STUDY OF THE NEMATODE COMMUNITY AS AN INDICATOR OF ENVIRONMENTAL DISTURBANCE IN COCOA (THEOBROMA CACAO L.) AGROECOSYSTEMS

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Abstract

Nematodes are part of soil food webs as decomposers, mycophages, bacteriophages, predators and phytoparasites; when soil disturbance and contamination occur, the populations of these trophic groups are affected, which are used to evaluate the health and quality of agroecosystems. This research aimed to evaluate the presence of the nematode community as an environmental indicator in two cocoa farming systems. Samples were taken in two areas where different farming systems are managed. In this selection, a plantation with conventional and associated management is included. For each farm, ten soil subsamples were taken by the extraction method of centrifugation and flotation in sugar. The results determined the presence of five species of phytoparasitic nematodes in the established systems, among which are: Criconemoides, Helicotylenchus, Hemicycliophora, Tylenchus and Meloidogyne, a beneficial genus identified as Mononchus was found, in the chemical analysis of soil there is no evidence of interaction of any element with the identified genera, but the percentage of organic matter is closely related to the presence of predatory nematodes in the systems that have agroecological management. Among the agricultural systems studied, plantations managed as monoculture cocoa (Theobroma cacao) are associated with phytonematodes and in plantations where cocoa and banana (Musa balbisiana) are associated, the presence of the beneficial nematode (predator) Mononchus is evident. These important organisms' taxonomic and functional diversity makes them indicators of soil conservation status in tropical systems studied.

Keywords: Maturity index, monocultures, trophic guild, ecological indicators.

Introduction

Theobroma cacao is an essential crop for the Ecuadorian economy and ranks fourth in agricultural exports with 293 487 t produced on 559 617 ha, mainly in El Oro, Guayas, Los Ríos, Manabí, Esmeraldas and Santo Domingo de los Tsáchilas (INEC, 2016). Moreover, this product constitutes the raw material for producing some products (Reynel et al., 2016; Solórzano and Balseca, 2017) due to its high content of unsaturated (34%) and saturated (60%) fats, which are derived from three main products: cocoa liquor, cocoa butter and cocoa powder (Arvelo et al., 2017).

Tropical agroforestry systems, such as cocoa systems, are characterized by complex associations of multiple functions and trees and crops of different ages. Therefore, they are a sustainable alternative to modern intensive agricultural systems or monocultures (Ngo et al., 2013).

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However, studies in Ghana (Africa) report a significant increase in cocoa crop yield when shade (timber, fruit, banana and other trees) was suppressed (Clough et al. 2009).

Managed agroecosystems tend to have low levels of biodiversity, internal interactions and feedback mechanisms. Human activities determine system inputs and outputs. Relationships between organisms are usually disturbed. Because of economic competition, agricultural soils become more and more polluted when chemical fertilizers, pesticides, etc., have been widely used. Therefore, monitoring and assessing agroecosystem soils' quality becomes important, particularly for farm managers who could modify their agricultural strategies (Duong et al., 2014).

Soil nematodes were considered reliable bioindicators of the state and processes of an ecosystem (Porazinskaa and Duncanb, 1999). This is because nematodes possess the most important characteristics of any prospective bioindicator: abundance in virtually all environments, diversity of life strategies, feeding habits, short and relatively well-defined life cycles (Yeates, 1999).

Nematodes vary in sensitivity to pollutants and environmental disturbances. In addition, the recent development of indices that integrate the responses of different taxa and trophic groups to disturbance provides a robust basis for analyzing faunal assemblages in the soil as in situ environmental assessment systems. For these reasons, several researchers have attempted to study relationships between nematode community structure and natural ecosystem succession or environmental disturbance (Ferris and Bongers, 2006).

In this research, nematode communities were studied in diverse agroecosystems located in different ecological zones and their participation as indicators of environmental disturbance.

Materials and methods

Description of the study area

The study was conducted in two areas of Ecuador with different characteristics (Table 1) in 2021. The Luz de América zone is located in the province of Santo Domingo de Los Tsáchilas (00° 24' 36" S; 79° 18' 43" W) at 274 masl with precipitation of 2860 mm, the average temperature of 24 °C, relative humidity of 85 % and heliophany of 680 daylight hours per year-1. The Patricia Pilar area is located in the province of Los Ríos (00° 35' 2" S; 79° 22' 00" W) at 246 masl. The average temperature is 24.6 ºC, heliophany 760.1 hours, light, year-1, relative humidity 82 %, annual precipitation 3337.1 mm year-1 (INAMHI, 2022).

Table 1. Soil characteristics of the areas and agricultural systems studied.

Parameters	Light of America		Patricia Pilar	
	Associate	Monoculture	Associate	Monoculture
Texture	Clay loam	Clay loam	Loamy loam	Loamy loam
pH	5.97	5.95	5.90	5.97
Organic matter (%)	8.29	7.78	7.26	5.82
NH_4 (ppm)	13.52	16.12	16.77	17.42
Phosphorus (ppm)	5.91	3.53	15.80	16.78
Potassium (meq/100ml)	0.37	0.29	0.28	0.38
Sulfur (ppm)	7.78	12.37	11.79	15.82

Cocoa growing systems

In order to study common cocoa systems in Ecuador, the following two types were evaluated: conventional cocoa farms without any shade (monoculture); conventional cocoa farms with shade trees and plantain (mixed or associated).

The mixed or associated cultivation system has cocoa variety CCN-51, with a planting spacing of 3.5 x 3.5, for a density of 815 plants ha-1, with an average age of 4 years; this crop is associated with Plantain (Musa balbisiana), without established arrangement, it is managed in an agroecological way, in this system, organic fertilizers are applied as Bocchi between 2.5 kg per plant, once a year and foliar biofertilizers based on manure, mountain microorganisms, the management of weeds is manual, and pest and disease control is done in an integrated manner, without the use of chemical molecules. In addition, this cultivation system has a type of permanent shade with forest trees of Laurel (Cordia alliodora) and certain citrus fruits such as oranges, lemons and mandarins, whose shade regulation does not have an established or planned design but is carried out according to the producer's criteria.

The monoculture system is cultivated with cocoa variety CCN-51 at a distance of 3m x 3m, has an average age of 7 years, is established as a monoculture and is managed conventionally. For the control of weeds, chemical control is used (Paraquat dichloride 276 g l-1) every two months, while pests and diseases are controlled with insecticides and fungicides. The most commonly used molecules for insect pest control on this farm are: (Lambdacyhalothrin, Chlorpyrifos, Cypermethrin, Diazinon and Dimethoate) and for fungus control (Pyraclostrobin, Azozystrobin, Cymoxanil, Mancozeb, Propamocarb and Benocor). Soil fertilizers are applied without calculating dosages, nor have soil chemical analyses been carried out to identify elements with deficiencies; the most commonly used fertilizer formulas by the farmer are: (15N-15P-15K and 18N-16P-0K).

Soil sampling

A total of 4 commercial cocoa plots were delimited in this study. The number and location of the plots (2 localities) and the type of system (mixed and monoculture) was determined according to the availability of land, and an attempt was made to cover each agroecological condition of each locality. Each plot consisted of 1 hectare.

Soil samples were collected from each plot at 20 cm depth in September 2021. At each location, ten sampling points were taken for each farming system, following a zig-zag pattern. Samples were taken at each site, and each sample, composed of approximately 400 g of soil, was placed in an individual plastic bag and immediately taken to the laboratory. Nematodes were extracted from each sample from 100 g of soil by decanting and gradient centrifugation with sugar media (Nic Smol, 2007). The total amount of nematodes were counted in the square counting dish. The nematodes were then collected and mounted (Smol, 2007).

They were identified down to taxonomic genera and classified into trophic guilds, including bacterivores, fungivores, omnivores, predators and parasites (Yeates et al., 1993).

Ecological indices and nematode diversity

The Shannon-Wiener diversity index of families was determined through the formula: Ht = -Σti*log2 *ti where ti is the proportion of the total sample corresponding to taxon i. It combines two components of diversity: the number of species and the equality or inequality of the distribution of individuals in the various species.

Family equity was also analyzed as Et = Ht / Htmáx where Htmáx = log2 (number of taxa). It compares the calculated diversity with the maximum possible diversity. It is not a measure influenced by abundant taxa or the number of taxa, as is the diversity index.

Finally, the maturity index (Bongers, 1990) was calculated with the formula MI= S vi pi, where vi is the c-p value in taxon i and pi is the proportion of the taxon in the sample. Phytophagous nematodes are excluded. This index determines the degree of success an ecosystem achieves and is based on the placement of nematode families into five c-p groups representing different survival strategies and ecological requirements. The maturity index (MI) is a simple equation that evaluates the nematode community in a given sample according to the proportion of colonizing and persistent individuals. The higher the environmental disturbance, the MI has values close to 1, while values above 3 suggest environmental stability.

The data analysis for each variable was performed with the statistical software NINJA: (Nematode Indicator Joint Analysis) (Sieriebriennikov et al., 2014).

RESULTS AND DISCUSSION

The 4550 individuals identified belonged to 6 genera, the most abundant of which were Helicotylenchus for the two ecological zones and agricultural systems, Tylenchus and Hemicycliophora, where the most significant presence was observed in the agricultural systems of Santo Domingo. On the other hand, Meloidogyne and Criconemoides were observed with a greater presence in the ecological zones of Los Ríos. The lowest number of individuals was expressed by the genus Mononchus with a total of 150 individuals present (Table 2). Studies by Bustamante et al. (2019) show that the highest abundance of nematodes recorded in the cocoa crop corresponds to the six genera found in this study.

Hemicycliophora, a phytoparasitic nematode that is very harmful in many crops, appears in the systems that are conventionally managed in Santo Domingo and Los Ríos and that are established as monocultures in both locations; another phytoparasite that is associated with the cocoa crop is Criconemoides, This was found in the associated system in the Santo Domingo area, with an average total density of 100 nematodes 100 cm-3 and in more significant quantities in the two systems in the Los Ríos locality with an average of 425 nematodes 100 cm-3 (Table 2).

Table 2. Nematode taxa identified number of individuals of the genera recorded.

Genres	Ecological zones			
	Light of America		Patricia Pilar	
	Associate	Monoculture	Associate	Monoculture
Helicotylenchus	400	550	350	550
Tylenchus	300	300	0	0
Mononchus	100	0	50	0
Criconemoides	100	0	150	550
Meloidogyne	50	50	400	100
Hemicycliophora	0	400	0	150
Total	950	1300	950	1350

Bacteriophages predominated, followed by the predatory guild, which reflects soils disturbed by the intensification and mismanagement of agricultural activities implemented in the research area (Table 3).

Few sites reflect the maintenance of trophic succession of these edaphic organisms to contribute to the distribution of nutrients and minerals in the soil. According to Ferris (2010), these organisms are responsible for 30% of nitrogen mineralization in the soil, being the leading ecosystem service, they provide.

Among the detailed genera is the presence of a beneficial nematode such as Mononchus of the Mononchidae family, characterized by biological control, is considered a predator that reduces the populations of phytoparasitic nematodes. It was found in the agroecological managed systems in the two locations studied in the association of cocoa (Theobroma cacao) with plantain (Musa balbisiana).

Regarding edaphic factors, the taxonomic groups did not show any affinity or relationship in the four localities and this agrees with the research conducted in coffee plantations by Arreaga et al. (2017), the ranges of difference that the percentage of organic matter presented in each of the systems of the different localities was notorious, this could be related to the type of management since the highest percentage of OM was located in the systems associated with the two study areas. Therefore, the criterion is shared with the previous author that the richness of the nematode communities could be changing, not in response to physical-chemical factors of the soil, but rather to agricultural management factors in each of the farms under study.

Table 3. Representation of the number of individuals per trophic guild in two ecological zones

Trophic guilds	Ecological zones			
	Light of America		Patricia Pilar	
	Associate	Monoculture	Associate	Monoculture
Bacteriophage	850	1300	900	1350
Predator	100	0	50	0

Concerning the diversity, dominance, and equity indices (Table 4), few differences are observed between the two types of systems. Although some studies indicate that alterations caused by the type of management can cause a decrease in the value of the diversity index, others report little or no difference between altered and undisturbed systems (Cedeño et al., 2022). In many cases, the type of agricultural system and their agricultural activities cause a decrease in the abundance of soil nematodes.

This discrepancy is because the changes are more evident in the composition of the community than in its ¬diversity. Although the mixed system did not have the highest diversity index, it does show the highest equity value, indicating a better distribution of the total number of individuals in the families present.

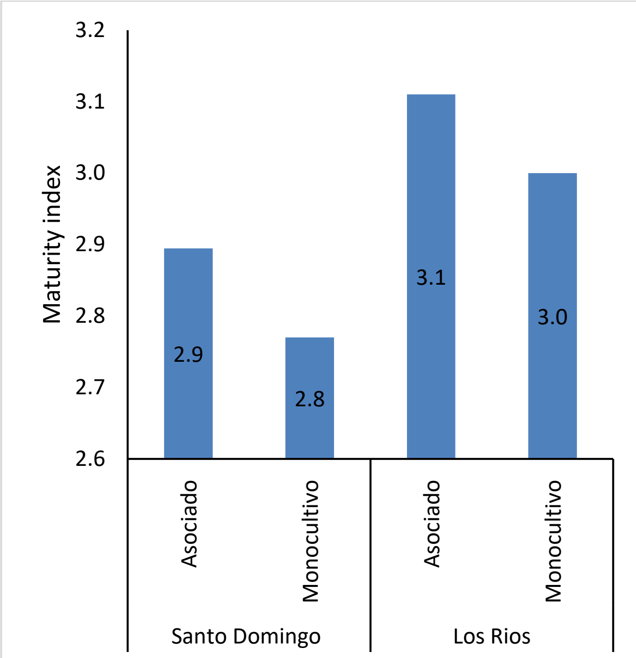
The number of phytonematodes found in the monoculture system was higher than in the mixed system for the two ecological zones; however, populations vary over time as they are attracted to the plants by plant exudates. In addition, other factors such as soil water content, pore size, or other biotic factors result in spatial distribution. Laasle et al. (2022) indicate that, due to the intimate relationship of phytonematodes with their host plants, they are inhabitants of the root-soil interface, which differs considerably from the rest of the soil mass. According to Bustamante (2019), phytoparasitic genera form a higher percentage of the nematofauna in agroecosystems than in natural ecosystems. This author indicates that nematodes such as Meloidogyne, Helicotylenchus, Tylenchus, Criconemoides, and Hemicycliophoracausa cause severe damage in cocoa agricultural systems, and this is the reason why large amounts of nematicides are applied in the tropical areas where they are grown.

Table 4. Variables calculated for the nematode community in two cocoa farming systems.

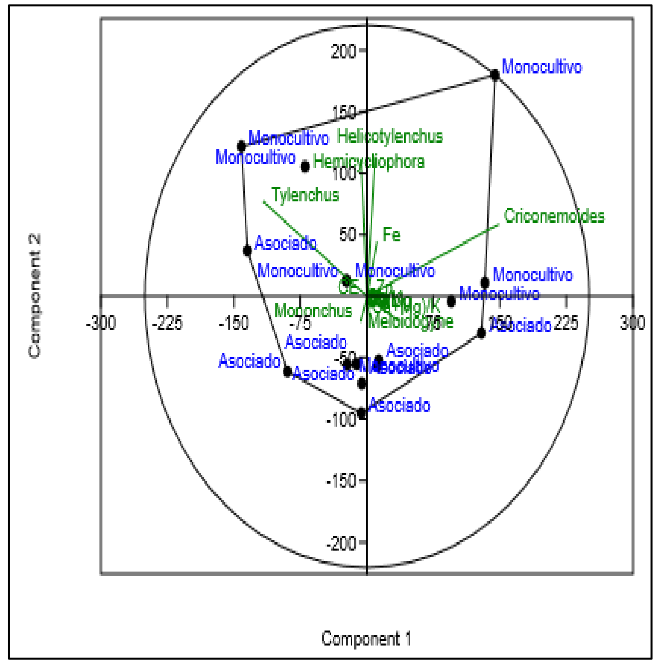
Parameters	Light of America		Patricia Pilar	
	Mixed	Monoculture	Mixed	Monoculture
Shannon Wiener Diversity	0.9	0.9	0.7	0.8
Family equity	0.9	1.0	0.7	0.9
Simpson 1-D dominance	0.6	0.6	0.4	0.5
Phytoparasitic nematodes (%)	89.5	100.0	94.5	100.0
Beneficial nematodes (%)	10.5	0.0	5.5	0.0

The Maturity index (Figure 1) allows us to determine each agricultural system's degree of conservation or disturbance. There are differences between localities and between systems; for the locality of Santo Domingo, the associated system reaches an index of 2.9 and the conventional system that is managed as a monoculture obtains 2.8; something similar is observed in the locality of Los Ríos, the highest index is directed towards the system that associates cocoa with banana with a value of 3.1, while the conventional system reaches a score of 3.0, these results reaffirm the approach of Varela (2018), who proposes that the maturity and food web indices have a descriptive function, and do not evaluate the participation of nematodes in the flow of energy, which are the resources that determine the size and activity of the food web. Eventual validation of this tool could help better to understand the interactions of ¬soil populations in agricultural ¬systems.

The results obtained in this study are in partial agreement with those obtained by other similar studies (Bhusal et al., 2015, Kergunteuil et al., 2016; Traunspurger et al., 2017), which point out that the role played by nematodes in the decomposition of organic matter and food webs could be higher in places located at higher altitudes, and that temperature is one of the factors that could explain this relationship.



Maturity index (MI) values consider the number of nematodes observed as a function of the agricultural system and ecological zones.



Principal component analysis (Biplot) between locations, agricultural systems, number of nematodes found and soil parameters.

The component analysis (Figure 2) shows the distribution of each of the nematodes found within the circle of correlations with the system, where abundance shows that the nematodes Helicotylenchus, Hemicycliophora, Tylenchus, Criconemoides and Meloidogyne are closely related to cocoa systems that are managed as monoculture, Criconemoides and Meloidogyne are closely related to cocoa systems managed as a monoculture. The nematode Mononchus is located in the quadrant governed by systems where cocoa (Theobroma cacao) and plantain (Musa balbisiana) are associated. This research shows nematodes' ecological and economic importance as environmental indicators in tropical systems since they can adapt to different environments. This same criterion is shared by Ferris et al. (2001), Yeates et al. (1999) in their research reports that the nematofauna provide an effective response to environments conserved or disturbed due to agronomic practices applied to agroecosystems.

Conclusions

Six nematode genera were identified (Helicotylenchus, Tylenchus, Mononchus, Criconemoides, Meloidogyne and Hemicycliophora), with an overall total of 4550 organisms. The Mononchus genus stands out for its trophic group. It is characterized as a predatory nematode. Its function in the soil is to act as a biological controller of other phytoparasitic nematodes, exclusively in the systems that have agroecological management in the two study zones and in association with the plantain crop (Musa balbisiana).

It is evident from the analysis of the maturity index that the cultural, technical and ecological management of agroecosystems has a notorious influence on the diversity of the nematofauna; it is concluded that the indiscriminate use of pesticides and the execution of inadequate cultural practices significantly reduces the biodiversity of beneficial nematodes allowing the abundance of phytoparasites.

The nematofauna provides factual information on the edaphic processes resulting from agricultural methods, so this group of organisms, easy to obtain, abundant and determined by morphological characters, represents a handy tool to measure the anthropogenic impact on the environmental health of agroecosystems.

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