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NEMATODE COMMUNITY STRUCTURES DEPICT SOIL HEALTH STATUS OF KOLA NUT TREE (COLA SPP.) FORESTS OF SOUTH-WEST NIGERIA

SUMMARY

Nematodes, the most prevalent multicellular organism in soil, hold a crucial position in the construction of the soil food web. They have been established as being excellent indicators of soil conditions which ultimately influence crop productivity. A survey was conducted to evaluate the soil nematode assemblages of kola tree forests in Oke Geu, Litaye, Ajue and Oniparaga villages of Ondo state, Nigeria. Soil samples were taken at random from all fields. The nematodes encountered were identified to generic level. A sum of 27 nematode genera, spanning 20 families, were discovered in sampled kola fields. Eight genera of plant parasitic nematodes were recovered, *Helicotylenchus* and *Paratylenchus* were the most abundant species. In line with all previous reports of free-living nematode assemblages in Nigeria, this study established that bacterivores were the most common nematodes. No significant difference ($P < 0.05$) was observed across all indices of ecosystem measured except with respect to the mean plant parasitic index and PPI/MI ratios. According to the food web study, 25% of the samples were plotted in quadrat B, reflecting mature and nutrient-rich soil conditions based on their metabolic footprints, while the remaining 75% were lotted in quadrat C, indicative of stable and fertile soil. Results further emphasized the importance of utilizing the concept of nematode c-p values in interpreting food web status of different soil habitats. The findings from this research added to the body of knowledge already available on the application of nematodes as markers of soil health and

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furthermore provides the first study of nematode assemblages in kola tree fields in West and Sub-Saharan Africa.

Keywords: Nematodes, kola nut, ecosystem functions, soil food web, metabolic footprint

INTRODUCTION

Cola nitida and *Cola acuminata* (Kola) belongs to the family Sterculiacea. They are large robust trees that are commercially grown in tropical climates of the world (Hutchinson and Dalziels 1963; Asogwa et al. 2006; Onaolapo and Onaolapo 2019; Jacob et al. 2024). Several varieties abound, with over 40 species known but the most common and of paramount importance being *C. nitida* and *C. acuminata* (Daramola, 1978; Oladokun, 1982; Odutayo et al. 2018; Jacob et al. 2024). They are generally found along west African coast, occurring predominantly in the Ghanaian and Ivorian forests (Hutchinson and Dalziels 1963; Opeke, 2005). The nuts could either be white, red or pink in colour (Nkemakolam, 2002; Nyadanu et al. 2020; Ichetaonye et al. 2024). Kola nuts are considered as a principal trade item to a great extent in west Africa and Nigeria for centuries (Mokwunye, 2009; Adesida et al. 2021). Nigeria produces up to 88% of total world kola nut, with 200,000 metric tonnes annually (Atanda et al. 2011; Oladigbolu et al. 2023). A multitude of Africans treasure and regard kola nuts symbolically in custom, tradition and heritage, because it exemplifies cordiality, bond and amity thus it is consumed at reunions, social and religious gatherings and more (Asogwa et al. 2012; Unya et al. 2021).

Scientifically, several medicinal merits are identified with kola nuts. It contains phytochemical compounds such as phenolics, theophylline, tannins, theobromine betanine and principally caffeine which is in a very high concentration. Kola nuts have been indicated in the treatment of some health challenges like diarrhoea, asthma, malaria, whooping cough, nausea and toothache (Obineche, 2017; Blades, 2000). It may be taken as a stimulant, poison antidote and tranquilizer (Adebayo and Oladele 2012; Aniwada and Ezema 2022). It serves as an excellent stimulant and eliminates fatigue. Kola nut pod husk is an important by product of nut processing with high nutritional value, it is employed as a feed additive in ruminants and poultry (Babatunde and Hamzat 2005; Lateef, 2023). Kola nut is also of high significance in pharmaceutical, textile, food, and beverage industries (Ogutuga, 1975; Olunloyo, 1979; Jaiyeola, 2001; Lateef, 2023; Ichetaonye et al. 2024). Kola nut trade is a thriving business in many local markets in Africa, where bulk trading of the nuts is directed to long distances as wholesale (Adesida et al. 2021). The recent scarcity of kola nuts which was occasioned by decline in yield observed within the last 12 years has been attributed to several factors such as increase in the number of unfruitful and sterile trees which was further linked to attack by field pests, pathogens, aging trees, reduced pollination and in some cases, poor soil conditions (Moshood, 2020; Azeez, 2021; Bello et al. 2022a).

Nematodes are known to be the most abundant multicellular organism in the soil found occupying the second, third and fourth trophic levels in the soil food web structure (Du Preez et al. 2022). They have been suggested as being excellent indicators of soil conditions which ultimately influence crop productivity (Ferris, 2010). Owing to their significant role in the soil food web, nematodes have been used to assess soil conditions in situ by evaluating their dynamics and interpreting their community structures (Young and Unc 2023). The integration of nematode feeding habits is represented by functional guilds (Yeates et al. 1993).

Furthermore, colonizer-persister (C-P) scales Bongers and Bongers (1998) was developed for studying faunal food web analysis while metabolic footprints were instituted (Ferris, 2010) for a more holistic study of soil health community dynamics based on nematode life strategies.

Bongers (1990) grouped nematodes on a continuum (r - to k- strategies) where r-strategies being the colonizers are those sensitive nematodes that occupy nutrient rich habitats and lay numerous small eggs. In contrast, during temporary circumstances of increased food supply, the k-strategies (persisters) scarcely react. According to this categorization, nematodes are divided into five groups on the C-P scale: carnivore nematodes, fungi feeders, omnivore nematodes, bacterivores and plant feeders. Moreover, Bongers and Bongers (1998) introduced the idea of functional guilds in an effort to incorporate nematode feeding groups into the life strategy principles. This comprises of species with comparable growth, metabolic, and reproductive traits and serving similar ecological roles. While the ecological significance and worth of soil nematodes have been acknowledged since the early 1960s (Banage, 1963), their role in controlling the densities of soil bacteria and fungi brought them to the forefront of the soil nutrient cycling process (Perez-Moreno and Read 2001; Hoorman, 2011). This gave rise to a more optimistic understanding of nematodes' functions in soil processes (Yeates, 2003). The dynamics of nematode communities and their significance have been researched on a variety of crops throughout the world (Schorpp and Schrader 2017; Tian *et al*. 2020). However, very little is known about abundance, distribution, and ecological services they offer within the agro-ecological systems of sub-Saharan Africa (SSA).

There are reports on several field and storage pests of kola nut in literature. However, despite the importance of this crop, information on its associated nematodes (both free living and plant parasitic) is sparse, hence the need for this study. Using kola tree fields as a case study, the current study looked at these factors in order to provide a good knowledge of how nematode community structures relate to soil health dynamics. Making decisions about soil conservation that will help SSA achieve stable and sustainable forest soil health depends on this understanding.

MATERIALS AND METHODS

Nematode sampling

Four farms on an expanse of 24 hectares (6 hectares each) located at Oke Geu, Ajue, Oniparaga and Litaye villages in Ondo state, Nigeria were sampled. An average of 820 kola nut trees in each farm was sampled separately between July and November, year 2021. The field on each farm was divided into rows and columns for easy sampling and adopting the systemic method of sampling. The top soil around each tree was scrapped to remove weeds and other particles. 820 individual samples were collected from the field. Soil samples were taken around the rhizosphere of each tree at a distance of 60 cm from the tree stem. Four points were sampled around each tree at a depth of 20 cm with a hand trowel. Samples from each tree was mixed to represent the particular tree. Each soil sample weighing approximately 1500 g was packed with label and then moved to the laboratory for evaluation. In the laboratory, the soil in each of the samples were thoroughly mixed and 200 cm^3 was taken out for nematode extraction by sieving and decanting following the method of Cobb (1918).

Nematode extraction, counting and identification

Composite soil samples were mixed and 200 cc sub-sample was taken from each and used for extraction. Nematode extraction from the sub-samples followed a modified sugar floatation technique (Jenkins, 1964). The recovered nematodes were placed in a Doncaster counting dish (Doncaster, 1962). Following procedure of Nico et al. (2002) 4% formaldehyde solution was introduced. Nematodes were identified by mounting them on glass microscopic slides with anhydrous glycerin. The University of Nebraska-Lincoln UNL Nematology Laboratory's interactive diagnostic keys were used in addition to the pictorial keys created by Jairaipuri and Ahmed (1992), Andrassy (2005), and Holovachov et al. (2009).

Frequency of occurrence and mean nematode abundance were expressed per field and collectively by calculating the Prominence values (PV) for each nematode genus identified according to DeWaele and Jordaan (1988) using the equation:

$$PV = \text{Population density } X\ \sqrt{\text{frequency of occurrence}}\ /10$$

Nematode diversity was expressed according to nematode feeding groups and their C-P scale values. Soil faunal (food web) structure was calculated according to Ferris et al. (2001; 2004). The structural, basal and enrichment indices trajectories were obtained from nematode abundance in guilds. The following equation was used in their calculations: Kbnb, where nb is the abundance of nematodes in each guild and kb is the weightings given to the guilds to indicate the features of the food web. Additionally, the following formulas were used to generate the structural indices (SI) and enrichment indices (EI):

$100x(e/(e+b))$ and $100x(s/(s+b))$, respectively.

The Nematode Indicator Joint Analysis (NINJA) software programme was used to determine the total biomass and metabolic footprints (Sieriebriennikov et al. 2014). Lastly, based on the nematode faunal composition of each field mapped in the faunal profile, the soil condition of each field sampled was categorized into quadrats A, B, C, and D (Ferris *et al*. 2001; 2004).

RESULTS AND DISCUSSION

Nematodes from all trophic levels (Plant feeders (PPNs), Bacterivores, Fungivores, Omnivores and Predators) were recovered from soils of kola tree fields sampled. A total of 27 genera from 20 families of nematodes were recovered from soil samples (Table 1; Fig 1). The most prevalent groups were omnivores (six genera), the predators (three genera), the fungivore, bacterivores and PPNs (each with eight genera). (Figs. 2A; 3A). C-p1–5 scale was used to represent the guilds, with c-p2 (10 genera) nematodes typically predominating (found in 54% of samples; see Figs. 2B and 3A). For the bacterivores group, Cephalobus was the most predominant (PV= 91.1; MPD= 101.3) found in 86.1% of soil samples. This was followed by Rhabditidae (PV= 79.9; MPD= 92.2) found occurring in 75% of the soil samples. The third most predominant Bacterivore was Eucephalobus (PV= 42.2; MPD= 67 %) recorded from 55.6% of the samples. Pseudacrobeles and Prsimatolaimus were the 2 least predominant Bacterivores from the kola field samples having PVs of 10.4 and 7.3 and found in 30.6% and 16.7% of the samples respectively. In terms of the fungivores, Aphelenchus was the most predominant (PV= 31.1; MPD= 38.4) found in 61.1% of samples. Regarding the omnivores from this study, Aporcelaimidae was the most predominant (PV= 46.8; MPD= 45.8) found occurring in 83.3% of the soil samples (Table 1). The second most predominant was Aporcelinus (PV= 25.4; MPD= 47.9) found present in 33.3 % of soil samples from the kola tree fields. The least predominant omnivores were Sectonema (PV= 10.5; MPD= 25.8) recovered from 16.7% of the samples. Result of plant parasitic nematodes (PPNs) from this study revealed that Helicotylenchus (PV= 63.2; MPD= 68.7) was the most predominant found occurring in 83.3% of soil samples while second most predominant PPN was Tylenchulus (PV= 17.4; MPD= 21.3) found occurring in 55.6% of the soil samples collected. Meloidogyne was the least predominant PPN recovered from the kola tree fields (PV= 5.3; MPD= 7.6) found in 47.2 % of the samples. Mononchus was the most predominant predatory nematode (PV= 45.8; MPD= 59.1) recovered from 61.1 % of the samples while the 2 least predominant predatory nematodes from the kola tree fields were Parahandrochus and Mylonchulus with PVs of 26.5 and 20.9 respectively (Table 1).

The c-p triangle and metabolic footprints were used to establish the ecological indices for the faunal analysis of nematode data (Fig. 4). Maturity indices ranged between 2.51 – 2.97 (Mean = 2.76) while mean Channel index (CI), Enrichment index (EI) and Structural index (SI) were 21.49, 45.33 and 77.98 respectively which indicates that most of the fields were nearly stable soil conditions without enrichment. Plant parasitic index/maturity index (PPI/MI) ratio ranged from 1.02 to 1.32 (mean =1.13) depicting a moderate proportion of PPNs to other nematode groups present. No significant difference ($P \leq 0.05$) was observed across all indices of ecosystem measured except in terms of the mean plant parasitic index and PPI/MI ratios (Table 2). Result of the food web analysis as depicted by the metabolic footprints showed that 25% of samples were plotted in quadrat B, representing maturing and enriched soil conditions while the rest 75 % lotted in quadrat C which was described as having stable and fertile soil conditions (Fig. 3A and B).

It has been determined that nematodes are excellent markers of the health of soil. The division of nematodes into trophic groups, feeding groups, food web/faunal analysis, and metabolic footprints of nematode groups have all significantly improved this technique (Yeates et al. 1993; Bongers and Bongers 1998; Ferris, 2010). Several studies have been conducted worldwide to provide insights into nematode community structures in different natural and agro-ecological systems (Schorpp and Schrader 2017; Tian et al. 2020).

Table 1. Calculated mean population densities (MPD), frequencies of occurrence (FO%), prominence values of individual nematode taxa recovered from kola tree fields in Nigeria.

Feeding group/Genus	***Family***	***Guilds***	***FO%***	***MPD***	***PV***
Bacterivores					
Cephalobus	*Cephalobidae*	Ba-2	86.1	101.3	91.1
Rhabditis	*Rhabditidae*	Ba-1	75.0	92.2	79.9
Eucephalobus	*Cephalobidae*	Ba-2	55.6	67.0	42.2
Acrobeles	*Cephalobidae*	Ba-2	66.7	45.4	35.1
Monhystera	*Monhysteridae*	Ba-2	44.4	44.1	29.4
Plectus	*Plectidae*	Ba-2	38.9	21.8	14.3
Pseudacrobeles	*Cephalobidae*	Ba-2	30.6	19.5	10.4
Prismatolaimus	*Prismatolaimidae*	Ba-3	16.7	19.2	7.3
Fungivores					
Aphelenchidae	*Aphelenchidae*	Fu-2	61.1	38.4	31.1
Paraphelenchus	*Aphelenchidae*	Fu-2	13.9	22.0	9.2
Omnivores					
Aporcelaimidae	*Aporcelaimidae*	Om-5	83.3	45.8	46.8
Aporcelinus	*Aporcelaimidae*	Om-5	33.3	47.9	25.4
Discolaimus	*Discolaimidae*	Om-4	36.1	35.4	21.3
Achromadorus	*Achromadoridae*	Om-3	52.8	28.7	20.8
Laimydorus	*Dorylaimidae*	Om-4	27.8	21.5	11.3
Sectonema	*Aporcelaimidae*	Om-5	16.7	25.8	10.5
Herbivores					
Helicotylenchus	*Hoplolaimidae*	PL-3	83.3	68.7	63.2
Paratylenchus	*Tylenchidae*	PL-3	55.6	21.3	17.4
Pratylenchus	*Pratylenchidae*	PL-3	61.1	16.4	13.1
Hoplolaimus	*Hoplolaimidae*	PL-3	33.3	21.3	12.3
Criconema	*Criconematdae*	PL-3	58.3	13.8	10.5
Xiphinema	*Longidoridae*	PL-5	75.0	10.6	9.1
Ditylenchus	*Anguinidae*	PL-2	69.4	9.8	8.2
Meloidogyne	*Meloidogynidae*	PL-3	47.2	7.6	5.3
Predators					
Mononchus	*Mononchidae*	Pr-4	61.1	59.1	45.8
Parahadronchus	*Mononchidae*	Pr-4	50.0	38.9	26.5
Mylonchulus	*Mylonchulidae*	Pr-4	47.2	31.5	20.9

Ba= Bacterivores; Fu= Fungivores; Om= Omnivores; PL= Plant feeders (PPN);
Pr= Predators

Table 2. Indices of ecosystem functions expressed by nematode taxa recovered from kola tree fields in Nigeria

Index name	Site A	Site B	Site C	Site D	ANOVA, p	Mean	SD
Maturity Index (MI)	2.76	2.97	2.8	2.51	0.32	2.76	0.19
Maturity Index 2-5 (MI2-5)	3.21	3.17	3.01	2.81	0.16	3.05	0.18
Sigma Maturity Index (SMI)	2.84	3.04	2.85	2.6	0.22	2.83	0.18
Plant Parasitic Index (PPI)	3.09	3.02	3.02	3.32	0.01	3.11	0.14
PPI/MI	1.12	1.02	1.08	1.32	0.03	1.13	0.75
Channel Index (CI)	19.92	20.37	36.6	9.07	0.37	21.49	11.35
Basal Index (BI)	12.66	17.87	19.07	19.97	0.43	17.39	3.27
Enrichment Index (EI)	65.1	29.97	40.42	45.82	0.10	45.33	14.73
Structure Index (SI)	82.32	80.16	77.87	71.57	0.21	77.98	4.64
Composite footprint (CF)	506.46	346.4	444.78	435.17	0.19	433.20	65.93
Enrichment footprint (EF)	173.7	57	95.93	104.83	0.12	107.87	48.56
Structure footprint (SF)	279.17	201.21	185.8	235.21	0.41	225.35	41.40
Total biomass (mg)	2.44	1.83	2.16	1.95	0.43	2.10	0.27

In Nigeria and west Africa, the very few information available about nematode community structure came from arable crops production systems. Previous studies from Nigeria which addressed free-living nematode community structures were those from Eche et al. (2013) who identified 43 genera of free-living nematodes from citrus, maize and yam fields of north central region of Nigeria. Also, Alabi et al. (2017) reported 4 genera from yam fields in southwest Nigeria. Therefore, to the best of our knowledge, no known study exists in respect of any forest ecological systems in Nigeria. Our present study is the first in this regard, using kola tree forests as a template for using nematode community structures in other forest ecosystem diversity and functioning studies in West Africa

In the current study, a total of 8 genera of plant parasitic nematodes with Helicotylenchus and Paratylenchus being the most predominant. Although, these two nematodes are highly destructive in many ornamental crops worldwide (Bell and Watson 2001: Davis et al. 2004; Howland and Quintanilla 2023), their predominance in kola tree fields is interesting being contrary to previous reports of PPNs abundance across southwest Nigeria where root-knots (Meloidogyne spp.) are known to be the most predominant PPNs (Bello et al. 2020). Therefore, build-up of high population of these two destructive nematodes must be discouraged since this might pose a threat to kola nut production in this region in the future.

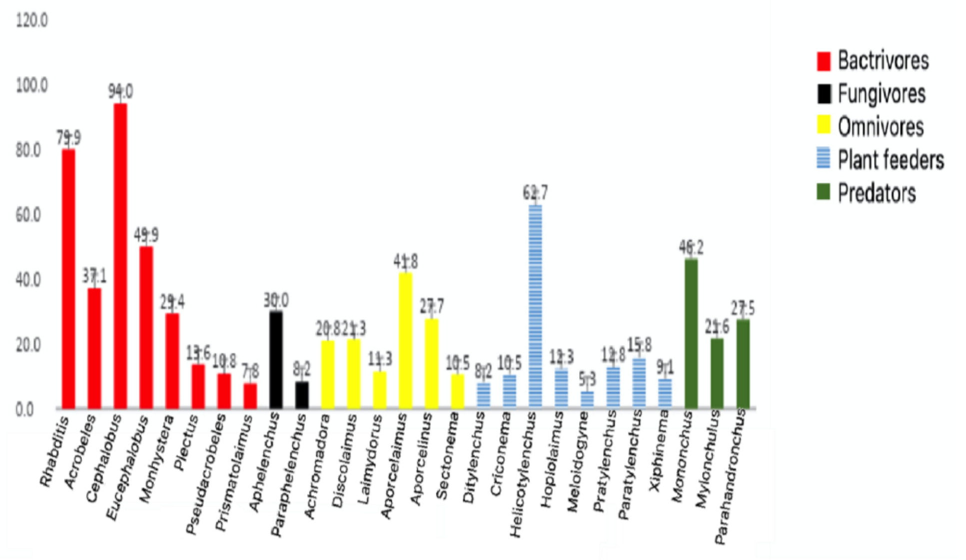

Figure 1: Mean prominent values of nematode taxa recovered from kola nut fields in Nigeria.

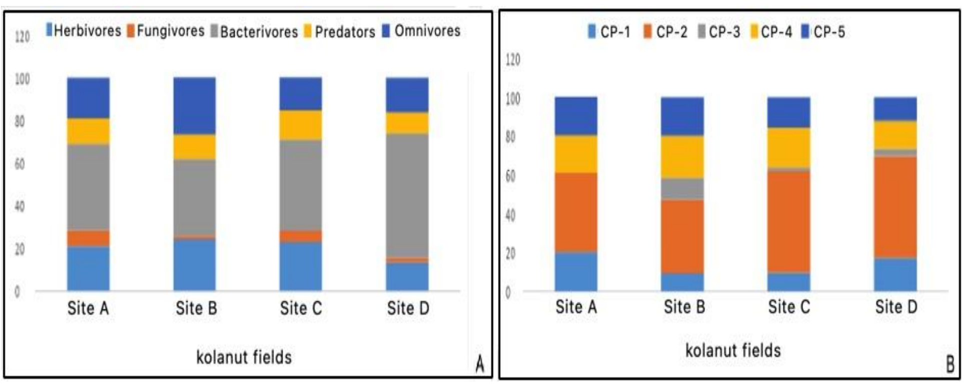

Figure. 2. A. Relative percentage of feeding type composition of nematode assemblages from kola fields in Nigeria. B. Relative percentage of colonizer-persister (C-P) structure of nematodes assemblages from kola fields in Nigeria

Bacterivores nematode group was the most predominant free-living nematodes from this current study which supports all earlier reports from Nigeria (Eche et al. 2013; Alabi et al. 2017). Generally, bacterivore nematodes have reproductive capability and short lifecycle which is a sign of enhanced soil conditions (Du Preez et al. 2022). Furthermore, it is also an established fact that community structures of soil organisms are largely dependent upon autotrophic inputs of plant as well as subsidiary inputs from other sources as depicted by enrichment and soil conservation practices (Ferris and Bongers 2006).

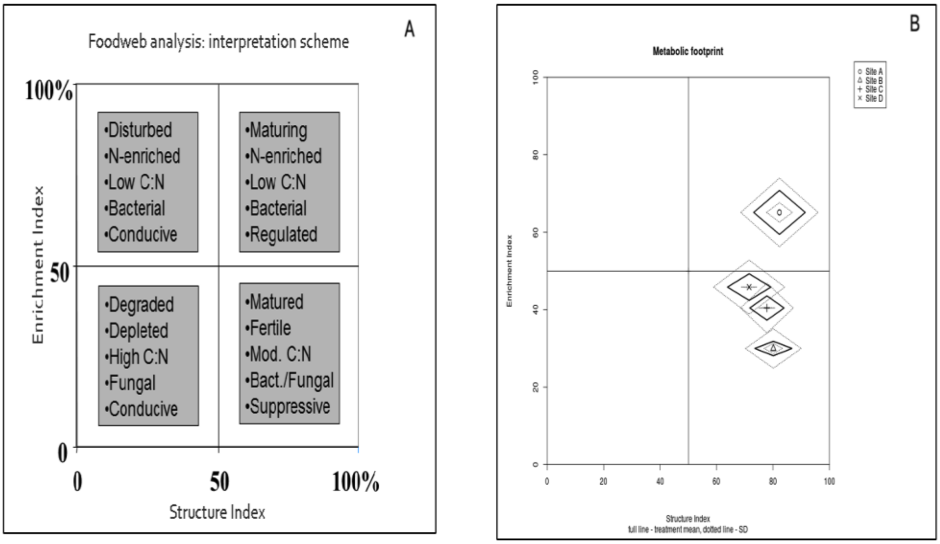

Figure 3. A. Food web analysis interpretation scheme. B. Metabolic footprints of nematodes assemblages from kola fields from Nigeria.

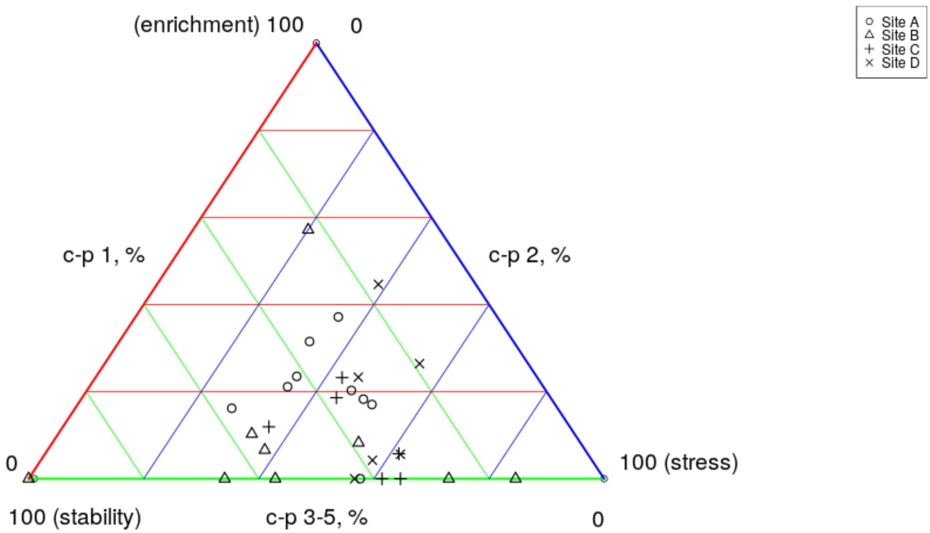

Figure 4. A c-p triangle of individual unweighted proportional representation of each c-p class represented in the nematode assemblage of kola fields in

The two most predominant free-living bacterivores nematodes from our current study: Cephalobus and Rhabditis, belong in the cp-2 and cp-1 groups respectively which is an indication of a mixture of both enriched and stressed soil conditions. Aporcelaimidae (cp-5) being the most predominant omnivores nematode recovered from this study confirms earlier reports of high abundance of the Aporcelaimidae from other cropping systems within south west Nigeria (Rashidifard et al. 2021; Bello et al. 2022b). Our findings further emphasize the importance of utilizing the concept of nematode c-p values in interpreting food web status of different soil habitats. The high mean maturity indices (MI) recorded from the fields is an indication of absence of N-fertilization or manuring since according to Bongers et al. (1997), MI values are known to decrease significantly as a result of manuring of any form.

Furthermore, Bongers et al. (1997) identified a correlation between PPI/MI ratio and soil disturbance. Therefore, we can say that the low PPI/MI ratio recorded from this current study is attributable to the low disturbance experienced due to the fact that no tillage activities was reported from any of the kola tree fields sampled in the last fifteen years. The proportionate contributions of the cp-3-5 nematodes to the cp-2-5 nematode groups are described by the structural index (SI) (Ferris et al. 2001). The high SI values recorded from our current study agrees with previous data from other forest ecosystems (Ferris et al. 2004; Cardosso et al. 2016). This also further buttresses the fact that EI, MI and SI values are important indices for measuring ecosystem functions using nematodes as indicators. Our findings further support the importance of utilizing the concept of nematode c-p values in interpreting food web status of different soil habitats. Majority (75 %), of the soil samples obtained from the kola tree fields plotted in quadrat C, was described as stable soil conditions, while the remaining 25% plotted in quadrat B represent maturing soil conditions. This implies that soils from most of the kola tree fields are in either a stable or maturing food web conditions which are characterized by low disturbance to moderate disturbance; also having a balanced bacterial mediated decomposition channels due to low C/N ratios. The results of our current study therefore agree with earlier reports which suggest a near balanced decomposition channels in most forest ecosystems that is devoid of disturbance (Waring and Running 2010).

Nematodes being the most abundant metazoan in the soil plays a crucial role in virtually all soil processes and has been identified as being an excellent indicator of soil conditions. The current study contributes to the existing information regarding the use of nematodes as soil health indicators and furthermore provides the first study of nematode assemblages in this case for kola tree fields in West and Sub-Saharan African region. Thus, comprehending that the dynamics of soil health is crucial to discerning how our ecosystem as a whole respond to problems with soil health.

CONCLUSION

Microbiome in soil consists of microbiota, macrofauna, mesofauna and microfauna, thus forming the complexity of food web. Flow of energy is supported in a properly arranged food web. This characterizes a perfect and healthy ecosystem that is primary to crop production. Food web state in soil is pivotal for forethought to achieve affordable management of cultivable lands. A representative of multiplex trophic zones in food web are nematodes. Hence projecting them as premium organisms in soil health evaluation. Helicotylenchus and Tylenchulus are the prominent pathogenic nematodes identified. More studies on the pathogenicity of the prominent plant parasitic nematodes on kola are needed in order to discourage their build up so as to avert impending problem in the future

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