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Original article (Orijinal araştırma)

A taxonomic study on nematode diversity of some terrestrial trees in Türkiye: a case study in Tekirdağ

Türkiye'deki bazı odunsu ağaç türlerinin nematod çeşitliliği üzerine taksonomik bir çalışma: Tekirdağ ili örneği

Lerzan ÖZTÜRK[1*]

Abstract

The current study was conducted in 2024 in Süleymanpaşa, Tekirdağ to determine the nematode biodiversity in the black poplar [*Populus nigra* L. (Malpighiales: Salicaceae)], Himalayan cedar [*Cedrus deodara (*Lamb.) G.Don (Pinales: Pinaceae)], cypress [*Cupressus sempervirens* L. (Pinales: Cupressaceae)], oriental plane [*Platanus orientalis* L. (Proteales: Platanaceae)], common ash [*Fraxinus excelsior* L. (Lamiales: Oleaceae)], stone pine [*Pinus pinea* L. (Pinales: Pinaceae)], and black locust [*Robinia pseudoacacia* L. (Fabales: Fabaceae)] growing areas. Soil samples were collected from the 0-60 cm soil depth of each tree rhizosphere. Nematodes were extracted using the centrifuge flotation method, and identifications were made using polytomous keys. In the study, 38 genera were identified in the rhizosphere soil of stone pine, 32 in Himalayan cedar, 36 in cypress, 34 in oriental plane, 31 in black locust, 27 in common ash, and 27 in black poplar. *Cephalobus* Bastian, 1865 (Rhabditida: Cephalobidae), *Aphelenchus* Bastian, 1865 (Aphelenchida: Aphelenchidae), *Filenchus* Andrassy, 1954 (Tylenchida: Tylenchidae), and *Geocenamus* Thorne & Malek, 1968 (Tylenchida: Merliniidae) were common genera across all tree species. Taxonomic diversity indices were calculated to compare nematode diversities at different taxonomic levels. The Shannon diversity index (H′) ranged from 2.99 to 3.41, Evenness (J′) from 0.90 to 0.95, Maturity index (MI) from 2.29 to 2.48, and Plant-parasitic index (PPI) from 2.84 to 3.02. Analysis by trophic groups revealed that plant parasitic and bacterial feeder nematodes were more prevalent in all tree species.

Keywords: Nematode fauna, soil, taxonomy, woody trees, Türkiye

Öz

Kara kavak [*Populus nigra* L. (Malpighiales: Salicaceae)], Himalaya sediri [*Cedrus deodara* (Lamb.) G.Don (Pinales: Pinaceae)], servi [*Cupressus sempervirens* L. (Malpighiales: Salicaceae)], Doğu çınarı [*Platanus orientalis* L. (Proteales: Platanaceae)], Adi dişbudak [*Fraxinus excelsior* L. (Lamiales: Oleaceae)], Karaçam [*Pinus pinea* L. (Pinales: Pinaceae)] ve Yalancı akasya [*Robinia pseudoacacia* L. (Fabales: Fabaceae)] ağaçlarının yetişme alanlarındaki nematod biyoçeşitliliğini belirlemek için 2024 yılında Süleymanpaşa Tekirdağ'da bir çalışma yapılmıştır. Her ağacın rizosferinde 0-60 cm derinlikten toprak örnekleri alınmıştır. Nematodlar, santrifüj yöntemi kullanılarak ekstrakte edilmiş ve teşhisleri polytomous anahtarlar kullanılarak yapılmıştır. Çalışmada, Karaçam rizosfer topraklarında 38 cins, Himalaya sedirinde 32 cins, servide 36 cins, doğu çınarında 34 cins, yalancı akasyada 31 cins, adi dişbudakta 27 cins ve kara kavakta 27 cins tespit edilmiştir. *Cephalobus* Bastian, 1865 *(*Rhabditida: Cephalobidae)*, Aphelenchus* Bastian, 1865 *(*Aphelenchida: Aphelenchidae), *Filenchus* Andrassy, 1954 *(*Tylenchida: Tylenchidae) ve *Geocenamus* Thorne & Malek, 1968 (Tylenchida: Merliniidae) tüm ağaç türlerinde bulunmuştur. Nematod çeşitliliklerini farklı taksonik düzeyde karşılaştırmak için birçok taksonomik çeşitlilik indeksi hesaplanmıştır. Shannon diversity index (H′) 2.99 ile 3.41 arasında, Evenness index (J′) 0.90 ile 0.95 arasında, Maturity (MI) 2.29 ile 2.48 arasında ve Plant-parasitic index (PPI) 2.84 ile 3.02 arasında değişmiştir. Trofik gruplara göre yapılan analizde, bitki paraziti ve bakteri ile beslenen nematodların tüm ağaç türlerinde yaygın olduğu belirlenmiştir.

Anahtar sözcükler: Nematod faunası, toprak, taksonomi, odunsu ağaçlar, Türkiye

[1] Namık Kemal University, Faculty of Arts and Sciences, 59200, Süleymanpaşa, Tekirdağ, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: lerzanozturk@gmail.com
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Introduction

Terrestrial trees are critical components of ecosystems worldwide, and forests established with these trees provide habitat for countless species and essential ecological services that sustain life on Earth. Covering 31% of the world's land area, forest ecosystems are crucial, containing elements vital for human life such as water, soil, and energy (Stanturf & Mansourian, 2020). Forests, housing approximately 60.000 tree species, offer habitats for plants, animals, and people, particularly in underdeveloped countries (Anonymous, 2020). They also yield resources like wood, fibre, oil, and fruit, supporting industries in timber, food, medicine, and cosmetics (Anonymous, 1993). Located at the crossroads of Europe and Asia in the northern hemisphere, Türkiye features several climate types including continental, Mediterranean, and Black Sea, each characterized by distinct precipitation patterns. Türkiye hosts 800 taxa of terrestrial tree species, with oak covering 29.4%, red pine 22.85%, and Scots pine 17.54% of the total forest area as of 2022. The reported area for the cultivation of these tree species in Tekirdağ province is 1.098.12 hectares (Anonymous, 2023). Many of these forest tree species are now being cultivated in parks, gardens, and other areas (Anonymous, 2022).

Trees harbour a diverse community of organisms within their ecosystems, both above and below ground. Some of these organisms can be harmful to trees, while others can coexist without causing harm.Hundreds of nematode species inhabit agricultural fields, pastures, soil, water, plant roots, and animals. Among these, approximately 4.100 species are known as plant parasites, capable of feeding on or inside roots of plants, and 27.000 nematodes with different feeding habitats were identified in soils (Decraemer & Hunt, 2006; Háněl & Čerevková, 2010). Damage by plant parasitic nematodes can be particularly significant in young seedlings and saplings, often drastically reducing their survival rates soon after planting. On the other hand, other free-living nematode species exhibit feeding behaviours such as omnivore, predator, fungivore and bacterivore, posing no threat to plants. These species play crucial roles in decomposing animal and plant organic matter in the soil and are essential components in the cycling of mineral nutrients in the soil. The population density of these free-living nematodes in soil provides insights into nutrient cycling, mineral content, productivity, and soil acidity, distinct from those influenced by plant-feeder nematodes (Yadav et al., 2018).

In the global context, extensive research has identified diverse nematode communities, often focusing on identifying *Bursaphelenchus xylophilus* (Steiner & Buhrer, 1934) Nickle, 1981 one of the most destructive nematodes (Karmezi et al., 2022). For instance, Lawton et al. (1998) documented 374 nematode species in Cameroon's tropical forests, highlighting the richness of nematode biodiversity in such environments. Similarly, Skwiercz (2012) reported 119 nematode species across various forest types in Poland, showcasing global variations in nematode fauna. In a study conducted on Mount Ararat in Türkiye, nematodes belonging to 62 genera were identified across various habitats, including mountain grasslands, wildflower meadows, riverbeds, marshes, and chalk grasslands (Çakmak et al., 2021). *Rotylenchus conicaudatus* Atighi et al., 2011 (Nematoda: Hoplolaimidae), *Heterodera trifolii* Goffart, 1932 and *Tylenchorhynchus mangiferae* Luqman & Khan, 1986 were nematodes identified for the first time at species level (Çakmak et al., 2019). In the other studies in Türkiye, *Bursaphelenchus* species have been identified in the provinces that located in Anatolian part of the country (Akbulut et al., 2006, 2008; Öztürk, 2019; Taşdemir et al., 2020).

In Türkiye, particularly in the Northwestern European part, there is limited data on nematode diversity associated with forest trees, with only two species identified so far: *Xiphinema pachtaicum* Tulaganov, 1938 in cypress, poplar, pine, acacia, and spruce, and *Xiphinema index* Thorne & Allen, 1950 in cypress (Öztürk et al., 2023). This underscores the necessity for comprehensive surveys to understand the nematode communities associated with forest trees. The present study aims to fill this gap by investigating nematode presence in habitats where terrestrial trees grow. The study categorize nematodes into different functional groups based on their feeding habits: fungivores (feeding on fungi), bacterivores (feeding on bacteria), predators (feeding on other nematodes or microorganisms), omnivores (feeding on various organic matter), and plant parasitics nematodes. By determining the diversity and functional roles of nematodes across 7 forest tree species, this study aims to provide valuable insights into soil ecology, and forest tree health management.

Materials and Methods

Nematological survey

The nematological survey was conducted in May 2024 in several locations in Tekirdağ province (Figure 1). As part of the study, soil samples were collected from woodlands, parks, and gardens in the Süleymanpaşa district, and surrounding villages. The survey focused on areas where various tree species are cultivated and 140 soil samples were collected. For each tree species, 20 soil samples were collected from 20 distinct locations (Table 1).

Figure 1. A map illustrating the survey locations in Süleymanpaşa district, its geographical position within the province, and its location in Turkey.

Table 1. The number of samples per tree species

Common name	Scientific name	Number of samples
Oriental plane	*Platanus orientalis* L.	20
Black poplar	*Populus nigra* L.	20
Black locust	*Robinia pseudo-acacia* L.	20
Cypress	*Cupressus sempervirens* L.	20
Common ash	*Fraxinus excelsior* L.	20
Himalayan cedar	*Cedrus deodara* (Lamb.) G.Don	20
Stone pine	*Pinus pinea* L.	20

Attention was paid to ensuring that there was at least a 1 km distance between the areas being sampled. According to the data obtained from the meteorological station at the Tekirdağ Viticulture Research Institute during the study year, the average air temperature in January was 6°C, the soil temperature was 8.2°C, and the precipitation was 56.4 mm. In February, the average air temperature was 9.4°C, the soil temperature was 9.6°C, and the precipitation was 34.8 mm. In March, the air temperature was recorded as 9.82°C, the soil temperature as 11.5°C, and the precipitation as 83 mm. In April, the average air temperature was 15.72°C, the soil temperature was 15.9°C, and the precipitation was 82.8 mm. In May, the air temperature increased to 16.25°C, while the soil temperature was 17.3°C, and the precipitation dropped to its lowest level at 27.8 mm. These data reflect the climatic conditions during the study period and played a crucial role in determining the timing of sampling.

In the sampled areas, trees of the same genus were found clustered together, with no other tree species present; only weeds were observed. The sampled trees were identified as follows: species identification was conducted using published diagnostic keys. Subsequent examinations focused on parameters such as leaf arrangement on the branch, leaf morphology (needle-like, compound, etc.), leaf margin characteristics (serrated or smooth), trunk color, trunk structure, bark morphology, tree height, and seed structure.

For species identification, the dichotomous keys of Schulz et al. (2005), Lavin et al. (2012), Lange (2014), Atha & Boom (2017), and Anonymous (2024a, b) were utilized. Additionally, expert engineers within Tekirdağ Metropolitan Municipality and Tekirdağ Viticulture Research Institute, possessing extensive knowledge and experience in species identification, was consulted. Furthermore, some sampled trees had species identification labels affixed by the Tekirdağ Metropolitan Municipality.

During the surveys, soil samples were collected from a depth of 0-60 cm within the canopy projection of the trees. At this stage, soil samples were collected from multiple points and combined into a single bag to create a composite sample. The sampled areas were non commercial areas. In poplar trees (Populus spp.), a spacing of 2.40-3 meters was observed between individual trees, whereas other tree species exhibited a spacing of 4.80 to 5 meters. Nematodes in the soil samples were extracted using the centrifuge flotation method described by Jenkins (1964). Initially, 200 grams of soil were placed in a bowl with water. The mixture was thoroughly mixed and passed through a 200-mesh sieve, followed by a 400-mesh sieve. The nematodes remaining at the bottom of the 400-mesh sieve were collected into a tube. The collected nematode suspension was first centrifuged at 1750 rpm for five minutes. Next, a solution containing 475 g/L of sugar was added to the tubes instead of water, and the suspension was centrifuged again for one minute. Finally, the suspension was passed through a 400-mesh sieve, and the remaining nematodes were collected. Female and if present male nematodes were then killed by heating to 55°C for slide preparation. The dead nematodes were fixed with solutions TAF (40% formaldehyde + triethanolamine + distilled water), Seinhorst I (glycerol + distilled water), and Seinhorst II (glycerol + ethanol), according to the method described by Seinhorst (1959), before being mounted on slides.

Nematodes were examined under a microscope at magnifications ranging from 10X to 100X. To identify at genus and species level polytomous keys from several sources, including Loof & Jairajpuri (1968), Choudhary & Jairajpuri (1984), Geraert & Raski (1987), Anderson (1979), Abolafia & Santiago (1996), Handoo & Golden (1989), Loof & Luc (1990), Brzeski (1991), Handoo et al. (2007), Scholze & Sudhaus (2011), Wu et al., (2016), and Tran et al. (2024), were used. Leica DM 1000 microscope was used in the examination and morphometric measurement of nematodes. The morphometric measurements were conducted using Leica Application Suite. The identified nematodes were then classified based on order, family, colonizer-persister (c-p) value, and feeding habitat. In some genera, such as *Laimidorus* and *Dorylaimus*, female individuals were not captured; therefore, identifications were limited to the genus level. The nematodes were classified based on Siddiqi (2000).

The number of samples in which the species or genus was recorded was divided by the total number of samples to calculate the frequency of occurrence (F%) of each species. A heat map of genera abundance was generated for each forest tree using XLSTAT software. Diversity indices, including Shannon-Wiener, Simpson’s Diversity, Richness, and Evenness, Maturity index (MI), Plant-parasitic index (PPI) were calculated for each terrestrial tree. MI stands for free-living nematodes that are omnivorous, predatory, or feed on bacteria and fungi, while PPI stands for plant-feeding nematodes (Bongers, 1990). The indices were calculated as follows (Pielou, 1966; Bongers, 1990; Neher & Darby, 2009; Öztürk & Avcı, 2023). Other indices were generated with Phyton 3.12, and Sieriebriennikov et al. (2014).

Shannon-Weiner $H' = \sum[(pi) \times log\ (pi)]$

Evenness $J' = \frac{\mathrm{H}\prime}{\ln(\mathrm{S})}$ Richness $R = \frac{S-1}{ln\ N}$

Simpson's Diversity index $1 - D = 1 - [\sum n\ (n-1)/N(N-1)]$

Pi: the abundance of species in the genera; S: total genera number; n_i: The number of nematodes in a genur; N: Total number of nematodes in sample belonging to all genera

Maturity index (only bacterial feeder, fungal feeder, omnivore and predator nematodes) $MI = \sum c(i) \times pi$

Plant-parasitic index (only Plant-parasitic nematodes) $PPI = \sum c(i) \times pi$

pi represents the ratio of free-living nematodes in group i to all collected individuals in the area

c (i) represents the colonizer-persister values of nematodes in the area

Results and Discussion

Nematode fauna composition in terrestrial trees

Forty-four nematode genera belonging to eight orders, 10 suborders, 16 superfamilies, 23 families, and 31 subfamilies were identified in terrestrial tree-growing areas in Tekirdağ. Order Tylenchida exhibited the highest overall values across all tree species, indicating its prevalence in these environments. In contrast, Triplonchida was less prominent, absent from the Oriental plane, and Common ash, suggesting a limited presence. Tylenchida dominated each tree species with values ranging from 34.2 to 44.1, comprising a significant portion of the nematode community. Following Tylenchida were Dorylaimida and Rhabditida, contributing nine and seven genera, respectively (Figure 2).

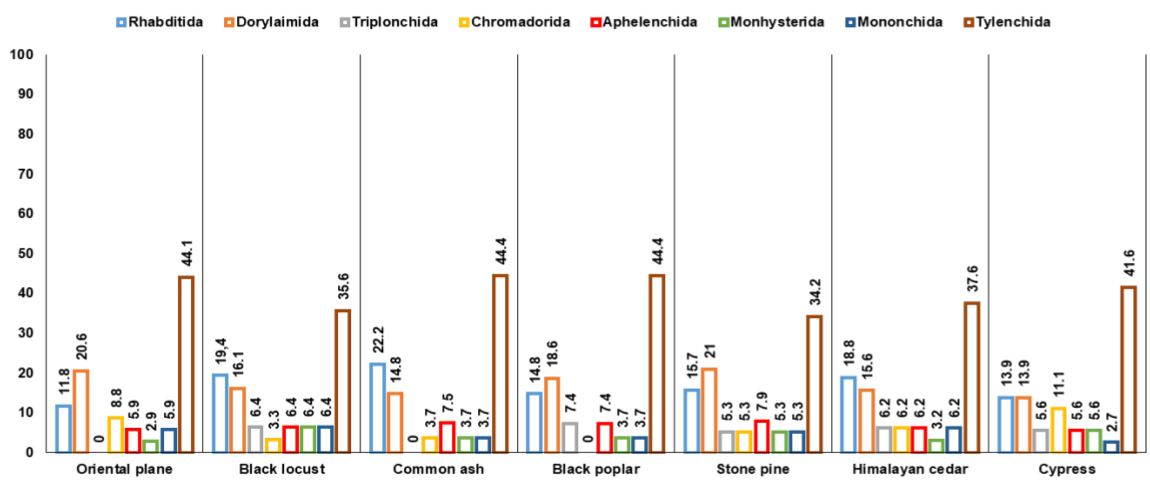

Figure 2. The proportion (%) of eight nematode orders among tree species.

The taxonomic classification of identified genera was represented in Table 2.

Table 2. Taxonomic classification of 44 nematode genera associated with terrestrial trees surveyed in Tekirdağ

Genera	Order	Suborder	Superfamily	Families	Subfamily
Acrobeles	Rhabditida	Cephalobina	Cephaloboidea	Cephalobidae	Cephalobinae
Alaimus	Dorylaimida	Dorylaimina	Alaimoidea	Alaimidae	Alaiminae
Achromadora	Chromadorida	Chromadorina	Chromadoroidea	Achromadoridae	Achromadorinae
Acrobeloides	Rhabditida	Cephalobina	Cephaloboidea	Cephalobidae	Cephalobinae
Aphelenchus	Aphelenchida	Aphelenchina	Aphelenchoidea	Aphelenchidae	Aphelenchinae
Aphelenchoides	Aphelenchida	Aphelenchina	Aphelenchoidea	Aphelenchioididae	Aphelenchoidinae
Aporcelaimellus	Dorylaimida	Dorylaimina	Dorylaimoidea	Aporcelaimidae	Aporcelaiminae
Basiria	Tylenchida	Tylenchina	Tylenchoidea	Tylenchidae	Boleodorinae
Boleodorus	Tylenchida	Tylenchina	Tylenchoidea	Tylenchidae	Boleodorinae
Cervidellus	Rhabditida	Cephalobina	Cephaloboidea	Cephalobidae	Cephalobinae
Cephalobus	Rhabditida	Cephalobina	Cephaloboidea	Cephalobidae	Cephalobinae
Clarkus	Mononchida	Mononchina	Mononchoidea	Mononchidae	Prionchulinae
Coslenchus	Tylenchida	Tylenchina	Tylenchoidea	Tylenchidae	Tylenchinae
Mesocriconema	Tylenchida	Tylenchina	Criconematoidea	Criconematidae	Criconematinae
Discolaimus	Dorylaimida	Dorylaimina	Dorylaimoidea	Qudsianematidae	Discolaiminae
Ditylenchus	Tylenchida	Tylenchina	Sphaerularioidea	Anguinidae	Anguininae
Dorylaimus	Dorylaimida	Dorylaimina	Dorylaimoidea	Dorylaimidae	Dorylaiminae
Filenchus	Tylenchida	Tylenchina	Tylenchoidea	Tylenchidae	Tylenchinae
Geomonhystera	Monhysterida	Monhysterina	Monhysteroidea	Monhysteridae	Monhysterinae
Geocenamus	Tylenchida	Tylenchina	Tylenchoidea	Merliinidae	Merliniinae
Helicotylenchus	Tylenchida	Tylenchina	Tylenchoidea	Hoplolaimidae	Hoplolaiminae
Laimydorus	Dorylaimida	Dorylaimina	Dorylaimoidea	Dorylaimidae	Laimydorinae
Mesodorylaimus	Dorylaimida	Dorylaimina	Dorylaimoidea	Dorylaimidae	Laimydorinae
Mesorhabditis	Rhabditida	Rhabditina	Rhabditoidea	Rhabditidae	Rhabditinae
Monhystera	Monhysterida	Monhysterina	Monhysteroidea	Monhysteridae	Monhysterinae
Mylonchulus	Mononchida	Mononchina	Mononchoidea	Mylonchulidae	Mylonchulinae
Plectus	Chromadorida	Chromadorina	Plectoidea	Plectidae	Plectinae
Panagrolaimus	Rhabditida	Rhabditina	Rhabditoidea	Rhabditidae	Panagrolaiminae
Prismatolaimus	Triplonchida	Tobrilina	Prismatoloidea	Prismatolaimidae	Prismatolaiminae
Prodorylaimus	Dorylaimida	Dorylaimina	Dorylaimoidea	Dorylaimidae	Laimidorinae
Psilenchus	Tylenchida	Tylenchina	Tylenchoidea	Tylenchidae	Psilenchinae
Pratylenchoides	Tylenchida	Tylenchina	Tylenchoidea	Merliniidae	Pratylenchoidinae
Pratylenchus	Tylenchida	Tylenchina	Tylenchoidea	Pratylenchidae	Pratylenchinae
Paratylenchus	Tylenchida	Tylenchina	Tylenchoidea	Tylenchulidae	Paratylenchinae
Rhabditis	Rhabditida	Rhabditina	Rhabditoidea	Rhabditidae	Rhabditinae
Rotylenchus	Tylenchida	Tylenchina	Tylenchoidea	Hoplolaimidae	Rotylenchulinae
Tripyla	Triplonchida	Tripylina	Tripyloidea	Tripylidae	Tripylinae
Tylenchus	Tylenchida	Tylenchina	Tylenchoidea	Tylenchidae	Tylenchinae
Tylenchorhynchus	Tylenchida	Tylenchina	Tylenchoidea	Telotylenchidae	Telotylenchinae
Tylencholaimus	Dorylaimida	Dorylaimina	Tylencholaimoidea	Tylencholaimidae	Tylencholaiminae
Tylocephalus	Chromadorida	Chromadorina	Plectoidea	Plectidae	Wilsonematinae
Xiphinema	Dorylaimida	Dorylaimina	Longidoroidea	Longidoridae	Xiphinematinae
Wilsonema	Chromadorida	Chromadorina	Plectoidea	Plectidae	Wilsonematinae
Zygotylenchus	Tylenchida	Tylenchina	Tylenchoidea	Pratylenchidae	Pratylenchinae

The nematode genera varied by tree species, with 38 genera found in Stone pine, 32 in Himalayan cedar, 36 in Cypress, 34 in Oriental plane, 31 in Black locust, and 27 each in Common ash and Black poplar. A diverse community of nematodes was identified, comprising 15 genera of bacterial feeder nematodes, such as *Tylocephalus* Crossman, 1933 and *Geomonhystera* Andrassy, 1981*,* four genera of fungal feeders, including *Ditylenchus* Filipjev, 1936 and *Tylencholaimus* De Man, 1876, five genera of omnivores like *Prodorylaimus* Andrassy, 1959 and *Laimydorus* Siddiqi, 1969*,* and five genera of predators, notably *Clarkus* Jairajpuri, 1970 and *Mylonchulus* Cobb, 1916. In the bacterial feeder group, species-level identification was achieved for 10 species, highlighting the richness of this group. In the plant-parasitic nematode genera found in the areas, 21 nematodes were identified at the species level. Of these, nine species, including *Boleodorus thylactus* Thorne, 1941, could feed on fungal cultures and therefore grouped as root-fungal feeders. The distribution of plant-parasitic nematodes varied significantly across tree species: 17 species were found in Stone pine, 17 in Himalayan cedar, 16 in Cypress, 19 in Oriental plane, 12 in Black locust, 15 in Common ash, and 13 in Black poplar . Among the plant-parasitic species, endoparasitic (13.6%), ectoparasitic (81.8%), and semi-endoparasitic (4.5%) nematodes were identified, with ectoparasitic nematodes being the most dominant across all tree species (Table 3, Figure 3).

Table 3. The nematodes recorded on a per-terrestrial tree (Sp: stone pine, Hc: Himalayan cedar, C: Cypress, As: Oriental plane, Bl: Black locust, Ca: Common ash, Bp: Black poplar)

Genera/species		**c-p**	**Feeding habitat**	**Sp**	**Hc**	**C**	**Op**	**Bl**	**Ca**	**Bp**
Acrobeles	*Acrobeles ciliatus*	2	Bacterial feeder	■	■	■	■	■	■	■
	Acrobeles complexus	2	Bacterial feeder	■		■	■	■		
	Acrobeles cylindricus	2	Bacterial feeder	■						
Alaimus	*Alaimus primitivus*	4	Bacterial feeder	■	■	■	■	■	■	■
Achromadora sp.		3	Bacterial feeder	■		■	■	■		
Acrobeloides	*Acrobeloides nanus*	2	Bacterial feeder	■	■	■	■	■	■	■
Cervidellus	*Cervidellus vexilliger*	2	Bacterial feeder	■	■			■	■	
Cephalobus	*Cephalobus persegnis*	2	Bacterial feeder	■	■	■	■	■	■	■
Geomonhystera	*Geomonhystera villosa*	1	Bacterial feeder	■	■	■		■	■	■
Mesorhabditis sp.		1	Bacterial feeder			■		■		
Monhystera sp.		2	Bacterial feeder	■		■	■	■		
Panagrolaimus	*Panagrolaimus rigidus*	1	Bacterial feeder	■	■				■	
Plectus sp.		2	Bacterial feeder		■	■				
Prismatolaimus	*Prismatolaimus intermedius*	3	Bacterial feeder	■	■	■		■		■
Rhabditis		1	Bacterial feeder	■	■	■	■	■	■	■
Tylocephalus	*Tylocephalus auriculatus*	2	Bacterial feeder	■		■	■			
Wilsonema	*Wilsonema schuurmanstekhoven*	2	Bacterial feeder	■	■	■	■		■	
Aphelenchus	*Aphelenchus avenae*	2	Fungal feeder	■	■	■	■	■	■	■
Aphelenchoides	*Aphelenchoides sacchari*	2	Fungal feeder	■	■	■	■	■	■	■
Ditylenchus	*Ditylenchus myceliophagus*	2	Fungal feeder	■	■	■	■	■	■	■
Tylencholaimus	*Tylencholaimus proximus*	4	Fungal feeder	■			■			
Aporcelaimellus	*Aporcelaimellus obscuroides*	5	Omnivore		■			■	■	■
Dorylaimus sp.		4	Omnivore	■	■	■	■	■	■	■
Laimydorus sp.		4	Omnivore	■		■	■			
Mesodorylaimus sp.		5	Omnivore	■		■	■	■		
Prodorylaimus sp.		5	Omnivore	■		■				■
Discolaimus sp.		4	Predator	■	■		■			

Table 3. continued

Genera/species		c-p	Feeding habitat	Sp	Hc	C	Op	Bl	Ca	Bp
Clarkus	*Clarkus papilatus*	4	Predator	■	■	■	■	■		
Mylonchulus sp.		4	Predator	■	■		■	■	■	■
Tripyla sp.		3	Predator	■	■	■		■		■
Basiria	*Basiria graminophila*	2	Root-fungal feeder [Ek]	■		■	■		■	
Boleodorus	*Boleodorus thylactus*	2	Root-fungal feeder [Ek]	■	■	■	■	■	■	■
Coslenchus	*Coslenchus costatus*	2	Root-fungal feeder [Ek]			■		■		
	Coslenchus turkeyensis	2	Root-fungal feeder [Ek]		■		■			
Filenchus	*Filenchus thornei*	2	Root-fungal feeder [Ek]	■	■	■	■	■	■	■
	Filenchus cylindricus	2	Root-fungal feeder [Ek]	■	■		■		■	
	Filenchus sheri	2	Root-fungal feeder [Ek]	■	■	■	■	■	■	■
Geocenamus	*Geocenamus brevidens*	3	Plant-parasitic [Ek]	■	■	■	■	■	■	■
Mesocriconema	*Mesocriconema xenoplax*	3	Plant-parasitic [Ek]	■	■	■	■		■	■
Helicotylenchus	*Helicotylenchus digonicus*	3	Plant-parasitic [Ek]	■	■	■	■	■	■	■
	Helicotylenchus pseudorbustus	3	Plant-parasitic [Ek]	■	■		■		■	
Psilenchus	*Psilenchus hilarulus*	2	Root-fungal feeder [Ek]	■	■		■	■	■	■
Pratylenchoides	*Pratylenchoides alkani*	3	Plant-parasitic [Sen]	■	■				■	■
Pratylenchus	*Pratylenchus thornei*	3	Plant-parasitic [En]	■	■	■	■			■
	Pratylenchus neglectus	3	Plant-parasitic [En]	■	■	■	■	■		
Paratylenchus	*Paratylenchus nainianus*	2	Plant-parasitic [Ek]	■	■	■	■	■	■	
Rotylenchus	*Rotylenchus cypriensis*	3	Plant-parasitic [Ek]	■	■	■	■	■		■
Tylenchus	*Tylenchus davainei*	2	Root-fungal feeder [Ek]	■		■	■			■
Tylenchorhynchus	*Tylenchorhynchus annulatus*	3	Plant-parasitic [Ek]			■	■		■	
Xiphinema	*Xiphinema pachtaicum*	5	Plant-parasitic [Ek]	■	■	■	■	■	■	■
	Xiphinema index	5	Plant-parasitic [Ek]		■					
Zygotylenchus	*Zygotylenchus guaverei*	3	Plant-parasitic [En]			■	■	■	■	■

* Ek: Ectoparasite En: Endoparasite Sen: Semi-endoparasitFigure 3. Comparative graph showing the % proportion of trophic groups in each terrestrial tree.

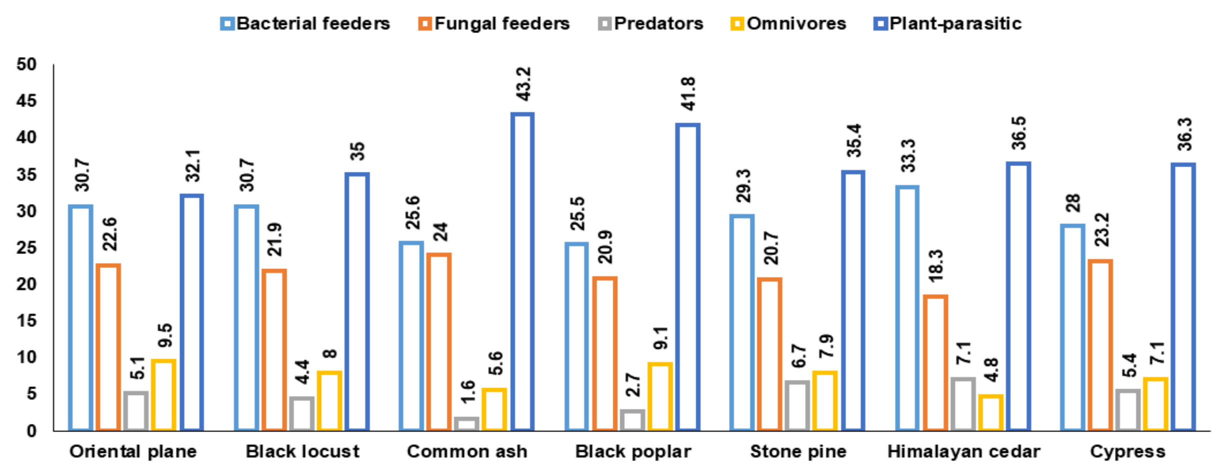

Figure 3. Comparative graph showing the proportion (%) of trophic groups in each terrestrial tree.

The identified nematodes were classified into five c-p classes (1-5) and five feeding groups. Species with c-p 2 values (22.2-29.6 %) and c-p 4 values (11.1-18.4%) were particularly prominent in the free-living nematode community across all tree species (Figure 4). The c-p 2 group primarily comprised nematodes from the families Cephalobidae and Plectidae. In contrast, the c-p 4 group included predatory and omnivore nematodes from the families Mononchidae and Dorylaimidae. Among the plant-parasitic nematode species, those with p-p 3 values (14.8-20.8%) were notably prevalent, belonging to the families Merliniidae, Pratylenchidae, and Hoplolaimidae.

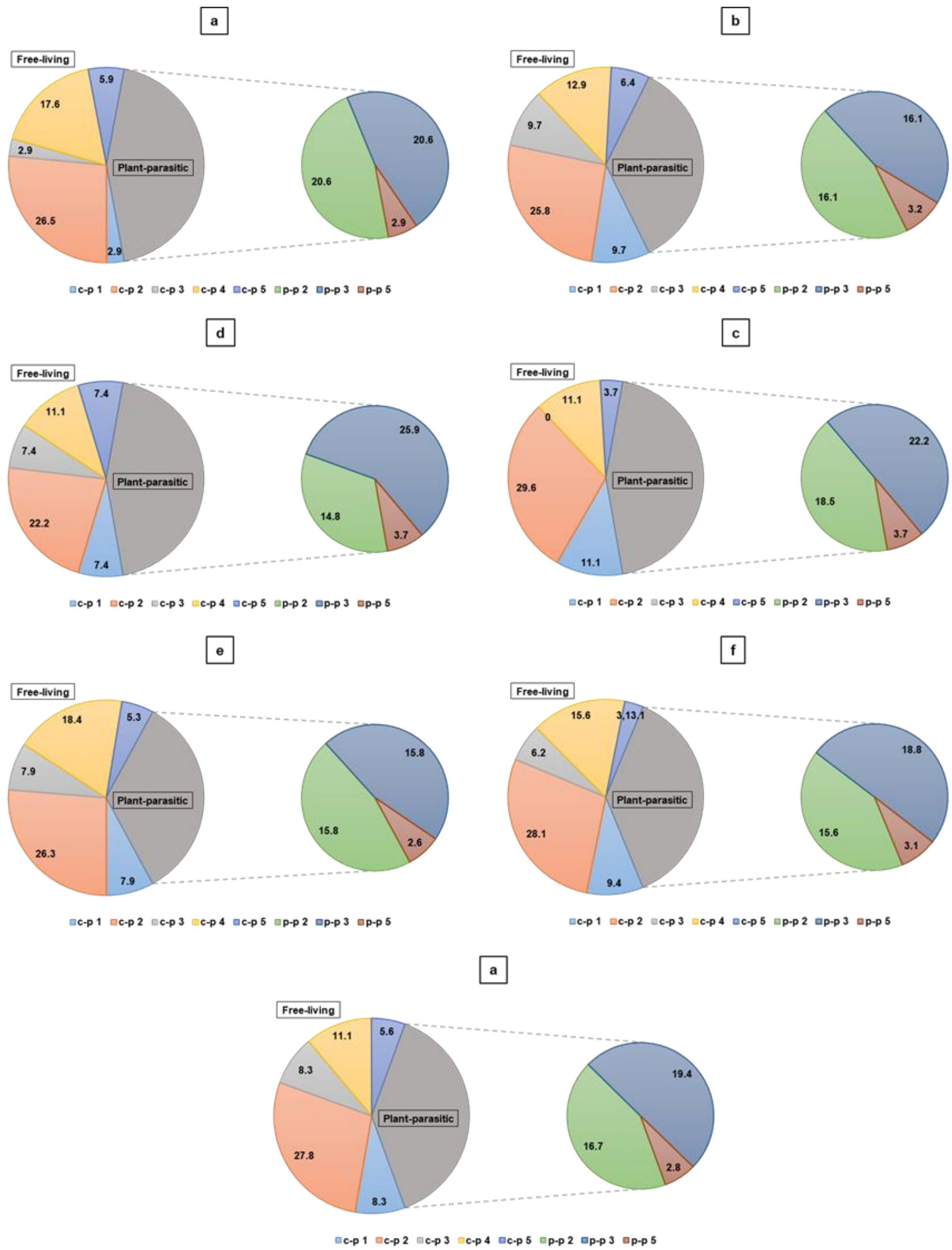

Figure 4. Comparative graph showing the proportion (%) of c-p groups in each terrestrial tree: a) Oriental plane; b) Black locust; c) Common ash; d) Black poplar; e) Stone pine; f) Himalayan cedar; g) Cypress.

Nematode genera such as *Acrobeles* von Linstow, 1877*, Alaimus* De Man, 1880*, Acrobeloides* Cobb, 1928*, Cephalobus, Aphelenchus, Aphelenchoides* Fischer, 1894*, Ditylenchus, Boleodorus* Thorne, 1941*, Helicotylenchus* Steiner*,* 1945, *Geocenamus,* and *Xiphinema* have been detected across all tree species (Figure 5). *Acrobeles ciliatus* Linstow, 1877*, Cephalobus persegnis* Bastian*,* 1865*,* and *Acrobeloides nanus* De Man, 1880 emerged as the most prevalent bacterial feeder nematodes in the study. Notably, *A. nanus* was identified as the most common species across all soil samples, with a remarkable occurrence rate of 94.2%, followed closely by *C. persignis* at 91.4% and *A. ciliatus* at 54%. These three species were consistently found in the soil of all tree species. Among fungal feeder species, *Aphelenchoides sacchari* Hooper, 1958 (80%), and *Aphelenchus avenae* Bastian, 1865 *(*96.4%) were the most frequently detected. Regarding plant-parasitic nematodes*, Geocenamus brevidens* stood out as the most abundant species, occurring in an impressive 98.2% of all soil samples. Additionally, six species *Boleodorus tylactus, Helicotylenchus digonicus* Perry, 1959*, Filenchus thornei (*Andrássy, 1954) Andrássy, 1963*, Filenchus sheri* Khan & Khan, 1978*,* and *Xiphinema pachtaicum* were consistently found across all tree species. In contrast, the *X. index* was found only in Himalayan cedar.

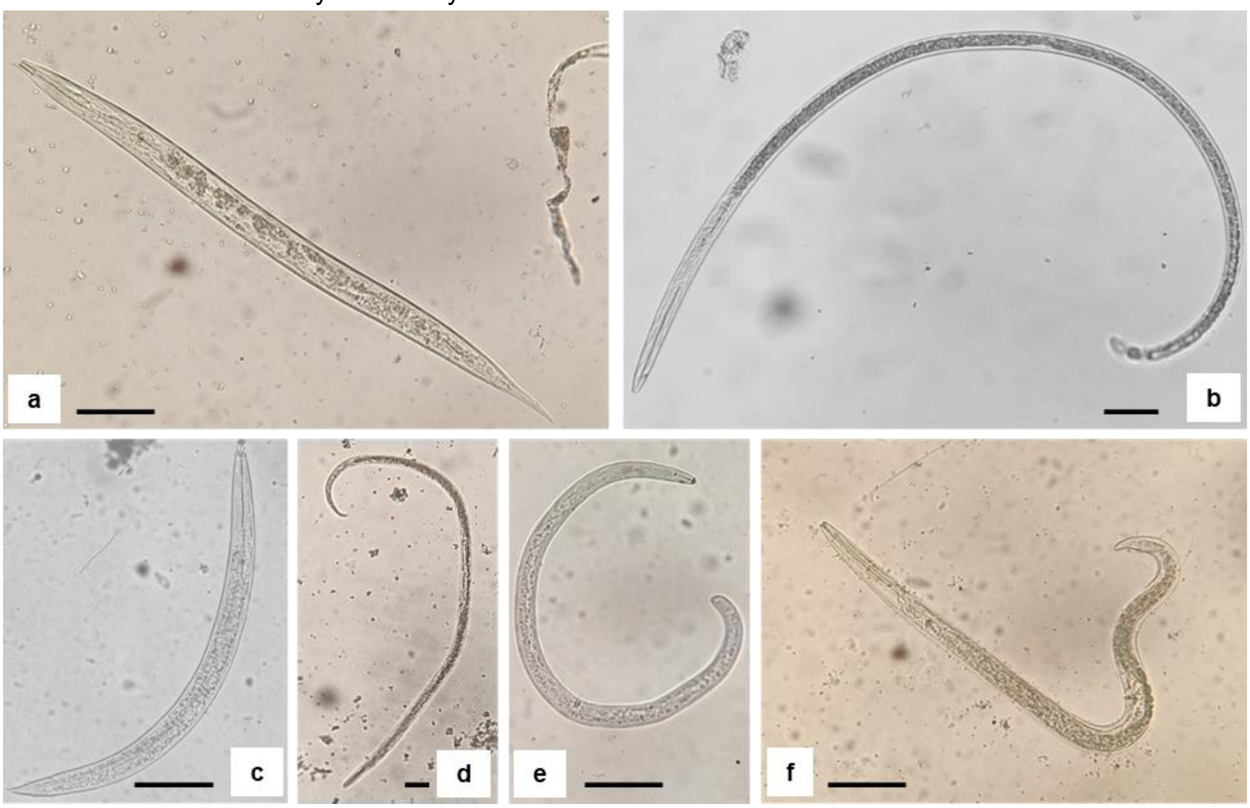

Figure 5. Nematode in this study from genera: a) *Rhabditis*; b) *Xiphinema*; c) *Acrobeles*; d) *Alaimus*; e) *Rotylenchus*; f) *Cephalobus* (a, c, e, f 20 µm; b 100 µm; d 200).

In terms of nematode density per 100 grams of soil samples, the average proportion of free-living nematodes in the total soil community ranged from 42% to 56.2%. In contrast, the proportion of plant-parasitic nematodes varied between 43.8% and 58%. Figure 6 illustrates the average nematode abundance found in 100 grams of soil, represented in a heat map generated in XLSTAT using Z scores. The dendrogram in the left axis represents terrestrial trees, and the axis at the bottom indicates identified nematode genera. A dendrogram at the top shows the clustering of these genera based on their abundance patterns. In the heat map, the colors green, red, and black convey varying levels of nematode abundance. A higher nematode populations were indicated with green color, suggesting a robust presence within the soil sample. In the heat map, the shades of red indicate that the nematode populations in the soil samples for certain genera are low. According to heatmap some nematodes exhibit a clear preference for particular tree species. *Psilenchus* is more abundant in Cypress and Black locust, while *Xiphinema* and Helicotylenchus are more prevalent in Oriental plane. Ditylenchus shows relatively higher abundance in Common ash and Black poplar. In contrast, some nematodes are more evenly distributed. Figure 6 shows that the least number of individuals were found in the black poplar, while the most were found in the species that were present. Genera clustered in the same clade in a heat map share quite close or similar abundance patterns. Results on this map were consistent with the previous data in Table 3.

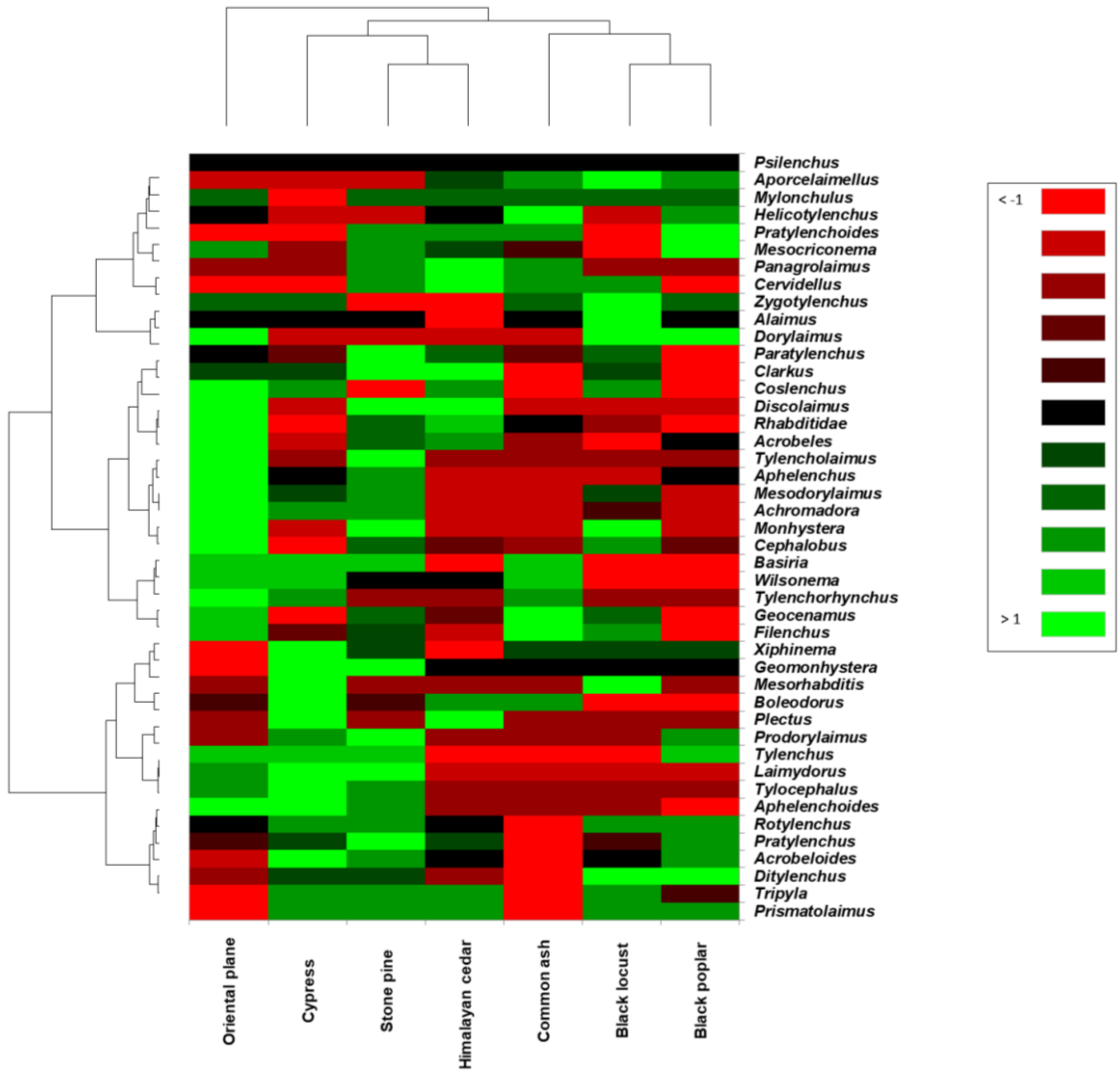

Figure 6. A heatmap illustrating the abundance of nematode genera found in sampled terrestrial trees. The names of the tree species are listed along the right axis, while the identified nematode genera are displayed along the bottom axis. A color key indicates the average nematode abundances per 100 grams of soil.

Nematode biodiversity indices in terrestrial tree growing areas

Table 4 summarises the biodiversity indices calculated using average nematode populations. There was significant variation in diversity, dominance, richness, and ecological indices among tree species, reflecting differences in soil biodiversity and ecosystem function.

Table 4. Average nematode diversity indices of terrestrial trees

Diversity indices	Oriental plane	Black locust	Common ash	Black poplar	Stone pine	Himalayan cedar	Cypress
Shannon-Weiner (H')	3.17	3.15	2.99	3.14	3.37	3.25	3.41
Evenness (J')	0.090	0.091	0.090	0.095	0.092	0.093	0.095
Simpson’s Diversity index (1-D)	0.059	0.056	0.072	0.047	0.040	0.043	0.036
Reciprocal Simpson index (1/D)	16.72	17.58	14.49	21.25	24.40	22.74	25.67
Maturity index (MI)	2.45	2.29	2.24	2.48	2.41	2.28	2.33
Maturity index 2-5 (MI)	2.61	2.46	2.42	2.58	2.57	2.52	2.49
Sigma maturity index (ΣMI)	2.61	2.50	2.54	2.71	2.57	2.48	2.51
Plant-parasitic index (PPI)	2.93	2.88	2.94	3.02	2.86	2.85	2.84
Berger-parker dominance index	0.12	0.13	0.16	0.09	0.10	0.09	0.10
Menhinick index	2.64	2.71	2.47	2.63	2.93	2.64	3.17
Margaleff richness index	6.46	6.16	5.44	5.58	7.22	5.96	7.20
Dominance (1-D)	0.94	0.94	0.92	0.95	0.95	0.95	0.96
Equitability index (J)	0.86	0.89	0.87	0.92	0.91	0.93	0.92
Channel index (CI)	46.27	42.86	45.45	58.97	42.11	30.67	43.53
Basal index (BI)	25.57	28.78	30.00	27.97	25.95	25.03	28.03
Enrichment index (EI)	53.17	54.69	56.41	47.56	53.90	60.48	54.84
Structure index (SI)	63.97	55.89	50.96	62.53	62.75	59.42	57.51
Bacterivore footprint	14.35	13.47	14.30	10.80	20.06	20.40	22.92
Fungivore footprint	2.53	2.46	2.12	2.13	2.90	1.87	3.15
Omnivore footprint	33.25	23.80	23.32	31.24	25.30	21.72	27.06
Predator footprint	4.84	4.06	0.91	1.96	7.09	6.84	4.26
Herbivore footprint	10.17	11.01	11.68	11.05	12.38	9.45	11.70

The Shannon-Weiner index (H'), which measures species diversity, was highest for cypress (3.41) and stone pine (3.37), demonstrating that these trees supported the most diverse communities. In contrast, common ash (2.99) had the lowest diversity, suggesting a more limited range of species. Evenness (J'), which assesses how evenly species were distributed, was highest in black poplar and cypress (0.095), showing a more balanced species composition. In contrast, oriental plane and common ash (0.090) had slightly lower evenness, meaning that some species were more dominant than others.

Simpson’s Diversity Index (1-D), which quantifies dominance, showed that cypress (0.036) and stone pine (0.040) had the lowest dominance values, meaning that no single species prevailed in these communities. Conversely, common ash (0.072) had the highest dominance, signifying that a few species were more abundant. The Reciprocal Simpson Index (1/D) followed the same trend, with cypress (25.67) and stone pine (24.40) having the highest values, revealing diverse communities, while common ash (14.49) was the least diverse.

Maturity indices (MI, MI 2-5, and ΣMI) provided insights into ecosystem stability. Black poplar (2.48) had the highest maturity index, emphasizing a more stable environment, whereas common ash (2.24) had the lowest, reflecting a younger or more disturbed ecosystem. The Plant-Parasitic Index (PPI) was highest in black poplar (3.02), confirming that plant-parasitic nematodes were more prevalent in its soil ecosystem, while cypress (2.84) had the lowest PPI, suggesting fewer plant-parasitic nematodes.

The Berger-Parker Dominance Index, which measures the dominance of the most abundant species, was highest for common ash (0.16), implying that its soil community was strongly influenced by a few species. In contrast, black poplar (0.09) had the lowest dominance, reflecting a more even species distribution. Species richness indices, such as the Menhinick and Margaleff indices, measure the number of species present. Cypress exhibited the highest richness values (Menhinick: 3.17, Margaleff: 7.20), closely followed by stone pine (Margaleff: 7.22), revealing that these trees hosted the most species. In contrast, common ash had the lowest richness (Menhinick: 2.47, Margaleff: 5.44), showing fewer species in its soil environment. The Equitability Index (J'), which evaluates species distribution fairness, was highest in Himalayan cedar (0.93) and black poplar (0.92), representing a more even species distribution. Oriental plane (0.86) had the lowest value, confirming that certain species were more dominant.

Ecological indices provided further insights into ecosystem functioning. The Channel Index (CI) was highest in black poplar (58.97), illustrating a well-structured food web, while Himalayan cedar (30.67) had the lowest, implying a less developed trophic structure. The Enrichment Index (EI), which measures nutrient cycling efficiency, was highest in Himalayan cedar (60.48), revealing a well-functioning microbial and nematode community, and lowest in black poplar (47.56). The Structure Index (SI), which reflects ecosystem complexity and resilience, was highest in oriental plane (63.97), highlighting a strong ecological framework, whereas common ash (50.96) had the lowest, suggesting a less stable community.

Trophic footprints, representing nematode community composition, varied significantly among trees. Cypress had the highest bacterivore footprint (22.92), proving high microbial activity in the soil, while black poplar had the lowest (10.80). The fungivore footprint was highest in cypress (3.15), showing strong fungal activity, and lowest in Himalayan cedar (1.87). The omnivore footprint, representing generalist feeders, was greatest in oriental plane (33.25) and lowest in Himalayan cedar (21.72). The predator footprint, indicating predatory nematode abundance, was highest in stone pine (7.09) and lowest in common ash (0.91), signifying limited predator presence in ash soils. Lastly, the herbivore footprint, representing plant-feeding nematodes, was highest in stone pine (12.38) and lowest in Himalayan cedar (9.45), confirming different levels of plant-nematode interactions.

From these results, cypress and stone pine emerged as the most diverse and ecologically stable tree species, with high species richness and low dominance, fostering a balanced and resilient soil ecosystem.

Discussions

At the end of the study, 44 nematode genera were identified, indicating rich biodiversity in Tekirdağ terrestrial forests. The presence of nematodes from different functional groups, including fungal feeders, bacterial feeders, predators and omnivores, indicates a complex food web. The differences in the number of genera associated with different tree species (e.g., 38 in pine vs. 27 in ash) may be due to factors such as plant root structure and nutrient availability, as well as soil conditions, soil moisture, and soil microorganisms. Research by Bongers & Ferris (1999) highlighted how different plant species can influence soil nematode communities. Their findings support the observation that pine maintains the most diverse nematode fauna with 38 genera, suggesting that tree species with specific root structures or nutrient profiles can significantly influence nematode diversity. Similarly, several published researches demonstrated that specific tree species can support different nematode communities, resembling similarity with the findings from Tekirdağ. In a study conducted in Greece, several nematode genera, including *Bursaphelenchus xylophilus, Clarkus,* and *Tylencholaimus,* were found in pine areas (Karmezi et al., 2022). In Bulgaria, 48 plant-parasitic nematode species were identified in the same tree species, with *Geocenamus brevidens, Helicotylenchus digonicus,* and *Geocenamus brevidens* being commonly found, similar to the results in Tekirdağ (Peneva & Choleva, 1994). In Brazil, 35 nematode genera from 5 trophic groups have been identified in forest areas. Similar to the fauna in Tekirdağ, genera such as Aphelenchoides and Rhabditis were found to be the most common and abundant. In another research in Slovakia, 51 nematode genera have been identified in forest areas consisting of various tree species, including *Fraxinus excelsior*. When nematodes were identified, it was observed that the number of free-living bacterivorous nematodes was greater (Cerevkova et al., 2021).,

The different nematode assemblages found in different tree species likely reflect differences in root exudates and soil nutrient profiles, which may influence nematode diversity and abundance. The lower number of nematode genera in some trees and locations in Tekirdağ may be due to predatory genera, such as Mylonchulus, that play a functional role in the suppression of the populations of other nematodes, including potential plant parasites. For instance, predators *Mylonchulus, Clarkus, Tripyla, Discolaimus,* and omnivores such as *Aporcelaimellus,* and *Dorylaimus* may feed on nematode genera *Aphelenchus, Aphelenchoides, Helicotylenchus, Plectus, Cephalobus, Acrobeloides, Rotylenchus,* and *Tylenchorrhynchus (*Small, 1987).

The higher prevalence of Tylenchida in all tree species revealed its importance in the province. Its significant values (ranging from 34.2 to 44.1) indicate its adaptability and ability to survive in various soil and plant conditions in Tekirdağ. The dominance of Tylenchida in the Tekirdağ study was consistent with the findings of Yeates, 1979, who noted that Tylenchida was common in various terrestrial ecosystems. Tylenchid species, such as *Mesocriconema xenoplax, Pratylenchus neglectus*, and *Pratylenchus thornei* are significant threats to plants due to their ability to cause substantial damage. These nematodes feed on roots, and cause deformation and lesions, leading to stunted growth and impaired nutrient uptake, which can severely affect plant health and agricultural productivity (Thompson & Clewett, 2021). Their presence in the soil not only disrupts root function but also makes plants more vulnerable to environmental stresses and diseases, ultimately resulting in reduced crop yields. Effective management strategies are essential to mitigate their impact and protect agricultural outputs.

The identification of 15 genera of bacterial feeders, four genera of fungal feeders, five genera of omnivores, and five genera of predators illustrates a well-structured nematode community in survey areas in Tekirdağ. Moreover, the bacterivore footprint is highest in Cypress (22.92), while the fungivore footprint is notably higher in Cypress (3.15). Predator footprints are significant in Stone Pine (7.09), reflecting a robust predator presence. Achieving species-level identification for 10 female species within the bacterial feeders emphasizes the richness and ecological significance of this group. Bacterial feeders play a crucial role in decomposing organic matter and regulating bacterial populations, thereby enhancing nutrient availability for plants. (Bardgett & van der Putten, 2014). Research has shown that the presence of diverse nematode communities is linked to improved soil structure and fertility, which is essential for sustainable agriculture (Pires et al., 2023). Their diversity is high in a healthy soil environment that can support a wide range of microbial activity. The presence of four genera of fungal feeders highlights their importance in the decomposition of organic materials, particularly in breaking down fungal biomass (Zhang et al., 2020). The identification of five genera of omnivores and five genera of predators indicates a biologically more community structure and soil food web (Ferris et al., 2012; Pires et al., 2023). Omnivorous nematodes can feed on both bacteria and other nematodes, facilitating energy transfer within the food web. Predators, on the other hand, play a critical role in controlling populations of bacterial and fungal feeders, helping to maintain balance within the ecosystem (Khan & Kim, 2007).

Seventeen diversity indices were calculated for each terrestrial tree. The higher Shannon-Weiner index values in Cypress (3.41) and Stone Pine (3.37) is the sign of a richer and more diverse nematode community (Krebs, 1985). This diversity can enhance ecosystem resilience, as varied species fulfill different ecological roles. Conversely, in Tekirdağ Common Ash (2.99) exhibits a lower Shannon-Weiner index. The Evenness values reveal a more balanced distribution of nematode species in Cypress and Black Poplar, which is critical for maintaining ecosystem stability. In a higher Evenness, no single species dominates the community (Pielou, 1966). The low Simpson's index values in Cypress and Stone Pine indicate a diverse community with lower dominance by any single species. This is advantageous for ecosystem functioning, as it allows for a more balanced interaction among species. In contrast, the higher dominance observed in Black Locust could imply that a few species may dominate (Díaz et al., 2007). All calculated indices highlight the ecological roles and interactions of nematodes associated with different tree species. The indices data in Tekirdağ suggest that trees like Cypress and Stone Pine support more diverse and balanced nematode communities, which contribute positively to ecosystem resilience. In contrast, species like Common Ash and Black Locust may face challenges due to lower diversity and higher dominance, respectively. Understanding these dynamics is crucial for managing forest ecosystems and enhancing soil health.

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