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Plant-Parasitic and Free-Living Nematode Community Associated with Oak Tree of Magoebaskloof Mountains, Limpopo Province, South Africa

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Abstract: A study was conducted in the mountains of Magoebaskloof, Limpopo Province, where oak trees grow along the banks of the Broederstroom River. This study revealed that 22 nematode genera were associated with oak trees (*Quercus robur*). The most frequently occurring nematodes were *Aphelenchus* sp. (100%) and *Plectus* sp. (100%), followed by *Helicotylenchus* sp. (90%). This study examined the relationship between nematodes and the physicochemical properties of the soil using Pearson correlation. It uncovered that the organic matter content (OMC) had a negative correlation with the number of *Panagrolaimus* sp. ($r = -0.770$) and *Hemicycliophora* sp. ($r = -0.674$). Conversely, the sand percentage positively correlated ($r = 0.695$) with the number of *Hemicycliophora* sp. The clay content of the soil showed a positive correlation ($r = 0.617$) with the number of *Ditylenchus*. Soil pH demonstrated a significant negative correlation with *Acrobeloides* sp. ($r = -0.877$). The canonical correspondence analysis (CCA) explained 63.3% of the relationship between nematodes and soil physicochemical properties. The CCA results indicated that *Ditylenchus* exhibited a positive correlation with OMC, while the *Panagrolaimus* and *Hemicycliophora* species showed a negative correlation with OMC. The results indicated that none of the soil sample sites were under stress. The soil food web analysis revealed that most soil samples were nutrient-enriched with a low C/N ratio. In conclusion, this study revealed that oak trees harbor a high diversity of plant-parasitic and free-living nematodes. The results suggest that soil nematodes, particularly free-living bacterivores, such as *Panagrolaimus*, can indicate organic matter content in the soil.

Keywords: indicator; forest trees; nematode; soil food web; population density

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1. Introduction

Forests, which are vital to the health of our planet, encompass an impressive 4000 km^2 of the Earth's terrestrial area, representing about 33% of the total land [1]. Oak trees (*Quercus* spp.), a type of evergreen plant belonging to the family Fagaceae, are grown in temperate and tropical regions across the globe. These majestic trees can be found in the Americas, Asia, Europe, and North Africa [2] and are particularly abundant in South Africa, where the *Q. robur* species thrives. Oak timber is renowned for its durability, strength, and hardness, making it a valuable material for many applications, including furniture, floors, building frames, and veneers [3].

Nematodes serve as essential players in critical soil processes, in which they contribute to the decomposition of organic matter, the release of minerals, and the cycling of nutrients. Any shifts in the composition of the nematode community hold the potential to exert a substantial impact on the overall functioning of ecosystems [4].

Within the soil particles, various feeding groups of nematodes live, including the plant-parasitic and free-living nematodes [5]. Free-living nematodes, such as those belonging to genera *Panagrolaimus*, *Plectus*, and *Acrobeloides* are bacterivores, which have stoma with a terminal bulb in the pharynx, that bear a valvular apparatus to squeeze the bacteria [6].

In contrast, some nematodes are fungivores, such as the *Aphelenchus* species [7], which have stylet to punch the fungal cells. In addition, plant-parasitic nematodes, such as *Helicotylenchus* spp. and *Hemicycliophora* spp., bear stylets, which assist them in penetrating the root and, therefore, negatively affects plant health and production [8].

In addition to recording the presence of the plant-parasitic nematode communities in soil, free-living bacterivore nematodes can offer valuable insights into the stability of the soil food web and its ecological functioning. One key example of this is the significant role that nematodes play in directly and indirectly influencing nitrogen cycling within soils [9].

Plant-parasitic nematodes can significantly impact forest trees, in which their effect can lead to various symptoms, including root necrosis, root gall, and hyperplasia, all of which can hinder the normal growth and development of trees [1]. Furthermore, soil-dwelling nematodes, like *Xiphinema*, can transmit viruses to healthy trees. Nematodes can also collaborate with fungi and bacteria, developing complex diseases in forest trees [10]. Additionally, *Pratylenchus penetrans* and *Meloidogyne* species [1] were reported to have severe damage to oak trees.

So far, in South Africa, various free-living and plant-parasitic nematodes have been reported to be associated with different crops [11].

In the heart of the Magoebaskloof mountain range in Limpopo Province, South Africa, oak trees grow alongside the Broederstroom River, significantly impacting the area's ecology. However, despite the importance of these trees, plant-parasitic nematodes have not been well studied in South African oak trees. Some plant-parasitic nematodes, such as *Paratylenchus* spp., *Criconema* spp., and *Longidorus* spp., were reported in association with *Quercus* species in South Africa, but their effects on oak trees were not fully understood [12]. Additionally, soil nematodes and their relationship with oak trees have not yet been surveyed in South Africa. Therefore, this study aims to investigate the relationship between nematodes and soil physicochemical properties in oak trees in Limpopo Province, South Africa.

2. Materials and Methods

2.1. Soil Sampling

The soil samples were collected from various locations in Magoebaskloof mountain near the Broederstroom River (GPS coordinates 23°49′19.3″ S 29°57′29.2″ E), specifically from the area around oak trees (*Quercus robur*) (Figure S1). The soil texture was determined following the method outlined by van Capelle et al. [13].

Sixty soil samples were collected from ten different sites (six subsamples for each site), each located 100 m apart. Next, any above-ground plant debris was removed, and soil samples (approximately 1 kg) were collected separately from each site, from a 0 to 30 cm depth. The soil samples were collected using a simple random technique. The subsamples were taken from each site to cover the root zone and to recover various groups of nematodes. The samples were placed in a cooler box and transported to the Nematology laboratory. Next, the soil samples were processed, and the nematodes were identified. The soil samples were collected in the fall season (April), during a period of average precipitation of 55.2 mm and an average temperature of 25 °C in the sampling area.

2.2. Nematode Diagnosing

A tray technique was used to extract nematodes from 200 g of soil from each location [14,15]. After extraction, the nematodes were counted using a Zeiss stereomicroscope (Discovery V8; Jena, Germany), and the nematodes were identified at the genus level using a VWR light microscope (Italy). Nematode specimens were preserved in a hot 4% formaldehyde solution and then transferred to anhydrous glycerin for identification. The nematode genera were identified using the classification provided by Andrássy [16], Geraert [17], and Shokoohi and Abolafia [18].

2.3. Soil Properties Analysis

Soil's chemical properties, including measuring the organic matter content (OMC), pH, and EC, were analyzed. The pH was measured using the Thermo Scientific Orion 3 Star pH Benchtop from Waltham, MA, USA, while the EC was measured using an EC meter. To quantify the organic matter content (OMC), the difference between the oven-dry soil mass and soil mass after combustion was divided by the oven-dry soil mass [19]. The soil samples were analyzed using AgriLASA standard protocols [20].

2.4. Statistical Analysis

This study aimed to determine the predominant nematode genera associated with oak trees in the Limpopo Province. To achieve this, the relationships between the nematode mean population density (MPD) and the frequency of occurrence (FO) of each identified genus were expressed as a prominence value (PV) for each locality. The PV was calculated using the following equation: PV = Population density $\times \sqrt{}$ frequency of occurrence [21].

The Shannon Index (H') [22] was calculated to assess nematode biodiversity. A Pearson correlation method in XLSTAT v.2021 [23] was used to compute a correlation between the soil physicochemical properties and the nematode community. Additionally, a canonical correspondence analysis (CCA) was conducted, based on the method by Zhang et al. [24], to evaluate the relationship between soil factors such as pH, EC, soil texture, OMC, and nematode.

To normalize the data of frequencies/percentages, we used log 10. The effectiveness of nematodes in oak tree soil health assessment was evaluated using the c-p (colonizer-persister) triangle and food web diagnostics through NINJA (Nematode Indicator Joint Analysis) online software [25]. The nematode indices were calculated through the online program Nematode Indicators Joint Analysis (NINJA) (https://shiny.wur.nl/ninja; accessed on 1 January 2024).

3. Results

3.1. Diversity of Nematodes

In the soil samples collected from ten different sites near oak trees, a total of 22 nematode genera were identified from all samples (refer Table 1).

Table 1. Mean abundance of nematode genera (per 200 g soil) associated with oak trees in Magoebaskloof, Limpopo Province, South Africa.

Nematode	C-p Class	P-p * Class	Site-1	Site-2	Site-3	Site-4	Site-5	Site-6	Site-7	Site-8	Site-9	Site-10	Feeding Type
Plant-Parasitic													
Basiria sp.	0	2	7	6	4	66	22	5	46	23	71	0	Root hair feeders
Boleodorus sp.	0	2	0	3	0	2	0	1	4	0	2	0	Root hair feeders
Helicotylenchus sp.	0	3	75	5	30	80	40	12	24	2	35	0	Semi-endoparasites
Hemicycliophora sp.	0	3	4	5	0	1	0	6	0	0	0	0	Ectoparasites
Meloidogyne sp.	0	3	7	0	0	4	0	0	6	0	4	0	Sedentary parasites
Paratrophurus sp.	0	3	0	0	0	0	0	0	9	0	49	2	Ectoparasites
Pratylenchus sp.	0	3	4	15	0	9	22	108	35	50	155	0	Migratory endoparasites
Rotylenchulus sp.	0	3	35	25	23	30	0	0	1	28	15	0	Sedentary parasites
Xiphinema sp.	0	5	0	0	0	5	0	1	0	2	2	0	Ectoparasites
Free-Living													
Aphelenchoides sp.	2	0	21	3	116	15	3	13	6	8	2	2	Fungivores
Aphelenchus sp.	2	0	2	6	22	11	2	15	19	13	18	0	Fungivores
Ditylenchus sp.	2	0	8	0	8	19	12	6	21	88	14	0	Fungivores
Acrobeles sp.	2	0	6	2	9	2	0	0	0	0	0	0	Bacterivores
Acrobeloides sp.	2	0	31	4	117	28	9	41	33	72	32	0	Bacterivores

Table 1. *Cont.*

Nematode	C-p Class	P-p * Class	Site-1	Site-2	Site-3	Site-4	Site-5	Site-6	Site-7	Site-8	Site-9	Site-10	Feeding Type
Alaimus sp.	4	0	6	1	0	0	0	6	0	0	0	0	Bacterivores
Panagrolaimus sp.	1	0	59	52	25	61	4	25	17	0	0	69	Bacterivores
Plectus sp.	2	0	19	6	3	3	11	8	8	4	2	5	Bacterivores
Prismatolaimus sp.	3	0	15	2	0	2	6	0	0	0	0	0	Bacterivores
Rhabditis sp.	1	0	6	3	0	17	6	76	8	5	3	0	Bacterivores
Aporcelaimellus sp.	5	0	13	6	30	25	7	0	2	70	10	2	Omnivores
Mylonchulus sp.	4	0	0	13	1	6	38	2	4	0	8	2	Predators
Tripylina sp.	3	0	25	21	78	52	36	27	16	4	2	0	Predators/Bacterivores

* P-p = plant-parasitic class; numbers for the sites are the average nematode density.

3.1.1. Plant-Parasitic Nematodes

Plant-parasitic nematodes were detected as nine genera, including *Basiria* sp., *Boleodorus* sp., *Helicotylenchus* sp., *Hemicycliophora* sp., *Meloidogyne* sp., *Paratrophurus* sp., *Pratylenchus* sp., *Rotylenchulus* sp., and *Xiphinema* sp. (Table 1).

3.1.2. Free-Living Nematodes

Free-living nematodes were detected as 13 genera, including *Aphelenchoides* sp., *Aphelenchus* sp., *Ditylenchus* sp., *Acrobeles* sp., *Acrobeloides* sp., *Alaimus* sp., *Panagrolaimus* sp., *Plectus* sp., *Prismatolaimus* sp., *Rhabditis* sp., *Aporcelaimellus* sp., *Mylonchulus* sp., and *Tripylina* sp. (Table 1).

Among all the nematodes genera identified, free-living nematodes, including *Aphelenchoides* sp. and *Panagrolaimus* sp., were found in all of the soil samples. However, the plant-parasitic nematodes, namely *Meloidogyne* sp., *Xiphinema* sp., and *Hemicycliophora* sp., were observed in low numbers in the soils (refer Table 1).

3.2. Indices of the Nematode Communities

3.2.1. Plant-Parasitic Nematodes

Helicotylenchus sp. (FO = 90%), and *Basiria* sp. (FO = 90%), followed by *Pratylenchus* (FO = 80%) were detected with the most frequency of occurrence. In contrast, the least frequent nematode was *Paratrophurus* sp., with a 20% frequency of occurrence. *Pratylenchus* sp. had the most prominence value (PV = 7.4), and *Boleodorus* sp. and *Xiphinema* sp. (PV = 0.3) had the lowest PV (Figure 1).

3.2.2. Free-Living Nematodes

The most frequent free-living nematodes associated with oak trees were *Aphelenchus* sp. and *Plectus* sp., with a 100% frequency of occurrence (Figure 1). In contrast, *Alaimus* (FO = 30%), *Acrobeles* (FO = 40%), and *Prismatolaimus* (FO = 40%) were detected with the lowest frequency of occurrence.

3.3. Correlation Between Soil Parameters and Nematodes

3.3.1. Plant-Parasitic Nematodes

The results indicated a significant correlation ($p < 0.05$) between sand ($r = 0.695$) and the number of *Hemicycliophora* sp. (Figure 2). The silt of the soil had a negative correlation with the number of *Hemicycliophora* sp. ($r = -0.618$). and *Hemicycliophora* sp. ($r = -0.674$). The EC of the soil had a negative correlation with the number of *Hemicycliophora* sp. ($r = -0.622$) (Figure 2). In addition, the result indicated that *Meloidogyne* sp. and *Pratylenchus* sp. had no significant correlation with the soil parameters. *Basiria* sp. showed a positive correlation ($r = 0.566$) with OMC (Figure 2).

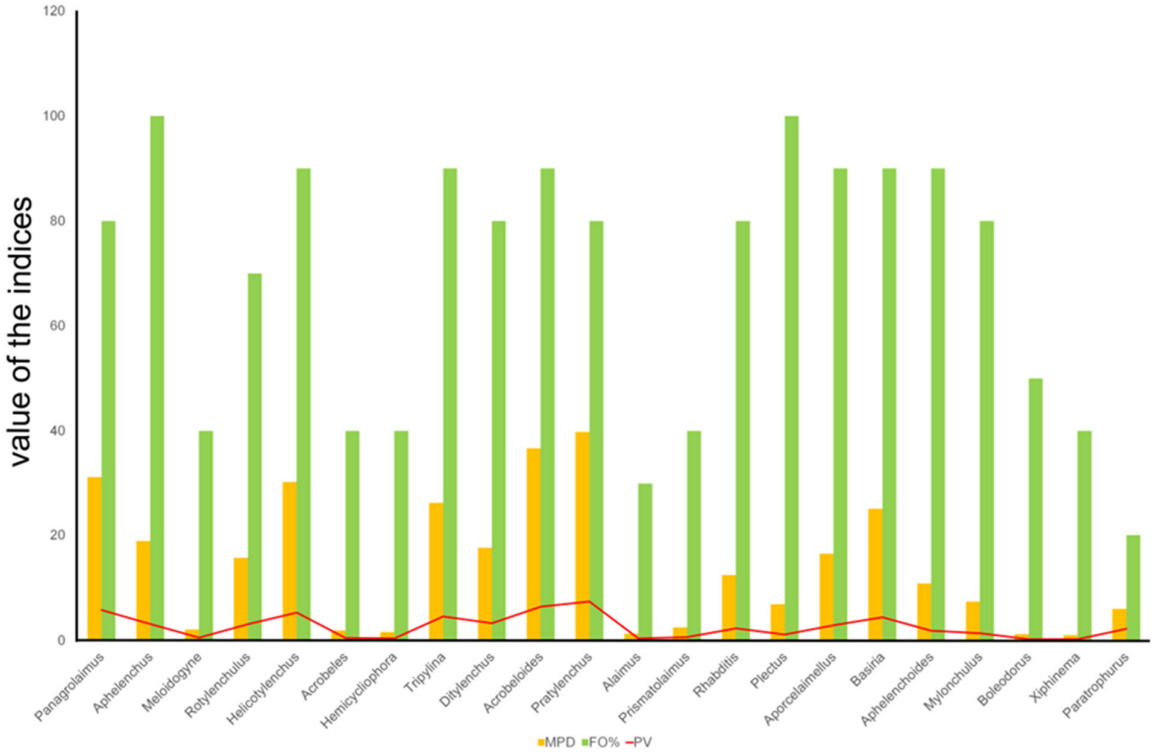


Figure 1. Nematodes mean population density (MPD), frequencies of occurrence (FO%), and prominence values (PVs) were studied in oak trees of Magoebaskloof, Limpopo Province, South Africa.

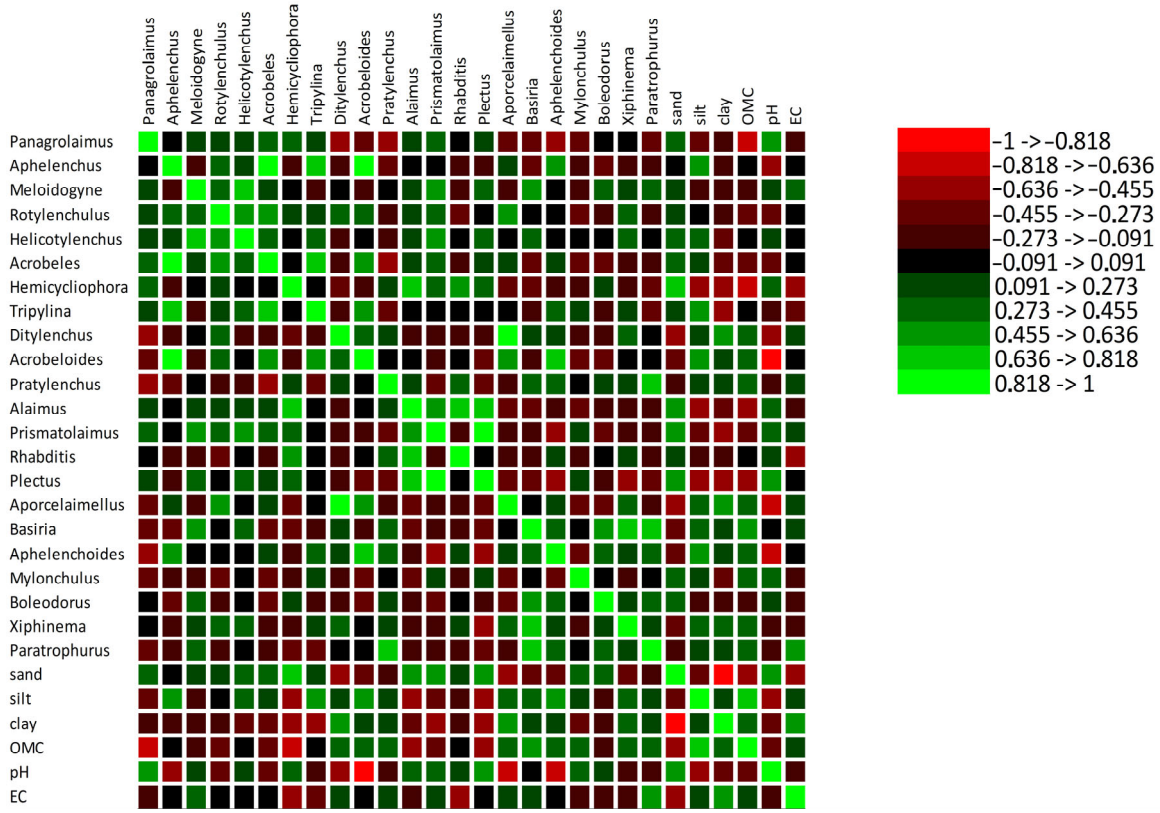


Figure 2. Correlation between soil variables and nematodes of oak trees of Magoebaskloof in Limpopo Province, South Africa.

3.3.2. Free-Living Nematodes

The result showed that the number of *Ditylenchus* sp. ($r = 0.617$) positively correlated with the soil's clay. OMC negatively correlated with the number of *Panagrolaimus* sp. ($r = -0.770$), In addition, the pH of the soil had a negative correlation with the number of *Acrobeloides* sp. ($r = -0.877$) and *Aporcelaimellus* sp. ($r = -0.771$) (Figure 2).

The results of the canonical correspondence analysis (CCA) showed an accumulated variability of 63.3% in the nematodes and sites analysis, with 35.3% for CCA1 and 28.0% for CCA2. The contribution of nematode genera and sites of the study to the CCA indicated that plant-parasitic nematodes, including *Meloidogyne* sp. and *Hemicycliophora* sp., were dominant in Sites-1, 2, and 4 with more soil sand.

The CCA (Figure 3) showed that *Hemicycliophora* sp. negatively correlated with OMC.

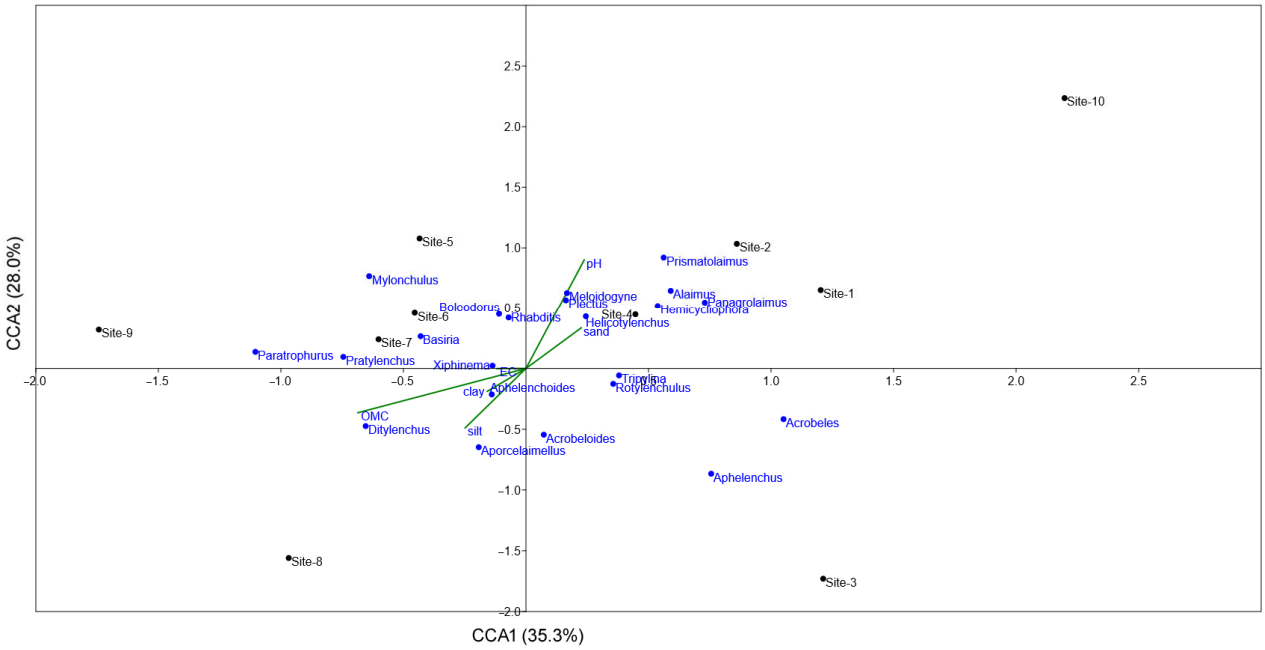


Figure 3. CCA plot of the relationship of the soil variables and nematodes associated with oak trees in Magoebaskloof, Limpopo Province, South Africa.

Free-living nematodes, including *Ditylenchus* sp., *Aphelenchoides* sp., and *Aporcelaimellus* sp., were dominant in Site-8 with more OMC, clay, and silt. In contrast, *Panagrolaimus* sp., was dominant in the Sites-1, 2, and 4 with more soil sand.

The CCA (Figure 3) showed that *Panagrolaimus* sp. negatively correlated with OMC. In contrast, EC of the soil had no significant effect on the numbers of nematodes associated with oak trees.

The community analysis showed a significant difference ($p < 0.001$) in the sigma maturity index, plant-parasitic index, channel index, enrichment index, and structure index among nematode numbers in the tested soil samples (Table 2). The values of sigma maturity (SMI) and plant-parasitic index (PPI) changed, indicating the abundance of free-living and plant-parasitic nematodes. These changes represent the vigor status of the host plant, ranging from 1.3 to 2.9 and from 2.6 to 3.0, respectively (Table 2). In contrast, channel and structure indexes were indicators of food web condition. Free-living nematodes, including *Prismatolaimus* sp. (c_p3; Sites-1, 2, 4, 5) and *Aporcelaimellus* sp. (C_p5; all sites except for Site-6), were observed with 40% and 90% of the frequency of occurrence, which is a sign of structured soil.

Table 2. Nematode community indices computed for nematode numbers associated with oak trees in Magoebaskloof, Limpopo Province, South Africa. Each data averages the individual per 200 g soil (mean ± S.D).

Index Name	Site-1	Site-2	Site-3	Site-4	Site-5	Site-6	Site-7	Site-8	Site-9	Site-10	*p* Value
Sigma Maturity Index	2.4 ± 0.1	2.4 ± 0.2	2.4 ± 0.2	2.5 ± 0.3	2.9 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	2.7 ± 0.2	2.3 ± 0.2	1.3 ± 0.2	<0.001
Plant-Parasitic Index	2.9 ± 0.1	2.9 ± 0.1	2.9 ± 0.1	2.7 ± 0.2	2.7 ± 0.3	2.9 ± 0.2	2.6 ± 0.1	2.6 ± 0.1	2.3 ± 0.2	3.0 ± 0.1	<0.001
Channel Index	10.7 ± 0.2	3.9 ± 0.1	59.4 ± 0.2	12.6 ± 0.1	29.8 ± 0.2	7.8 ± 0.2	31.5 ± 0.1	84.5 ± 0.1	73.9 ± 0.1	0.7 ± 0.1	<0.001
Enrichment Index	76.9 ± 0.1	91.6 ± 0.3	47.2 ± 0.2	82.1 ± 0.1	60.6 ± 0.3	84.1 ± 0.1	62.7 ± 0.1	41.1 ± 0.1	40.4 ± 0.2	97.5 ± 0.1	<0.001
Structure Index	69.2 ± 0.1	87.4 ± 0.2	57.2 ± 0.2	79.5 ± 0.4	88.7 ± 0.3	52.8 ± 0.2	42.6 ± 0.1	70.7 ± 0.1	59.3 ± 0.3	74.6 ± 0.1	<0.001
Shannon Index H′	2.5 ± 0.2	2.0 ± 0.3	2.5 ± 0.1	2.3 ± 0.2	2.1 ± 0.1	2.5 ± 0.1	2.0 ± 0.2	2.1 ± 0.3	0.7 ± 0.1	2.4 ± 0.1	<0.001
Total Biomass, mg	1.0 ± 0.1	0.4 ± 0.1	0.7 ± 0.3	1.0 ± 0.2	0.3 ± 0.1	0.7 ± 0.3	0.7 ± 0.2	1.4 ± 0.1	0.7 ± 0.2	0.1 ± 0.1	<0.001
Herbivores, % of total	38.5	33.1	12.2	45	38.5	37.8	48.3	68.2	91.4	2.4	-
Fungivores, % of total	9	5.1	31.3	10.3	7.8	9.7	17.8	13.1	3.2	2.4	-
Bacterivores, % of total	41.4	39.3	33	25.8	16.5	44.3	25.5	9.8	3.5	90.2	-
Predators/Omnivores, % of total	11.1	22.5	23.4	18.9	37.2	8.2	8.5	8.9	1.9	4.9	-

The free-living nematodes genera, including *Aphelenchoides* sp. (C_p2), *Aphelenchus* sp. (C_p2), *Ditylenchus* sp. (C_p2), *Acrobeles* sp. (C_p2), *Acrobeloides* sp. (C_p2), *Alaimus* sp. (C_p2), *Panagrolaimus* sp. (C_p1), *Plectus* sp. (C_p2), and *Rhabditis* sp. (C_p1) were observed in the soil samples, which are indicators of the enrichment of the soil.

Total nematode biomass ranged from 0.1 to 1.4 mg per individual across all Magoebaskloof soil samples surveyed. The plant-parasitic index was the highest (3.0), where *Rotylenchulus* sp., *Helicotylenchus* sp., *Pratylenchus* sp., and *Hemicycliophora* sp. were found in more numbers than other soil samples (Tables 1 and 2).

The c-p status of free-living nematodes can be a useful indicator of soil health in oak trees in Magoebaskloof, located in the Limpopo Province. Most soil samples taken from Magoebaskloof were close to enrichment conditions, as c-p 1–5 (which are nematodes) tended to increase by 80% in Site-5 (Figure 4). In contrast, basal nematodes (c-p 2) increased, while soil enrichment decreased by 100% in Site-10. None of the soils from Magoebaskloof showed 100% stress condition. Oak soils (Sites-1, 2, 4, 6) were enriched and stabled. Sites 7–9 were close to stress conditions due to plant-parasitic nematodes found in those soils (Figure 4).

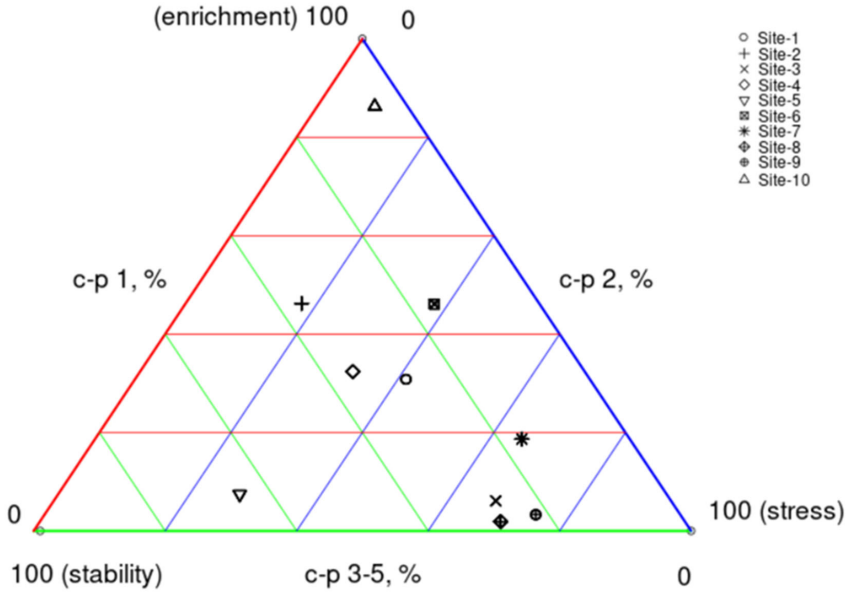


Figure 4. The c-p (colonizer–persister) triangle depicting soil status based on the c-p groups of nematodes found in oak trees in Magoebaskloof, Limpopo Province, South Africa.

The food web analysis (Figure 5) was performed for all nematode taxa identified in the soil samples. This analysis indicated the soil health status of each site based on the nematode communities present (Figure 5). Most of the soil samples for oak trees (Sites-1, 2, 4, 5, 6, and 10) were shown to be mature and N-enriched (60%), Site-7 showed disturbed soil (10%), and Sites-3, 8, and 9 (30%) showed degraded soil, a high C/N ratio, and stressed condition.

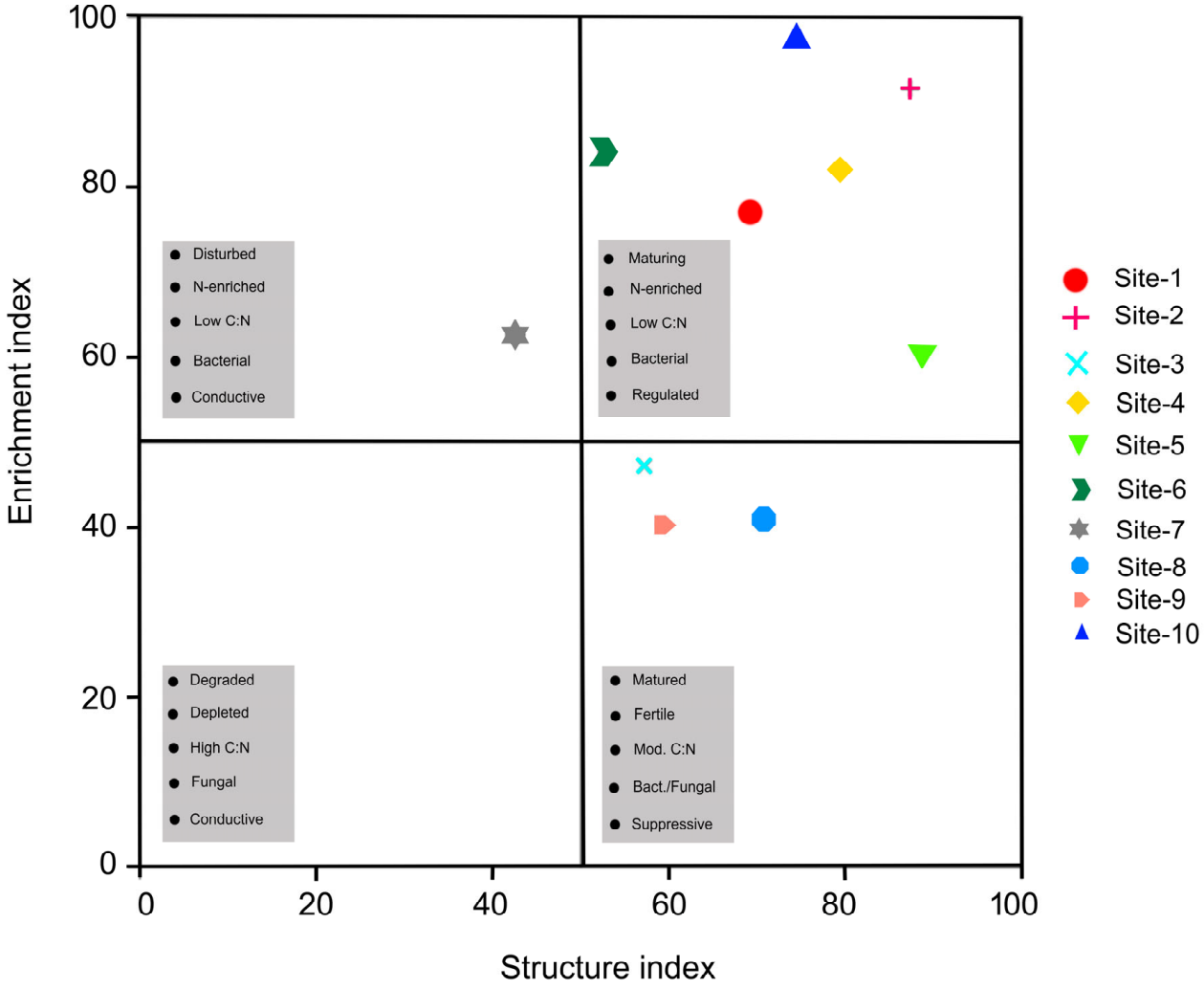


Figure 5. Food web analysis of various soil samples collected from oak trees in Magoebaskloof, Limpopo Province, South Africa, and their position as soil health bioindicators.

4. Discussion

The present study conducted in Limpopo Province, South Africa, shows that oak trees in the area are associated with various nematodes, including both plant-parasitic and free-living types. Various types of nematodes are involved in the food web of the soil [26]. *Meloidogyne* species, known to threaten oak trees [1,27], were found in this study, confirming previous reports. In addition, *Hemicycliophora*, *Mesocriconema*, and *Paratrichodorus* nematodes were also previously associated with oak trees in the region [12]. In addition, *Pratylenchus* was reported as a decline reason of oak trees [1]. The present study found *Hemicycliophora* in association with oak trees as well. This is the first extensive study on the nematodes associated with oak trees in Limpopo Province; therefore, several other nematodes than those found in previous works were found.

Within plant-parasitic nematodes, the *Helicotylenchus* and *Pratylenchus* species were the most distributed with a 90 and 80% frequency of occurrence. *Pratylenchus* is one of the most economically critical plant-parasitic nematodes genera, which cause damage and

yield loss in various crops [1,28]. The results of the current study demonstrate that various feeding groups of free-living nematodes, including bacterivores, fungivores, predators, and omnivores, were linked to oak trees.

Soil parameters, including pH, negatively correlated with the number of nematodes genera in the soil, including the *Aphelenchoides*, *Acrobeloides*, and *Aporcelaimellus* species. The previous work showed a positive correlation between pH and *Acrobeloides* associated with tomatoes and kiwi [29,30]. The differences in the host and soil environment cause variations in the nematode community. Oak trees grow naturally close to a river and have access to more water, food sources, and organic matter content than those grown in agricultural lands. Moreover, different species of *Acrobeloides* might respond differently to soil pH. On the other hand, *Aphelenchoides* exhibited the same reaction in various environments, implying that most *Aphelenchoides* species have a similar strategy in dealing with soil pH.

Due to the soil texture, most nematode genera thrive in sandy or loamy soils. The silt percentage of the soil showed a negative correlation with the total number of nematodes in soybeans in Arkansas [31]. The same result was reported in the relationship between the number of *Pratylenchus*, *Nanidorus*, and *Xiphinema* associated with tomatoes in South Africa [29]. The same result was obtained in the present study, in which silt had a negative correlation with the number of *Hemicycliophora*, *Alaimus*, *Plectus*, and *Panagrolaimus*. Some of the studies showed a positive correlation of silt with the number of *Pratylenchus*, while the present study showed no correlation with *Pratylenchus* in oak trees. Plant-parasitic nematodes require adequate soil moisture and pore space to facilitate their movement toward plant roots, where they feed and reproduce. Without these conditions, their ability to cause damage and harm to plants may be reduced. Therefore, to ensure successful plant development, it is vital to regularly assess the presence of plant-parasitic nematodes and the overall soil conditions [31]. Soil clay content is a crucial factor affecting the number of nematodes in the soil. Previous studies have shown that *Ditylenchus* nematodes positively correlate with clay content [29], which was also observed in the present study. The presence of nematodes has been observed to be more prominent in soil with high clay content in okara [32]. This is attributed to the influence of clay content on the soil's water holding capacity and nutrient availability [33]. Therefore, a high clay content facilitates an environment conducive to the proliferation of nematodes, leading to an increase in their population within the soil. On the other hand, *Acrobeles* nematodes negatively correlated with soil clay content [29], which was also observed in the present study.

Organic matter is crucial in determining the diversity and distribution of nematodes in soil. A study conducted in Brazil found that low nematode densities were associated with soil organic matter (SOM) in soybean roots [34]. Similarly, another study showed that the number of nematodes was correlated with the amount of organic matter in the soil [33]. However, the relationship between organic matter and soil nematodes can be positive or negative.

In Brazil, soil organic matter was found to negatively correlate with the total number of nematodes and *Pratylenchus* in soybean soil studied by Dias-Arieira et al. [34]. In contrast, our study showed a positive correlation between the number of *Pratylenchus* and the organic matter content in oak trees. This could be due to the proximity of oak trees to the river, which leads to higher organic matter content in the soil. Additionally, certain species of *Pratylenchus*, such as *P. brachyurus*, exhibit a higher reproductive rate in maize compared to soybeans. This is due to the fact that maize is a poor host, allowing the nematode to thrive inside the root [35].

Furthermore, the soil organic matter content in soybean soil showed a positive correlation with *Xiphinema*, which is consistent with the findings of our study. According to a recent study, there is a clear negative relationship between the amount of organic matter in the soil and the density of nematodes, particularly the dominant families of Cephalobidae and *Pristionchus*, in farmlands and grasslands across the Netherlands [36]. The same result was obtained in the present study. This finding suggests that higher levels of organic matter in soil may help to reduce the number of nematodes present. Additionally,

previous research has shown that high soil moisture levels negatively impact bacterivores and predator nematodes [37,38]. One possible explanation is that moist soil aggregates provide a more favorable environment for these nematodes and their prey, as is the case with oak trees along the riverbank.

The sigma maturity index (SMI) measures the level of environmental disturbance in non-agricultural soil, according to Sanchez-Moreno and Ferris [39]. The findings from the study on nematodes associated with oak trees showed that only Site-10 had an SMI score of 1.3, which is lower than the ideal score of 2. This suggests that the oak tree in Site-10 is not fully matured, and the soil is disturbed. On the other hand, the SMI scores in all the other sites were over 2, indicating that the soil is matured and has a proper soil food web.

The plant-parasitic index (PPI) indicates the presence of plant-parasitic nematodes in the study sites [39]. The results show that the PPI score is higher than 2 in all sites. This indicates that plant-parasitic nematodes, such as *Meloidogyne* and *Xiphinema*, are associated with the oak tree root system. However, due to the low number of plant-parasitic nematodes in the soil, the PPI scores were not high, and the damage caused was not significant.

The channel index (CI) indicates the decomposition rate of soil organic matter [39]. The results showed that at Sites-3, 8, and 9, the CI was higher than 50, which indicates that bacterivores were more dominant, leading to a more rapid decomposition of organic materials. On the other hand, the CI was lower than 50 at the rest of the sites, suggesting that fungivore nematodes were dominant, and a lower rate of organic matter decomposition was expected.

The enrichment index is a measure that helps us understand the amount of food available in soil samples [39]. By analyzing the results of this study, we can see that the majority of the sites had a value greater than 60, which indicates a high level of food availability in the soil that supports oak trees. This suggests that the soil of these oak trees is highly enriched with nutrients that support the growth and maintenance of the trees. The enrichment index is an essential tool for understanding the health and vitality of soil ecosystems, and its results can help us make informed decisions about land use, conservation, and ecological management.

The structure index is a scientific tool that measures the degree of land disturbance caused by various ecological and environmental factors [38]. It plays a vital role in identifying the condition of soil, particularly in areas with natural and disturbed lands. The present study has revealed that a significant portion of the soil in the region has a value of higher than 60 in the structure index, suggesting that oak trees in the area have faced relatively less damage due to factors such as salinity, tillage, pollution, etc. This finding implies that the soil in the region has a good structure and is less prone to erosion and other forms of damage, which can benefit the environment and the sustainable management of natural resources.

The Shannon index is used to measure community species diversity, with a higher value indicating a greater number of different nematode species [29]. This study found high nematode diversity in all locations except Site-9, indicating a thriving and diverse community associated with okra.

The faunal profile is a visual representation of a particular area's enrichment index (EI) and structure index (SI). It is divided into four quadrants, in which the oak tree had its food webs predominantly present the soil with a low C/N ratio, which means that the soil has a balanced decomposition channel and is matured. The presence of food webs in this quadrant indicates that it provides a suitable environment for the oak tree to grow. The location of oak trees in an area encourages a natural decomposition process in the soil, where bacterivores and fungivores break down organic matter and increase the presence of microorganisms. As a result, the soil's maturity and enrichment index are positively impacted [40]. Moreover, a study revealed that adding effective microbes derived from yeast, raw milk, and decomposing leaves to the forest soil can further enhance its maturity and structure index [40]. Therefore, an increase in soil microbes leads to an increase in

bacterivore and fungivore nematodes. This accelerates the decomposition of soil organic matter, increases mineralization, and releases nutrients for plant growth [41].

This demonstrates the importance of maintaining a healthy soil ecosystem for optimal plant growth and environmental health.

The results of research work on oak trees from Portugal [42] have provided valuable insights into the feeding behavior of nematodes, revealing that bacterivores, followed by fungivores, are the dominant feeding groups. This finding was further confirmed in the present study, which found that bacterivores were the most prevalent group in 50% of the sampling sites, followed by herbivores. At the same time, fungivores were the third group of nematodes associated with oak trees. Moreover, the previous study conducted in Portugal [42] revealed that *Hemicycliophora*, *Helicotylenchus*, *Xiphinema*, and *Pratylenchus* were present among the plant-parasitic nematodes. The current study confirms the presence of these taxa and further highlights the remarkable diversity of nematodes associated with oak trees in Limpopo Province, South Africa. This study's findings suggest that these nematodes play a crucial role in the ecological balance of the region and could have significant implications for the management and conservation of oak tree species. Further study is needed to understand better the impact of these nematodes on the health and growth of oak trees and their interactions with other organisms in the ecosystem.

5. Conclusions

The oak trees in the Magoebaskloof mountains are a vital part of the local ecosystem. To ensure the health of the trees and the soil in which they grow, it is essential to understand the diversity of the nematodes present. Our survey found a diverse range of nematodes associated with oak trees, with free-living bacterivores being the most dominant. The soil around oak trees was found to be stable and low in stress, with organic matter negatively correlating with nematode numbers. This suggests that measuring the level of organic matter in the soil can be a helpful way to monitor soil health over time. In particular, the number of *Panagrolaimus* nematodes in the soil was strongly affected by organic matter levels, making it a potential bioindicator of soil health in oak trees. More research is required to investigate the seasonal variations of nematodes and their ecological role in the soil.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d16110673/s1, Figure S1. Sampling location of oak trees in Magoebaskloof mountain, Limpopo Province, South Africa. A: South Africa map; B: Sampling sites; C: Oak tree leaves at sampling sites; D: Oak tree seed at sampling sites; E, F: Oak trees.

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