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Original article (Orijinal araştırma)

Biodiversity and distribution of soil nematodes at surroundings of the ancient city of Prusias ad Hypium, Konuralp, Düzce (Türkiye)[1]

Prusias ad Hypium (Konuralp) Antik Kenti, Düzce (Türkiye)'deki toprak nematodların biyolojik çeşitliliği ve dağılımı

Taylan ÇAKMAK[2*]

Abstract

The diversity and distribution of soil nematodes were studied in Prusias ad Hypium (Konuralp) Ancient City, Düzce (Türkiye) in 2021. A total of 1.388 individuals were discovered within 17 families, 25 genera and 30 species. Species richness and the nematode abundance show closely related distribution at each ecological characteristic and the number of species reaches a maximum where low disturbance of soil is observed. Nematodes feeding with bacteria are dominating the total community (53%) in all sampling sites, followed by omnivorous (31.6%), plant parasitic (6%), fungivorous (4.8%) and predatory nematodes (4.6%).

Keywords: Community analysis, distribution, ecology, habitat

Öz

Bu çalışmada, 2021 yılında Düzce İli (Türkiye) Prusias (Konuralp) Antik Kenti ve çevresinde yaşayan toprak nematodlarının çeşitliliği ve yayılışları araştırılmıştır. 17 familya, 25 cins ve 30 türe ait toplam 1.388 birey tespit edilmiştir. Örnek alınan alanlara göre nematod çeşitliliği (tür zenginliği) ve nematod sayısı, düşük insan etkisinin gözlemlendiği yerlerde maksimum sayıya ulaştığı, habitatlar arası yakın ilişkili bir dağılım göstermiştir. Tüm örnekleme alanlarında bakterivor nematodlar (%53) nematod topluluğuna sayıca hakimdir. Bakterivor nematodları sayıca omnivor nematodlar (%31,6), bitki paraziti nematodlar (%6), fungivor nematodlar (%4,8) ve predatör nematodlar (%4,6) takip etmektedir.

Anahtar sözcükler: Topluluk analizi, dağılım, ekoloji, habitat

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Introduction

Many biogeographic reasons explain the biodiversity of Türkiye. Türkiye is at the intersection of Asia and Europe and has a diversity of geographic features and climatic conditions, consequently the country has rich biodiversity. As a result of the ice ages, which lasted between 1.8 million and 10 thousand years ago, a wide range of species richness evolved and is distributed throughout the country. The map of biodiversity hotspots defined on a global scale indicates the rich species diversity found in Türkiye (Demirsoy, 1998).

Nematodes are animals that comprise an important part of ecdysozoan with a wide range of life strategies and feeding habits. They have adapted to many environmental conditions by being able to feed on many other species. These features involve a crucial role in soil nematodes in the food webs in soil. According to biodiversity and conservation studies, nematodes (Zhang, 2012), currently identified with some 25 to 30 thousand named species, constitute the highest diversity group with 1 million species (Hugot et al., 2001) in terms of estimated species number after Arthropoda. Free-living animals comprise the most abundant group of them, which inhabit soils and (both freshwater and marine) sediments (Yeates et al*.,* 1993). Other species have become plant and animal (including human) parasites, causing important diseases and pests (Lee, 2002). Their adaptability, diversity and abundance make nematodes useful bioindicators of soil health (Wilson & Khakouli-Duarte, 2009). Various ecological indices (Bongers, 1990; Bongers et al., 1997; Ferris & Bongers, 2009) have been proposed to use them to monitor natural (successional) or artificial (disturbances caused by human activities) changes that occurred in these systems (Bongers & Ferris, 1999).

The ancient city Prusias ad Hypium (Konuralp) is located in Düzce Province (40°54’20" N, 31°08’53" E), of Türkiye between Sakarya and Bolu Provinces in the Western Black Sea Region. It is unique place having the only antique theater in Black Sea Region with an archeological park. Today, the ancient city is located in a town called Konuralp. The natural habitat is mainly dominated by forest and cultivated trees such as *Fagus sylvatica* L. and *Quercus robur* f. *fastigiata* (Lam.) O. Schwarz (Fagales: Fagaceae). The main agriculture in the region is *Corylus avellana* L. (Fagales: Betulaceae) trees. Also, wild plant meadow and mountain grassland are common plants of the landscape. In Düzce, which reflects the characteristics of the climate of the Western Black Sea Region surrounded by dense forest areas, studies on the diversity of soil nematodes are quite limited. Therefore, as might be expected, nematode species distribution, ecology and diversity in the surrounding areas of Prusias Ancient City are largely unknown. This study aimed to examine the soil nematode fauna surrounding areas of Prusias ad Hypium. More specifically, characterization of the soil nematode fauna, description of distribution patterns of nematode species, and analysis of soil maturity indices associated with nematode assemblages in the studied area.

Materials and Methods

Sampling

This study was conducted at Düzce University, Faculty of Agriculture, Department of Agricultural Biotechnology. Soil samples were collected in Konuralp, Düzce, Türkiye, between July and September 2021 (Figure 1). The sampling was conducted along three different eco-habitats, namely forest (Sessiz Bahçe, 1-10), farms (surroundings of antique theater, 11-20) and fruit orchards (21-30). These zones were determined in order to cover different habitats in the region. Thirty soil samples were collected from three sampling locations (Table 1). Samples were collected from a depth of 30 cm from a 15 x 15 cm plot. Soil samples were then placed in zip-lock sample bags, kept in a mobile refrigerator during transport, and taken to the laboratory for nematode extraction.

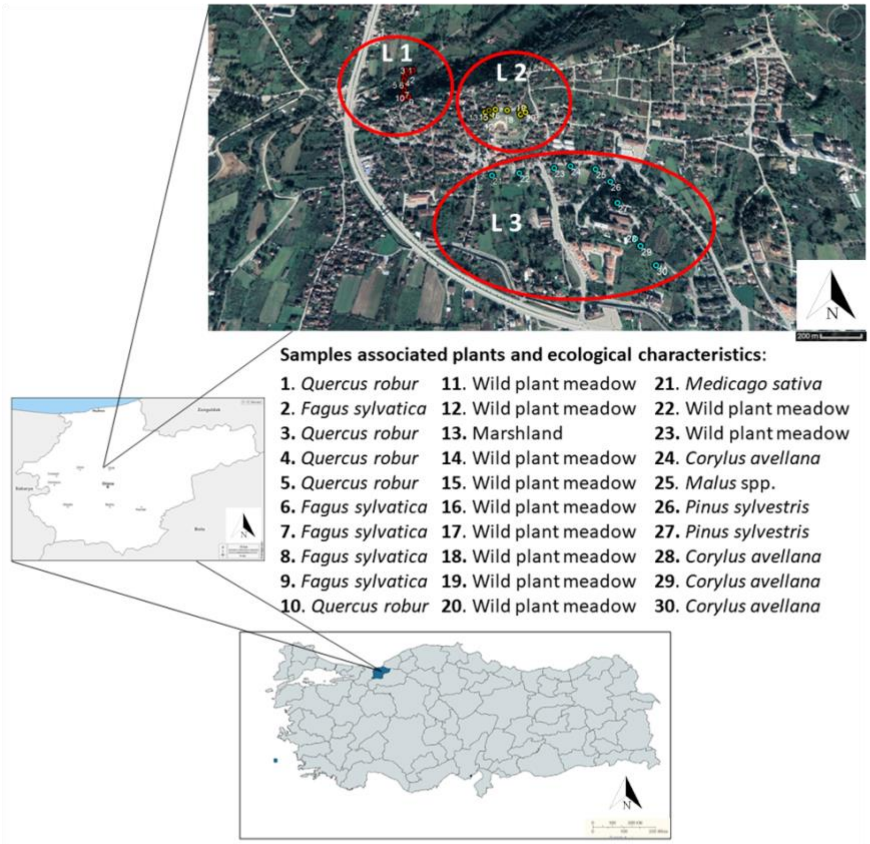

Figure 1. Location of the sampling sites; L1: Sessiz Bahçe natural park, L2: Surrounding area of Prusias Antique Theatre, L3: Orchards around the town.

Table 1. Associated plants and geographical coordinates of the sampling sites

Sample	Associated plant	Coordinates	Sample	Associated plant	Coordinates
1 (L1)	*Quercus robur*	40°54'27" N, 31°8'41" E	16 (L2)	Wild plant meadow	40°54'23" N, 31°8'54" E
2 (L1)	*Fagus sylvatica*	40°54'27" N, 31°8'40" E	17 (L2)	Wild plant meadow	40°54'23" N, 31°8'55" E
3 (L1)	*Quercus robur*	40°54'27" N, 31°8'40" E	18 (L2)	Wild plant meadow	40°54'22" N, 31°8'56" E
4 (L1)	*Quercus robur*	40°54'27" N, 31°8'40" E	19 (L2)	Wild plant meadow	40°54'23" N, 31°8'56" E
5 (L1)	*Quercus robur*	40°54'26" N, 31°8'40" E	20 (L2)	Wild plant meadow	40°54'23" N, 31°8'56" E
6 (L1)	*Fagus sylvatica*	40°54'26" N, 31°8'40" E	21 (L3)	*Medicago sativa*	40°54'16" N, 31°8'52" E
7 (L1)	*Fagus sylvatica*	40°54'25" N, 31°8'40" E	22 (L3)	Wild plant meadow	40°54'16" N, 31°8'56" E
8 (L1)	*Fagus sylvatica*	40°54'24" N, 31°8'40" E	23 (L3)	Wild plant meadow	40°54'17" N, 31°09'00" E
9 (L1)	*Fagus sylvatica*	40°54'24" N, 31°8'41" E	24 (L3)	*Corylus avellana*	40°54'17" N, 31°09'20" E
10 (L1)	*Quercus robur*	40°54'24" N, 31°8'40" E	25 (L3)	*Malus* spp.	40°54'17" N, 31°09'05" E
11 (L2)	Wild plant meadow	40°54'22" N, 31°8'51" E	26 (L3)	*Pinus sylvestris*	40°54'15" N, 31°9'07" E
12 (L2)	Wild plant meadow	40°54'22" N, 31°8'52" E	27 (L3)	*Pinus sylvestris*	40°54'13" N, 31°09'08" E
13 (L2)	Wild plant meadow	40°54'23" N, 31°8'51" E	28 (L3)	*Corylus avellana*	40°54'10" N, 31°09'10" E
14 (L2)	Wild plant meadow	40°54'23" N, 31°8'51" E	29 (L3)	*Corylus avellana*	40°54'09" N, 31°09'10" E
15 (L2)	Wild plant meadow	40°54'23" N, 31°8'52" E	30 (L3)	*Corylus avellana*	40°54'07" N, 31°09'12" E

Nematode extraction

The modified Baermann (1917) funnel technique was used to extract nematodes. Rock and large organic particles were separated from the samples. A 100-g subsample of soil was used for extraction. Samples were incubated on plastic mesh sieves covered with paper towel. Live nematodes were collected after 48 h and placed in 4% formalin solution. Then, each extract was labeled with the relevant sample number and transferred to Düzce University Faculty of Agriculture, Department of Agricultural Biotechnology Nematology Laboratory in plastic tubes.

Slide preparation of nematodes

After obtaining the nematodes, all nematodes were rinsed with distilled water to remove possible residues and transferred to 1.25 cm deep staining blocks containing 96% ethanol and incubated at 40°C. Then, a few drops of glycerol in 4% formalin (1:99) were added and left at room temperature overnight. The next morning, a few drops of glycerol in 95% ethanol were added and two-thirds of its cavity was covered with a glass lid. A few drops of the glycerol-ethanol (5:95) solution were added every 2 h for a gradual transition to glycerol. At the end of the day, two drops of glycerol-ethanol (50:50) were added to the staining block (Seinhorst, 1959). The next day, individual nematodes were covered with glycerol and permanent glass slides prepared (Yoder et al., 2006).

Identification and data analyses

Identification and observations of nematodes were made under an Olympus CH microscope (Olympus Optical, Tokyo, Japan). Using the keys of the classification provided by De Ley and Blaxter (2004) with additional information from Hodda et al. (2006) and Andrássy (2002, 2005, 2009), nematodes were identified to genus level. Nematode life cycle properties according to colonizer-persister classification (1-5) were obtained based on Bongers (1990) and Bongers and Ferris (1999). The classification of feeding types was established in line with to Yeates et al. (1993). To indicate the maturity degree referring to the nematode community composition in the ecosystem, several maturity indices were calculated according to Ferris & Bongers (2009). The nematode indicator joint analysis calculation system (Sieriebriennikov et al., 2014) was used for diagnostics on feeding types, maturity indices and food web structure. Diversity indices were calculated by performing the Shannon-Wiener index and Simpson Index.

Results and Discussion

A total of 1,388 nematodes have been collected and identified. These belonged to 30 species, 25 genera, 17 families and 6 orders (Table 2). About 26 to 76 individuals per 100 g of soil were collected from each sampling site (Table 3).

Table 2. Number of nematodes taxa collected from Prusias ad Hypium, Türkiye

Class	Order/Suborder	Family	Genera	Species	Specimen
Enoplea	Enoplida	1	1	1	2
	Mononchida	1	2	2	65
	Dorylaimida	6	8	10	522
Chromadorea	Monhysterida	1	3	4	269
	Plectida	2	3	3	359
Rhabditida	Tylenchina	6	8	10	171
Total		17	25	30	1388

Table 3. Species abundance and occurrence

Genus Name	Phylum: Family	Total Abundance	Relative Abundance	% Occurrence	C-p Class
Anaplectus Coninck & Schuurmans Stekhoven, 1933	Plectidae	256	18.5	100	2
Mesodorylaimus Andrássy, 1959	Dorylaimidae	212	15.3	93	4
Geomonhystera Andrássy, 1981	Monhysteridae	206	14.9	86	2
Eudorylaimus Andrássy, 1959	Dorylaimidae	150	10.8	83	4
Plectus Bastian, 1865	Plectidae	99	7.14	83	2
Panagrolaimus Fuchs, 1930	Rhabditidae	63	4.55	53	1
Monhystera de Man, 1881	Monhysteridae	59	4.26	30	2
Aporcelaimus Thorne et Swanger, 1936	Aporcelaimidae	44	3.17	30	5
Tylencholaimus De Man, 1876	Tylencholaimidae	41	2.96	40	4
Eucephalobus Steiner, 1936	Cephalobidae	38	2.74	30	2
Belondira Thorne, 1939	Belondiridae	34	2.45	30	0
Mylonchulus Cobb, 1916	Mylonchulidae	33	2.38	46	4
Clarkus Jairajpuri, 1970	Mononchidae	32	2.31	37	4
Hoplolaimus Daday, 1905	Hoplolaimidae	23	1.66	17	0
Aporcelaimellus Heyns, 1965	Aporcelaimidae	21	1.52	20	5
Pungentus Thorne & Swanger, 1936	Nordiidae	20	1.44	23	4
Tylenchorhynchus Cobb, 1913	Tylenchida	18	1.30	7	0
Aphelenchoides Fischer, 1894	Aphelenchida	16	1.15	17	2
Tylenchus Bastian, 1865	Tylenchida	9	0.65	10	0
Eumonhystera Andrássy, 1981	Monhysteridae	4	0.29	13	2
Alaimus de Man, 1880	Alaimidae	4	0.29	3	4
Acrobeloides Cobb, 1924	Cephalobidae	3	0.22	10	2
Tylocephalus Crossman, 1933	Plectidae	2	0.14	7	2
Cervidellus Thorne, 1937	Cephalobidae	1	0.07	7	2

Community analysis

The mean of Shannon-Wiener index from 30 samples was 1.74 ± 0.15 with this index indicating higher diversity in the sampling zone around the town (1.86 ± 0.24), then around Prusias ad Hypium (1.73 ± 0.42) and the forested area (1.62 ± 0.18). The average value of the maturity index was 2.86 ± 0.21. However, any significant differences based on ANOVA ($p > 0.05$) were detected in terms of diversity and maturity indices between the sampling sites. Different maturity indices (sigma maturity and maturity 2-5) showed a similar trend of variability on the different sampling sites (average value of sigma maturity was 2.90 ± 0.22, and maturity 2-5 was 2.94 ± 0.19). Enrichment Index (20 ± 11) showed a high variability between the sampling sites. Structure index (74 ± 7.0) revealed that most of the samples (83%) shows highly mature soil profile.

There were five samples (7, 11, 12, 19 and 26) with semi-endoparasites predominant among the plant parasites, two samples (15 and 23) with epidermal/root hair-feeders predominant and in the remaining samples the ectoparasites were predominant a (Table 4).

Table 4. Maturity, maturity 2-5, Shannon-Wiener, sigma maturity, enrichment and structure indices of all sampling sites

Sample	Index					
	Maturity	Maturity 2-5	Sigma Maturity	Shannon-Wiener	Enrichment	Structure
1	3.4	3.5	3.4	1.9	26.7	91.1
2	2.6	2.6	2.6	1.9	0	62.6
3	2.6	2.8	2.6	1.7	35.3	71.2
4	2.5	2.6	2.6	1.2	21.1	61.5
5	3.6	3.7	3.6	1.9	34.8	91.7
6	3.0	3.0	3.0	1.2	0	80.4
7	3.2	3.2	3.2	1.8	0	84.7
8	2.7	3.0	2.7	2.1	55.0	78.3
9	3.9	3.9	4.3	1.8	0	97.8
10	2.2	2.2	2.2	0.8	0	32.8
11	2.8	2.8	2.9	1.6	0	74.1
12	3.5	3.5	3.5	1.7	0	92.3
13	2.9	2.9	2.9	1.6	0	75.3
14	2.4	2.5	2.4	1.7	25.8	53.8
15	2.6	2.6	2.5	1.9	13.3	64.9
16	2.9	2.9	2.9	1.4	13.8	77.9
17	2.4	2.5	2.6	1.8	25.8	54.9
18	2.1	2.5	2.1	1.6	71.6	59.6
19	2.7	3.1	2.8	2.1	64.9	82.2
20	3.0	3.1	3.1	1.9	25.0	81.7
21	3.1	3.2	3.1	2.1	13.3	81.8
22	2.6	2.8	2.6	2.2	47.8	71.5
23	2.6	2.8	2.6	1.9	40.0	71.0
24	2.9	3.3	2.9	1.8	71.4	88.5
25	2.9	2.9	3.2	2.0	0	76.3
26	2.6	2.6	2.6	1.8	23.3	61.0
27	3.1	3.1	3.1	1.5	0	82.5
28	3.1	3.1	3.1	1.5	0	81.9
29	3.3	3.3	3.3	2.0	0	85.6
30	2.5	2.5	2.6	1.7	3.9	56.1
Mean ± SD	2.86 ± 0.21	2.94 ± 0.19	2.9 ± 0.22	1.74 ± 0.15	20 ± 11	74 ± 7

Feeding type composition

For each sample, the relative abundance of feeding types is presented (Figure 2). Bacterivorous nematodes dominated the community (53%) in all sampling sites. This was followed by omnivores (32%), plant parasites (6%), fungivores (5%) and predatory nematodes (5%). There was no clear correlation between nematode communities and ecological characteristics. Omnivore and predatory nematodes were detected more frequently in sampling sites of similar ecological characteristics. Fungivore nematodes were of low abundance in all sampling zones.

Of the plant parasitic nematodes, the ectoparasites were the most abundant (56%) followed by semi-endoparasites (29%). Epidermal/root hair feeders were the third most common group at 15% and algal/lichen/moss feeders are following with 9.5%. Migratory endoparasites and sedentary endoparasites were not found in any samples.

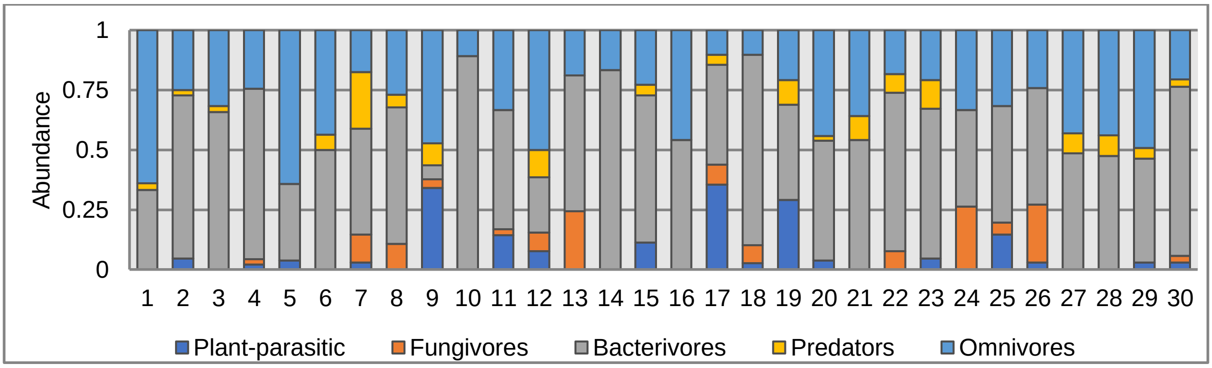

Figure 2. Relative abundance of feeding types in the sampling sites.

Community structure of free-living nematode assemblages

Based on the life cycle properties, free-living nematodes were classified from c-p 1 (colonizers, enrichment opportunists) to c-p 5 (persisters with long life cycle) groups. The free-living nematode community in Prusias ad Hypium did has a recognizable pattern. Opportunistic taxa (c-p = 1) are re-evaluated because they are considered enrichment opportunists and their population densities increase rapidly in response to addition of nutrients to soil and may not necessarily reflect long-term changes in soil ecological condition. Those with c–p values between 2 and 5 are more stable temporally and may provide relatively long-term information about environmental conditions (Hodda et al., 2006). Sampling sites were dominated by colonizer nematodes with c-p value 1-2 (57%). Enrichment opportunist nematodes with c-p value 4-5 were present 43% of the sampling sites (Figure 3).

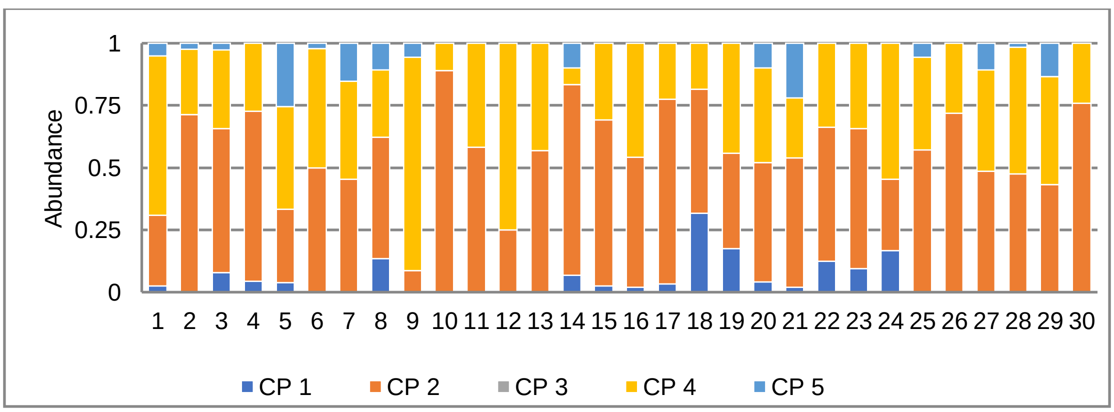

Figure 3. Coloniser-persister structure of free-living nematode assemblages. Maturity Indices (MI) are computed as a weighted mean frequency, $\sum\left(\frac{vi\,X\,fi}{n}\right)$ where *vi* is the c–p value assigned to family, *fi* is the frequency of family *i* in sample, and *n* is the total number of individuals in a sample (Bongers, 1990).

Food web analysis

The analysis of food web structure using the colonizer-persister maturity concept allows evaluation soil community structure and enrichment state of the samples (Ferris et al., 2001). Only one sample (10) showed a poor profile in terms of the structure of the soil. Four of 30 samples resulted in position within the maturing, N-enriched, low C:N cycle, high bacterial suppressive and regulated soil classification, while 25 soil samples resulted within a matured, fertile, moderate cycle of C/N, bacterial/fungal and suppressive soil type (Figure 6).

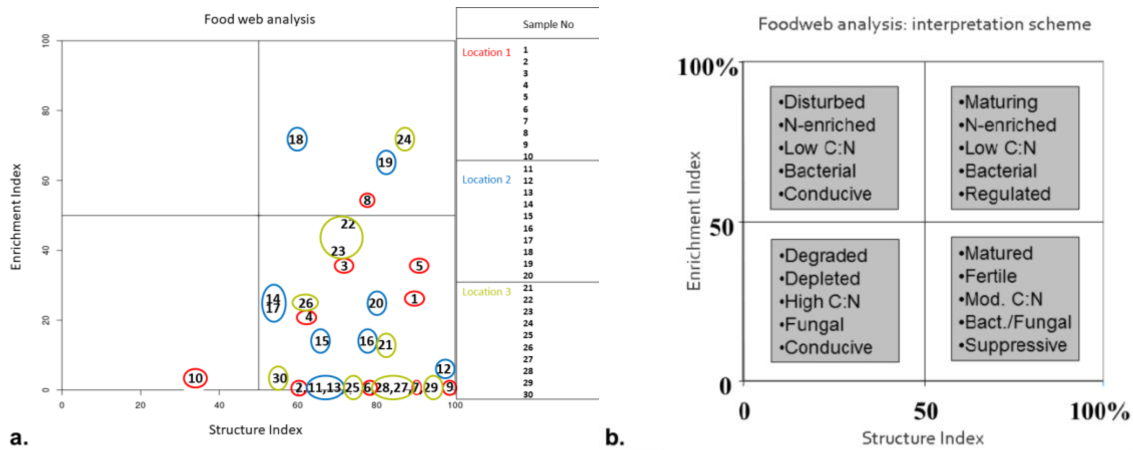

Figure 4. a) Food web analysis of 30 sample sites and b) the interpretation scheme (Ferris et al., 2001).

Discussion

Faunistic

In recent years, there have been a global trend to examine how nematode communities behave in the soil food web by monitoring their role in the ecosystem. In Türkiye, soil nematode diversity, especially free-living forms, are so far poorly investigated. This study aimed to determine soil nematode fauna in one location of the West Black Sea Region using a holistic approach and to identify all forms of soil nematodes. *Anaplectus, Cervidellus, Pungentus* and *Tylocephalus* genera were some of a few records that have been reported in the entire country. This original study, therefore not only significantly expands on what is known about nematode genera in Türkiye, but also improves knowledge of the geographic records in the Western Black Sea Region of Türkiye.

Previously, Yıldız et al. (2021) and Imren et al. (2015) investigated nematode diversity in three habitat types around Bolu Province, Türkiye. Most of the genera has similar patterns in both Düzce and Bolu but they represented by different species in general. Although most of these taxa are globally distributed, it is clear that most of the free-living nematode species are not well known in Türkiye. Regarding the total diversity, this study provides a contribution on the importance of faunistic studies of soil nematodes.

Species distribution

An integrative approach to the species relative abundance and occurrence was used for the determination of species distribution patterns. The outcomes of this study revealed a widespread group of nematodes (*Anaplectus, Mesodorylaimus, Geomonhystera, Eudorylaimus* and *Plectus*) that were most abundant and frequent, and this contrast with the least abundant/frequetn group (*Eumonhystera, Alaimus, Acrobeloides, Tylocephalus* and *Cervidellus*). However, some genera had different distributions. For example, *Hoplolaimus* and *Tylenchorhynchus* may occur abundantly but less frequently within the samples since these taxa occur with high numbers in a limited number of soil samples. Host plant availability may explain this situation as this is the main limiting factor for the distribution of plant parasitic nematodes.

Nematode abundance

The environmental variables are optimal for a nematode survey during the late summer season according to Yeates & Bird (1994) and Stamou et al. (2005). In this survey, the total nematode abundance ranged from 26 to 76 individuals per 100 g of soil, so the sampling time was suitable. The faunistic results, however, show similarities with the characteristics of a temperate humid climate and environmental features at the genus level (Andrássy, 2005; 2009). However, future identification to species level might demonstrate variation and richness within these genera.

Nematode community

The trophic groups in the nematode assemblages varied between the sampled zones. Mean abundance and fraction of bacterivorous nematodes did not differ significantly between sites (Figure 2). A high number of bacterivorous nematodes were found in all sampling sites. The order Dorylaimida appeared as the most diverse group in terms of genera richness. Predatory nematodes were found at all sampling sites and are classified as a persister group, which have a long life and tend to occur at mature and fertile soils. Also, little variation was seen in the abundance of omnivorous, fungivorous and plant parasitic nematodes. Also, maturity indices had similar values at all sites and did not differ significantly between sites. Schnürer et al. (1986) and Yeates (2007) considered that the effect of local, regional, and seasonal differences (soil chemical differences, texture and structure, moisture, and organic matter) and environmental disturbances caused by humans were the most important factors affecting the nematode community. Although Prusias ad Hypium is a protected area, such disturbances may affect the nematode community structure around the town, since it is a permanent settlement for humans. Overall, nematode assemblages showed similar trends by means of maturity indices between 30 sampling sites which is divided into 3 groups namely forest (1–10), farms (11–20) and fruit orchards (21–30). The impact of environmental changes did not show any consistent pattern at the surroundings of Prusias ad Hypium Ancient City. The soil properties may be studied further for explaining this matter.

Soil food web analysis

Nematodes have many advantages as a bioindicators in assessing soils. Nematodes high abundance make statistically valid sampling possible and their broad range of biodiversity facilitates observing expanded physiologies and feeding types that are spread to any environmental conditions worldwide. This definitely positions them at a key positions in the food webs of soil. Their high range of tolerance occurs under all climatic conditions in pristine to highly polluted habitats; include tolerant species and sensitive species. Life-cycle times ranging from 6 days to over 2 years, and rapid responses to disturbance and enrichment provides for wide perspectives when monitoring soil and biological indicators for changes in the environment and soil health.

Soil food web analysis revealed the dominance of highly structured and enriched soil profiles around the Prusias ad Hypium according to nematofauna data (Ferris et al., 2001). Most of the samples clustered within the moderate C:N cycle and suppressive profile of soil structure, which indicates high similarities on the soil characteristics of the area around the antique city. This contribution provides data on the fauna of nematodes in Prusias and its surroundings, the structure of trophic groups, colonizer-persister groups in the nematode community, maturity and soil food web analysis which reveals that the knowledge related to the diversity of nematodes might give beneficial for conservation of biodiversity and soil health monitoring.

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