Rec. zool. Surv. India: Vol. 122(2)/163–171, 2022
DOI: 10.26515/rzsi/v122/i2/2022/168238

ISSN (Online) : 2581-8686
ISSN (Print) : 0375-1511

Community analysis of soil-inhabiting nematodes in natural vegetations of Singalila National Park, West Bengal (India)

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Abstract

Analysis of soil samples collected from the natural vegetations of Singalila National Park of Darjeeling District, West Bengal, yielded 50 genera under 9 orders and 25 families of soil-inhabiting nematodes. In terms of taxonomic diversity, Order Tylenchida showed maximum diversity (28%), followed by Rhabditida and Dorylaimida (22%), Mononchida (10%), Enoplida (6%), Aphelenchida and Plectida (4%), Triplonchida and Chromadorida (2%). In terms of abundance, the highest group was Rhabditida (31%) followed by Tylenchida (30%), Dorylaimida (18%), Plectida (7%), Chromadorida (5%), Aphelenchida (4%), Mononchida and Triplonchida (2%). The least abundant group was Enoplida (1%). In terms of the trophic diversity, the plant parasites represent the highest number of generic diversity (30%) followed by bacterivores (28%), predators (18%), omnivores (16%) and fungivores (8%). Bacterial feeders dominated in abundance (38%) followed by plant-parasites (34%), omnivores (13%), fungivores (8%) and predators (7%). In the present study, the values of Shannon-Weaver (H) and Maturity Index (MI) was 2.26 and 2.25 respectively depicting that the study area is less disturbed and has a high nematode diversity. PPI/ MI value is low showing the mature ecosystem in the region. Food web indices CI, EI, and SI showed that the study area was structured and enriched with resources. This study is a pioneer study in the area on soil-inhabiting nematodes and provides the baseline data which will be useful in future long-term monitoring studies in the region.

Keywords: Abundance, Darjeeling State, Nematode Diversity, Food Web Indices, Maturity Indices

Introduction

Soil organisms are an important resource and provide a wide range of necessary functions for the long-term management of soil ecosystem. Among soil fauna, nematodes (Bongers, 1990) collembola (Frampton, 1997) and mites (Ruf, 1998) are considered as significant bioindicator, however, nematodes have been studied most often since they possess several characteristics that make them successful ecological indicators (Freckman, 1988) as they occupy a vital position in the soil food web (Neher, 2001). Besides, soil nematodes can be placed into at least five or more functional or trophic groups based upon their feeding habits (Yeates *et al*., 1993).

Nematodes have been successful in adapting to every ecosystem, from soils to freshwater to marine ecosystem, and they have been reported from polar regions to the tropics and are found from highest to lowest elevations, even in oceanic trenches and also within the earth's lithosphere (Borgonie *et al*., 2011). Recently, Shatilovich *et al.* (2018) have reported the viable soil nematodes from the samples of Pleistocene permafrost deposits of the Kolyma River Lowland that corresponds to 30000-40000 years. They can also occur in uncommon places in beer malts, vinegar, and water-filled cracks in the earth's crust. They are omnipresent in all habitats that provide available organic carbon sources (Bongers & Ferris, 1999). Soil nematodes are regarded as one of the key agents in some crucial soil processes as they contribute directly to the biogeochemistry of soils by regulating processes such as decomposition, mineralization, and nutrient cycling (Neher, 2001). Bacterial and fungal feeders are essential in the decomposition of organic matter and recycling of nutrients in the soil. They hold the crucial position in soil food chain as decomposers, plant parasites, omnivores

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Article Received on: 01.02.2022 Accepted on: 27.09.2022

and predators at various trophic levels in soil (Yeates *et al*., 1993). Any variation in the habitat that affects their feed source or environmental factors will be reflected in soil inhabiting nematode diversity (Bonger, 1990).

Several studies have been reported on the community analysis of soil nematodes from forests like Bassus, 1962, 1964; Johnson *et al*., 1972; Yeates 1972, 1996; Boag, 1974; Sohlenius, 1977, 1982; Wasilewska, 1979; Sohlenius and Wasilewska, 1984; Zell, 1989; Ruess, 1995; Ruess *et al*., 1996; Neher *et al*., 2005; Yeates, 2007; Baniyamuddin *et al*., 2007; Háněl, 2008; Rizvi, 2008, Tomar and Ahmad, 2009; Tong *et al*., 2010; Butenko *et al*., 2017; Kitagami *et al.* 2017 and Kashyap *et. al.*, 2021. However, no data is available on the community analysis of soil-inhabiting nematodes from Singalila National Park, West Bengal and hence the present study was undertaken to find the diversity and abundance of soil inhabiting nematodes occurring in the Singalila National Park of Darjeeling District, (West Bengal) India.

Material and Methods

Soil Sampling comprised Forest litter and Grassland from an altitude from 2250-3090 M

For the present study, soil samples were collected from natural vegetations of Singalila National Park (27° 13'15" N to 22° 01'46" N and 83° 01'91" E to 98° 07' 54" E) spread over Darjeeling district, West Bengal. It is located on the Singalila Ridge, at an elevation of more than 7000 feet above sea level. In 1986, the park was designated as a wildlife sanctuary, and in 1992, it was designated as a National Park. On the north, it is bounded by the state of Sikkim, while on the west, it is bordered by Nepal. The park is a part of Eastern Himalayas and includes three separate biomes ranging from subalpine to subtropical.

Soil sampling was done from 2250 m to 3090 m altitude and the area of grasslands, small trees and forest litter were mainly selected. Random sampling was done up to a depth of 20 cm or more and total 17 composite soil samples were collected from different areas of the National Park (Tumbling, Money Bhanjan, Kalapokhri, Lamitura and Garibass). The collected soil samples were then stored in polythene bag and properly labelled. After reaching laboratory, nematodes were extracted from samples by using Cobb's sieving and decanting method (Cobb, 1918) followed by modified Baermann's funnel technique (Christie & Perry, 1951). They were then fixed using fixative (95 parts of 30% alcohol and 5 parts of glycerol) and placed in a desiccator containing anhydrous Calcium Chloride for dehydration (Seinhorst, 1959). Nematodes were then mounted on glass slide in anhydrous glycerine and sealed by wax ring method of De Maeseneer and D' Herde (1963). The prepared slides were then studied under microscopes (Olympus BX 53 DIC Olympus Microscope). Soil nematodes were counted and identified up to generic level and were allotted to trophic groups namely, Bacterivores, Fungivores, Plant-Parasites, Omnivores and Predators (Yeates *et al*., 1993) and c-p groups according to Bongers (1990).

Community analysis and diversity indices were calculated

Frequency N: the number of samples in which the genus was present.

Absolute frequency (AF): (Frequency of the genus)/ total number of samples X 100.

Mean density (D): Number of nematode specimens of the genus counted in samples/total number of the samples collected.

Relative density (RD) %: Mean density of the genus / Sum of the mean density of all nematode genera X 100.

Shannon's diversity H'-Σ pi ln pi;

Maturity indices were calculated based on c-p values assigned to different genera of soil nematodes (Bongers, 1990);

PPI- Plant-parasitic nematodes; Nematode Channel Ratio NCR - B/B+F;

Enrichment index (EI) - (e/e+b) x100,

Structure index (SI) - (s/s+b) x100 and

Channel ratio (CI) to understand the dynamics of soil food web.

Where Pi - proportion of individual of Taxon I in the total population; b, e & s represents sum products of assigned weights & number of individuals of all genera.

Results

The present study revealed a total of 50 genera belonging to 9 orders and 25 families identified from the samples collected from natural vegetations of Singalila National Park, West Bengal. In terms of genera, plant parasitic nematodes represented the highest number (30%), followed by bacterivores (28%), predatory (18%), omnivores (16%) and fungivores (8%). In terms of trophic abundance, bacterivores represented the highest number (38%), followed by plant parasites (34%), omnivores (13%), fungivores (8%) and predatory nematodes (7%).

Nematode Diversity

In terms of abundance, the most diverse order is Rhabditida (30.6%) followed by Tylenchida (29%), Dorylaimida (17.8%), Plectida (7.07%), Chromadorida (4.7%), Aphelenchida (4.4%), Triplonchida (2%), Mononchida (2%) and Enoplida (1%). In terms of genera, Tylenchida (28%), were the most frequent with 14 genera followed by Rhabditida (22%), Dorylaimida (22%), Mononchida (10%), Enoplida (6%), Aphelenchida, Plectida (4% each), Chromadorida and Triplonchida (2% each).

Frequency: Helicotylenchus was the most dominant genus with the highest frequency of occurrence (58%) and relative density (11.2%) per 100 cm^3 of soil.

Among the predators, genus *Aporcelaimellus* was the most prevalent with absolute frequency (N= 9 and AF= 52.9%), whereas the least frequent genera were *Mononchus, Iotonchus* and *Ironus* (N=1 and AF=5.88%). Among bacterivores, genus *Plectus* was recorded in all the samples analysed with N= 4 and AF=23.5% as the highest, whereas the least frequent genera were *Alaimus, Bursilla, Macrolaimellus, Nothoacrobeles, Prismatolaimus, Pseudacrobeles* and *Zeldia* with N= 1 and AF= 5.88%.

Among plant parasites, most frequent genus was *Helicotylenchus* (N=10, AF=58.8%) and least frequent were *Aglenchus, Aorolaimus*, *Cephalenchus, Criconema, Psilenchus* and *Hemicriconemoides (*N=1, AF=5.88%). The most frequent genus among omnivores was *Thornenema* and *Eudorylaimus* with N=7 and AF=41.1%, while *Skibbenema* were least frequent genera with AF=15% each. Among fungivores, the most frequent genus was *Filenchus* with N=5 and AF=29.4% and least frequent were *Tylenchus* with N=1 and AF=5.88% each.

Trophic Relationship among Soil Nematodes

Frequency: Among the nematode community analysed in the vegetations of Singalila National Park, Omnivores were found to be the most prevalent group in the entire nematode community with N=3.88 and coefficient of variation (CV) =174%, AF=22.7 (CV=173.6%). Fungal Feeders were also quite prevalent with N=3.25 (CV=190.6%) and AF=19.12 (CV=190.2%), followed by plant parasites with N=2.87 (CV=113.4%) and AF=16.86 (CV=113.2%); Predators with N=2.56 (CV=101.9%) and AF=15.03 (CV=101.9%) and Bacterivores were least frequent in the community with N=1.93(CV=180.4%) and AF=11.34 (CV=180%).

Density: Bacterivores were the most dominant group in the entire nematode community in Singalila National Park with D=1.9 (CV=101.6%) and Relative Density (RD) of 2.72 (CV=101.9%), whereas Predators were the least dominant with D=0.52 (73.2%) and RD=0.74 (CV=72.5%).

Nematode Community Indices

The maturity index (MI) ranged from 1.78 – 4.0 (2.25 ± 0.29), Shannon Diversity Index (H') varied from 1.64 – 2.76 (2.26 ± 0.14), the Plant Parasitic Index (PPI) varied from 0.6 – 2.24 (0.96 ± 0.18) and the PPI/MI varied from 0.2-3.02 (0.73 ± 0.23). The Structure Index (SI) was found to be 40 – 100 (80.62 ± 8.24) while the Enrichment Index (EI) ranged from 31.57 – 96.37 (64.24 ± 9.25) and Channel Index (CI) ranges varies between 3.78-71.04(10.34 ± 6.99) (Table 4).

Table 1. Nematode community structure in natural vegetations of Singalila National Park. (N = 17)

Bacterivores	Frequency	AF%	MD	RD%
Acrobeloides	3	17.64706	3.294118	4.718014
Achromadora	2	11.76471	0.705882	1.011003
Alaimus	1	5.882353	0.235294	0.337001
Bursilla	1	5.882353	0.235294	0.337001
Chiloplacus	3	17.64706	2.117647	3.033009
Chronogaster	2	11.76471	1.176471	1.685005
Macrolaimellus	1	5.882353	0.470588	0.674002

Mesorhabditis	3	17.64706	6.117647	8.762027
Nothoacrobeles	1	5.882353	0.941176	1.348004
Plectus	4	23.52941	3.764706	5.392016
Prismatolaimus	1	5.882353	1.647059	2.359007
Protorhabditis	3	17.64706	4.705882	6.740021
Pseudacrobeles	1	5.882353	0.235294	0.337001
Zeldia	1	5.882353	0.941176	1.348004
Fungivores				
Tylenchus	1	5.882353	0.117647	0.168501
Filenchus	5	29.41176	2.352941	3.37001
Aphelenchus	4	23.52941	1.117647	1.600755
Aphelenchoides	3	17.64706	2	2.864509
Plant parasites				
Aglenchus	1	5.882353	0.470588	0.674002
Aorolaimus	1	5.882353	0.529412	0.758252
Atetylenchus	4	23.52941	1.882353	2.696008
Cephalenchus	1	5.882353	1.411765	2.022006
Criconema	1	5.882353	0.058824	0.08425
Helicotylenchus	10	58.82353	7.882353	11.28953
Hemicriconemoides	1	5.882353	0.058824	0.08425
Hoplolaimus	5	29.41176	0.941176	1.348004
Pratylenchus	2	11.76471	0.705882	1.011003
Psilenchus	1	5.882353	0.235294	0.337001
Rotylenchulus	2	11.76471	2	2.864509
Rotylenchus	5	29.41176	2.235294	3.20151
Trophurus	2	11.76471	2.882353	4.128263
Tylenchorhynchus	5	29.41176	2.058824	2.948759
Xiphinema	2	11.76471	0.470588	0.674002
Omnivores				
Thornenema	7	41.17647	2.647059	3.791262
Sicaguttur	3	17.64706	0.588235	0.842503
Willinema	2	11.76471	0.411765	0.589752
Laimydorus	5	29.41176	2.294118	3.28576
Skibbenema	1	5.882353	0.176471	0.252751
Mesodorylaimus	3	17.64706	1.588235	2.274757
Eudorylaimus	7	41.17647	1.058824	1.516505
Dorylaimus	3	17.64706	0.411765	0.589752
Predators				
Clarkus	2	11.76471	0.411765	0.589752
Paramylonchulus	2	11.76471	0.588235	0.842503
Mononchus	1	5.882353	0.058824	0.08425

Mylonchulus	2	11.76471	0.294118	0.421251
Iotonchus	1	5.882353	0.058824	0.08425
Tripylina	2	11.76471	0.294118	0.421251
Ironus	1	5.882353	0.117647	0.168501
Aporcelaimellus	9	52.94118	2.352941	3.37001
Nygolaimus	3	17.64706	0.470588	0.674002

Table 2. Community relationship between different trophic groups of nematodes. (BF: Bacterivores; FF: Fungivores; PP: Plant Parasites; Om: Omnivores; PR: Predators)

Values	BF	CV%	FF	CV%	PP	CV%	Om	CV%	PR	CV%
Frequency	1.93 ± 0.29	180.4	3.25± 0.85	190.06	2.87 ± 0.65	113.44	3.88 ± 0.79	174	2.56 ± 0.84	101.99
AF%	11.34 ± 1.68	180	19.12±5.02	190.25	16.86± 3.85	113.23	22.79± 4.64	173.6	15.03 ± 4.91	101.97
MD	1.9 ± 0.5	101.6	1.4 ± 0.5	140	1.59 ± 0.5	81.122	1.15 ± 0.33	123.7	0.52 ± 0.24	73.239
RD%	2.72 ± 0.71	101.9	2 ± 0.72	139.86	2.27 ± 0.72	81.071	1.64 ± 0.47	122.4	0.74 ± 0.34	72.549

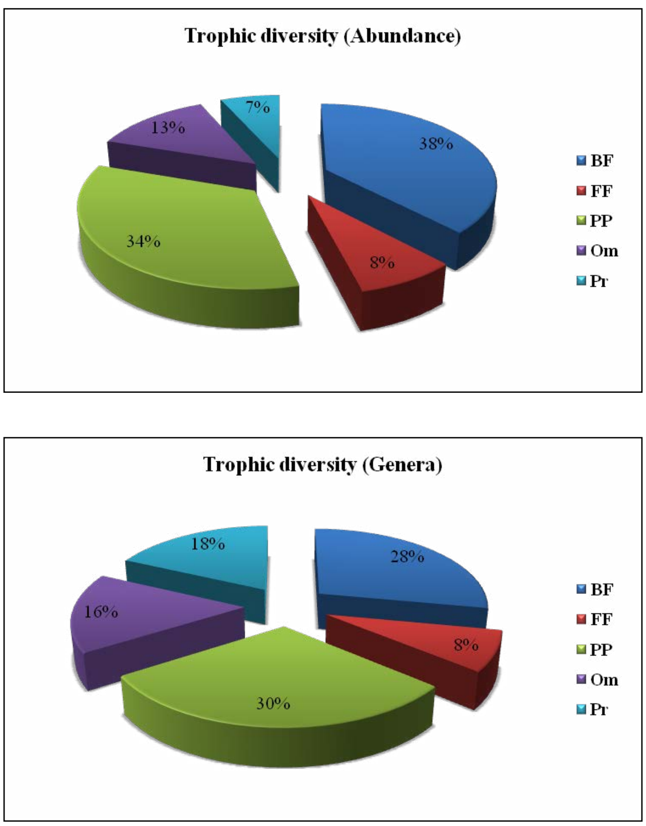

Figure 1. Trophic diversity (abundance and genera) of nematodes in Singalila National Park.

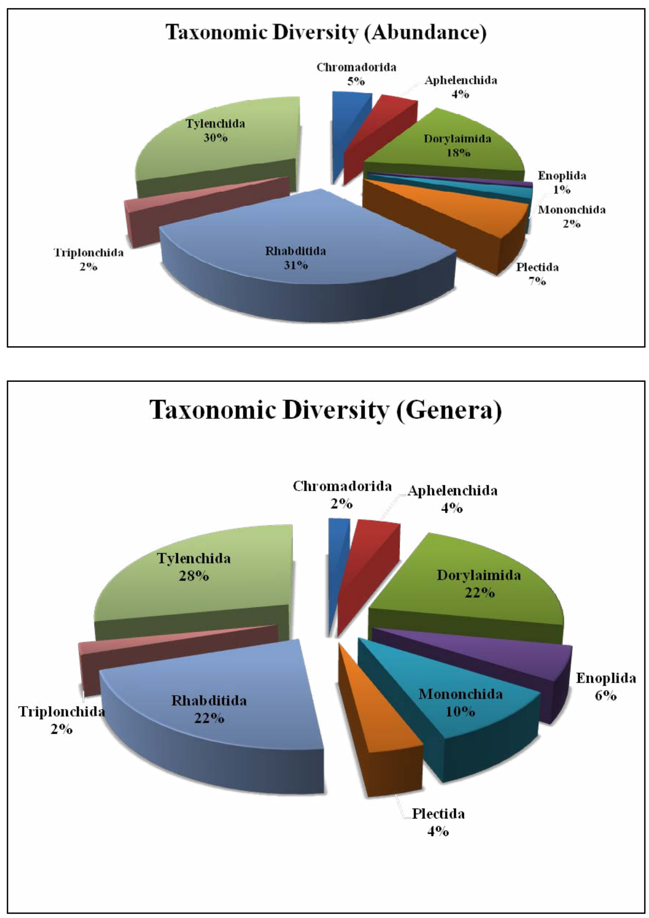

Figure 2. Taxonomic diversity (abundance and genera) of nematode orders in Singalila National Park.

Table 3. Various ecological indices of nematodes in Singalila National Park (Mean±SE)

MI	2.25 ± 0.29
MI	2.25 ± 0.29
H'	2.26 ± 0.14
Simp	0.75 ± 0.03
Invsimp	5.17 ± 0.71
J	0.86 ± 0.02
PPI	0.91 ± 0.18
PPI/MI	0.73 ± 0.23
Richness	8.71 ± 1.81
EI	64.24 ± 9.25
CI	10.34 ± 6.99
SI	80.62 ± 8.24

Discussion

The present study showed a good diversity of soil inhabiting nematodes with a total of 50 genera belonging to 9 orders and 25 families identified from the soil samples collected from natural vegetations of Singalila National Park. Among the trophic group, plant parasites represent the highest number of generic diversity and bacterivores representing highest in abundance. Among the taxonomic groups, Order Tylenchida dominated in terms of number of genera, and Rhabditida, in terms of abundance. Bacterial feeders dominated in abundance followed by plant-parasites in the forest (Nisa *et al*., 2021). Our result is in agreement with previous studies on soil inhabiting nematode community in forest region (Yeates, 1996; Yeates *et al*., 2000; Neher *et al*., 2005; Baniyamuddin *et al*., 2007; Vaid *et al*., 2014; Kumar & Ahmad 2017).

Shannon's diversity index (H') reflects the soil nematodes diversity in an ecosystem. Higher H' value indicates diverse ecosystem while low value indicates less diverse ecosystem. Previous studies reported disparity of H' value in different habitats. Study recorded variation in H' value for nematode diversity for banana crop, pasture and forest as 1.35, 1.97, 2.07 respectively (Pattison *et al*. 2004). In the present study, the values of H' was 2.26±0.14. This showed that study area has decent nematode diversity and less disturbed, which is supported by Maturity index value which showed stable nematode community in this region. Result is supported by previous studies in spruce forests of the Beskydy Mountains and Vihorlat Mountains (Hanel, 1996; Hanel & Cerevkova, 2010; Kumar & Ahmad, 2017).

The PPI indicates plant parasitic nematode resources in soil. Higher values reflect less disturbance in the community and low values shows higher disturbance in the area (Bongers 1990, 1999). In the present study, PPI value is higher indicating that the park is somewhat less disturbed. The PPI/MI ratio is also indicating the soil nutrient status (Bongers *et al*., 1997). In present study, PPI/ MI value is low showing the mature ecosystem in the region.

Food web indices CI, EI, and SI reflect on the soil food web dynamics and the status of ecosystem (if it is enriched, structured or stressed) (Ferris *et al*., 2001). EI provides a mean of movement of resources in the soil food web (Ferris *et al*., 2004). Present study showed that the study area was enriched with resources.

SI describes whether soil ecosystem is structured / matured (high SI) or disturbed (low SI). It has been reported in earlier studies that generally in fallow soils and forests the value of SI are higher which may due to high abundance of omnivores and predators suggesting a food web with more trophic linkages (Ferris & Matute, 2003). SI value in the study was high (SI 80.62) that is supported by previous studies (Ferris *et al*., 2001; Berkelmans *et al*., 2003, Kumar & Ahmad, 2017) which says that high values of SI reflects less disturbed status of the studied ecosystems. The high values of MI and H' also showed structured soil inhabiting nematode assemblages.

Conclusion

It may be concluded that among soil microbiota, soil inhabiting nematodes are considered as one of the groups of soil biota as bioindicators of soil health status. Community structure of soil nematodes vary with ecosystem, abiotic and biotic conditions, altitude and vegetation. From above discussion, the natural vegeations of Singalila National Park, West Bengal has structured and mature ecosystem. This conclusion is well supported by various classified nematode feeders, c-p groups and various nematode community indices indicating a stable and structured nematode community of the ecosystem. This study provides the baseline data and will be helpful in further long term monitoring studies in the region. Furthermore, studies are required in relation with altitude and vegetation to better understand the relationship between above ground and below ground system in the region.

Acknowledgements

We are thankful to the Director, Zoological Survey of India, for providing facilities and encouragements. We acknowledge the permission from Forest Department of Singalila National Park for conducting survey. Special thank are due to Dr P.C. Pathania for bringing the soil samples from Singalila survey.

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