



Impact of Agricultural Land Use on Nematode Diversity and Soil Quality in Dalmada, South Africa

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Article

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Abstract: During a survey on soil nematode diversity, the soil samples were collected from Field-1 (3-months-not-used land), Field-2 (5-years-not-disturbed land), Field-3 (the rhizosphere of tomatoes), and Field-4 (natural land, not disturbed for 50 years), in Dalmada, Limpopo Province, South Africa. A total of 25 nematode genera were found to be associated with the surveyed plant species. The result showed Acrobeloides, Aphelenchus, Aporcella, Ditylenchus, Mesorhabditis, Pratylenchus, and Rotylenchus with a 100% frequency of occurrence. Meloidogyne was detected only in association with Field-3, with a low frequency of occurrence (25%). The study of the relationship between nematodes with physicochemical properties in the soil using Pearson correlation revealed that phosphate of the soil had a positive correlation (r = 0.977) with *Bitylenchus* and *Pseudacrobeles* species. In contrast, pH strongly correlated with Nanidorus (r = 0.928), Trypilina (r = 0.925), Xiphinema (r = 0.925), and Zeldia (r = 0.860). The principal component analysis placed Field-4 and Field-3 in two groups, indicating the biodiversity dynamics among the two locations. Soil texture showed that clay was correlated with Rotylenchulus. In contrast, soil texture had no effect on Meloidogyne. The Shannon index was the lowest (1.7) for Field-1 in Dalmada compared to the other Fields, indicating lower nematode diversity. The structure index showed that Field-2 was disturbed with a low C:N ratio. In contrast, Field-3 and Field-4 had suppressive soil but matured and fertile. The network analysis showed that Panagrolaimus was only found in Field-4 and was the most engaging genus describing soil quality in the soil system in Dalmada. In conclusion, Field-2 showed a high diversity of free-living nematodes than the disturbed land of tomatoes. Additionally, plant-parasitic nematodes numbered more in the rhizosphere of tomatoes. The results suggest that the soil nematodes, especially free-living bacterivores, may mediate the effects of ecosystem disturbance on soil health.

Keywords: nematodes; physicochemical properties; soil health; tomato

1. Introduction

Any life in soil or water is represented by microorganisms such as bacteria and eukaryotic organisms such as fungi, protists, microarthropods, nematodes, and earthworms [1]. Soil nematodes are the main organisms that are critical in the soil food web [2]. As classified by Yeates [3], nematodes are grouped into herbivores (plant parasitic nematodes), bacterivores, fungivores, omnivores, and predators. Additionally, in association with other soil organisms, nematodes play an essential role in the decomposition of organic matter for soil health [4]. Moreover, some nematodes are studied as soil health indicators. Therefore, nematodes also provide information about the abundance and activity of other soil organisms and thus have been used as indicators to study soil food web conditions [5–7], soil biodiversity, and ecosystem functioning [6,7]. Furthermore, nematodes are valuable indicators of soil ecosystem health, pollution, and eutrophication in aquatic habitats [8]. Different land-use types experience various disturbances and management practices resulting in soil quality or health changes. However, the effect of land-use changes on the biodiversity of soil organisms has not been investigated in Limpopo Province, South Africa.



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Copyright: © 2023 by the author. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). The tomato is a very important vegetable crop in South Africa. Tomatoes are the second most important vegetable commodity after potatoes, planted on about 6000 hectares [9]. Tomatoes contribute about 24% of the total vegetable production in South Africa. Plant-parasitic nematodes have previously been studied as a threat to crops in Limpopo Province [9–14]. Additionally, root-knot nematodes are the main plant-parasitic nematode that causes a yield loss of tomatoes in Limpopo Province [9]. However, despite all the information on nematode diversity, how does human activity affect the biodiversity of nematodes in the soil?

Agricultural land use means using land to produce agricultural products for human or animal consumption, including livestock raising operations, croplands, orchards, pastures, greenhouses, plant nurseries, and farms [15]. Land-use change has already been shown to impact nematode diversity [15]. For example, crop rotation and cover crops affect microbial composition [16]. Physical impacts such as tillage and compaction affect root pathogenic fungi, protozoa, collembola, and earthworm communities [17]. Additionally, various agricultural land uses indicated changes in the nematode diversity in India [18]. Moreover, intensive agriculture affects the food web by changing the nematode community of the European soil [19].

Furthermore, intensive land use turns nematode diversity into specialization [20]. However, the nematode diversity of different land uses have not yet been studied in Limpopo Province. Therefore, the purposes of the study were (1) to assess the nematode community affected by agricultural land use and (2) to evaluate the relationship between nematodes with selected physicochemical properties in the soil.

2. Materials and Methods

2.1. Soil Sampling

Soil samples were collected from different locations in Dalmada designated Field-1 (3-months-not-used land), Field-2 (5-years-not-disturbed land), Field-3 (the rhizosphere of a tomato), and Field-4 (natural land, not disturbed for 50 years) (Table 1; Figure 1). Soil texture was determined according to van Capelle et al. [17].

Table 1. Localities of the soil samples of different sites in Dalmada, Limpopo Province, South Africa.

Sampling Site	Site	GPS Coordinates
Field-1	3 months not cultivated, prepared for tomato plantation	S: 23°53′44.96″; E: 29°32′52.844″
Field-2	Free land not cultivated for 5 years	S: 23°53′44.086″; E: 29°32′47.463″
Field-3	Tomato field	S: 23°53′44.411″; E: 29°32′55.192″
Field-4	Natural land, not disturbed for 50 years	S: 23°53'39.567"; E: 29°32'53.417"



Figure 1. Sampling location of four fields in Dalmada, Limpopo Province, South Africa. (**A**) Field-1 = land prepared for tomatoes, not cultivated for 3 months; (**B**) Field-2 = land not cultivated for 5 years; (**C**) Field-3 = tomato fields; (**D**) Field-4 = natural land, not disturbed for 50 years.

Sixty samples were collected randomly from the locations (Table 1). After removing the aboveground plant debris, fifteen soil samples from each site were collected separately from 0 to 30 cm below the soil surface. The subsamples cover each location for the root zone and roots to recover the various groups of nematodes. Samples stored at 4 °C in cooler boxes were transferred to the Nematology laboratory of the Aquaculture Research Unit (the University of Limpopo, South Africa). The soil samples were processed to identify the nematodes and soil parameters analyses on the same day.

2.2. Nematode Identification

Nematodes were extracted from 200 g of soil from each location on the same collection day using a modified tray technique [13,21]. The nematodes were counted with a stereomicroscope (Zeiss; Discovery V8; Oberkochen; Germany), and their genera identification was finalized using a light microscope (Zeiss, Lab.A1; AX10; Oberkochen, Germany). Nematodes were then fixed with a hot 4% formaldehyde solution and transferred to anhydrous glycerin [22] for species identification. The nematode genera were identified according to the classification provided by Andrássy [23], Geraert [24], and Shokoohi and Abolafia [25].

2.3. Soil Properties Analysis

Chemical properties of soil, including ammonia, nitrate, phosphate, and hardiness, were evaluated at the Aquaculture Research unit laboratory using a Hach spectrophotometric device (Loveland, CO, USA) based on the protocol instructed by the company. In addition, the pH was measured using Thermo Scientific Orion 3 Star pH Benchtop (Waltham, MA, USA). The samples were analyzed for potassium (K) (Method 3120 B and EPA 200.7) using the standard APHA [26] method. Total nitrogen (N) using test number 1.14537.0001; ammonia and ammonium using test number 1.14752.0001/1.14752.0002/1.00683.0001; and total phosphate (P) using test number 1.14848.0001 were measured. Nitrate was analyzed according to Cadmium reduction method no. 8171 of DOC316.53.01069 [27]. Phosphate, ammonia, and copper were analyzed according to USEPA PhosVer 3, and the results were obtained [27]. All soil properties were analyzed using spectrophotometry. The hardiness of the extracted water from the tray method of the tested samples [13] also was analyzed using the Calcium and Magnesium—Calmagite Colorimetric Method 8030 of DOC316.53.01043 [27].

2.4. Statistical Analysis

The relationships between nematode mean population density (MPD) and frequency of occurrence (FO) of each nematode genus identified were expressed as prominence value (PV) for each locality to determine which genera were predominant in Dalmada, Limpopo Province. The PV was calculated using the equation [28].

PV = Population density $\times \sqrt{}$ frequency of occurrence

Nematode biodiversity index, including Shannon Index (H') [29] was calculated. Finally, to evaluate the soil factors' relationship, including pH, ammonia, nitrate, phosphate, hardiness, locality, and nematode, a principal component analysis (PCA) was conducted based on Renčo et al. [30]. A principal component analysis (PCA) utilizing XLSTAT [31] was used to ordinate the sites by the abundance of nematode genera. Soil properties were used as supplementary variables to identify relationships with the abundances of the main nematode genera. The scores' values were determined for each variable based on each of the principal components, and the scores for the first two components were used to form a two-dimensional plot (PC1 and PC2) based on eigenvalues given with the software XLSTAT. The data of frequencies/percentages were normalized using log 10. Additionally, the following indices were calculated:

- Frequency of Occurrence (FO%) = (Number of samples containing a genus/number of total samples) × 100;
- (2) Relative Abundance (RA) = Total number of individuals of a particular genus per g soil and root sample in all samples/number of samples, including those with zero counts for that genus;
- (3) Population Density (PD) = Mean number of individuals of a particular genus/number of positive samples;
- (4) Absolute frequency (AF) = Number of samples containing a genus/Total Number of samples collected × 100;
- (5) Absolute Density (AD%) = Density of the genus/Total No: of samples collected \times 1001;
- (6) Relative Frequency (RF%) = Frequency of the species/Total frequency of all the species × 100.

The potential of nematodes as effective soil health bio-indicators in Dalmada fields was assessed using the c-p (colonizer-persister) triangle and food web diagnostics using NINJA (Nematode Indicator Joint Analysis) online software. The identified nematode genera were grouped into bacterivores, omnivores, fungivores, herbivores, and predators for the ecological indices, function indices, and metabolic footprints. The maturity index (MI), Maturity index of nematodes in cp2-5 (MI2-5), and plant-parasitic index (PPI) were calculated for each site. Channel index (CI), basal index (BI), enrichment index (EI), and structure index (SI) were also computed. The Enrichment Index and Structure Index were plotted into a fauna profile which depicts the structure and state of the soil food web as either disturbed, degraded, maturing, or structured. The nematode metabolic footprints were also examined. The metabolic footprints and indices were computed using the online program Nematode Indicators Joint Analysis (NINJA) (https://shiny.wur.nl/ninja; accessed on 21 April 2023) [32].

2.5. Data Visualization

The network of nematode genera compositions in different land-use types was analyzed using Gephi 0.10.1 202301172018 software [33]. First, each one of the soil sample places had their latitude and longitude inserted into the table as a site node and, using the Geo Layout plugin, were distributed in space accordingly and marked to be settled. After that, the remaining nodes representing the nematodes found in this study were inserted, and their placements were calculated with the Yifan Hu layout, which placed nodes based on the strength of connections each one had to the locked nodes of the site. In this way, stronger relationships led to the node representing the nematode being closer to the site node. The thickness of the connecting lines also represented the strength of the connections. Yifan Hu layout also, by its inherent characteristics, made the visualization of single-connected nodes easier to identify.

3. Results

3.1. Analysis of the Nematode Communities

Twenty-five species belonging to 25 genera were identified (Table 2). Fifteen species were identified in Field-4, whereas 13 species were identified in Field-3 in association with tomatoes (Table 2). From the overall genera recovered, *Aporcelaimus*, *Filenchus*, and *Panagrolaimus* were detected only in Field-2; *Nanidorus*, *Trypilina*, and *Xiphinema* only in Field-4; and Meloidogyne only in Field-1 (Table 2).

Table 2. Mean abundance of nematode genera associated with different soils in Dalmada, Limpopo Province, South Africa. [Field-1 = land prepared for tomatoes, not cultivated for 3 months; Field-2 = land not cultivated for 5 years; Field-3 = tomato fields; Field-4 = natural land, not disturbed for 50 years; C-p = colonizer-persister; P-p = plant-parasite].

Genus	C-p Class	P-p Class	Feeding Group	Field-1	Field-2	Field-3	Field-4
Acrobeles	2	0	Bacterivores	113.1	0	4.8	99.4
Acrobeloides	2	0	Bacterivores	18.3	96.2	38.3	40.8
Aphelenchoides	2	0	Fungivores	49	138.9	4.6	0
Aphelenchus	2	0	Fungivores	34.3	133.2	28.7	44.5
Aporcella	5	0	Omnivores	68.5	27.1	66.5	70.6
Aporcelaimus	5	0	Omnivores	0	32	0	0
Bitylenchus	0	3	Herbivores—ectoparasites	0	5.5	84.3	0
Cervidellus	2	0	Bacterivores	4.7	4.7	0	4.1
Diphtherophora	3	0	Fungivores	6.1	11.4	0	9.4
Ditylenchus	2	0	Fungivores	88.1	112.1	46.7	86.7
Filenchus	2	0	Fungivores	0	8.4	0	0
Meloidogyne	0	3	Herbivores—sedentary parasites	22.2	0	0	0
Mesorhabditis	1	0	Bacterivores	34.4	132.8	155.3	4.9
Nanidorus	0	4	Herbivores—ectoparasites	0	0	0	13.7
Panagrolaimus	1	0	Bacterivores	0	5.3	0	0
Plectus	2	0	Bacterivores	5.3	0	7.7	0
Pratylenchus	0	3	Herbivores—semi-endoparasites	4.9	4	9.9	20.5
Prismatolaimus	3	0	Bacterivores	0	12.2	10.7	13.5
Pseudacrobeles	2	0	Bacterivores	5	0	49.5	0
Rotylenchus	0	3	Herbivores-migratory endoparasites	10.5	13	61.5	53.3
Rotylenchulus	0	3	Herbivores-migratory endoparasites	6.1	4.6	0	0
Trypilina	2	0	Bacterivores	0	0	0	9.1
Wilsonema	2	0	Bacterivores	0	22.8	0	0
Xiphinema	0	5	Herbivores—ectoparasites	0	0	0	16.9
Zeldia	2	0	Bacterivores	5.1	5.1	0	11.7

Three plant-parasitic species (PPN), including *Bitylenchus ventrosignatus*, *Rotylenchus brevicaudatus*, and *Rotylenchulus parvus* were identified from the soil samples of tomatoes (Table 2). Variations were observed for MPD in all nematode species over the soil sites (Table 3). Out of the 60 soil samples from the four locations, the most prevalent nematode encountered were *Acrobeloides*, *Aphelenchoides*, *Aporcella*, *Ditylenchus*, *Mesorhabditis*, *Pratylenchus*, and *Rotylenchus* with 100% of FO%. The most PV (1.4) was estimated for *Acrobeles*, *Ditylenchus*, and *Mesorhabditis* (Table 3).

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Genus	FO%	RA	PD	PV	RF
Acrobeles	75%	54.3	1.6	1.4	9.3
Acrobeloides	100%	48.4	0.8	0.8	8.3
Aphelenchoides	75%	48.1	1.4	1.2	8.3
Aphelenchus	100%	60.2	1.0	1.0	10.3
Aporcelaimus	25%	8.0	2.1	1.1	1.4
Aporcella	100%	58.2	1.0	1.0	10.0
Bitylenchus	50%	22.4	1.5	1.1	3.9
Cervidellus	75%	3.4	0.1	0.1	0.6
Diphtherophora	75%	6.7	0.2	0.2	1.2
Ditylenchus	100%	83.4	1.4	1.4	14.3
Filenchus	25%	2.1	0.6	0.3	0.4
Meloidogyne	25%	5.6	1.5	0.7	1.0
Nanidorus	25%	3.4	0.9	0.5	0.6
Panagrolaimus	25%	1.3	0.4	0.2	0.2
Plectus	50%	3.3	0.2	0.2	0.6
Pratylenchus	100%	9.8	0.2	0.2	1.7
Prismatolaimus	75%	9.1	0.3	0.2	1.6
Pseudacrobeles	50%	13.6	0.9	0.6	2.3
Rhabditis	100%	81.9	1.4	1.4	14.1
Rotylenchus	100%	34.6	0.6	0.6	5.9
Rotylenchulus	50%	2.7	0.2	0.1	0.5
Trypilina	25%	2.3	0.6	0.3	0.4
Wilsonema	25%	5.7	1.5	0.8	1.0
Xiphinema	25%	4.2	1.1	0.6	0.7
Zeldia	75%	5.5	0.2	0.1	0.9

Table 3. Community analysis of nematodes associated with different sites in Dalmada, Limpopo Province, South Africa. [FO = Frequency of Occurrence; RA = Relative Abundance; PD = Population Density; PV = Prominence Value; RF = Relative Frequency].

3.2. Indices of the Nematode Communities

Changes in the sigma maturity index and plant-parasitic index statuses of Field-4 and Field-3 were significant (Table 4). The indices of the nematode communities were generally inconsistent between sites due to their ability to identify ecosystem disturbance (Table 4). The Shannon index (H') identified an ecosystem disturbance of Field-1. The lowest Shannon index was recorded in Field-1 (H' = 1.8), which was lower than in the tomato field. In contrast, MI was the highest in Field-4 (2.6) compared to the other sites. The total nematode biomass (p < 0.001) was the highest (2.5 ± 0.3) in Field-1. In contrast, the metabolic activities (metabolic footprints) of various nematode guilds (herbivores, bacterivores, fungivores, predators, and omnivores) identified ecosystem disturbance well throughout the study, in which the fungivores and bacterivores footprints were 47.1 and 77.9, respectively, for Field-2, which are the highest among the sites studied. Additionally, Basal Index (BI), Enrichment Index (EI), Structure Index (SI), and composite and structure footprints distinguish between Field-4 (the natural land) and the other sites throughout the study.

Index Name	Field-1	Field-2	Field-3	Field-4	<i>p</i> -Value
Basal Index	31.5 ± 1.6	26.3 ± 0.5	13.6 ± 1.1	31.7 ± 1.0	< 0.001
Bacterivore Footprint	44.7 ± 2.5	77.9 ± 3.2	61.2 ± 5.6	48.0 ± 2.6	< 0.001
Channel Index	60.7 ± 2.9	39.1 ± 1.3	11.5 ± 1.2	86.1 ± 3.4	< 0.001
Composite Footprint	385.0 ± 42.4	216.8 ± 9.4	166.5 ± 13.7	176.8 ± 4.2	< 0.001
Enrichment Footprint	31.4 ± 5.0	81.3 ± 1.7	48.5 ± 5.1	21.7 ± 1.7	< 0.001
Enrichment Index	46.8 ± 3.1	65.9 ± 0.8	79.4 ± 1.6	33.8 ± 1.3	< 0.001
Fungal/Bacterial	0.9 ± 0.1	1.4 ± 0.0	0.3 ± 0.0	0.7 ± 0.0	< 0.001
Fungivore Footprint	25.7 ± 3.4	47.1 ± 1.0	11.9 ± 0.9	22.2 ± 1.8	< 0.001
Herbivore Footprint	247.1 ± 41.4	5.2 ± 1.1	25.2 ± 3.8	36.7 ± 3.4	< 0.001
Maturity Index	2.4 ± 0.1	2.1 ± 0.0	2.2 ± 0.1	2.6 ± 0.0	< 0.001
Maturity Index 2-5	2.5 ± 0.0	2.4 ± 0.0	2.8 ± 0.1	2.6 ± 0.0	< 0.001
Omnivore Footprint	67.6 ± 6.4	30.6 ± 6.5	68.2 ± 9.5	69.9 ± 2.6	< 0.001
Plant Parasitic Index	2.9 ± 0.0	2.8 ± 0.0	3.0 ± 0.0	3.4 ± 0.0	< 0.001
Shannon Index (H')	1.8 ± 0.0	2.2 ± 0.0	2.2 ± 0.0	2.2 ± 0.0	< 0.001
Sigma Maturity Index	2.5 ± 0.1	2.1 ± 0.0	2.4 ± 0.1	2.8 ± 0.0	< 0.001
Structure Footprint	68.8 ± 6.4	90.6 ± 7.5	69.8 ± 9.6	73.7 ± 2.5	< 0.001
Structure Index	56.4 ± 2.4	46.4 ± 2.0	71.1 ± 3.6	62.2 ± 1.5	< 0.001
Total Biomass, mg	2.5 ± 0.3	0.8 ± 0.1	0.6 ± 0.1	0.7 ± 0.0	< 0.001
Total Number, Individual	486.9 ± 31.5	795.4 ± 17.6	568.3 ± 43.2	501.3 ± 14.6	< 0.001

The c-p status of free-living nematodes can be an important indicator of soil status in tomatoes and natural field in Dalmada, Limpopo Province. The c-p triangle was established for all nematodes obtained from different localities (Figure 2). The majority of soil samples taken from Dalmada were close to stress conditions, as c-p 3–5, which are the structure nematodes, tend to decrease drastically by 80% (Figure 2). On the other hand, basal nematodes (c-p 2) were increased alongside the decrease of soil enrichment by 60%. None of the soil from Dalmada showed stable condition. In contrast, tomato field (Field-3) showed enriched soil up to 50–60% (Figure 2) compared to the other fields.



Figure 2. The c–p (colonizer–persister) triangle depicting soil status regarding nematode c–p groups' evolution in Dalmada Fields, Limpopo Province, South Africa.

The food web analysis (Figure 3) was conducted for all nematode taxa identified in all soil samples. This analysis highlighted the soil health status of each site in the function of

occurred nematode communities (Figure 3). Field-3 (tomato) was shown to have mature, N-enriched (80%), regulated soils with a bacterial feature. Field-2 was shown to be disturbed (50%) and conductive with a low C:N ratio.





On the other hand, most samples of Field-1 and Field-4 were mature (55–60%) and fertile with a moderate C:N ratio and bacterial/fungal features. Metabolic footprint analysis was conducted for all sites to explain the latter analysis further, and soils are clearly distinguished regarding their soil health (Figure 4). Soil samples from Field-3 (tomato) were distinctively highly enriched (75%) and matured with a high abundance of bacterial feeders. Furthermore, fertile soils were accurately presented in Field-1 (58%) and Field-4 (62%) (natural land). Soil disturbance in the Dalmada fields was highlighted mainly in Field-2, with 48% of disturbance. Overall, the result indicated that the soil samples of Dalmada were more structured than enrichment.



Figure 4. Nematode food web diagnostics and their position as soil health bioindicators. Metabolic footprint showing the amplitudes of nematode enrichment and structure indices throughout Dalmada Fields, Limpopo Province, South Africa.

3.3. Correlation of the Selected Soil Parameter with Nematodes

The soil type was loamy sand (Field-1), sandy loam (Field-2), sandy loam (Field-3), and sand (Field-4), which were distinguished based on the soil texture (Table 5).

Table 5. Soil texture of the sites studied in Dalmada, Limpopo Province, South Africa.

Site	Clay%	Silt%	Sand%
Field-1	1.7	19	79.3
Field-2	6	38	56
Field-3	1.9	27.5	70.6
Field-4	1.7	8.6	89.7

The results indicated a positive correlation (p < 0.05) between clay and *Acrobeloides* (r = 0.947), *Aphelenchus*, and *Wilsonema* (r = 0.922). In contrast, clay showed a strong negative correlation (p < 0.05) with *Aporcella* (r = -0.933). Sand showed a strong negative correlation (p < 0.05) with *Aporcella* (r = -0.829), *Mesorhabditis*, (r = -0.828), and *Wilsonema* (r = -0.825). Copper showed a positive correlation with *Rotylenchus* (r = 0.547) and a negative correlation with *Rotylenchulus* (r = -0.926). The pH of the soil showed a positive correlation only with *Nanidorus* (r = 0.928), *Trypilina* (r = 0.925), *Xiphinema* (r = 0.925), and *Zeldia* (r = 0.860). Phosphorus showed a positive correlation with *Pseudacrobeles* (r = 0.988) and *Bitylenchus* (r = -0.839) and *Ditylenchus* (r = -0.833). Nitrate showed a positive correlation with *Rotylenchulus* (r = -0.839) and *Ditylenchus* (r = -0.862). Finally, ammonia showed a positive correlation with *Rotylenchulus* (r = -0.862), and *Xiphinema* (r = -0.862). Finally, ammonia showed a positive correlation with *Rotylenchulus* (r = -0.862). Finally, ammonia showed a positive correlation with *Rotylenchulus* (r = -0.862). Finally, ammonia showed a positive correlation with *Rotylenchulus* (r = -0.935) (Figure 5).



Figure 5. Correlation of the selective soil parameters with the nematodes associated with different fields of Dalmada in Limpopo Province, South Africa.

3.4. Relationship of Soil Parameters and Nematodes

The principal component analysis (PCA) (Figure 6) explains 73.9% of the variation, 43.4% explained by PC1 and 30.5% explained by PC2, in which the soil chemical had a sig-

nificant effect on the distribution of the nematode species. The contribution of nematodes, soil variables, and study sites to the analysis indicates that *Acrobeloides, Aphelenchoides, Aphelenchus, Aporcelaimus, Filenchus, Panagrolaimus, Rotylenchulus,* and *Wilsonema* were dominant in substrates characterized by clay. In contrast, *Bitylenchus, Plectus,* and *Pseudacrobeles* were dominant in substrates characterized by phosphate. Additionally, *Mesorhabditis* was prevalent in the site with a greater silt percentage. The results indicate that pH was correlated with *Zeldia.* Moreover, sand was more correlated with *Pratylenchus* in natural soil.



Biplot (axes PC1 and PC2: 73.9 %)

Figure 6. Biplot of principal component analysis (PCA) of the nematodes' species in different fields of Dalmada, Limpopo Province, South Africa. [Field-1 = land prepared for tomatoes, not cultivated for 3 months; Field-2 = land not cultivated for 5 years; Field-3 = Tomato fields; Field-4 = Natural land, not disturbed for 50 years].

3.5. Data Visualization

A network projection was performed using Gephi to show the distribution of nematodes found on the different soil samples considering the geolocation of the sites (Figure 7). The proximity of Field-4 and Field-2 was apparent with an opposite group of the other sites, including Field-3 (tomato) and Field-1. Each of the sites had nematodes that were exclusively found in them. One emphasis was found on *Panagrolaimus* and *Wilsonema*, two of the bacterivores' nematodes found with a solid connection to Field-2. *Meloidogyne* was only connected with Field-1, while *Trypilina* and *Xiphinema* had relationships with Field-4 (natural land) only. *Acrobeles complexus* had a good connection with Field-4 and Field-1. The remaining nematodes were more distributed and were found between the four sites of sampling. The network analysis also indicated a strong relationship between *Aphelenchus, Aphelenchoides*, and *Ditylenchus* and Field-2. It was also of note that *Aporcella*, an omnivore, had a strong connection with Field-4 and Field-1.



Figure 7. Network analysis of the relationship of nematodes and fields of the study in Dalmada, Limpopo Province, South Africa. [1: Field-1 = land prepared for tomatoes, not cultivated for 3 months; 2: Field-2 = land not cultivated for 5 years; 3: Field-3 = tomato fields; 4: Field-4 = natural land, not disturbed for 50 years].

4. Discussion

Different agricultural lands affect nematode diversity [34]. Additionally, various agricultural lands change the composition, diversity, and habitat structure of understory plant species, potentially affecting soil biology [35]. This phenomenon explains the dynamics of nematodes in Dalmada in Limpopo Province, South Africa. Nematodes are the central part of the food web in soil ecosystems as they impact biological activity in the soil [36]. Soil as a habitat for nematodes can be changed due to various agricultural practices such as monoculture, tillage, drainage, application of agrochemicals, irrigation, and organic mulch [37]. The results indicated that nematode abundance was higher in high-input organic systems than in perennial cropping systems, while species diversity was most remarkable under minimum tillage treatments [38]. The same result was obtained in the present study as the diversity of nematodes was lower in Field-1 (1.75) with higher tillage than in Field-2 (2.24) and Field-4 (2.22) with lower tillage. According to Yeates et al. [37], nematode diversity was more greatly affected by long-term human effects on the soil. This phenomenon happens in Field-3, where tomatoes were cultivated for a long time and there is a lower diversity index (2.1) than Field-4 (natural land).

On the other hand, intensive land use decreases the nematode diversity [39]. They have indicated that herbivores, bacterivores, and omnivores were higher in organic vegetable fields than in conventional. Moreover, the present result agrees with previous studies [40]. The EI indicates the prevalence of opportunistic species, whereas the metabolic footprint

measures the carbon utilization of component taxa [41]. Previous studies report crop residue retention increases, EI and SI [42]. The same result was obtained in the present study, where in Field-1 and Field-4, higher EI and SI were observed than in the rest of the soil samples. In Field-1, the land was prepared for tomato plantation, which was left for three months. The residue of the previous tomato plantation was left in the field, so the SI and EI were higher than the rest. The same scenario applies to Field-4, a natural land with lots of plant residue to increase the SI and EI.

Furthermore, nematode community composition within the soil reflects the soil food web's changes. The fungivores/bacterivores (F/B) ratio describes the decomposition pathway in detritus food webs [43], in which smaller ratios were associated with faster rates of decomposition and nutrient turnover [44]. In the present study, F/B ratio was lower for Field-3 (tomato), indicating a quicker decomposition rate of plant debris than in other fields. However, Field-2 (the field left for 5 years) had the highest F/B ratio of the samples, indicating slow decomposition and nutrient turnover.

The PPI and MI indices are valuable indicators used to evaluate agricultural ecosystem conditions [45]. In the present study, the PPI was higher in natural land than others, and the MI was higher in natural land than the rest. The nematode MI [46] focused on the sensitivity of different taxa to the stress pattern and was scaled from 1 to 5 values. These indices measure the degree of disturbance that occurs in a particular ecosystem. This disturbance can interrupt the ecological succession of a soil community, and, hence, its history can be distinguished [38]. Freckman and Ettema [38] highlighted that both the MI and PPI ($2 \le PPI \le 5$) were mainly used to study the effects of disturbances on nematode communities in agroecosystems. Low values indicate a high disturbance alongside the vital presence of colonizers.

On the other hand, increased values define common disturbance patterns with a high degree of persistent nematodes. The previous work showed that organic farming had higher PPI and MI than conventional farming (Sanchez-Moreno et al., 2018). In the present study, agricultural land affected both PPI and MI. The same finding was observed by Bongers et al. [39], as they indicated agricultural land and management practices affect the nematode community assemblage. However, sampling time may affect the MI, regardless of the soil type, as indicated by Neher [44]. Regarding the CI, differences were observed between the prospected localities so long as all decomposition channels of organic matter were different depending on the abundance of fungivores and bacterivores. Laasli et al. [47] mentioned that disturbed systems have a decomposition aspect dominated by fungi organisms. Therefore, fungi decomposition was considered slow compared to the bacteria counterpart. The same result was obtained in the present study as the fungivores were fewer than bacterivores in all fields. In addition, soil texture affects the nematode distribution in the soil [44]. Overall, the maturity index, based on the nematode fauna, is proposed as an assessment of the condition of the soil ecosystem [5]. Additionally, Maturity index (MI) is the most sensitive of the indices of the degree of disturbance of the soil ecosystem [47]. In Field-1 and Field-4, a predominance of omnivores was observed, indicative of healthy soil. This result agrees with those reported by Laasli et al. [47], who pointed out that the decrease or absence of these trophic groups indicates deterioration in the health of the soil. Additionally, according to Bongers [46], the presence and, more particularly, the abundance of omnivores nematodes represent an undisturbed soil. A functional analysis of the soil's food web condition may include rates of soil respiration, organic matter decomposition, biologically mediated mineralization, and other processes [5] so that a small extent of carbon transfers to a higher trophic level [5]. The PCA plot showed the effect of soil parameters on the distribution of nematodes. Laasli et al. [47] indicated that clay had a significant role in the distribution of *Panagrolaimus*. The same result was obtained in the present study. Moreover, Pratylenchus was observed in correlation with sand particles in the soil. The result agrees with Laasli et al.'s finding [47].

In addition, nematodes have long served as bioindicators, and the ecological indices of nematode communities reveal natural and human-induced changes in soil ecosystems [48].

The use of soil nematodes as health indicators offers many advantages. Their use for assessing soils has increased because of their high diversity and abundance and their essential and various roles in ecosystem function [49]. The diversity, community structures, and functions of soil nematodes are critical indices in soil health; the anthropogenic disturbances in agriculture (tillage inversion, cropping patterns, and nutrient management) may influence soil nematodes [50]. The network analysis of the trophic group of nematodes and different fields showed a specific connection between the nematode and the soil. The network analysis of the present study showed that *Trypilina* was connected with Field-4, a natural land that has not been disturbed for 50 years. In addition, *Panagrolaimus* had a connection with Field-2, which was 5-years not disturbed and cultivated. The network analysis implies that bacterivore nematodes can be used as soil bio-indicators of a soil's status.

5. Conclusions

In this study, the biodiversity of nematodes was affected by agricultural land use. Natural land showed a higher diversity index, despite human activities, than other agricultural lands. Additionally, the field with tomatoes showed less biodiversity and a low stability of the soil condition. *Meloidogyne*, as the most dangerous plant-parasitic nematode, only correlated with Field-1 where the land was not cultivated for 3-months. On the other hand, *Pratylenchus* was observed in all studied sites. Moreover, in Field-3, where the land was used for tomato production, fewer bacterivore nematodes were detected. Therefore, the results imply that the natural environment bears a higher soil quality with the proper number of beneficial nematodes. Agricultural land use and climate change factors, including temperature and precipitation, change the nematode diversity and soil health quality. Furthermore, it can be concluded that free-living nematodes such as *Panagrolaimus* and *Trypilina* can be used as an indicator of pollution and soil condition as these species were only found in natural 5-years-not-cultivated land and natural land.

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