

Habitat-specific differences in soil nematode diversity and community and their entomopathogenic activity

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Abstract

Soil nematodes are essential components of terrestrial ecosystems, playing critical roles in nutrient cycling, soil structure formation, and ecosystem functioning. They also act as bioindicators, reflecting soil health and ecological changes due to their sensitivity to environmental factors. Studying soil nematode biodiversity is crucial for understanding their roles in nutrient cycling, ecosystem functioning, and their responses to environmental changes and importantly their entomopathogenic activity. It also provides insights into soil health and helps develop sustainable land management practices. This study explores the diversity and community composition of soil nematodes across grassland, shrub, and forest habitats at S.R.T.M. University, Nanded, Maharashtra, India. A total of 58 genera, representing 11 orders, were identified using morphological and molecular techniques. The nematode community composition varied significantly among habitats, with bacterial feeders dominant in shrubs, fungal feeders in grasslands, and predatory nematodes in forests. The findings highlight the ecological importance of nematodes as bioindicators of soil health and underscore the need for integrated methodologies to enhance taxonomic accuracy and biodiversity assessment. In terms of nematode diversity, nematodes of the order Dorylaimida were found to be the most diverse 40% followed by Tylenchida 25%, Rhabditida 16%, Mononchida 6%, Aphelenchida, Chromadorida Monhysterida and Plectida with 3.5% each. The entomopathogenic activity of isolated nematodes namely Eudorylaimus, Thornenema, Mylonchulus, Nygolaimus and Oionchus were recorded against the insect larva and revealed that about 8.62% of the isolated nematodes having moderate entomopathogenic activity.

Keywords: Soil nematodes, biodiversity, grassland, forest, shrubs, entomopathogenic activity

Introduction

Soil nematodes are one of the most diverse and abundant groups of invertebrates inhabiting terrestrial ecosystems (Coleman *et al.*, 2024) [4]. These microscopic organisms play crucial roles in nutrient cycling, soil structure formation, and ecosystem functioning. Nematodes occupy various trophic levels, ranging from bacterivores, fungivores, and omnivores to plant-parasites and predators, making them valuable bioindicators of soil health and ecological changes (Mourya *et al.*, 2023) [12]. The diversity and community composition of soil nematodes are influenced by a range of biotic and abiotic factors, including soil type, organic matter content, vegetation cover, and anthropogenic activities (Lazarova *et al.*, 2021 and Gattoni *et al.*, 2022) [7, 11].

Biodiversity study in the University area, on the bank of Godavari is a thrust area of research (Darak *et al.* 2021) [5].

Despite their ecological importance, soil nematode diversity remains understudied in many regions due to their cryptic nature and the challenges associated with their identification. Advances in molecular techniques, such as metabarcoding and environmental DNA (eDNA) analysis, have revolutionized the study of nematode communities, enabling researchers to uncover patterns of diversity and the impacts of environmental gradients on nematode assemblages (Thomassen *et al.*, 2024) [16].

This study aims to explore soil nematode diversity and community composition across different land-use types, investigating the relationship between nematode diversity and soil health indicators. We hypothesize that nematode diversity and trophic group composition will vary significantly between natural, agricultural, and urban soils,

reflecting the impacts of human activity and habitat modification.

Materials and Methodology

Study Sites

The study was conducted at S.R.T.M. University, located in Nanded, Maharashtra state, India.

S.R.T.M. University was established in 1994 and is situated at coordinates 19°06'08.4"N 77°17'05.3"E. Based on the weather of Maharashtra, Nanded district comes under the Marathwada region. The climate of the study area can be characterized as mainly dry but with variations across seasons. The winter season occurs between October to January and has an average temperature of around 26°C. In contrast, the summer months of March to May are characterized by very hot days, with an average temperature of around 33°C. This region is characterized by hill locks and dense forest vegetation, creating a unique natural environment within the university campus. The study area at S.R.T.M. University provides an ideal location to investigate the diversity and community composition of soil nematodes due to their geographical features, climate variability, and diverse vegetation cover.

Soil Sampling

A total 15 soil samples were collected from different habitats. From each plot, composite soil samples were obtained by collecting five subsamples from a depth of 0-15 cm using a soil auger. Samples were stored in sterile plastic bags, transported to the laboratory on ice, and processed within 48 hours.

Nematode Extraction, Identification and Enumeration

The soil samples were processed using standard methods for nematode extraction. The procedure followed a combination of Cobb's (1918) sieving and decantation techniques, along with a modified Baermann's funnel approach. Initially, the soil was placed in a bucket and thoroughly mixed with a small amount of water to create a soil-water mixture. By hand, lumps of soil were broken down, and any extraneous materials, such as stones and debris, were carefully removed from the sample. This preparation ensured a clean and homogenous soil sample suitable for nematode extraction and subsequent analysis. The fixed nematode specimens were observed under a microscope for identification. The total number of nematode specimens in each sample was determined using a nematode counting dish under a stereomicroscope. Subsequently, the specimens were carefully packed in plastic vials for further study and analysis. Nematodes were mounted on temporary slides for morphological identification, and key taxonomic characteristics were observed under a compound microscope.

Soil Physicochemical Analysis

Soil physicochemical properties, including pH, organic matter content, and nutrient levels (N, P, K), were measured following standard protocols (Page *et al.*, 1982). Soil

microbial biomass was assessed using the fumigation-extraction method.

Entomopathogenic activity of Nematodes:

Greater wax moth (*Galleria mellonella*) and mosquito larvae were distributed in the Petri plates. Nematode suspension were prepared in sterile distilled water. Insect larvae in the petri dishes lined with moist filter paper. 1ml of nematode suspension was introduced to each dish and ensured that the larvae should be exposed to the nematodes. Control groups were kept in which only distilled water was introduced instead of nematode suspension. All plates were kept for incubation at 25°C for 48–72 hours followed by observation of larvae for the signs of infection. Mortality was recorded.

Results and Discussion

Study area

Soil samples were collected from the Swami Ramanand Teerth Marathwada University, Nanded Campus, as shown in Figure 1. Several researchers did their research on the plants and animal species in the Nanded district of Maharashtra. Among them, Tambre and Chavan (2016) recorded 10 species of snakes from the SRTM University area whereas, Gulrez and Chavan (2022) recorded 17 ant species belonging to 15 genera of six families from the Nanded district. But no records are known about nematofauna at S.R.T.M. University, Nanded area.



Fig 1: Map of the study area: Premises of Swami Ramanand Teerth Marathwada University, Nanded (Source: Google Maps)

Soil sampling

A total of 15 soil samples were collected from the sampling sites with the help of special equipment as shown in figure 2. Based on their sampling sites, they were labelled as G1, G2, G3, G4 and G5 for Grassland ecosystem, S6, S7, S8, S9 and S10 for shrubs and F11, F12, F13, F14 and F15 for the Forest ecosystem. Wang *et al.* (2021) [17] studied changes in the composition of soil nematode assemblages and their effects on soil microbial food webs along fen, bush, and forest environments. They detected 36 genera; nematode assemblages and soil characteristics differed significantly among habitats, while richness, abundance, and diversity increased across successional stages. The study showed that the change in trophic dominance shifted from bacterial channel dominance in fen to omnivore predators' top-down impact in the forest. It's worth further research on such taxa since *Udonchus* and *Eudorylaimus* might act as markers of vegetation succession as well as soil food web dynamics.



Fig 2: Soil sample collection at sampling site of the S. R. T. M. University Campus

Nematode Extraction, Identification and Enumeration

The soil samples were processed and nematodes were extracted with standard protocol as shown in Figure 3. Morphological features were considered for the identification of the nematodes collected from the soil samples as shown in figure 4. Based on the feeding mode of nematode, soil nematode populations were categorized into five trophic groups: Bacterivores (Ba), Fungivores (Fu), Predators (P), Omnivores (Om), Plant Parasites (Pp) (Zhong *et al.*, 2016) [18], and nematodes also classified based on the life strategies are colonizers and persisters proposed by Quist *et al.*, (2019) [15]. The nematode diversity and ecology were studied by employing the following parameters: Frequency (N): It is the total number of samples in which a particular genus was present. Absolute Frequency (AF %): It is the frequency of a genus/Total number of counted samples X 100. Pun *et al.* (2024) studied the different identifications and quantification methods developed for plant-parasitic nematodes using different methodologies. Morphology and molecular techniques are widely debated based on advantages and some drawbacks, but morphological identification, however fundamental to this end, cannot keep up with the same exactness in the identified or scaled species brought by Next-generation sequencing techniques. It has been observed that judicious integration of state-of-the-art molecular approaches and classical morphological study enhances knowledge of the PPNs and their interaction with microbes and plants at large. Emerging technologies like machine learning and genomic studies hold great promise for not only enhancing the accuracy in identification, and finding genetic markers, but also advancing basic research on various aspects of nematode parasitism; successful implementation in the future highly depends upon effective data management and its evaluation.

Research on benthic sediments reveals a million-nematode species, even though only a few thousand have been documented in the scientific literature. The diversity of terrestrial nematodes is not well documented. There are still many unclassified nematode species. Therefore, for all

individuals and species, a reliable and transferable identification system is necessary. Morphology has always been a key component of nematode taxonomy. All higher-level nematode classifications must include morphology, which is also frequently used to rapidly identify species. Additional taxonomic procedures are needed for intra-specific grouping in order to distinguish between nematode species, subspecies, races, pathotypes, and strains. There is a great need for biological inventories and biodiversity studies, yet there are not enough researchers. Identification of an organism can be aided by the use of a molecular marker derived from a part of its genome (Nisa *et al.*, 2022) [14].

The quantity of nematodes per 100 g of soil in each sample was used to quantify nematode abundance. The nematode diversity and community analysis were calculated to observe the prevalence groups or genera of the soil samples. A total of 58 genera belonging to eleven different orders were identified. The ranges of nematode genera varied from 17-26 per sample in grasslands, 12-22 in shrubs and 20-23 in forests, most of the samples the range was more than 18 genera. The detailed diversity and quantitative description of nematodes investigated in this study is mentioned in the Table. 1.



Fig 3: A. Soil sample examination and processing. B. Nematode samples were collected in a sealed container

Table 1: Nematode diversity among the 15 soil samples of three different habitats and their entomopathogenic activity

Sample No. Nematode Genera with Feeding Habits	G1	G2	G3	G4	G5	S6	S7	S8	S9	S10	F11	F12	F13	F14	F15	Generic Abundance	Entomopathogenic Activity %	
Bacterial Feeder Nematode-14																	G	M
1. Achromadora	3	10	2	7	9	0	0	0	3	0	3	10	6	3	0	56	-	-
2. Amphidelus	0	0	0	0	0	0	2	4	0	0	0	0	0	0	0	6	-	-
3. Anaplectus	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	5	-	-
4. Cephalobus	5	62	3	45	32	14	14	24	4	0	2	6	32	4	0	247	-	-
5. Chiloplacus	0	0	0	0	0	8	8	0	4	0	1	2	6	4	0	33	-	-
6. Chronogaster	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	10	-	-
7. Eucephalobus	10	10	4	22	8	6	12	16	2	8	3	28	6	2	8	145	-	-
8. Eumonhystera	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	6	-	-
9. Mesorhabditis	0	0	0	0	0	16	0	12	8	4	0	0	0	8	4	52	-	-
10. Monhystera	0	0	0	0	0	1	0	0	2	0	0	0	0	2	0	5	-	-
11. Plectus	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	8	-	-
12. Prismatolaimus	5	10	2	12	0	22	6	12	22	24	2	24	12	22	24	199	-	-
13. Zeldia	5	10	0	20	0	0	2	0	0	0	3	0	4	0	0	44	-	-
14. Ethmolaimus	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0	12	-	-
Fungal Feeder Nematodes-09																		
15. Belondira	0	0	0	0	0	0	0	0	0	36	0	0	0	0	0	36	-	-
16. Dorylaimellus	22	160	16	50	155	59	14	0	5	28	5	4	24	5	28	575	-	-
17. Dorylaimoides	0	0	0	7	48	3	0	0	0	0	0	0	0	0	0	58	-	-
18. Proleptonchus	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	-	-
19. Tylencholaimus	0	0	0	0	8	1	4	60	0	4	5	0	0	0	4	86	-	-

20. Tylencholaimellus	6	0	1	0	9	0	0	0	0	0	0	0	0	0	16	-	-	
21. Tyleptus	15	0	0	0	0	0	0	0	0	0	0	10	0	0	25	-	-	
22. Aphelenchus	15	10	2	10	9	4	40	0	10	8	2	4	8	10	8	140	-	-
23. Aphelenchoides	0	0	3	0	0	10	40	0	8	16	4	14	6	8	16	125	-	-
Omnivore Nematodes-15																		
24. Amphidorylaimus	0	0	0	0	0	6	0	0	3	4	0	0	2	3	4	22	-	-
25. Aporcelaimellus	0	0	0	0	0	1	0	0	0	0	4	18	4	0	0	27	-	-
26. Crassolaibium	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	-	-
27. Eudorylaimus	5	0	1	0	27	4	0	0	0	0	1	16	6	0	0	60	50	60
28. Discolaimium	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	4	-	-
29. Discolaimoides	3	0	0	0	24	2	0	0	0	0	2	0	0	0	0	31	-	-
30. Discolaimus	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	-	-
31. Kochinema	4	0	2	0	0	0	0	0	0	0	0	0	0	0	0	6	-	-
32. Labronema	0	25	0	0	9	0	0	0	0	0	0	0	0	0	0	34	-	-
33. Mesodorylaimus	0	0	0	0	24	0	6	8	0	0	0	6	0	0	0	44	-	-
34. Moshajia	30	10	0	30	42	31	12	0	5	8	0	2	4	5	8	187	-	-
35. Lagenonema	6	390	6	3	38	0	2	0	0	32	0	4	28	0	32	541	-	-
36. Oriverutus	0	0	1	0	16	0	2	0	2	0	4	0	0	2	0	27	-	-
37. Sicorinema	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	9	-	-
38. Thornenema	50	20	21	3	210	0	34	0	3	16	2	24	6	3	16	408	40	60
Predatory Nematodes-04																		
39. Mylonchulus	8	0	0	0	8	2	28	0	4	0	3	46	12	4	0	115	60	70
40. Clarkus	0	0	0	0	0	0	0	0	8	1	0	0	0	8	17	-	-	
41. Nygolaimus	0	0	0	0	9	0	0	0	4	0	0	0	0	4	17	60	60	
42. Oionchus	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	4	50	80
Plant Parasitic Nematodes-17																		
43. Basiria	0	0	0	0	0	0	68	2	8	0	0	0	2	8	88	-	-	
44. Criconema	0	0	0	4	0	0	0	0	0	0	0	0	0	0	4	-	-	
45. Ditylenchus	3	45	5	40	0	0	2	4	2	4	4	4	6	2	4	125	-	-
46. Filenchus	0	0	4	41	27	4	0	10	12	4	12	0	0	0	4	1120	-	-
47. Helicotylenchus	25	120	44	15	60	0	0	0	4	0	3	18	20	4	0	313	-	-
48. Hemicycliophora	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	-	-
49. Hemicriconemoides	0	0	0	0	8	0	0	0	0	4	0	0	0	0	4	16	-	-
50. Hoplolaimus	0	30	1	0	27	0	0	0	0	0	0	0	0	0	0	58	-	-
51. Meloidogyn sp.	0	0	0	0	0	0	0	0	3	0	0	0	0	3	0	6	-	-
52. Merlinius	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	10	-	-
53. Pratylenchus	3	20	3	50	0	0	0	0	0	470	0	0	0	0	0	546	-	-
54. Rotylenchus	5	0	0	9	9	1	90	0	12	0	43	6	2	12	470	659	-	-
55. Scutellonema	0	10	2	0	0	0	0	0	0	0	0	0	0	0	0	12	-	-
56. Tylenchorhynchus	0	0	0	10	9	0	0	0	0	0	2	4	0	0	0	25	-	-
57. Xiphinema	0	0	0	0	0	0	12	0	60	1	0	0	0	0	60	133	-	-
58. Tylenchus	0	0	0	0	0	3	0	0	0	0	0	0	4	0	0	7	-	-
No. of genera	21	17	19	18	26	21	20	12	21	19	22	20	23	21	20			
Nematode Abundance in each Sample	231	952	123	386	835	199	328	1244	112	758	101	245	222	112	722	6570		

G- Greater wax moth Larvae M- Mosquito Larvae



Fig 4: Identification of the nematodes based on morphological features

In terms of nematode diversity, nematodes of the order Dorylaimida were found to be the most diverse 40% (n=23 genera) followed by Tylenchida 25% (n=15), Rhabditida 16% (n =6), Mononchida 6% (n=4), Aphelenchida,

Chromadorida Monhysterida and Plectida with 3.5% (n=2 each) as shown in figure 5.

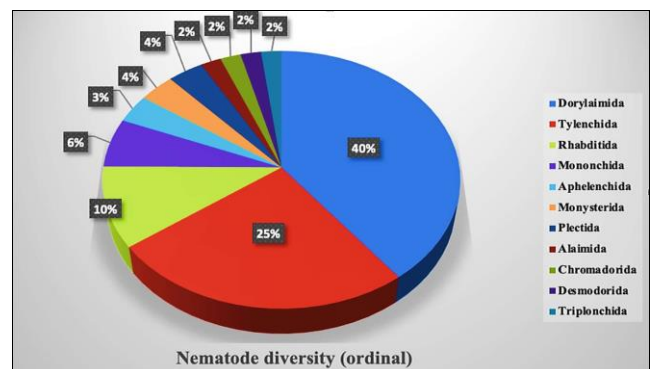


Fig 5: Nematode Diversity (Ordinal)

Among three different habitats, the bacterial feeder nematodes were found to be the most diverse in shrubs and

less diverse in grassland. Whereas fungal feeder nematodes were found to be most diverse in grassland and less diverse in shrubs. Whereas the omnivore's nematodes were found to be most diverse in grassland and less diverse in forest. The predatory nematodes were found to be most diverse in forests and less diverse in grassland. The plant parasitic nematodes were found to be most diverse in grassland and forest and slightly less diverse in shrubs (Figures 6 and 7).

In 120 field plots in desert, semiarid, and mesic grasslands, Franco *et al.*, 2022 [6] experimentally decreased and increased growing season precipitation for two years. Researchers then evaluated the effects of precipitation controls on the richness of the nematode genus, community structure, and C footprint. At all sites, nematode diversity and evenness decreased over time as annual precipitation increased; however, the mechanism underlying these temporal responses varied for moist and dry grasslands. While the observed decreases in dominant colonizer taxa and the negative precipitation–diversity relationship may have resulted from increases in the population of predaceous taxa with increasing precipitation in mesic conditions, drought-adapted rare taxa were lost in arid and semiarid sites as precipitation increased.

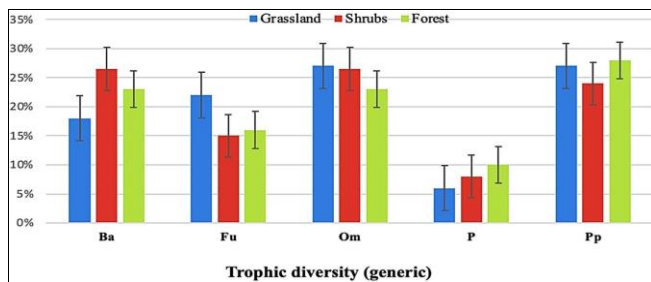


Fig 6: Trophic diversity of nematodes (generic diversity)

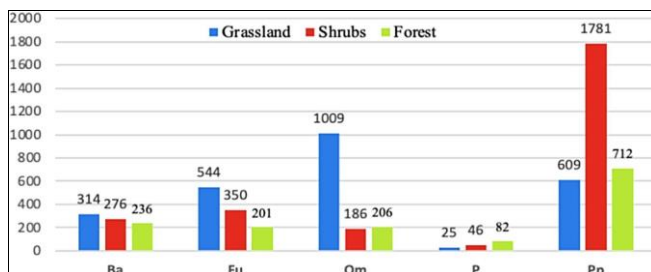


Fig 7: Nematode Abundance based on the Trophic Groups.

Entomopathogenic activity of Nematodes:

The study identified nematodes with potential entomopathogenic activity, including Eudorylaimus, Thornenema, Mylonchulus, Nygolaimus, and Oionchus (Table.1). These nematodes exhibit diverse mechanisms for targeting insect pests. Predatory nematodes such as Mylonchulus and Nygolaimus directly attack soil-dwelling insect larvae by piercing the cuticle and consuming internal contents (Bilgrami and Gaugler, 2004) [3]. Omnivorous nematodes like Eudorylaimus and Thornenema display opportunistic predatory behavior, combining microbial interactions with physical feeding strategies (Mukharjee and Ray, 2024 and Laasli *et al.*, 2024) [10]. Additionally, Oionchus has been reported to feed on soft-bodied insect larvae, contributing to pest suppression in soil ecosystems. Entomopathogenic activity is mediated through either direct predation or symbiotic microbial associations (Kaltenpoth and Engl, 2014) [9]. For instance, Mylonchulus and

Nygolaimus immobilize their prey by mechanical disruption, followed by enzymatic digestion (Ahmad, 1990) [2]. While symbiotic bacteria such as Xenorhabdus and Photorhabdus are widely reported in Rhabditida nematodes like Steinernema and Heterorhabditis, the role of bacterial associations in the identified genera remains an area for further investigation (Abd-Elgawad, 2022) [1]. These nematodes are effective against soil-dwelling pests like *Galleria mellonella* (greater wax moth larvae) and scarabaeid grubs, which are significant agricultural pests (Hazir *et al.*, 2003) [8].

The study underscores the importance of nematodes with entomopathogenic potential in integrated pest management (IPM). Their ability to naturally regulate pest populations highlights their ecological significance and their role as a sustainable alternative to chemical pesticides. Further research is needed to characterize the symbiotic relationships, enzymatic profiles, and specific target pest ranges of these nematodes to optimize their use in pest control strategies.

Conclusions

The study highlights the diversity and ecological significance of soil nematodes, demonstrating their roles as bioindicators of soil health across grassland, shrub, and forest habitats at S.R.T.M. University. A total of 58 nematode genera from 11 orders were identified, with significant variation in diversity, trophic composition, and abundance across habitats. Morphological and molecular approaches, integrated with emerging technologies such as machine learning, provide a promising pathway for improving nematode identification and understanding their interactions with soil ecosystems. Five of 58 nematodes show entomopathogenic activities indicating the nematodes can be a good bio-insecticidal substitute to harmful chemical insecticidal compounds. These findings underscore the need for further research on unclassified nematode species, effective data management, and the exploration of nematode biodiversity to support sustainable agricultural and ecological practices.

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