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Distribution, frequency and occurrence of cereal nematodes on the Central Anatolian Plateau in Turkey and their relationship with soil physicochemical properties

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Summary – The distribution of important plant-parasitic and free-living nematodes in the cereal production areas of the Central Anatolian Plateau (CAP) of Turkey was investigated with systematic surveys. Two important plant-parasitic nematode groups were found widely distributed; cereal-cyst nematodes (78.3%) and root-lesion nematodes (42.6%). Cereal cyst nematodes (CCN) were identified as *Heterodera filipjevi* in 18 provinces. *Heterodera latipons* was found in only one province. *Pratylenchus thornei* and *P. neglectus* were the most widely distributed species of root-lesion nematodes. Other frequently recorded plant-parasitic nematodes belonged to the genera *Geocenamus* (52.4%), *Pratylenchoides* (35.6%), *Helicotylenchus* (29.7%) and *Paratylenchus* (19.2%). Konya on the southern CAP had a significantly high incidence of *P. neglectus* as well as free-living nematodes. The incidence of CCN was greatest in areas of sandy soils on the CAP, with densities of up to 95 cysts (100 g soil)$^{-1}$. Population densities of *Geocenamus*, *Pratylenchus* and *Pratylenchoides* were high in some locations. Soil physicochemical properties were investigated for their relationship to nematode distribution. There was a slight positive correlation of *P. thornei* and clay content; conversely, there was a significant negative correlation of *P. neglectus* with clay and a positive correlation with sand. Electrical conductivity (EC) was positively correlated with *P. neglectus*. Nematodes in the genera *Helicotylenchus*, *Paratylenchus*, *Trophurus* and *Tylenchorhynchus* were only recorded at low population densities in the sampled area. By contrast, nematodes in the genera *Aphelenchus*, *Aphelenchoides*, *Ditylenchus*, *Dorylaimus*, *Tylenchus* and bacterivorous genera had relatively high populations. Total free-living nematodes were positively correlated with EC and zinc (Zn) concentration. The Zn content of soil was generally at a level deficient for plant growth.

Keywords – barley, electrical conductivity, *Heterodera filipjevi*, *Heterodera latipons*, iron, nematode survey, organic matter, pH, *Pratylenchus* spp., texture, wheat, zinc.

Turkey is one of the ten largest wheat-producing countries in the world, with total production of 18 million tonnes (Anon., 2011a). Average grain yield is around 2.5 t ha^{-1} (Anon., 2011b). Cereal monoculture is practised under an annual fallow system. Plants are often under water stress (average annual rainfall 300-450 mm) and need limited supplementary irrigation (Anon., 2011c).

The soil of the Central Anatolian Plateau (CAP) and transitional zone is red brown, loamy clay, pH 6.8-8.3. The soil depth varies from very shallow (<20 cm) to deep (>90 cm) (Anon., 2011d). Soils are characterised by low organic matter (<1%) and available phosphorus, but they are rich in calcium and sufficient in potassium (Eyüpoglu *et al*., 1998). Over a range of 1028 soil samples

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investigated in Konya and Karaman provinces, 49.7% were deficient in iron (Fe) (<2.5 ppm) and 65.8% in zinc (Zn) (<0.5 ppm) (Isık *et al*., 1999). Boron toxicity is a well-known nutritional constraint for cereal culture in some regions of CAP (Gezgin *et al*., 2002), especially for wheat and barley grown in zinc deficient soils (Alkan *et al*., 1998). Zinc deficiency is mostly seen in high pH (>8), low organic matter (<1%) and loamy clay textured soils (Eyüpoglu *et al*., 1998).

Plant-parasitic nematodes are one of the important biotic constraints to cereal production in Turkey and yield losses of up to 50% due to nematodes are documented for wheat on CAP (Nicol *et al*., 2004). Cereal cyst nematodes were first recorded for Turkey in east Anatolia (Yüksel, 1973). *Heterodera filipjevi* Madzhidow, 1981, *H. latipons* Franklin, 1969 and *H. mani* Mathews occur frequently in CAP (Enneli *et al.*, 1994; Rumpenhorst *et al*., 1996; Oztürk *et al*., 1998; Abidou *et al*., 2005). *Pratylenchus crenatus* Loof, 1960, *P. fallax* Seinhorst, *P. neglectus* (Rensch, 1924), *P. penetrans* (Cobb) and *P. thornei* Sher & Allen, 1953 were reported in east and southeast Anatolia (Yüksel, 1974; Imren, 2007), *P. thornei* was also recorded in the Aegean and Thrace regions (Misirlioglu & Pehlivan, 2007).

Yüksel (1977) first recorded *Pratylenchoides alkani* Yüksel, 1977 and *P. erzurumensis* Yüksel, 1977 in the east Anatolian region. *Pratylenchoides laticauda* Braun & Loof was found in the south Mediterranean region (Elekcioglu, 1992, 1996) and *P. sheri* in the southeast Anatolian region (Imren, 2007). *Merlinius brevidens* (Allen) was found in the south Mediterranean (Elekcioglu, 1992, 1996), southeast Anatolian (Imren, 2007), Thrace and Aegean regions (Misirlioglu & Pehlivan, 2007). *Merlinius microdorus* (Geraert) was recorded from the south Mediterranean region (Elekcioglu, 1992, 1996).

Several authors have reported the relationships between soil characteristics, the distribution of nematodes and the severity of attacks by the pathogenic species. Sandy loam soils are the best for *H. avenae* development (Swarup & Sossa-Moss, 1990), and soybean cyst nematode population density was correlated positively with sandy soils and negatively with clay and silty soils (Avendano *et al*., 2004). By contrast, *P. thornei* develops well in heavy textured, loamy clay soils around the world (Nicol, 1991). Soil organic matter content has an indirect effect on soil nematode communities by increasing the residential microbial population and Rhabditidae are indicative of organic enrichment (Bongers, 1999). Vellidis *et al.* (2006) observed significantly high positive correlations between cereal yield and electrical conductivity (EC) and used EC for prediction of the nematode prone areas.

The pathogenicity of *H. sacchari* on rice was greater in pots containing sandy soil than in pots of loamy clay soil (Coyne & Plowright, 2000). Cyst and lesion nematodes have greater damage potential where the plant growth is stressed, *i.e*., with poor soil nutrition or structure, high temperatures or water stress (Barker & Noe, 1987; Nicol & Ortis-Monasterio, 2004) or where other plant pathogens occur (Taheri *et al*., 1994).

The objectives of the current study were to investigate the distribution of plant-parasitic and other nematode feeding groups in soils used for cereal production on the CAP, and their relationships with several physical and chemical characteristics of the soils sampled.

Materials and methods

Sampling locations and methods

A total of 286 samples (soil and plant) were collected systematically during March-April in 2003, 2004 and 2005, at the tillering stage of the wheat and barley crops. Soil samples were collected from fields adjacent to the roadside at intervals of about 10 km. From each field, a 2 kg bulk soil sample was taken consisting of 15 subsamples, which were collected by using a 2 cm diam. auger to a depth of 20 cm in a zigzag sampling pattern. Five plants with stems and roots were taken and added to each sample. The elevation, latitude and longitude for each sampling site were recorded using the global positioning system (GPS). Geographical coordinates for each field were mapped with ArcGIS™ (ESRI, Ankara, Turkey). Ninety-nine soil samples were collected from Afyon, Eskisehir, Konya, Kütahya and Nigde provinces in 2003; 78 from Ankara, Burdur, Corum, Denizli, Isparta, Kırıkkale, Kırsehir, Usak and Yozgat provinces in 2004, and 99 from Ankara, Bilecik, Bolu, Kayseri, Nigde, Sivas and Yozgat provinces in 2005 (Fig. 1). The cysts of *Heterodera*, gathered each year during the spring period, were of bad quality and unusable for molecular identification. For molecular identification of *Heterodera* specimens, 117 of the more infested sites were resampled in June 2007 at the same GPS locations.

Soil physicochemical analysis

Soil parameters including pH, texture, EC, organic matter, iron and zinc contents were analysed according

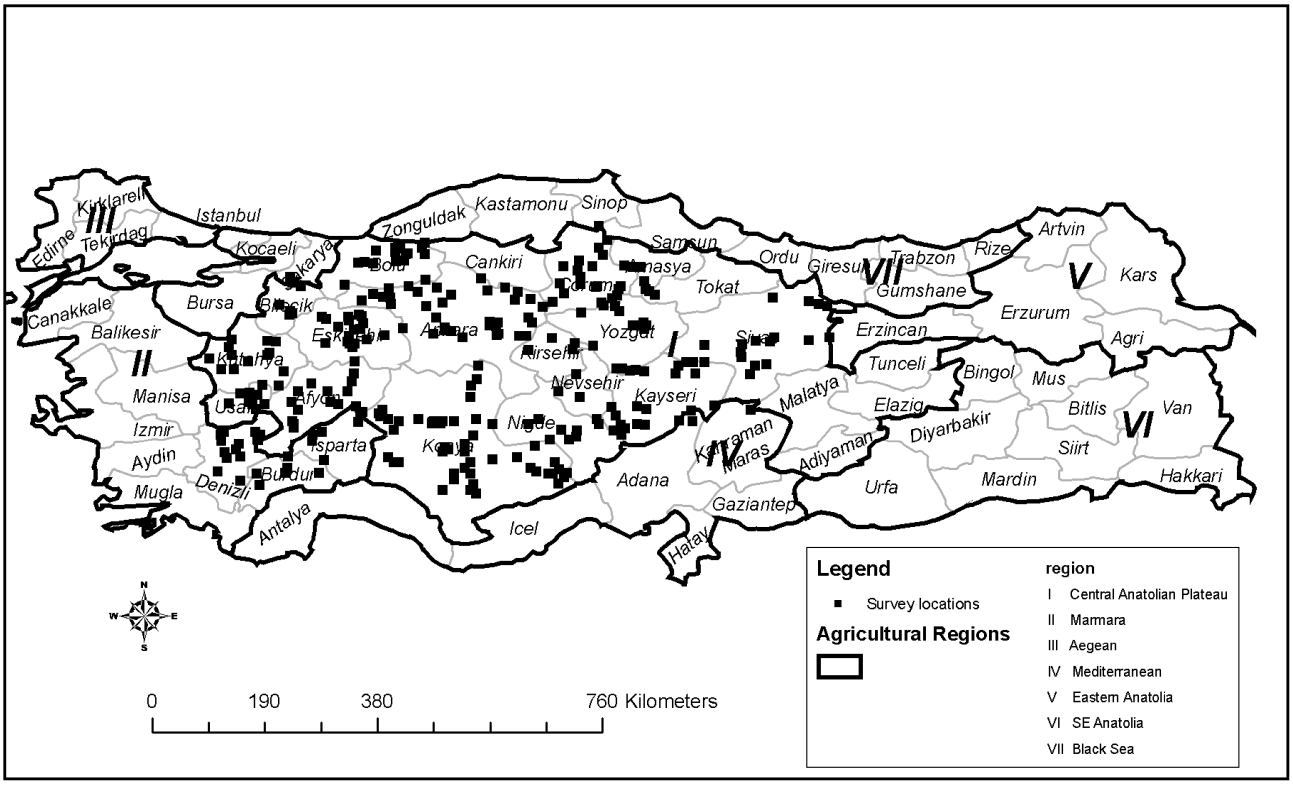


Fig. 1. *The 286 GPS soil sampling points on the Central Anatolian Plateau of Turkey over the 3-year period of 2003-2005.*

to standard methods. Soil pH was determined using a glass electrode pH meter in saturated soil, according to Richards (1954). Sand, silt and clay contents (%) were fractionated using Bouyoucos hydrometer methodology (Uzunoglu, 1992). EC was measured as dS m^{-1} according to Oztan and Ulgen (1961). Organic matter (%) was determined using the modified Walkley-Black method (Ulgen & Atesalp, 1972). Iron (Fe) and Zn contents were analysed according to Lindsay and Norvell (1978).

Nematode extraction and identification

Migratory nematodes were extracted from 200 g of soil from each sample using the modified Whitehead tray technique (Whitehead & Hemming, 1965) and then counted under a light microscope (40×) after being keyed to genus level on morphological features. Soil moisture content was measured by drying 100 g of soil from each sample in oven at 110°C for 3 days. Numbers of nematodes are given per 100 g of dry soil and the genera are grouped according to trophic groups described by Yeates *et al.* (1993).

Nematode from plant roots were extracted by misting (Stirling *et al.*, 1999). Numbers of nematodes recovered were very low and, consequently, have not been included in the results. Permanent slides of the migratory nematodes and second-stage juveniles (J2) of cereal cyst nematodes (CCN) were prepared from all available specimens according to Hooper (1986). Identification of the specimens was performed by the Laboratory of Nematology (I. Halil Elekcioglu, Cukurova University, Turkey) on morphology and morphometric characters. Cysts of *Heterodera* were extracted, using the modified Fenwick Can method (Fenwick, 1940; Stirling *et al*., 1999) from 250 g of soil under constant water flow (2 l min^{-1}). The numbers of cysts, with or without eggs, were counted under a dissecting microscope at 20× magnification.

Permanent slides of cyst vulva and J2 were prepared according to Hooper (1986) for identification of the CCN species using morphology and morphometric characters. Identification of CCN was additionally performed with the PCR-RFLP technique (Bekal *et al*., 1997; Subbotin *et al*., 1999, 2003; Tanha Maafi *et al.*, 2003; Madani *et al.*, 2004).

DNA was extracted from ten individual cysts, including eggs and second-stage juveniles (J2), separately from each location using a Promega kit (Promega, Fitchburg, WI, USA). DNA of the samples was digested using four restriction enzymes (*Alu*I, *Hae*III, *Pst*I and *Rsa*I). The species were identified by comparing the restriction fragment lengths of samples with those of defined populations. The defined populations of *H. avenae*, *H. filipjevi* and *H. latipons* were provided by Roger Rivoal and Sylvie Valette, INRA Le Rheu, France.

Nematodes were classified into the following categories according to Yeates *et al.* (1993): plant-parasitic nematodes, into sedentary and migratory endoparasitic or ectoparasitic groups, and free-living nematodes into different trophic groups.

Statistical analysis

Distribution patterns and frequency of nematodes were defined in 18 provinces of the CAP at the genus level. Numbers of samples containing certain genera of nematode were expressed as frequency of the total samples taken from that province. Principal component analysis and correlation of associations (Anon., 1994) were used to explore the relationships of the different nematode groups and abiotic soil factors.

In order to determine any significant spatial distribution patterns for the different nematode species, geo-spatial statistics were used. All sampling locations (with or without nematodes) were included in the analysis and tests for spatial auto-correlation were undertaken using Moran's I statistic, with subsequent hot-spot analysis carried out using the Getis-Ord Gi* statistic for local spatial auto-correlation if global spatial auto-correlation was detected (Ord & Getis, 1995). All tests were implemented within the spatial statistics component of ArcGIS^{TM} (ESRI). Moran's I statistics were run using a range of search radius distances (15 to 500 km) in order to determine the distance at which spatial auto-correlation, based on Z values, was most significant. The metric of this test is a Z score, which is used to determine significance levels. A Z score >2 indicates highly significant ($P < 0.01$) clustering, $Z < -2$ highly significant dispersed ($P < 0.01$) and Z around 0 indicates random distribution. Fixed search distances were used and all points given equal weighting.

Soil physicochemical properties, frequency and population densities per 100 g of dry soil of each nematode genus in provinces were analysed using ANOVA, Each Pair and Student's t-test (SAS Institute, 1985, Cary, NC, USA).

Results

Physicochemical characteristics of soils

Most of the soil physicochemical properties differed in the 18 provinces surveyed (Table 1). The average value of pH was 7.44 with the highest in Corum (pH 7.66) and the lowest (pH 6.97) in Bolu. The highest EC was recorded in Konya (2.52 dS m^{-1}) and the lowest in Kütahya (0.59 dS m^{-1}) with a mean EC value of 1.10 dS m^{-1}. Soil organic matter contents ranged from 0.23% (Denizli) to 1.82% (Kütahya) for a 1.12% average. Zinc contents ranged from 0.28 ppm (Denizli) to 1.50 ppm (Bolu) with 0.64 ppm average. Iron contents ranged from 1.16 ppm (Corum) to 18.10 ppm (Bolu) with 4.31 ppm average. Average contents (%) of sand, silt and clay were 40.53, 27.36 and 32.10, respectively. These characteristics differed widely according to the provinces and more particularly between Nigde and Burdur, which show opposite values in sand or silt-clay contents, with a lighter soil in Nigde than in Burdur.

Species identification of key plant-parasitic nematodes

Thirteen nematode genera were identified from the soil samples in the study. These belong to the orders Tylenchida (Thorne, 1949), Aphelenchida (Siddiqi, 1980) and Dorylaimida (Pearse, 1942). Only two CCN species were found to occur on the CAP. The more common is *H. filipjevi* present in all provinces except Burdur and Kutahya; *H. latipons* was found only in Yozgat.

Five species of *Pratylenchus* were recorded. *Pratylenchus thornei* was the most frequent, found in all provinces except Afyon, Isparta, Kayseri and Kirikkale; *P. neglectus* was found in nine provinces (Bolu, Burdur, Corum, Denizli, Kayseri, Nigde, Sivas, Usak and Yozgat); *P. crenatus* in Ankara and Bolu; *P. scribneri* Steiner, 1943 in Bolu and Eskisehir; and *P. loosi* Loof, 1960 in Ankara.

Five species of *Pratylenchoides* were identified from the soil samples. *Pratylenchoides alkani* was found in all provinces except Afyon, Kirikkale, Kirsehir and Nigde; *P. erzurumensis* was recorded in Ankara, Bilecik, Bolu, Burdur, Denizli and Konya; *P. variabilis* Sher, 1970 in Konya; *P. crenicauda* Winslow, 1958 in Denizli; and *P. ritteri* Sher, 1970 in Corum, Denizli and Eskisehir.

Two species of *Geocenamus* were obtained: *Geocenamus microdorus* (Geraert, 1966) in Bolu, Burdur, Denizli, Kirikkale, Konya, Usak and Yozgat, and *G. brevidens* (Allen, 1955) in Burdur, Denizli, Isparta and Konya.

Table 1. *Average soil physiochemical properties of samples collected in 18 provinces over 3 years, 2003-2005.*

Province	pH	Electrical conductivity (dS m^{-1})	Organic matter (%)	Fe (ppm)	Zn (ppm)	Sand (%)	Silt (%)	Clay (%)
Afyon	7.29	0.79	1.64	4.81	0.78	41.87	27.76	30.35
Ankara	7.64	0.69	1.09	3.73	0.63	36.64	25.94	37.43
Bilecik	7.51	2.49	1.16	6.65	0.76	36.68	29.46	33.87
Bolu	6.97^{+}	1.02	1.13	18.10*	1.50*	37.53	28.87	33.60
Burdur	7.44	0.67	0.84	2.94	0.46	28.27^{+}	32.35*	39.38
Corum	7.66	1.08	1.40	1.16^{+}	0.44	30.87	28.91	40.22
Denizli	7.52	0.66	0.23^{+}	3.02	0.28^{+}	33.10	30.59	36.31
Eskisehir	7.35	1.50	1.70	2.27	0.48	43.24	24.74	32.02
Isparta	7.30	0.94	0.95	6.35	0.47	29.48	27.84	42.68*
Kayseri	7.33	0.67	0.70	3.95	1.25	46.63	23.99	29.38
Kirikkale	7.57	1.11	0.80	1.95	0.37	53.76	24.62	21.65
Kirsehir	7.45	1.36	1.61	2.50	0.50	48.69	25.05	26.25
Konya	7.48	2.52*	1.18	2.43	0.72	40.84	28.83	30.32
Kutahya	7.53	0.59^{+}	1.82*	2.64	0.55	37.14	29.53	33.33
Nigde	7.36	1.28	0.57	2.12	0.63	62.25*	22.89^{+}	14.86^{+}
Sivas	7.58*	0.60	0.91	8.28	0.67	41.29	28.74	29.97
Usak	7.55	0.66	1.18	2.13	0.39	37.36	26.57	36.07
Yozgat	7.46	1.28	1.18	2.62	0.56	43.95	25.89	30.16
Mean	7.44	1.10	1.12	4.31	0.64	40.53	27.36	32.10

*, significantly highest value; +, significantly lowest value.

Tylenchorhynchus was represented by four species: *Tylenchorhynchus striatus* Allen, 1955 was found in Ankara, *T. parvus* (Allen, 1955) in Eskisehir and Yozgat, *T. mamillatus* Tobar-Jimenez, 1966 in Burdur and Kirsehir and *T. latus* Allen, 1955 in Konya.

Frequency and distribution of cereal nematodes

Among the plant-parasitic nematodes, cysts of CCN occurred at the highest frequency (78.3%) in surveyed area. The second most frequent were the ectoparasitic plant-parasitic nematodes, with *Geocenamus* (52.5%) the most frequent genus. Next were the migratory endoparasitic *Pratylenchus* (42.7%) and *Pratylenchoides* (35.7%). Ectoparasitic plant-parasitic nematodes; *Helicotylenchus* spp. (29.7%), *Paratylenchus* spp. (19.2%), *Trophurus* spp. (5.2%) and *Tylenchorhynchus* spp. (4.9%) occurred at the lowest frequency ($P < 0.0001$) in the 18 provinces (Table 2). Spatial analysis showed that CCN cysts were significantly clustered in the central and southern part of the CAP with a corresponding absence in the southwestern part of the CAP (Fig. 2). The highest CCN frequency ($P < 0.02$) was in Bilecik, Isparta, Kirsehir and Kütahya (Table 2). In contrast to cyst frequency, the frequency of J2 of CCN was significantly lower ($P < 0.0001$) in 6.6% of soil samples.

There was no significant difference between provinces for frequency of *Pratylenchus* spp. However, individual species distribution differed. *Pratylenchus neglectus* had a highly significant hot-spot cluster of high incidence of the species in the south of the CAP around Konya (Fig. 4), while *P. thornei* was randomly distributed across the CAP. Concomitant infestations of cereal cyst nematode and root-lesion nematodes were detected in 33.9% of sampling sites, particularly in Bilecik and Kirsehir (Table 2).

The frequencies of hyphal, epidermal cell and root hair feeders (*Ditylenchus* spp. (62.2%), *Aphelenchoides* spp. (54.2%), *Aphelenchus* spp. (43.0%), *Tylenchus* spp. (37.4%)), omnivorous (*Dorylaimus* spp. (49.3%)), and bacterivorous nematodes (82.0%) were significantly high in cereal fields surveyed compared with other nematodes. Bacterivorous nematodes were found at the highest frequency in 18 provinces ($P < 0.0001$) (Table 2). Eight genera of bacterivorous nematodes were observed: *Acrobeles*, *Acrobeloides*, *Cephalobus*, *Cervidellus*, *Eu-*

Table 2. *Frequency of soil samples with nematodes (genus level) in 18 provinces of CAP.*

Province	Sampling year	Number of samples	CCN cysts	CCN second-stage juveniles	*Pratylenchus* spp.	Cyst + lesion nematodes	*Ditylenchus*	*Aphelenchoides*	*Geocenamus*	*Dorylaimus*	*Aphelenchus*	*Tylenchus*	*Pratylenchoides*	*Helicotylenchus*	*Paratylenchus*	*Trophurus*	*Tylenchorhynchus*	Total bacterivorous nematodes
Afyon	2003	17	15	0	6	4	0^{+}	0^{+}	0^{+}	0	0	0	0^{+}	0	0	0	0	0^{+}
Ankara	2004-2005	29	24	6	14	13	22	16	23*	20	11	17	18*	14	11	0	0	27*
Bilecik	2005	7	7*	0	4	4	4	4	4	7	1	6	5*	2	0	0	0	7
Bolu	2005	15	11	1	9	6	5	10	8	13	8	10	8	7	7	11	0	14*
Burdur	2004	5	5	0	1	1	5*	2	2	4	3	2	3	1	0	0	0	5*
Corum	2004	20	13	1	5	3	13	6	14	12	10	8	12	9	1	0	0	20*
Denizli	2004	16	10	1	9	8	14	10	4^{+}	5	10	9	9	6	3	0	0	16*
Eskisehir	2003	17	15	0	9	8	15	9	10	3	9	9	8	3	2	0	4	16*
Isparta	2004	7	7*	1	1	1	7*	3	4	3	3	1	4	3	2	0	1	7*
Kayseri	2005	19	15	3	15	12	18	18*	14*	19	5	4	8	12	8	2	0	19
Kirikkale	2004	7	5	0	0	0	2	3	5	5	3	1	1	0	1	0	0	7*
Kirsehir	2004	6	6*	1	3	3	6*	2	5*	2	4	2	0^{+}	1	3	0	1	6
Konya	2003	49	37	1	18	13	30	34	23	14	25	8	10	9	4	0	3	43*
Kutahya	2003	13	6^{+}	0	6	3	5	5	5	3	5	3	3	3	1	0	1	6^{+}
Nigde	2003/2005	11	9	1	8	6	9	11*	7	9	7	6	1	3	3	0	1	10*
Sivas	2005	20	17	0	5	4	5^{+}	5^{+}	5^{+}	5	5	4	1^{+}	4	3	2	1	6
Usak	2004	11	8	1	4	4	7	6	7	8	5	4	5	4	4	0	0	10*
Yozgat	2004/2005	17	14	2	5	4	11	11	10	9	9	13	6	4	2	0	2	16*
Total number of samples		286	224	19	122	97	178	155	150	141	123	107	102	85	55	15	14	235
% samples containing nematodes			78.3	6.6	42.7	33.9	62.2	54.2	52.5	49.3	43.0	37.4	35.7	29.7	19.2	5.2	4.9	82.2

CCN, cereal cyst nematodes; *, significantly highest value; +, significantly lowest value.

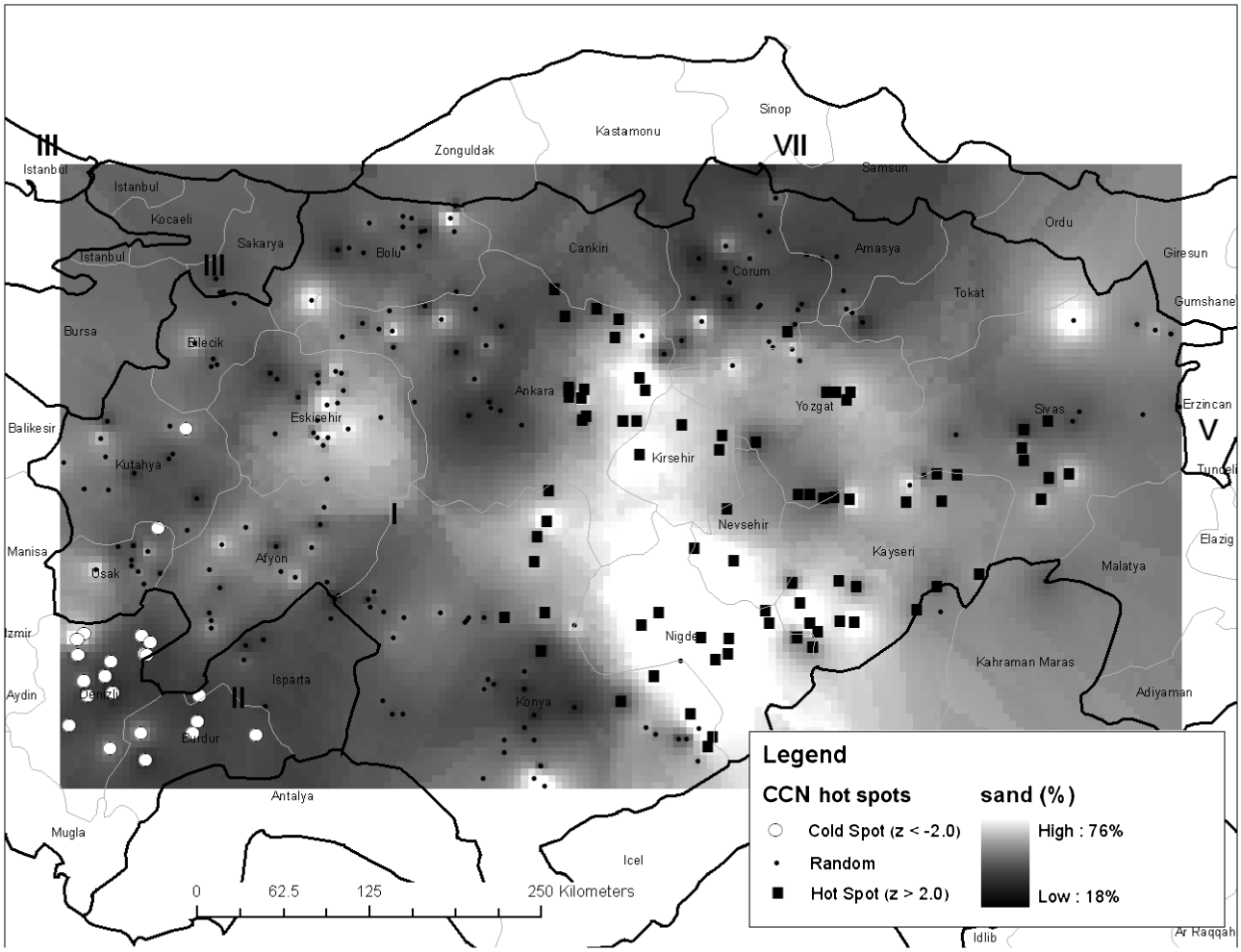


Fig. 2. *Relationship between percentage sand content in the soil and total cyst population. Black squares indicate significant spatial clustering of high cyst populations ('hot spots', GetisOrd Gi* P < 0.001), white circles indicate significant spatial clustering of low cyst populations ('cold spots', GetisOrd Gi* P < 0.001).*

cephalobus, *Monhystera*, *Rhabditis* and *Wilsonema.* Equivalent analysis of the distribution patterns of hyphal, epidermal cell and root hair feeder, omnivorous and bacterivorous nematode species revealed highly significant ($P < 0.001$) clusters for the trophic groups in Konya (data not shown but closely aligned with that of *P. neglectus*).

The frequency of *Geocenamus* spp. was significantly higher in the samples from Ankara, Kayseri and Kirsehir and, conversely, significantly lower ($P < 0.0001$) in Afyon, Denizli and Sivas (Table 2). *Pratylenchoides* spp. occurred at significantly higher ($P < 0.0001$) frequency in Ankara and Bilecik and lower frequency in Afyon, Kirsehir and Sivas (Table 2).

Total bacterivorous nematodes occurred at significantly higher frequency in most of the provinces including Ankara, Bolu, Burdur, Corum, Denizli, Eskisehir, Isparta, Kirikkale, Konya, Nigde, Usak and Yozgat, whereas the frequency of total bacterivorous nematodes was significantly lower ($P < 0.0001$) in Afyon and Kütahya (Table 2).

Ditylenchus spp. occurred at significantly higher frequency ($P < 0.0001$) in Burdur, Isparta and Kirsehir and in lower frequency ($P < 0.0001$) in Afyon and Sivas. *Aphelenchoides* spp. occurred at significantly higher frequency ($P < 0.0001$) in Kayseri and Nigde and in lower frequency ($P < 0.0001$) in Afyon and Sivas.

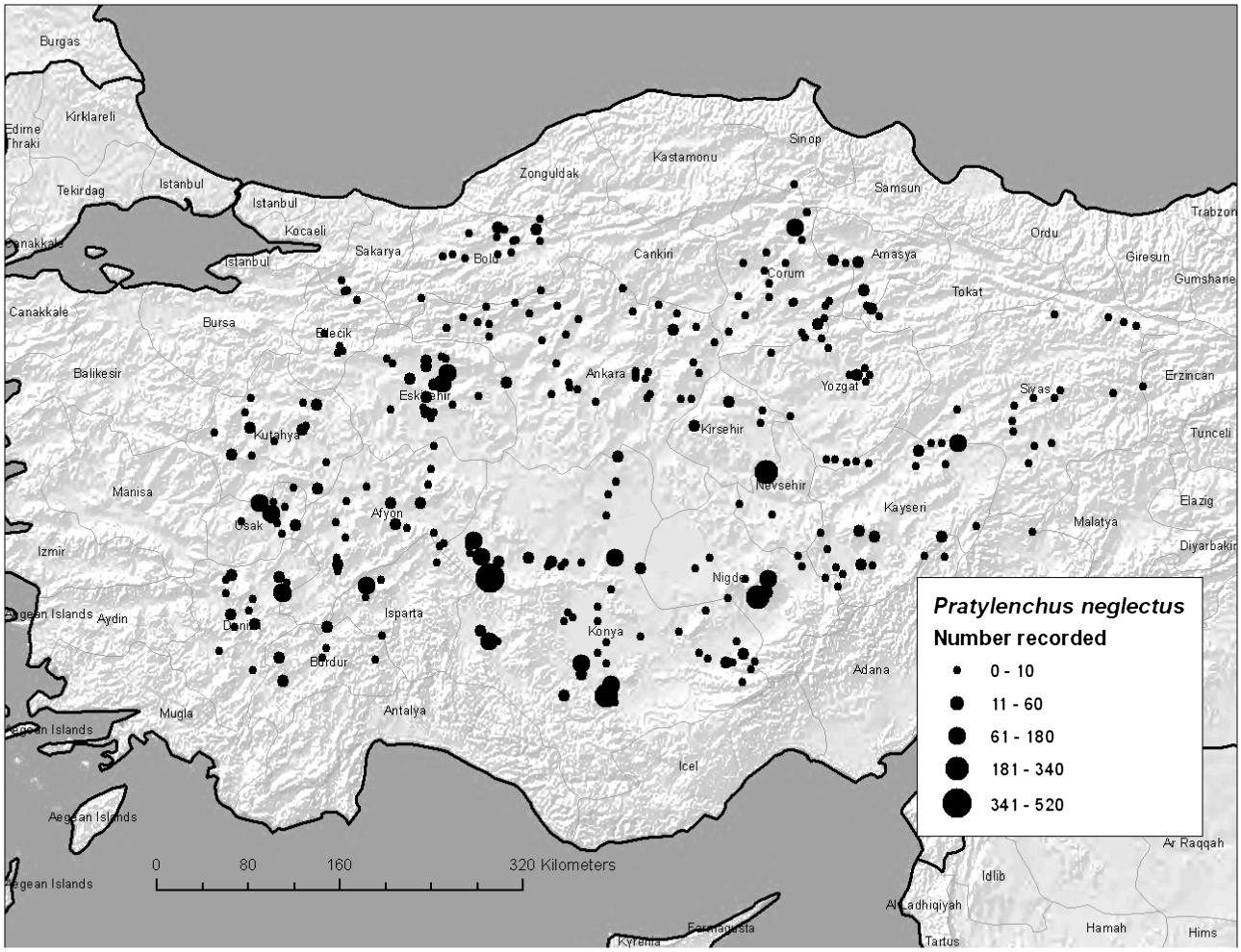


Fig. 3. *Distribution of* Pratylenchus neglectus *in the CAP according to point pattern analysis. Symbols are scaled by number of nematodes (200 g soil)*$^{-1}$.

Relationship between soil characteristics and the distribution of nematodes

Spatial analysis, combining results from the cluster point pattern analysis with interpolated soil property data derived from surveys, indicated a spatial relationship between CCN cyst distribution and sand content. The strong coincidence of areas with significant incidence of CCN and soils with a high sand content, or the converse, is illustrated in Figure 2. This relationship was supported by principal component analysis (PCA) which revealed significant correlations ($P < 0.05$) between number of cysts in soil and sand content. There was also a negative correlation ($P < 0.10$) between Fe concentration and cyst nematode density (Fig. 5). For *Pratylenchus*, there was a slight positive correlation ($P < 0.10$) between *P. thornei* and clay content. By contrast, there was a significant negative correlation ($P < 0.05$) of *P. neglectus* with clay content and a slightly positive correlation ($P < 0.10$) with sand. There was a positive correlation ($P < 0.05$) between EC and *P. neglectus* population density (Fig. 5). There was no relationship found between other species of plant-parasitic nematode and investigated soil parameters. The group of epidermal cell, root hair, hyphal feeder and bacterivorous nematodes was positively correlated ($P < 0.01$) with EC and soil zinc (Fig. 5).

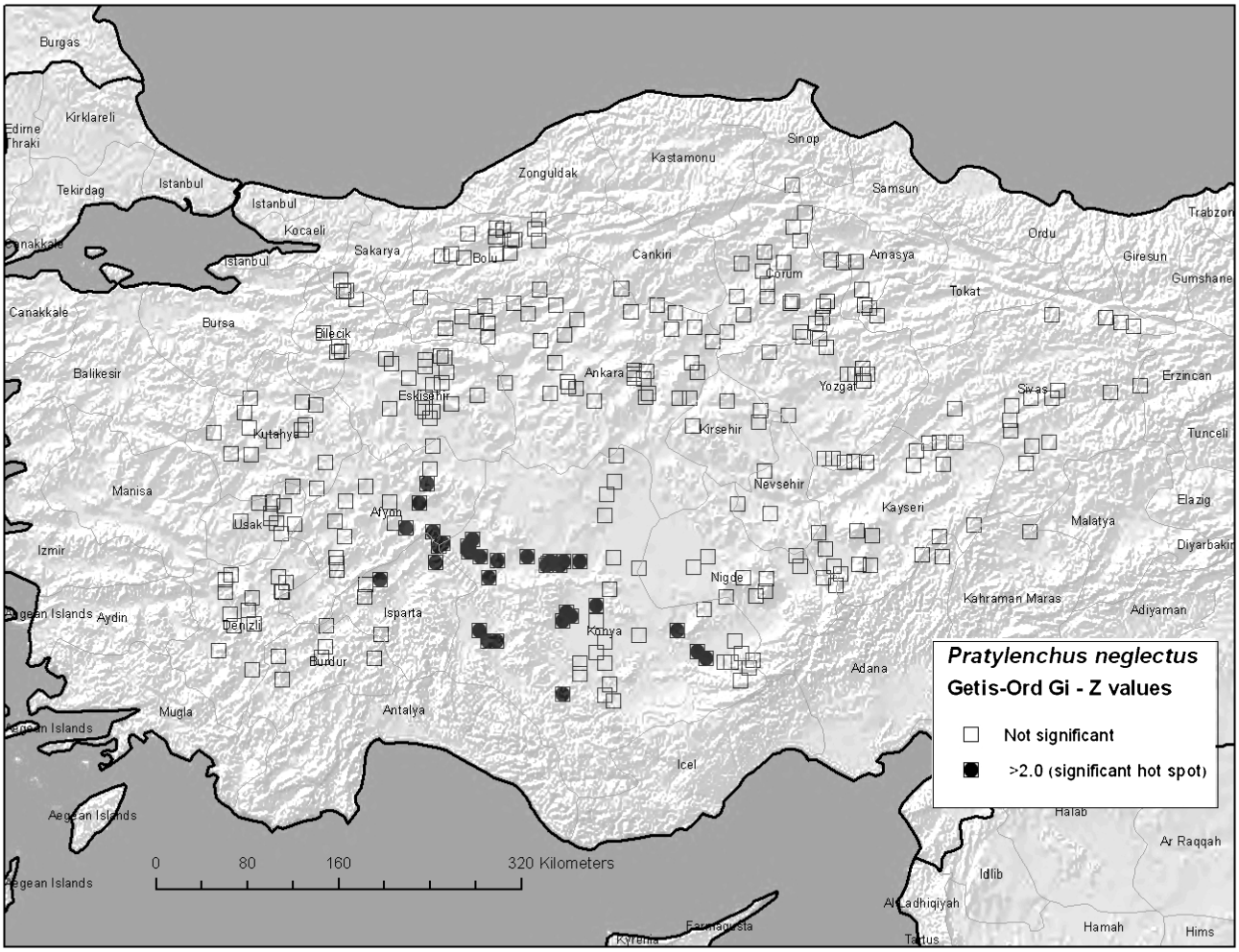


Fig. 4. *Spatial pattern analysis of* Pratylenchus neglectus*. Black squares indicates high nematode incidence ('hot spots', GetisOrd Gi* P < 0.001), white squares indicates low nematode incidence.*

POPULATION DENSITY OF NEMATODES

The highest ($P < 0.006$) CCN cyst density was obtained in Kirsehir, Yozgat and Nigde; an average of 18, 12 and ten cysts was found in 100 g of dry soil and populations ranged from five to 40, zero to 95 and zero to 44 cysts (100 g dry soil)$^{-1}$ in the sampled fields in these provinces, respectively. Cyst densities were also high in Eskisehir (max. 39), Kirikkale (max. 37), Sivas (max. 34), Isparta and Ankara (max. 31); the average density was 6-9 cysts (100 g dry soil)$^{-1}$. The lowest ($P < 0.006$) densities for CCN cysts were recorded in Kütahya (average one cyst).

The population densities of *Pratylenchus* spp. were not significantly different among provinces. However, higher population densities were obtained in Konya (max. 274), Nigde (max. 140), Kirsehir (max. 119), Sivas (max. 113), Denizli (max. 69) and Eskisehir (max. 52). The average population density in all surveyed sites was 14-29 nematodes (100 g dry soil)$^{-1}$ (Table 3). Using survey data, the individual nematode species incidence by site was mapped to obtain a visual impression of distribution patterns. Data for *P. neglectus* are shown in Figure 3, with site symbols scaled according to *P. neglectus* numbers recorded.

Maximum ($P < 0.0001$) population densities were recorded for *Geocenamus* spp. among plant-parasitic nematodes in all provinces. Population densities of *Geocenamus* spp. were also significantly different ($P < 0.0002$) among provinces, with a range of means of six to 68

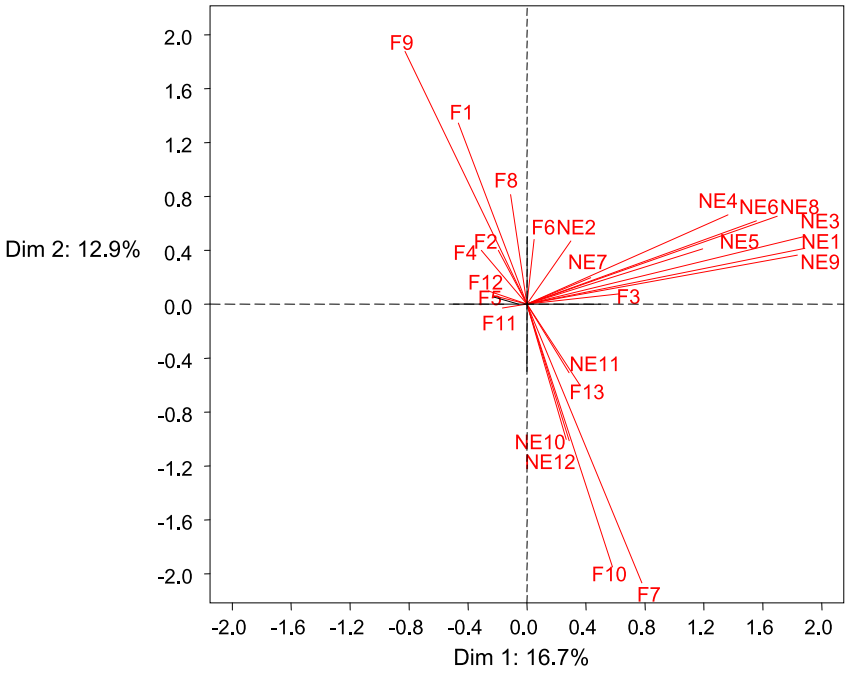


Fig. 5. *Principal component analysis of abiotic factors and soil nematodes. Variables of abiotic factors and nematode groups used in principal component and correlation analysis: F1: % saturation; F2: pH in saturated soil; F3: EC in extract (dS m^{-1}); F4: organic matter; F5: Fe (ppm); F6: Zn (ppm); F7: sand (%); F8: silt (%); F9: clay (%); F10: Soil class; F11: cereal region of Turkey; F12: region of Turkey; F13: rotation; NE1: number of* Pratylenchus neglectus *(200 g soil)$^{-1}$; NE2: number of* Pratylenchus thornei *(200 g soil)$^{-1}$; NE3: total* P. neglectus *and* P. thornei *(200 g soil)$^{-1}$; NE4: total plant-parasitic nematodes (200 g soil)$^{-1}$; NE5: total epidermal cell; root hair and hyphal feeder nematodes (200 g soil)$^{-1}$; NE6: total bacterivorous nematodes (200 g soil)$^{-1}$; NE7: total predator and omnivorous nematodes (200 g soil)$^{-1}$; NE8: total free living nematodes (200 g soil)$^{-1}$; NE9: total* Pratylenchus *spp. (200 g soil)$^{-1}$; NE10: number of empty cereal cyst nematode (CCN) cysts (250 g dry soil)$^{-1}$; NE11: number of full CCN cysts with eggs (250 g dry soil)$^{-1}$; NE12: total cyst population (full and empty) (250 g dry soil)$^{-1}$). This figure is published in colour in the online edition of this journal, which can be accessed* via *http://www.brill.com/nemy*

nematodes (100 g dry soil)$^{-1}$. The maximum population density obtained was 359 nematodes (100 g dry soil)$^{-1}$ in Corum.

Pratylenchoides spp. population densities were high ($P < 0.0001$) among all provinces, with the highest population of 749 nematodes (100 g dry soil)$^{-1}$ from Yozgat. Population densities of *Pratylenchoides* spp. were also higher ($P < 0.03$) in Denizli (max. 450), Ankara (max. 274), Isparta (max. 256), Corum (max. 207), Sivas (max. 201), Bilecik (max. 134) and Usak (max. 133), with an overall mean of 10-58 nematodes (100 g dry soil)$^{-1}$.

Population densities of *Helicotylenchus* spp. were high in some provinces; Usak (max. 302), Yozgat (193), Konya (max. 141), Isparta (max. 134) and Denizli (max. 127), with a mean of 6-33 nematodes (100 g dry soil)$^{-1}$, but low in other provinces ranging between zero and 63 nematodes (100 g dry soil)$^{-1}$. However, there was no significant difference between provinces for *Helicotylenchus* spp. Population densities of *Paratylenchus* spp. were also not significantly different among provinces. Densities of this genus were lower in comparison to other plant-parasitic nematodes. The highest population densities were in Kayseri (max. 171) and Konya (max. 157). The mean population density in these locations was 14 and seven nematodes (100 g dry soil)$^{-1}$. *Trophurus* spp. were only recorded in three provinces with a range of two to 25 nematodes (100 g dry soil)$^{-1}$. The highest ($P < 0.0001$) population level recorded was 171 nematodes (100 g dry soil)$^{-1}$ in Bolu. *Tylenchorhynchus* spp. was recorded in eight provinces with relatively low population densities and there was no significant difference among provinces. The maximum population density was 67 nematodes (100 g dry soil)$^{-1}$ in Konya and the mean

Table 3. *Average population densities and range (in brackets) of nematodes (nematode genus (100 g dry soil)$^{-1}$) in 18 provinces.*

Province	CCN cysts	*Pratylenchus*	*Geocenamus*	*Pratylenchoides*	*Helicotylenchus*	*Paratylenchus*	*Trophurus*	*Tylenchorhynchus*	*Tylenchus*	*Ditylenchus*	*Aphelenchus*	*Aphelenchoides*	*Dorylaimus*	Total bacterivorous nematodes
Afyon	4 (0-18)	5 (0-32)	0^{+}	0^{+}	0	0	0^{+}	0	0^{+}	0^{+}	0^{+}	0^{+}	0^{+}	0^{+}
Ankara	6 (0-31)	5 (0-39)	30 (0-135)	29 (0-274)	7 (0-47)	7 (0-45)	0^{+}	0	8 (0-77)	38 (0-172)	11 (0-119)	16 (0-84)	11 (0-26)	130 (0-644)
Bilecik	3 (1-7)	9 (0-30)	29 (0-66)	30 (0-134)	3 (0-12)	0	0^{+}	0	23 (0-54)	8 (0-24)	1^{+} (0-6)	9 (0-30)	23 (6-47)	52 (0-116)
Bolu	3 (0-21)	4 (0-25)	9 (0-39)	7 (0-36)	4 (0-19)	5 (0-32)	25* (0-171)	0	42* (0-156)	8 (0-65)	5 (0-13)	10 (0-31)	16 (0-47)	256 (18-1358)
Burdur	2 (0-5)	5 (0-23)	7 (0-22)	27 (0-65)	2 (0-11)	0	0^{+}	0	16 (0-58)	61 (12-116)	52 (0-128)	27 (0-81)	16 (0-36)	185 (111-406)
Corum	2 (0-16)	5 (0-45)	67 (0-359)	47 (0-207)	10 (0-47)	2 (0-34)	0^{+}	0	21 (0-109)	25 (0-90)	17 (0-134)	11 (0-55)	27 (0-106)	177 (23-424)
Denizli	2 (0-10)	17 (0-69)	6 (0-42)	58 (0-450)	16 (0-127)	3 (0-23)	0^{+}	0	28 (0-171)	57 (0-137)	35 (0-127)	28 (0-118)	14 (0-84)	170 (48-327)
Eskisehir	9 (0-39)	14 (0-52)	40 (0-195)	19 (0-95)	3 (0-31)	2 (0-20)	0^{+}	7 (0-54)	21 (0-107)	78* (0-375)	32 (0-154)	26 (0-72)	4 (0-31)	117 (0-300)
Isparta	7 (1-31)	6 (0-45)	30 (0-111)	58 (0-256)	31 (0-134)	23 (0-36)	0^{+}	5 (0-34)	2^{+} (0-12)	61 (22-75)	97* (0-524)	16 (0-67)	17 (0-50)	176 (114-290)
Kayseri	7 (0-17)	9 (0-37)	24 (0-62)	9 (0-86)	11 (0-60)	14 (0-171)	2^{+} (0-39)	0	7 (0-63)	58 (0-181)	7 (0-78)	99 (0-441)	88* (6-240)	176 (0-589)
Kirikkale	7 (0-37)	0	29 (0-63)	3 (0-21)	0	8 (0-54)	0^{+}	0	5 (0-33)	5^{+} (0-21)	8 (0-22)	9^{+} (0-22)	21 (0-59)	67^{+} (22-142)
Kirsehir	18* (5-40)	29 (0-119)	34 (0-76)	0	9 (0-55)	18 (0-65)	0^{+}	4 (0-22)	25 (0-76)	43 (11-119)	31 (0-119)	68 (0-302)	14 (0-65)	204 (0-532)
Konya	5 (0-26)	22 (0-274)	22 (0-198)	7 (0-98)	6 (0-141)	7 (0-157)	0^{+}	3 (0-67)	6 (0-54)	77 (0-647)	46 (0-324)	119* (0-1228)	7 (0-89)	460* (0-1740)
Kutahya	1^{+} (0-4)	7 (0-23)	10 (0-32)	4 (0-21)	9 (0-63)	3 (0-41)	0^{+}	4 (0-52)	4 (0-21)	31 (0-174)	25 (0-129)	18 (0-115)	2^{+} (0-11)	74 (0-269)
Nigde	10 (0-44)	26 (0-140)	68* (0-290)	0	4 (0-18)	5 (0-23)	0^{+}	1 (0-12)	12 (0-54)	39 (0-86)	30 (0-195)	95 (6-781)	32 (0-98)	281 (95-583)
Sivas	7 (0-34)	8 (0-113)	15 (0-94)	10 (0-201)	2 (0-18)	3 (0-43)	3^{+} (0-57)	1 (0-12)	6 (0-57)	12 (0-91)	4 (0-30)	18 (0-196)	14 (0-103)	296 (0-1659)
Usak	4 (0-28)	12 (0-60)	46 (0-134)	26 (0-133)	33 (0-302)	12 (0-58)	0^{+}	0	16 (0-109)	44 (0-290)	37 (0-134)	27 (0-97)	22 (0-61)	268 (44-851)
Yozgat	12 (0-95)	4 (0-23)	36 (0-113)	74* (0-749)	28 (0-193)	2 (0-22)	0^{+}	2 (0-22)	24 (0-101)	39 (0-284)	22 (0-91)	27 (0-138)	22 (0-96)	150 (0-346)

CCN, cereal cyst nematodes; *, significantly highest population level; +, significantly lowest population level.

population density ranged between one and seven nematodes (100 g dry soil)$^{-1}$ (Table 3).

Population densities of hyphal, epidermal cell and root hair feeders, omnivorous and bacterivorous nematodes were significantly higher ($P < 0.0001$) than those of plant-parasitic nematodes. The highest ($P < 0.0001$) population densities found were of bacterivorous nematodes followed by *Aphelenchoides* spp. and *Ditylenchus* spp., and the lowest ($P < 0.0001$) population densities were for *Aphelenchus* spp., *Dorylaimus* spp. and *Ty-*

lenchus spp. Mean population densities were up to 97 nematodes (100 g dry soil)$^{-1}$ (Isparta) for *Aphelenchus* spp. ($P < 0.0023$) and 119 nematodes (100 g dry soil)$^{-1}$ (Konya) for *Aphelenchoides* spp. ($P < 0.0007$). *Ditylenchus* spp. and *Tylenchus* spp. population densities averaged 0-78 nematodes (100 g dry soil)$^{-1}$ (max. in Eskisehir, $P < 0.0033$) and 0-42 nematodes (100 g dry soil)$^{-1}$ (max. in Bolu, $P < 0.0001$), respectively. The mean *Dorylaimus* spp. population density was between 0-88 nematodes (100 g dry soil)$^{-1}$ (max. in Kayseri, $P < 0.0001$) (Table 3). The population densities of bacterivorous nematodes in soil samples varied widely, averaging 0-460 nematodes (100 g dry soil)$^{-1}$ and ranged from zero to 1740 nematodes (100 g dry soil)$^{-1}$ in Afyon (lowest, $P < 0.0001$) and Konya (highest, $P < 0.0001$).

Discussion

The soil in the sampled area was neutral to slight alkaline, with a pH of between 6.97 and 7.66, and an organic matter content of less than 2%. The Fe and Zn contents of 4.31 and 0.64 ppm, respectively, are adequate levels; levels below 2.5 for Fe and 0.5 ppm for Zn are deficient for plant growth (Anon., 2011e). Higher organic matter content areas were low in EC and this situation was most pronounced in Kütahya.

Cereal cyst nematodes were widely distributed in CAP in Turkey, but the distribution was found to be clustered. Both spatial analysis and PCA confirmed the association of cereal cyst nematodes with sand content of the soils, similar to *H. avenae* as documented previously by Swarup and Soss-Moss (1990). Findings that soybean cyst nematode, *H. glycines*, increased iron deficiency chlorosis symptoms of plants (Chen *et al*., 2007) are consistent with the negative relationship observed in the present study between CCN cysts and iron content of soil.

Both shrunken and empty cysts were found in the samples. CCN J2 emergence and infection of plant roots takes place from November to April under rainfed winter wheat conditions in CAP in Turkey (Sahin *et al*., 2008). Therefore, J2 could have already entered plant roots and, consequently, J2 were found at only low densities in soil at the sampling time of March-April. The distribution of *avenae* group species, mainly *H. filipjevi*, in cereal growing areas in CAP is in agreement with the previous studies by Enneli *et al*. (1994), Rumpenhorst *et al.* (1996), Öztürk *et al.* (1998) and Abidou *et al.* (2005). *Heterodera latipons* was found at only one location in Yozgat province. Therefore, only one species was found at each location, which contrasts with the findings of Abidou *et al.* (2005) and Rumpenhorst *et al.* (1996), who reported multiple CCN species infestations at Turkish sites.

Distribution of *avenae* group cyst nematodes in Turkey is strongly related to climatic differences among the geographic regions. *Heterodera latipons* is adapted to Mediterranean climatic conditions, whilst *H. avenae* and *H. filipjevi* develop in more temperate climates (Nicol *et al*., 2003). Southeast Anatolian, Thrace and Aegean regions have a Mediterranean climate, while the Central Anatolian region has a temperate climate. Although infestation of *H. avenae* has been found in Thrace, Aegean (Misirlioglu & Pehlivan, 2007), the southeast Anatolian (Imren, 2007) and the east Anatolian region (Yüksel, 1973), it is not found in CAP. *Heterodera latipons* has been found frequently in the southeast Anatolian region in Turkey (Abidou, *et al*., 2005; Imren, 2007).

Assuming an average number of eggs per cyst of 150 conservatively, then the number of eggs or J2 of *H. filipjevi* is up to 142 (g dry soil)$^{-1}$ (the maximum cyst density found was 95 cysts (100 g dry soil)$^{-1}$). Economic densities for yield loss in the range of 10 to 40 eggs and J2 (g soil)$^{-1}$ for *H. avenae* were given by Swarup and Sosa-Moss (1990). Therefore, *H. filipjevi* at many locations on the CAP is present in population densities that are damaging for cereal.

Root-lesion nematode species were also common in the CAP, with *P. neglectus* and *P. thornei* the most widely distributed species throughout the CAP. The results obtained from the present study on the affinity of *P. thornei* with clay soils and, conversely, the affinity of *P. neglectus* with sandy soils, support previous findings that the distribution patterns of *Pratylenchus* spp. depends on soil texture (Grandison & Wallace, 1974; Nicol, 1991; Mc Sorley & Frederick, 2002; Thompson *et al*., 2010). These two species were commonly found together in sampling locations in the present study. Economic damage threshold for *P. thornei* is 42 individuals (100 cm soil)$^{-1}$, as defined by Van Gundy *et al.* (1974) in Mexico. Yield losses caused by *P. thornei* at above damaging population densities occur in the east Mediterranean region in Turkey (Elekcioglu & Gözel, 1997, 1998). In many locations, populations of *Pratylenchus* spp. are above the threshold for economic damage. When it is considered that 34% of locations have both cereal-cyst and root-lesion nematodes, potential damage could be more severe in these locations. Wheat is a poor host for *P. scribneri* and *P. crenatus* and a non-host for *P. loosi* (Rich *et al*., 1977; Anon., 2011f,

g), and hence these species are probably not of great economic importance in cereals.

Geocenamus spp. was widely distributed and had high population densities in many provinces surveyed, and could be damaging to cereals in these CAP locations. *Geocenamus brevidens* was shown to be damaging on wheat in Oregon (Smiley *et al*., 2006) and the east Mediterranean region in Turkey (Elekcioglu & Gozel, 1998). *Geocenamus microdorus* was the most prevalent species identified from seven provinces in CAP, whilst *G. brevidens* was recorded from four provinces. However, *G. brevidens* has been recorded from other regions in Turkey, including the south Mediterranean, southeast Anatolian, Thrace and Aegean regions (Elekcioglu, 1992, 1996; Imren, 2007; Misirlioglu & Pehlivan, 2007).

Pratylenchoides spp. and *Tylenchorhynchus* spp. could be important for cereals on the CAP, as was shown for *P. ritteri* and *Zygotylenchus guevarai* in France, Italy and Spain (Griffin, 1989). Among the five species of *Pratylenchoides* observed in CAP, *P. alkani* and *P. erzurumensis* are the most prevalent species observed in 14 and six provinces, respectively, first being identified in the east and southeast Anatolian region by Yüksel (1977).

While some species of the genera *Aphelenchus*, *Aphelenchoides*, *Ditylenchus* and *Dorylaimus*, and other tylenchid nematodes are migratory endoparasitic and epidermal cell and root hair feeder on plants, other species of these genera also feed on microbial material in the soil (Yeates *et al*., 1993). Konya province appeared to be particularly suitable for *P. neglectus*, hyphal-feeding and bacterivorous nematodes. The nature of the conditions determining these high incidences is associated with high EC. High EC values in this area and positive correlation between these nematodes and EC support this relationship. Soil microorganisms can be enriched, depending on the beneficial increase of the minerals and EC, allowing bacterivorous and hyphal-feeding nematode populations to increase. The widespread distribution and high abundance of bacterivorous and hyphal-feeding nematode genera indicated that the organic matter composition of the surveyed area is suitable for microbial-mediated nutrient cycling and thus for crop production. However, soil organic matter content was not directly correlated to free-living nematode fauna in the surveyed area to the same extent as EC. Hence, EC is seen as a more suitable determinant for the soil free-living nematode fauna and consequently for plant health. Bacterivorous nematodes are beneficial nematodes affecting nutrient cycling and indirectly crop plant development (Neher, 2001). Their occurrence in this study is positively correlated with zinc concentration and EC, which supports this relationship as suggested previously by Graham and Webb (1991); zinc reduces disease development both directly and indirectly. There is no doubt that the crop plant plays an important role in the structuring of the nematode communities.

It is clear from this study that cereal cyst nematode, *H. filipjevi*, and the two root-lesion nematode species, *P. thornei* and *P. neglectus*, have a widespread distribution strongly related to the soil type in the rainfed cereal-cropping zone of the Turkish Anatolian Plateau. *Heterodera filipjevi* would be damaging in many cases as would probably the two species of root-lesion nematodes. Further investigation is needed to assess *Geocenamus* spp., *Helicotylenchus* spp., *Paratylenchus* spp., *Pratylenchoides* spp. and *Tylenchorhynchus* spp. damage on cereals as their population densities appeared high. Higher population densities of bacterivorous and hyphal-feeding nematodes are positive indicators for soil health, showing that the soils in cereal-growing areas in CAP have biological populations favouring soil nutrient cycling and thus crop growth.

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