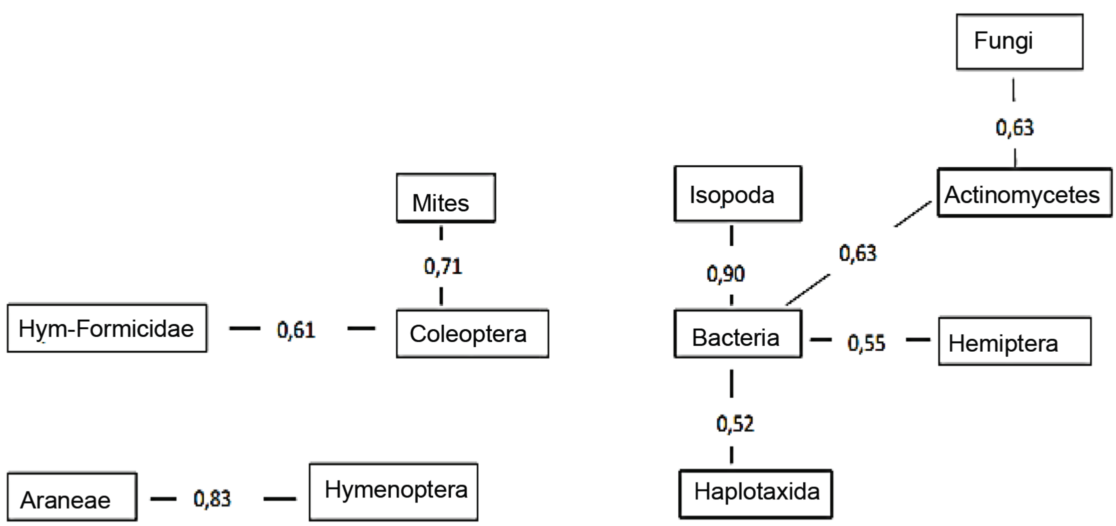

Fig. 3. Correlations among the edaphic biota groups.

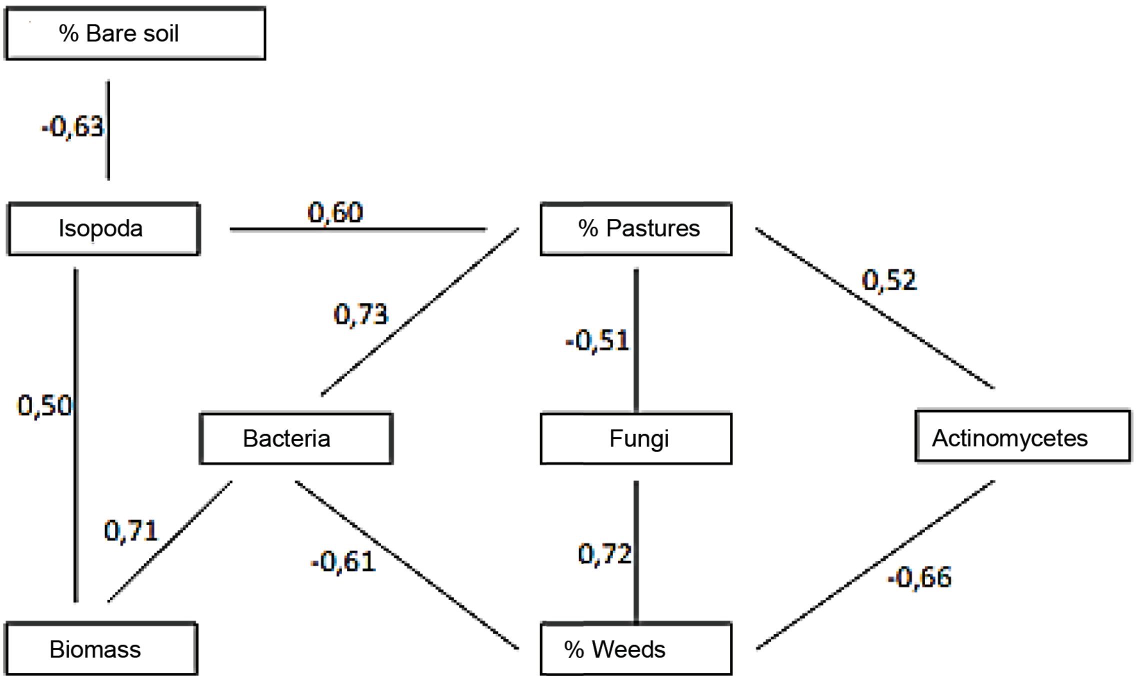

Fig. 4. Correlations between edaphic biota and vegetation variables.

Rodríguez-Suárez *et al*. (2018) in the evaluation of edaphic macrofauna in different land uses of the Colombian Amazon, including pastures and silvopastoral systems, referred that the principal component analysis separated natural forest as the land use with the highest diversity of macrofauna, where orders Isopoda and Gastropoda were abundant. According to these authors, the results indicate that higher tree diversity may result in greater heterogeneity and quantity of leaf litter and, consequently, greater availability of food and microhabitats, as well as better edaphic and microclimatic conditions for the development of macrofauna.

Rodríguez (2020) reported that earthworm abundance and biomass were positively related to rotation intensity and legume proportion indices in agroecosystems in Argentina. Singh *et al.* (2021) reported that, in Germany, in different grassland types, earthworm communities were significantly affected. The lowest abundance and biomass could be seen in grassland subject to intensive use, due to the lower diversity of plants present. Guaca *et al*. (2019) reported a significant effect of vegetation (forests, forest plantation, silvopastoral system and pastureland) on soil microbiological properties in the Colombian Amazon.

Selection of indicators. The orders of the edaphic macrofauna Isopoda and Haplotaxida were selected as indicators of good quality of the edaphic environment, because according to the analysis of canonical correlations they were the ones that had the best relationship with the physical and chemical properties of soils, as well as with the variables of the plant component in the studied pasturelands. In addition, as biological indicators they fulfill certain characteristics that support them for this purpose: great aptitude for speciation, short life cycle, little dispersal power due to their adaptation to edaphic life and different soil types, feeding habits related to the degradation of organic matter and predictable response to changes in the environment (Cabrera-Dávila, 2019). These organisms are easy to collect and identify, as they can be seen with the naked eye. In addition, laboratory equipment, reagents, and specialized personnel are not needed for their determination, which is a limitation for physical and chemical indicators.

Several studies suggest the use of earthworms as indicators of soil quality (Mekonen, 2019; Siebert *et al*., 2019; Rodríguez, 2020). In Cuba, Cabrera-Dávila (2019) proposed the earthworms/ants ratio as a fauna indicator. In land uses with greater soil cover, as is the case of forests, this ratio was higher than one; while in use systems with a higher degree of disturbance the values were close to zero. The authors point out the practical usefulness of this indicator, because the involved groups are easy to identify and do not require specialized knowledge. Likewise, the families belonging to Isopoda are defined as indicator taxa: Philosciidae in primary forests and Trachelipidae and Armadillidae in agroforestry systems.

In spite of the above-stated facts, Ramírez *et al*. (2019) recognize that the identification and study of these organisms as bioindicators of soil quality and ecosystem biodiversity remains a universal problem, and that macro-invertebrate communities vary in their composition, abundance and richness, depending on the state of disturbance caused by land use change. Therefore, they are indicators of the disturbance and impact of different forms of management.

Conclusions

There were significant canonical correlations between the physical and chemical properties of the soil with the edaphic biota, and of the latter with the plant component and among the biological variables themselves. From the last ones, it was possible to suggest relationships between these elements in the studied pastureland agroecosystems, which help to explain the dynamics of these soil organisms with the physical and chemical properties and with the plant component. The orders Haplotaxida and Isopoda are proposed as soil quality indicators, selected and validated from the analysis of canonical correlations between physical and chemical edaphic properties and the plant component. In addition, they are easy to sample and identify, which can be carried out by farmers, researchers and other specialists.

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Conflict of interest

There is no conflict of interest among the authors.

Authors’ contribution

- Licet Chávez-Suárez: Designed the research, directed the field work, carried out the analysis and interpretation of the results and wrote the paper.
- Idalmis Rodríguez-García: Participated in the research design, analysis and interpretation of the results and writing of the paper.
- Verena Torres-Cárdenas: Performed the statistical analysis of the results and collaborated in their analysis and interpretation.
- Diocles Benítez-Jiménez: Collaborated in the research design and with the statistical analysis and interpretation of the results.
- Alexander Álvarez Fonseca: Participated in the research design, took field samples and collaborated in writing the paper.

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