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Article

Ecological Interaction in the Proliferation of Phytoparasitic Nematodes in Coffee var. Typica

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Abstract: Phytoparasitic nematodes are a common problem in coffee production worldwide. In Peru, the proliferation of phytoparasitic nematodes in *Coffea arabica* L. var. Typica has negative impacts on coffee production and huge economic losses. The objective of this study was to determine the ecological interactions that influence the proliferation of phytoparasitic nematodes in *C. arabica* var. Typica in Quillabamba, Cusco, Peru. The density of phytoparasitic nematodes in the soil and root was evaluated using the modified Baermann channel method. Identification of genera and species of phytoparasitic nematodes was carried out in the different samples, based on descriptions and taxonomic keys. Physical and chemical parameters of the soil were evaluated. Principal component analysis (PCA) and a correlation network were employed to distribute the different species of nematodes. The variables soil texture, organic matter, pH, altitude, temperature and humidity were used. During the sampling, several genera of plant-parasitic nematodes were identified in addition to various categories of nematodes. *Meloidogyne exigua*, *Pratylenchus coffeae*, *Allotrichodorus longispiculis* and *Helicotylenchus longicaudatus* are among the specific species of nematodes identified. The genus with the highest incidence was *Meloidogyne* spp. with 77.3% in soil and 96% in roots, reaching an average density of 340 nematodes in 100 g of soil and 368 nematodes in 1 g of roots. The PCA results show that 63.7% of the variability of the data is retained in the first two components. PCA shows that the sampled areas were differentiated and grouped with the evaluated characteristics, while the nematodes overlap in the representation of the analysis, thus making it difficult to select them. Pearson's correlation showed a negative correlation between altitude and soil nematode density (−0.22), suggesting that conditions at higher altitudes may be more unfavorable for nematode growth. In addition, a positive correlation was found between soil pH and organic matter (0.93, $p < 0.001$) and may influence nematode density. The factors that influence the density of nematodes in the soil and plant roots are multiple and complex, and their understanding is essential for the success of coffee production. The finding of the current study may be useful in the development of sustainable strategies for managing nematodes in coffee network production.

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1. Introduction

Coffee (*Coffea arabica* L.) is one of the most important economic activities in many tropical countries [1]. The largest coffee producer in the world is Brazil, with 2,993,780 tons produced in 2021, followed by Vietnam (1,845,032.98 t), Indonesia (765,415 t), Colombia (560,340 t), Ethiopia (456,000 t), Honduras (400,674.42 t) and Peru (365,582.14 t) according to FAO data [2].

Among the coffee species cultivated in Peru is *Coffea arabica* L. var. Typica, which provides high quality coffee production [3]. Peru ranks seventh among coffee-producing countries according to the FAO [2]. This position in this world ranking is determined, among other factors, by susceptibility to different pests such as *Hemileia vastratix* [1,4,5] and nematodes [6,7]. For producers who cultivate small plots, and commonly to those in Peru, these pathogenic agents cause many losses that are associated with low investment in their production, a lack of knowledge and incorrect identification and management [7,8].

Nematodes are microscopic organisms that play an important role in soil ecology, particularly in causing significant damage to agricultural crops [9,10]. These microscopic organisms penetrate the roots of the coffee plant, causing damage at the root level and, as a consequence, decrease coffee bean production [11–13]. Knowing the ecological factors that influence the development of phytoparasitic nematodes associated with this crop is very important. Studies have shown that the interaction between nematodes and their environment is complex [10,14]. This interaction is influenced by many factors, such as soil chemical composition, moisture, temperature, nematode population density, etc. [14].

Soil ecology is a key factor influencing the development of phytoparasitic nematodes associated with coffee cultivation [15–17]. Soil chemical properties, such as pH, nutrient availability and soil texture, can significantly affect the population of phytoparasitic nematodes in coffee cultivation. Decraemer and Hunt [10] show that phytoparasitic nematodes can thrive in acidic and nutrient-poor soils, which shows the adaptive capacity of these pathogens. Low nutrient (phosphorus, calcium, magnesium, potassium and molybdenum) availability can increase the solubilization of zinc, copper, iron, manganese and aluminum, which, depending on soil management and applied fertilizers, can reach toxic levels for plants [10]. Nematodes have been shown to be extremely sensitive organisms to changes in soil moisture and temperature [17,18]. They are able to adjust their metabolism and activity in response to changes in soil temperature and moisture [19]. It has been described that the optimum humidity for nematode survival and reproduction varies according to the species and can range from 40% to 80% [10]. Studies associated with coffee have determined that the ideal temperature for the proliferation of phytoparasitic nematodes is between 25 °C and 30 °C, although this also varies according to the species of nematode and the geographical location of the coffee crop [20].

Nematode population density is also a critical factor influencing the survival, proliferation and damage that phytoparasitic nematodes cause to the coffee crop. Nematodes are organisms that tend to proliferate rapidly under favorable conditions, and this can lead to overpopulation in the soil [21,22]. For this reason, it is important to understand the factors that affect nematode population density and to develop integrated management strategies to control their proliferation [23].

Ecological factors (soil chemical composition, moisture, temperature and nematode population density) are critical to understanding the development of phytoparasitic nematodes associated with coffee cultivation. The knowledge of these factors is important to develop effective integrated management strategies to control the proliferation of nematodes and minimize their impact on coffee production. The objective of this study was to determine the ecological interactions that influence the proliferation of phytoparasitic nematodes in *Coffea arabica* var. Typica in Quillabamba, Cusco, Peru.

2. Materials and Methods

2.1. Study Area

The study was carried out in coffee production farms in Quillabamba, Cusco region, Peru. Samples were collected in eight *C. arabica* L. var. Typica producing areas: Quebrada Honda (latitude: S 12°49.5824′, longitude: W 072°39.58′), Huayanay Centro 01 (latitude: S 12°48.6521′, longitude: W 072°38.6914′), Huayanay Centro 02 (latitude: S 12°48.9447′, longitude: W 072°38.9014′), Huayanay Alto 01 (latitude: S 12°49.3773′, longitude: W 072°38.5427′), Huayanay Alto 02 (latitude: S 12°48.9207′, longitude: W 072°38.7688′), Empalizada (latitude: S 12°48.3601′, longitude: W 072°37.7248′), Aguilayoc (latitude: S 12°47.6869′, longitude: W 072°38.0907′) and Huayanay (latitude: S 12°48.4666′, longitude: W 072°38.9343′).

2.2. Climatic Conditions

To obtain specific data on humidity, altitude and temperature, in situ measurements were made using a thermohygrometer to obtain accurate measurements of environmental factors. In addition, a GPS was used to determine the altitude of each sampling site. These in situ measurement instruments allowed us to obtain a detailed characterization of the environmental factors relevant to the study.

2.3. Soil Analysis

The following parameters were determined for the eight coffee-producing zones: soil texture, organic matter content and pH. Soil texture was determined using the hydrometer method. The organic matter content was determined using the Walkley and Black method (% O.M.) [24]. For the determination of pH, the potentiometer method was used, soil water ratio 1:1 in the saturated paste, as described by Rhoades [25].

2.4. Soil and Root Sampling for Nematode Analysis

In each zone, soil and root samples were randomly collected, sampling was performed with a zigzag pattern using a small shovel. In each evaluated plot, 20 subsample points were taken under the canopy of the coffee trees at a depth of 15 cm, and at the same time, the coffee roots were collected according to the recommendations of Coyne, Nicol and Claudius-Cole [9]. The subsamples were homogenized to form a sample of approximately 1 kg of soil and 100 g of roots per evaluated plot. Each sample was placed in a polyethylene bag properly coded and georeferenced in each sampling field using a portable GPS unit.

2.5. Soil and Root Nematode Analysis

Soil and root samples extraction was carried out using the modified Baermann tray method [26]. Soil and root samples were placed in trays, then the sample was covered with filter paper and water was added to the container at a water/sample ratio of 1:2 (volume/weight) for the extraction of nematodes from the soil. Each sample was processed in duplicate, and after 48 h of resting, the sediments were extracted and then passed through a 500 micron sieve. The sediments extracted in the sieve were placed in Petri dishes for further analysis or identification.

2.6. Identification of Phytoparasitic Nematodes

Individual soil and root samples were used to identify the phytoparasitic nematodes associated with coffee cultivation. Nine identification keys described in the literature were used for the characterization (Table 1).

Table 1. List of identification keys used in the determination of phytopathogenic nematode species.

Phytonematodes	Identification of Key Phytonematodes
Meloidogyne spp.	Durán Mora [27]
Pratylenchus spp.	Oliveira, Santos and Silva [28]
Allotrichodorus spp.	Rashid, De Waele and Coomans [29]
Helicotylenchus spp.	Mancini & Moretti [30]
Trichodorus spp.	Handoo and Golden [31]
Dorylaimidos spp.	Coomans [32]
Rhabditidos spp.	Giblin-Davis, Williams and Bekal [33]
Mononchids spp.	Loof and Coomans [34]
Criconematidos spp.	Taylor and Netscher [35]

2.7. Statistical Analysis

The association between the proliferation of phytoparasitic nematodes in the different zones and the relationship with the variables that were estimated according to Pearson correlation analysis with the aid of statistical software Rbio were previously determined [36]. Correlation networks were established to better visualize the general correlations existing between the variables evaluated, and the specific correlations associated with each of the nematode species described in the work. Principal component analyses (PCA) for the interpretation of the results were performed. These analyses were performed using Rbio software version 166 for Windows (Rbio Software version 166, UFV, Viçosa, MG, Brazil).

3. Results

3.1. Characterization of the Environmental, Physical and Chemical Factors of the Collection Areas

The interaction between nematodes and the environment determines the degree of the dissemination of these pathogens in coffee crop for a given location. Soil and root samples were taken to characterize nematode species associated with the coffee cultivar. The main characteristics of the soils of eight locations are shown in (Table 2). The data show that the different regions manifest altitudes ranging from 1400 m.a.s.l. (Aguilayoc) to 1866 m.a.s.l. (Huayanay Alto 01). The temperature for the different sampling sites had maximum values for Huayanay Center 02 of 28.3 °C and minimum for Huayanay Alto 01 with 21.3 °C (Table 2). Minimum humidity values of 67% were recorded in Quebrada Honda and maximum values of 85.4% in Aguilayoc. As for soil texture, only two soil textures (sandy loam and sandy clay loam) were found in the eight regions evaluated; both soil textures were represented in 50% of the sites (Table 2). The pH value of the different soil samples was close to 5. If we consider that the content in Huayanay Alto 01 was 7.53% and in Huayanay Centro 01 was 3.22%, the organic matter of the different sites would show variability upon quantification (Table 2).

Table 2. Physical and chemical analysis of the soil in the different sampling zones.

Region	Altitude (masl)	T * (°C)	Humidity (%)	Soil Texture	pH (KCl)	SOM (%)
Quebrada Honda	1755	23.4	67	Sandy loam	5.15	4.12
Huayanay High 01	1866	21.3	84.6	Sandy loam	5.47	7.53
Stockade	1676	25.8	80.9	Sandy clay loam	5.32	4.89
Huayanay Center 01	1579	28.1	84.6	Sandy clay loam	5.18	3.22
Huayanay	1515	27.4	78.5	Sandy clay loam	5.25	3.35
Huayanay High 02	1632	22.5	82.7	Sandy loam	5.51	6.81
Aguilayoc	1400	24.2	85.4	Sandy clay loam	5.42	6.11
Huayanay Center 02	1642	28.3	84.4	Sandy loam	5.22	3.33

* T: Average temperature per sampling zone, SOM: organic matter.

3.2. Identification of Phytoparasitic Nematodes

Three genera of phytoparasitic nematodes of importance for coffee cultivation in var. Typica were identified, *Pratylenchus* spp., *Helicotylenchus* spp. and *Meloidogyne* spp., in addition to the following categories: *Trichodoridae* spp., *Dorylaimidae* spp., *Rhabditides* spp., *Mononchidae* spp. and *Criconematides* spp.

A detailed study of the genus *Meloidogyne* resulted in the identification of the species *Meloidogyne exigua* (Figure 1). The morphological characteristics of females include a rounded to hexagonal perineal pattern, with a dorsal arch that can vary from low to rounded to slightly high and quadrangular. The lateral fields are inconspicuous, and some striae near the anus are curved and thick. In some patterns, transverse striae can be observed in the lateroanal region. As for the males, it was observed that they present vermiform bodies without folds and a rounded to blunt posterior end. The cephalic region is sclerotized, and both the cephalic region and the corpus anuli are delineated in the same way. The cephalic region is ringless, and the labial disc and median lips fuse to form a continuous low cephalic capsule with a shallow groove in the middle (Figure 1).

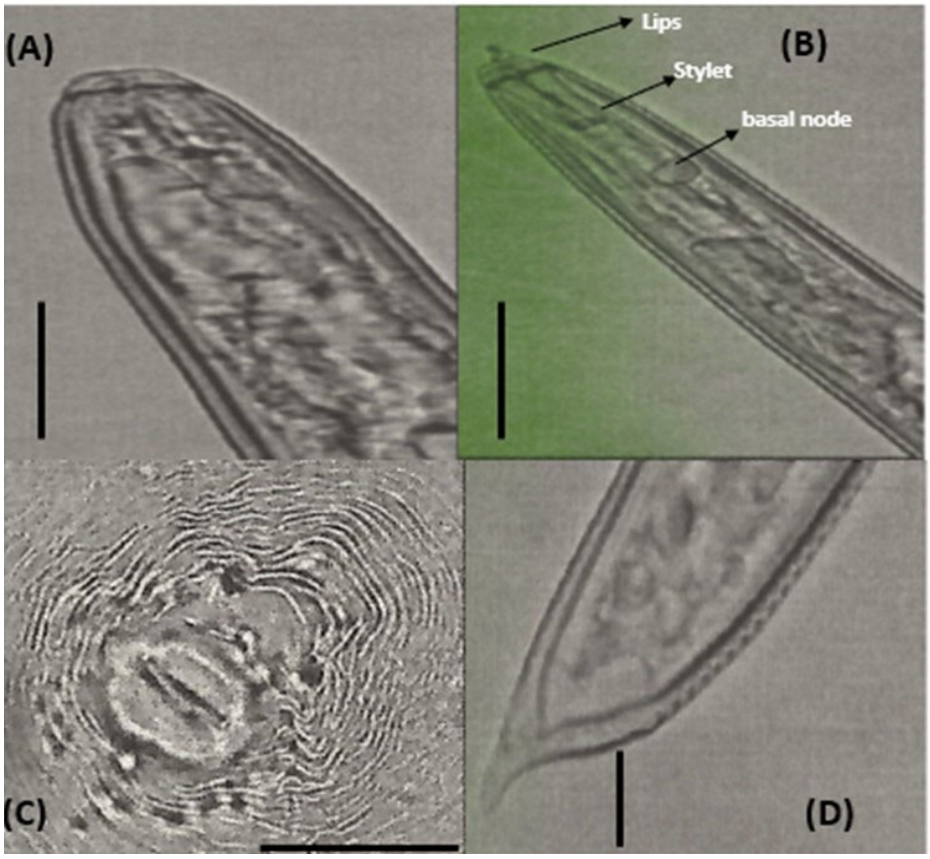

Figure 1. Morphological characterization of *Meloidogyne exigua* (**A**) anterior region of a male, (**B**) anterior region of second stage juvenile, highlighting the stylet and mouth, (**C**) perineal pattern of females and (**D**) posterior region of third instar juveniles (bar = 20 µm).

Regarding the genus *Pratylenchus*, the identification of females of this genus with a labial region was observed during the two-year study, which allows an easy identification of the species (Figure 2). The observed population mainly composed of parthenogenetic individuals, and only one male was found within this population. The tail of the species presents mainly a truncated shape, although some individuals with hemispherical and smooth tail ends were also observed. The position of the vulva was measured with respect to the total body length and was found to be in the range of 76.5–81.7%. The stipe length was measured to be 17.05 mm. From the results obtained, it is concluded that the species studied is *Pratylenchus coffeae* (Figure 2).

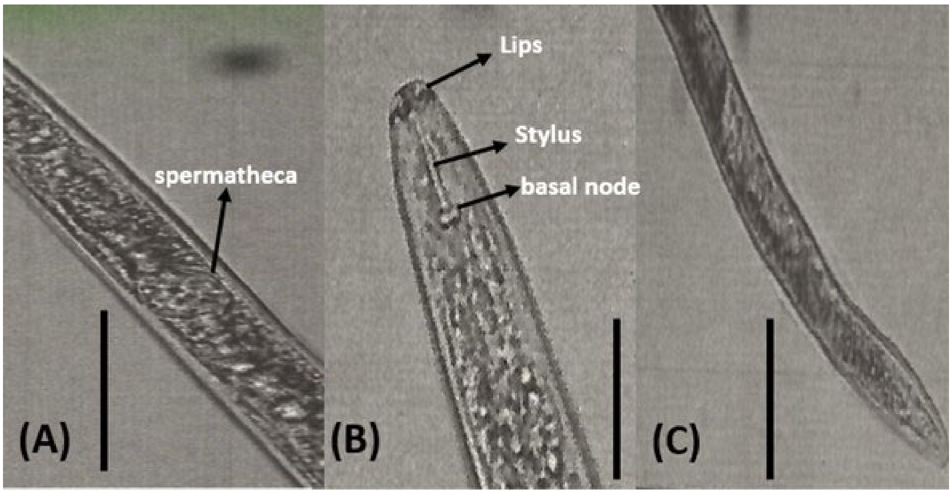

Figure 2. Morphological characterization of *Pratylenchus coffeae*: (**A**) spermatheca, highlighting its structure and location within the female nematode; (**B**) anterior region of the female, highlighting the stylet and mouth; (**C**) posterior region of the female tail (bar = 20 µm).

The species *Helicotylenchus longicaudatus* was also identified. It was observed that this species presents a tail with a long ventral projection, with an average c′ of 1.8. In addition, it was not possible to distinguish the presence of functional spermatheca, suggesting that this structure is not functional in this species. As for the labial region of the females, it was found to be smooth. These characteristics are congruent with those of the species *H. longicaudatus* (Figure 3).

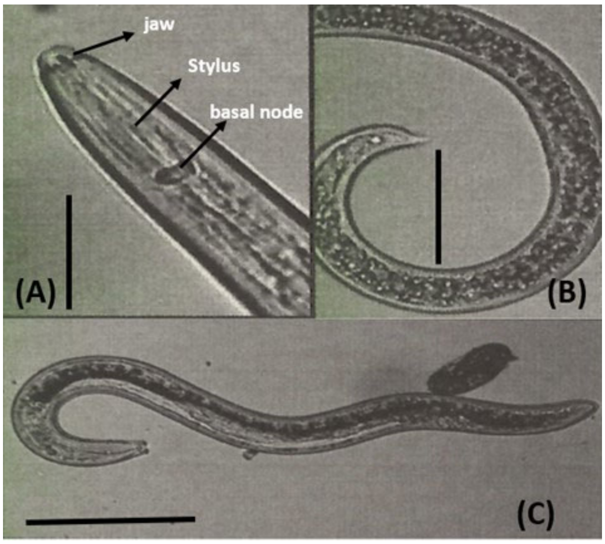

Figure 3. Morphological characterization of *Helicotylenchus longicaudatus*: (**A**) anterior region of a male, showing the stylet and mouth with flattened lips; (**B**) posterior region of the male, exhibiting the tail with anal opening and a ventral projection; (**C**) complete female (bar = 20 µm).

3.3. Incidence of Phytoparasitic Nematodes in the Soil

The incidence of phytoparasitic nematodes in soils of *C. arabica* L. var. Typica within eight different areas is shown in Table 3. Three genera of phytoparasitic nematodes were observed: *Meloidogyne* spp., *Helicotylenchus* spp. and *Pratylenchus* spp. The genus *Meloidogyne* spp. was found to be the most prevalent in all areas, especially in the Aguilayoc zone, which had the highest incidence (77.3%), followed by the Huayanay Centro 02 zone, with 74%. The presence of other categories of nematodes such as *Dorylaimidae* spp., *Trichodoridae* spp., *Mononchidae* spp., *Rhabditidae* spp. and *Criconematidae* spp. was observed in different proportions in each zone (Table 3). Although the genera *Meloidogyne* spp. and Helicotylenchus spp. did not show high incidence, their presence in the evaluated areas is important and should be considered in phytosanitary management decisions for coffee cultivation. In particular, the incidence of *Dorylaimidae* spp. was the genera that showed the second highest distribution whenever *Meloidogyne* spp. were present, suggesting that this group of nematodes could be an important factor in the plant–nematode interaction in these areas. Therefore, future studies should consider the detailed evaluation of the biology and ecology of these groups of phytoparasitic nematodes, in order to provide more complete and accurate information for the implementation of effective phytosanitary management strategies in coffee crops.

Table 3. Population of phytoparasitic nematodes by genus, identified in 100 g of soil according to the study areas.

Genre	**Huayanay High 1**		**Huayanay High 2**		**Huayanay Center 1**		**Huayanay Center 2**		**Huayanay**		**Quebrada Honda**		**Aguilayoc**		**Stockade**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Pratylenchus spp.	20	11.1	-	-	-	-	-	-	-	-	-	-	10	2.3	-	-
Trichodorides spp.	40	22.3	20	5	20	7.6	-	-	30	11.1	-	-	-	-	-	-
Dorylaimidae spp.	100	55.5	140	35	100	38.4	-	-	60	22.2	80	30.8	80	18.2	60	42.8
Rhabditidae spp.	20	11.1	20	5	-	-	20	7.4	40	14.8	-	-	-	-	20	14.4
Meloidogyne spp.	-	-	160	40	120	46.4	200	74	120	44.4	120	46.2	340	77.3	60	42.8
Mononchids spp.	-	-	60	15	-	-	10	3.7	-	-	-	-	-	-	-	-
Crichonematides spp.	-	-	-	-	20	7.6	-	-	-	-	20	7.6	-	-	-	-
Helicotylenchus spp.	-	-	-	-	-	-	40	14.9	20	7.5	40	15.4	10	2.2	-	-
TOTAL	180	100	400	100	260	100	270	100	270	100	260	100	440	100	140	100

3.4. Incidence of Phytoparasitic Nematodes on Roots

The incidence of phytoparasitic nematodes in coffee roots was estimated and is shown in (Table 4). The same three genera of nematodes that were identified in soil were recognized as: *Meloidogyne* spp., *Pratylenchus* spp. and *Helicotylenchus* spp. (Table 3). In addition, only one category *Rhabditidae* spp. was identified as opposed to the five that were detected in soil samples. The results revealed that among the populations studied, the genus *Meloidogyne* spp., which possesses the highest roots (Table 4), was present in 100% of the collection sites. The incidence of this genera showed a high prevalence (99.1%) in Aguilayoc, followed by the Quebrada Honda region, with a high incidence of 96%, suggesting that these locations may require additional control measures to prevent the infestation of coffee roots. The second most prevalent genus of phytoparasitic nematodes was *Pratylenchus* spp., which showed the highest incidence in Huayanay Alto 01 (34.3%), followed by Huayanay Centro 02 with 15.6%. A third genus, *Helicotylenchus* spp., was found only in Quebrada Honda, with a low incidence (4%) (Table 4). Among the categories of phytoparasitic nematodes, only the category *Rhabditidae* spp. was found, with a high incidence in Huayanay Alto 01 (48.6%) and present in six of the eight sites sampled.

Table 4. Nematode population by genus, identified in 1 g of root according to the study areas.

Genre	Huayanay High 1		Huayanay High 2		Huayanay Center 1		Huayanay Center 2		Huayanay		Quebrada Honda		Aguilayoc		Stockade	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Pratylenchus spp.	24	34.3	14	5.9	-	-	20	15.6	-	-	-	-	2	0.9	8	3.9
Rhabditidae spp.	34	48.6	36	15.3	12	13.1	38	29.6	22	5.6	-	-	-	-	26	12.9
Meloidogyne spp.	12	17.1	186	78.8	80	86.9	70	54.8	368	94.4	96	96	230	99.1	168	83.2
Helicotylenchus spp.	-	-	-	-	-	-	-	-	-	-	4	4	-	-	-	-
TOTAL	70	100	236	100	92	100	128	100	390	100	100	100	232	100	202	100

3.5. PCA Analysis

Principal component analysis (PCA) was performed to examine the relationship between different ecophysiological factors related to coffee cultivation and the proliferation of nematodes in the soil and plant roots (Figure 4). Ten different variables were used for this analysis, including geographic region, the presence of phytoparasitic nematodes, altitude, nematode density in soil and roots, soil texture, soil pH, soil organic matter, temperature and humidity.

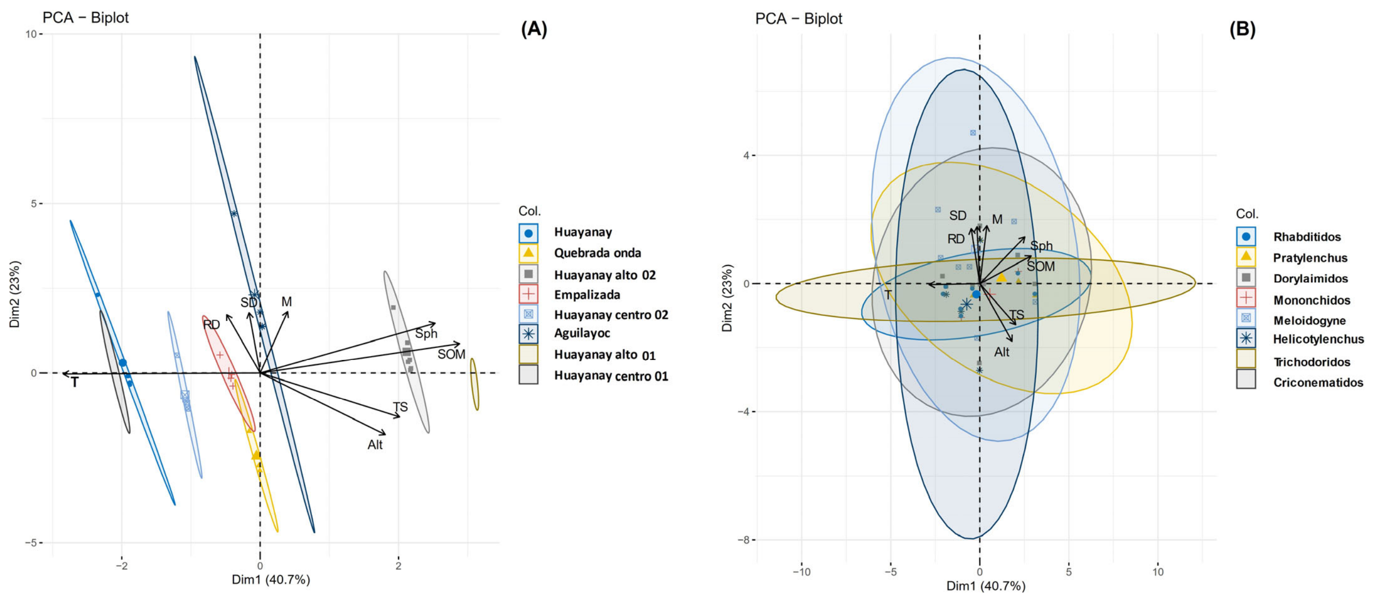

Figure 4. Graphical representation of the result of a principal component analysis obtained by comparing geographical regions (**A**) and phytoparasitic nematodes' genera and category (**B**) when considering values of eight variables for coffee cultivation. Variables evaluated: Altitude above sea level (Alt), density of phytoparasitic nematodes in the soil (SD), density of phytoparasitic nematodes in the root (RD), soil texture (TS), soil pH (Sph), soil organic matter (SOM), zone temperature (T) and zone humidity (M).

The PCA shows that 63.7% of the variability of all data is retained in the first two components (Figure 4). When considering the distribution of the eight collection sites (Figure 4A), it is observed that there is a large variability among them and that they are associated with the characteristics evaluated. Observing the figure, we can affirm that SD, RD and M are associated with the Aguilayoc site (Figure 4A), while Huayanay Alto 02 is associated with Sph and SOM. Associated with the zone temperature are Huayanay Alto 01 and Huayanay. This association observed in Figure 4A is what determines the behavior in each of the sampled sites.

When considering the eight phytoparasitic nematodes' genera and category that were identified and characterized, the degree of their association is shown in Figure 4B. There is an overlap between all the phytoparasitic nematodes' genera and category in relation to the evaluated variables, which shows that the evaluated parameters are determinant in the dissemination of most of the described genera and category (Figure 4B).

3.6. Pearson Correlation Analysis

Pearson correlation coefficients were estimated based on the eight characteristics evaluated in coffee (Figure 5). The highest coefficient value was obtained for the variables: SpH × SOM (0.93, $p < 0.001$), SMO × T (−0.91, $p < 0.001$), SpH × T (−0.74, $p < 0.001$) and TS × Alt (0.67, $p < 0.001$). Altitude correlated positively with SOM (0.35, $p < 0.05$) and negatively with T (−0.50, $p < 0.01$), and it manifested low correlations with the other variables (Figure 6). This suggests that climatic and edaphic conditions at higher altitudes may be more favorable for nematode growth and reproduction in the soil. Furthermore, this correlation may be an indication that environmental factors that influence altitude, such as temperature and organic matter content, may be favoring nematode growth.

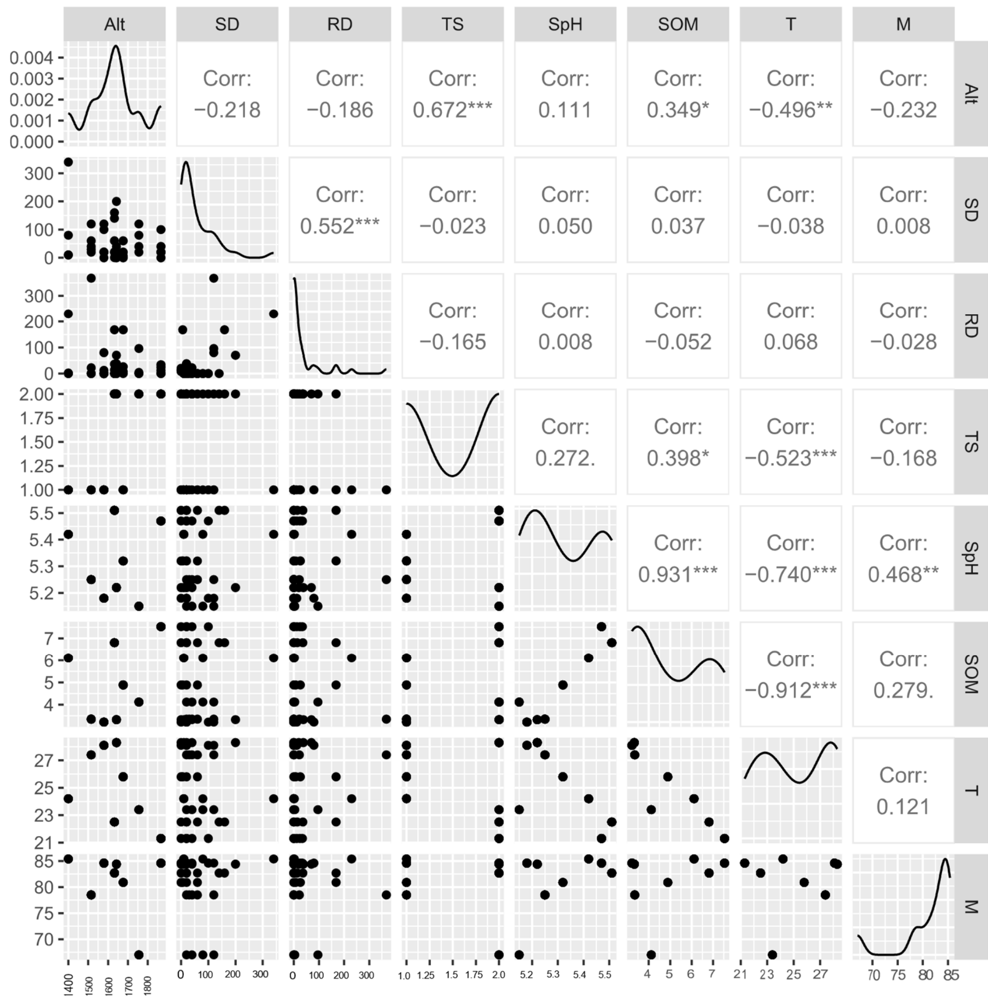

Figure 5. Pearson correlation and scatter plot obtained when comparing eight characteristics evaluated in coffee var. Typica: altitude above sea level (Alt), density of phytoparasitic nematodes in the soil (SD), density of phytoparasitic nematodes in the root (RD), soil texture (TS), soil pH (Sph), soil organic matter (SOM), temperature of the zone (T) and humidity of the zone (M). Correlation values with symbols *, ** and *** represent significant differences at 0.5, 0.1 and 0.01%, respectively.

SD showed moderate (0.55) and highly significant ($p < 0.001$) correlations only with RD, evidencing that there is a high chance that whenever a density of nematodes is found in the soil, they are present in the roots as well. TS was positively correlated with SOM (0.40, $p < 0.05$) and was highly significant, but of negative magnitude with T (−0.52, $p < 0.001$). For SpH, apart from the variables that manifested high correlations (Figure 5), M also exerted an effect on this variable (0.47, $p < 0.01$).

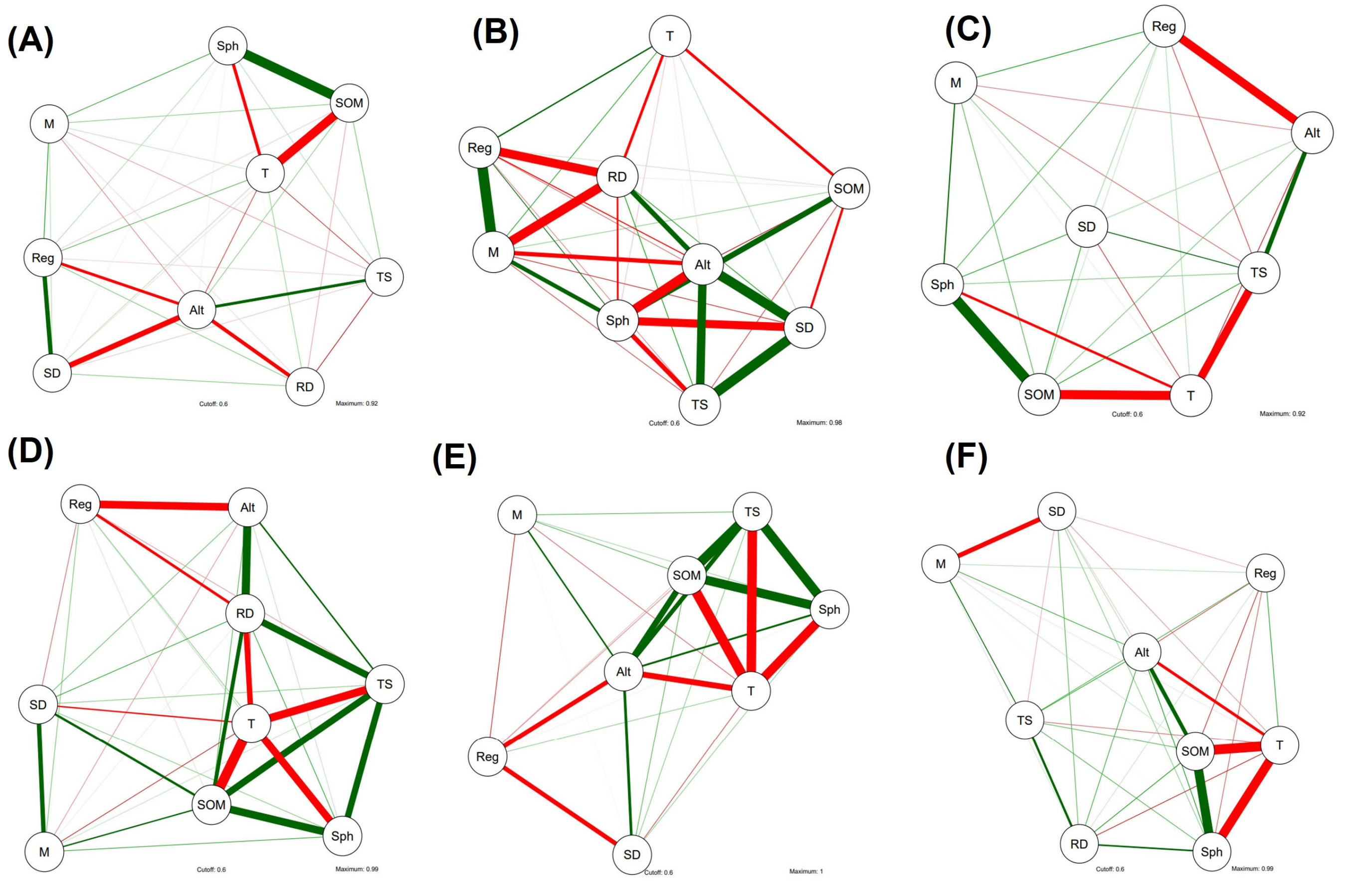

Figure 6. Correlation network obtained by establishing Pearson corrections: region (Reg), altitude above sea level (Alt), density of phytoparasitic nematodes in soil (SD), density of phytoparasitic nematodes in roots (RD), soil texture (TS), soil pH (Sph), soil organic matter (SOM), temperature of the area (T) and humidity of the area (M). The different networks obtained are in relation to the phytoparasitic nematodes genera and categories: (**A**) *Meloidogyne*, (**B**) *Helicotylenchus*, (**C**) *Dorylaimidos*, (**D**) *Pratylenchus*, (**E**) *Trichodoridos* and (**F**) *Rhabditidos*. The thickness of the line between thicker, closer to a correlation value of 1 or −1, depending on the color. Red and green lines represent negative and positive correlations, respectively.

3.7. Correlation Networks Established for the Different Nematodes Described

To verify the interaction of each of the nematodes described in this study in relation to the quantified variables, correlation networks were carried out and are shown in Figure 6. A total of the eight phytoparasitic nematodes described in this work, the category *Criconematidos* spp. and that of *Mononchidos* spp. were discarded for this analysis because they presented only two pieces of information in each of the evaluated variables. For the genera *Meloidogyne* spp., individuals were found in the eight sampled zones. High positive correlations were obtained between SpH and SOM, Reg and SD, Alt and TS, while high negative correlations were found when evaluating the variables SOM and T, Reg and Alt, Alt and SD, and Alt and RD (Figure 6A).

For the genera *Helicotylenchus* spp., the current study was able to identify individuals within this genera in four sampled areas, which showed high positive correlations ($p > 0.6$) when considering the following pairs of variables: Reg and M; Alt and SD; Alt and TS; Alt and SOM; Alt and RD; and Sph and M (Figure 6B). These results show that Alt was a determinant factor in the manifestation of this genera if we consider that the variation of this variable alters at the same time of the nematode densities (SD and RD), the soil texture (TS) and the organic matter content (SOM).

For the category *Dorylaimidos* spp., the study allowed the identification of individuals in seven zones, which showed high positive correlations ($p > 0.6$) when only the combination of the variables Alt and TS, and Sph and SOM were considered (Figure 6C). Negative ($R > 0.6$) and significant correlations for this category were obtained when combining: Alt and Reg; T and TS; and T and SOM. For the genus *Pratylenchus* spp., the study allowed the identification of four zones, which showed high positive correlations ($R > 0.6$) when considering the combination of the variables: Alt and RD; RD and TS; TS and SOM; TS and SOM; TS and Sph; Sph and SOM; SD and SOM; and SD and M (Figure 6D). Negative ($R > 0.6$) and significant correlations for this genera were obtained when combining: T and RD; T and TS, T and Sph; T and SOM; and Reg and Alt (Figure 6D). For the category *Trichodoridos* spp., the study allowed the identification of four zones, which showed high positive correlations ($R > 0.6$) when considering the combination of the variables: TS and Sph; TS and SOM; TS and Alt; and SOM and Sph (Figure 6E). Negative ($R > 0.6$) and significant correlations for this genera were obtained when combining: TS and T; T and SOM, T and Sph; T and Alt; Reg and Alt; and Reg and SD (Figure 6E). For the category *Rhabditidos* spp., the study allowed the identification of seven zones, which showed high positive correlations ($R > 0.6$) when considering the combination of the variables: SOM and Sph; and Alt and SOM (Figure 6F). Negative ($R > 0.6$) and significant correlations for this category were obtained when combining T and Sph; T and SOM, T and Alt; and M and SD (Figure 6F). These results show that for each of the described genera and category, a different interaction pattern was obtained, which determines their distribution and pathogenicity in the evaluated pigtail sites.

4. Discussion

In recent years, significant losses in coffee yields have been recorded and farmers have been forced to rotate and replace this cultivar (Typica) with other crops in Quillabamba [6,7]. The wide susceptibility of this variety to pests and diseases is the main motivation for the development of the present research where the results indicate a high degree of incidence of phytoparasitic nematodes [1,10]. Nematode damage reduces coffee production and is often only detected when the coffee plant is in an advanced stage of the disease, making it difficult to manage. This type of damage hinders the possibility of identifying the causal agent and initiating its control, often associated with latent infections, making management difficult for farmers who are unable to recognize them with the naked eye alone [37].

According to our findings in the current investigation, eight and four different genera of nematodes were found to be associated with soil and root samples, respectively, within eight regions of Quillabamba-Cusco. Among the genera, the genus *Meloidogyne* was the most present (detected in most of the collection sites), regardless of the type of sample (soil or root). Nematodes of the genus *Meloidogyne* are commonly found in coffee-growing soils worldwide [37–40]. This is because *Meloidogyne* spp. has a wide capacity to adapt to different environments, such as those described in the present work, where the regions in which it was detected vary in terms of altitude above or at sea level, soil texture, temperature, humidity, pH and organic matter content (Table 2). In addition to this genus, the phytoparasitic nematodes *Helicotylenchus* and *Pratylenchus* were also detected in smaller proportions, distributed in the different regions of Quillabamba-Cusco, coinciding with accounts that have previously reported on the coffee-cultivated soils where these genera were found [41,42].

Other groups of nematodes were found in this study, with emphasis on the presence of the category *Dorylaimidae* spp. found only in the soil samples of all the areas evaluated. Some authors point out that nematodes of this category may be important for the biological control of other phytoparasitic nematodes, such as *Meloidogyne* spp. [43,44]. The presence of this category *Dorylaimidae* spp., which is always associated with the presence of *Meloidogyne* spp., may also be a confirmation of the activity it exerts as a biological control, present in the soil of seven of the eight locations evaluated in soil samples for the cultivation of coffee cv. Typica, which could have implications for the phytosanitary management of plantations in future work.

The associations that were observed within the PCA (Figure 4) and correlation network (Figure 5) studies revealed that there are local ecological factors affecting the proliferation of nematodes, thus determining the occurrence of some species in some locations and their absence in others. Several authors have pointed out the importance of ecological interactions in the proliferation of phytoparasitic nematodes in agricultural soils [45,46]. This interaction is influenced by factors such as soil chemical composition, moisture, temperature, nematode population density, etc. [14]. In coffee production areas, different agricultural management practices such as crop rotation and the application of biological products based on microorganisms that control nematodes [47–50] can affect the abundance and distribution of nematodes in the soil [51].

When evaluated for the presence of phytoparasitic nematodes in the roots, it was found that as in the soil, the genus *Meloidogyne* spp. was the most prevalent in coffee roots, with the highest average population (Table 4). This result agrees with the findings of Kushwaha et al. [52], Pereira et al. [53] and Vieira et al. [54], who also found *Meloidogyne* spp. as the most distributed genus when characterizing coffee-growing soils worldwide. The high levels of *Meloidogyne* spp. in the Quebrada Honda (96%) and Huayanay (94.4%) regions suggest that additional control measures are needed to reduce or rectify the existing infestation in coffee roots, as suggested by Rajendran and Ojiambo [55] and Ribeiro et al. [56] in previous studies. The phytoparasitic nematode *Pratylenchus* spp. was the second most prevalent genus, with high incidence in Huayanay Alto (34.3%) and Huayanay Centro 02 (15.6%). A third genus identified in root samples was *Helicotylenchus* spp., which was found in only one collection site (Quebrada Honda) with a low incidence of only 4% (Table 4). Although a high prevalence of *Helicotylenchus* spp. was not observed, it is important to note that these nematodes can also cause significant damage to the roots of coffee plants, as described by Zhu et al. [57].

Within the categories described in this work, the significant distribution of phytoparasitic nematodes *Rhabditidae* spp. can be highlighted, with a high incidence in Huayanay Alto 01 (48.6%) and a similar incidence in Huayanay Centro 2 (29.6%), despite its presence in only six of the eight locations evaluated (Table 4). The results suggest that farmers should implement control measures to reduce the incidence of these nematodes and prevent damage to coffee plant roots, as recommended by Gutiérrez-Gutiérrez et al. [58] and Molina-Bravo et al. [59].

The relationship between ecological factors and the distribution of phytoparasitic nematodes in coffee (*Coffea arabica* L. var. Typica)-growing areas highlight the importance of understanding ecological interactions (Figure 6) and biodiversity in these areas (Table 2). Temperature, humidity, soil texture and soil pH are key factors that can influence the composition and structure of the community of microorganisms present in the soil that favor or harm plants and the availability of nutrients for the different organisms in the ecosystem. The results of the study indicate that areas with lower temperatures and higher humidity (High Huayanay) are conducive to the development and propagation of phytoparasitic nematodes in the soil, which can affect the availability of nutrients for plants by the damage they promote in the roots and in the assimilation of nutrients, determining the productivity of these plants in these ecosystems. However, the presence of a high level of organic matter in the soil (Table 2) could indicate a lower presence of phytoparasitic nematodes in some regions. Likewise, it was observed that nematode density decreases with increasing altitude (Figure 6), which could be related to the average temperature and humidity in each region (Table 2). Moreover, it was also found that for each of the nematodes described, there is a change in the relationship between the variables evaluated when observing the correlations (Figure 5), which may determine their density in soils and roots. These findings are important and constitute the basis for understanding and improving the necessary management of the natural resources and the conservation of biodiversity in coffee-producing areas [60,61].

Within the characterization that was performed in the eight coffee var. Typica-producing zones, soil texture was one of the variables evaluated. Previous studies have shown that soil texture determines the presence of phytoparasitic nematodes [62]. Clay loam soils have a higher capacity to retain water and nutrients, which may favor plant growth and, therefore, the presence of phytoparasitic nematodes [63]. On the other hand, sandy loam soils are more porous and allow better drainage, which can reduce the presence of phytoparasitic nematodes [62]. The presence and proliferation of phytoparasitic nematodes in the soils of the regions studied are influenced by various ecological factors, which, when linked to soil texture, can determine their ability to promote damage to coffee. Knowing and managing these factors is important in order to design integrated pest management strategies in the region that allow for a greater production of the culture in these regions.

Pearson correlations (Figure 6) suggest negative correlations between altitude and soil nematode density, contradicting findings from other previous studies. For example, Alfaro-Lucas et al. [64] found that areas with lower temperatures and higher humidity are conducive to the development and spread of phytoparasitic nematodes in soil. Similarly Molinari et al. [65] found a higher nematode diversity in low-altitude coffee fields in Brazil, while higher-altitude fields had a lower nematode diversity. A high positive (0.93) and significant ($p < 0.001$) correlation between soil pH and soil organic matter content (Figure 6) was found in the present study. Other previous studies have found that acidic soils are usually richer in organic matter due to a decreased microbial activity, leading to an accumulation of soil organic matter [66,67]. Considering our results, the soils of Quillabamba-Cusco manifest, in a general way, a behavior contrary to what has been reported in relation to the combination of ph and organic matter content. The positive correlation (0.52, $p < 0.001$) between root nematode density and soil nematode density (Figure 6) is also consistent with the existing literature. Other studies have shown that nematode density in soil can affect nematode density in plant roots [68,69].

The multivariate analyses that were performed to better interpret the data obtained show that there is a clear relationship between the density of nematodes in the soil and in the roots, together with the different factors being analyzed (Figures 4–6). Within the factors, a stronger correlation was evidenced between nematode density in the soil with soil texture (*Helicotylenchus*, Figure 6B) and soil organic matter (*Helicotylenchus*, Figure 6B and *Pratylenchus*, Figure 6D). The factors that have the strongest correlation with root nematode density are soil moisture (*Helicotylenchus*, Figure 6B) and temperature (*Helicotylenchus*, Figure 6B, *Pratylenchus*, Figure 6D and *Rhabditidos*, Figure 6F). If we consider that the soil texture and soil organic matter have a significant correlation with soil nematode density, producers can implement agricultural practices that increase soil organic matter and, therefore, reduce nematode density [70]. Furthermore, if soil moisture and temperature are correlated with nematode density in roots, as evidenced by the results of this work, growers can also consider measures such as irrigation management and the use of mulches to maintain moisture at adequate levels in the soil, as already reported by Bebber et al. [71].

These results are consistent with the findings of other studies and support the idea that several environmental factors, including altitude, soil pH, organic matter, soil nematode density and soil moisture, influence the distribution of phytoparasitic nematodes in coffee production systems, and that there is a differential response in relation to each of the genus described. However, it is important to keep in mind that these results provide general knowledge, and more detailed studies are needed to fully understand the ecological interactions that influence the presence of nematodes in the soil and on coffee plants.

In addition, several studies have consistently shown that the presence of plant-parasitic nematodes in coffee-growing regions can cause significant economic losses. According to the study by Melakeberhan et al. [72], these organisms have the ability to drastically reduce coffee production by negatively affecting the health and yield of the plants. Plant-parasitic nematodes feed on the roots of coffee plants, causing general weakening, a decreased ability to absorb nutrients and water, as well as visible symptoms such as wilting and limited growth [73]. These negative effects on coffee production can have a direct impact on the income of producers and on the local economy of coffee-growing regions, as indicated by Eskes and Engels [74] in their study on the economic impact of phytoparasitic nematodes in the coffee industry. In this sense, the proper management of the presence of plant-parasitic nematodes is crucial to minimize economic losses and guarantee the sustainability of coffee production. Authors such as Coyne et al. [9] have proposed the use of integrated pest management practices such as crop rotation, the selection of resistant varieties and biological control as effective strategies to reduce the incidence of plant-parasitic nematodes in coffee plantations.

5. Conclusions

The study conducted in eight producing areas of *Coffea arabica* L. var. Typica identified three genera of phytoparasitic nematodes, with *Meloidogyne* spp. being the most prevalent in soil and roots. The density of nematodes in soil and plant roots was influenced by several factors, such as organic matter, soil texture, humidity, temperature and altitude, and their understanding is essential for successful coffee production. The results of this study may be useful for farmers and researchers in developing more effective and sustainable strategies for managing nematodes and improving coffee quality. Further studies are needed to better understand the ecological interactions that influence the proliferation of phytoparasitic nematodes and the means to mitigate their negative effects on coffee production.

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