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Community structure of soil inhabiting nematodes in an apple orchard at Bandipore, Kashmir, India

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Abstract
Community structure of soil inhabiting nematodes from a fifty year apple orchard (*Pyrus mallus*) was studied. Nematode roles were assessed in terms of generic frequency, diversity, density and trophic diversity, as well as some common ecological indices. Ten randomly selected composite samples yielded 37 genera belonging to 8 orders. They were allotted different trophic groups on the basis of their feeding habits. Total nematode numbers extracted averaged 637/100 cc of soil. Of the total population, 43% were herbivores, 32.1% bacteriovores, 11% were predators, 7% omnivores and 6% fungivores. Their mean density ranged from .5-50 per 100cc of soil in the entire nematode population studied. Identified nematode genera were assigned c-p values, most of which belonged to cp 3 and 4 (18 genera), thus giving an idea of stability of nematode populations in the studied apple orchard.

Keywords: Abundance, ecological diversity, ecological indices, *Pyrus mallus*, generic diversity, nematode trophic groups

1. Introduction

Soil is home to many vertebrate and invertebrate species. Invertebrate species largely outnumber the vertebrate species. The functional characteristics of these invertebrates generally depend upon the soil characteristics and thus any change in soil condition effect the population dynamics of these invertebrates and are easily reflected in various forms.

Soil microfauna, such as protozoa and nematodes, are important constituents of soil food webs [1]. Their activities regulate the size and function of fungal bacterial populations in the soil [2, 3], plant community composition [4] and rates of carbon [5] and nitrogen [6] turnover. Nematodes are of particular interest because they are the most numerous soil mesofauna and occupy all trophic consumer levels within the soil food web. Therefore, their community structure can provide important insights regarding many aspects of ecosystem function [7, 8].

The assemblage of plant and soil nematode species occurring in a natural or a managed ecosystem constitutes the nematode community. These communities are sensitive to changes in food supply [9] and environment [13, 14]. Thus, communities also have a significant role in regulating decomposition and nutrient cycling [15] and occupy a central position in the soil food web [16]. When attributes of soil nematode communities are quantified through measures such as diversity index [17] or maturity index, an indication of relative soil biological or ecological health is obtained, which can be used as one measure to address issues of change in ecological condition of soils in agricultural systems.

Since nematodes are so abundant and omnipresent in ecosystems, they serve as elegant indicators of environmental disturbance [20-24]. Nematodes posses the most important attributes of any prospective bioindicator [25]; abundance in virtually all environments, diversity of life strategies and feeding habits [26, 27] short life cycles, and relatively well-defined sampling procedures. Several attempts have been made by researchers to develop relationships between nematode community structure and succession of natural ecosystems or environmental disturbance [28-31].

Soil microbes play an important role in plant nutrient cycling in organic farming [32]. Additions of organic matter to soil are expected to increase numbers of bacteriovores and fungivorous nematodes and decrease numbers of plant-parasitic nematodes [33, 34]. Applications of manure add organic matter and microbes, a source of food for the nematodes [35, 36]. When bacteria are plentiful in soil, bacteriovorus nematodes may discharge amino acids in substantial amounts. However, as bacterial population decrease, nematodes begin to starve, and protein catabolism for maintenance energy requirements leads to increased ammonium excretion by nematodes. Nitrogen content appears to be an important measure of potential microbial activity and, subsequently, the rate of decomposition [37].

Although numbers of studies have been done on various soil nematode communities, those in orchards have not largely been studied yet [38, 39]. The aim of present study was to investigate nematode communities and temporal changes in the nematode fauna of apple orchards of district Bandipora. Bandipora is one of the twenty-two districts of J & K located 56 km from Srinagar city towards its north. Specifically, population dynamics, faunal analysis, and community composition of soil nematodes have been studied to assess the role nematodes as indicators of soil condition. Nematodes were isolated using standard techniques, generic level identification and counting was done in the laboratory. Statistical analysis of the data has been done to analyze the frequency of distribution, dominance, density, prominence value and importance value of different trophic and taxonomic groups and the relationship between the groups. Diversity indices such as Shanon-Wiener Index, Maturity index, Plant Parasitic Index and Nematode Channel Ratio were also calculated.

2. Materials and Methods

Soil description, soil sampling and processing

Study area was an Apple orchard (*Pyrus mallus*) in village Patushai of District Bandipora which is about sixty kms from Srinagar city. The study was done from April 2014 to November 2014. Apple trees have been in the orchard for over last thirty years. The approximate area of the field was1450 m^2, having 80-90 trees in all with wide spacing. Soil samples were collected from a distance of about two feet from the tree trunk. Five cores were collected from a depth of 10-15 cm using a steel corer of $1cm^2$ cross sectional area, around the circumference of each tree. These samples were mixed to make a composite sample and from this 100 cc of soil were taken for further processing. The soil samples were processed by [40] sieving and decantation and modified Baermann's funnel technique. Extraction for recovery of nematodes was made after 24 hrs.

The soil was sandy loam and the pH of soil ranged from 7.5-8.5. Chemical parameters such as available carbon, potash and phosphates were determined in a Government soil laboratory: available carbon = 0.21%; available potash = 156 kg per hectare; available phosphates = 7 kg per hectare.

2.1 Identification of nematode genera

Glass slides containing about two hundred nematodes per sample were prepared for identification. Identification up to generic level was done mainly using [42-46]. Trophic groups were allocated according to [47] and cp groups after [18].

2.2 Counting of nematodes

Population count of nematodes was made using a Syracuse counting dish. The suspension was made homogenous by bubbling with pipette thoroughly before taking 2 ml of nematode suspension in the dish for counting. Counting of each sample was done three times and a mean was obtained. The final population was obtained by multiplying the final quantity of nematode suspension (50 ml) with mean number of nematodes counted and dividing by the quantity of suspension used for counting (2 ml).

2.3 Community Analysis

Following parameter were used for community analysis of various nematodes genera from the region using techniques of [48].

2.4 Abbreviations used

Frequency (N): Frequency of nematode genus (*i. e.* the number of samples in which the genus was present).

Absolute Frequency (AF %): (Frequency of the genus) × 100/ total number of samples counted.

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

Relative Density (RD %): Mean density of the genus × 100 / sum of mean density of all nematode genera.

Diversity Indices: Following diversity indices were calculated:

Shannon-Wiener Index (H') = -∑ Pi in Pi

Maturity Index

Plant Parasitic Index (PPI) =∑ PPi Xi/∑ Xi

Nematode Channel Ratio (NCR) =B /B+F

Where,

Pi = proportion of individual of taxon i in the total population;

PPi= cp values assigned to taxon I according to [18].

Xi = abundance of taxon i in the sample

B = abundance of bacteriovore nematodes

F = abundance of fungivore nematodes

Statistical analysis was done with the help of a computer program statistica, specdive and curve expert. Significance levels were studied by students test.

3. Results

3.1 Diversity of Nematode genera

Soil samples collected from apple orchards in Bandipora yielded thirty seven nematode genera with bacteriovores representing highest number (46%), followed by herbivores (21%), omnivore (19%), fungivore (9%) and predators (5%). In terms of individual abundance, the fungal feeders were the dominant group (64%), followed by herbivore (14%), omnivore (12%), fungal feeders (8%) and predators (2%) (Fig.1. A&B). In terms of taxonomic group among the thirty seven genera identified, the order Rhabditida represented (59%), followed by Tylenchida (15%), Dorylaimida (13%), Aphelenchida (6%), Areolaimida (4%), Mononchida and Alaimida (1%) each and Monhysterida (0.33%). In terms of abundance also Rhabditida was highest (59%), followed by Tylenchida (13%), Dorylaimida (13%), Aphelenchida (6%), Areolaimida (4%), Alaimida and Mononchida (1%) each and Monhysterida (0.33%). A minimum of four and maximum of twenty- five genera per sample were recorded with most of the sample containing 12-18 genera. In terms of individual abundance, 141-686 specimens per soil sample were recorded (Fig.2. C&D) with most of the samples containing 200-300 individuals.

3.2 Frequency

Rhabditis was the most frequent genus among the Bacteriovores with a frequency of 9/10, absolute frequency of 90% and relative frequency of 8.91%, whereas *Amphidelus* was least frequent 1/10, with an absolute frequency 10% and relative frequency 0.99%. The genus *Eudorylaimus* was the most frequent genus among the omnivores with a frequency 7/10, absolute frequency of 70% and relative frequency of 23.33%, whereas, *Ecumenicus* was least frequent 2/10, with absolute frequency 20% and relative frequency 6.66%. Among the eight genera of plant parasitic nematodes and suspected plant parasitic nematodes recorded, *cephalenchus* was the most frequent genus 9/10 with an absolute frequency 90% and relative frequency16.98% whereas *Psilenchus* 3/10 with an absolute frequency 30% and relative frequency of 5.66% was least frequent. *Aphelenchoides* was the most frequent genus 9/10, with an absolute frequency 90% and relative frequency of 39.13%. Least frequent genus was *Dorylaimellus* 6/10, AF = 60% and RF = 26.08%. Predators were represented by only two generas in the study i.e., *Mononchus* and *Mylonchulus*. Genus *Mylonchulus* was more prevalent (2/10) with an absolute frequency 20% and relative frequency of 6.66%, whereas *Mononchus* was least frequent genus (1/10) having an absolute frequency 10% and relative frequency 3.33%.

3.3 Mean density

Among Bacteriovores *Rhabditis* was also the most dominant genus in the group with relative density of 19.63%. Least dominant genera was *Amphidelus* (0.3/per soil sample), RD = 0.3%. Among omnivores *Amphidorylaimus* was the most dominant genus (MD = 17.6%) with a relative density of 7.21%. The least dominant genera was *Crassl abium* (MD = 1.2%), with a relative density of 0.49%. *Helicotylenchus* was the most dominant genus in this group with a mean density of (MD = 9.9) and relative density of 4.05%. *Aphelenchoides* was also the most dominant genus in this group (MD = 9.4/soil sample) with a relative density 3.85%, while *Dorylaimellus* was least frequent genus with relative density of 0.98%.

Table 1: Population structure of soil inhabiting nematodes of Apple orchard

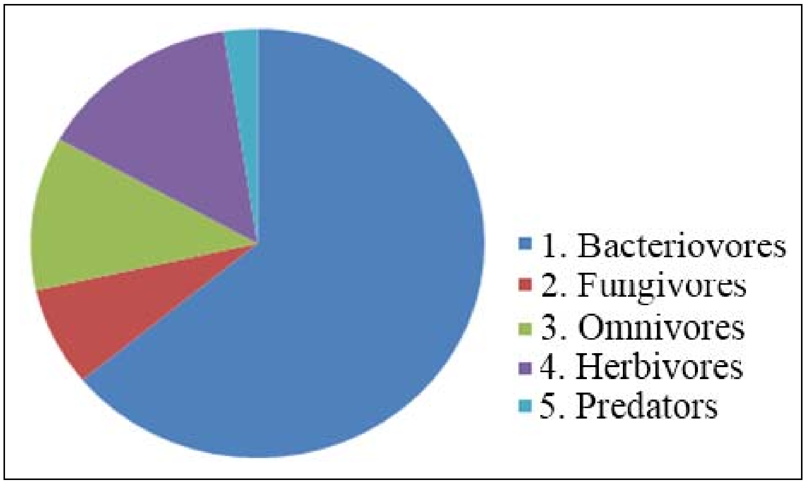
List of Genera	CP Value	N	AF%	MD	RD%
Bacteriovores					
Rhabditis	1	9	90	47.9	19.63
Mesorhabditis	1	8	80	27	11.06
Cuticularia	1	7	70	3.2	1.31
Caenorhabditis	1	8	80	19.9	8.15
Coarctadera	1	6	60	2.1	0.86
Protorhabditis	1	6	60	16.8	6.88
Panagrolaimus	1	8	80	14.6	5.98
Rhabditonema	1	8	80	9.4	3.9
Eucephalobus	2	9	90	10	4.01
Acrobeloodes	2	6	60	7.6	3.11
Plectus	2	3	30	0.9	0.36
Chiloplectus	2	4	40	0.1	0.40
Geomonhystra	2	3	30	0.9	0.53
Leptolaimus	3	9	90	8.4	3.44
Alaimus	4	3	30	0.5	0.20
Amphidelus	4	1	10	0.3	0.12
Paramphidelus	4	3	30	1.6	0.16
Omnivorous					
Crassolabium	4	3	30	1.2	0.49
Eudorylaimus	4	7	70	3.4	1.39
Mesodorylaimus	4	5	50	3.9	1.59
Eucumenicus	4	3	30	2	0.81
Amphidorylaimus	4	3	30	17.6	7.21
Dorylaimoides	4	6	60	2.88	1.18
Herbivores					
Cephalenchus	3	9	90	6.6	2.7
Pratylenchoides	3	4	40	4.75	1.94
Paratylenchus	3	6	60	6	2.45
Ditylenchus	3	8	80	6.8	2.78
Psilenchus	3	3	30	1.3	0.53
Hemicricinemoides	3	8	80	5.1	2.09
Helicotylenchus	3	8	80	9.9	4.05
Hoplolaimus	3	7	70	5.1	2.09
Fungivores					
Aphelenchus	2	9	90	7.1	2.9
Aphelenchoides	2	10	100	9.4	3.85
Dorylaimellus	5	6	60	2.4	0.98
Predators					
Mononchus	5	1	10	0.4	2.6
Mylonchulus	5	2	20	0.16	1.06

4. Nematode Community Dynamics

The diversity of nematode fauna in agroecosystems and their relationships to soil processes suggests that they are potential bioindicators. However, the effects of plants, soil types and nematode biogeography mean a 'functional group' may be a better indicator than particular nematodes. Permanent grassland may be regarded as providing a baseline for nematode diversity in a given soil. The relative abundance of fungal-feeding and bacterial-feeding nematodes serves as sensitive indicator of management changes (Yeates & Bongers, 1999). For assessing the community dynamics and role of nematodes in the agroecosystems, various indices such as Shannon's diversity index (H'), Maturity index (MI) including plant parasitic families, Maturity index 2-5 (MI25, excluding ba1 functional guild), Plant parasitic index (PPI), Nematode channel ratio (NCR) and PPI/MI were calculated. The percent abundance of cephalobids, tylenchids, dorylaims and other nematodes were also obtained.

Food web diagnostics of the ecosystem was studied in terms of Enrichment index (EI), Structure index (SI) and Basal index (BI) following the weighed faunal analysis concept of [20].

Trophic Diversity (Abundance)



Trophic Diversity (Genera)

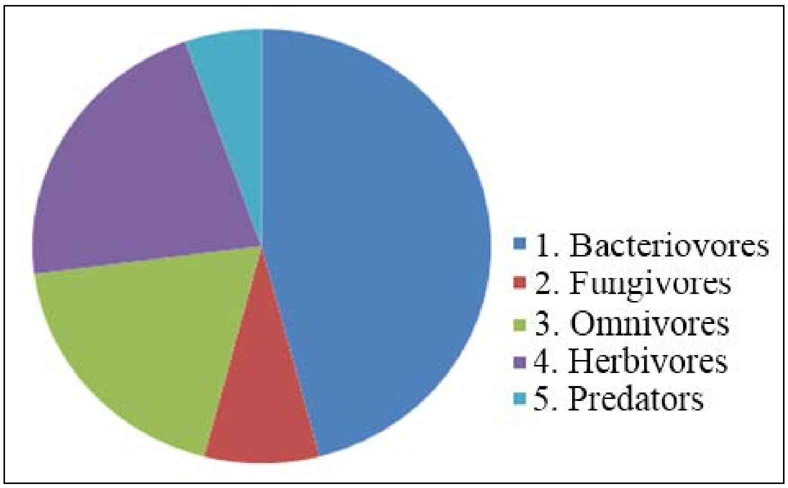
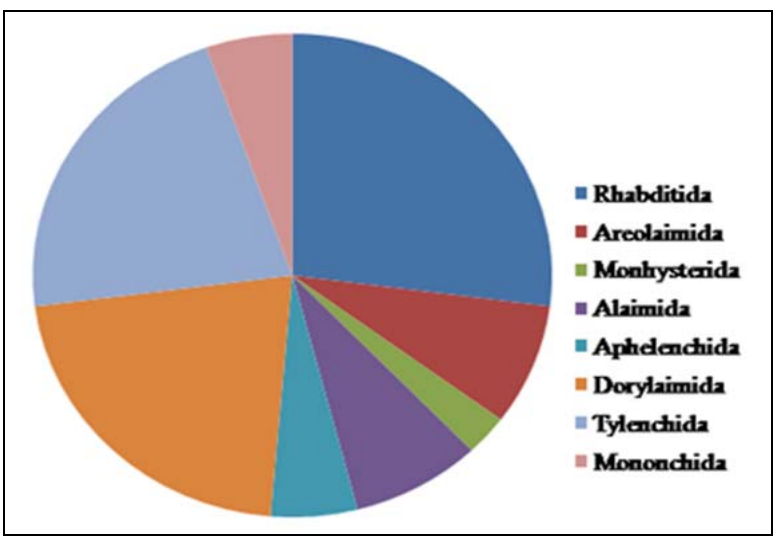


Fig 1: Trophic diversity and abundance of nematodes in an apple orchard (A & B).

Ordinal Diversity (Abundance)



Ordinal Diversity (Genera)

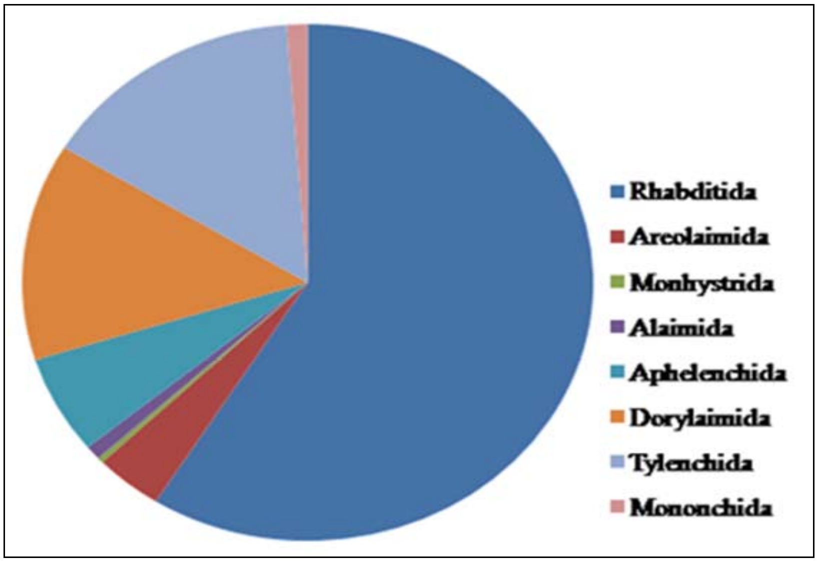


Fig 2: Ordinal diversity and abundance of nematodes in an Apple orchard (C & D).

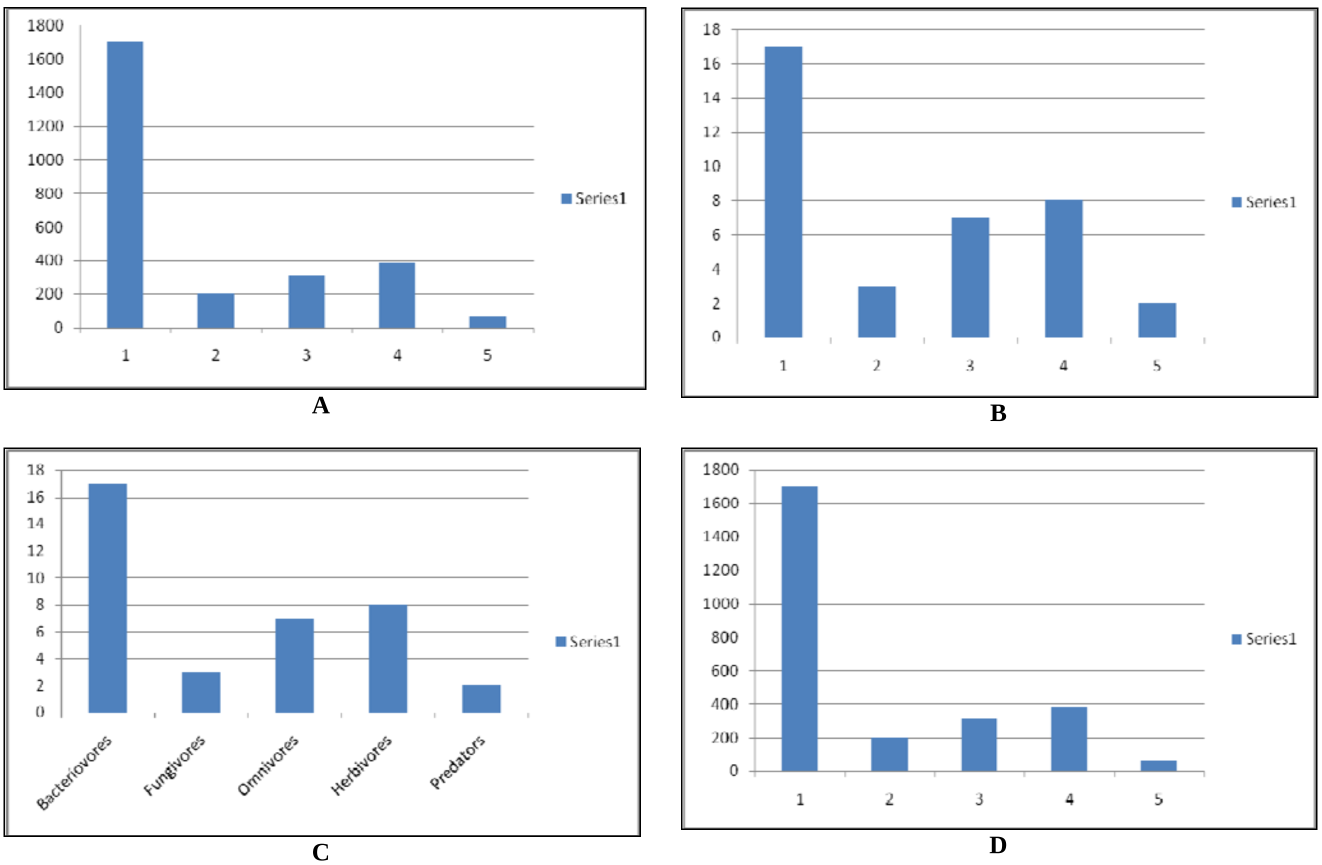


Fig 3: Community relationship between different feeding groups in A, B-Density, C, D-Frequency

5. Discussion

Although nematodes co-exist together in different ecosystems [49], their frequency, density and diversity vary depending upon ecological and edaphic factors [50, 51]. Soil mineral concentration, soil type, pore spaces and root exudates could be other factors, which influence generic coexistence and density of nematodes. Present study revealed a great deal of generic diversity within entire nematode community. A total of 37 genera were identified, representing bacteriovores (17 genera), Herbivores (8 genera), Omnivores (7 genera), Fungivores (3 genera) and Predators (2 genera).

As a trophic group, Bacteriovores dominated other nematode communities. [52] Found 16 nematode genera in farmland ecosystem, [53] recorded up to 36 genera in deciduous forest and [49] reported 6-228 species from grassland. Similarly, present study showed mean nematode density at 0.5-50 which was more than [52, 53] but less than that reported by [12] and, [31]. Such differences may be explained by the fact that nematodes coexist together in different ecosystems [49]; but their frequency, density and diversity. Varies depending upon ecological (Structural heterogeneity, competition, predation, host and soil disturbance) and edaphic factors (mineral and organic matter, pore spaces and moisture, below ground litter and root exudates) [50, 51]. Soil moisture concentration, soil type, pore spaces and root exudates could be other factors which influence generic coexistence, density of nematodes. These factors and their role in generic diversity ecology and coexistence of nematode communities are required to be explained.

As a nematode community, bacteriovores nematodes dominated other nematode Communities *viz*., herbivores, omnivores, fungivores and predators. However fungal feeder showed predominance over phytophagous, bacteriovorus/free living and predatory nematode communities.

Phytophagous nematodes are the best known of the soil organisms because of the damage they cause to agricultural crops; such as decreasing plant production, disrupting plant nutrient and water transfer, besides decreasing the quantity and quality of the produce. In agricultural soils, greater diversity of trophic groups may be correlated with an increase in the frequency of less abundant trophic groups e.g., fungivorous, omnivorous and predators relative to that of generally more abundant trophic groups, for example, bacteriophagous /free-living and phytophagous [54]. Present study showed bacteriovorus were more dominant, and frequent. The reason for this could be their small body structure and easy availability of food. The other reason of dominance of bacterial feeders in this study may be due to use of fertilizers and other manures that add excessive nutrient to the soil. This increases the no. of nematodes that are capable of responding quickly to increased food supply and therefore, increase the enrichment index. Increased nutrient availability may increase root growth, which in turn may increase the resources available for the herbivores nematodes. Due to this reason, though herbivores constituted an important component of the soil ecosystem of apple orchards, in agreement with Y [55]. Among the herbivore nematodes of apple orchards, most dominant genera were *Cephalenchus, Pratylenchoides, Paratylenchus, Ditylenchus, Psilenchus, Hemicriconemoides, Helictylenchus and Hoplolaimus.*

The dominance of Rhabditis may be attributed to higher bacterial production and high nitrate concentration. Cluster analysis of abundance between different trophic groups and prey-predator populations revealed a close relationship between various sample sites.

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