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NEMATODE COMMUNITY STRUCTURE AS INFLUENCED BY LAND USE AND INTENSITY OF CULTIVATION

[INFLUENCIA DEL USO DEL SUELO Y LA INTENSIDAD DE CULTIVO SOBRE LA ESTRUCTURA DE LA COMUNIDAD DE NEMATODOS]

J.W. Kimenju*, N.K. Karanja, G.K. Mutua, B.M. Rimberia and P.M. Wachira

University of Nairobi, P. O. Box 30197-00100, Nairobi, Kenya.
Email: wkimenju@yahoo.com
**Corresponding author*

SUMMARY

This study was conducted to determine the effect of land use and intensity of land cultivation on the nematode community structure. The land use types represented in the study sites were natural forest, plantation forest, tea, coffee, napier grass, agroforestry, fallow and annual crop cultivation dominated by maize intercropped with beans. Nematode diversity and abundance decreased with intensity of land cultivation, with the natural forest being regarded as the benchmark. The decrease in nematode diversity was assessed using Shannon, Simpson and species richness indices and was used to reflect the underlying changes in physical, chemical and biological properties of soil environment. The highest maturity indices for free-living and plant parasitic index were recorded in the natural forest and intensively cultivated land under annual crops (maize/beans), respectively. Plant parasitic nematodes were predominant in soils that were under agricultural production while saprofagic nematodes dominated the forested land as exemplified by the ratios of free-living to plant parasitic which were, 5.18 and 0.54 in the natural forest and annual crop production systems respectively. Changes in the nematode community structure, as exhibited by diversity indices, may be a reflection of real differences in the soil characteristics and changes in ecosystem functions.

Key words: Abundance; diversity; richness and maturity index.

INTRODUCTION

Nematodes are small worm-like organisms which are present in almost all soil habitats where they interact directly and indirectly with plants and other microfauna, regulating decomposition and release of nutrients to the plants (Colman *et al.,* 1984). They are ubiquitous and have diverse feeding behaviors and life strategies ranging from colonizers to persistors (Bongers, 1990; Yeates, 1999). Due to their diversity in feeding habits, nematodes are an integral part of the food webs in soil ecosystems (Yeates *et al.,* 1993). In almost every soil sample, nematodes from five trophic levels namely bacteriovores, fungivores, herbivores, predators and omnivores are usually represented (Freckman and Baldwin, 1990). Phytophagous nematodes (herbivores) are the most intensively studied group because of their economic importance as biotic constraints to crop production. However, as the role of soil nematodes in regulating soil bacterial and fungal populations and thus cycling of major soil nutrients becomes clear, a more inclusive view of nematodes is becoming established (Yeates and Bongers, 1999). Consequently, focus is shifting from plant parasitic nematodes to the entire nematode community in the soil.

The diversity of nematodes in agro-ecosystems and the total abundance of members of different trophic levels are largely controlled by the biophysical, chemical and hydrological conditions of the soil (Yeates and Bongers, 1999). The soil as a habitat for nematodes can be changed through management practices such as monoculture, tillage, drainage, application of agrochemicals, irrigation and organic mulch (Freckman and Ettema, 1993; Yeates, 1999). For instance, nematode abundance was higher in high organic input systems than in perennial cropping systems while species diversity was greatest under minimum tillage treatments (Freckman and Ettema, 1993) According to Yeates (1999), nematode diversity tends to be greatest in ecosystems experiencing long-term human interference and changes in nematode community may be a reflection of changes in soil and ecological processes. Nematodes interact with other soil organisms in complex food webs to provide essential functions and ecosystem services which include maintenance of soil structure, carbon sequestration, bio-control of pests and diseases, soil detoxification and nutrient cycling.

The last decade has witnessed increased sensitivity to loss of diversity as a result of pollution, agricultural intensification, greenhouse effect, modification of

global carbon and nitrogen cycles (Asner *et al.*, 1997). The status of below-ground biodiversity is however, not conclusively documented and little is known of the effects of land use on the diversity especially in the tropics. Given the ease of recovering nematodes from soil coupled with the relatively quick identification to an acceptable taxonomic level makes them potential indicators of the impact of changing land use and soil conditions (Yeates and Bongers, 1999). This study was, therefore, undertaken to establish the effect of land use and agro-ecosystem management on the nematode community structure.

MATREIAL AND METHODS

The study was conducted in two benchmark sites namely Embu in the highlands of central Kenya and Taita Taveta in the coastal highlands. The soil in Embu was classified as humic nitisoils while Taita Taveta soils were classified as humic cambisols (Jaetzold and Schmidt, 1983; FAO, 1989). Soil samples were taken from 60 sampling points, distributed among the main land use types at each benchmark site. The sampling points were marked using a grid and were 200m apart. At each sampling point, two vertically crossing lines and two concentric circles of radius 3 and 6m were drawn. An auger was used to take four samples from the 0-20 cm soil depth in the small circle and eight in the outer circle (Wachira *et al.*, 2008)

The 12 sub-samples were mixed homogeneously to constitute a composite sample from which 500g of soil was taken, placed in a plastic bag, sealed and then kept under cool conditions. The samples were transported to the laboratory in a cool box and stored at 10^oC.

Nematodes were extracted from the soil using the sieving and centrifugation techniques (Jenkins, 1964). A soil sub-sample, $200cm^3$, was drawn from the composite sample and placed in a bucket to which five litres of water was added. The suspension was agitated by stirring and allowed to sediment for two minutes. It was then poured through a 60-mesh screen and nematodes were collected on a 400-mesh screen. The nematode suspension was further clarified using the modified centrifugal and sugar flotation method. The suspension was spinned at 3500 rpm for 4 minutes and the supernatant was discarded. The residue in the centrifuge tubes were then re-suspended in 48% sugar solution and spinned again at 1000 rpm for two minutes. Nematodes were collected by pouring the supernatant through a 400-mesh sieve.

The nematodes were killed by heating the suspension in a water bath at 50-70 ^oC and then fixed with Golden solution which was made by mixing 40% formaldehyde, glycerine and distilled water in the ratio of 8:2:90 as described Hopper (1970). The nematodes were enumerated by pipetting 2ml of the suspension into a counting slide. The total number was recorded as the mean of three counts. For glycerine infiltration, the nematode suspension was reduced to 3ml, to which 7 ml of Seinhorst solution I (96% alcohol: glycerine: distilled water) was added. The suspension was then placed in a desiccator at 43^oC overnight. The suspension was then dried at the same temperature to reduce the volume which was then adjusted to 10ml using Seinhorst solution II (96% alcohol: glycerine at the ratio of 95:5), and then incubated overnight. The process was repeated three times at the same temperature for at least 48 hours to evaporate all the alcohol. After this process the nematodes from the dish were mounted on the slides. Twenty five nematodes from the slides in each sample were selected for identification to genus level under a compound microscope at a magnification of 400-1000.

The nematode families and genera were assigned to trophic groups (bacterial and fungi feeders, plant parasites, omnivores and predators as described by Yeates *et al.* (1993). Taxonomic groups were also assigned to colonizer-persistor c-p values, according to Bongers (1990).

The data were presented according to the following parameters; total abundance, trophic groups, genus richness index ($d = (S-1) \log N$, where S = Number of genera and N = total number of nematodes, Simpsons diversity index ($Ds = 1- \sum(Pi)^2$, where Pi = percent of genus "ι" in the total abundance). Shannon Wiener's diversity index ($H' = - \sum P_i \log_2 P_i$), evennessess of Simpson's diversity index ($Es = Ds/ Dsmax$ where $Ds_{max} = 1- 1/s$).

The maturity index (MI) based only on free-living nematodes and the plant parasitic index (PPI) (including plant parasites only) were both calculated using the formula illustrated by Bongers (1990), $\sum v_i \times f_i$ where, v_i = c-p value from 1 to 5 for the taxon"i" and f_i = relative frequency of taxon "i" but the opportunist nematodes excluded in the calculation of PPI, which was used in calculation of pollution induced stress factors and the PPI/MI ratio to assess soil fertility (Bongers and Bongers, 1998). All analyses were based on the relative abundance of nematode genus and analysis of variance. Divers and GenStat statistical packages were used for data analysis.

On soil analysis, available nutrient elements (PK Na Ca Mg and Mn) were analyzed through Mehlich double Acid method. Total organic carbon was estimated through Calorimetric method where the carbon concentration was read on the spectrophotometer at 600nm. Total nitrogen was estimated through Kjeldahl method where soil samples

were digested with concentrated sulphuric acid containing potassium sulphate, selenium and copper sulphate hydrated at approximately 350^0c. Total nitrogen was determined calorimetrically on a flow analyzer. Soil pH – Water was determined in 1:1 (w/v) soil – water suspension with pH meter. The Extractable phosphorus was estimated through olsen method (for soils with pH 7.0 and above). The dried soil samples were extracted in a ratio (w/v) with 0.5 M sodium bicarbonate solution at pH 8.5. Phosphorus in the extract was determined spectrophotometrically.

Statistical analysis

All the data collected was subjected to analysis of variance using GENSTAT Discovery Edition 3 computer package

RESULTS

Nematodes from 25 genera and 21 different families were recovered from the main land use types represented in Embu and Taita benchmark sites (Table 1a and 1b). The nematodes were classified as herbivores (PF), bacteriovores (BF), fungivores (FF), omnivores (OM) and predatory (PR). Herbivores were predominant in the agroecosystems while bacteriovores, fungivores, omnivores and predatory nematodes dominated in the natural and plantation forests.

Table 1a. Distribution of nematode communities in different land use systems in Embu.

				Land use types					
Family	Genera	C-P[a]	Trophic group[b]	Tea	Coffee	Napier	Maize	Natural forest	Plantation forest
Hoplolaimidae	*Helicotylenchus*	*3*	PF[b]	84	135	54	158	19	78
Tylenchidae	*Tylenchulus*	2	PF	4	0	16	0	15	11
Meloidogynidae	*Meloidogyne*	3	PF	26	113	6	113	38	35
Pratylenchidae	*Pratylenchus*	3	PF	24	86	20	172	9	22
Tylenchidae	*Tylenchus*	2	PF	74	10	62	60	104	72
Belonolaimidae	*Tylenchorhynchus*	3	PF	23	24	7	25	25	21
Hoplolaimidae	*Scutellonema*	3	PF	18	40	34	82	14	29
Hoplolaimidae	*Rotylenchus*	3	PF	16	4	0	0	9	4
Hoplolaimidae	*Hoplolaimus*	3	PF	20	14	5	113	23	44
Criconematidae	*Criconema*	3	PF	118	25	170	16	84	110
Criconematidae	*Hemicriconemoides*	3	PF	25	23	40	8	23	45
Longidoridae	*Xiphinema*	5	PF	5	0	24	0	88	67
Trichodoridae	*Trichodorus*	4	PF	0	12	5	15	10	14
Longidoridae	*Longidorus*	5	PF	4	1	15	0	103	51
Hemicyclophoridae	*Hemicycliphora*	3	PF	0	0	0	5	51	11
Cephalobidae	*Acrobeles*	2	BF[c]	1	0	38	0	134	91
Monochidae	*Mononchus*	4	PR[d]	4	12	46	0	84	89
Rhabditidae	*Rhabditis*	1	BF	9	14	14	8	180	59
Cyatholaimidae	*Chromadora*	3	OM[e]	0	1	9	8	213	86
Cephalobidae	*Cephalobus*	2	BF	4	0	6	0	64	17
Bunonematidae	*Bunonema*	1	BF	0	0	4	0	20	3
	Prodorylaimus	5	OM	0	0	0	0	43	2
Aphelenchoididae	*Aphelenchoides*	2	FF	9	0	0	11	54	16
Cephalobidae	*Eucephalobus*	2	BF	0	5	9	0	9	17

[a]Colonizer-persistor scale I-5 where cp 1 are colonizers characterized by short generation time and cp 5 are persisters characterized by long generation time (Bongers, 1990). [b]PF = plant feeders, BF = bacterial feeders, PR = Predacious OM=omnivores, FF=fungal feeders.

Table 1b. Distribution of nematode communities in different land use systems in Taita Taveta.

Family	Genera	C-P[a]	Trophic group[b]	Land use types: Veg	Coffee	Fallow	Maize	Natural forest	Plantation forest
Hoplolaimidae	*Helicotylenchus*	*3*	PF[b]	131	109	70	96	29	19
Tylenchidae	*Tylenchulus*	2	PF	18	112	8	9	80	68
Meloidogynidae	*Meloidogyne*	3	PF	127	54	59	190	7	21
Pratylenchidae	*Pratylenchus*	3	PF	29	41	69	6	14	0
Tylenchidae	*Tylenchus*	2	PF	41	42	18	85	16	10
Belonolaimidae	*Tylenchorhynchus*	3	PF	70	117	30	60	48	0
Hoplolaimidae	*Scutellonema*	3	PF	144	96	107	115	14	38
Hoplolaimidae	*Rotylenchus*	3	PF	46	90	110	89	38	0
Hoplolaimidae	*Hoplolaimus*	3	PF	56	39	122	59	60	20
Criconematidae	*Criconema*	3	PF	26	9	66	11	73	12
Criconematidae	*Hemicriconemoides*	3	PF	26	3	38	1	4	29
Longidoridae	*Xiphinema*	5	PF	0	3	26	18	73	37
Trichodoridae	*Trichodorus*	4	PF	24	14	29	68	28	23
Longidoridae	*Longidorus*	5	PF	0	3	32	3	55	23
Hemicyclophoridae	*Hemicycliphora*	3	PF	0	0	2	5	12	1
Cephalobidae	*Acrobeles*	2	BF[c]	9	58	94	41	111	0
Monochidae	*Mononchus*	4	PR[d]	20	6	95	7	57	139
Rhabditidae	*Rhabditis*	1	BF	56	21	4	7	157	21
Cyatholaimidae	*Chromadora*	3	OM[e]	32	40	31	13	93	85
Aphelenchoididae	*Aphelenchoides*	2	FF	4	56	102	23	98	91
Cephalobidae	*Eucephalobus*	2	BF	0	15	46	0	12	38
Qudsinematidae	*Labronema*	4	OM	1	55	8	0	12	0
Plectidae	*Plectus*	2	BF	41	74	102	28	14	0
Nygolaimidae	*Nygolaimus*	5	PR	28	51	8	0	61	3
Aphelenchidae	*Aphelenchus*	2	FF	0	39	4	1	106	149

[a]Colonizer-persistor scale I-5 where cp 1 are colonizers characterized by short generation time and cp 5 are persisters characterized by long generation time (Bongers, 1990). [b]PF = plant feeders, BF = bacterial feeders, PR = Predacious OM=omnivores, FF=fungal feeders. Veg. = vegetable.

The total nematode numbers varied significantly ($P<0.05$) among the land use systems at the two benchmark sites (Table 2). Nematode abundance was highest in the natural forest followed by planted forest while it was lowest in the coffee fields in the Embu benchmark. Among the agricultural land uses, nematode abundance was highest in maize followed by tea and least under coffee. Genus richness was significantly ($P< 0.05$) higher in the natural forest, followed by plantation forest in Embu. In agro-ecosystems, genus richness was lowest in maize/bean and vegetable production systems in Embu and Taita Taveta, respectively.

The abundance of plant parasitic nematodes was highest under maize and coffee in Embu and Taita-Taveta, respectively (Table 3).The ratio of free-living nematodes to plant parasitic nematodes (R) showed a general decline in land under agricultural use, reflecting dominance by plant parasitic nematodes. The proportion of free-living to plant parasitic nematodes was highest in the natural forests followed by the plantation forests at both sites.

Shannon diversity indices were variable among the land use systems under test (Table 4). The Shannon diversity index was higher in natural forest and planted forests as compared to intensively cultivated systems under annual crops. Among the agricultural land uses, Shannon indices were higher in tea and napier than in coffee and maize systems. Simpson's index showed a similar trend where diversity was highest in the natural forest followed by the plantation forest. In agricultural

land, Simpson's diversity index was highest in tea, intermediate under napier and lowest under maize.

Table 2. Genus richness and relative abundance of nematodes across the land uses in Embu and Taita benchmark sites.

	Embu			Taita-Taveta	
Land use	Genus richness	Abundance	Land use	Genus richness	Abundance
Plantation forest	6.68	832	Plantation forest	4.64	902
Coffee	3.87	446	Coffee	5.39	1338
Maize	2.68	667	Maize	4.97	1082
Napier	5.58	492	Fallow	7.27	1288
Natural forest	6.89	1039	Natural forest	8.23	1379
Tea	5.10	449	Vegetable	4.11	1067
LSD ($p_{<0.05}$)	0.8	101	LSD ($p_{<0.05}$)	0.78	231

Table 3. Comparison of plant parasitic and free-living nematodes in different land use systems.

	Embu			Taita-Taveta		
Land use	PPN	FL	FL:PPN	Land use	PPN	FL	FL:PPN
Plantation forest	440.8	592.5	1.34	Plantation forest	353	526	1.49
Coffee	587.8	428.7	0.73	Coffee	948	419	0.44
Maize	672.5	360.5	0.54	Maize	938	120	0.13
Napier	508.8	480.0	0.94	Fallow	796	458	0.57
Natural forest	197.5	931.0	4.71	Natural forest	189	759	4.01
Tea	547.5	447.5	0.82	Vegetable	915	191	0.21
LSD ($p_{<0.05}$)	23.8	30.0		LSD ($p_{<0.05}$)	122	137	

PPN-Plant parasitic nematodes, FL-Free-living nematodes, FL: PPN-Ratio of free-living to plant parasitic nematodes

Table 4. Effect of land use on nematode communities measured using Shannon and Simpson indices in Embu and Taita Taveta benchmark sites.

	Embu benchmark site			Taita benchmark site	
Land use	Shannon Index	Simpson Index	Land use	Shannon Index	Simpson Index
Plantation forest	2.722	0.928	Plantation forest	2.394	0.891
Coffee	2.005	0.831	Coffee	2.565	0.909
Maize	1.692	0.780	Maize	2.637	0.884
Napier	2.322	0.860	Fallow	2.782	0.929
Natural forest	2.722	0.919	Natural forest	2.883	0.938
Tea	2.274	0.865	Vegetable	2.362	0.890
LSD ($p_{<0.05}$)	0.188	0.040	LSD ($p_{<0.05}$)	0.159	0.022

The maturity index (MI) was significantly ($P<0.05$) variable among the land uses in the two sites (Table 5). The highest MI was recorded in the natural forests while the lowest was in coffee and vegetable fields in Embu and Taita, respectively. Within the land under agricultural use, MI was higher in tea compared to maize and coffee. The maturity index minus opportunists (MINO) was also found to be higher in the natural forest when compared to the other land uses. Among the land uses under crop cultivation, MINO was highest in maize/bean than in the other crops in Embu. Plant parasitic index (PPI) was also variable among the land uses, being highest in the intensively cultivated land under crops (maize/beans) in both benchmark sites.

Table 5. Maturity indices of nematode communities in the Embu and Taita Benchmark sites.

	Embu benchmark					Taita benchmark			
Land use	MI	MINO	PPI	PPI/MI	Land use	MI	MINO	PPI	PPI/MI
Plantation forest	3.033	2.92	2.46	0.81	Plantation forest	2.86	3.12	1.27	0.44
Coffee	0.151	0.14	1.81	12.0	Coffee	2.37	2.61	2.99	1.26
Maize	0.180	1.60	2.82	15.67	Maize	0.49	0.53	3.17	6.47
Napier	1.045	1.00	1.72	1.65	Fallow	2.28	2.56	2.74	1.20
Natural forest	5.515	5.02	2.42	0.44	Natural forest	3.65	3.74	2.22	0.61
Tea	0.209	0.13	1.51	5.21	Vegetable	0.49	0.53	3.05	6.22
LSD ($p_{<0.05}$)	0.086	0.02	0.07		LSD ($p_{<0.05}$)	0.01	0.12	0.03	

DISCUSSION

This study has revealed that nematode diversity decreases with intensity of land cultivation or soil disturbance. The natural forest can be considered as the benchmark since it has the highest diversity and abundance of nematodes of different trophic levels. Natural forest ecosystems are characterized by long-term freedom from human interference including application of agrochemicals have high aboveground diversity and soil organic matter content. Disturbance of the natural forest through felling of indigenous trees, followed by establishment of single species plantations resulted in a decline in nematode abundance and species richness. According to Bloemers *et al*., (1997), disturbance not only changes species but also the species composition. Considering the biological characteristics of nematodes and the diversity exhibited within their community, variability would be expected in the response of members of different trophic levels to disturbance. Indeed, free-living nematodes are more sensitive to ecosystem disturbances making them potential bio-indicators of the changes in the soil environment (Bongers and Bongers, 1998).

Agricultural intensification is usually associated with increased disturbance of the soil through tillage, indiscriminate use of mineral fertilizers and pesticides, manipulation of organic residues and planting of a narrow range of plant genotypes or complete monoculture (Yeates *et al*., 1999). In the long-run these attributes inevitably interfere with the functions of any ecosystem (Giller *et al*., 1997). Among other fundamental ecosystems functions, biological control of pests and diseases such as plant parasitic nematodes is disrupted resulting into population build-up. Some of the available options to reverse the trend include diversified cropping systems encompassing multiple cropping and crop rotation, conservation agriculture (based on minimum tillage and cover cropping) and organic farming.

It is not in doubt that plant density and heterogeneity of plant communities vary with levels of human interference with natural forests and management practices in agro-ecosystems. Given that nematodes are heterotrophs, plants play both direct and indirect roles in structuring of their communities because they ultimately depend on them (Yeates, 1999). Consequently, different land use types result in different plant community structures and ultimately in different decomposition and nutrient cycling pathways (Cadish and Giller, 1997). Rhizosphere processes link plants to the soil and root-feeding nematodes are known to increase the supply of carbon from roots to the soil microbial biomass (Young, 1998; Yeates, 1999). For example, an experiment on extended clean fallow revealed that removal of all plants had a suppressive impact on predacious, bacterial and fungal feeding nematodes (Wardle *et al*., 1999). Additional evidence has been adduced showing that values for both diversity and maturity indices were higher in mixed species grass swards than under monoculture (Wasilewska, 1995).

The plant community at any site directly affects herbivorous nematodes. The correlation between plant host and nematode population growth is particularly strong in host specific herbivorous nematodes (Yeates, 1999). Phytonematodes with broad host ranges feed on many crop and non-crop plants where their effects are usually neglected. The effect of plants on decomposer components (macro and micro flora feeding nematodes) is indirect because they do not feed directly on the plants present.

An increase in the proportion of plant parasitic nematodes (herbivores) was associated with increase in ecosystem disturbance. The trend denotes increased dominance of herbivorous nematodes with increase in agricultural intensification. These changes in nematode community structure could be indicative of wide ranging changes in physical, chemical and

biological properties of the soil. A study by Kandji *et al*. (2001) demonstrated that changes in soil characteristics particularly the physio-chemical properties influence the abundance, distribution and structure of nematode communities. According to Yeates and Bongers (1999), the decrease in diversity of the nematode faunae with increasing intensity of cultivation can be attributed to physical disturbance, change in quantity and quality of organic matter being returned to the soil and increase in numbers of specific plant feeding nematodes that are favoured by the crops selected.

Nematode abundance was higher in the maize/bean land use compared to monocultures under coffee, napier or tea. High inputs of agrochemicals particularly pesticides in coffee and fertilizers in tea can be the main contributing factors. In addition, monocultures tend to favour certain groups of nematodes. In a related study on the effect of human intervention on nematode communities, Freckman and Ettema (1993) reported that nematode abundance was higher under annual crops compared with perennial cropping systems. These differences may be a reflection of the changes that are attributed to monoculture and its influence on availability of suitable food especially for the plant parasitic nematodes. Genus richness was lowest in land under annual crop cultivation which could be rated as the most disturbed ecosystem. This was consistent with findings by Bouwman and Zwart (1994) who reported that crop fields receiving agrochemical and tillage inputs had increased total nematode biomass which was dominated by herbivores.

CONCLUSION

The study has revealed that land use has an effect on the nematode community structure. Increase in soil disturbance results in increase of plant parasitic nematodes. This could be attributed to the increased feeding sites for the nematodes for example the many plant root produced by a crop due to fertilizer application. Also intensive agriculture is characterized with cultivation of different crops which supports may different nematodes. Mono cropping results in decline of nematode numbers and also species richness. This particular observation could be used as one of the cultural methods of nematodes control on farms.

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