



Nematode diversity and its association with soil properties in monocrop pigeon pea

Samuel Maina, Hannah Karuri^{*}, Julius Mugweru

Department of Biological Sciences, University of Embu, P.O Box 6-60100, Embu, Kenya

ARTICLE INFO

Keywords:

Cajanus cajan

Nematode community structure

Shannon diversity

ABSTRACT

Pigeon pea is a versatile pulse crop grown in semi-arid regions of Kenya; however, its production is affected by plant-parasitic nematodes. The current study was undertaken to investigate the diversity of nematodes and the influence of soil properties on their diversity in monocrop pigeon pea fields in Mbeere North, Embu County, Kenya. Soil samples were collected from Gatunguru B, Gwakaithi, Itururi, Kambungu, Kanyueri, Karigiri, Mbangua and Njarange regions. From each field, soil samples were collected from a depth of 25 cm using W-shaped sampling pattern. The nematodes were identified to the genus level using morphological features. In total, 46 nematode genera assigned to five trophic levels were identified across the eight regions. Abundance of *Meloidogyne*, *Rotylenchulus*, *Longidorus*, *Acrobeloides*, *Cervidellus*, *Panagrolaimus*, *Prismatolaimus* and *Wilsonema* varied markedly among the eight regions. Bacterivores belonging to colonizer-persister group 2 were the most prevalent group. There were no differences in Pielou's evenness, genus richness, Shannon and Simpson diversity indices across the regions. Canonical correspondence analysis indicated significant correlations between certain nematode genera and soil attributes with the first two axes accounting for 56.65% of the variance. *Acrobeloides* correlated positively with Mg, C, Mn and N, and negatively with Fe. The occurrence of *Hoplolaimus* and *Mesorhynchitis* was associated negatively with soil pH, clay and Ca, and positively with sand. The present work reveals a high abundance of economically important PPN in monocrop pigeon pea which necessitates that appropriate nematode management programs are implemented.

1. Introduction

Pigeon pea (*Cajanus cajan*) is a versatile and valuable pulse crop that is mainly cultivated in developing countries in sub-Saharan Africa and Asia [1]. Pigeon pea can be grown in diverse environments in different cropping systems due to its unique characteristics such as drought tolerance, nitrogen-fixing ability, low input requirements and wide temporal variation in maturation period (90–300 days) [2]. It is a cheap source of essential nutrients such as proteins, minerals, vitamins and carbohydrates. It is also used as feed for fish, livestock, pigs and poultry [3,4]. Pigeon pea is grown on around 6.10 million ha in the world with an annual mean production of 0.82 t/ha [5]. In Kenya, pigeon pea ranks as the third most important crop after common beans and cowpeas and its grown on estimated acreage of about 133,525 ha [6,7]. From this area, the average pigeon pea yield is about 0.54 t/ha which is relatively lower than the potential yield (1.5–2.5 t/ha) [8]. Total yield production and productivity of pigeon pea are quite low despite a considerable increase in acreage under pigeon pea cultivation [9,10].

The main cause of low yields in pigeon pea is linked to its susceptibility to several pests and diseases [11]. Among the pests, plant-parasitic nematodes (PPN) such as *Meloidogyne* spp., *Heterodera* spp., *Rotylenchulus* spp., and *Pratylenchus* spp. are responsible for enormous economic losses in pigeon pea [12]. *Meloidogyne* spp. induces root galls, followed by a change in cell morphology, resulting in the accumulation of giant cells in the root cortex. *Heterodera* spp. form syncytia in the root stele characterized by pearly appearance while *Rotylenchulus* spp. induces dirty root disease. *Pratylenchus* spp. stimulates root necrotic lesions [9,13]. Worldwide, PPN are important pests causing huge crop losses of US \$ 173 billion each year [14]. In the US, *Heterodera glycines* causes substantial yield reductions in soybean annually [15,16]. *Meloidogyne* spp. remains one of the most damaging nematodes in agriculture attacking a wide array of crops such as chickpea, pigeon pea and green grams [12]. It causes about 21% yield losses in chickpea in India per year amounting to ₹ 4867.27 million [17]. *Rotylenchulus* spp. causes 21.19%, 32.84% and 80% yield losses in green gram, pigeon pea and chickpea, respectively [17]. On a global scale, *Pratylenchus* spp. are

^{*} Corresponding author.

E-mail address: hwkaruri@gmail.com (H. Karuri).

considered as the third most damaging nematode for numerous crops, including legumes, maize, sugarcane and potatoes [18–20] although their impact is often overlooked. Kimenju et al. [21] projected that *Pratylenchus* spp. can cause >10% in maize yield losses in East Africa. Furthermore, *Meloidogyne* spp. and *Heterodera cajani* have been observed to cause 8–35% and 14–30.1% yield losses in pigeon pea, respectively [22]. Singh [23] and Abd-Elgawad and Askary [24] reported that PPN accounts for around 12.3–13.2% of the yield loss in pigeon pea per annum. In terms of research, pigeon pea has often been regarded as an “orphan crop” compared with the other pulse crops [3,25]. Similarly, compared to other pests in pigeon pea, PPN has been neglected in terms of research despite their detrimental effects in various crops [26].

Unlike PPN, free-living nematodes (FLN) are beneficial organisms that constitute 60–80% of the entire soil nematode community [27]. Free-living nematodes occupy various trophic levels as primary, secondary and tertiary consumers within the soil food web [28], hence linking below- and above-ground processes in terms of soil fertility and plant productivity [29]. These nematode feeding groups can affect ecological processes either directly or indirectly. It is well documented that FLN greatly enhances plant growth indirectly by improving nutrient availability and uptake through the soil microbial loop mechanism [30, 31]. For instance, certain FLN feed on soil microbes such as bacteria and fungi and in the process they release excess nutrients that are taken up by the plants [31,32]. The activity and density of these nematodes can be regulated by predatory nematodes further modifying availability of nutrients [33]. Due to their ecological importance, FLN have been explored in several ecosystems including farmland agroecosystems [34, 35], forests [36] and grasslands [37]. Trap et al. [38] recorded significant increase in uptake of P by *Pinus pinaster* due to the presence of *Rhabditis* spp. *Acrobeloides* spp. mediated increased uptake of unlabeled P by rice [30] and under different agricultural practices it affected the plant biomass and nutrient content [32]. The abundance of nematodes is affected by environmental factors such as soil attributes including clay, organic matter, bulk density, moisture levels, nitrogen, magnesium and potassium [39,40]. The close link between diversity, structure and abundance of FLN with soil environment makes these nematodes a useful basis for assessing the quality of the soil food web and nutrients availability and uptake by plants [41].

There is a paucity of information regarding the abundance, diversity and distribution of FLN in African agro-ecosystems [34,42]. Information on nematodes associated with pigeon pea in Kenya is scarce despite the known harmful impacts of PPN and beneficial values of FLN. Given the lack of this basic information, the present work was undertaken to assess the PPN and FLN genera associated with pigeon pea in Kenya. We also aimed to establish the relationship between the structure of the nematode community of pigeon pea with soil physico-chemical characteristics.

2. Materials and methods

2.1. Site description and sampling

This work was conducted in Mbeere North sub-county, Embu County, Kenya (Table 1). The mean rainfall and temperature recorded in the area during the sampling period was 550 mm and 20 °C, respectively. We sampled fields (0.5–2 acres) that were cultivated with mono crop pigeon pea for more than six years under similar farming practices. The most commonly practiced type of soil management in the area is the input of organic amendment (cow manure) at a rate of 4640 kg/ha. A total of 24 pigeon pea fields in eight regions of Mbeere North sub-county were sampled in January 2021. In each field, three soil samples were taken using a soil auger (3.5 cm in diameter) at a depth of 25 cm. Each soil sample was collected along 3 separate W-shaped “sample walks”, consisting of 30 sampling points. The distance between the two sampling points was 10 m. From each “sample walk”, three 250 g soil composite cores were taken for nematode extraction, whereas 1 kg soil

Table 1

Soil sampling sites in Gatunguru B, Gwakaithi, Itururi, Kambungu, Kanyueri, Karigiri, Mbangua and Njarange, Embu County, Kenya.

Province	County	Region	Sampling Field	Latitude	Longitude
Eastern	Embu	Gatunguru B	F1	0°29'26.7"S	37°43'31.4"E
		Gwakaithi	F2	0°27'55.9"S	37°44'01.0"E
		Itururi	F3	0°30'16.8"S	37°45'18.2"E
		Kambungu	F4	0°28'21.3"S	37°48'43.5"E
		Kanyueri	F5	0°27'14.5"S	37°45'22.8"E
		Karigiri	F6	0°28'10.1"S	37°41'49.6"E
		Mbangua	F7	0°27'53.5"S	37°48'42.2"E
		Njarange	F8	0°27'36.7"S	37°48'35.1"E

sample was used for soil physicochemical analysis [43] (Table 1).

2.2. Nematode processing and enumeration

A 250 g soil sample was placed in filter trays for nematode extraction following the modified Baermann method for 48 hours [44]. After extraction, nematodes were heat-killed and fixed in a Golden solution according to the protocol by Hopper et al. [44]. The extracted nematodes were counted and identified to the genus level using a compound microscope (model DM750 Leica, Wetzlar, Germany). Nematode genera were grouped into five trophic groups [45]. Following this classification, the identified nematode genera in each region were ordered into herbivores, fungivores, omnivores, bacterivores and predators. Nematode genera within each trophic level were further assigned to respective colonizer-persister (cp) groups [33]. The value of c-p ranges from cp-1 (r-strategists or colonizers) to cp-5 (K-strategists or persisters). Those in cp-1 are characterized by high fecundity, short generation times and short lifecycles. Those in cp-5 have longer generation times, lower fecundity and longer life cycles [33]. The Shannon diversity, genus richness, Simpson diversity and Pielou's evenness were determined for each region.

2.3. Soil physico-chemical analysis and weather data

The soil physico-chemical characteristics including soil pH, total organic carbon, nitrogen, sand, clay, silt, pH, phosphorus, potassium, calcium, magnesium, manganese, copper, iron, zinc, and sodium were evaluated at the Kenya Agriculture and Livestock Research Organization, National Agricultural Research Laboratories as described [46–49]. Temperature and rainfall data were obtained from the Kenya Meteorological Department, Kenya.

2.4. Data analysis

Prior to analysis, nematode abundance data was evaluated for normality using the Shapiro-Wilk test. To fulfil the criteria for statistical analysis, data transformation ($\log(x+1)$) was executed where necessary. Differences in nematode genera abundances and diversity across the eight regions were determined by use of one-way analysis of variance (ANOVA) using R software. The Shannon diversity, genus richness, Simpson diversity and Pielou's evenness in each region were computed with the use of vegan library in R [50]. The canonical correspondence analysis (CCA) was employed to explore the distribution of nematode genera abundance in relation to soil physico-chemical attributes using the R package vegan [50].

3. Results

Overall, 46 nematode genera assigned to five trophic groups were identified across the eight regions as given in Table 2. Of these, bacterivores represented by 17 genera were the most dominant, followed by

Table 2

Nematode genera abundance (mean \pm standard error) in 250 g of soil collected from Gatunguru B, Gwakaithi, Itururi, Kambungu, Kanyuuri, Karigiri, Mbangua and Njarange regions, Embu County, Kenya.

Genus	Cp - value	Gatunguru B	Gwakaithi	Itururi	Kambungu	Kanyuuri	Karigiri	Mbangua	Njarange	F - value	P - value
		Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE		
Herbivores											
<i>Helicotylenchus</i>	3	4.3 \pm 4.3	47.7 \pm 24.1	0.0 \pm 0.0	39.0 \pm 15.0	34.7 \pm 28.4	0.0 \pm 0.0	4.3 \pm 4.3	4.3 \pm 4.3	2.068	0.108
<i>Hoplolaimus</i>	3	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	4.3 \pm 4.3	0.0 \pm 0.0	0.0 \pm 0.0	1.000	0.466
<i>Longidorus</i>	5	0.0 \pm 0.0	8.7 \pm 8.7	43.3 \pm 26.4	34.7 \pm 8.7	34.7 \pm 21.7	4.3 \pm 4.3	39.0 \pm 13.0	34.7 \pm 15.6	3.402	0.020*
<i>Malenchus</i>	2	0.0 \pm 0.0	0.0 \pm 0.0	8.7 \pm 8.7	4.3 \pm 4.3	13.0 \pm 13.0	0.0 \pm 0.0	13.0 \pm 13.0	0.0 \pm 0.0	0.583	0.760
<i>Meloidogyne</i>	3	338.0 \pm 318.5	104.0 \pm 45.7	4.3 \pm 4.3	0.0 \pm 0.0	86.7 \pm 44.0	472.3 \pm 165.3	8.7 \pm 4.3	17.3 \pm 11.5	3.523	0.018*
<i>Pratylenchus</i>	3	30.3 \pm 18.9	17.3 \pm 11.5	8.7 \pm 8.7	0.0 \pm 0.0	0.0 \pm 0.0	34.7 \pm 8.7	17.3 \pm 17.3	138.7 \pm 138.7	1.141	0.387
<i>Rotylenchulus</i>	3	86.7 \pm 61.1	21.7 \pm 15.6	0.0 \pm 0.0	17.3 \pm 111.5	130.0 \pm 66.7	34.7 \pm 21.7	4.3 \pm 4.3	0.0 \pm 0.0	5.755	0.002**
<i>Scutellonema</i>	3	164.7 \pm 90.5	43.3 \pm 43.3	104.0 \pm 64.1	17.3 \pm 17.3	34.7 \pm 18.9	17.3 \pm 8.7	82.3 \pm 75.9	13.0 \pm 7.5	0.316	0.936
<i>Trichodorus</i>	4	0.0 \pm 0.0	0.0 \pm 0.0	4.3 \pm 4.3	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	39.0 \pm 27.1	2.362	0.073
<i>Tylenchorhynchus</i>	3	0.0 \pm 0.0	0.0 \pm 0.0	8.7 \pm 4.3	0.0 \pm 0.0	0.0 \pm 0.0	8.7 \pm 8.7	0.0 \pm 0.0	0.0 \pm 0.0	2.214	0.089
<i>Tylenchulus</i>	3	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	4.3 \pm 4.3	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	1.000	0.466
<i>Tylenchus</i>	2	99.7 \pm 8.7	34.7 \pm 8.7	69.3 \pm 26.4	26.0 \pm 7.5	30.3 \pm 15.6	34.7 \pm 21.7	43.3 \pm 24.1	30.3 \pm 18.9	1.227	0.344
<i>Xiphinema</i>	5	4.3 \pm 4.3	13.0 \pm 7.5	8.7 \pm 8.7	8.7 \pm 4.3	8.7 \pm 4.3	4.3 \pm 4.3	17.3 \pm 11.5	21.7 \pm 15.6	0.327	0.931
Bacterivores											
<i>Acrobeles</i>	2	82.3 \pm 18.9	134.3 \pm 65.6	26.0 \pm 19.9	99.7 \pm 68.1	65.0 \pm 19.9	130.0 \pm 91.3	17.3 \pm 4.3	30.3 \pm 24.1	1.808	0.155
<i>Acroboloides</i>	2	0.0 \pm 0.0	34.7 \pm 17.3	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	4.000	0.010*
<i>Alaimus</i>	4	26.0 \pm 7.5	30.3 \pm 17.3	13.0 \pm 13.0	13.0 \pm 7.5	4.3 \pm 4.3	39.0 \pm 26.0	17.3 \pm 17.3	4.3 \pm 4.3	1.440	0.257
<i>Cephalobus</i>	2	238.3 \pm 119.7	628.3 \pm 462.3	121.3 \pm 56.8	160.3 \pm 95.6	177.7 \pm 11.5	372.7 \pm 86.7	47.7 \pm 21.7	134.3 \pm 85.4	1.489	0.240
<i>Cervidellus</i>	2	34.7 \pm 22.9	43.3 \pm 17.3	0.0 \pm 0.0	4.3 \pm 4.3	8.7 \pm 8.7	21.7 \pm 8.7	8.7 \pm 8.7	0.0 \pm 0.0	3.102	0.029*
<i>Chiloplacus</i>	2	0.0 \pm 0.0	0.0 \pm 0.0	4.3 \pm 4.3	30.3 \pm 30.3	39.0 \pm 13.0	52.0 \pm 27.1	0.0 \pm 0.0	17.3 \pm 17.3	1.760	0.165
<i>Drilocephalobus</i>	2	4.3 \pm 4.3	4.3 \pm 4.3	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.857	0.559
<i>Eucephalobus</i>	2	30.3 \pm 11.5	39.0 \pm 7.5	4.3 \pm 4.3	13.0 \pm 7.5	13.0 \pm 13.0	26.0 \pm 13.0	4.3 \pm 4.3	0.0 \pm 0.0	1.909	0.135
<i>Geomonhystera</i>	2	4.3 \pm 4.3	34.7 \pm 11.5	43.3 \pm 18.9	65.0 \pm 37.5	60.7 \pm 60.7	30.3 \pm 11.5	8.7 \pm 8.7	52.0 \pm 27.1	1.063	0.429
<i>Heterocephalobus</i>	2	117.0 \pm 41.8	60.7 \pm 24.1	52.0 \pm 7.5	60.7 \pm 42.7	52.0 \pm 13.0	130.0 \pm 22.5	69.3 \pm 4.3	86.7 \pm 45.9	0.602	0.746
<i>Mesorhabditis</i>	1	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	13.0 \pm 13.0	0.0 \pm 0.0	0.0 \pm 0.0	1.000	0.466
<i>Panagrolaimus</i>	1	95.3 \pm 26.4	17.3 \pm 4.3	8.7 \pm 8.7	43.3 \pm 18.9	39.0 \pm 15.0	0.0 \pm 0.0	0.0 \pm 0.0	8.7 \pm 8.7	11.531	<0.001***
<i>Plectus</i>	2	13.0 \pm 7.5	60.7 \pm 24.1	26.0 \pm 15.0	43.3 \pm 21.7	30.3 \pm 11.5	34.7 \pm 4.3	86.7 \pm 34.7	65.0 \pm 52.5	0.796	0.602
<i>Prismatolaimus</i>	3	34.7 \pm 11.5	60.7 \pm 18.9	56.3 \pm 11.5	86.7 \pm 11.5	8.7 \pm 8.7	47.7 \pm 17.3	17.3 \pm 17.3	65.0 \pm 32.7	3.861	0.012*
<i>Protorhabditis</i>	1	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	4.3 \pm 4.3	0.0 \pm 0.0	1.000	0.466
<i>Rhabditis</i>	1	34.7 \pm 18.9	30.3 \pm 18.9	8.7 \pm 8.7	108.3 \pm 53.2	34.7 \pm 34.7	8.7 \pm 8.7	8.7 \pm 8.7	26.0 \pm 26.0	0.968	0.486
<i>Wilsonema</i>	2	13.0 \pm 0.0	26.0 \pm 7.5	4.3 \pm 4.3	4.3 \pm 4.3	34.7 \pm 11.5	8.7 \pm 8.7	0.0 \pm 0.0	8.7 \pm 8.7	3.534	0.017*
Fungivores											
<i>Aphelenchoides</i>	2	30.3 \pm 17.3	30.3 \pm 18.9	34.7 \pm 28.4	73.7 \pm 34.7	13.0 \pm 13.0	43.3 \pm 15.6	8.7 \pm 4.3	13.0 \pm 7.5	1.041	0.442
<i>Aphelenchus</i>	2	78.0 \pm 19.9	78.0 \pm 37.5	30.3 \pm 11.5	82.3 \pm 17.3	151.7 \pm 52.7	312.0 \pm 208.1	34.7 \pm 8.7	56.3 \pm 50.0	2.061	0.110
<i>Filenchus</i>	2	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	4.3 \pm 4.3	8.7 \pm 8.7	0.0 \pm 0.0	0.857	0.559
Omnivores											
<i>Aporcelaimellus</i>	5	4.3 \pm 4.3	0.0 \pm 0.0	4.3 \pm 4.3	0.0 \pm 0.0	0.0 \pm 0.0	4.3 \pm 4.3	0.0 \pm 0.0	4.3 \pm 4.3	0.571	0.769
<i>Dorylaimellus</i>	5	17.3 \pm 11.5	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	4.3 \pm 4.3	8.7 \pm 8.7	0.0 \pm 0.0	0.0 \pm 0.0	1.691	0.182
<i>Eudorylaimus</i>	4	0.0 \pm 0.0	60.7 \pm 4.3	34.7 \pm 11.5	65.0 \pm 7.5	60.7 \pm 33.8	73.7 \pm 55.3	43.3 \pm 4.3	17.3 \pm 11.5	2.367	0.073
<i>Labronema</i>	4	26.0 \pm 13.0	39.0 \pm 19.9	21.7 \pm 11.5	30.3 \pm 8.7	34.7 \pm 4.3	43.3 \pm 15.6	13.0 \pm 7.5	43.3 \pm 11.5	1.138	0.389
<i>Mesodorylaimus</i>	4	4.3 \pm 4.3	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	4.3 \pm 4.3	0.0 \pm 0.0	8.7 \pm 4.3	17.3 \pm 11.5	1.654	0.191
<i>Prodorylaimus</i>	4	0.0 \pm 0.0	0.0 \pm 0.0	52.0 \pm 19.9	0.0 \pm 0.0	21.7 \pm 21.7	8.7 \pm 8.7	34.7 \pm 22.9	30.3 \pm 24.1	2.550	0.057

(continued on next page)

Table 2 (continued)

Genus	Cp - value	Gatunguru B	Gwakaithi	Itururi	Kambungu	Kanyuери	Karigiri	Mbangua	Njarange	F - value	P - value
		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE		
<i>Pungentus</i>	4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	4.3 ± 4.3	1.000	0.466
Predators											
<i>Discolaimus</i>	5	0.0 ± 0.0	4.3 ± 4.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.000	0.466
<i>Mononchus</i>	4	34.7 ± 34.7	0.0 ± 0.0	39.0 ± 13.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	4.3 ± 4.3	4.3 ± 4.3	2.283	0.081
<i>Mylonchulus</i>	4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	21.7 ± 21.7	0.0 ± 0.0	8.7 ± 8.7	0.0 ± 0.0	0.863	0.555
<i>Nygotaimus</i>	5	34.7 ± 8.7	8.7 ± 4.3	69.3 ± 18.9	52.0 ± 26.0	4.3 ± 4.3	69.3 ± 24.1	21.7 ± 8.7	60.7 ± 26.4	2.450	0.065
<i>Prionchulus</i>	4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	8.7 ± 8.7	0.0 ± 0.0	0.0 ± 0.0	73.7 ± 45.2	4.3 ± 4.3	2.077	0.107
<i>Tripyla</i>	3	0.0 ± 0.0	4.3 ± 4.3	30.3 ± 15.6	4.3 ± 4.3	4.3 ± 4.3	0.0 ± 0.0	0.0 ± 0.0	13.0 ± 13.0	1.005	0.463

Cp = colonizer-persister scale (Cp1-5). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

herbivores (13 genera), omnivores (7 genera), predators (6 genera) and fungivores (3 genera). There was a significant difference in the abundance of *Longidorus* ($P = 0.020$), *Meloidogyne* ($P = 0.018$), *Rotylenchulus* ($P = 0.002$), *Acrobeloides* ($P = 0.010$), *Cervidellus* ($P = 0.029$), *Panagrolaimus* ($P < 0.001$), *Prismatolaimus* ($P = 0.012$) and *Wilsonema* ($P = 0.017$). Among the herbivores, *Meloidogyne*, *Rotylenchulus* and *Longidorus* were predominant in Karigiri, Kanyuери and Itururi, respectively. For bacterivores, both *Cervidellus* and *Acrobeloides* were most prevalent in Gwakaithi while *Panagrolaimus*, *Prismatolaimus* and *Wilsonema* were most abundant in Gatunguru B, Kambungu and Kanyuери regions, respectively. No significant differences were recorded for genera belonging to fungivores, omnivores and predators. Following c-p classification, most herbivores were assigned to cp-3 except *Longidorus*, *Malenchus*, *Trichodorus*, *Tylenchus* and *Xiphinema*. Bacterivores were mainly categorized in the cp-2 group apart from *Alaimus*, *Mesorhabditis*, *Panagrolaimus*, *Protorhabditis*, *Rhabditis* and *Prismatolaimus*, whereas fungivores belonged to the cp-2 group. Both omnivores and predators belonged to cp4-5 guilds except *Tripyla* as recorded in Table 2.

Across the eight regions, there were no significant differences in Shannon diversity (ranging from 2.34 in Karigiri to 2.67 in Itururi), Simpson diversity (0.82; Gwakaithi – 0.91; Itururi), genus richness (18.33; Njarange – 23.00; Gwakaithi) and Pielou's evenness (0.76; Karigiri – 0.90; Itururi).

Soil physico-chemical properties across the eight regions are presented in Table 3. The canonical correspondence analysis (CCA) was used to establish the relationship between soil variables and obtained nematode genera abundance. Some soil variables significantly correlated with abundance of certain nematode genera as shown in Fig. 1. The

genera *Hoplolaimus* and *Mesorhabditis* correlated positively with sand and negatively with Ca, pH and clay. The abundance of *Acrobeloides* correlated positively with Mg, C, Mn and N, and negatively with Fe. Occurrence of *Discolaimus*, *Tylenchorhynchus*, *Mononchus*, *Aporcelaimellus* and *Tripyla* was positively associated with Fe and negatively with Mn, C, N and Mg. The first (eigenvalue = 0.12) and second (eigenvalue = 0.08) axes accounted for 33.24% and 23.41% of the total variance, respectively.

4. Discussion

The PPN genera identified in this study belonged to the orders Tylenchomorpha, Dorylaimida and Triplonchida [51]. Plant parasitic nematodes cause annual yield losses of US \$ 100–173 billion [14], more than that of invasive insects (about US \$ 70 billion) [52]. Several economically important PPN are associated with pulse crops including pigeon pea. These include *Meloidogyne* spp., *Heterodera* spp., *Paratylenchus* spp., *Rotylenchulus* spp., *Tylenchorhynchus* spp. and *Helicotylenchus* spp [9,53]. In this study, the 13 PPN genera recovered across the eight regions were observed in previous studies [54]. Sharma et al. [55] recorded 25 PPN associated with pigeon pea in Kenya, while Abuzar and Haseeb [56] reported 5 genera from pigeon pea fields in India. The variation in number of genera between these studies could probably be due to the fact that pigeon pea has been identified as a good host for several ecto- and endo-parasitic nematodes [11]. Sedentary endoparasite, *Meloidogyne* spp., is among the most economically important PPN with an extensive host range (>3000 plants species) [20, 57] including pigeon pea [9]. In the present study, high abundance of

Table 3

Soil physico-chemical attributes in Gatunguru B, Gwakaithi, Itururi, Kambungu, Kanyuери, Karigiri, Mbangua and Njarange regions, Embu County, Kenya.

Soil properties	Gatunguru B	Gwakaithi	Itururi	Kambungu	Kanyuери	Karigiri	Mbangua	Njarange
Soil pH	5.75	6.01	6.40	7.08	6.58	5.49	6.91	7.08
Total Nitrogen (%)	0.11	0.12	0.10	0.11	0.18	0.11	0.13	0.11
Total Organic Carbon (%)	1.16	1.29	0.88	1.20	2.06	1.24	1.49	1.20
Phosphorous ppm	33.00	40.67	60.00	53.00	46.00	29.33	50.67	53.00
Potassium meq%	1.04	0.81	0.52	1.00	1.68	0.87	1.23	1.00
Calcium meq%	2.20	1.93	2.60	5.00	5.60	1.67	5.20	5.00
Magnesium meq%	2.41	2.99	2.44	2.56	4.11	2.26	3.08	2.56
Manganese meq%	0.87	0.79	0.44	0.76	1.05	0.93	0.86	0.76
Copper ppm	1.00	0.33	0.64	2.78	0.25	1.20	1.94	2.78
Iron ppm	23.20	27.57	27.5	24.90	20.00	26.13	23.27	24.90
Zinc ppm	5.17	4.95	6.73	3.85	20.10	5.25	9.27	3.85
Sodium meq%	0.20	0.28	0.20	0.16	0.18	0.23	0.17	0.16
Sand	60.00	57.33	48.00	42.00	48.00	62.67	44.00	42.00
Clay	26.00	34.67	44.00	50.00	38.00	26.00	46.00	50.00
Silt	14.00	8.00	8.00	8.00	14.00	11.33	10.00	8.00

Note: meq = milliequivalent. ppm = parts-per million.

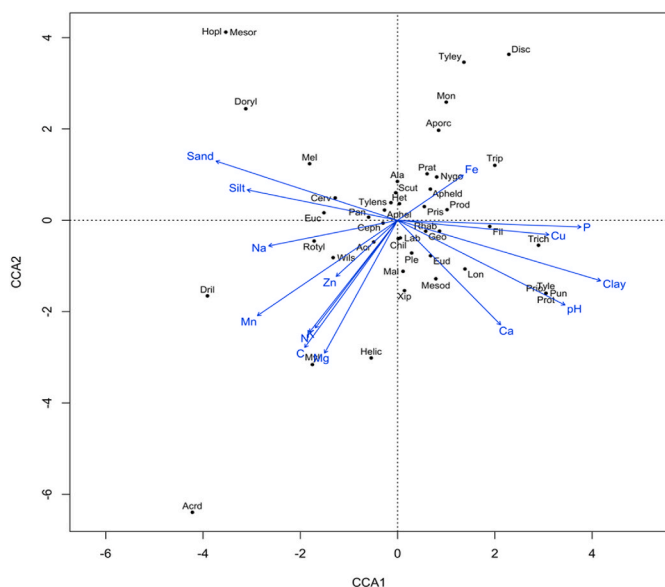


Fig. 1. Canonical correspondence analysis (CCA) of 46 nematode genera in Gatunguru B, Gwakaithi, Itururi, Kambungu, Kanyueri, Karigiri, Mbanga and Njarange regions using soil physico-chemical attributes (Carbon (C), Nitrogen (N), Calcium (Ca), Copper (Cu), Zinc (Zn), Sodium (Na), Magnesium (Mg), Phosphorus (P), Iron (Fe), clay, Silt, Sand, Potassium (K), Manganese (Mn) and soil Ph (pH)) marked by blue arrows. The first and second axes explain a cumulative variance of 56.65%. Nematode genera abbreviations include: Acr, *Acrobes*; Acrd, *Acrobeloides*; Ala, *Alaimus*; Apheld, *Aphelenchoides*; Aphel, *Aphelenchus*; Aporc, *Aporcelaimellus*; Ceph, *Cephalobus*; Cerv, *Cervidellus*; Chil, *Chiloplacus*; Disc, *Discolaimus*; Dril, *Drilocephalobus*; Doryl, *Dorylaimellus*; Euc, *Eucephalobus*; Eud, *Eudorylaimus*; Fil, *Filenchus*; Geo, *Geomonhystera*; Helic, *Helicotylenchus*; Het, *Heterocephalobus*; Hopl, *Hoplolaimus*; Lab, *Labronema*; Lon, *Longidorus*; Mal, *Malenchus*; Mel, *Meloidogyne*; Mesod, *Mesodorylaimus*; Mesor, *Mesorhabditis*; Myl, *Mylonchulus*; Mon, *Mononchus*; Nygo, *Nygolaimus*; Pan, *Panagrolaimus*; Ple, *Plectus*; Prat, *Pratylenchus*; Prio, *Prionchulus*; Pris, *Prismatolaimus*; Prod, *Prodorylaimus*; Prot, *Protorhabditis*; Pun, *Pungentus*; Rhab, *Rhabditis*; Rotyl, *Rotylenchulus*; Scut, *Scutellonema*; Trich, *Trichodorus*; Trip, *Tripyla*; Tyley, *Tylenchorhynchus*; Tylens, *Tylenchus*; Tyle, *Tylenchulus*; Wils, *Wilsonema*; and Xip, *Xiphinema*. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Meloidogyne corresponded with the results observed on pigeon pea in Malawi [58], India [58], and Brazil [53]. About 8–35% pigeon pea yield losses due to *Meloidogyne* spp. have been documented [11]. Additionally, a significant decline in plant growth characteristics such as yield, plant weight and height, chlorophyll content, number of pods, bulk density and root nodulation due to *Meloidogyne* spp. has been reported in pigeon pea [59]. *Meloidogyne incognita* along with either *Macrophomina phaseolina* or *Fusarium oxysporum* reduced plant growth parameters (plant height, shoot length, yield, number of pods, and fresh and dry weight), physio-biochemical attributes (carotenoids, chlorophyll, N, P and K contents), photosynthetic rate, transpiration rate and stomatal conductance in chickpea [60] and tomatoes [61].

Rotylenchulus is considered a significant pest of pigeon pea, resulting in yield losses ranging from 14% to 29% depending on the crop growth period, soil type, climatic factors and initial pathogenic level [11,22]. *Rotylenchulus* being the second most predominant genera in this study was consistent with previous reports [62]. This nematode has been found in association with pigeon pea in India [23], Belize [63], Fiji and Jamaica [64]. It has been reported to induce stunted growth, progressive dieback of main stem and twigs, yellowing of new leaves and premature death in pigeon pea [65]. *Longidorus*, also prevalent in the current work, has been shown to attack pulse crops especially chickpea, thereby acting as a vector for several important plant viruses [9]. Migratory endoparasites (*Scutellonema* and *Pratylenchus*) occurred in high abundance (not

statistically different) across the eight regions. The two nematodes have been recorded as important nematode pests in pigeon pea in Brazil, USA, Zimbabwe, Jamaica, India and Kenya [23,53,55,64]. In maize, *Pratylenchus* spp. reduced plant height, chlorophyll content, shoot and root weight as well as destroying cortical parenchyma and epidermal tissues resulting in severe root necrosis [20,66].

Free living nematodes are crucial in the decomposition of organic materials and recycling of soil nutrients [67]. They also play an important role in plant growth [31,32]. For example, *Prionchulus*, *Discolaimus* and *Tripyla* have been found to improve plant growth through the release of nitrogen compounds that are a byproduct of feeding on soil microorganisms [27,68]. The current work provides data on FLN from monocrop pigeon pea in a semi-arid agroecosystem. Bacterivores (*Panagrolaimus*, *Prismatolaimus*, *Cervidellus*, *Acrobeloides* and *Wilsonema*) were the most abundant as previously reported [34,69]. In another study, bacterivores dominated by family Rhabditidae were documented in maize cropping systems in Nigeria [70]. Bacterivores assigned to the cp-2 group were the most prevalent in this study which is in agreement with agro-ecosystems subjected to varying human activities [71]. Bongers and Bongers [72] stated that cp-2 nematodes (also regarded as general opportunists) can survive in stressed environments such as resource poor or enriched ecosystems. Diversity indices did not significantly vary across the eight regions probably due to the similar crop type [73]. However, crop type significantly influenced diversity indices in a nematode community in perennial agro-ecosystems compared to other factors such as rainfall [74].

Nematode community assemblages identified in this study varied in abundance, distribution and composition among the eight sampled regions, as previously reported [75]. The differences observed between pigeon pea fields herein could be partially explained by soil variables [76,77]. Kandji et al. [40] recorded a significant role of soil characteristics namely clay, organic matter and soil bulk density on nematode diversity, abundance and distribution. In the current study, the occurrence of *Acrobeloides* was positively associated with Mg, Mn, C and N, and negatively with Fe. Yavuzaslanoglu et al. [78] found that *Acrobeloides* was positively correlated with Mn and negatively with Mg. Elsewhere, Liang et al. [79] reported that the abundance of *Acrobeloides* was positively associated with C and N. Soil pH is regarded as a crucial factor in shaping the structure and abundance of nematode assemblages [80, 81]. In this study, the occurrence of *Hoplolaimus* and *Mesorhabditis* was negatively correlated with pH, clay and Ca, as well as positively associated with sand. Similarly, Matute [82] noted a negative relationship between soil pH with bacterivores (*Mesorhabditis*, *Rhabditis* and *Panagrolaimus*) and herbivores (*Hoplolaimus*, *Meloidogyne* and *Helicotylenchus*). Mashela et al. [83], however, reported that sand negatively affected the densities of *Hoplolaimus*. In another study, *Hoplolaimus* population was positively related to clay [84]. *Tylenchorhynchus* abundance in the present study was negatively affected by Mg, C and N as recently recorded [85]. The observed variation in the relationship between specific nematode genera and certain soil attributes in this study compared to other studies may be due to land-use effects among other factors [79].

5. Conclusion

In this study, forty-six nematode genera were identified in monocrop pigeon pea, dominated by bacterivores. The analysis of the relationship between nematode genera abundance and soil physico-chemical properties revealed that soil pH, clay and Ca negatively affected the abundance of genera *Hoplolaimus* and *Mesorhabditis*. Conversely, Mg, Mn and N positively influenced the occurrence of *Acrobeloides* whereas Fe negatively affected its abundance. This study provides baseline data which can be useful in designing sound and effective nematode management programs. However, further research on soil health management practices that enhance higher order nematodes with the ability to suppress PPN is needed in order to reduce PPN spread and improve

pigeon pea yields.

Funding

This work was supported by the CIRCLE research uptake fund.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

We thank the farmers from Embu County who contributed to the current study.

References

- J.A. Bernabe, A Promising Vegetable Pigeonpea (*Cajanus Cajan* L.) Variety Released for Ilocos Region, 2021. Philippines.
- R.K. Saxena, K.B. Saxena, R.K. Varshney, Pigeonpea (*Cajanus cajan* L. Millsp.): an ideal crop for sustainable agriculture, in: *Advances in Plant Breeding Strategies: Legumes*, Springer, 2019, pp. 409–429.
- R. Sultana, K.B. Saxena, R.R. Kumar, D. Kumar, M. Kirti, A. Pratap, in: S.B. T.-T. B, the P. Gupta (Eds.), Chapter 10 - Pigeonpea, Woodhead Publishing, 2021, pp. 217–240.
- S.C. Rao, S.W. Coleman, H.S. Mayeux, Forage production and nutritive value of selected pigeonpea ecotypes in the southern great plains, *Crop Sci.* 42 (4) (2002) 1259–1263.
- FAOSTAT, Production/Yield Quantities of Pigeon Pea in World +(Total), 2020. <https://www.fao.org/faostat/en/#data/QCL/visualize>. (Accessed 25 May 2022).
- J.D. Ojwang, R. Nyankanga, N.G. Rao, J. Imungi, Evaluation of vegetable pigeonpea [*Cajanus cajan* (L.) Millsp.] genotypes for yield stability, *CABI Agric. Biosci.* 2 (1) (2021) 41, <https://doi.org/10.1186/s43170-021-00061-8>.
- Ministry of Agriculture, Economic Review of Agriculture, Central Planning and Monitoring Unit, MoA, Ministry of Agriculture, 2015. Nairobi, Kenya, 2015.
- A.O. Esilaba, D. Nyongesa, M. Okoti, M. Otipa, L. Wasilwa, KCEP-CRAL pigeon peas extension manual, in: Kenya Agricultural and Livestock Research Organization, Elsevier, Nairobi, Kenya, 2021.
- T.H. Askary, Diversity of plant parasitic nematodes in pulses, in: *Plant Biodiversity: Monitoring, Assessment and Conservation*, CAB International, 2017, pp. 239–274.
- S. Pande, M. Sharma, U. Naga Mangla, R. Ghosh, G. Sundaresan, Phytophthora blight of Pigeonpea [*Cajanus cajan* (L.) Millsp.]: an updating review of biology, pathogenicity and disease management, *Crop Protect.* 30 (8) (2011) 951–957, <https://doi.org/10.1016/j.cropro.2011.03.031>.
- C.B.K. Sangma, in: B.P. Singh, G. Singh, K. Kumar, S.C. Nayak, N. Srinivasa (Eds.), *Soil and Crop Health Management for the Cultivation of Pigeon Pea: an Overview of Management Practices BT - Management of Fungal Pathogens in Pulses: Current Status and Future Challenges*, Springer International Publishing, Cham, 2020, pp. 143–167.
- P.P. Reddy, Pulse crops, in: *Nematode Diseases of Crops and Their Management*, Springer, 2021, pp. 67–95.
- R.A. Sikora, J. Desaeer, L. Molendijk, Integrated Nematode Management: State-Of-The-Art and Visions for the Future, CABI, 2022.
- E. Gamalero, B.R. Glick, The use of plant growth-promoting bacteria to prevent nematode damage to plants, *Biology (Basel)*. 9 (11) (2020), <https://doi.org/10.3390/biology9110381>.
- G.L. Tylka, C.C. Marett, Known distribution of the soybean cyst nematode, *Heterodera glycines*, in the United States and Canada in 2020, *Plant Health Prog.* 22 (1) (2021) 72–74.
- A.Y. Bandara, D.K. Weerasooriya, C.A. Bradley, T.W. Allen, P.D. Esker, Dissecting the economic impact of soybean diseases in the United States over two decades, *PLoS One* 15 (4) (2020), e0231141, <https://doi.org/10.1371/journal.pone.0231141> [Online]. Available:.
- R.K. Wallia, P.K. Chakraborty, Nematodes Problems of Crops in India," ICAR-All india Coord, Res. Proj. nematodes Agric., New Delhi, 2018.
- M. Kantor, Z. Handoo, C. Kantor, L. Carta, Top ten most important US-regulated and emerging plant-parasitic nematodes, *Horticulturae* 8 (3) (2022) 208.
- A.H. Mc Donald, D. De Waele, H. Fourie, Nematode pests of maize and other cereal crops, in: H. Fourie, V.W. Spaull, R.K. Jones, M.S. Daneel, D. De Waele (Eds.), *Nematology in South Africa: A View from the 21st Century*, Springer, 2017, pp. 183–199 (p. 569). Cham, Switz.: Springer.
- H.S. Jones, J. T. A. Haegeman, E.G.J. Danchin, R.N. Gaur and, J. Helder, M.G. K. Jones, T. Kikuchi, R. Manzanilla-Lopez, J.E. Palomares-Rius, W.M.L. Wesemael, Perry, Top 10 plant-parasitic nematodes in molecular plant pathology, *Mol. Plant Pathol.* 14 (9) (2013) 946–961.
- J.W. Kimenju, P.K. Wendot, A.K. Thuo, Parasitic Nematodes in Smallholder Maize Cropping Systems in East Africa," *Integr*, 48, *Nematode Manag. State-of-the-art visions Futur*, 2022.
- M.V. Reddy, T.N. Raju, S.B. Sharma, Y.L. Nene, D. McDonald, S. Pande, M. Sharma, *Handbook of Pigeonpea Diseases (Revised)*. Information Bulletin No. 42, International Crops Research Institute for the Semi-Arid Tropics, 2012.
- B. Singh, Status of phytoparasitic nematodes associated with pigeonpea in bundelkhand region of Uttar Pradesh, *Indian J. Nematol.* 45 (1) (2015) 39–42.
- M.M.M. Abd-Elgawad, T.H. Askary, Impact of phytonematodes on agriculture economy, in: *Biocontrol Agents of Phytonematodes*, CABI, Wallington, 2015, pp. 1–49.
- R.K. Varshney, R.K. Saxena, S.A. Jackson, The pigeonpea genome: an overview, in: *The Pigeonpea Genome*, Springer, 2017, pp. 1–4.
- D.L. Coyne, L. Cortada, J.J. Dalzell, A.O. Claudius-Cole, S. Haukeland, N. Luambano, H. Talwana, Plant-parasitic nematodes and food security in Sub-Saharan Africa, *Annu. Rev. Phytopathol.* 56 (2018) 381–403.
- D.A. Neher, Ecology of plant and free-living nematodes in natural and agricultural soil, *Annu. Rev. Phytopathol.* (2010) 371–394.
- D.A. Neher, Role of nematodes in soil health and their use as indicators, *J. Nematol.* 33 (4) (2001) 161–168.
- J. Trap, M. Bonkowski, C. Plassard, C. Villenave, E. Blanchart, Ecological importance of soil bacterivores for ecosystem functions, *Plant Soil* 398 (1) (2016) 1–24, <https://doi.org/10.1007/s11104-015-2671-6>.
- M.P. Ranoarisoa, C. Morel, A. Andriamananjara, C. Jourdan, L. Bernard, T. Becquer, L. Rabeharisoa, K. Rahajahalilaza, C. Plassard, E. Blanchart, J. Trap, Effects of a bacterivorous nematode on rice 32P uptake and root architecture in a high P-sorbing ferrallitic soil, *Soil Biol. Biochem.* 122 (2018) 39–49, <https://doi.org/10.1016/j.soilbio.2018.04.002>.
- M.T. Gebremikael, H. Steel, D. Buchan, W. Bert, S. De Neve, Nematodes enhance plant growth and nutrient uptake under C and N-rich conditions, *Sci. Rep.* 6 (2016), 32862.
- J. Trap, M.P. Ranoarisoa, S. Raharijaona, L. Rabeharisoa, C. Plassard, E.H. Mayad, L. Bernard, T. Becquer, E. Blanchart, Agricultural practices modulate the beneficial activity of bacterial-feeding nematodes for plant growth and nutrition: evidence from an original intact soil core technique, *Sustainability* 13 (13) (2021), <https://doi.org/10.3390/su13137181>.
- H. Sánchez-Moreno, S. Ferris, Nematode ecology and soil health, in: R.A. Sikora, D. Coyne, J. Hallmann, P. Timper (Eds.), *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*, third ed., CAB International, 2018, pp. 62–83.
- T.T. Bello, D.L. Coyne, H. Fourie, Free-living nematode assemblages in the rhizosphere of watermelon plants in Nigeria: a baseline study, *Nematology* 22 (1) (2019) 23–37.
- M.K. Sekhukhune, E. Shokoohi, M.Y. Maila, P.W. Mashela, Free-living nematodes associated with kiwifruit and effect of soil chemical properties on their diversity, *Biologia (Bratisl.)*. 77 (2) (2022) 435–444, <https://doi.org/10.1007/s11756-021-00956-x>.
- H. Karuri, Nematode community structure and functional guilds differ in tea fields and tropical forest, *Geoderma* 392 (2021), 115006, <https://doi.org/10.1016/j.geoderma.2021.115006>.
- J. Liu, Y. Chen, C. Du, X. Liu, Q. Ma, X. Zhang, D. Wang, Interactive effects of nitrogen addition and litter on soil nematodes in grassland, *Eur. J. Soil Sci.* 70 (3) (2019) 697–706.
- J. Trap, P.M. Ranoarisoa, U. Irshad, C. Plassard, Richness of rhizosphere organisms affects plant P nutrition according to P source and mobility, *Agriculture* 11 (2) (2021), <https://doi.org/10.3390/agriculture11020157>.
- U.N. Nielsen, E. Ayres, D.H. Wall, G. Li, R.D. Bardgett, T. Wu, J.R. Garey, Global-scale patterns of assemblage structure of soil nematodes in relation to climate and ecosystem properties, *Global Ecol. Biogeogr.* 23 (9) (2014) 968–978.
- S.T. Kandji, C.K.P.O. Ogo, A. Albrecht, Diversity of plant-parasitic nematodes and their relationships with some soil physico-chemical characteristics in improved fallows in western Kenya, *Appl. Soil Ecol.* 18 (2) (2001) 143–157.
- B.S. Griffiths, J. Römcke, R.M. Schmelz, A. Scheffczyk, J.H. Faber, J. Bloem, G. Pérès, D. Cluzeau, A. Chabbi, M. Suhadolc, J.P. Sousa, Selecting cost effective and policy-relevant biological indicators for European monitoring of soil biodiversity and ecosystem function, *Ecol. Indic.* 69 (2016) 213–223, <https://doi.org/10.1016/j.ecolind.2016.04.023>.
- F. Durand, W. Marais, E. Venter, A. Swart, J. Habig, A. Dippenaar-Schoeman, E. Ueckermann, R. Jacobs, C. Jansen van Rensburg, L. Tiedt, Die karst-ekologie van die Bakwenagrot (Gauteng): oorspronklike navorsing," *Suid-Afrikaanse Tydsk. vir natuurwetenskap en Tegnol* 31 (1) (2012) 1–17.
- R. Wiesel, L. Daniell, T.J. King, D. Neilson, Determination of the optimal soil sample size to accurately characterise nematode communities in soil, *Soil Biol. Biochem.* 80 (October) (2015) 89–91.
- D.J. Hooper, J. Hallmann, S.A. Subbotin, Methods for extraction, processing and detection of plant and soil nematodes, in: M. Luc, R.A. Sikora, J. Bridge (Eds.), *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*, CAB International, Wallingford, 2005, pp. 53–86.
- S.S. Yeates, G.W. Bongers, T.D. De Goede, R.G.M. Freckman, D.W. Georgieva, Feeding habits in soil nematode families and genera an outline for soil ecologists, *J. Nematol.* 25 (3) (1993) 315–331.
- M.L. Jackson, *Soil Chemical Analysis*. Verlag: Prentice Hall, Inc, John Wiley & Sons, Ltd, 1959. Englewood Cliffs, NJ. 1958, 498 S. DM 39.40, vol. 85, no. 3.
- J.L. Smith, J.W. Doran, Measurement and use of pH and electrical conductivity for soil quality analysis, *Methods for Assessing Soil Quality* (1997) 169–185, <https://doi.org/10.2136/sssaspecpub49.c10>.
- R.N. Gallaher, C.O. Weldon, J.G. Futral, An aluminum block digester for plant and soil analysis, *Soil Sci. Soc. Am. J.* 39 (4) (1975) 803–806.
- G.W. Gee, J.W. Bauder, A. Klute, *Methods of Soil Analysis, Part 1, Physical and Mineralogical Methods*, Soil Sci. Soc. Am. Am. Soc. Agron., 1986.

- [50] F.J. Oksanen, G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGinn, H. Wagner, Vegan: Community Ecology Package. R Package Version 2.5-3, 2018. <http://CRAN.R-project.org/>.
- [51] D.J. Hunt, J.E. Palomares-Rius, R.H. Manzanilla-López, in: R. Sikora, D. Coyne, J. Hallmann, P. Timper (Eds.), Identification, Morphology and Biology of Plant Parasitic Nematodes, Plant Parasit. Nematodes Subtrop. Trop. Agric, third ed., CABI Publ, Wallingford, England, 2018, pp. 20–61, <https://doi.org/10.1079/9781786391247.0020>.
- [52] C.J. Bradshaw, B. Leroy, C. Bellard, D. Roiz, C. Albert, A. Fournier, M. Barbet-Massin, J.M. Salles, F. Simard, F. Courchamp, Massive yet grossly underestimated global costs of invasive insects, Nat. Commun. 7 (1) (2016) 1–8.
- [53] R.A. Sikora, N. Greco, J.F.V. Silva, Nematode parasites of food legumes, Plant Parasit. Nematodes Subtrop. Trop. Agric. (2018) 259–318.
- [54] Y.Y. Min, K. Toyota, Plant-parasitic nematodes in some economically important crops in Myanmar – species, possible damage and control measures, Nematology 20 (1) (2018) 1–13, <https://doi.org/10.1163/15685411-00003133>.
- [55] S.B. Sharma, M.R. Siddiqi, P. Omanga, L. Singh, Distribution and potential importance of plant-parasitic nematodes associated with pigeonpea in Kenya, Afro-Asian J. Nematol. 3 (2) (1993) 182–187.
- [56] S. Abuzar, A. Haseeb, Bio-management of plant-parasitic nematodes in pigeon pea field crop using neem-based products and manurial treatments, World Appl. Sci. J. 7 (7) (2009) 881–884.
- [57] S.K. Singh, M. Hodda, G.J. Ash, Plant-parasitic nematodes of potential phytosanitary importance, their main hosts and reported yield losses, EPPO Bull. 43 (2) (2013) 334–374.
- [58] M. V Reddy, P. Subrahmanyam, A.A. Likoswe, V.W. Saka, W.A.B. Msuku, Pigeonpea diseases in Malawi, Plant Prot. 41 (2) (1993) 101–106.
- [59] T.H. Askary, Effect of different inoculum levels of root-knot nematode *M. javanica* on pigeonpea (*Cajanus cajan* L. Millsp.) cv. UPAS 120, Indian J. Ecol. 38 (2) (2011) 284–285.
- [60] A. Sumbul, I. Mahmood, Interactive effect of *Meloidogyne incognita* and *Macrophomina phaseolina* on the development of root–rot disease complex in relation to growth and physiological attributes of chickpea, Hell. Plant Prot. J. 13 (2020).
- [61] A. Maqsood, H. Wu, M. Kamran, H. Altaf, A. Mustafa, S. Ahmar, N.T.T. Hong, K. Tariq, Q. He, J.T. Chen, Variations in growth, physiology, and antioxidative defense responses of two tomato (*Solanum lycopersicum* L.) cultivars after Co-infection of *Fusarium oxysporum* and *Meloidogyne incognita*, Agronomy 10 (2) (2020), <https://doi.org/10.3390/agronomy10020159>.
- [62] K.S. Varaprasad, S.B. Sharma, T.R. Loknathan, Nematode constraints to pigeonpea and chickpea in Vidarbha region of Maharashtra in India, Int. J. Nematol. 7 (2) (1997) 152–157.
- [63] J. Bridge, D.J. Hunt, P. Hunt, Plant-parasitic Nematodes of Crops in Belize, Nematropica, 1996, pp. 111–119.
- [64] S.B. Sharma, D. McDonald, Global status of nematode problems of groundnut, pigeonpea, chickpea, sorghum and pearl millet, and suggestions for future work, Crop Protect. 9 (6) (1990) 453–458.
- [65] S.S. Ali, B. Singh, Nematodes of Pigeonpea and Their Management, Adv. Pigeonpea Res. DK Agencies Pvt Limited, New Delhi, India, 2005, pp. 284–314.
- [66] A. Sikandar, T.A. Khanum, Y. Wang, Biodiversity and community analysis of plant-parasitic and free-living nematodes associated with maize and other rotational crops from Punjab, Pakistan, Life 11 (12) (2021) 1426.
- [67] S. Maina, H. Karuri, R.N. Ng'endo, Free-living nematode assemblages associated with maize residues and their ecological significance, J. Nematol. 53 (2021).
- [68] M. M Tahat, K.M. Alananbeh, Y.A. Othman, D.I. Leskovar, Soil health and sustainable agriculture, Sustainability 12 (12) (2020) 4859.
- [69] A. Mbatyoti, M.S. Daneel, A. Swart, D. de Waele, H. Fourie, Terrestrial non-parasitic nematode assemblages associated with glyphosate-tolerant and conventional soybean-based cropping systems, J. Nematol. 50 (3) (2018) 243.
- [70] E.N. Mary, I. Emmanuel, N. Oyema, S.A. Yoila, M.B. Victoria, Effect of long term soil management practices on nematode population in an Alfisol under continuous maize in Northern Guinea Savanna of Nigeria, Int. J. Agric. Pol. Res. 1 (2013) 80–88.
- [71] D.W. Freckman, C.H. Ettema, Assessing nematode communities in agroecosystems of varying human intervention, Agric. Ecosyst. Environ. 45 (3–4) (1993) 239–261.
- [72] T. Bongers, M. Bongers, Functional diversity of nematodes, Appl. Soil Ecol. 10 (3) (1998) 239–251.
- [73] L. Wang, Y. Wang, W. Xiu, B. Tan, G. Li, J. Zhao, D. Yang, G. Zhang, Y. Zhang, Responses of soil microbial and nematode communities to various cover crop patterns in a tea garden of China, Int. J. Environ. Res. Publ. Health 19 (5) (2022), <https://doi.org/10.3390/ijerph19052695>.
- [74] R. McSorley, Relationship of crop and rainfall to soil nematode community structure in perennial agroecosystems, Appl. Soil Ecol. 6 (2) (1997) 147–159, [https://doi.org/10.1016/S0929-1393\(97\)00001-2](https://doi.org/10.1016/S0929-1393(97)00001-2).
- [75] F. Franco-Navarro, D. Godinez-Vidal, Soil nematodes associated with different land uses in the Los Tuxtlas biosphere reserve, Veracruz, Mexico, Rev. Mex. Biodivers. 88 (1) (2017) 136–145, <https://doi.org/10.1016/j.rmb.2017.01.002>.
- [76] G. Krif, F. Mokrini, A.E. Aissami, S.E. Laasli, M. Imren, G. Özer, T. Paulitz, R. Lahlali, A.A. Dababat, Diversity and management strategies of plant parasitic nematodes in Moroccan organic farming and their relationship with soil physico-chemical properties, Agriculture 10 (10) (2020), <https://doi.org/10.3390/agriculture10100447>.
- [77] N. Garcia, E. Grenier, A. Buisson, L. Folcher, Diversity of plant parasitic nematodes characterized from fields of the French national monitoring programme for the Columbia root-knot nematode, PLoS One 17 (3) (2022), e0265070, <https://doi.org/10.1371/journal.pone.0265070> [Online]. Available:.
- [78] E. Yavuzaslanoglu, O.A. Sonmezoglu, N. Genc, Z.M. Akar, A. Ocal, M.S. Karaca, I. H. Elekcioğlu, V.S. Ozsoy, M. Aydogdu, Occurrence and abundance of nematodes on onion in Turkey and their relationship with soil physicochemical properties, Nematology 21 (10) (2019) 1063–1079.
- [79] W. Liang, X. Zhang, Q. Li, Y. Jiang, W. Ou, D.A. Neher, Vertical distribution of bacterivorous nematodes under different land uses, J. Nematol. 37 (3) (2005) 254–258 [Online]. Available: <https://pubmed.ncbi.nlm.nih.gov/19262869>.
- [80] Y. Wang, J. Qiao, C. He, Z. Wang, W. Luo, L. Sheng, Towards multi-level biomonitoring of nematodes to assess risk of nitrogen and phosphorus pollution in Jinchuan Wetland of Northeast China, Ecotoxicology 24 (10) (2015) 2190–2199, <https://doi.org/10.1007/s10646-015-1550-5>.
- [81] C.C. Mishra, M.C. Dash, Effects of soil amendments on soil moisture, pH, microbial population, soil respiration and nematodes, Ann. Biol. 3 (2) (1987) 17–27.
- [82] M.M. Matute, Soil nematodes of *Brassica rapa*: influence of temperature and pH, Adv. Nat. Sci. 6 (4) (2013) 20–26.
- [83] P. Mashela, R. McSorley, L.W. Duncan, R.A. Dunn, Correlation of *Belonolaimus longicaudatus*, *Hoplolaimus Galeatus*, and Soil Texture with Yield of Alyceclover (*Alysicarpus* spp.), Nematropica, 1991, pp. 177–184.
- [84] R.F. Myers, R.E. Wagner, P.M. Halisky, Relationship between cultural factors and nematodes on merion Kentucky bluegrass, J. Nematol. 24 (1) (1992) 205–212 [Online]. Available: <https://pubmed.ncbi.nlm.nih.gov/19283225>.
- [85] I. Filali Alaoui, M. Ait Hamza, H. Benjlil, A. Idhmdia, A. Braimi, E. Mzough, A. Hallouti, K. Basaid, J.N. Furze, I.A. Zasada, T. Paulitz, Phyt parasitic nematodes of organic vegetables in the argan biosphere of Souss-Massa (Southern Morocco), Environ. Sci. Pollut. Res. 28 (45) (2021) 64166–64180.