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Diversity and community composition of nematode communities in horticultural and banana crops

Diversidad y composición de la comunidad de nemátodos en cultivos hortícolas y banano

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Abstract: Horticultural and musaceous crops are of great economic importance in Ecuador. Although there are studies on the association of phytopathogenic nematodes and the damage they cause, little research has been done on the diversity and composition of nematodes in these crops. The present study evaluates the diversity, composition and abundance of the nematode community related to soil physicochemical characteristics, reports Shannon H' diversity and nematode community structure, based on their trophic groups, colonizer-persistent (c-p) community structure and maturity index (MI). This work presents results from soils in horticultural and banana plantations in the provinces of Azuay (highlands), El Oro and Guayas (coast), respectively. The results showed average organic matter (OM%), temperature (T°C) and humidity (H%) variables with significant differences ($P<0.05$) between sampling regions. No organisms of the mycophagous trophic group were found throughout the study. Genera such as Tylenchus, Helycotylenchus and Pratylenchus, represented more than 50% of the total of those collected. However, a notable difference was observed in the predatory trophic group, with a higher abundance in the coastal region than in the highlands. In general, the Costa and Sierra growing regions had a considerable impact on abundance and physicochemical variables. Climate had an important effect on the distribution and diversity of nematodes, including those of the predatory trophic group. The abundance of the herbivore trophic group could be mediated by the presence of the predator trophic group.

Keywords: Nematode, diversity, abundance, structure, trophic

Resumen: Los cultivos hortícolas y de musáceas representan una gran importancia económica para el Ecuador. Aunque existen estudios sobre la asociación de nemátodos fitopatógenos y los perjuicios que causan, poco se ha investigado sobre la diversidad y composición de nemátodos en dichos cultivos. El presente estudio evalúa la diversidad, composición y abundancia de la comunidad de nemátodos relacionado con las características físico-químicas del suelo, reporta la diversidad de Shannon H' y la estructura de la comunidad de nemátodos, a partir de sus grupos tróficos, estructura de la comunidad colonizadores – persistentes (c-p) e índice de madurez (IM). Este trabajo presenta resultados de suelos en plantaciones hortícolas y de banano en las provincias del Azuay (sierra), El Oro y Guayas (costa) respectivamente. Los resultados demostraron promedios en las variables materia orgánica (MO%), temperatura (T°C) y humedad (H%) con diferencias significativas ($P<0.05$) entre las regiones de muestreo. No se encontraron organismos del grupo trófico micofago en todo el estudio. Géneros como Tylenchus, Helycotylenchus y Pratylenchus, representaron más del 50% del total de las colectadas. Pero se pudo observar una notable diferencia del grupo trófico predador con una abundancia mayor en la región Costa que en la Sierra. En general, las regiones de cultivo Costa y Sierra tuvieron un impacto considerable en la abundancia y variables físico-químicas. El clima presento un efecto importante en la distribución y diversidad de nemátodos, entre ellos los del grupo trófico predador. La abundancia del grupo trófico herbívoro pudo verse mediada por la presencia del grupo trófico predador.

Palabras clave: Nemátodo, diversidad, abundancia, estructura, trófico

Introduction

Nematodes are among the highest proportion microorganisms as they account for 80% of multicellular organisms and are also localized in most terrestrial ecosystems (Bardgett and Van Der Putten, 2014; Eisenhauer and Guerra, 2019; van den Hoogen et al., 2019).. They are considered model organisms for understanding soil ecology due to their trophic behavior and life form (Liu, Hu and Li, 2019). To understand this ecology, there are tools among which are the study of the diversity and composition of nematodes (Yan et al., 2021). From this community structure of nematodes based on their diversity and genus composition, the behavior of nematode trophic groups during winter seasons has been studied, but more research is still required (Girgan et al., 2021).. Even to measure their diversity and composition, variables such as height above sea level, temperature, humidity, crop type (short cycle or perennial) and even the level of intensive agriculture, are considered indicators of community structure (Dong et al., 2017; Emery et al., 2017)..

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This specific study of nematode communities has proven to be of great importance, both to know the richness of these microorganisms in megadiverse countries such as Ecuador or for the correlation of crop productivity with the different species that inhabit the soil (Altamirano-Benavides and Yanez-Moretta, 2016; Fan et al., 2020).. Depending on the trophic group, nematodes can be considered indicators on soil quality in crops. Since depending on the species composition, they provide indirect soil information. (Ney et al., 2019). Hence, nematode community structure in soil is important for the ecological functioning of agroecosystems and are a fundamental part in agricultural sustainability (Sánchez-Moreno et al., 2018).. They detect an imbalance in the correct biotic structure due to the use of pesticides, fertilizers, crop rotation, perennial crops, among others (Sánchez-Moreno, 2018).

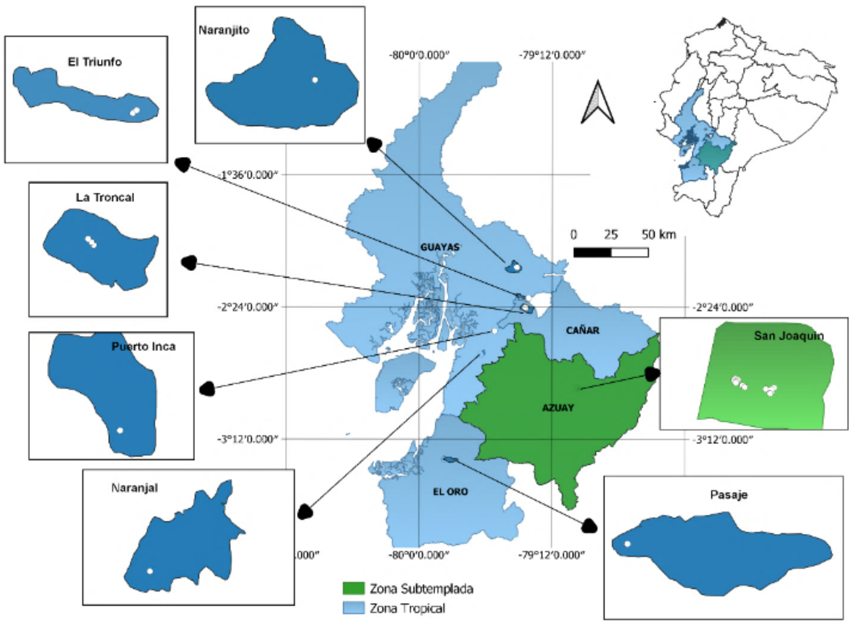
Among the soils that present the ideal characteristics for the investigation of nematode diversity are soils with musaceous and vegetable crops. These are among the most important and economically interesting crops worldwide as well as a great source of nutrients (Dias, 2012; Pareek, 2015; Brown et al., 2017; Campos and Caligari, 2017).. These crops can be affected by phytopathogenic nematodes that are considered as pest organisms of horticultural crops (Parvatha, 2013). However, for the control of these pests, intensive use of pesticides is seen to be necessary especially in conventional crops (do Amaral et al., 2018), affecting the nutritional characteristics in crop products. This use of pesticides would be justified by the high infestation of nematodes in crops and the need for effective chemical spraying for the control of this or other types of pests (Greco et al., 2020). This is why crop management has a direct effect on soil biology and can be used as an indicator for proper crop management (Pattison et al., 2020). (Pattison et al., 2018).. Crops in which different communities can be found either by their intensive use or even by the location where they are found.

In this research work, an analysis of the diversity and species composition of nematode species is carried out (Moreno and Talavera M., 2013). (Moreno and Talavera M., 2013).The objective was to describe the existing communities of nematodes in horticultural and banana crops in two zones, the highlands and the coast, respectively.

Materials and methods

The localities correspond to the Sierra and Costa climatic zones. In the Sierra region, soil samples were obtained from horticultural crops in the town of San Joaquín in the city of Cuenca, Azuay province. While in the coastal region, soil samples were collected from musaceous crops in the localities of El Triunfo, Naranjito, Puerto Inca and Naranjal in the province of Guayas, as well as Pasaje in the province of El Oro and La Troncal in the province of Cañar, Ecuador, as shown in Figure 1.

Figure 1. Map showing the location of the sampling points in the study area.



The study was developed during the months of February to May 2019. The study sites were randomly selected present in the locations mentioned above, this due to the economic importance of the crop in each region (Maffei et al., 2016).. Soil samples were taken in each farm, at the same time field interviews were conducted consisting of questions related to fertilizer management, water source, crop rotation and pest treatment. The crops were also selected according to their commercial interest due to the wide diversity of nematodes that use these plants as hosts and the level of damage they can cause by infecting the crops (Sivasubramaniam, Sivasubramaniam, et al., 2000).

(Sivasubramaniam, Hariharan and Zakeel, 2020).. The crops tested in the Sierra region were: garlic (Allium sativum), cabbage (Brassica oleracea Capitata group), broccoli (Brassica oleracea Italica group), lettuce (Lactuca sativa), carrot (Daucus carota), artichoke (Cynara cardunculus Scolymus group). In the coastal region, banana (Musa AAA) was used. In all cases, plantations at different stages of development were evaluated.

A total of 21 soil samples representing each of the crops sampled were collected. Twelve subsamples were taken from each sampling site, to obtain a composite and homogeneous sample of approximately 600 g. Soil samples were taken at a distance of 10 cm to 30 cm from the base of the stem at a depth of 20 cm to 30 cm and transported in plastic bags to the laboratory. The samples were stored in coolers to protect them from sunlight. Soil properties analyzed included soil temperature (T°C), percent moisture (H%), pH and soil organic matter (OM). To obtain OM, 100 g of soil (out of approximately 600 g) were sent to and analyzed in the soil laboratory of the School of Mechanical Engineering and Production Sciences, Escuela Superior Politécnica del Litoral, Gustavo Galindo Velasco campus. Soil T°C and H% were measured using a ProCheck portable data receiver and reader and a GS3 sensor. The pH was analyzed using a Luster Leaf 1847 Rapitest Digital Plus Soil pH Meter.

Nematodes were extracted from 200 g of fresh soil from the initial sample, divided into 2 extraction trays with 100 g each. The method used for the extraction of nematodes from the soil was the modified Whitehead trays (Whitehead and Hemming, 1965). (Whitehead and Hemming, 1965). It consists of a filter that was placed directly over the trays containing the water. The bottom of the filter was presented in such a way as to allow the soil to be completely wetted, then incubated for 48 hours. The collected suspension was filtered using a 230 mesh sieve and placed in a conical polypropylene tube. The nematodes in suspension were selected and sorted using a stereomicroscope.

Nematode abundance was measured as individuals per 200 g of soil. After extraction, the sediment was transferred to Petri dishes where the total number of nematodes per sample was counted with a BOECO model BST-606 stereo-zoom-microscope. Subsequently, the individuals were fixed with the use of formalin glycerin (40% formaldehyde 10 ml - Glycerin 1 ml - Distilled water 89 ml) and subsequent mounting of each individual in the object-holder plates. The nematodes in each sample were counted with the aid of a motorized research microscope OLYMPUS model BX63 and identified by genus (Bongers, 1989; Hunt, 1989). (Bongers, 1989; Hunt, 2008a, 2008b, 2008c; Andrássy, 2010) and then classified into bacterivore, plant parasite (herbivore), omnivore and predator feeding groups based on Yeates et al. (1993). In addition, nematodes were classified into the persistent colonizers group (cp1 - cp5) as an indicator of ecosystem structure and function (Bongers, 1990a; De Goede, Bongers and Ettema, 1993; Yeates et al., 1993)based on the successional state and functioning of the nematode community in soil (Bongers, 1990b; Ferris, Bongers and De Goede, 2001)..

Identification was based on examination of the caudal 'tail' and cranial 'head' limbs of each organism, primarily the cranial retractile points 'mouth; denticles; stipe; stoma; internal, dorsal, and ventral teeth', length and shape of the esophagus, characteristic parts of some genera (van Wyk and Mayhew, 2013).. In addition, genus-specific taxonomic keys were used, based on scientific papers on nematode identification (Odontopharynx and Man, 1989; Bostroêm, 1991; Vovlas, 1992; Mahato, 1997; Ahmad and Shaheen, 2004; Jana, Chatterjee and Manna, 2008; Tahseen and Rajan, 2009; Andrássy, 2010; Nusrat, Anjum and Ahmad, 2013; Shokoohi et al., et al., 2013, 2016; Carta and Skantar, 2014; Inserra et al., 2014; Alvarez-Ortega et al., 2016; Kolombia et al., 2017; Leduc and Zhao, 2017; Phani et al., 2018; Sikora, 2018; Kanzaki, Ekino and Masuya, 2019)..

The following nematode community indices were calculated: genus richness, nematode abundance, c-p community structure and IM maturity index were calculated based on data obtained using the program NINJA (Sieriebriennikov, Ferris and de Goede, 2014).. Diversity was calculated using the Shannon-Wienner index (H') which confers greater weight to uncommon species. It allows the nematode community to have a very even abundance distribution. In addition, it minimizes the specific diversity of nematodes in small samples such as the vegetable crops sampled (Krebs, 1999). (Krebs, 1999).

$$H' = \sum_{i=1}^{S} p_i log p_i$$

Where the factor S corresponds to the number of species (species richness), pi the proportion of individuals of species i with respect to

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the total number of individuals, ni the number of individuals of species i, and N the number of all individuals of all species.

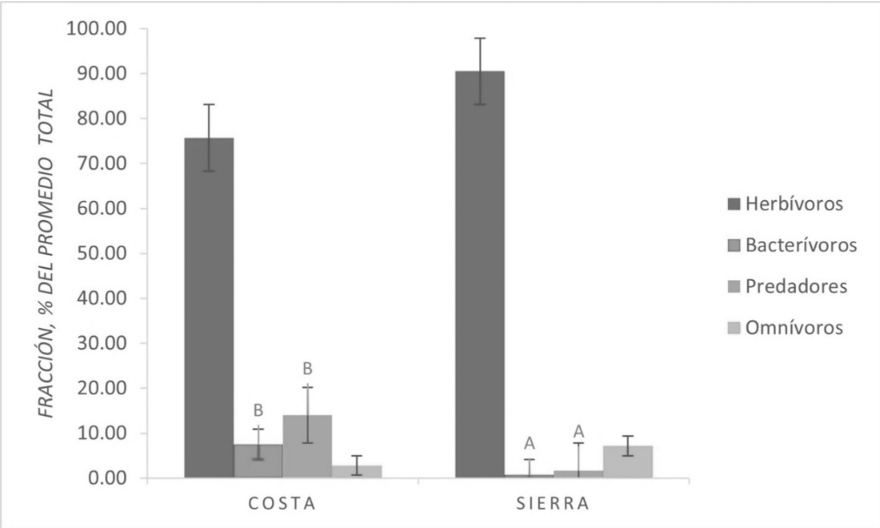
ANOVA analysis of variance was performed to compare the difference ($P<0.05$) of the results throughout the study and thus observe the effects of physicochemical variables with diversity indices, nematode abundance and maturity indices. Significant differences in main effects were analyzed by paired comparison using Tukey's test. All statistical tests were performed using InfoStat statistical software version 2020I(www.infostat.com.ar).

3. Result

An average abundance of 29 nematodes was found in the coastal region, while in the sierra region it was higher with an average of 107 nematodes. Of the total of 29 genera of nematodes in the herbivorous, omnivorous, predatory and bacterivorous trophic groups, the genera with the greatest number of individuals were Tylenchus, Helycotylenchus and Pratylenchus, both in samples of horticultural crops and bananas. In horticultural crops, the genera Helycotylenchus, Tylenchus and Pratylenchus had the highest number of individuals with 488, 436 and 111 individuals, respectively. In banana crops, the genus Helycotylenchus and the genus Radopholus also had the highest number of individuals with 163 individuals in both cases, while the genus Tylenchus had 103 individuals. All genera belong to the herbivorous trophic group.

As shown in Figure 2, no genera of the fungivore trophic guild were found. In the two sampling regions, only the averages of the bacterivore (Coast: 7.46 ± 1.47, Highlands: 0.69 ± 1.28) and predator (Coast: 14.01 ± 2.85, Highlands: 1.66 ± 2.47) guilds showed significant differences ($P<0.05$). Two ranges of significance were found for both the bacterivore and predator trophic groups.

Figure 2. Abundance in percentage of the total average of nematodes of four trophic groups of the sampling regions Coast with horticultural crops and Highlands with banana crops (individuals per 200 g of soil). All values are means + ES, n = 21. Letters A, B indicate significant differences between treatments (Tukey's test α = 0.05).



Of the percentages of c-p structure of free-living nematodes, only c-p1, c-p2 and c-p4 presented significant differences (P<0.05) with the following averages according to sampling zone: C-p1 (Coast: 32.16 ± 6.48, Sierra: 0.64 ± 5.62); c-p2 (Coast: 9.44 ± 2.47, Sierra: 0.80 ± 2.14) and c-p4 (Coast: 45.88 ± 11.94, Sierra: 81.89 ± 10.34). Regarding the range of significance for the c-p percentages, each region was grouped in different ranges. The MI, H' and the c-p3 - c-p5 structure did not present significant differences in their averages (P<0.05) in this study.

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Table 1. *Abundance of soil nematodes (dominant and frequent genera, trophic groups), physicochemical variables and ecological indices for the study of the nematode community. Values are shown with mean and standard error (mean±SE).*

Parameter	Coastal Region	Sierra Region
	Banana Crops	Horticultural Crops
Abundance of the most prominent genres		
Helycotylenchus	18.11±10.58	40.67±9.17
Tylenchus	11.44±9.82	36.33±8.51
Pratylenchus	8.00±4.06	9.25±3.51
Physical-chemical variables		
pH	6.33±0.14	6.55±0.12
MO%1	3.39±0.38A	8.58±0.33B
T°C1	30.56±0.22B	20.78±0.19A
H%1	4.95±0.91A	21.42±0.79B
Shannon Index H'	1.38±0.13	1.13±0.11
Maturity index IM	2.82±0.43	3.30±0.37
C-p11	32.16±6.48B	0.64±5.62A

C-p21	9.44±2.47B	0.80±2.14A
C-p3	7.41±4.81	0.00±4.16
C-p41	45.88±11.94A	81.89±10.4B
C-p5	5.13±2.83	0.00±2.45

1Values followed by different letters in the same row represent a significant difference according to Tukey's test (α = 0.05).

The physicochemical variable pH did not show significant differences ($P<0.05$) in the sampling zones. On the other hand, organic matter, temperature and humidity did show significant differences in the two sampling regions, as shown in Table 1. Temperature is positively related to diversity, while humidity and organic matter are reflected in diversity, such is the case that it is related to the regions sampled, since the significant differences in the averages of the Coast region show that the lower the percentage of humidity, the better the diversity in comparison with the humidity of the Sierra region where humidity is higher but diversity is lower than in the Coast region.

The lower diversity of nematodes in horticultural crops in the Sierra region compared to the diversity of banana crops in the Costa region may be due to the altitude of this region. As reported by studies on species richness, at higher altitudes, species richness generally decreases, as is the case with most of the soil fauna (McCain and Grytnes, 2002). (McCain and Grytnes, 2010; Vittoz et al., 2010; Mumladze et al., 2015).. Contrary to this statement according to K ergunteuil et al. (2016)., both diversity and abundance of nematodes increase with altitude. Although nematode abundance is indeed higher in horticultural crops in the Sierra region compared to banana crops in the Costa region, diversity is not. It is deduced then that the type of short-cycle crops such as vegetables does not allow the complete life cycle of persistent species such as predatory nematodes. In addition, the increase in H% observed (21%) in the Sierra region is related to the abundance of nematodes, especially the abundance of herbivorous species. This is due to the higher frequency and amount of rainfall in

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the Sierra region (Todd, Blair and Milliken, 2000). (Todd, Blair and Milliken, 1999; Landesman, Treonis and Dighton, 2011).This is mainly due to the flow of water in the crop irrigation system, data obtained from the interviews conducted.

According to work done by. W ardle & Yeates, (1993) and Zhao & Neher, (2014) concerning competition in food webs and soil energy pathways, at higher altitude conditions, enhances the flow of fungal energy within ecosystems. Despite not having found fungivorous genera, it is believed that they could be present, but in a minimal amount, perhaps due to the lack of fungivorous organisms that have been controlled by genera such as Tylenchus spp. and Iotonchus spp. Species of these genera would also feed on fungi and not only on plants as reported by certain studies (Okada et al., 2002; Tsuda & Futai, 2000).

Regardless of the region, type of crop or type of management, the genera of herbivorous nematodes were more abundant, thus demonstrating their phytopathogenic potential as pests. Even for the control of phytophagous nematodes such as those of the genus Tylenchus, Helycotylenchus, Pratylenchus and Radopholus, found in greater numbers in this study, could justify the possible use of agrochemicals on crops. Even this trophic group of herbivorous nematodes could displace other trophic groups due to the abundance that would occupy space for other organisms as is the case of predatory nematodes that are important as biological control (Chen et al., 2013).

The values obtained for H' diversity do not represent an optimal diversity (Shannon H' diversity greater than 2), possibly due to the type of perennial crop such as musaceae, since they present low organic carbon inputs from plant residues, as stated by B engtsson et al. (2005).

The relationship between diversity H' with the percentage of humidity, as well as T (°C) and MO may be due to the climate factor of the sampling zones, which would indicate greater diversity in the coastal region compared to the Sierra region. Authors such as Gillingham et al. (2012) and Suggitt et al. (2011) mention the influence of temperature directly on the distribution of species, including nematodes, according to the characteristics of the habitat, which is reflected in the relationship between temperature and the study areas. Likewise, the results obtained in relation to temperature and organic matter are related to those obtained by other studies (Bhusal, Tsiafouro, and others). (Bhusal, Tsiafouli and Sgardelis, 2015; Kergunteuil et al., 2016; Traunspurger et al., 2017; Varela Benavides, 2018)where they indicate the importance of nematodes in the decomposition of organic matter that could be higher in areas located at higher altitudes, where temperature is the key factor to understand this relationship.

Finally, the results obtained on the number of nematodes found in class c-p4, which refers to nematode genera with a long life cycle, larger and very sensitive to disturbances, generally encompasses predatory and omnivorous nematodes (Bongers, 1990a). (Bongers, 1990a). These results indicate more complex trophic networks in the Sierra region with short cycle crops than in the Costa region with musaceous crops, which determines a stability in the nematode community and therefore soil conservation, giving similar results presented in studies of nematode community structure in agroecosystems with agroecological crops (Salas and Achinelly, 1990a). (Salas and Achinelly, 2020)..

4. Conclusions

Differences in the abundance and diversity of nematodes were highly dependent on the region and its humidity and temperature variables, together with organic matter, which can have an important effect on the distribution of nematodes and even on the infestation of herbivorous nematodes. The abundance of predatory nematodes may be controlled by the type of short-cycle crop and moist climatic conditions. However, these conditions can be beneficial for nematodes of the herbivorous trophic group. The use of nematodes as an environmental indicator seems to be more promising depending on climatic conditions and crop type, but they certainly help to understand soil ecology in crops.

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