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Nematode Occurrence and Distribution in an Organically-Managed Soybean Field

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ABSTRACT
Optimum soil fertility, structure and the presence of substrates among others have been noted to influence the spatial and temporal distribution of nematodes in agricultural soils. Experiments to investigate nematode distribution on soybean field amended with six different composts were carried out in 2009 and repeated in 2010. Composts prepared from fresh plant leaves of: Chromolaena odorata, Tithonia diversifolia and Carica papaya stacked with poultry manure and cattle manure were applied at the rate of 6 tha^{-1} two weeks before planting. The experiment was laid out in a Randomized Complete Block Design with six replicates. The treatment included the six composts while untreated plots served as the control (no compost added). Soil was sampled for nematodes at the depth of 15cm prior to the application of organic amendments and around root zone of the plants (Rhizosphere) 60days after planting from 250 gram soil. Results showed that population of plant-parasites (Pp) were most abundant on the two location followed by omnivores (Om) and fungivores (Fu) and least of bacterivores (Ba) in the order (Pp > Om > Fu > Ba). Population of Meloidogyne spp. was significantly (P<0.05) lower on the top 0-15cm depth of all amended plots than the control plot. Population of omnivores (Dorylaims) and nematodes of less economic importance on soybean also increased. Population of Meloidogyne spp. was more abundant on control plots that amended plots. This offers a predictive way of selectively rotating crops on farming systems combining legume and non-legume in rotation.

KEYWORDS: *Nematode distribution", substrates", composts", crop rotation".*

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I. INTRODUCTION

The ubiquitous nature and bioactivities of nematodes makes them ecologically important in agricultural lands. Favored by the inherent characteristics endowed other living creatures by nature; they also have capacity to carry out all physical and physiological processes such as reproduction, feeding, excretion, response to stimuli as well as movement from one location to the other. Distribution of nematodes distance above sea level has been well studied (Wu *et al*, 2008; Niblack and Bernard, 1985). However, an intensely managed agricultural system have been suspected by (Ferris and Ferris, 1974) to support abundance of plant-feeding nematodes than do non-agricultural systems As key members in the soil food web (Wu *et al*., 2008), nematodes have been reported to contribute immensely to the rate of decomposition of plant litter and turnover of nutrients from soil organic matter (Wu *et al*., 2008) and many researchers have investigated into nematode communities in natural ecosystems and agro ecosystems including variation associated with management practices (Liang *et al*., 2002; Okada and Harada, 2007) Besides being a source of substrate on which soil micro-organisms feed, organic amendments have been appraised for its potency to reduce the population of plant-parasitic nematode of major economic importance mitigating large scale soybean production in the tropics (Atungwu *et al*., 2012) when used as a management option against root-knot nematodes whose magnitude of yield loss on the crop has been put at up to 100% (Atungwu and Lawal, 2009) in infested soybean fields, this in line with the postulate of Freckman and Ethema (1993) affirms that nematode population in this system can respond in a predictable way to ecosystem disturbance during land preparation and cultural practices involving incorporation of organic materials into the soil. It is therefore imperative to examine the impact of these organic products on the various species of nematodes inhabiting agricultural soils to which it is applied. This could complement the global efforts on the crusade of environmental safety with respect to soil conservation and climate as nematode community on agricultural soils can be used as indicators of climate change (Bongers, 1990). This study therefore intends to evaluate the species of nematode that could possibly be encountered on soybean fields particularly where organic agriculture is being adopted relative to inorganic systems.

II. MATERIALS AND METHOD

Description and time of experimentation

The experiments were located in Ibadan, Oyo and Abeokuta, Ogun Southwestern States of Nigeria from June, 2009 and repeated till October, 2010 respectively.

Soil sampling

Sampling involved collection of soil samples with the use of soil auger at 0-15cm depth before planting and sixty days after sowing of the seeds which commensurate with the peak of vegetative growth of the plants. Six sub- samples were collected per plot in a random pattern close to the rhizosphere of the growing soybean plants. These were bulked and mixed homogenously to form a representative sample from which 250 grams weight was measured for nematode assay. Samples were filled and labeled in a polythene bags and were carefully arranged in a plastic boxes for onward transportation to the laboratory under low temperature.

Determination of soil physical and chemical properties

Soil physical characteristics in terms of particle size distribution were evaluated through the Bouyoucous hydrometer method (1951). PH of the soil in water was obtained through by electronic method while percentage organic carbon was determined through the procedure of Walkley Black (1934). Total Nitrogen (N) was obtained by Macro-Kjeldahl digestion method, available Phosphorus by Bray- 1 method, Sodium (Na) and Potassium (K) through Flame photometer method.

Compost preparation and composition

Composting was carried out using fresh plant leaves of *Chromolaena odorata*, *Tithonia diversifolia*, and *Carica papaya* and animal materials: Cattle manure, Poultry manure and the fresh plants were constituted in ratio 3:1 in order to ensure an optimum C: N ratio required for rapid decomposition of the substrates within the constructed compost stacks. This process involved: collection of plant and animal materials. These materials were sorted carefully manually in order to get rid of non-biodegradables objects therein and later chopped into pieces of 15 to 20 cm with a sharp cutlass in order to reduce size of plant materials while at the same time increasing the surface area for ease of decomposition. The plant materials as well as the animal manures were stacked in layers while good stacking density was maintained within the holding units which were constructed using bamboo poles of 2 m length erected in a rectangular manner at 1 m × 1 m × 1 m × 1 m apart with adequate moisture. After stacking, regular turning fortnightly with the use of spade ensured homogenous mixing and distribution of substrates and heat within the holding units. The compost was harvested at exactly six months, a period long enough for complete decomposition. This was cured by air-drying, packed into polythene bags and labeled immediately for ease of identification among the different composts prepared.

Land preparation

Since the greatest diversity of nematodes is found under undisturbed soil and uncultivated pasture represents the greatest diversity of nematode community, land preparation was carried out in a way that posed minimum disturbance on the nematode community. The land was cleared with a cutlass and later tilled with a hoe. After this, the field was measured out using a measuring tape and plots afterwards were marked out with the use of split bamboo as pegs at 1.5 m x 1.5 m each. Each plot contained four of 1.5 m length ridges with 0.5 m alley way between one plot and the other.

Application of composts

The composts applied at the rate of six tones per hectare (6tha) were mechanically worked into the top 15cm depth of the soil (Atungwu and Lawal, 2009). It was left to mineralize for fourteen (14) days before planting commenced.

Planting of soybean

Viable soybean seeds were planted at the spacing of 75cm x 5cm to achieve a plant population of 533,333plants per hectare on plots of 3m x 4m size.

Extraction of nematodes from the soil

The modified Whitehead and Hemming (1965) tray method was used for nematode extraction. This involved the use of two sieves of 25 cm diameter with a sandwich of tissue paper between them. The set was placed in a plastic tray of larger diameter. Samples were thoroughly mixed; 250 g was measured into the sieves while clean water was gently poured into the plastic tray via the gap between the tray and the sieves until water just touches the bottom of the sieves. The set-up was left undisturbed in the laboratory for 18 hours for maximum nematode recovery. The suspension was poured into Nalgene bottles, topped-up to the factory fixed level and left for five hours (5 hours) for the nematodes to settle at the bottom. The supernatant was removed by siphoning with hose (rubber tubing) which was fixed to the spout of the bottle. This process continued until it was automatically broken. The nematode suspension was temporary stored in loosely closed McCartney bottles and labeled accordingly using paper tapes. Counting was achieved through the use of a stereomicroscope while identification of species was done with a compound microscope at the magnification of X40.

Data analysis

The data collected on the number of each genera of nematodes from 250 gram sampled soil was subjected to Analysis of Variance using SAS (2001) and descriptive statistics with Microsoft Excel.

III. RESULTS

Soil properties

Physical properties of soil in the two experimental fields comprised a very high proportion of sand (above 75%), with relatively low silt and clay making it characteristic sandy-loam through the experimental period. There was a slight change in pH value implying that soil was slightly acidic, but more in the second experiment than the first. Macro-nutrients, Sodium (Na^{++}), Potassium (K^{+}) and Calcium (Ca^{++}) at initial stage was lower comparatively, but, available P was higher at the final stage of the experiment than initial. This was similarly observed of the percentages (%) of Calcium, Nitrogen and Organic matter in both experiments.

Nematode index

Results from the laboratory process of extraction revealed the predominance of the following nematodes as inhabitants of the ecosystem: Meloidogyne spp., Pratylenchus spp., Tylenchus spp., Rotylenchulus spp., Helicotylenchus spp., Xiphinema spp., Hoplolaimus spp., Monochid spp., Aphelenchoides spp., Dorylaimus spp., Paratylenchus spp., Heterodera spp.

Nematode frequency

Nematode genera identified with all the amended plots were majorly plant-parasitic (herbivores) with minimum proportion (%) of *Meloidogyne* spp. and *Heterodera* spp . parasitic on soybean. Only plots amended with Cattle manure + *Chromolena odorata* , Cattle manure + *Carica papaya*, and the control plots of the experiment 1 showed traces of *Heterodera* . *Aphelenchoides* was the only representative of the Fungivores while *Dorylaimus* spp. represented omnivores throughout the experimental period.

Effect of organic amendment on nematode frequency

Composts comprising Cattle manure + *Carica papaya* supported higher population of *Trichodorus* spp. With apparently fewer in the control plots while none was found in control plots. Other amended plots do not support the nematode (fig. 1). *Dorylaimus* spp. was found to a high degree in all plots but, mostly on plots treated with Poultry manure + *Carica papaya.* Monochids (OM) population was higher in Cattle manure + *Carica papaya*, followed by Cattle manure + *Chromolena ododrata* and Cattle manure + *Thitonia diversifolia* than other compost types. This implied that manure from cattle source irrespective of plant material with which it was composted enhanced higher omnivore population than do others with least population in the control. *Hoplolaimus* and *Xiphinema* spp. Were pevailent to a small degree on plots treated with Cattle manure + *Chromolena odorata* while small percentage of *Criconemoides* were found in Cattle manure + *Chromolena odorata* and Poultry manure + *Thitonia diversifolia* .Every plot supported *Rotylenchulus* spp., *Prarylenchus* spp., *Tylenchulus* and *Meloidogyne* spp. Control plots had higher *Meloidogyne* spp. population than the others. *Helicotylenchus* spp. Appeared in all plots except the control plots and one comprising Cattle manure + *Chromolena odorata* in experiment 1.Distribution of nematodes on the field used for the second experiment also followed an irregular pattern of nematode spread. *Dorylaims* (Omnivores), *Aphelenchoides* (Fungivores), *Helicotylenchus*, *Tylenchulus*, and *Meloidogyne* spps (Plant parasites) were found on all amended plots except *M. incognita*, most abundant on control plots.

Table 1: Physical and chemical characteristics of the soil in the experimental fields

Soil Properties	Initial properties Experiment 1	Experiment 2	Final properties Experiment 1	Experiment 2
PHYSICAL				
% Sand	75.60	71.60	75.60	71.70
% Silt	13.60	13.60	13.60	13.60
% Clay	10.80	14.80	10.81	14.90
CHEMICAL				
PH (Water)	6.77	6.87	6.84	6.80
Na mg /kg	0.22	0.21	0.22	0.22
K mg /kg	0.38	0.32	0.41	0.39
Ca mg /kg	1.58	3.14	2.20	3.15
Mg mg /kg	0.37	0.58	0.35	0.60
H^+ mg /kg	0.07	0.07	0.07	0.06
Available P mg / kg	7.27	4.06	8.00	4.80
% C	0.66	0.62	0.90	0.82
% N	0.07	0.06	1.30	2.30
% O. M.	1.14	1.07	1.62	1.80
Textural Class				Sandy loam

Source: Atungwu *et al*., 2012

Table 2:Percentage (%) of the total number of nematode genera in 250 gram of sampled soil

EXPERIMENT 1

Nematode Genus	Compost types C1	C2	C3	C4	C5	C6	C7
Meloidogyne spp.	7.4	17.2	8.2	20.6	18.4	8.2	27.9
Pratylenchus spp.	29.6	17.2	20.5	20.6	15.8	20.4	13.9
Tylenchus spp.	11.1	6.9	6.8	8.6	13.2	8.2	9.3
Rotylenchulus spp.	11.1	6.9	6.8	14.3	5.7	10.2	16.3
Helicotylenchus spp.	3.7	10.3	6.8	0.0	8.6	18.0	0.0
Xiphinema spp.	0.0	0.0	0.0	0.0	2.9	0.0	0.0
Hoplolaimus spp.	0.0	3.4	0.0	0.0	0.0	2.0	0.0\
Monochids	0.0	0.0	0.0	0.0	2.9	0.0	0.0
Aphelenchoides spp.	11.1	3.4	2.3	11.4	5.7	10.2	4.7
Dorylaimus spp.	7.4	6.9	9.1	17.6	13.2	8.2	14.0
Paratylenchus spp.	18.5	27.6	29.5	17.1	15.8	12.2	9.3
Heterodera spp.	0.0	0.0	0.0	9.0	0.0	2.4	4.7

LEGEND

Mi– *Meloidogyne* Xi – *Xiphinema* Do– *Dorylaimus*
Pr - *Pratylenchus.* Cr– *Criconemoides* . Tr – *Trichodorus*
Ty – *Tylenchulus.* Ho– *Hoplolaimus.* He - *Heterodera*
Ro – *Rotylenchulus.* Mo - *Monochids*
He – *Helicotylenchus.* Ap –*Aphelenchoides*
C1 - Poultry manure + *Chromolaena odorata*
C2 - Poultry manure + *Tithonia diversifolia*
C3 - Poultry manure + *Carica papaya*
C4 - Cattle manure + *Chromolaena odorata*
C5 - Cattle manure + *Tithonia diversifolia*
C6 - Cattle manure + *Carica papaya*
C7 = No Compost (Control)

Table 3:Percentage (%) of the total number of nematode genera in 250 gram of sampled soil

EXPERIMENT 2

Nematode	Compost types						
Genus	C1	C2	C3	C4	C5	C6	C7
Meloidogyne spp.	21.9	17.5	20.8	20.8	18.3	16.2	39.7
Pratylenchus spp.	6.3	22.9	0.0	3.8	20.0	23.5	8.8
Tylenchus spp.	18.8	7.5	18.2	7.6	16.7	13.2	8.8
Rotylenchulus spp.	14.3	1.0	6.8	9.3	6.6	10.3	7.4
Helicotylenchus spp.	2.9	5.0	11.4	0.0	8.3	1.5	13.2
Xiphinema spp.	0.0	2.5	2.3	0.0	0.0	0.0	0.0
Hoplolaimus spp.	0.0	0.0	2.3	1.9	0.0	2.9	1.5
Monochid spp.	0.0	0.0	0.0	0.0	0.0	1.3	0.0
Aphelenchoides spp.	14.3	0.0	0.0	11.3	6.7	14.7	4.4
Dorylaimus spp.	8.6	12.5	11.4	7.6	10.0	8.8	10.3
Paratylenchus spp.	8.6	25.0	22.7	37.2	13.3	7.4	5.9
Heterodera spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0

LEGEND

Mi– *Meloidogyne* Xi – *Xiphinema* Do– *Dorylaimus*
Pr - *Pratylenchus.* Cr– *Criconemoides* . Tr – *Trichodorus*
Ty – *Tylenchulus.* Ho– *Hoplolaimus.* He - *Heterodera*
Ro – *Rotylenchulus.* Mo - *Monochids*
He – *Helicotylenchus.* Ap –*Aphelenchoides*
C1 - Poultry manure + *Chromolaena odorata*
C2 - Poultry manure + *Tithonia diversifolia*
C3 - Poultry manure + *Carica papaya*
C4 - Cattle manure + *Chromolaena odorata*
C5 - Cattle manure + *Tithonia diversifolia*
C6 - Cattle manure + *Carica papaya*
C7 - No Compost (Control)

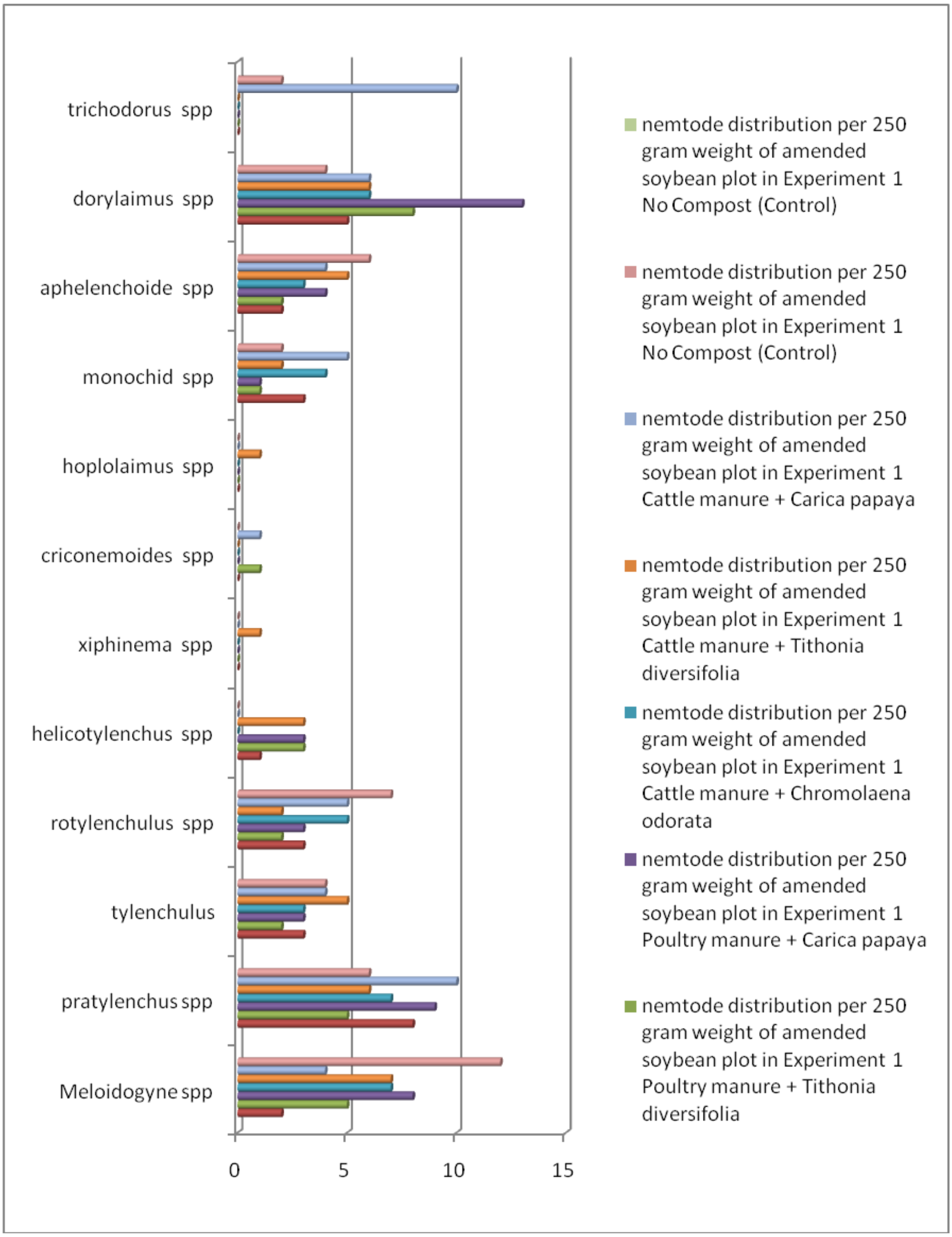

Figure 1: Distribution chart of nematodes genera on compost amended plot in experiment 1

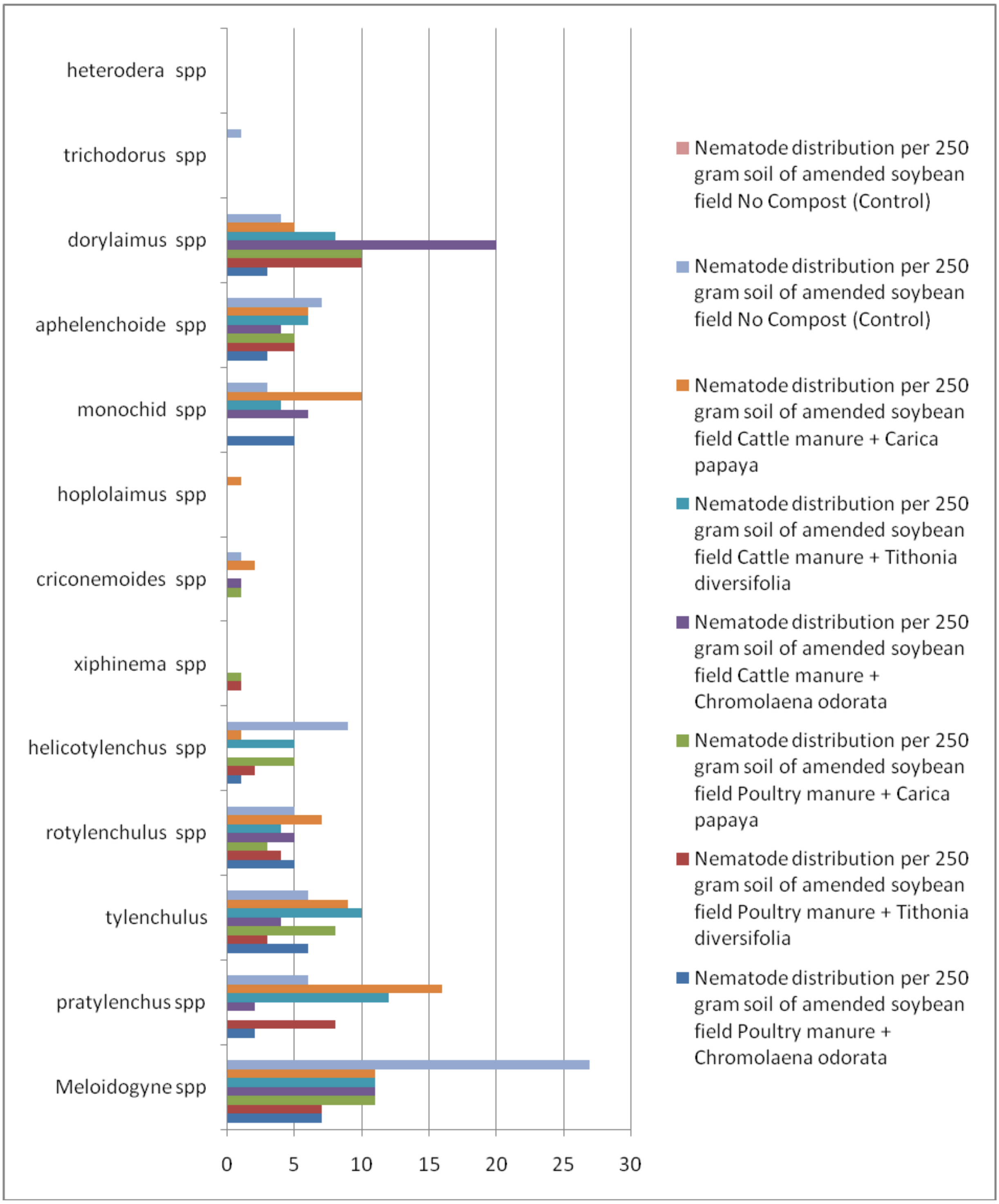

Figure 2: Distribution chart of nematodes genera on compost amended plot in experiment 2

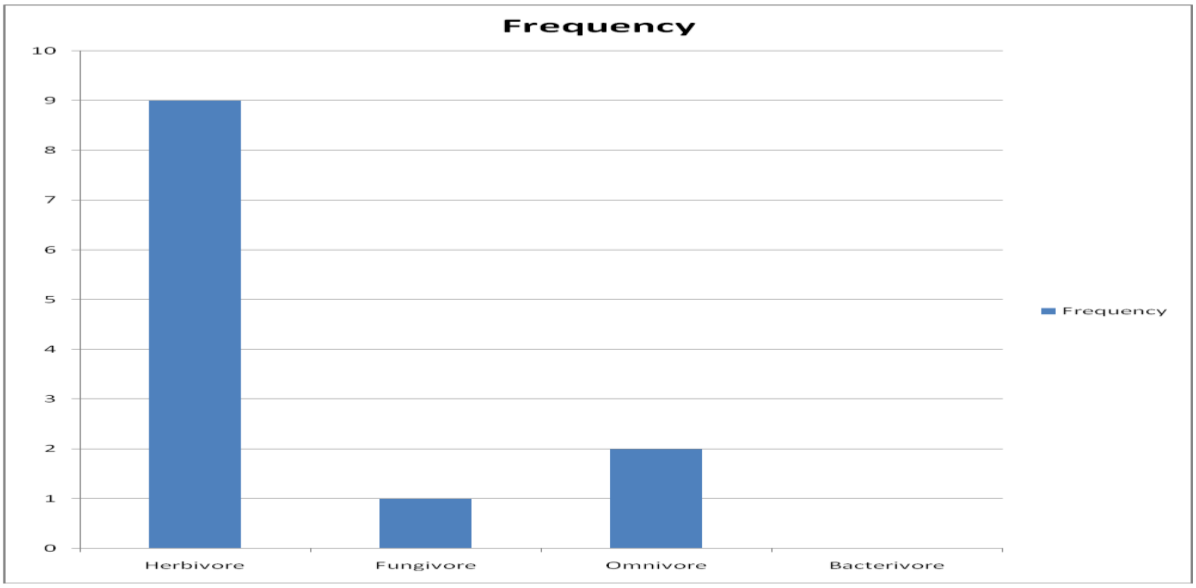

Figure 3: Frequency of trophic groups

IV. DISCUSSION

Prevention of pest situation associated with mono- and continuous cropping suggests the use of appropriated management options. Integrated method of pest management (IPM) comprising the use of compatible options such as soil amendment with crop rotation in soybean production in tropics leads to improvement in soil physical and chemical properties of the soil, increase in microbial population most importantly, nematodes. Distribution of nematodes in the soil is influenced among others by availability of substrate on which microbes feed, soil moisture, temperature and moisture among others. It is therefore important that the knowledge of types of nematodes present and the proportion of their population be considered when planning integrated management programme.The experimental sites used for this experiment showed different genera of nematodes with substantial amount of plant-parasitic nematode in association with soybean in Nigeria. This correlates with the findings of Niblack and Bernard (1985) where Dogwood, Maple and peach nurseries sampled in Tennessee revealed the presence of trophic groups comprising of plant-parasites, fungivores, omnivores and predators. The distribution of nematodes in wetland soils different distance from Boihea Sea has also been reported by Wu *et al.*, (2008). Application of composted materials from different plant and animal sources on a soybean plots led to the development of a complex ecosystem which was found to be constituted by varying population of nematode species each with different feeding characteristics. Roles played by these organisms was presumed to either be symbiotic when they tend to complement activities of other organisms, degradative when aid decomposition of organic materials, or parasitic when they are to inhabit, feed and cause harm to the hosts. All the experimental plots used for this experiment a higher population of plant-parasitic nematodes but most of which are not parasitic on soybeans but fewer of root-knot and cyst nematodes which are parasitic on the crop.Development of microbial population in the organically- amended agricultural soils has also been linked with the boost in substrates which serves as a source of food for these micro-organisms thereby enhancing their reproduction and multiplication in time and space.Results from this trial shows that various genera of nematodes can be prevalent in soybean fields managed organically with a change in the soil properties as reported by Ferris (2003) where mineral N concentration in an organically-managed soil increased bacterivore nematodes more than fungivore and plant-parasites. This is likely to be a predictive mean of selecting crops in rotation systems.

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