**DIVERSITY OF NEMATODES AND EARTHWORMS ASSOCIATED WITH TROPICAL TREES IN AN AFRICAN ARBORETUM**

**ABSTRACT**

In order to maintain soil fertility and quality, sustainable agriculture, and ecosystem processes, the population and diversity of soil organisms are crucial. This study was carried out at the Arboretum of the Faculty of Agriculture, University of Port Harcourt, and examined the diversity and population of nematodes and earthworms under a few chosen trees. Samples of soil, roots, and earthworms were randomly taken from the rhizosphere of trees at a depth of 0–30 cm using a spade and hand trowel. The extraction tray method was used to extract nematodes, the hand sorting and ethological method was used to collect and count earthworms, and the analysis of variance was performed on the data collected, and least significant difference was used to separate the means. Results showed significant difference in earthworm population across all trees with the highest earthworm population observed under *Annona muricata* (25.8), while lowest earthworm population was recorded under *Irvingia gabonensis*. Nematode population ranged from 3.0 – 53.0 while earthworm population ranged from 7 – 25.8 under the various tree species. Four (4) nematode species were identified, and *Dorylaimida spp* was the most predominant nematode. All five selected tropical tree species had effect on nematode community structure and earthworm population but *Tectona grandis* exerted the greatest effect. These impacts may be due to passive byproducts of nutrient intake, root shape, shearing habitat preference by trees and soil organisms, or active selection for soil microbes by plants through root exudates. These trees could be used in sustainable agriculture and agroforestry.

**Keywords:** Arboretum, Earthworm, Nematode, Population Structure, Diversity, Tree Species

**INTRODUCTION**

Soil microbes are an essential component of forest ecosystems due to their important roles in the majority of nutrient transformations in forest soils (DeGannes *et al*., 2016). As a result, numerous studies have focused on the relationships between the above- and below-ground elements of forest ecosystems, with a particular emphasis on determining how different tree species affect the composition of soil microbial communities and earthworms (Clark, 2021; Shao *et al*., 2023).

Like microorganisms, earthworms have been studied for a long time because they are important organisms in forest ecosystems, gardens, and arboretums, and they also contribute significantly to soil fauna in a wide range of soil types and climates (Reynolds, 2011; Myburgh, 2017; Moore *et al*., 2022; Reynolds, 2024). They add organic material to the soil and influence the activity of other soil organisms (Clark, 2021), and according to Gillespie (2018), earthworms have a direct or indirect role in the biodegradation process, stabilization through humus fractions, and other soil activities. Environmental factors that control earthworm communities in forests are litter characteristics and soil properties, particularly clay content, pH, base saturation, soil moisture content, organic matter content, and aluminium (Al) toxicity (De Wandeler *et al.,* 2016).

Nematodes are a very varied group of invertebrates and the most prevalent multicellular critters in soil (van den Hoogen, 2019). They may live freely in soil, freshwater, marine settings, and even in odd locations like vinegar, beer malts, and water-filled fissures deep below the Earth's crust, and can even live as parasites in animals and plants (Shah & Mahamood, 2017; Bailey, 2020). Functional variety has become more and more well-known in recent years (Escalas *et al*., 2019), and nematodes in this context are generally categorized as omnivorous, predatory, fungal, bacterial, and plant-feeding (van den Hoogen, 2019). Nematodes that feed on bacteria and fungi, release ammonium (NH4+) and nitrogen (N) in excess of their structural and metabolic requirements, which can raise the availability of nutrients for plants (Ingham, 2016; Shao *et al*., 2023). However, by consuming plant roots or root hairs, plant-feeding nematodes can reduce the primary productivity of plants (Escalas *et al*., 2019). The degree of top-down regulation and the complexity of the soil food web can also be reflected in the relative abundance of predatory and omnivorous nematodes, which can then affect how lower trophic-level worms affect plant development (Shao *et al*., 2023). Nematodes are a vital component of the soil ecosystem (Mburu *et al*., 2020), and are involved in several critical soil processes (Pehlivan *et al*., 2020). They play a crucial role in mineralizing, or releasing, nutrients in forms that plants can use, just like protozoa do (Ingham, 2016). Nematodes are also parasites and predators on earthworms (Reynolds, 2021).

Trees have an impact on soil properties through a variety of processes, such as root penetration, the addition of organic matter through root litter, and the exudation of ions and organic compounds (De Gannes *et al*., 2016). Trees can also change the soil microbial composition and/or community organization by altering the litter chemistry and site microclimate, and they affect the surrounding soil by releasing or secreting various substances called rhizodeposites, which are mostly composed of carbohydrates, organic acids, secondary metabolites, and amino acids (Bardgett & Caruso, 2020). The rhizosphere, with its interwoven network of plant roots, soil, and a varied collection of bacteria, fungus, eukaryotes, and archaea, is without a doubt the most complex microhabitat (Hakim *et al*., 2021). It is well known that the biomass, activity, and communities of live microorganisms associated with trees vary depending on the species and rhizosphere (Lladó *et al.,* 2018; Bardgett & Caruso, 2020).

Trees have a significant impact on the population, composition, and structure of soil organisms (Hakim *et al*., 2021), which their diversity and abundance serve as ecological factors to assess soil health and productivity because of their potential to improve biotic and abiotic stress resistance, promote plant growth, remediate contaminated soils, recycle nutrients, control soil fertility and weather, mineralize rock, and perform other tasks that reduce the need for fertilizers and pesticides in agriculture (Bardgett & Caruso, 2020). This study therefore aim to access nematode and earthworm diversity and population under selected trees, and identify the precise trees that best support the growth of nematode and earthworm. This will assist in giving the general agricultural community helpful information about particular trees that can be used in agroforestry in order to sustain agricultural productivity and enhance soil fertility.

**MATERIALS AND METHODS**

**Study Area**

This study was carried out at the University of Port Harcourt's Forestry and Wildlife Arboretum unit, Faculty of Agriculture between May and June 2024. The site is situated between latitudes 400° 31 N and 50° 00 N and longitudes 6° 45 E and 70° 00 E, with an average temperature of 27°C, a relative humidity of 78%, and an average rainfall of 2500–4000 mm.

**Selected Trees**

The selected trees for this study are *Annona muricata* L., 1753 (Soursop), *Gmelina arborea* Roxb. 1814 (Beechwood), *Tectona grandis* L.f., 1781 (Teak), *Irvingia* *gabonensis* (Aubry-Lecomte ex O'Rorke) Baill., 1867 (Bush mango), and *Nauclea diderrichii* (De Wild.) Merr. (Bilinga), 1915.

**Soil and Root Sampling**

Soil and tender root samples were collected from the rhizosphere of each of the selected tropical tree species (*Gmelina arborea, Tectona grandis, Annona muricata, Irvingia gabonensis* and *Nauclea diderrichii*), using a spade and hand trowel at a depth of 0-30cm. To create a single bulk sample for a tree species, samples were taken from five tree stands. Five bulked samples were taken from each tree species, and twenty-five bulked soil and root samples were gathered in total. After being collected, the samples were put into clearly labelled polythene bags and promptly taken to the laboratory for nematode extraction.

**Nematodes Extraction**

Nematode extraction was done at the Crop Protection Laboratory, Department of Crop and Soil Science, Faculty of Agriculture, University of Port Harcourt. The extraction tray method was used to remove nematodes from the soil samples (Coyne *et al*., 2007). Every bulked sample that was gathered was put in a separate dish, sieved to get rid of any dirt or stones, and then thoroughly blended to ensure homogeneity. 200 milliliters of soil samples were poured onto a piece of face tissue that had been placed in a plastic sieve with an extraction tray underneath as part of the extraction process. By the side, water was added to the extraction tray. After the extraction setup had been in place for 48 hours, the sieve was taken out and the water was transferred into a beaker. The suspension was then left for a day to settle. After the solution was decanted, the nematode-containing suspension was transferred into a sampling container and kept in the refrigerator until it was time for identification and counting. After that, the nematode suspension sample bottles were delivered to the Nematode Research Laboratory Institute for Agriculture in Ibadan, Oyo State, Nigeria, for counting and identification.

**Identification and Counting of Nematodes**

A 2-milliliter aliquot of nematode suspension was pipetted into a Doncaster counting dish. In order to identify and count free-living and plant parasite nematodes using Bell Key, the Doncaster counting dish was alternatively put under a dissecting microscope and a compound microscope. The total number of free-living and plant parasitic nematodes in each sample was determined by multiplying the mean number of nematodes determined from the suspension by the total volume of the suspension.

**Earthworm Sampling**

By using an ethological method in conjunction with hand sorting, earthworms were extracted from the soil (DIN ISO, 2006). This was accomplished by creating a quadrant that was 30 cm long and 30 cm wide. After removing the earthworms from the designated soil using 0.5% formalin, the earthworms were collected after a 30-cm excavation was made with a shovel. To create a single bulk sample for a tree species, earthworm samples were taken from the rhizosphere of five tree stands; five bulked samples were taken from each tree species. Twenty-five samples of bulked earthworms were gathered. After being gathered, the earthworms were placed in sample collection vials with proper labels. After being cleaned with tap water, the earthworms were brought to the laboratory to be counted.

**Statistical Analysis**

Analysis of variance (ANOVA) was performed on the data gathered from the different parameters at P ≤ 0.05. At 5%, the least significant difference was used to separate the mean differences.

**RESULTS**

**Nematode Population under the Selected Trees**

Results of the mean nematode population are presented in figure 1. Nematode population ranged from 3.0 – 53.0 (p ≤ 0.05). *Tectona grandis* had the highest mean population of nematodes across the five tree species, and this was significantly higher than those of the other tree species. *Annona muricata* had the second highest population (21.75) and it was significantly higher than *Gmelina arborea* (9.75), *Irvingia gabonensis* (6.0) and *Nauclea diderrichii* (3.0). *Gmelina arborea* had the third highest population and it was significantly higher than *Irvingia gabonensis* and *Nauclea diderrichii. Nauclea diderrichii* hadthelowestpopulation, which was not significantly different from the nematode population of *Irvingia gabonensis.* Nematode population followed the order; *Nauclea diderrichii* (3.0) < *Irvingia gabonensis* (6.0) < *Gmelina arborea* (9.75) < *Annona muricata* (21.75) < *Tectona grandis* (53.0) for the selected trees, respectively.

**Figure 1: Nematode Population**

Means with the same letters were not significantly different at p ≤ 0.05

**Earthworm Population under the Selected Trees**

Results of the mean earthworm population are presented in figure 2. Earthworm mean population ranged from 7.0 – 25.8. *Annona muricata* had the highest population (25.8); it was not significantly different from the earthworm population of *Gmelina arborea* (21.8)*,* but it was significantly higher than those of *Nauclea diderrichii* (15.0)*, Tectona grandis* (7.8)*,* and *Irvingia gabonensis* (7.0). *Gmelina arborea* had the second highest population and it was significantly higher than those of *Nauclea diderrichii,* *Tectona grandis* and *Irvingia gabonensis.* *Irvingia gabonensis* had the lowest earthworm population of 7.0 (Table.1). Earthworm population followed the order; *Irvingia gabonensis* (7.0) < *Tecton agrandis* (7.8) < *Nauclea diderrichii* (15.0) < *Gmelina arborea* (21.8) < *Annona muricata* (25.8) for the selected trees respectively.

**Figure 2: Nematode Population**

Means with the same letters were not significantly different at p ≤ 0.05

**Diversity and Distributions of Nematodes under the selected Tree Species**

Results on the diversity and distribution of some nematode species identified across the selected trees species is presented on Table 1. Four (4) nematode species were extracted and identified across the selected tree species. The nematode species include: *Helicotylenchus sp., Dorylaimida sp., Monochus sp.,* and *Rhabitida sp.*  Only *Dorylaimida sp.* was found across all five tree species. All four nematode species were found under *Tectona grandis* thus it had thehighest nematode diversity followed by *Annona muricata.* Three nematode species (*Helicotylenchus sp., Dorylaimida sp.,* and *Monochus sp.*) were found under *Annona muricata* whereas *Gmelina arborea, Irvingia gabonensis* and *Nauclea diderrichii* had two nematode species each. *Dorylaimida sp.* and *Helicotylenchus sp.* occurred across *Irvingia gabonensis* and *Nauclea diderrichii,* while *Dorylaimida sp. and Monochus sp.* were found under *Gmelina arbor*

**Table 1: Diversity and Distributions of Nematodes across the Tree Species**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Tree Species** | ***Helicotylenchus spp*** | ***Dorylaimida spp*** | ***Monochus spp*** | ***Rhabitida spp*** |
| *Gmelina arborea* | 0.0 | 30.0b | 9.0a | 0.0 |
| *Tectona grandis* | 96.0a | 96.0a | 5.0b | 15.0a |
| *Annona muricata* | 63.0b | 21.0c | 3.0b | 0.0 |
| *Irvingia gabonensis* | 6.0c | 12.0d | 0.0 | 6.0b |
| *Nauclea diderrichii* | 6.0c | 6.0d | 0.0 | 0.0 |

Means with the same letters were not significantly different at p ≤ 0.05

**DISCUSSION**

Nematode population ranged from 3.0 – 53.0 (p ≤ 0.05). *Tectona grandis* had the highest mean population of nematodes across the five tree species, and this was significantly higher than those of the other tree species. *Annona muricata* had the second highest population and it was significantly higher than *Gmelina arborea*, *Irvingia gabonensis* and *Nauclea diderrichii*. Nematode population followed the order; *Nauclea diderrichii* < *Irvingia gabonensis* < *Gmelina arborea* < *Annona muricata* < *Tectona grandis* for the selected trees respectively (Figure 1). The variations in nematode population across the different trees have been linked to several factors. For instance, Reynolds (1972) illustrated that the tree species has a significant effect on the distribution and density of earthworm species in the soil under the tree species. Franco *et al*. (2019) reported that availability of food and water supplies, together with an appropriate temperature, often comprise the major elements that control nematode abundance and spread, Liu *et al*. (2019) maintained that soil porosity and water potential control nematode migration, development, and survival in soil, and Shao *et al*. (2023) posited that the amount of organic carbon in the soil had the biggest impact on nematode variety and abundance.

For diversity and distribution, four (4) nematode species were extracted and identified across the selected tree species and include: *Helicotylenchus sp., Dorylaimida sp., Monochus sp.,* and *Rhabitida sp.*  Only *Dorylaimida sp.* was found across all five tree species. All four nematode species were found under *Tectona grandis* thus it had thehighest nematode diversity followed by *Annona muricata.* Three nematode species (*Helicotylenchus sp., Dorylaimida sp.,* and *Monochus sp.*) were found under *Annona muricata* whereas *Gmelina arborea, Irvingia gabonensis* and *Nauclea diderrichii* had two nematode species each. *Dorylaimida sp.* and *Helicotylenchus sp.* occurred across *Irvingia gabonensis* and *Nauclea diderrichii,* while *Dorylaimida sp. and Monochus sp.* were found under *Gmelina arbor* (Table 1). Some of these species were reported in the study by Shao *et al*. (2023). Nematode diversity may fluctuate as a result of variations in precipitation, for example, which might modify the makeup of soil nematode communities (Franco *et al*., 2019). Also, nematodes frequently suffer when there is less aeration in saturated soil, and it has been demonstrated that many nematodes move and develop at their fastest rates when the soil water content shifts from saturated to somewhat drier states (Liu *et al*., 2019). Similarly, Shao *et al*. (2023) reported that nematode alpha-diversity, biomass, and abundance were more significantly influenced by mean annual precipitation in tropical zones, mean annual temperature and total soil phosphorus content in temperate zones, and soil pH in warm-temperate zones.

The physical, chemical, and biological characteristics of soils are influenced by the variety and activities of earthworms, one of the most significant soil biotas (Mulia *et al*., 2021). Because of their physical activity, soil pores are created, which facilitates the soil's water and nutrient dynamics. Earthworms help microbes like bacteria and fungus carry out further decomposition processes by recycling organic materials (Mulia *et al*., 2021). Earthworms have an impact on ecosystem services and soil health through these activities (Mulia *et al*., 2021). Due to differences in soil temperature, moisture content, surface litter abundance, vegetation kinds, land use management, and human activities, earthworm species' populations and diversity differ throughout terrestrial environments (Singh *et al*., 2016). Because of this, earthworms are sensitive to changes in land usage, and generally speaking, more extensive anthropogenic interventions decrease earthworm diversity in their environments (Dewi & Senge, 2015).

In this study, the soils under the selected tree species were dominated by the endogeic species *Pontoscolex corethrurus* (Rhinodrilidae)*, Hyperiodrilus africanus* (Eudrilidae)and little of *Amynthas sp.* (Megascolecidae, the jumping worms). Results from earthworm population showed that the soils under the various trees sampled had distinct earthworm population and soils under *Annona muricata* had highest earthworm population (Figure 1). Similarly, of the six species that were ubiquitous in every environment that was examined by Mulia *et al*. (2021), *Pontoscolex corethrurus* was uncommon in natural forests but predominately found in habitats with high levels of human activity. The high earthworm population identified in soils under *Annona muricata* in this study, likely could be due to the high moisture content of the soils under *Annona muricata* which was reported by Schelfhout *et al*. (2017). In contrast to soils under *Irvingia gabonensis* had the lowest earthworm population. This could be attributed to the assertion by Augusto *et al.* (2015) that soils under *Irvingia gabonensis* have low moisture content which could contribute to low forest floor decomposition rates and direct negative impacts on the activity of soil biota, hence the low earthworm population.

Furthermore, literature has also shown that inherent variations in the quality of leaf litter among tree species essentially produce distinct soil conditions, both directly through the litter's chemical makeup and indirectly through its effects on the size and makeup of earthworm communities, particularly burrowing (endogeic and anecic) earthworm communities (Schelfhout *et al*., 2017). Consequently, they are typically linked to moist soils. The studies by Marsden *et al*. (2020) and Mulia *et al*. (2021) showed that earthworm variety decreased as a result of natural forests being converted into various land uses, which may have a significant impact on the two communes' soil health and ecosystem functioning, and tree-based farming systems like agroforestry had a higher richness of earthworms among the human-disturbed ecosystems. Additionally, higher earthworm diversity is produced in tree-based systems by improved microclimate conditions, such as lower soil temperature and higher soil humidity, minimum plot management techniques like ploughing, and a greater supply of organic matter from above- and below-ground litter (Cardinael *et al*., 2019). These elements also produce differences in earthworm diversity across regions close to and distant from trees in agroforestry, such as between crop alley and tree row in the alley-cropping system (Cardinael *et al*., 2019).

**CONCLUSION**

The study's findings indicate that the link between nematodes, earthworms, and trees is extremely dynamic and varies depending on the species of tree. This means that different tree species have distinct effects on the variety and composition of nematodes in their immediate environment. Even though each of the chosen tree species had a unique impact on the nematode and earthworm population, composition, and structure surrounding them, *Tectona grandis* had the greatest beneficial impact on community structure, followed by *Annona muricata* due to its larger earthworm population than the others. The plant should be regarded by farmers and the agricultural community at large as a good tree species for agro-forestry if the goal is to improve soil quality and productivity because soils under *Tectona grandis* were found to have more diverse and populous nematode species and earthworms, which is indicative of a fertile soil.

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