

## Original article (Orijinal araştırma)

# A survey in sunflower fields in Tekirdağ (Türkiye) to determine soil health with nematode-based diversity indices

Tekirdağ ili (Türkiye) ayçiçeği tarlalarında nematod çeşitlilik indeksleri ile toprak sağlığının belirlenmesine yönelik bir araştırma

Lerzan ÖZTÜRK<sup>1</sup> \* 

Gürkan Güvenç AVCI<sup>2</sup> 

## Abstract

In this study conducted in 2021-2022, nematode community structure was investigated in sunflower fields in Tekirdağ to determine sampled fields' physical characteristics based on nematode biodiversity indices. For this purpose, soils collected from 37 sunflower fields were examined for nematode presence. In the soils, 34 genera of fungivore [3 genera, e.g., *Aphelenchoides* Fischer, 1894 (Aphelenchida: Aphelenchoididae)], bacterivore [9 genera, e.g., *Acrobeloides* Cobb, 1924 (Rhabditida: Cephalobidae)], omnivore [4 genera, e.g., *Dorylaimus* Dujardin, 1845 (Dorylaimida: Dorylaimidae)], predator [2 genera, e.g., *Seinura* Fuchs, 1931 (Aphelenchida: Aphelenchoididae)], and plant-parasitic [17 genera, e.g., *Pratylenchus* Filipjev, 1936 (Tylenchida: Pratylenchidae)] nematodes were recovered by the modified Baermann Funnel method. The dominant nematodes were fungivores and plant-parasitics occurring in all fields. Among 17 plant-parasitic nematodes identified at a species level, the most economically important species were *Longidorus elongatus* Mikoletzky, 1922 (Dorylaimida: Longidoridae), *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 (Tylenchida: Anguinidae), *Pratylenchus thornei* Sher & Allen 1953, and *Pratylenchus zea* Graham, 1951 (Tylenchida: Pratylenchidae). Maturity indices calculated to estimate soil characteristics in fields were  $2.33 \pm 0.36$ , and the value was determined to be  $<3$  in most fields except for two fields. Food web analysis revealed that 76.3% of soils had worsened physical characteristics and a high C: N ratio. The characteristics of soils in two fields were enriched, and the others were fertile.

**Keywords:** *Helianthus annuus*, nematode community analysis, soil health

## Öz

2021-2022 yıllarında yürütülen bu çalışmada, Tekirdağ ili ayçiçeği tarlalarında nematod biyoçeşitlilik indeksleri kullanılarak örneklenen tarlaların fiziksel özelliklerini belirlemek için nematod komünite yapısı incelenmiştir. Bu amaçla 37 ayçiçeği tarlasından toplanan topraklarda nematod varlığı incelenmiştir. Toprakta 34 cins fungivor [3 cins, örneğin *Aphelenchoides* Fischer, 1894 (Aphelenchida: Aphelenchoididae)], bakterivor [9 cins, örneğin *Acrobeloides* Cobb, 1924 (Aphelenchida: Aphelenchoididae)], omnivor [4 cins, örneğin *Dorylaimus* Dujardin, 1845 (Dorylaimida: Dorylaimidae)], predatör [2 cins, örneğin *Seinura* Fuchs, 1931 (Aphelenchida: Aphelenchoididae)] ve bitki paraziti [17 cins, örn., *Pratylenchus* Filipjev, 1936 (Tylenchida: Pratylenchidae)] nematodları içeren 34 cins nematod modifiye edilmiş Baermann Huni yöntemiyle izole edilmiştir. Tüm alanlarda fungivor ve bakterivor türler baskın bulunmuştur. Tür düzeyinde tespit edilen 17 bitki paraziti nematod arasında ekonomik açıdan en önemli türler *Longidorus elongatus* Mikoletzky, 1922 (Dorylaimida: Longidoridae), *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 (Tylenchida: Anguinidae), *Pratylenchus thornei* Sher & Allen 1953 ve *Pratylenchus zea* Graham, 1951 (Tylenchida: Pratylenchidae) olmuştur. Tarlalarda toprak özelliklerini tahmin etmek için hesaplanan olgunluk indeksleri  $2.33 \pm 0.36$  olup, iki alan dışında çoğu alanda değer  $<3$  olarak belirlenmiştir. Besin ağı analizi, toprakların %76.3'ünün fiziksel özelliklerinin kötüleştiğini ve yüksek bir C:N oranına sahip olduğunu ortaya çıkarmıştır. İki tarlada ise toprak özellikleri zenginleştirilmiş, diğerleri ise verimli olarak saptanmıştır.

**Anahtar sözcükler:** *Helianthus annuus*, nematod komünite analizi, toprak sağlığı

<sup>1</sup> Viticulture Research Institute, 59200, Tekirdağ, Türkiye

<sup>2</sup> Atatürk Soil and Water Agricultural Meteorology Research Institute, 39060, Kırklareli, Türkiye

\* Corresponding author (Sorumlu yazar) e-mail: lerzanzoturk@gmail.com

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## Introduction

Sunflower is the most-grown oil seed plant in Türkiye with the large cultivation area. The plant is preferred due to its adaptation to dry and irrigated soil conditions and suitability to mechanization during the vegetation period. Sunflower oil corresponds to 69% of the vegetable oil production in the country and constitutes 32% of the total consumed oil (Yağmur et al., 2021). Each plant has 22-55% total oil content, and the oil contains saturated and unsaturated fatty acids (Akkaya, 2018). In addition to processing it into oil, sunflower seeds are consumed as a snack or animal feed. An essential part of the sunflower fields planted in Türkiye is located in provinces such as Edirne, Tekirdağ, and Kırklareli in Thrace.

As with many cultivated plants, there are many organisms in sunflower soils, and soil health in cultivation areas can be determined using their diversity and interaction with the environment. Soil health is the soil's continuous function in the ecosystem. It is described as the continuous processing potential of the soil to maintain biological productivity in cultural soils, improve the quality of the soil environment, and protect living organisms. Besides affecting the development of plants, soil health affects the life cycles of beneficial microorganisms in the soil. It causes tremendous damage to the living things that consume sunflower products (Doran & Zeiss, 2000).

There are many indicators of soil health worldwide, and nematodes are considered among them. Nematodes have an important place regarding the number of species and population. An average of 385 nematode species are named annually, and 25.000 identified species are included in 2.271 genera and 256 families (Anderson, 2008; Hodda, 2011). They are classified into different trophic groups according to their feeding patterns. Nematodes with different trophic groups are associated with plants, bacteria, fungi, and other soil faunas and are considered an essential component of the soil food web. For instance, by moving nematodes in the soil, the permeability and water infiltration increase, allowing the soil and organic residues to mix. Soil nematodes are responsible for recycling nutrients and minerals in the environment. The soil physical condition was assessed using Diversity indexes such as Shannon-Weiner, Basal, Structure, and Enrichment calculated using the trophic structures and functional guilds of nematodes (Bongers, 1990).

Many nematode species in sunflower fields are mentioned in the literature, and the most damaging species to plant growth are *Meloidogyne* (Tylenchida: Heteroderidae) species. The other genera found in sunflower plantations include several ectoparasitic and endoparasitic nematodes like *Helicotylenchus* Steiner, 1945 (Tylenchida: Hoplolaimidae), *Pratylenchus* (Tylenchida: Pratylenchidae), *Quinisulcius* (Tylenchida: Belonolaimidae), and *Xiphinema* (Dorylaimida: Longidoridae) (Fourie et al., 2017). In our country, in the study conducted in 2000, 30 plant-parasitic species belonging to 9 families and 24 genera were identified. Still, free-living species have not been studied (Kepenekçi, 2001).

The current study investigated plant-parasitic and free-living nematode diversity, and the soil physical properties were determined by calculating some diversity indices. This is the most recent study in which nematode community structure was determined together with free-living nematodes.

## Materials and Methods

### The study area and soil collection

An intensive nematode survey was conducted between 2021-2022 in 11 districts in Tekirdağ province of Thrace, Türkiye. During the study, randomly selected 37 sunflower fields with at least 15km distance were visited (Figure 1, Table 1). The latitudes of the fields were between 40°52'-41°19'S, longitudes were between 27°6'-28°0'W, and elevations ranged between 3 and 258 meters. The large-scale non-irrigated sunflower production was dominant in the visited fields. The weather temperature during the survey was 25-28°C, and the average monthly total precipitation was 24.2 mm. Six soil subsamples were collected from different points in each field by moving in a zigzag pattern. The soil depth of sampling was 0-60 cm, and an average of 1 kg of soil was taken per field.



Figure 1. Map representing study area in Tekirdağ.

Table 1. The total production, survey locations, and total acreage of sunflower fields in Tekirdağ

Provinces/ID	Districts	Production area (ha)	Total production (ton)
Çerkezköy (Çer)	Merkez	1.896	3.092
Çorlu (Çor)	Sarılar, Seymen, Yenice	12.619	21.929
Ergene (Erg)	Ahimehmet, Misinli, Velimeşe	14.388	27.733
Hayrabolu (Hay)	Dambaslar, Soylu, Susuzmüsellim	36.108	69.666
Kapaklı (Kap)	Bahçeağıl, Yanık ağıl	4.128	7.171
Malkara (Mal)	Evrenbey, İbribey, Karamurat	33.483	64.729
Marmara Ereğlisi (Mar)	Türkmenli, Yakuplu, Yeniçiftlik	4.782	10.1162
Muratlı (Mur)	Arzulu, İnanlı, Kırkkepenekli, Yurtbekler	13.431	35.253
Saray (Sar)	Büyükyoncalı	14.351	29.060
Süleymanpaşa (Sül)	Barbaros, Bıyıklı, İncecik, Köseilyas, Mahramlı	31.882	61.598
Şarköy (Şar)	Beyoğlu, İshaklı	2.688	5.177

### Nematode recovery and identification

The nematodes in each field soil were extracted from 100 cm<sup>3</sup> of the sample by the Baermann Funnel technique. In this method, soil cores were placed in sieves with a single layer of filter paper, filled with water, and incubated for 24 hours. The next day, nematodes that migrated to water were collected by sieving on 400 mesh sieves. The nematodes were counted with 1 ml of the extracted suspension at 10X magnification under the microscope. Free-living nematodes were identified at the genus level, and plant parasitic and Aphelenchids were identified at the species level by examining the female's morphologic features like stylet shape, vulva position, tail shape, and longitudinal striations. The slides of nematode females were prepared by heat-killing and processing in TAF [(7%) formaldehyde + (2 %) triethanolamine + (91%) distilled water)], Seinhorst I (1 ml glycerin + 79 ml distilled water), and Seinhorst II (5 ml glycerin + 95 ml ethanol) solutions. Processed nematodes were fixed on a glycerin-dropped slide by the wax ring method (Seinhorst, 1959). Plant parasitic species were identified with published references (Geraert & Raski, 1987; Brezski, 1991; Loof & Luc, 1993; Castillo & Volvas, 2005, Handoo et al., 2007).

### The nematode trophic groups, diversity indices, nematode-soil health relation

Nematodes extracted from sunflower fields were subjected to several diversity and food web analyses to determine the health status of soils in sampled areas. The Shannon-Weiner Diversity Index, Evenness, and Richness were calculated to evaluate the diversity index of nematode fauna in fields. The formulas used to calculate the indices were as follows (Pielou, 1966; Neher & Darby, 2009). Additionally, Principal component analysis (PCA) was performed with the XSLSTAT 2022 software to the data obtained from the soils taken from each field. Abundance and frequency were analyzed with PCA.

$$\text{Shannon-Weiner Index (H')}: H' = -\sum [(p_i) \times \log(p_i)]$$

$$\text{Pielou's Evenness Index (J')}: J' = H' / \ln(S)$$

$$\text{Genera Richness Index (GR)}: GR = S - 1 / \ln N$$

Pi: the proportion of individuals in genera; S: the number of genera; N: the number of all identified nematodes.

The extracted nematodes were divided into bacterivore, fungivore, predator, omnivore, and plant-parasitic based on the feeding habitat. Soon they were classified by colonizer-persister values. The nematode community and soil food web were analyzed with Maturity (MI), Maturity (MI)2-5, Plant-parasite (PPI), Basal (BI), Channel (CI), Enrichment (EI), and Structure (SI) indices calculated according to Ferris et al. (2001). MI was used to assess disturbance in soil; lower values were considered as more disturbed, and higher values as less disturbed soils. The MI2-5 value was used to indicate soil health status. Other indices were computed to determine the state of the food web in the soil. While the EI indicates nutrient enrichment and availability, the SI value gives information about the food web structure. The BI also shows the soil food web; higher values indicate worsened conditions. CI was used to indicate whether organic matter decomposition was related to bacterial or fungal feeder nematodes. Fungivore nematodes dominated the decomposition at high CI values, while bacterivores played a primary role in decomposition at low CI values (Ferris et al., 2001).

Nematode Indicators Joint Analysis (NINJA) online software was used to prepare the c-p triangle and soil food-web scheme (Sieriebriennikov et al., 2014). Based on nematode diversity the fields were placed in different Quadrats (Figure 2) (Ferris, 2001)

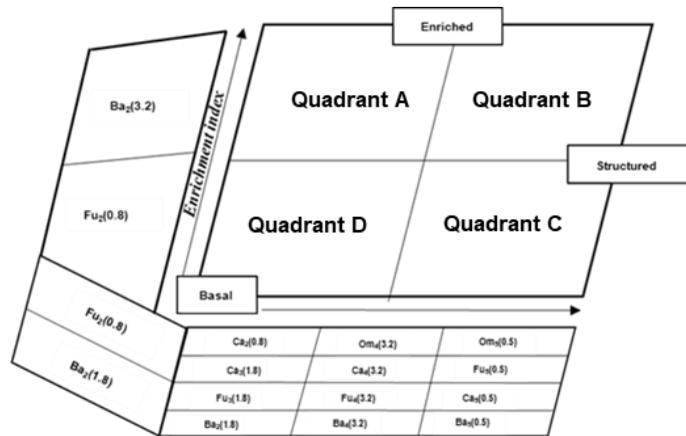


Figure 2. Scheme representing soil food-web. Quadrant A: disturbed and enriched; Quadrant B: enriched and maturing; Quadrant C: Matured; Quadrant D: Degraded (Ferris et al., 2001).

## Results

### The nematode genera, distribution, and trophic groups

In this study, 37 genera of nematodes were identified in 37 sunflower fields in Tekirdağ. Nematodes belonged to 8 orders (Aphelenchida, Chromadorida, Dorylaimida, Rhabditida, Mononchida, Triplonchida, and Tylenchida), 8 suborders, and 22 families (Figure 3).

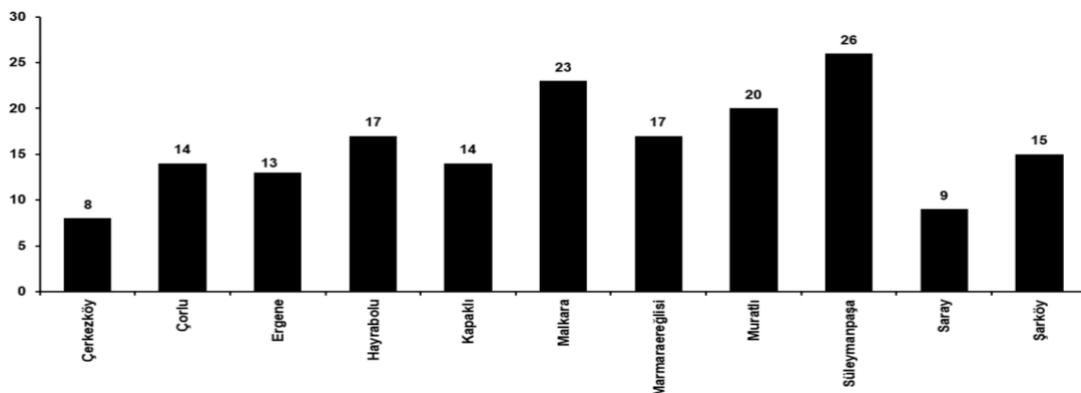


Figure 3. The number of genera identified in sunflower fields in 11 districts.

Specimens were classified into five trophic groups by feeding habitat. Of these, nine genera were bacterivores, three were fungivores, six were predators, and three were omnivores. Fungivore and bacterivores, omnivores, and predators were grouped under free-living nematodes. The plant-parasitic nematodes belonged to 17 genera and included a plant-parasitic species (*Ditylenchus dipsaci* Kühn, 1857) from the fungivore genera *Ditylenchus*. The most common nematodes in sunflower fields were bacterivores, followed by fungi feeders, plant-parasitic, omnivores, and predators. In the sunflower fields, fungivores dominated three samples, while bacterivores were prominent in five and plant feeders in eight samples. A higher population of fungi and bacterivores were found in Süleymanpaşa and Kapaklı (Figure 4). The lowest rate of plant parasitic nematodes in soil samples was 3.6%, and the highest was 69.1%. Omnivores were found only in 86.5 % and predators in 43.2% of the fields. In some locations, the free-living nematode population was high and plant-parasitic nematode were low in number.

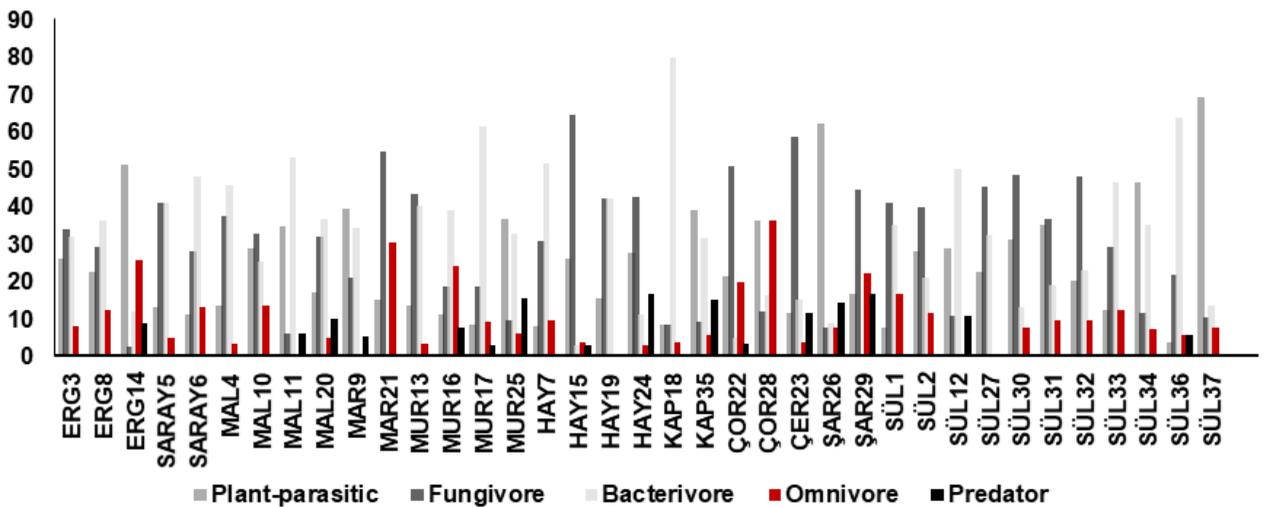


Figure 4. The % proportion of nematodes with different feeding habitats in 37 sunflower fields surveyed in Tekirdağ.

Identified free-living nematodes were classified under nine suborders and 14 families (Table 2). The greater abundance of *Ditylenchus* Filipjev, 1936 (fungivore), *Acrobeloides* Cobb, 1924 (bacterivore), *Cephalobus* Bastian, 1865 (Rhabditida: Cephalobidae (bacterivore), *Mesodorylaimus* Andrassy, 1959 (Dorylamida: Dorylaimidae) (bacterivore) was observed in sampled areas. An average of  $69.3 \pm 35.8$  (23-234) free-living nematode individuals were found per 100 cm<sup>3</sup> of soil in each soil sample.

Twenty-two plant-parasitic nematode species belonging to two suborders and 11 families were identified in sunflower fields in Tekirdağ (Table 3). The abundance of plant parasitic nematodes in all soil samples ranged from 41 to 234 individuals per 100 cm<sup>3</sup> soil. Of the recovered species, *Filenchus filiformis* Ebsary, 1991 (Tylenchida: Tylenchidae), *Geocenamus tesellatus* (Goodey, 1952) Brzeski, 1991 (Tylenchida: Merliniidae), *Tylenchorhynchus annulatus* Cobb, 1913 (Tylenchida: Belonolaimidae) and *Pratylenchus zaeae* Graham, 1951 were the most widespread. In contrast, *Longidorus elongatus*, *Paratrophurus acristylus* Siddiqi & Siddiqi, 1983 (Tylenchida: Telotylenchidae), and *Xiphinema pachtaicum* (Dorylaimida: Longidoridae) were found to be rare.

Table 2. Data representing taxonomic classification, c-p values, the occurrence, and abundance of free-living nematodes in sunflower fields in Tekirdağ (ba: bacterivore; fu: fungivore; om: omnivore; pr: predator)

Genera / species	Functional guild	Suborder	Families	Occurrence (%)	Abundance (min-max nematodes/ 100 cm <sup>3</sup> soil)
<i>Achromadora</i> Cobb, 1913	Ba3	Chromadorina	Achromadoridae	5.4	1-2
<i>Acrobeles</i> Linstow, 1877	Ba2	Cephalobina	Cephalobidae	27.0	3-21
<i>Acrobelloides</i> Cobb, 1924	Ba2	Cephalobina	Cephalobidae	81.0	2-20
<i>Alaimus</i> de Man, 1880	Ba4	Dorylaimina	Alaimidae	10.8	1-3
<i>Cephalobus</i> Bastian, 1865	Ba2	Rhabditina	Cephalobidae	67.5	3-53
<i>Cervidellus</i> Thorne, 1937	Ba2	Cephalobina	Cephalobidae	17.1	2-3
<i>Monhystera</i> Bastian, 1865	Ba2	Monhysterina	Monhysteridae	8.1	2-5
<i>Plectus</i> Bastian, 1865	Ba2	Chromadorina	Plectidae	2.7	2
<i>Rhabditis</i> Dujardin, 1845	Ba1	Rhabditina	Rhabditidae	14.3	2-11
<i>Wilsonema</i> Cobb, 1913	Ba2	Chromadorina	Plectidae	5.4	2
<i>Aphelenchoides clarus</i> Thorne & Malek, 1968	Fu2	Aphelenchina	Aphelenchoididae	8.2	3-15
<i>Aphelenchoides sacchari</i> Hooper, 1958	Fu2	Aphelenchina	Aphelenchoididae	35.1	7-27
<i>Aphelenchoides obtusus</i> Thorne & Malek, 1968	Fu2	Aphelenchina	Aphelenchoididae	5.4	3-7
<i>Aphelenchus avenae</i> Bastian, 1865	Fu2	Aphelenchina	Aphelenchidae	48.7	3-14
<i>Ditylenchus geraerti</i> (Paramonov, 1970)	Fu2	Tylenchina	Anguinidae	70.2	3-21
<i>Aporcelaimellus</i> Heyns, 1965	Om5	Dorylaimina	Aporcelaimidae	24.3	2-3
<i>Dorylaimus</i> Dujardin, 1845	Om4	Dorylaimina	Dorylaimidae	40.5	1-4
<i>Eudorylaimus</i> Andrásy, 1959	Om4	Dorylaimina	Qudsianematidae	16.2	2-8
<i>Mesodorylaimus</i> Andrásy, 1959	Om4	Dorylaimina	Dorylaimidae	59.4	3-28
<i>Prodorylaimus</i> Andrásy, 1959	Om4	Dorylaimina	Dorylaimidae	5.4	2-3
<i>Clarkus</i> Jairajpuri, 1970	Pr4	Mononchina	Mononchidae	2.7	7
<i>Seinura</i> Fuchs, 1931	Pr4	Aphelenchina	Aphelenchoididae	8.1	2
<i>Tripyla</i> Bastian, 1865	Pr3	Tripylina	Tripylidae	8.1	4-10

As well in Principle Component Analysis (PCA), nematode species nematodes were grouped based on frequency as dominant (4 genera), frequent (6 genera), infrequent (15 genera), and rare (12 genera) (Figure 5).

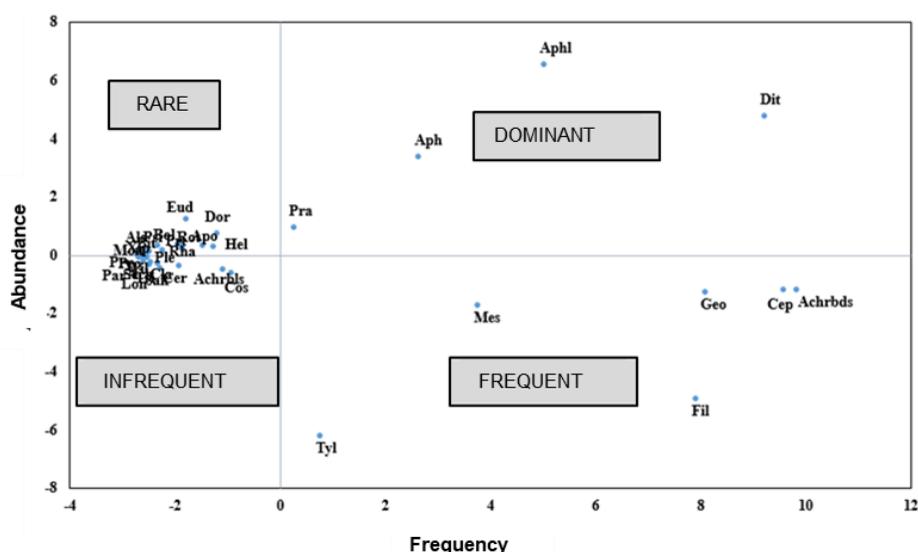


Figure 5. Principle component analysis (PCA) of nematode distribution pattern in sunflower fields in Tekirdağ. Abundance and frequency scheme of nematode genera. (Ach: *Acromodora*; Achrbis: *Acrobeles*; Achrbds: *Acrobelloides*; Ala: *Alaimus*; Aphl: *Aphelenchoides*; Aph: *Aphelenchus*; Bit: *Bitylenchus*; Bol: *Boleodorus*; Cep: *Cephalobus*; Cer: *Cervidellus*; Cos: *Coslenchus*; Dit: *Ditylenchus*; Dor: *Dorylaimus*; Eud: *Eudorylaimus*; Fil: *Filenchus*; Geo: *Geocenamus*; Hel: *Helicotylenchus*; Lon: *Longidorus*; Mes: *Mesodorylaimus*; Mon: *Monhystera*; Pro: *Prodorylaimus*; Psi: *Psilenchus*; Par: *Paratylenchus*; Pry: *Paratylenchus*; Ple: *Plectus*; Pra: *Pratylenchus*; Prt: *Pratylenchoides*; Rha: *Rhabditis*; Rot: *Rotylenchus*; Sak: *Sakia*; Sei: *Seinura*; Tyl: *Tylenchorhynchus*; Tri: *Tripyla*; Xip: *Xiphinema*).

Table 3. Data representing taxonomic classification, c-p values, the occurrence and abundance of plant-parasitic nematodes in sunflower fields in Tekirdağ (Pp: Plant-parasitic; RHF: Root hair feeder)

Genera / species	Functional guild	Suborder	Families	Occurrence (%)	Abundance (min-max nematodes/ 100 cm <sup>3</sup> soil)
<i>Bitylenchus parvus</i> Jairajpuri 1982	Pp3/RHF	Tylenchina	Dolichodoridae	5.4	3-6
<i>Boleodorus thylactus</i> Thorne, 1941	Pp2/RHF	Tylenchina	Boleodorinae	16.2	2-3
<i>Coslenchus alacinatus</i> Siddiqi, 1981	Pp2/RHF	Tylenchina	Tylenchidae	27.0	2-5
<i>Ditylenchus dipsaci</i> (Kühn, 1857) Filipjev, 1936	PP2	Tylenchina	Anguinidae	18.9	3-17
<i>Filenchus filliformis</i> Ebsary, 1991	Pp2/RHF	Tylenchina	Tylenchidae	51.3	7-23
<i>Filenchus thornei</i> (Andrassy, 1954) Andrassy, 1963	Pp2/RHF	Tylenchina	Tylenchidae	24.3	3-5
<i>Filenchus cylindricus</i> (Thorne & Malek, 1968)	Pp2/RHF	Tylenchina	Tylenchidae	13.5	3-6
<i>Geocenamus tesellatus</i> (Goodey, 1952) Brzeski, 1991	Pp3	Tylenchina	Merliniidae	43.2	2-32
<i>Geocenamus brevidens</i> (Allen, 1955) Siddiqi	Pp3	Tylenchina	Merliniidae	24.3	4-60
<i>Helicotylenchus digonicus</i> Perry, 1959	Pp3	Tylenchina	Hoplolaimidae	21.6	8
<i>Helicotylenchus tunisiensis</i> Siddiqi, 1964	Pp3	Tylenchina	Hoplolaimidae	5.4	3-4
<i>Longidorus elongatus</i> Mikoletzky, 1922	Pp5	Dorylaimina	Longidoridae	2.7	2
<i>Paratrophurus acristylus</i> Siddiqi & Siddiqui, 1983	Pp3	Tylenchina	Telotylenchidae	2.7	2
<i>Pratylenchus thornei</i> Sher & Allen 1953	Pp3	Tylenchina	Pratylenchidae	13.5	2-17
<i>Pratylenchus zaeae</i> Graham, 1951	Pp3	Tylenchina	Pratylenchidae	29.7	4-7
<i>Pratylenchoides alkani</i> Yüksel, 1977	Pp3	Tylenchina	Pratylenchidae	11.4	2-3
<i>Psilenchus hilarulus</i> de Man, 1921	Pp2	Tylenchina	Boleodorinae	8.1	1-2
<i>Rotylenchus buxophilus</i> Golden, 1956	Pp3	Tylenchina	Hoplolaimidae	10.8	3-4
<i>Paratylenchus rotundicephalus</i> Bajaj, 1988	Pp3	Tylenchina	Paratylenchidae	5.4	2
<i>Sakia allii</i> Suryawanshi, 1971	Pp2/RHF	Tylenchina	Boleodorinae	5.4	5
<i>Tylenchorhynchus annulatus</i> Cobb, 1913	Pp3	Tylenchina	Belonolaimidae	29.7	5-105
<i>Xiphinema pachtaicum</i> Tulaganov, 1938	P-P5	Dorylaimina	Longidoridae	2.7	3

Omnivore, predator, fungivore, and bacterivore free-living nematodes recovered from sunflower fields in Tekirdağ were grouped from 1 to 5 depending on the colonizer-persister (c-p) values. Genera from c-p2 were prominent among free-living nematodes. The % fraction of c-p2 nematodes in 37 soil samples ranged between 43.75 and 100. In the sampled fields in Tekirdağ, enrichment opportunist nematodes in the c-p1 class were found only in 6 fields (Figure 6). Furthermore, nematodes with c-p3 values were detected in 6 areas, c-p4 in 34 areas and c-p5 values in 9 areas.

Plant-parasitic nematodes belonged to three c-p groups. The prevalence of c-p2, c-p3, and c-p5 among all sampled fields were 45.9%, 100%, and 5.4%, respectively. The nematodes in 20 fields belonged to c-p3 only. Parallel to the frequent occurrence of the c-p3 class in survey areas, the population was also higher in soils. *Geocenamus* was the dominant genus in c-p3, while *Filenchus* was the most common genus in c-p2. The c-p5 nematode was detected only in two locations in Süleymanpaşa, and two species *Longidorus elongatus*, and *Xiphinema pachtaicum*, from this class were identified (Figure 7).

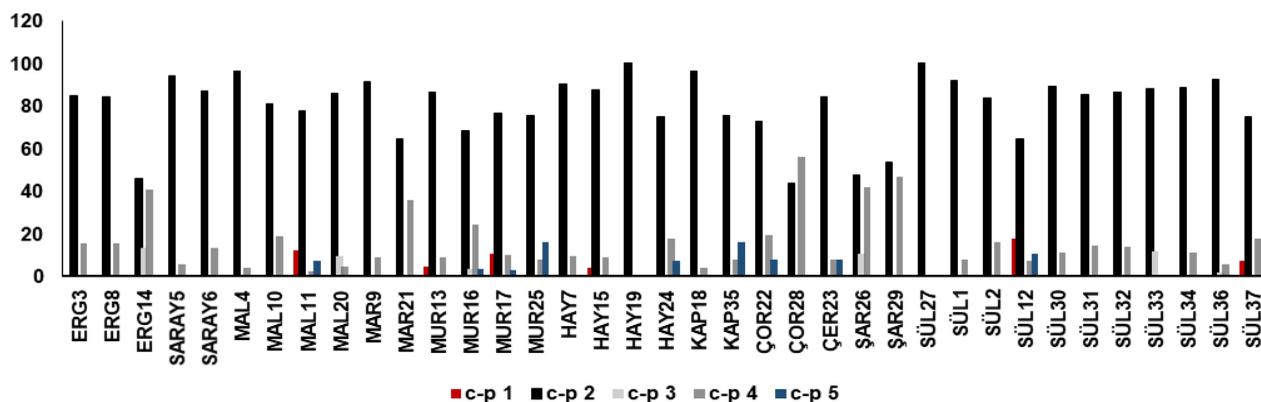


Figure 6. The % proportion of free-living nematodes with different colonizer-persister values in 37 sunflower fields surveyed in Tekirdağ.

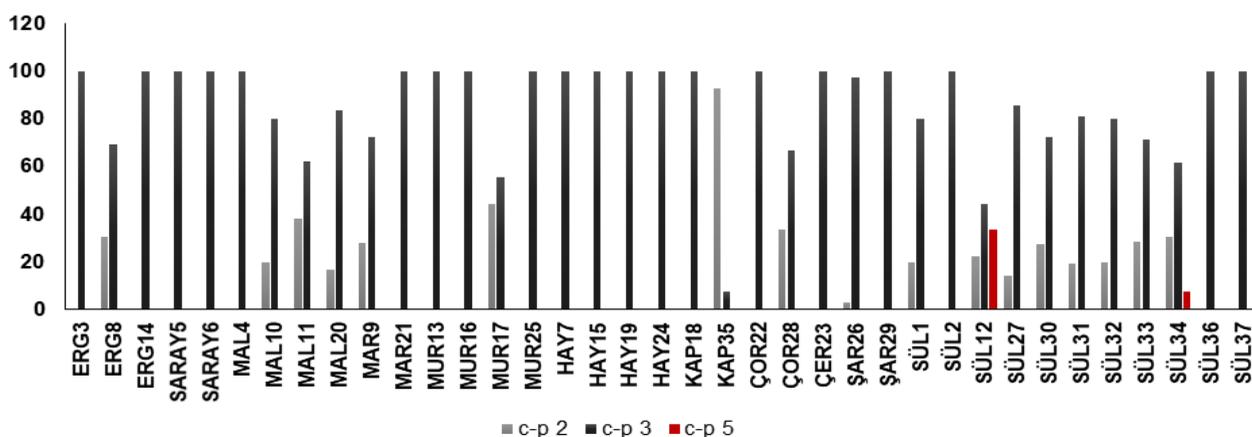


Figure 7. The % proportion of plant-parasitic nematodes with different colonizer-persister values collected from 37 surveyed sunflower fields in Tekirdağ.

### Nematode diversity indices, soil food web, soil health

Several indices indicating nematode diversity and soil health were calculated for sunflower fields. As seen in Figure 8, The Maturity index (MI) and, Maturity index (MI)<sub>2-5</sub> in fields differed; the mean value was  $2.35 \pm 0.26$  (2-3.12) for MI and  $2.37 \pm 0.28$  (2-3.12) for (MI)<sub>2-5</sub>. The MI value of all fields was  $\geq 2$  in. The lowest value (2) was observed in two, and the highest was (3.12) in one field. Consequently, as the soils with low MI index were examined, the abundance of bacterivore and fungivore from the c-p 1-2 group was high compared to the other trophic groups. On the contrary, in soil with an MI value above 3, the ratio of plant, fungivore, and bacterivore nematodes was counted as 36%, 12%, and 16%. The mean PPI value [ $2.91 \pm 0.15$  (2.6-3.4)] was also found to be low in sunflower fields where endoparasitic, semi-endoparasitic, and large-sized species like *X. pachtaicum* from Longidoridae are not common.

The Shannon-Weiner Diversity Index (H') was  $1.9 \pm 0.3$  (1-2.57). Pielou's Evenness Index (J') was  $0.89 \pm 0.06$  (0.7-0.98). The genera richness ranged between 4-17 (Figure 9).

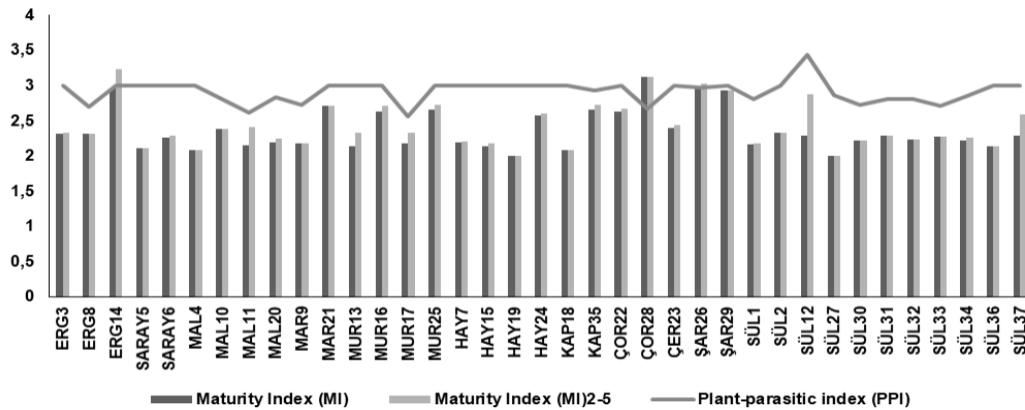


Figure 8. Maturity index (MI), Maturity index (MI)<sub>2-5</sub>, and Plant-parasitic (PPI) values of 37 sunflower fields surveyed in Tekirdağ.

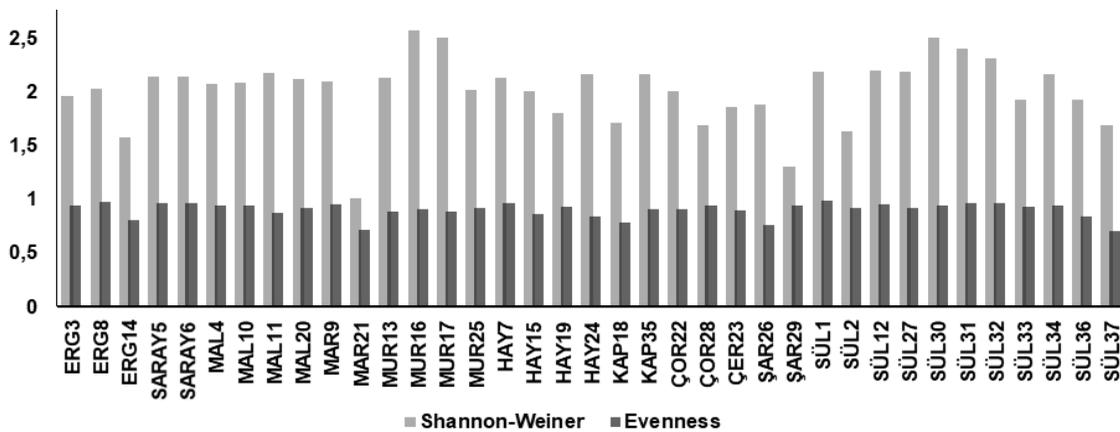


Figure 9. Shannon-Weiner diversity ( $H'$ ) and Evenness indices of nematode diversity of 37 sunflower fields surveyed in Tekirdağ.

In each field, Basal (BI), Channel (CI), Enrichment (EI), Plant parasite (PPI), and Structure (SI) indices were calculated (Figure 10). The mean CI was determined as  $92.7 \pm 18.6$  (33.3-100), which was calculated as  $>50$  in 91.9% of the fields. On the other hand, the average BI was found to be  $41.6 \pm 14.8$  (15.2-66.7). This value was calculated as  $\leq 30$  in 11 fields, 30-60 in 22 fields, and  $\geq 60$  in five fields. The mean EI indicating nutrient enrichment was  $37.49 \pm 10.2$  (13.6-51.1), and 64.9% of fields had values under 40. Again, our average Structure Index value, determined as  $43.13 \pm 21.3$  (0-83.7), was very low in 11 fields.

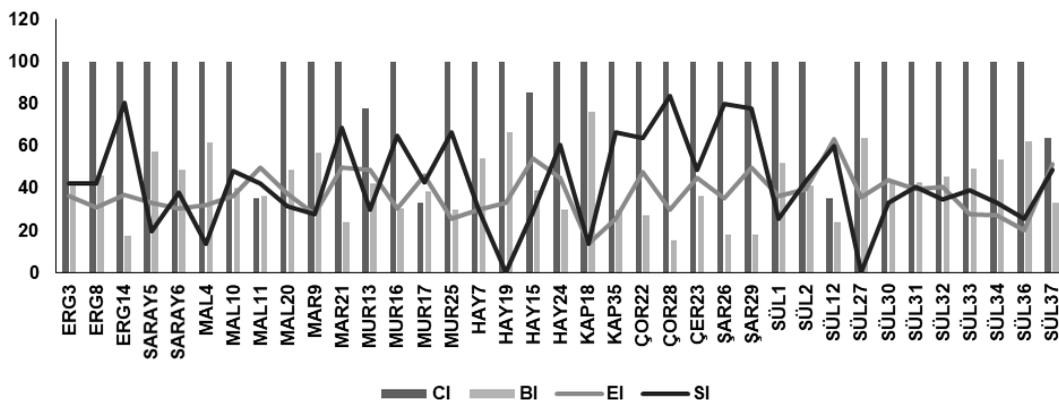
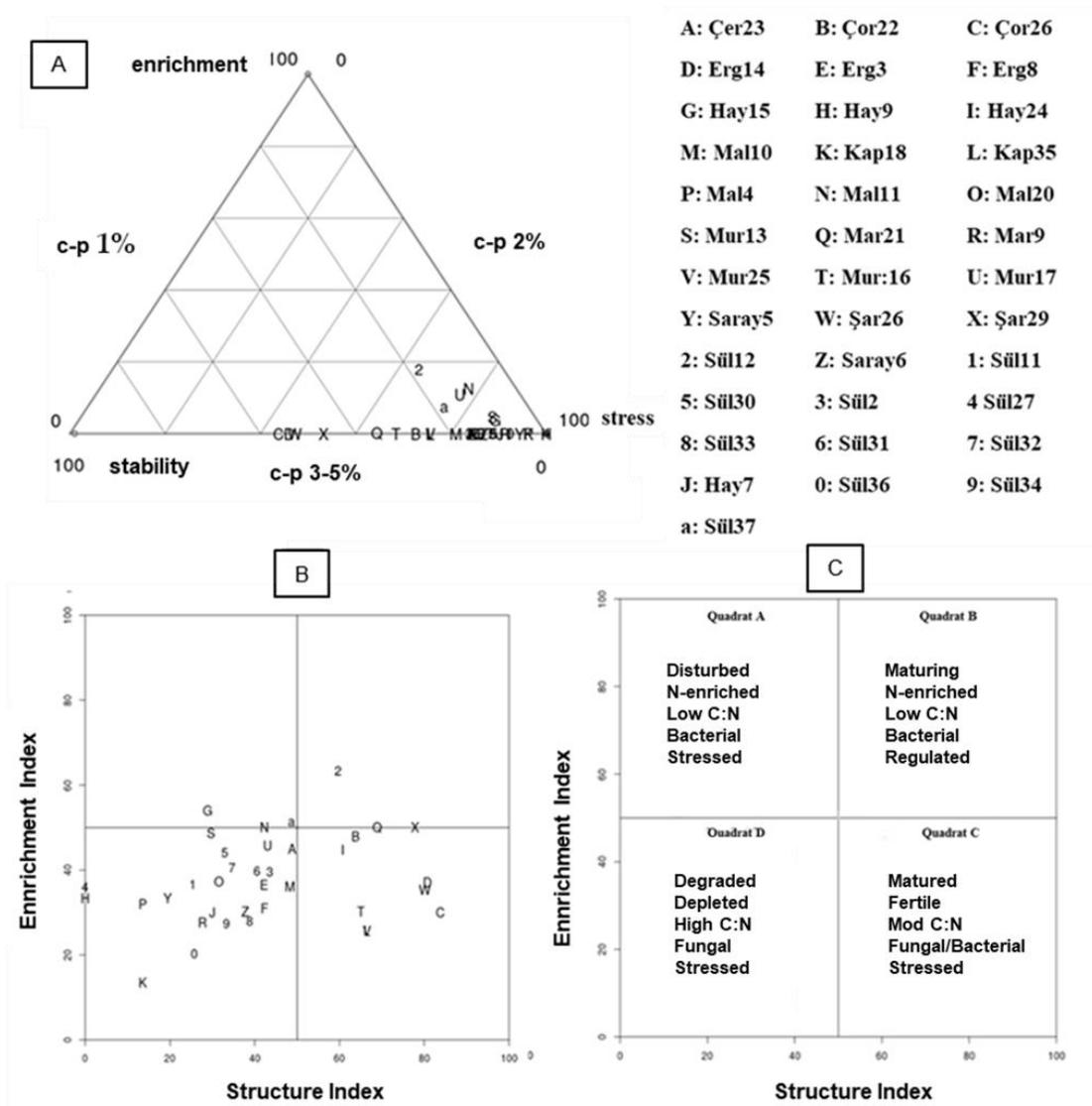


Figure 10. Channel (CI), Basal (BI), Enrichment (EI), and Structure (SI) Indices of nematode diversity of 37 sunflower fields surveyed in Tekirdağ.

The c-p triangle plot prepared using c-p values of nematodes revealed that most of the nematodes in sunflower fields in Tekirdağ belonged to the c-p2 and c-p3-5 classes. As the soil food web scheme was examined, the difference in the soil-food web in surveyed sunflower fields in Tekirdağ can be seen. The fields were replaced in four quadrats. Twenty-three (62.1 %) fields are located in the D quadrat. For instance, fields in Süleymanpasa, Saray, Çerkezkoç, Malkara, and Marmara Ereğlisi were placed in group D. Based on the explanations of the scheme of Ferris et al. (2001), the soil conditions of 23 fields in this quadrat were considered degraded, and stressed, containing high levels of C: N and the fungal decomposition is dominant. In this study, the species and population numbers of nematodes feeding on fungi and bacteria have come to the fore in the fields located in the D quadrat, confirming this. Fungivore *D. geraerti*, *A. avenae*, and *Aphelenchoides* species have been detected commonly in these fields. However, seven (18.9 %) fields in quadrat C were undisturbed and fertile, containing more bacterivore and fungivore species. On the contrary, fields in quadrat A (Hay15, Mal 11, Sül37) and quadrat B (Mar21, Sül12, Şar29) had strongly or slightly disturbed soils with N-enriched characteristics. In these soils, bacterivore nematodes were dominant (Figure 11).



**Figure 11.** A) c-p triangle representing soil status of sunflower fields based on c-p group; B) the metabolic footprint of surveyed sunflower fields; C) structure and enrichment status of the soil food web from sunflower fields.

## Discussion

Nematodes in Phylum Nematoda are one of the most abundant organisms in the soil ecosystem. Their number of genera and species is also higher than other pests. Ritz et al. (2009) identified 183 biological indicators to assess soil conditions, including parameters such as the biodiversity indices of nematodes, number of taxa, and abundance. Since at least one genus of nematodes is present in every soil, they can be used to measure the state of the structure of the soil food web (Pothula et al., 2022).

In the study carried out in the sunflower fields in Tekirdağ province, free-living bacterivore and fungivore nematodes, which adapt well to different soil conditions, were commonly distributed. At least one genus of these nematodes was detected in each sunflower field. Accordingly, *Acrobeloides*, *Cephalobus*, and *Aphelenchus*, a highly occurred genera in Tekirdağ, were reported to be common in agricultural areas in several countries as well as in Türkiye (Bongers, 1990; Yıldız, 2007; Yıldız et al., 2017; Çetintaş, 2017). Identified free-living soil nematodes have been reported to have different contributions to organic material decomposition and structure improvement in soil. Nematodes restore minerals and other nutrients to the soil, which they decompose from bacteria, and other substrates by feeding. This affects the soil's amount of elements like N, P, K, and Mg (Yeates et al., 1993; Yadaw et al., 2018). For instance, *Tripyla* and *Clarkus*, which have a prevalence of 8.1% and 2.4% in sunflower fields in Tekirdağ, respectively, feed on the soil microbes and lead to N release. There is a balance in fertile, well-developed soils in the populations of these nematodes, which are in different trophic groups and have different functions (Tahat et al., 2020).

In addition to free-living species, several plant parasitic nematodes were present in soil samples in Tekirdağ. According to the feeding strategy, nematodes were grouped as ectoparasites, semi-endoparasites, and migratory endoparasites. Among ectoparasites *Boleodorus*, *Filenchus*, *Paratylenchus*, and *Sakia* feed on plant root hairs, and feeding these species with several fungi has been mentioned in several studies (Yeates, 1993). Ectoparasites, detected in 94.6% of all examined areas, were found to be the most common in survey areas. Endoparasites were found only in 20 samples. Of the recovered species, *F. filiformis* Ebsary, 1991, *G. tesellatus* (Goodey, 1952) Brzeski, 1991, *T. annulatus* Cobb, 1913 and *P. zaeae* Graham, 1951 were the most widespread. Commonly detected nematodes were weak plant-parasitic species that did not cause plant damage or yield loss, while populations of all species remained below the economic damage threshold. For example, *P. zea* has been reported as a poor host of sunflower genotypes, and the population of the nematode was found low (33/250 cm<sup>3</sup> soil) in previous studies. For this reason, rotation with sunflower is recommended in the control of *P. zaeae*, which is very harmful to corn plants (Bolton & Waele, 1989). Likewise, in a previous study dating back to 2001 by Kepenekçi, 19 plant parasitic nematode species were identified in sunflower fields in Türkiye, and as in our study, *G. tesellatus*, *P. zaeae*, and *C. allacinatus* were the most common species in survey locations. Unlike the study of Kepenekçi (2001), in our study, species from plant-parasitic *B. thylactus*, *D. dipsaci*, *G. brevidens*, and *G. tesellatus*, *L. elongatus*, *P. acristylus*, *P. hilarulus*, and *X. pachtaicum* were found for the first time in sunflower fields in Tekirdağ. Among them, *L. elongatus* has been reported as a vector of viruses in some plants, but no data were found about its damage in sunflowers. (Brown et al., 1995). *D. dipsaci*, can parasitize more than 450 plants, especially onions and garlic (Greco et al., 1993).

The soil conditions of surveyed sunflower fields in Tekirdağ were estimated based on identified soil nematode diversity and related indices. In most of the fields calculated, MI and MI2-5 values were under 3. Values between 2-3 represent disturbance, stress, and a weak food web structure, and an MI of more than three indicates quality structured soil with a good food web (Du Preez et al., 2022). As a matter of fact, 21 fields we sampled in Tekirdağ were found to have degraded structures. The Channel index (BI) value of more than 50 in 34 fields indicates organic matter decomposition, mainly with fungivore nematodes (Sánchez-Moreno & Ferris (2007). Indeed, in sunflower fields, fungivore nematodes such as *Aphelenchus*, *Aphelenchoides*, and *Ditylenchus* genus dominate soils with a CI value of 50 and above. Meanwhile,

according to Sánchez-Moreno & Ferris (2007), a higher Enrichment index (EI) indicates the bacterial decomposition of organic material, and a value of around 35 was found in 24 fields. Again, Structure and Basal indices were found to be less than 60. Even if the SI value was 0 in two fields, assuming that an index closes to 30 or low is considered a disrupted food web and high values as improved. In soils with higher EI and lower SI values, fertility is high. In contrast, soils with higher SI values are more suppressive to opportunistic species (Sánchez-Moreno & Ferris, 2018).

According to the c-p triangle, most of the nematodes in survey areas belonged to the c-p2 group. Short lifecycles and high reproduction characterize the identified c-p2 and c-p3 nematodes (Zhou et al., 2023). The c-p 1 group nematodes were low in numbers and not highly distributed. The abundance of these nematodes in the soil can be used to predict organic matter content and nitrogen mineralization in the soil food web. Since soils with low c-p1 nematode species and individuals are characterized as poor in organic matter (Ciobanu & Popovici, 2017). According to scheme of Ferris (2001), surveyed sunflower fields in Tekirdağ were generally located in Quadrats C and D in the soil food web scheme. Fungivores were common in Quadrat D and fungivores/bacterivores in Quadrat C. In these soil diversity conditions C: N amount is quite similar to the food source, and nearly half of C is consumed mostly by fungivores in respiration (Ferris, 1998). Additionally, bacterivore and fungivore nematodes increase the nitrogen content in the soil (Ferris, 2010). After the nematodes feed on organic residues, CO<sub>2</sub> and NH<sub>4</sub> are released, thus increasing soil fertility. The nematodes retain 16% of the nitrogen, and 84% is released into the soil. Of the released N bacterivore nematodes consume much more for survival (Khanum & Mahmood, 2021).

The present study provides information on the nematode community status and the relationship between the present nematode species, soil structure, and soil food web in sunflower fields in Tekirdağ. Knowing the nematode community structure in agroecosystems will promote the development of new policies to protect soil health.

## References

- Akkaya, M. R., 2018. Prediction of fatty acid composition of sunflower seeds by near-infrared reflectance spectroscopy. *Journal of Food Science and Technology*, 55 (6): 2318-2325.
- Anderson, C. R., 2000. *Nematode Parasites of Vertebrates: Their Development and Transmission*. CABI Publishing, England, 633 pp.
- Bolton, C. & D. De Waele, 1989. Host suitability of sunflower hybrids to *Pratylenchus zaeae*. *Journal of Nematology*, 21 (4S): 682-685.
- Bongers, R., 1990. The maturity index: An ecological measure of environmental disturbance based on nematode species composition. *Oecologia*, 83 (1): 14-19.
- Brown, D. J. F., W. M. Robinson & D. L. Trudgill, 1995. Transmission of viruses by plant nematodes. *Annual Review of Phytopathology*, 33: 223-249.
- Brzeski, M. W., 1991. Review of the genus *Ditylenchus* Filipjev, 1936 (Nematoda: Anguinidae). *Revue Nematology*, 14 (1): 9-59.
- Castillo, P. & N. Vovlas, 2005. *Bionomics and Identification of Rotylenchus Species*. Nematology Monographs and Perspectives, 3, Leiden, The Netherlands: Brill Academic Publishers, Leiden-Boston, USA, 377 pp.
- Ciobanu, M. & I. Popovici, 2017. Structural and functional diversity of nematode fauna associated with habitats located in the Natura 2000 site Apuseni (Romania). *Russian Journal of Nematology*, 25 (1): 23-36.
- Çetintaş, R., 2017. Kahramanmaraş Sütçü İmam Üniversitesi meyve bahçesindeki nematodlar ve trofik grupları. *KSÜ Doğa Bilimleri Dergisi*, 13 (1): 34-41 (in Turkish with abstract in English).
- Doran, J. W. & M. R. Zeiss, 2000. Soil health and sustainability: managing the biotic component of soil quality. *Applied Soil Ecology*, 15: 3-11.

- Du Preez, G., M. Daneel, R.G.M. De Goede, M. J. Du Toit, H. Ferris, H. Fourie, S. A. Geisen, T. Kakouli-Duarte, G. W. Korthals, S. Sanchez-Moreno & J. H. Schmidt, 2022. Nematode-based indices in soil ecology: Application, utility and future directions. *Soil Biology and Biochemistry*, 169: 108640 (1-14).
- Ferris, H., R. C. Venette, H. R. van der Meulen & S. S. Lau, 1998. Nitrogen mineralization by bacterial-feeding nematodes: Verification and measurement. *Plant and Soil*, 203 (2): 159-171.
- Ferris, H., T. Bongers & R. G. M. de Goede, 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. *Applied Soil Ecology*, 18 (1): 13-29.
- Ferris, H. & T. Bongers, 2009. "Indices Developed Specifically for Analysis of Nematode Assemblages, 124-145". In: *Nematodes as Environmental Indicators*. (Eds. M.J. Wilson & T. Kakouli Duarte). CABI Publishing, England, 326 pp.
- Ferris, H., 2010. Contribution of nematodes to the structure and function of the soil food web. *Journal of Nematology*, 42 (1): 63-67.
- Fourie, H., A. Donald, S. Steenkamp & D. De Waele. 2017. "Nematode Pests of Leguminous and Oilseed Crops, 201-230". In: *Nematology in South Africa: A View from the 21st Century*. (Eds. H. Fourie, V. Spaull, R., Jones, M., Daneel & D. De Waele) Springer, Cham, Switzerland, 569 pp.
- Geraert, E. & D. J. Raski, 1987. A reappraisal of *Tylenchina* (Nemata). *Revue Nematologia*, 10 (2): 143-161.
- Greco N., 1993. Epidemiology and management of *Ditylenchus dipsaci* on vegetable crops in Southern Italy. Institute of Nematologia Agraria, Bari, Italy. *Nematropica*, 23 (2): 247-251.
- Handoo, Z. A., A. Khan & S. Islam, 2007. A key and diagnostic compendium to the species of the genus *Merlinius* Siddiqi, 1970 (Nematoda: Tylenchida) with description of *Merlinius khuzdarensis* n. sp. associated with date palm. *Nematology*, 9 (2): 251-260.
- Hodda, M., 2011. Phylum Nematoda Cobb, 1932. *Zootaxa*, 3148 (1): 63-95.
- Kepenekçi, İ., 2001. Taxonomic investigations on the species of Tylenchida (Nematoda) in sunflower (*Helianthus annuus* L.) fields in Marmara Region. *Plant Protection Bulletin*, 41 (3): 101-134.
- Khanum, T. A. & N. Mehmood, 2021. Bacterial feeding nematodes use for nitrogen mineralization and plant production. *Acta Scientific Pharmaceutical Sciences*, 5 (5): 02-06.
- Loof, P. A. A. & M. Luc, 1993. A revised polytomous key for the identification of species of the genus *Xiphinema* Cobb, 1913 (Nematoda: Longidoridae) with exclusion of the *X. americanum*-group. *Systematic Parasitology*, 24 (3): 185-189.
- Neher, D. A. & B. J. Darby, 2009. "General Community Indices That Can Be Used for Analysis of Nematode Assemblages, 107-123". *Nematodes as Environmental Indicators* (Eds. M. Wilson & T. Kakouli-Duarte) CABI Publishing, England, 338 pp.
- Pielou, E. C., 1966. The measurement of diversity in different types of biological collections. *Journal of Theoretical Biology*, 13: 131-144.
- Pothula, S. K., G. Phillips & C. E. Bernard. 2022. Increasing levels of physical disturbance affect soil nematode community composition in a previously undisturbed ecosystem. *Journal of Nematology*, 54 (1): 1-55.
- Ritz, K., H. I. J. Black, C. D. Campbell, J. A. Harris & C. Wood, 2009. Selecting the biological indicators for monitoring soils: A framework for balancing scientific and technical opinion to assist policy development. *Ecological Indicators*, 9 (6): 1212-1221.
- Sánchez-Moreno, S. & H. Ferris, 2007. Suppressive service of the soil food web: Effects of environmental management. *Agriculture, Ecosystem and Environment*, 119 (1-2): 75-87.
- Sanchez-Moreno, S. & H. Ferris, 2018. "Nematode Ecology and Soil Health, 62-86". In: *Plant-Parasitic Nematodes in Subtropical; and Tropical Agriculture* (Eds. R. A. Sikora, D. Coyne, J. Hallmann & P. Timper) CABI Publishing, England, 888 pp.
- Seinhorst, J. W., 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica*, 4 (1): 67-69.
- Sieriebriennikov, B., H. Ferris & R. G. M. de Goede, 2014. NINJA: An automated calculation system for nematode-based biological monitoring. *European Journal of Soil Biology*, 61: 90-93.

- Tahat, M. M., M. K., Alananbeh, Y. A. Othman & D. I. Leskovar, 2020. Soil health and sustainable agriculture. *Sustainability*, 12 (12): 4859 (1-26).
- Yadav, S., J. Patil & R. Kanwar, 2018. The role of free-living nematode population in the organic matter recycling. *International Journal of Current Microbiology and Applied Sciences*, 7 (6): 2726-2734.
- Yağmur, B., B. Okur & N. Okur, 2021. The effect of the applications of humic acid and potassium on content of nutrient and oil of seed and yield of sunflower. *ISPEC Journal of Agricultural Sciences*, 5 (1): 156-167.
- Yeates, G. W., T. D. Bongers, R. G. M. de Goede, D. W. Freckman & S. S. Georgieva, 1993. Feeding habits in soil nematode families and genera - an outline for soil ecologists. *Journal of Nematology*, 25 (3): 315-331.
- Yıldız, Ş., 2007. Şanlıurfa İli Nematod Faunası ve Biyoçeşitliliği Üzerine Araştırmalar. Ç.Ü. Fen Bilimleri Enstitüsü, Bitki Koruma Anabilim Dalı (Unpublished) PhD Thesis, 102 pp (in Turkish with abstract in English).
- Yıldız, Ş., M. İmren & N. Duman, 2017. Nematode biodiversity in cereal growing areas of Bolu, Türkiye. *Turkish Journal of Entomology*, 41 (2): 159-168.
- Zhou, Y., H. Zheng, D. Gao & J. Zhao, 2023. Population dynamics and feeding preferences of three bacterial-feeding nematodes on different bacteria species. *Agronomy*, 13 (7): 1808.