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Nematode biodiversity in cereal growing areas of Bolu, Turkey

Bolu ili buğday alanlarında nematod biyoçeşitliliği

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Summary

Agricultural fields usually contain both plant parasitic and beneficial free-living nematodes. Plant parasitic nematodes have a negative impact on plant productivity and quality traits, whereas free-living nematodes can have beneficial effects on the agricultural soils health. This study was conducted to investigate the diversity of both plant parasitic nematodes and beneficial free-living nematode. Soil samples were collected in 2015 from wheat growing areas of Bolu Province to investigate soil nematode diversity. Bolu Province was divided into two sub-areas according to elevational. Forty-three nematode taxa were found in the samples; 13 taxa plant parasites, 12 bacterivores, 4 fungivores, 10 omnivores and 4 predators. Relative distribution of nematode trophic groups indicated a bacterivore dominated community, followed by plant parasites, fungivores and omnivores; predators represented only a small proportion. Free-living nematodes, especially bacterivores of basal fauna members and large bodied omnivore members were in good condition in abundance and diversity. General community and maturity indices were calculated for each sample and for the two sub-areas. They produced narrow range values with no significant differences. The study revealed that soil food web in wheat growing areas of the province was in fair to good condition based on nematode diversity.

Keywords: Bioindicator, diversity, nematodes, trophic groups, wheat

Özet

Tarım arazileri hem bitki paraziti nematodlar hem de serbest yaşayan yararlı nematodları birlikte barındırmaktadır. Bitki paraziti nematodlar bitki kalite parametreleri üzerinde olumsuz etkiler doğururken, serbest yaşayan nematodlar ise yaşamsal faaliyetleri sonucu olumlu etkilere sahiptir. Dolayısıyla, çalışma hem bitki paraziti nematodların çeşitlilik yapısı hem de yararlı nematodların çeşitlilik yapısını incelemek üzere yürütülmüştür. Bu amaçla, 2015 yılında Bolu ili buğday alanlarından toprak örnekleri toplanmıştır. Bolu ili rakım farklılıkları göz önüne alınarak iki farklı alt-bölgeye ayrılmıştır. Çalışma alanında, 13 bitki paraziti, 12 bakterivor, 4 fungivor, 4 predatör ve 10 omnivor gruba ait olmak üzere, toplam 43 nematod taksonu tespit edilmiştir. Nematod trofik gruplarının oransal dağılımlarına göre, bakterivorların baskın olduğu bir kommunite yapısına sahip olduğunu, bunu bitki parazitleri, fungivorlar, omnivorlar izlemiş, predatörler ise oldukça düşük bir orana sahip olduğu saptanmıştır. Serbest yaşayan nematodlardan, özellikle temel fauna bileşeni olan bakterivor ve iri cüsseli omnivore nematodların hem çeşitlilik hem de yoğunluk bakımından iyi durumda olduğu görülmüştür. Genel kommunite ve nematode maturity indisleri her iki alt-bölgeden alınan toprak örnekleri için hesaplanmış, indis değerleri arasında ise istatistikî olarak bir farklılığın görülmediği, dar bir aralıkta dağıldığı saptanmıştır. Çalışma sonuçlarına göre, nematode biyoçeşitlilik yapısına dayanılarak elde edilen bilgiler ışığında, Bolu ili buğday alanlarındaki toprak besin ağı orta ve iyi dereceler arasında yer aldığı sonucuna varılmıştır.

Anahtar sözcükler: Biyoindikatör, çeşitlilik, nematodlar, trofik gruplar, buğday

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Introduction

Soil contains a relatively large number of different groups of organisms that maintain ecological services through their physical and metabolic activities. Some of the ecological functions of soil organisms are decomposition of organic matter from plant and animals, cycling of minerals and nutrients, redistribution of minerals and nutrients, reservoirs of minerals and nutrients in their bodies, sequestration of carbon, detoxification of pollutants (mostly by micro fauna), regulation of soil structure, community self-regulation and biological regulation or suppression of pest species (Ferris, 2016). The diversity, abundance and functions of soil biota in the soil food web determine the quality of the services or so called soil health.

Nematodes are one of the most abundant and diverse microscopic animals in the soil environment and they are represented at different soil food-web levels interacting with soil biota in multiple ways (Freckman & Ettema, 1993; Ritz & Trudgill; 1999; Ferris et al., 2001; De Deyn et al., 2004; Viketoft et al., 2011). Nematodes are known to be among the most taxa rich organismal group on earth, having with an estimated 500 000 to 1 000 000 species. Only about 20 000 species have been described, and the systematic literature is widely dispersed (Hodda, 2007).

To date, the most extensively studied group of nematodes have been the agricultural pest nematodes that cause economic loses by damaging plants. However, a vast range of ecologically beneficial free-living nematodes are also present in the same soil environment that is inhabited by plant parasitic nematodes. Nematodes are usually divided in to five trophic groups including plant parasitic (herbivores), bacterial feeding (bacterivores), fungal feeding (fungivores), nematodes that feed on other nematodes (predators) and nematodes that can feed on plants, fungi, bacteria and other nematodes (omnivores). These groups are associated with their food by examining the morphology of their feeding apertures (Yeates et al., 1993; Bongers & Bongers, 1998; Neher, 2001).

Nematodes live in water-filled soil pores exposed to physical, chemical and biological changes in soil conditions that affect them and change their composition. Their dynamic community composition, long life and their ease of extraction, examination and allocations to trophic groups have made them one of the most preferred indicators of soil biodiversity around the world over the last two decades (Bongers, 1990; Yeates, 2003; Mulder et al., 2005). It is accepted that a healthy soil should contain an abundant and a diverse community of free-living nematodes but less abundant and less diverse composition of plant parasitic nematodes.

The health of a soil ecosystem can be estimated by examining the diversity and abundance of nematodes. As proposed by Bongers (1990), nematode communities have been examined by using a specifically developed maturity index (MI) and the generalist community diversity indices.

Little information is available on nematodes in agricultural soils of Bolu Province. The most recent and the extensive study on the subject was that of Imren et al. (2015), which determined the occurrence of the plant parasitic nematodes associated with cereal-production areas of Bolu Province. However, there has been no comprehensive study of nematode communities including free-living species in the region. Since the nematode assemblage of every region tend to change with the variations in ecosystem components, geographical differences and climate, locally focused studies are valuable to assess nematode biodiversity and its consequences.

Through this study, it is expected that the baseline data will be provided for the future nematological studies in this area. The objective of this study was to investigate the status of the nematode community composition in relation to soil conditions in wheat growing areas of Bolu Province, Turkey.

Material and Methods

Study site and sampling

Bolu Province, covering about 1% of the total land area of Turkey, is located in the Western Black Sea Region with an 8.276 km^2 land area, of which, 55% is forests and 18% cultivated agricultural land. The climate in the area is characterized by wet cold winters and hot dry summers. Average annual temperature and annual precipitation is estimated as 10.9°C and 573 mm, respectively. Due to the geographical and climatic features, agricultural production in the province is limited to a few main crops including wheat, potato and sugar beet. Wheat is the most commonly grown crop of the province because of its best suited to the region's precipitation (Anonymous, 2016).

In order to investigate nematode community, structure a total of 26 soil samples were collected in wheat growing areas of Bolu Province from June to August 2015. Sampling sites were categorized into two sub-areas to compare community variables. Area 1 was around Bolu City and Area 2 included Gerede, Dörtdivan and Yeniçağa Districts. The districts in Area 2 were at least 7-10 km apart from each other and, and have similar elevation, soil type and topographical features. The two study areas were about 60 km apart separated by mountains, therefore they had significant differences in elevation (726 and 1475 m for Area 1 and 2, respectively).

Each soil sample was collected from a visually determined section (0.5 ha) of a field from 10-20 cm depth using a 25-mm soil auger. Each sample consisted of 10-15 soil cores totaling approximately 1-1.5 kg in weight. Samples were placed in labeled plastic bags and transported to the laboratory in insulated boxes.

Nematode extraction

Nematodes were extracted from individual soil samples by using a modified Baermen funnel technique (Whitehead & Hemming, 1965). Subsamples (100 g) of homogenized soil were placed in 15-cm plastic Petri dishes and water added until the sample was covered. After 48 h, nematodes that had migrated into the water in the lower part of dish, which was then transferred to 100 ml measuring cylindrical and the nematodes allowed settle for 8 h. The volume was then reduced to 15 ml by discarding excessive water from the Petri dish. Finally, nematode containing suspension were rinsed into 15 ml and transferred to plastic Falcon tubes for storage at 4°C until assessed.

Identification and grouping of nematodes

The water was removed from the tubes by pastor pipette concentrating the nematodes in 1 ml. For counting, the nematode suspension was mixed with a micropipette and 100 µl transferred to a glass slide for examination under light microscope at 100X magnification. Nematodes were identified mostly to genera; however, four taxa were only identified to family, and they were also allocated to trophic group based on morphological structure of mouth parts specialization for feeding habit and diet.

Nematode community analyses

Nematode community structure was analyzed in relation to the site differences especially the distance between the sampling sites and two locations in wheat fields (alpha and beta diversity). Diversity indices, the summarized numerical expression of many taxa have been calculated to assess the diversity of nematode communities in soil. Generalist diversity indices such as the number of taxa (SR), the Shannon diversity index (H'), the Shannon evenness index (E), Hills N1 and N2, Simpsons D; MI family and trophic diversity [bacterivore to fungivore ratio, Ba/(Ba+Fu)] indices developed specifically for nematode biodiversity and trophic diversity indices were computed as described in (Neher & Darby, 2009). Colonizers-persisters (C-P) groups, based on the r-K reproductive strategy, were placed on a 1-5 scale based on their reproductive capacities (Bongers, 1990).

Statistical analysis

Diversity indices were calculated and log transformation applied to all nematode abundance data to examine any significance between the locations prior to performing ANOVA and T-test in SPSS at $P \leq 0.05$ significance level.

Results and Discussion

Nematode faunal structure

Forty-three nematode taxa were found in the samples; 13 plant taxa plant parasities, 12 bacterivores, 4 fungivores, 10 omnivores and 4 predators. The abundance of nematodes at taxon level ranged from 3 to 390 (individuals/100 g soil) and abundance among the samples ranged from 531 to 2145 (individuals/100 g soil). The list of nematode genera, their abundance and frequencies are given in in Table 1.

Table 1. Nematode abundance (average number of individuals/100 g of soil), frequency (percent occurrence in samples) and associated colonizers-persisters (C-P) scale values of the nematode genera

Nematodes	Area 1		Area 2		C-P value
	Abundance	Occurence (%)	Abundance	Occurence (%)	
Plant Parasitic					
Filenchus Andrassy, 1954	74.4±10.6	68.8	60.4±12.5	51.5	2
Helicotylenchus Steiner, 1945	6.9±2.5	43.8	13.2±3.8	30.9	3
Heterodera Schmidt, 1871	0.6±0.6	6.3	1.2±0.9	10.3	3
Merlinius Siddiqi, 1970	38.8±7.3	68.8	21.7±5.6	61.8	3
Pratylenchoides Winslovv, 1958	26.3±5.5	50	5.2±1.2	20.6	3
Paratylenchus Micoletzky, 1922	18.8±4.7	43.8	3.1±2.0	20.6	2
Paratrophurus Arias, 1970	0.6±0.6	6.3	0±0.0	0	3
Pratylenchus Filipjev, 1936	20.6±5.5	62.5	6.5±2.9	41.2	3
Psilenchus De MAN, 1921	0.3±0.3	6.3	0.5±0.5	10.3	2
Rotylenchus Linford &Oliveira, 1940	0.3±0.3	6.3	0±0.0	0	3
Trophurus Loof, 1956	0,1±0.0	6.3	0±0.0	0	3
Tylenchorhynchus Cobb, 1913	48.8±5.8	68.8	39.3±7.2	61.8	3
Tylenchus Bastian, 1865	22.5±5.1	31.3	46.4±5.8	61.8	2
Bacterivores					
Rhabditis Dujardin, 1845	28.1±7.9	68.8	31.1±9.2	92.7	1
Monhysteridae	77.5±13	93.8	83.3±11.4	100	2
Cephalobus Bastian, 1865	105.6±9.4	100	76.7±11.3	92.7	2
Eucephalobus Steiner 1936	91.3±7.3	93.8	110.2±11.2	100	2
Acrobeloides Thorne, 1937	105.6±16.2	100	51.8±17.6	92.7	2
Achromadora Cobb, 1913	0±0.0	0	4.3±2.5	20.6	3
Cervidellus Thorne, 1937	2.5±1.4	18.8	0±0.0	0	2
Alaimidae	1.3±1.2	6.3	0±0.0	0	4
Alaimus de Man, 1880	5.0±2.0	37.5	3.5±1.4	30.9	4
Wilsonema Cobb, 1913	3.8±1.7	25	1.1±0.9	10.3	2
Plectus Bastian, 1865	68.8±8.8	87.5	95.6±12.5	100	2
Prismatolaimus de Man, 1880	0.6±0.6	6.3	1.0±0.9	10.3	3

Table 1. (Continued)

Nematodes	Area 1		Area 2		C-P value
	Abundance	Occurence (%)	Abundance	Occurence (%)	
Fungivores					
Aphelenchoides Fischer, 1894	107.5±7.3	100	79.6±7.4	100	2
Aphelenchus Bastian, 1865	63.8±9.3	93.8	37.2±10.7	72.1	
Ditylenchus Filipjev, 1936	68.8±11.7	93.8	37.3±7.3	72.1	2
Tylencholaimus de Man, 1876	3.1±2.1	12.5	0±0.0	0	4
Predators					
Mononchus Bastian, 1865	6.3±3.4	25	10.2±2.4	72.1	4
Clarkus Jairajpuri, 1970	0±0.0	0	1.1±0.9	10.3	4
Seinura Fuchs, 1931	0±0.0	0	1.3±0.6	20.6	4
Tripyla Bastian, 1865	0.7±0.0	7.2	0±0.0	0	3
Omnivores					
Dorylaimidae	2.5±2.4	6.3	0±0.0	0	4
Actinolaimidae	7.5±3.6	25	1.8±0.9	10.3	5
Dorylaimus Dujardin, 1845	1.3±1.2	6.3	0±0.0	0	4
Campydora Cobb, 1920	1.3±1.2	6.3	0±0.0	0	4
Mesodorylaimus Andrassy, 1959	0.3±0.3	6.3	0.5±0.5	10.3	4
Prodorylaimus Andrassy, 1959	11.9±4.0	50	13.6±2.8	82.4	4
Aporcelaimus Thorne & Swanger, 1936	4.4±4.2	6.3	11.3±4.3	20.6	5
Aporcelaimellus Heyns, 1965	38.8±5.3	87.5	46.0±6.0	82.4	5
Eudorylaimus Andrassy, 1959	5.6±5.4	6.3	0±0.0	0	4
Belondira Thorne, 1939	21.3±8.5	56.3	38.3±9.9	61.8	5

Although the study area was divided into sub-areas based on the altitude differences, the results revealed no significant differences on nematode community parameters between the sub-areas, such as SR, abundance and occurrence in the samples. However, previous studies from various parts of the world have indicated that altitude was an important parameter in shaping soil nematode communities, regarding characteristics like distribution, species richness and abundance (Hoschitz & Kaufmann, 2004; Háněl & Čerevková, 2010; Zhang et al., 2012; Tsiafouli et al., 2017).

The maximum plant parasitic nematode abundance in the study was 210 individuals/100 g soil. Fourteen genera of plant parasitic nematodes were identified. Most abundant and common genera were *Filenchus, Tylenchorhynchus, Merlinius, Pratylenchus* and *Tylenchus*, in that order (Table 2). There was no significant difference between the two areas in terms of the abundances of plant parasitic nematodes.

Abundances of plant parasitic nematodes in our study were not as high as those of the study by Bao & Neher (2011), in which the abundance of plant parasitic nematodes ranged 719 to 3,578 individuals/100 cm^3 of dry soil from Vermont, USA. Bulluck et al. (2002) also reported that plant parasitic nematodes were the most abundant group and ranged from 83 to 88% of the total community. However, Akyazi et al., 2014 reported lower abundance values of plant parasitic nematodes from Ordu Province in Central Black Sea Region of Turkey. Low plant parasitic nematode densities in agricultural soils are considered a good indicator of soil health indicating relative minimal damage and consequent less economic loses on crops.

Table 2. Nematode maturity indices and general community indices (Neher & Darby, 2009) for two wheat growing areas in Bolu Province, Turkey

Indices	Area 1	Area 2
SR	17.13±05	16.4±0.1
PPI	2.51±0.0	2.38±0.1
FI (PPI/MI)	1.07±0.0	1.01±0.1
ΣMI	1.74±0.1	1.84±0.1
MI	2.37±0.1	2.41±0.1
ΣMI 2-5	2.69±0.1	2.82±0.1
MI 2-5	2.40±0.1	2.47±0.1
Ba/(Ba+Fu)	0.66±0.0	0.73±0.0
H' (Shannon)	2.52±0.0	2.47±0.1
E (H/Hmax)	0.8±0.0	0.8±0.0

Bacterivores consisted of 11 genera in one family and were the dominant nematode fauna in both areas, with no significant difference in abundance between the areas. The genera *Cephalobus, Acrobeloides, Eucephalobus* and family Monhysteridae, in that order, were the most abundant and the widespread bacterivores (Table 2). These findings are consistent with those of Ettema & Bongers (1993), Neher (1999) and Yeates (2003), who reported that Cephalobidae and Rhabditidae were the most abundant families in agricultural soils. These nematodes are the basal faunal members that are stress tolerant and long-lived groups in the C-P 2 group (Bongers & Bongers, 1998). Therefore, they are fairly common in various agroecosystems throughout the seasons even if other free-living nematode groups are missing.

In both study areas, Rhabditids were found to be widespread (with 28 and 31 individuals/100 g soil in Area 1 and 2, respectively) but their abundance was not significantly different. Their presence is considered to be an indication of the presence of decomposing organic matter or recent chemical fertilization. Given their opportunistic nature, rapidly increasing or decreasing in number with changes in nutrient availability (especially nitrogen), and their short life cycle, they placed in the C-P 1 group (Ferris, 2001). Ferris et al. (2004) reported that Rhabditid nematodes tended to have low population numbers when soil organic matter was inadequate. Thus, their presence and wide distribution in the study areas suggest that wheat growing soils of Bolu Province contained decomposing organic matter when the samples were taken. However, this was not the case in wheat growing areas of Central and South Eastern Anatolian where Rhabditidae were relatively scarce (Şahin et al., 2009; Yildiz, 2012; Yıldız & Elekçioğlu, 2012). Relative low annual precipitation can reduce organic matter decomposition as a result of the slow activity of microfauna in soil (Laakso et al., 2000).

However, there have been various studies that report findings consistent with our results, supporting the view that bacterivore nematodes are the most abundant nematode trophic group in agricultural soils (Porazinska et al. 1999; Carmen & Zaborski, 2014; Tsiafouli et al. 2017).

Fungivores were represented by *Aphelenchoides, Ditylenchus, Aphelenchus* and *Tylencholaimus,* and their abundance listed in Table 2. The abundance and the distribution of *Aphelenchoides* was not significantly different between the study areas, however, *Aphelenchus* and *Ditylenchus* had a slightly lower abundance in Area 2 ($P \leq 0.05$). The fungivore trophic group was the third most abundant (Figure 1).

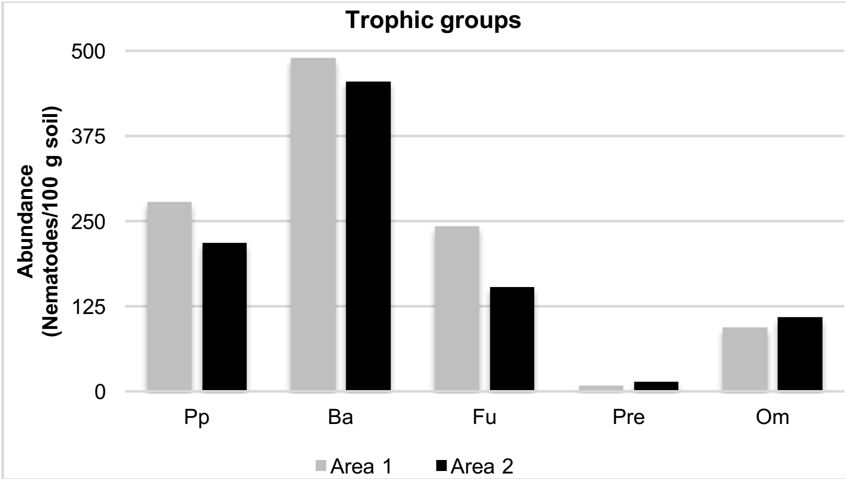

Figure 1. Trophic structure of nematode fauna in two wheat growing areas in Bolu Province, Turkey, based on abundance.

Fungivore nematodes feed on soil fungi that are important in the decomposition of high C:N ratio organic matter (Liang et al., 2009), such as dry wood or straw, thus the diversity and abundance of these nematodes is influenced by the soil organic matter quantity, sufficient soil humidity and soil temperature.

Predators were the least abundant and distributed trophic group in the study (Table 2, Figure 1). Since the predator species in a community occupy the narrow top portion of the trophic pyramid, it is expected that they well be detected in low numbers in a community. Predatory nematodes of C-P 5 have larger body, longer life cycle and extreme sensitivity to soil disturbances, such as heavy tillage in agricultural areas. Predatory nematodes are more likely to be abundant and diverse in undisturbed natural ecosystems, such as grasslands and pastures (Bongers & Bongers, 1998; Georgieva et al., 2002).

The omnivore group consisted of eight genera in two families in Area 1 and five genera and one family in Area 2. *Aporcelaimellus* and *Belondira* were the most abundant and the common nematodes in both areas. Other genera were present with in lower numbers (Table 2). Omnivorous nematodes have similar behavioral and structural patterns to those of predators in agroecosystem and undisturbed natural ecosystems due to their similar features and also being in the C-P 5 group (Bongers & Bongers, 1998). However, they differ from predatory nematodes in their broader diet. Due to their permeable cuticle, large bodies, long life and low fecundity, omnivores are considered to be the soil nematode group most sensitive to stress conditions (Shao et al., 2008).

Relative distribution of nematode trophic groups of the study areas indicated a bacterivore dominated community, followed by plant parasitic nematodes, then fungivores and omnivores, with predators the least abundant group (Figure 1).

Nematode diversity

C-P groups displayed a very similar distribution pattern in both areas (Figure 2). The results revealed that the differences between the areas, especially the altitude, did not have any significant influence on the abundance of the C-P groups. C-P groups of nematodes are based on the life history similarities, referred to the *r-K* strategist principles, to be able to condense information and to facilitate interpretations about these complex species rich nematode communities. Also, functional groups of nematodes can be considered as groups of species that have similar functions in ecosystem services even if they belong to distant taxonomic groups (Bongers & Bongers, 1998; Schratzberger et al., 2007).

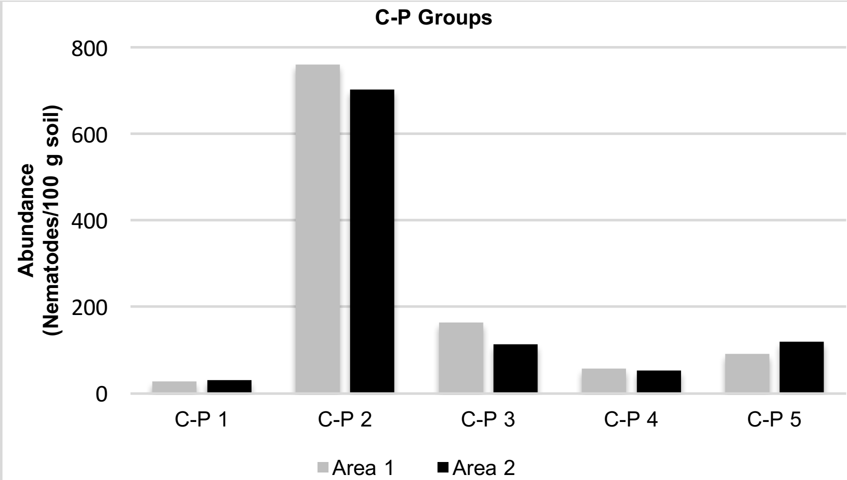

Figure 2. C-P group abundances of nematode communities in two wheat growing areas in Bolu Province, Turkey.

The findings of the study indicate that there was no significant difference in nematode community and maturity indices among the samples (alfa diversity) or between the areas (beta diversity). The average values of indices in both areas were: SR, 17 and 16; H', 2.52 and 2.47; E, 1.56 and 1.54; Ba/(Ba+Fu), 0.66 and 0.73; PPI, 2.51 and 2.38; MI, 2.38 and 2.41; MI 2-5 2.40 and 2.47 (Table 2).

The stability of the index values across the study area, either among the samples or between the sub-areas, suggest that nematode community structure also share common characteristics in these two areas and there was no obvious influencing factor detected over the nematodes in any of the sampled sites. This can be attributed to the commonalities in the use and history of the fields sampled. These fields had primarily non-irrigated, low intensive agricultural regimes that limit causative factors on land properties and ultimately nematode community structure.

To measure nematode community and soil environment interactions, general community indices and specifically developed maturity indices for nematode faunal analysis have been extensively used to obtain information and monitor soil condition from the seminal work of Bongers (1990) through to the latest study by Tsiafouli et al. (2017). Community indices are referred to assess SR, the relative abundances of the species and the evenness in a community H’, Simpsons index and Hills index. Whereas, MI family has been the most reliable tool to assess the degree of stress in soil due to its greater sensitivity (Li et al, 2005). Trophic group ratio indices such as bacterivore to fungivore ratios are preferred to assess impact of soil environment over the nematode community depending on the nematode community changes in the diversity and numbers (Neher & Darby, 2009).

In conclusion, a well-established mature nematode community is also considered to be good indicator of the wider soil biota and, ultimately, soil health. In Bolu Province, nematode fauna is dominated by bacterial-feeding, plant parasitic, fungal-feeding and omnivorous nematodes. Its nematode community is well balanced and diverse structure; consequently, indicating a healthy environment for soil biota and agricultural production.

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