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Plant-parasitic nematodes associated with maize, and their relationship with soil properties in southern and southwestern Ethiopia

Abebe W. ASEFFA [1], Beira H. MERESSA [1], Tadele TEFERA [2], Kristina K. GREEN [3], Peter ANDERSON [3,*] and Esayas MENDESIL [1,*]

[1] *College of Agriculture & Veterinary Medicine, Jimma University, P.O. Box 307, Jimma, Ethiopia*
[2] *International Center of Insect Physiology & Ecology (ICIPE), P.O. Box 5689, Addis Ababa, Ethiopia*
[3] *Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Box 190, 234 22 Lomma, Sweden*
ORCID iDs: Aseffa: 0000-0003-1408-1262; Meressa: 0000-0001-9554-5393; Tefera: 0000-0001-6373-0842; Green: 0000-0002-1820-2036; Anderson: 0000-0003-4105-8236; Mendesil: 0000-0002-9915-643X

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Summary – Maize is an important cereal crop in sub-Saharan Africa (SSA). Plant-parasitic nematodes significantly impact maize production. However, research on maize nematodes in SSA is limited. The current study aimed to assess the occurrences and densities of nematodes associated with maize and to assess the correlation of some soil physicochemical properties with nematodes in southern and southwestern Ethiopia. One hundred and eighty-six soil and root samples were collected from 62 maize fields (31 from Hawassa Zuria and 31 from Mana districts). Eleven plant-parasitic nematode genera were identified. The most important genera of plant-parasitic nematodes identified were *Meloidogyne* and *Pratylenchus*, followed by *Helicotylenchus* and *Tylenchorhynchus*. The nematodes *Pratylenchus* and *Criconemoides* were significantly and positively associated with pH, soil organic carbon (SOC) and soil organic matter (SOM). Additionally, *Tylenchorhynchus*, *Pratylenchus* and *Rotylenchulus* were significantly positively correlated with soil available phosphorus (SAP). However, *Criconemoides* and *Meloidogyne* had a strong negative correlation with soil bulk density (SBD). This study provides useful baseline information on maize-associated nematodes, and will be useful for agricultural policy makers, private farmers, non-governmental organisations and agricultural extension workers enabling them to establish sustainable maize nematode management strategies.

Keywords – correlation, density, frequency, *Helicotylenchus*, maize, *Meloidogyne*, *Pratylenchus*, soil physicochemical properties, *Tylenchorhynchus*, *Zea mays*.

Maize (*Zea mays* L.) is the most important cereal crop worldwide (Allie *et al.*, 2020). It plays an important role in the global economy and contributes the lion's share of world agriculture trade as a source of food, feed and various raw materials for industrial products (Abdulfeta & Aseged, 2021). According to USDA (2021), the main global maize producer countries are the USA, China, Brazil and Argentina, representing 64.63% of world production (Wang & Hu, 2021).

Maize is the most important staple cereal crop in sub-Saharan Africa (SSA) (Shiferaw *et al.*, 2011; Keba, 2020) and more than 300 million Africans depend on maize as their main staple food crop (IITA, 2022). It accounts for 30-50% of low-income household expenditures in Africa, and more than 30% of people's calorie intake in SSA comes from maize (IITA, 2022). Subsequently, several African countries that depend on maize as a staple food crop have adopted agricultural policies to maintain steady commodity supply through increased crop production and productivity (Shiferaw *et al.*, 2011; Smale *et al.*, 2011; IITA, 2022). For example, Ethiopia is Africa's fourth largest maize producer (CSA, 2018; Keba, 2020). Maize is the second most widely cultivated crop in the country under various agroecologies and socioeconomic

* Corresponding authors, e-mails: peter.anderson@slu.se; emendesil@yahoo.com

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conditions, largely under a rain-fed production system produced primarily by smallholder farms that account for more than 95% of the total area and maize production (Abate *et al*., 2015).

Although it is a strategic crop, fluctuation in maize yield is a common worldwide phenomenon attributed to many biotic and abiotic factors (Macauley & Ramadjita, 2015). Previous studies have shown that plant diseases and pests often affect maize cultivation, including those caused by soil-borne fungi, such as *Rhizoctonia solani*, *Fusarium* spp., and plant-parasitic nematodes (Soenartiningsih *et al*., 2015). Several major genera of plant-parasitic nematodes have been reported in association with maize agroecosystems. The most economically important are root-knot nematodes (*Meloidogyne* spp.) and lesion nematodes (*Pratylenchus* spp.), plus several others, such as spiral nematodes (*Helicotylenchus* and *Scutellonema*) and *Tylenchorhynchus* (Mc Donald *et al*., 2017). Global crop yield losses due to plant-parasitic nematodes are estimated to be 12.6% or US$ 216 billion annually (Nyaku *et al*., 2017). In SSA up to 50% maize yield loss caused by *Meloidogyne*s spp. had been reported (Coyne *et al*., 2018). Nematodes are influenced by soil physicochemical properties of soil pH, soil organic carbon, soil organic matter and soil available phosphorus (Villenave *et al*., 2013; Nisa *et al*., 2021).

In Africa, particularly in Ethiopia, limited research on nematodes associated with vegetables, ornamental cut flowers, and coffee has been carried out so far (Mekete *et al*., 2008; Meressa *et al*., 2014, 2018; Abebe *et al*., 2015; Aseffa *et al*., 2018; Singh *et al*., 2018, 2023; Meressa & Aseffa, 2022). However, research and information on cereal crop nematodes, particularly maize nematode, is lacking although the country is a potential maize producer in SSA. Therefore, this research assessed the occurrences and densities of nematodes associated with maize, and evaluated the relationship between soil properties and nematode densities in two districts in south and southwestern Ethiopia representing two different agroecologies.

Materials and methods

Description of the study area

The study was carried out during the year 2021-2022 in Hawassa Zuria of the Sidama region and Mana districts of the Jimma zone of the Oromia region in southern and southwestern Ethiopia, respectively (Fig. 1). The districts are among the main areas for maize production in the country, covering different agroecologies. Mana district (7°67′N, 37°7′E) is located at an altitude of 1400-2610 m a.s.l. The mean annual temperature and rainfall of the district are 20.5°C and 1525 mm, respectively, and the type of soil in the area is Nitisol with a pH of 4.5-5.5 (ARDO, 2008). The Hawassa Zuria district (6°56′16″ and 7°9′10″N latitude; 38°21′41″ and 38°24′15″E longitude) is located at an altitude of 1630-2000 m a.s.l. (NMA, 2017). Hawassa Zuria has a mean annual rainfall of 955 mm and a mean annual temperature of 20°C and the type of soil in the area is Nitisol (SZPED, 2004).

Hawassa Zuria is part of the Great East African Rift Valley, where the maize farms are open fields without forest remnants. The farming systems practised in the districts are predominantly maize mono-cropping; while some farmers also practise intercropping, mostly with haricot beans, cabbages and yam. However, Mana district is partly surrounded by East African Afromontane forests and is found in the Ethiopian highlands. The area is among the main coffee-producing districts in the country. Maize and teff are the main cereal crops grown in the area. The main varieties of maize grown in the districts include ‘BH660’, ‘BH661’, ‘Limmu’ and local varieties.

Selection of the site and sample collection

Site selection and soil sampling were carried out in farmers’ maize fields from June to October 2021. Sampling was done during the flowering stage of maize. Sixty-two maize farms (31 from Hawassa Zuria and 31 from Mana district) were selected for soil and root sampling. Three 5 m × 5 m plots were randomly assigned to each farm where samples were collected. From each plot, 20 soil cores were collected. Three composite soil samples, each weighing *ca* 500 g, and root samples, each weighing *ca* 200 g, were randomly collected from the maize rhizosphere of each farm at depths of 20-40 cm using a 3 cm diam. soil augur resulting in 362 samples (a total of 186 soil samples and 186 root samples). Geographic coordinates for each farm were recorded using Garmin GPS. The soil and root samples were sealed in a plastic bag and carefully transported to the Jimma University College of Agriculture and Veterinary Medicine Plant Disease Diagnostic Laboratory for the extraction of nematodes.

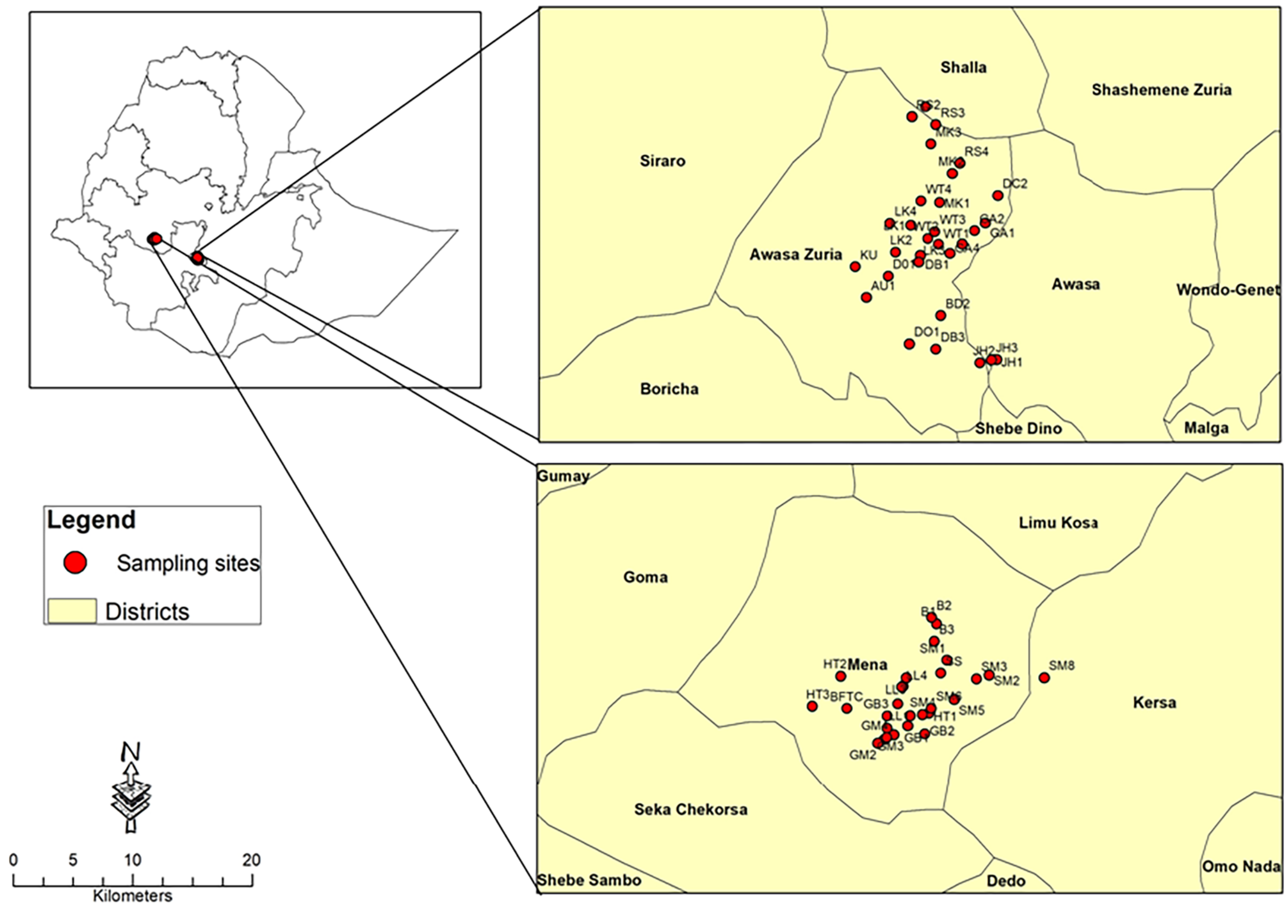

Fig. 1. Map of soil sampling sites in south and south western Ethiopia.

NEMATODE EXTRACTION FROM SOIL AND ROOT SAMPLES

Soil nematode extraction involved processing 100 ml of soil using the modified Baermann tray method. The setup was incubated at room temperature for 48 h (Hooper *et al.*, 2005). The nematodes were collected on a 20 μm aperture stainless steel sieve and transferred to a 100 ml plastic cup and stored in the refrigerator at 4-5°C until the nematodes could be identified to the genus level and enumerated. For root nematodes, a similar procedure was followed, where roots were carefully washed, and 30 g from each sample were chopped in to 1-2 cm pieces. These root pieces were then incubated for 72 h following the same protocol as for soil nematode extraction.

IDENTIFICATION AND QUANTIFICATION OF NEMATODES

The nematodes were identified and quantified from a 1 ml aliquot nematode suspensions under a light compound microscope (AmScope MU300) at 10-40× magnification using a nematode counting slide chamber. The identification was made based on nematode morphology (Hooper, 1990; Hunt *et al.*, 2005; Karssen & Moens, 2006).

SOIL PHYSICOCHEMICAL PROPERTY ANALYSIS

For soil physicochemical property analysis, soil pH, soil organic carbon (SOC), soil organic matter (SOM), soil available phosphorus (SAP) and soil bulk density (SBD) were measured for all maize fields. The pH of the soil was determined in a water suspension at 1:2.5 soil:liquid ratio (w/v) potentiometrically using a glasscalomel combination electrode (Van Reeuwijk, 1992). The Walkley & Black (1934) wet digestion method was used to determine the soil organic carbon content, and the percentage of soil organic matter was obtained by multiplying the percentage of soil organic carbon by a factor of 1.724 following the assumption that soil organic matter is composed of 58% carbon. The soil available phosphorus was determined using 1MHCl and 1M NH4F solutions as an extractant using a spectrophotometer at 882 nm following the method described by Van Reeuwijk (2002). Soil bulk density was determined by dividing the dry soil mass by its volume using a metal ring.

Data analysis

Data for the soil physicochemical variables, population density, and frequency of occurrence of nematodes were checked for normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests. The soil physicochemical variables were analysed untransformed because they met the data normality assumptions, while the population density of nematode was transformed using $\log_{10}(x)$. All descriptive statistics and data normality tests were performed using SPSS version 20 software. The population density (PD) of each nematode was calculated using the following formula: PD = (total number of nematodes $(100\ \text{ml soil})^{-1}$)/(number of positive samples). Frequency of occurrence (FO) for each nematode was calculated with the formula: FO = (number of positive samples containing nematode/number of collected samples) × 100. Prominence value (PV) for each nematode was calculated with the formula: PV = (nematode population density $\times \sqrt{\text{FO}})/10$(De Waele & Jordaan, 1988). Principal component analyses (PCA) and the Pearson correlation were performed using R Studio 2022.12.0 and run in R version 4.2.1. The nematode ecological diversities for Shannon-Weaver diversity index (H'), Evenness (J), Margalef richness (SR) and Dominance (D) were executed using PAST software (Hammer *et al.*, 2001).

Results

Occurrence and density of nematodes in maize rhizosphere and root samples

Plant-parasitic nematodes were ubiquitous across all the surveyed sites and all the soil samples, highlighting their presence in maize cultivation. Analysis of the data revealed the association of maize with 11 nematodes genera (Table 1). Results in Table 1 illustrate higher population densities of plant-parasitic nematodes, such as *Meloidogyne*, *Pratylenchus*, *Helicotylenchus* and *Tylenchorhynchus*, in the Hawassa Zuria district with respective densities of 585, 285, 190 and 157 indiv. $(100\ \text{ml soil})^{-1}$. These nematodes exhibited frequency of occurrences (FO) ranging from 43 to 100%, and prominence value (PV) ranging from 125 to 582. By contrast, the

Table 1. Population density (PD), frequency of occurrence (FO) and prominence value (PV) of nematodes $(100\ \text{ml soil})^{-1}$ and $(30\ \text{g root})^{-1}$ from Hawassa Zuria and Mana districts of Ethiopia.

Nematode genus	Nematode population density $(100\ \text{ml soil})^{-1}$						Nematode population density $(30\ \text{g root})^{-1}$					
	PD		FO(%)		PV		PD		FO(%)		PV	
	Hawassa Zuria	Mana	Hawassa Zuria	Mana	Hawassa Zuria	Mana	Hawassa Zuria	Mana	Hawassa Zuria	Mana	Hawassa Zuria	Mana
Pratylenchus	285	136	100	97	285	134	499	204	93	93	481	197
Meloidogyne	585	81	99	83	582	74	102	35	85	48	94	23
Tylenchorhynchus	157	48	92	39	151	30	–	–	–	–	–	–
Helicotylenchus	190	63	43	55	125	47	–	–	–	–	–	–
Scutellonema	14	234	5	79	3	208	–	–	–	–	–	–
Tylenchidae	33	65	39	79	21	58	–	–	–	–	–	–
Longidorus	42	30	27	30	22	16	–	–	–	–	–	–
Trichodorus	55	39	60	40	43	25	–	–	–	–	–	–
Rotylenchulus	72	70	76	77	63	61	–	–	–	–	–	–
Criconemoides	42	17	81	18	38	7	–	–	–	–	–	–
Xiphinema	19	26	20	23	8	12	–	–	–	–	–	–

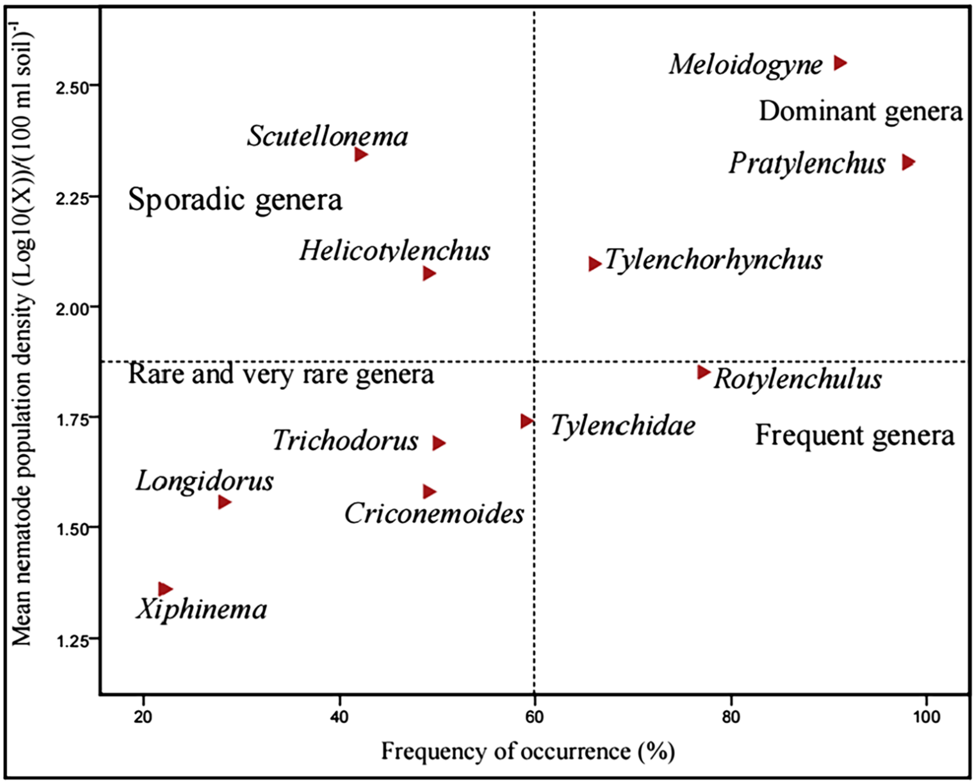

Fig. 2. The overall mean population density and frequency of occurrence of nematodes (100 ml soil)$^{-1}$ found in maize rhizosphere at Hawassa Zuria and Mana districts of Ethiopia.

Mana district displayed higher densities and occurrences of *Scutellonema* and *Pratylenchus* from the soil samples: 234 and 136 indiv. (100 ml soil)$^{-1}$; FO 79% and 97%; and PV 208 and 134, respectively. Other nematode genera encountered showed a low population density and occurrence. Of all the nematodes detected from overall soil samples, *Meloidogyne* was the most prominent followed by *Pratylenchus* and *Tylenchorhynchus* (Fig. 2).

The root samples contained two endoparasitic nematode genera, *Pratylenchus* and *Meloidogyne*. *Pratylenchus* was notably abundant in root samples from both districts with population densities of 499 and 204 indiv. (30 g root)$^{-1}$, compared with *Meloidogyne*, which exhibited lower densities of 102 and 35 indiv. (30 g root)$^{-1}$, respectively. The frequencies of occurrence of *Pratylenchus* in root samples were consistent across both districts, whereas the occurrence of *Meloidogyne* varied. Furthermore, *Pratylenchus* emerged as the more prominent nematode genus compared to *Meloidogyne* in the maize root samples (Table 1).

Relationship between soil physicochemical properties and nematodes

The relationship between physicochemical properties of the soil and the nematode genera was evaluated using PCA (Fig. 3). Results revealed that PC1 and PC2 accounted for 27 and 12.8% of the variations, respectively. The PC1 axis exhibited positive correlation with nematode genera such as *Criconemoides*, *Pratylenchus*, *Rotylenchulus*, *Tylenchorhynchus*, *Meloidogyne*, *Xiphinema* and *Trichodorus*, and to all soil physicochemical properties except SBD. Genera such as *Helicotylenchus*, *Scutellonema*, *Tylenchidae* and *Longidorus* were associated with negative PC values. The PC2 axis was positively related to genera such as *Scutellonema*, *Tylenchidae*, *Xiphinema* and *Trichodorus*, and soil physicochemical properties of SAP, SBD and pH. The negative PC value was related to genera such as *Meloidogyne*, *Criconemoides* and SOC, SOM. The nematodes *Pratylenchus*, *Tylenchorhynchus*, *Xiphinema* and *Trichodorus* were more associated with soil available phosphorus

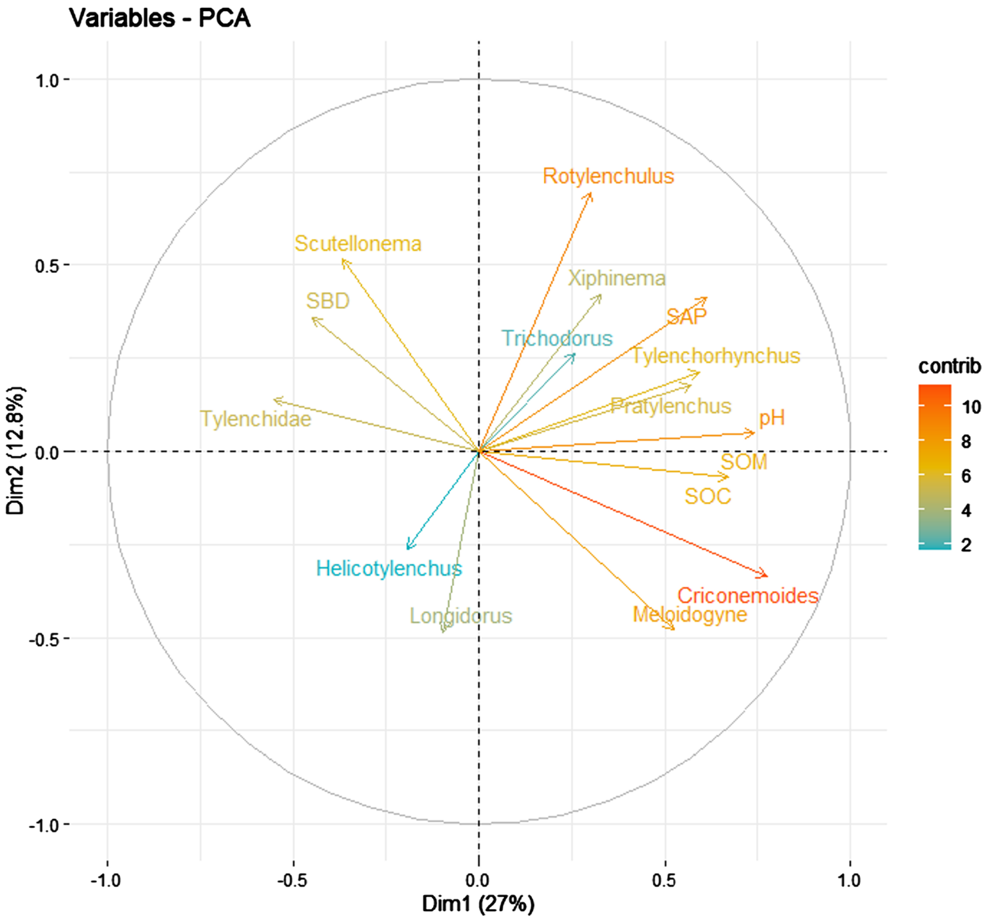

Fig. 3. Principal Component Analysis (PCA) of relationship between soil physicochemical properties (soil organic carbon (SOC); soil organic matter (SOM); soil available phosphorus (SAP); soil bulk density (SBD)) and nematode genera in maize rhizosphere at Hawassa Zuria and Mana districts of Ethiopia. Percentage of variance explained was 27% and 12% for PC1 and PC2, respectively.

(SAP) and pH. Remarkably, a robust association between SBD and *Scutellonema* was revealed. The significant relationship was rigorously evaluated through a correlation plot
(Fig. 4).

The relation between the nematodes and physicochemical properties of the soil was assessed using a correlation plot. Results showed a significant and positive correlation between *Pratylenchus* and *Criconemoides* with pH, SOC and SOM, indicating a strong association between these nematodes and the soil's chemical composition. The nematode *Tylenchorhynchus Pratylenchus* and *Rotylenchulus* showed a significant positive correlation with SAP. Moreover, *Scutellonema* and *Tylenchorhynchus* exhibited a significant positive correlation with SBD and pH, respectively. However, *Tylenchidae* showed a significant negative correlation with pH, SOC and SOM. Furthermore, *Criconemoides* and *Meloidogyne* had a strong negative correlation with SBD (Fig. 4).

Nematode community ecological indices

There were disparities in nematode ecological indices assessed across the two districts (Table 2). Notably, for individual nematodes, the minimum was observed in HT2 contrasting sharply with the maximum count in WT4, with 304 and 2934, respectively, in the Mana and Hawassa Zuria districts ($F_{1,60}$ = 15.7, P = 0.004). Moreover, dominance showed the highest mean observed

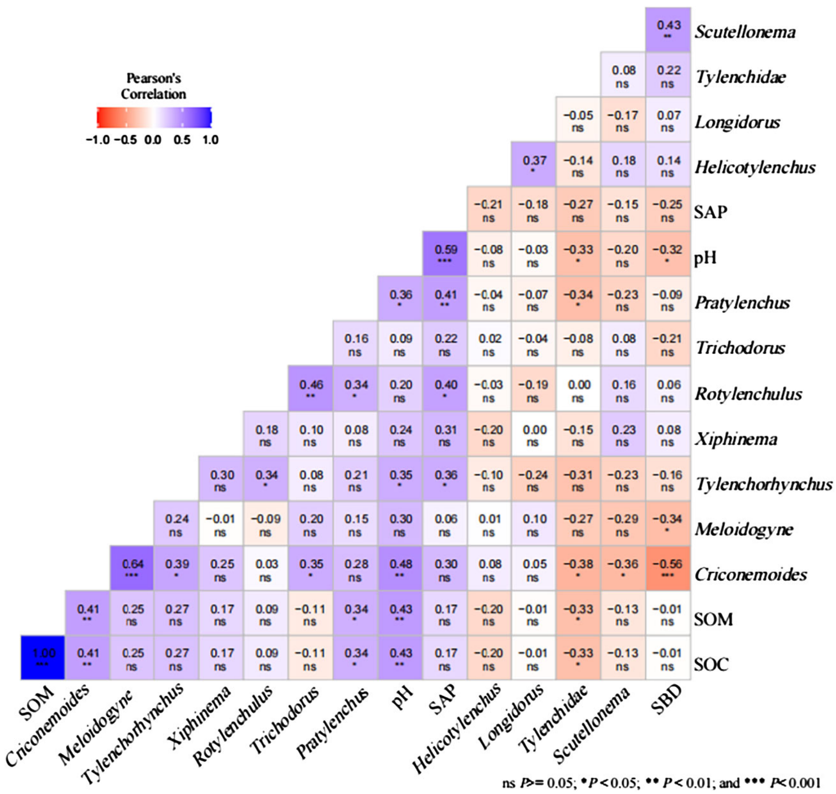

Fig. 4. Correlation of nematode genera with soil physicochemical properties and nematode genera in maize rhizosphere at Hawassa Zuria and Mana districts of Ethiopia. The scale bars on the top-left shows the significance level (strong positive correlation towards scale bar value of 1.0); ns = not significant.

Table 2. Ecological indices of nematode communities in surveyed soil samples of maize fields from the Hawassa Zuria and Mana districts of Ethiopia. Dominance (D), Shannon-Weaver diversity index (H'), Evenness (J) and Margalef richness (SR).

Diversity indices	Mana (mean ± SE)	Hawassa Zuria (mean ± SE)	P value	F
Number of taxa	(5 = Min, 11 = Max)	(5 = Min, 10 = Max)		
Individuals	618 ± 74^{b}	1276 ± 125^{a}	0.004	15.7
Dominance (D)	0.2 ± 0.01^{b}	0.4 ± 0.03^{a}	0.001	13.2
Shannon (H')	2.0 ± 0.04^{a}	1.4 ± 0.05^{b}	0.001	18.8
Evenness (J)	0.8 ± 0.02^{a}	0.5 ± 0.02^{b}	0.003	16.5
Margalef richness (SR)	1.1 ± 0.03^{a}	0.9 ± 0.03^{b}	0.001	12.8

Different letters (column) indicate significant differences among the districts, $P < 0.05$ (Tukey HSD test).

in the Hawassa Zuria district (0.33) ($F_{1,60} = 13.2$, $P = 0.001$). *Meloidogyne* exhibited the highest dominance at MK3, while *Xiphinema* at site GM2 showed the lowest dominance level. The mean Shannon-Weaver diversity indices (H') ($F_{1,60} = 18.8$, $P = 0.001$), Evenness (J) ($F_{1,60} = 16.5$, $P = 0.003$) and Richness ($F_{1,60} = 12.8$, $P = 0.001$) were higher in the Mana district compared with the other district.

Discussion

This study indicated considerable populations of plant-parasitic nematodes associated with maize. The pronounced occurrences and population densities of the endo-parasitic nematodes, *Pratylenchus* and *Meloidogyne*, may be attributable to the maize mono-cropping system practised in the study areas, and to the broad host range of these nematodes. This finding aligns with previous research indicating that mono-cropping agroecosystems facilitate the buildup of specific nematodes by providing favourable conditions and availability of specific resources (Yeates & Bongers, 1999; Kimenju *et al.*, 2009; Moura & Franzener, 2017). Whilst there have been no reported instances of maize damage caused by *Pratylenchus* and *Meloidogyne* in Ethiopia, previous studies have shown that maize is a suitable host for these two nematode genera (De Waele & Jordaan, 1988; Koenning *et al.*, 1999; Mueller *et al.*, 2016, 2020; Mc Donald *et al.*, 2017; Coyne *et al.*, 2018; Pretorius, 2018; Simon *et al.*, 2018; Allie *et al.*, 2020). Therefore, our study underscores the significance of *Pratylenchus* and *Meloidogyne* as major pests in maize production.

The study further showed that *Tylenchorhynchus* and *Helicotylenchus* had a high frequency of occurrence and population densities, indicating that they may cause damage to maize crops. This finding is consistent with previous studies by Mueller *et al.* (2016, 2020) and Mirsam *et al.* (2020), which documented that these nematode are responsible for yield losses in maize. On the other hand, *Xiphinema* and *Longidorus* had low occurrences and densities, implying that they may not be prevalent nematodes in maize fields. This finding corroborates previous studies by Atandi *et al.* (2017) and Sikandar *et al.* (2021) that also reported a low occurrence and abundance of *Longidorus* and *Xiphinema* in maize fields in Kenya. However, Mueller *et al.* (2016, 2020) reported significant maize yield loss due to *Longidorus*, indicating that the damage potential of these nematodes could vary depending on geographical locations and other factors.

Scutellonema was sporadically detected at high densities, suggesting it is unlikely to be a major pest in the study area. Kraus-Schmidt & Lewis (1979) also noted that cereals, such as maize and sorghum, are not typically hosts for this nematode. However, other studies such as Walters (1979) and De Waele & Jordaan (1988) found a high occurrences of *Scutellonema* in maize from South Africa, and a recent study in Kenya also indicated a high population of *Scutellonema* that negatively impacts maize productivity (Maina *et al.*, 2020), suggesting that the pathogenicity of this nematode may vary by location. Damage caused by *Rotylenchulus* has been reported in multiple tropical crops and was frequently encountered in this study, indicating the potential pest status of this nematode. Sumbul *et al.* (2015) previously recovered a high occurrence of *Rotylenchulus* in maize fields, legumes and oil-seed crops.

In the present study, while the nematode *Tylenchidae*, *Trichodorus*, and *Criconemoides* occurred less frequently and with lower densities, their potential to cause significant damage to maize crops in various countries has been documented (CABI, 2020). These findings emphasise the importance of considering location and species differences in the significance of the pathogenicity of nematodes in crops.

The density of plant-parasitic nematodes, including *Pratylenchus*, *Tylenchorhynchus*, *Rotylenchulus* and *Criconemoides*, revealed a significant and positive relationship with soil pH, SOC, SOM, and SAP. By contrast, *Xiphinema* showed a non-significant positive correlation with all soil parameters. This might be likely due to the impact of soil nutrients on microbial activity, which is essential for plant growth. Previous research has also highlighted the importance of soil pH in the regulation of soil microbial communities in agricultural soils (Neher *et al.*, 2005; Zhong *et al.*, 2010). However, we also observed a significant negative correlation between *Tylenchidae* and pH, SOC and SOM, similar to the findings by Kesba & Al-Shalaby (2008) and Laasli *et al.* (2022) showing that soil organic matter and pH can negatively affect nematode density. Nematode occurrences and densities can vary temporally and spatially, influenced by the soil conditions and the cultivated crop. Therefore, the significant of nematode observed in our study is likely attributable to a favourable soil environment and the host plant, maize (Stevens *et al.*, 2010; Liu *et al.*, 2016).

In conclusion, although information on maize-associated nematodes remains limited, our study provides valuable insight into their occurrences and densities in Ethiopia. It demonstrates the diversity of plant-parasitic nematodes with the dominant and important genera of *Meloidogyne* and *Pratylenchus* indicating the potential threat posed by the maize mono-cropping in the districts, with implications for maize yield loss. Thus, the observed high densities and occurrences of these nematodes signal their pest status and raises concerns for maize production in Ethiopia. Given that maize is a staple food crop in Ethiopia, emphasis should be given to proactive measures, such as crop diversification through crop rotation, to minimise the impact of plant-parasitic nematodes on maize cultivation. Our study offers crucial baseline information to policy makers, private farmers and agricultural extension workers. However, more research is essential to identify all nematode species associated with maize and their potential damage in all maize-growing areas in Ethiopia to formulate sustainable management strategies and ensure productivity of the crop in the country.

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