RESEARCH ARTICLE

A Study on Community Diversity of Soil-Inhabiting Nematodes in Lower Forest Area of Gulmarg of District Baramulla, Jammu and Kashmir, India

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Abstract The current study involved the assessment and enumeration of soil-inhabiting nematode diversity in lower Gulmarg forest area (34.05°N 74.38°E) of district Baramulla in Jammu and Kashmir, India. Fifty samples of soil were collected at random from lower Gulmarg natural forest area and were processed to extract and isolate different nematodes. After screening the samples, 30 nematode genera were recorded. Of the total population, 57.8% were bacterivores, 22.23% omnivores, 11.95% predators, 5.08% plant-parasitic and 2.85% fungivores. On the basis of mean density, *Cuticularia* was the most dominant genus while *Caenorhabditis* was the least. According to the trophic grouping, bacterial feeders represent the highest percentage (50%), followed by omnivores (16.66%), plant-parasitic (13.33%), predatory (13.33%) and fungivores (6.66%). Bacterivores were the most dominant groups among all the isolated nematodes because the study soil was found to be mildly acidic (mean pH = 6–6.3), hence favoured the bacterial decomposition pathway in this lower forest area. Shannon-Weaner diversity index showed that this region has a high nematode diversity of 3.02 while the Maturity index showed fewer (2.63) disturbances in this region. Moreover, all the 30 isolated genera were assigned different c-*p* values according to Bongers (Oecologia 83:14–19, 1990) which showed nematode population stability in the given area.

Keywords Nematode · Bacterivores · Diversity indices · Forest · Gulmarg · Jammu and Kashmir, India

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Introduction

Nematodes are eel-like, mostly microscopic and ubiquitous organisms. They are the most successful invertebrate organisms after insects and occupy lower position in taxonomic hierarchy. They are highly adapted to and capable of living in almost any possible habitat ranging from deep sea trenches to highest elevations, from tropical regions to polar habitats, etc. Being the most abundant metazoans on the earth, nematodes form four of every five multicellular organisms on this planet (Gunapala 1998). They occupy a key position in soil food web. They help in decomposition and mineralization (Beare et al. 1992). On the basis of trophic grouping, nematodes may be bacterivores, predators, fungivores, omnivores and plant-parasitic (Yeates et al. 1993). Soil environment, which involves various factors like organic matter (Wasilewska 1970; Hendrix et al. 1990; Griffths et al. 1994), density of nematodes with bulk (Jones and Thomasson 1976), provides a clear idea about the nematode activity, their trophic structure and abundance.

All the nematode species occurring in a particular natural or managed habitat constitutes the nematode community of that very habitat or area. These communities are very much sensitive to changes in different parameters like pH, salinity, food supply (Yeates 1987), and environment (Samoiloff MR 1987; Wasilewska 1989). The soil habitat characteristics play a pivotal role in structuring the biological and functional characteristics of these nematodes. Thus, any change in the environment of their habitat affects their population dynamics. The nematodes play a pivotal role in regulating the nutrient cycling (Anderson et al. 1983) and organic matter decomposition and play a key role in the food web of soil habitat (Moore 1991). The biological and ecological health of the soil is obtained by

the quantification of different attributes of soil nematodes through different indices which have been used in the present work.

In India, 10 bio-geographic zones are found. They are grouped into 26 provinces (Rodgers and Panwar 1988). A number of investigators have worked on the nematode diversity in Indian forests such as natural forests of Arunachal Pradesh (Baniyamuddin et al. 2007), Sal forests of Dehradun (Rizvi 2008) and DKG forest of district Poonch, Jammu and Kashmir (Vaid et al. 2014). Gulmarg (34.05°N 74.38°E) is a famous skiing destination 56 km away from Srinagar, lying in the district Baramulla of North Kashmir, Jammu and Kashmir, India. It lies in the lap of Pir Panjal Range of Western Himalayas at an altitude of 2645 m. This natural meadow remains covered with snow during winters. The soil of Gulmarg meadow comprises of lime stones, sandstones, lacustrine deposits, glacial deposits, etc. Gulmarg is famous for diversified vegetation. The Gulmarg area has a rich vegetation cover comprising of conifers—*Cedrus deodara, Pinus griffithii, Abies pindrow, Aesculus indica*), etc. The major shrubs inhabiting the area are *Indigofera heterantha, Sorbaria tomentosa*, etc. Dicotyledonous herbs like *Rumex patientia* also make their presence in the ground cover. The study area was chosen to explore the nematode diversity since the said area is very much unexplored in this respect.

Materials and Methods

Collection of Samples

A survey was conducted during April-August, 2018 for enumerating the diversity and community structure of soil nematodes at lower forest areas of Gulmarg (34.05°N 74.38°E) (Fig. 1). Sampling design was random and fifty soil samples were collected randomly from the lower forest area of Gulmarg using trowel. These samples were collected in air tight plastic bags in order to retain the moisture, and the bags were labelled with names of the collection sites, temperatures at the time of collection, and dates.

Processing of Samples

After the collection of soil samples, various techniques involving sieving and decantation technique of Cobb and modified funnel technique of Baermann were used for extraction and isolation of nematodes in the laboratory. The extracted and isolated nematodes from the soil samples were killed and fixed by using hot FA 4:1 fixative and then dehydrated in glycerine–alcohol (5 parts of glycerine + 95 parts of 30% ethanol) (Seinhorst 1959). Finally, anhydrous glycerine was used to mount the nematodes.

Identification of Isolated Nematodes

After fixation and dehydration, permanent slides were made by wax ring method using the paraffin wax and then Olympus BX51 DIC microscope was put in use to study these permanent.

slides. The identification of isolated and extracted nematodes was done by using relevant literature (Andrássy 1983; Jairajpuri and Ahmed 1992 and Ahmed 1996) upto the generic level.

Nematode Counting

The nematode abundance of each sample was counted by preparing slides of the nematodes in order to identify the various genera. Syracuse counting dish was also used to count the nematodes.

Community analysis: The nematode diversity and ecology was studied by employing 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 $\times$ 100.

$$AF = \frac{Genus\ frequency}{Total\ Number\ of\ counted\ samples} \times 100$$

Relative Frequency (RF %): It is the frequency of a particular genus / Total frequency of all genera $\times$ 100.

$$RF = \frac{Frequency\ of\ genus}{Total\ frequency\ of\ all\ genera} \times 100$$

Mean density (MD): It is the number of nematode specimens of a particular genus counted in all samples / total number of the samples counted.

$$MD = \frac{Total\ number\ of\ individuals\ of\ a\ particular\ genus\ counted\ in\ all\ samples}{Total\ Number\ of\ samples\ counted}$$

Relative density (RD %): It is the mean density of a particular genus / sum of mean density of all nematode genera $\times$ 100 (Tomar et al. 2006).

$$RD = \frac{Mean\ density\ of\ a\ particular\ genus}{Sum\ of\ mean\ density\ of\ all\ nematode\ genera} \times 100$$

Diversity indices: Following diversity indices were calculated:

Shannon-Weaner Index (H') = $-\Sigma Pi\ (ln\ Pi)$ (Shannon and Weaner 1949)

Fig. 1 Sampling site of lower Gulmarg forest area

Simpson index (D) $= \dfrac{\sum n(n-1)}{N(N-1)}$

Menhinicks index $(D_{Mn}) = S/N$

Margalefs index $(D_{Mg}) = S - 1/ln\,N$

where Pi = proportion of individual of taxon i in the total population, S = Number of species recorded, N = Total number of individuals in the sample

Maturity index (MI) $= 1/N\,\Sigma\,(CP)\,ni$

where N = Total number of individuals of all species. ni = Total number of individuals of species i.

Wasilewska index (WI) $=$ Genera of $(BF + FF)/PP$

where BF = Bacterial feeders, FF = Fungal feeders, PP = Plant parasitic

Plant Parasitic index (PPI) $= 1/N\,\Sigma\,(CP)\,ni$

where ni is the total number individuals of plant parasitic nematodes.

Statistical Analysis of Data

For analysis, One-way Analysis of Variance (ANOVA) model was used, where the abundance of nematode groups was taken as response and feeding groups as factor. The significance between nematode groups was considered at $p \geq 0.05$.

Results

Diversity of Nematode Genera

In this nematode community diversity study of lower Gulmarg forest area in Baramulla district, we obtained 30 nematode genera with bacterivores representing the highest number (57.8%), followed by omnivores (22.23%), predators (11.95%), parasitic (5.08%), and fungivores (2.85%) (Fig. 2). In terms of taxonomic grouping, the order Rhabditida represents 44.33% of all collected specimens, followed by Dorylaimida (23.33%), Tylenchida (16.66%), Mononchida (10%) and Aphelenchida (3.33%) (Fig. 3).

On the basis of trophic grouping, bacterivores represented 50% of the collected nematodes, followed by omnivores (16.66%), parasites (13.33%), predators (13.33%) and fungivores (6.66%) (Fig. 4).

The most dominant genus among the isolated nematodes was *Cuticularia*, with a frequency of 32%. It was followed by *Mesorhabditis* (30%). The least common genus, on the
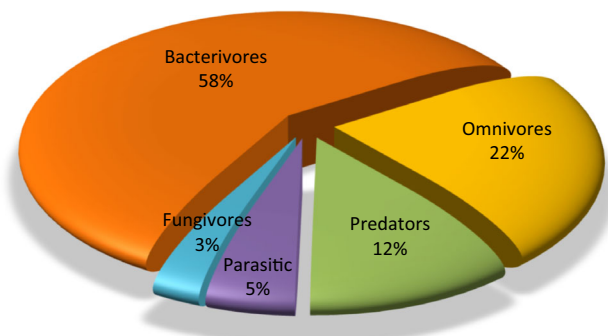

Fig. 2 Nematode generic diversity

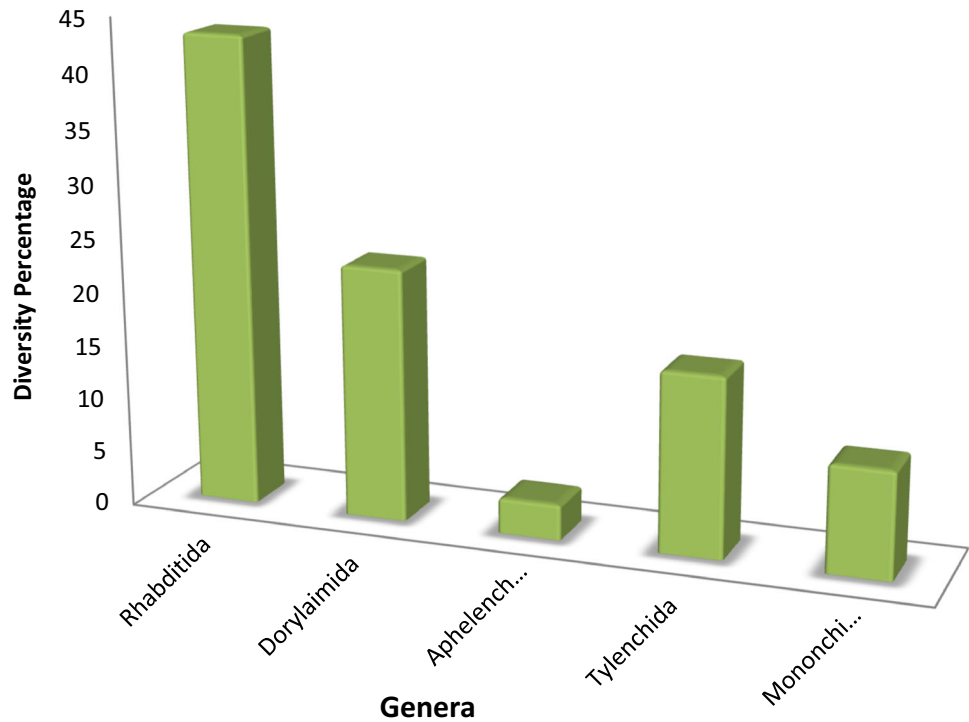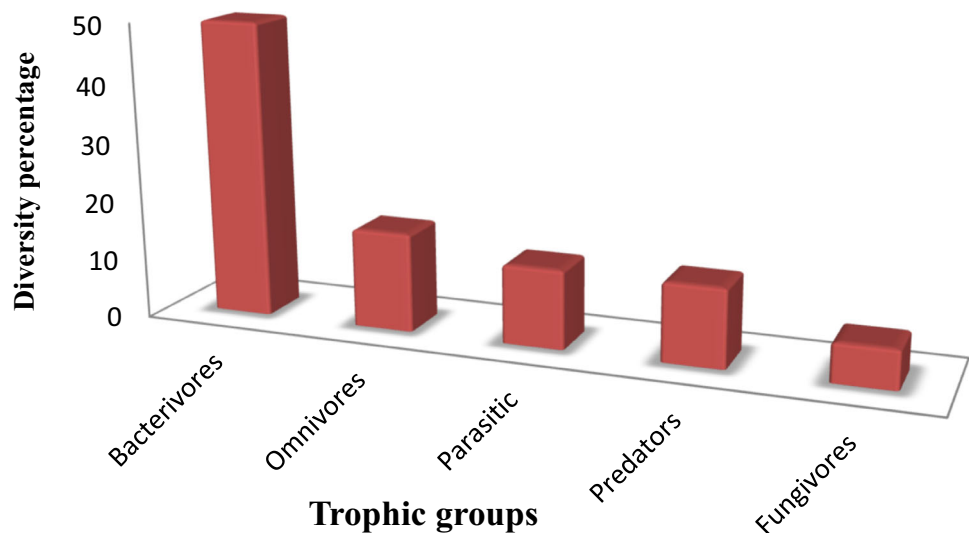**Fig. 3** Nematode diversity on the basis of taxonomic groupings

Fig. 4 Diversity of the collected nematodes on the basis of trophic groupings

other hand, was *Caenorhabditis* with a frequency of only 2%.

Frequency

Among the bacterivore nematodes, *Cuticularia* was the most frequent genus with Absolute Frequency (AF %) of 64%, followed by 60% AF of *Mesorhabditis*; whereas the *Caenorhabditis* was the least frequent genus with AF of 4%. *Dorylaimus*, the most frequent genus among omnivores with AF% of 52% was the dominant one and the least

frequent genus among all the nematodes was predatory *Torbilus* (AF of 18%).

Hoplolaimus was the most frequent genus among plant parasitic nematodes (PPN), with absolute frequency of 20%, whereas *Trichodorus* was the least frequent genus having absolute frequency of 10%. Among predatory nematodes, *Mononchus* was the dominating genus with 44% absolute frequency. *Dorylamellus*, with AF of 18% was the most frequent genus among the fungivore nematodes (Table 1).

Table 1 Population structure of soil-inhabiting nematodes in lower Gulmarg forest area of district Baramulla, Jammu and Kashmir, India

S. No	Nematode genera	C-p value	N	AF%	RF%	MD	RD%
Bacterivores							
1	*Cuticularia*	1	32	64	8.22622108	7.4	14.1437308869
2	*Mesorhabditis*	1	30	60	7.712082262	6.0	11.4678899083
3	*Rhabditis*	1	09	18	2.313624679	1.26	2.4082568807
4	*Pelodera*	1	17	34	4.370179949	0.86	1.6437308869
5	*Caenorhabditis*	1	02	04	0.514138817	0.18	0.3440366972
6	*Rhabditella*	1	06	12	1.542416452	0.56	1.0703363914
7	*Teratorhabditis*	1	08	16	2.05655527	0.74	1.4143730887
8	*Bursilla*	1	11	22	2.827763496	0.94	1.7966360856
9	*Curviditis*	1	11	22	2.827763496	1.44	2.752293578
10	*Bunonema*	1	07	14	1.799485861	0.86	1.6473308869
11	*Panagrolaimus*	1	12	24	3.084832905	2.4	4.5871559633
12	*Diploscapter*	1	14	28	3.598971722	1.9	3.6314984709
13	*Protorhabditis*	1	25	50	6.426735219	4.4	8.4097859327
14	*Rhabpanus*	1	05	10	1.285347044	0.8	1.5290519878
15	*Acrobeles*	2	04	08	1.028277635	0.7	1.3379204893
Predatory							
16	*Prionchulus*	4	11	22	2.827763496	0.8	1.5290519878
17	*Clarkus*	4	18	36	4.627249357	0.9	1.7201834862
18	*Mononchus*	4	22	44	5.655526992	1.4	2.6758409786
19	*Mylonchulus*	4	21	42	5.398457584	3.2	6.1162079511
Plant parasitic							
20	*Trichodorus*	4	05	10	1.285347044	0.76	1.4525993884
21	*Hoplolaimus*	3	10	20	2.570694087	0.9	1.7201834862
22	*Pratylenchus*	3	08	16	2.05655527	0.52	0.993883792
23	*Helicotylenchus*	3	09	18	2.313624679	0.5	0.9556574924
Omnivores							
24	*Dorylaimus*	4	26	52	6.683804627	4.6	8.7920489297
25	*Tornaria*	4	16	32	4.11311054	1.2	2.2935779817
26	*Nygolaimoides*	4	11	22	2.827763496	1.6	3.0581039755
27	*Torbilus*	3	09	18	2.313624679	0.9	1.7201834862
28	*Mesodorylaimus*	4	14	28	3.598971722	3.4	6.498470948
Fungivores							
29	*Dorylamellus*	5	09	18	2.313624679	0.4	0.7645259938
30	*Aphelenchus*	2	07	14	1.799485861	1.1	2.1024464832

C-p value = Colonizer-persister value, *N* = Frequency, *AF* = Absolute frequency, *RF* = Relative frequency, *MD* = Mean density, *RD* = Relative density

Mean Density (MD) and Relative Density (RD %)

Among the plant parasitic nematodes, *Hoplolaimus* was the most dominant genus having the MD of 0.9 and RD of 1.72% while as the genus *Helicotylenchus* was the least dominant with MD of 0.5 and RD of 0.95%. *Mylonchulus* with MD of 3.2 and RD of 6.11% was the most prominent genus among the predatory nematodes and *Mononchus* (MD = 1.4, RD = 2.67%), *Clarkus* (MD = 0.9, RD = 1.72%) and *Prionchulus* (MD = 0.8, RD = 1.52%) were the least dominant genera among the predatory nematodes.

Among the bacterivore nematodes, *Cuticularia* with MD of 7.4 was the most dominant genus with RD of 14.14% while as the least dominant genus was *Caenorhabditis* with MD = 0.18 and relative density of 0.34%. Among the omnivore nematodes, *Dorylaimus* was the most dominant genus with MD of 4.6 and with RD of 8.79% whereas the least dominant genera was *Torbilus* with MD of 0.9 and RD of 1.72% and among fungivores, *Aphelenchus* with MD of 1.1 and RD of 2.10% was the dominant genus.

One-way Analysis of Variance (ANOVA) was also used for all the nematodes comprising of bacterivores, predators,

plant-parasitic, omnivores and fungivores with the mean 12.86, 18, 32, 15.5 and 8 respectively among these trophic groups. The *p* value was 0.351346 which is more than 0.05 and hence statistically not significant.

Discussion and Conclusion

Soil ecosystems inhabit and support a rich diversity of fungi, bacteria, algae, protozoa, arthropods, mites, nematodes, etc. Nematodes contribute directly or indirectly to the biogeochemistry of soil by regulating decomposition and nutrient cycling. There are many organisms which act as bio-indicators of the soil health. Based on the abundance, genetic diversity and the variability of niches, nematodes are considered as the most studied biological indicators to make predictions about past or present soil conditions (Nehar 2001). Nematodes are omnipresent, inhabiting almost every possible habitat ranging from deep ocean trenches to the highest peak mountains, marine to fresh water, polar to tropics, etc. Their diversity and abundance depends upon ecological and edaphic factors like soil pH, nutrient content, moisture, etc. The other factors which influence their richness and diversity are soil type, pore spaces, root secretions, ploughing and tilling, anthropogenic disturbances, grazing and pesticides. In the present study, the number of genera identified was 30 representing bacterivorous nematodes (15 genera), omnivorous nematodes (5 genera), herbivorous nematodes (4 genera), predatory nematodes (4 genera) and fungivorous nematodes (2 genera). Among these, bacterivores represent the highest number of genera. Since the soil was found to be mildly acidic, it supported the bacterial growth and as a result bacterivore nematode represented the highest number. Again, fungivorous nematodes were least abundant because they thrive well in more acidic soils (Alexander 1977 and Wasilewska 1979).

On the basis of taxonomic groupings, Order Rhabditida dominated in terms of genera number as well as the abundance. It is followed by Orders Dorylaimida, Mononchida and Tylenchida. The low dominance and less abundance of Dorylaimida and Mononchida could be due to disturbances in the studied area. As for as the soil physico-chemical conditions and disturbances are concerned, Dorylaimida and Mononchida are the most sensitive nematode groups (Forge and Simard 2001) and they are often considered as indicators of soil health (Thomas 1978; Sohlenius and Wasilewska 1984). Considering these, the present study points to the possible degradation of soil quality in the studied area. On the basis of trophic grouping, bacterivores dominated the most in terms of abundance and density, followed by omnivores, parasitic nematodes and predatory nematodes. In this region, bacterial

Table 2 Summary of nematode diversity indices in lower Gulmarg forest area of Baramulla District, Jammu Kashmir, India

S. No	Ecological indices	Values
01	Shannon-Weaner Index (H')	3.02
02	Simpson index (D)	0.06
03	Menhinicks index (D_{Mn})	0.58
04	Margalefs index (D_{Mg})	3.68
05	Maturity Index (MI)	2.63
06	Plant Parasitic Index (PPI)	0.33
07	Wasilewska index (WI)	4.25

decomposition pathway is predominant than the fungal pathway, may be due to the less acidic (pH = 6–6.3) conditions in the soil. This is in accordance with Alexander (1977) and Wasilewska (1979) that forest areas with more acidic soils, show dominance of the fungal pathways.

Bongers (1990) studied that the different diversity indices play a pivotal role in indicating the condition or ecological health of forest soils. The value of Shannon-Weaner Index (3.02) exhibits the high nematode diversity. The current study of various diversity indices, like Margalefs index (D_{Mg}), Menhinicks index (D_{Mn}) indicates that this region has high nematode species richness of 3.68 and 0.58 respectively (Table 2). These results agree with E. P. Odum (1969), those forests with high H' values are at mature stage. The nematode 'maturity index' also plays a key role in providing useful information regarding the direction of change within a particular soil type. The lower value of Wasilewska index signifies that the current studying area is dominated by bacterivore nematodes.

In conclusion, our study on lower forest area of Gulmarg revealed rich diversity of Rhabditid nematodes and low diversity of Mononchid nematodes. Apparently, this could be attributed to anthropogenic disturbances among which tourism might have played a key role. As for as the nematode diversity is concerned, the present study can be considered as an initial step to study this forest area. Further research on this forest area is warranted in order to fully explore the biodiversity of this region.

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