

## Impact of Land Use Changes on Nematode Diversity and Abundance

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### Abstract

This study was conducted to determine the effect of land use on 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 crops dominated by maize and beans. Nematode diversity and abundance decreased with intensity of land cultivation or human interference, 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 (MI) for free-living and plant parasitic index (PPI) were recorded in the natural forest and intensively cultivated land under annual crops (maize/beans) respectively. Herbivorous 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 ecosystems respectively. Changes in the nematode community structure as exhibited by diversity indices may be a reflection of real differences in soil and ecosystem functions.

**Keywords:** Abundance, community, diversity, land-use, richness and maturity index.

### Introduction

Nematodes are small worm-like organisms which are present in almost all agro-ecosystems where they interact directly and indirectly with plants and other microfauna, regulating decomposition and release of nutrients to the plants (Colman *et al.*, 1984). Nematodes are ubiquitous and have diverse feeding behaviors and life strategies ranging from colonizers to persistors (Bongers, 1990; Yeates, 1999). Due to their diversity in biological and particularly 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 positive 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 input organic 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 belowground 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 ability to identify them 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 changes in land use and agro-ecosystems management on nematode community structure.

### **Methodology**

The study was conducted in two benchmark sites namely Embu in the highlands of central Kenya and the coastal highlands in Taita-Taveta. The soil in Embu is classified as Humic Nitisols (FAO, 1989) and Humic Cambisols (Jaetzold and Schmidt, 1983) in Taita Taveta. Soil samples were taken from 60 pre-determined sampling points, distributed among the main land use types at each benchmark sites. 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 depth in the small circle and eight in the outer circle as shown in the figure below.

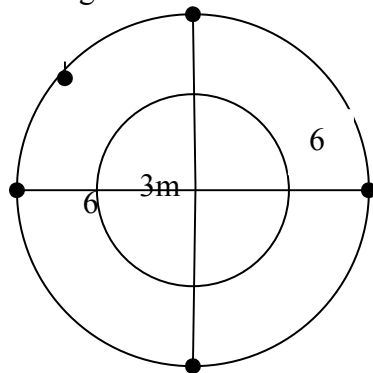


Figure 1: Schematic representation of the 12 soil sampling points

The 12 sub-samples were mixed homogeneously to constitute a composite sample from which 500g of soil were taken, placed in a plastic bag, sealed and then kept under shade. The samples were then transported to the laboratory in a cool box and stored at 4°C. Nematodes were extracted from the soil using the sieving and centrifugation techniques (Jenkins, 1964). A soil sub-sample, 200cm<sup>3</sup>, was drawn from the composite sample and placed in a bucket to which two litres of water was added. The suspension was agitated for about 30 seconds and allowed to sediment for 2 minutes, 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 centrifugation sugar flotation method. By this method, the suspension is spinned at 3500 rpm for 4 minutes and the supernatant discarded. The residue in the centrifugal tubes were then re-suspended in 48% sugar solution and spinned once again at 1000 rpm for 2 minutes. Nematodes were then collected by pouring the supernatant through a 400-mesh sieve. The nematodes were heat killed in a water bath at 50-70 °C and fixed with Golden solution (40% formaldehyde: glycerine: distilled water mixed in the ratio of 8:2:90) using the method by 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, and 7ml of Seinhorst1 solution (96% alcohol: Glycerine: distilled water) was added. The suspension was then placed in a desiccator at 43°C over night. The suspension was then dried at the same temperature to reduce the volume which was then adjusted to 10ml using Seinhorst 11 solution (96% alcohol: glycerine: 95:5 parts respectively), and the dish incubated overnight again. The process was repeated three times, with the dish being maintained 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. One hundred nematodes from the slides in each sample were randomly 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) (Yeates *et al.*, 1993). Taxonomic groups were also assigned to colonizer-persister c-p values according to Bongers, (1990). The data were presented according to the following parameters; total abundance, trophic groups, species richness index ( $d = (S-1) \log N$ , where  $S$  = Number of genera and  $N$  = total number of nematodes, Simpsons diversity index ( $D_s = 1 - \sum(P_i)^2$ , where  $P_i$  = percent of genus "i" in the total abundance). Shannon Wiener's diversity index ( $H' = - \sum P_i \log_2 P_i$ ), evenness of Simpson's diversity index ( $E_s = D_s / D_{s_{max}}$  where  $D_{s_{max}} = 1 - 1/s$ ). 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 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 & Bongers, 1998). All analyses were based on the relative abundance of nematode genus and analysis of variance conducted on the data sets. Divers and GenStat statistical packages were used for data analysis.

## 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 b). The nematodes could be grouped into four main trophic levels: herbivores (PF), bacteriovores (BF), fungivores (FF), omnivores (OM) and predatory (PR) nematodes. Herbivores were predominant in the agroecosystems while bacteriovores, fungivores, omnivores and predatory nematodes dominated in the natural and plantation forests.

**Table 1a: Nematode communities and their distribution in the different land use systems in Embu**

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

<sup>a</sup>Colonizer-persister 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). <sup>b</sup>Plant feeders <sup>c</sup>Bacteriovores <sup>d</sup>Predacious <sup>e</sup>Omnivores

**Table 1b: Nematode communities and their distribution in the different land use systems in Taita-Taveta**

Family	Genera	Trophic C-P <sup>a</sup> group	Land use types					
			Veg	Coffee	Fallow	Maize	Natural forest	Plantation forest
Hoplolaimidae	<b>Helicotylenchus</b>	3 PF <sup>b</sup>	131	109	70	96	29	19

Tylenchidae	<i>Tylenchulus</i>	2	PF	18	112	8	9	80	68
Meloidogynidae	<i>Meloidogyne</i>	3	PF	127	54	59	190	7	21
Pratylenchidae	<i>Pratylenchus</i>	3	PF	29	41	69	6	14	0
Tylenchidae	<i>Tylenchus</i>	2	PF	41	42	18	85	16	10
Belonolaimidae	<i>Tylenchorhynchus</i>	3	PF	70	117	30	60	48	0
Hoplolaimidae	<i>Scutellonema</i>	3	PF	144	96	107	115	14	38
Hoplolaimidae	<i>Rotylenchus</i>	3	PF	46	90	110	89	38	0
Hoplolaimidae	<i>Hoplolaimus</i>	3	PF	56	39	122	59	60	20
Criconematidae	<i>Criconema</i>	3	PF	26	9	66	11	73	12
Criconematidae	<i>Hemicriconemoides</i>	3	PF	26	3	38	1	4	29
Longidoridae	<i>Xiphinema</i>	5	PF	0	3	26	18	73	37
Trichodoridae	<i>Trichodorus</i>	4	PF	24	14	29	68	28	23
Longidoridae	<i>Longidorus</i>	5	PF	0	3	32	3	55	23
Hemicyclophoridae	<i>Hemicycliphora</i>	3	PF	0	0	2	5	12	1
Cephalobidae	<i>Acrobeles</i>	2	BF <sup>c</sup>	9	58	94	41	111	0
Monochidae	<i>Mononchus</i>	4	PR <sup>d</sup>	20	6	95	7	57	139
Rhabditidae	<i>Rhabditis</i>	1	BF	56	21	4	7	157	21
Cyatholaimidae	<i>Chromadora</i>	3	OM <sup>e</sup>	32	40	31	13	93	85
Aphelenchoididae	<i>Aphelenchoides</i>	2	FF	4	56	102	23	98	91
Cephalobidae	<i>Eucephalobus</i>	2	BF	0	15	46	0	12	38
Qudsinematidae	<i>Labronema</i>	4	OM	1	55	8	0	12	0
Plectidae	<i>Plectus</i>	2	BF	41	74	102	28	14	0
Nygolaimidae	<i>Nygolaimus</i>	5	PR	28	51	8	0	61	3
Aphelenchidae	<i>Aphelenchus</i>	2	FF	0	39	4	1	106	149

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 system in the Embu benchmark. Among the agricultural land uses, nematode abundance was highest in maize followed by tea and least under coffee. Species richness (SR) was found to be significantly ( $P < 0.05$ ) higher in the natural forest and closely followed by plantation forest in Embu. Species richness indices in agro-ecosystems were lowest maize and vegetables in Embu and Taita, respectively.

**Table 2: Species richness and relative abundance of nematodes across the land uses in Embu and Taita benchmark sites<sup>x</sup>**

Land use	Embu <sup>x</sup>		Land use	Taita-Taveta <sup>x</sup>	
	Species richness	Abundance		Species richness	Abundance
Abundance					

					4.64 c
					5.39 c
<b>Plantation forest</b>				Plantation forest	4.97 c 902 ab
Coffee	6.68 a	832 b		Coffee	7.27 b 1338 a
Maize				Maize	8.23 a 1082 ab
Napier	3.87 d	446 d		Fallow	4.11 cd 1288 a
Natural forest	2.68 e	667 c		Natural forest	0.78 1379 a
Tea	5.58 b	492 d		Vegetable	1067 ab
LSD (p<0.05)	6.89 a	1039 a		LSD (p<0.05)	231
	5.10 b	449 d			
	0.8	101			

<sup>x</sup> Means followed by different letters are significantly different

Plant parasitic nematode numbers were highest under maize in Embu, coffee and maize in Taita-Taveta (Table 3, Fig. 2a & b). The ratio of free-living nematodes to plant parasitic nematodes (R) showed a general decline for the land use systems under agricultural use reflecting dominance by plant parasitic nematodes. The proportions of free-living to plant parasitic nematodes was highest in the natural forests followed by the plantation forests at both sites.

**Table 3. Comparison of plant parasitic to free-living nematodes for different land use systems<sup>a</sup>**

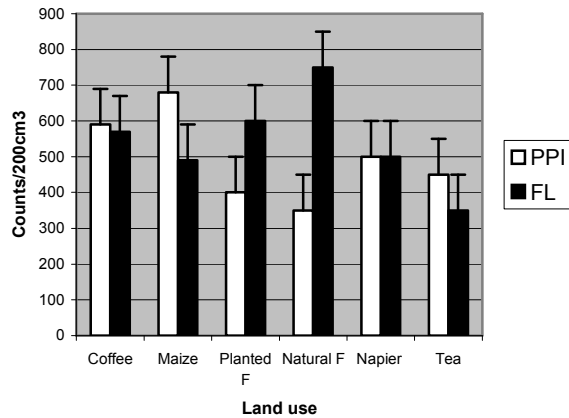
Land use	Embu			Taita-Taveta		
	Land use	R		Land use	R	
<b>Plantation forest</b>						
		440.8 e			353 b	
		587.8 b			948 a	
Coffee		672.5 a		Plantation forest	938 a	526 b 1.49
Maize		508.8 d	592.5 b 1.34	Coffee	796 ab	419 b 0.44
Napier		197.5 f	428.7 d 0.73	Maize	189 b	120 c 0.13
Natural forest		547.5 c	360.5 e 0.54	Fallow	915 a	458 b 0.57
Tea		23.8	480.0 c 0.94	Natural forest	122	759 a 4.01
LSD (p<0.05)			931.0 a 4.71	Vegetable		191 c 0.21
			447.5 d 0.82	LSD (p<0.05)		137
			30.0			

<sup>a</sup> Means followed by different letters are significantly different

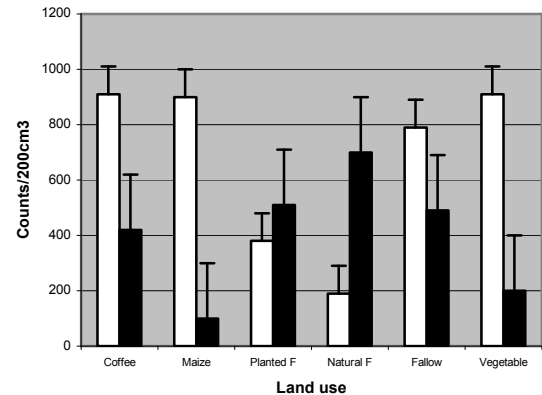
**PPN-Plant parasitic nematodes, FL-Free-living nematodes, FL: PPN-Ratio of free-living to plant parasitic nematodes**

Plant parasitic nematodes were dominant in maize, coffee, and tea systems while napier had almost equal numbers of plant parasitic and free-living nematodes (Figure 2a and b). Plant parasitic nematode populations were highest in maize and least in natural forest while the inverse was the case for the free-living nematodes. Free-living nematodes under natural and plantation forests were dominant.

a). Embu



b). Taita-Taveta



PPN-Plant parasitic nematodes, FL-Free living nematodes

**Figure 2. Abundance of plant parasitic and free-living nematodes in Taita-Taveta and Embu benchmark sites**

Shannon diversity indices were variable among the land use systems (Table 4). The Shannon diversity 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 Diversity index showed a similar trend where diversity was highest in the natural forest followed by the plantation forest. Under agriculture practices, Simpson's Diversity index was highest in tea, intermediate under napier and coffee and lowest under maize.

Table 4: Effect of land use on nematode communities measured using Shannon and Simpson indices\*

Embu			Taita		
Land use	Shannon Index	Simpson Index	Land use	Shannon Index	Simpson Index
<b>Plantation forest</b>			Plantation forest	2.394 b	0.891 b
Coffee	2.722 a	0.928 a	Coffee	2.565 a	0.909 b
Maize	2.005 c	0.831 d	Maize	2.637 a	0.884 d
Napier	1.692 d	0.780 e	Fallow	2.782 a	0.929 a
Natural forest	2.322 b	0.860 d	Natural forest	2.883 a	0.938 a
Tea	2.722 a	0.919 b	Vegetable	2.362 b	0.890 bc
LSD ( $p < 0.05$ )	2.274 b	0.865 c			
	0.188	0.040	LSD ( $p < 0.05$ )	0.159	0.022

\*Means followed by different letters are significantly different.

## Discussion

This study has revealed that nematode diversity decreases with intensity of land cultivation or human interference. 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 frequently 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 monotypes (Yeates *et al.*, 1999). These attributes inevitably interfere, in the long-run, with the functions of any ecosystem (Giller *et al.*, 1997). Among other fundamental ecosystems functions, biological control of pests and diseases such as plant nematodes is disrupted resulting into population build-up. Some of the available options to reverse the trend include diversified agricultural systems (based on multiple cropping and crop rotation), conservation agriculture (based on minimum tillage and cover cropping) and organic farming. It is anticipated that plant density and heterogeneity of plant communities vary with levels of human interference in natural settings and management requirements in agro-ecosystems. In accordance to expectations, plants play both direct and indirect roles in structuring of the nematode communities because nematodes are heterotrophs and therefore ultimately depend on autographs such as higher plants (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 can be obtained from a report by Wasilewska (1995) that shows that values for both diversity and maturity indices were higher in mixed species grass swards than under monoculture. 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 a wide crop and non-crop plants where their effects are usually neglected. The effects of plants on decomposer components (micro 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 management 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 while the others are rendered homeless. In a related study on the effect of human intervention on nematode communities, Freckman and Ettema (1993) also reported that nematode abundance was higher under annual crops compared with perennial cropping systems. The 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. Species richness was lowest in the annual cropland use 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.

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