

# Distribution of nematodes in wetland soils with different distance from the Bohai sea

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## ABSTRACT

In order to elucidate the distribution of soil nematodes in coastal wetlands and the effect of different distance from the sea line on soil nematode communities, we investigated the community structure of soil nematodes in one wetland perpendicularly oriented from Bohai sea coastline. In June 2006, soil samples were collected from the Yellow River Delta wetlands, in Dongying city of Shandong Province, China. Soil nematode communities were analyzed at the depths of 0–10 and 10–20 cm. The results showed that plant parasite nematodes were the most abundant trophic groups in both depths and at four sites. The average relative abundance was 91.33% of the nematode community. Several ecological indices which reflected soil nematode community structure, diversity, maturity and plant parasitism were compared in these four sites. The results indicated that the maturity index (MI) and plant parasitism index (PPI) were more sensitive than the other indices for assessing the response of soil nematode communities to soil of coastal wetland.

**Keywords:** soil nematode; distribution; coastline; salt content

Nematodes are the most numerous of all metazoans. They constitute one of the most numerically important components of the soil fauna (Warwick et al. 2002) and thus significantly impact nutrient cycling and primary productivity in diverse ecosystems. Moreover, nematode populations can respond in predictable ways to ecosystem disturbance (Freckman and Ettema 1993). Therefore, nematode community composition can be used as sensitive indicators of ecosystem change (Bongers 1990). For example, nematode communities significantly respond to soil physiochemical conditions, such as temperature, moisture content (Pen-Mouratov et al. 2004), and organic matter (Yeates and Coleman 1982). In recent years, many researchers have investigated nematode community in natural ecosystems and agroecosystems, including variations associated with land management differences (Liang et al. 2002, Okada and Harada 2007) and changes of aboveground vegetation (Bongers and Ferris 1999, Wu et al. 2005). While the nematode communities in different succession stage marenmma remains to be investigated

and little information is available concerning the effects of salt content on the distribution of soil nematodes in marenmma.

Comparison of biological activity along gradients is a powerful tool to understand soil biodiversity. There have been many studies along altitudinal or successional gradients (Coûteaux et al. 2002) and fertility gradients (Yeates et al. 2004). Coastal wetlands play an important role in contributing detritus to estuarine food webs (Haines and Montague 1979). As a key member in soil food webs, nematode affect the decomposition rate of plant litter and the turnover of nutrients from soil organic matter. So it probably plays the same important roles in coastal wetlands. In order to elucidate soil nematode community structure in the wetlands with different distance away from coastline and nematode responses to soil salt content, we investigated the horizontal and vertical variability of soil nematodes in wetland soil, the relationship between the variability and soil salinity, organic matter content and total nitrogen content in wetland soil.

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## MATERIAL AND METHODS

### Study area and environmental conditions

The work was conducted in the Yellow River delta in Dongying city of northeastern Shandong Province (118°33'–119°20'E, 37°35'–38°12'N) (Figure 1). The climate in this area is warm-temperate continental monsoon climate with dry and windy spring, hot and rainy summer, cool and clear autumn, and a cold, dry and snowless winter. The mean annual temperature is 11.9°C, with an average annual precipitation of 592 mm. The soil consists of coastal saline soils.

Four experimental sites were selected according to the typical over ground vegetation formed by different succession stage of coastal soil. Site 1 was bare soil, which was about 6 km away from the coastline and only a few *Suaeda salsa* occurred. This site always inundated by sea water during the tidal cycle (not every tidal cycle). Site 2 was about 8 km away from the coastline. The dominant vegetation in this site was *Suaeda salsa* L. spp., and companion species were *Tamarix* L. spp., *Phragmites australis*. Site 3 was about 11.5 km away from the coastline. In site 3, the dominant vegetation was *Tamarix* L. spp., companion species were *Suaeda salsa* L. spp., *Phragmites australis* spp., *Imonium aureum* L. spp. and *Cynanchum* spp. Site 4 was about 16 km away from the coast. The main vegetation was *Imperata cylindrical* spp.; companion species included *Aeluropus sinensis*, *Apocynum venetum* L. spp., *Limonium sinense* (Girard) Kuntze spp., *Cirsium setosum* (Willd.) Bess. ex Bieb. spp., *Phragmites australis* (Cav.) Trin. ex Steud. spp., *Sonchus oleraceus* L. Soil physicochemical characteristics were given in Table 1.

### Sampling procedure and sample analysis

The soil samples were taken at depths of 0–10 cm and 10–20 cm and collected from the four sites



Figure 1. Study areas shown in the map (the dot in the map is sampling areas)

in June 2006. At each site, three plots (5 m × 5 m) were selected randomly, ten cores (5 cm inner diameter) of soil from each plot were collected randomly with a soil auger and put in a plastic bag, mixed together into a composite sample (around 1000 g) of 0–10 cm and 10–20 cm respectively. Three replicate soil samples were collected from each site. Each sample was placed in an individual plastic bag and then was sealed. All samples were kept at 4°C for biological and chemical analysis.

Soil samples were gently and fully mixed by hand and then divided into aliquots for analyzing soil nematodes, soil moistures, salt content and organic matter determinations. Soil samples were dried at 105°C for gravimetric determination of soil water content. Soil organic matter was analyzed by the potassium dichromate-volumetric method. Soil total N was determined by the method of the Kjeldahl (Bao 2000). Soil salt content was measured by conductance (Collins et al. 2000).

Nematodes were extracted from a 100 g soil sample with a sugar flotation and centrifugation procedure and preserved in TAF (triethanolamin formalin) (Li et al. 2007a), all extracted nematodes in each sample were counted and were identified according to order, family, and genus (if possi-

Table 1. Physicochemical properties of the coastal wetland in the study

| Site | Distance from coastline (km) | Soil salt content (g/kg) |         | Soil water content (%) |          | Organic matter content (g/kg) | Total nitrogen content (g/kg) |
|------|------------------------------|--------------------------|---------|------------------------|----------|-------------------------------|-------------------------------|
|      |                              | 0–20 cm                  | 0–10 cm | 0–10 cm                | 10–20 cm | 0–20 cm                       | 0–20 cm                       |
| 1    | 6.0                          | 18.25*                   | 27.20   | 27.20                  | 27.73    | 2.40                          | 0.21                          |
| 2    | 8.0                          | 14.32                    | 24.95   | 24.95                  | 30.08    | 4.48                          | 0.25                          |
| 3    | 11.5                         | 12.18                    | 17.28   | 17.28                  | 19.50    | 5.93                          | 0.33                          |
| 4    | 16.0                         | 2.82                     | 20.47   | 20.47                  | 21.10    | 15.90                         | 0.75                          |

\*the values are the means of three replicates

ble) using a compound microscope. Nematode populations were expressed as per 100 g dry soil (Li et al. 2007a).

### Ecological indices and statistical analysis

The nematode community was analyzed by the following approaches:

- (1) absolute numbers of individuals per 100 g dry soil;
- (2) trophic structure: the classification of trophic groups was assigned to bacterivores (B), fungivores (F), plant parasites (PP), and omnivores-predators (OP) based on known feeding habits or stoma and esophageal morphology (Yin 1998, Xie 1999);
- (3) diversity and ecological indices of the soil nematode community were calculated by the following formulae:

Shannon-Wiener index:

$$H' = - \sum_{i=1}^s p_i \ln(p_i) \quad (\text{Pen-Mouratov and Steinberger 2005})$$

Simpson diversity ( $S_g$ ) (which gives more weight to common genera):

$$S_g = 1 / \sum p_i^2 \quad (\text{Freckman and Ettema 1993})$$

where:  $p_i$  is the proportion of the  $i^{\text{th}}$  taxon individual in the total population and  $S$  is the number of total population in the  $i^{\text{th}}$  sample

Maturity index (MI) and plant parasite index (PPI):

$$MI = \sum_{i=1}^n v(i) \cdot f(i)$$

$$PPI = \sum_{i=1}^n v(i) \cdot f(i)$$

where:  $v(i)$  is the c-p (colonizer-persister) (MI for non-plant parasites and PPI for plant parasites) value of taxon  $i$  (Bongers 1990, Freckman and Ettema 1993), and  $f(i)$  is the frequency of that taxon in a sample

All data were subjected to the statistical analysis of variance using the SAS model (one-way ANOVA). Additionally, a correlation analysis was conducted using SPSS 12.0 to determine the relationships between soil salt content versus total nematodes and the trophic groups. Differences with  $P < 0.05$  were considered significant.

## RESULTS AND DISCUSSION

### Nematode community structure

In our study, 34 families/genera were found in four stations (Table 2). The number of nematodes in one soil sample ranged from 11 to 4809 individuals/100 g dry soil in the study. The average number of nematodes reached the maximum ( $4\,319 \pm 396$ ) at 0–10 cm depth in site 4, and the minimum ( $13.5 \pm 2.0$ ) was found at 10–20 cm depth in site 1 (Table 3). The total number of nematodes in site 4 was significantly higher than in the other sites ( $P < 0.0001$ ;  $df = 3.11$ ). The number of soil nematodes in different sites correlated negatively with soil salt content, and the correlation was  $r = -0.9323^{**}$  ( $P < 0.01$ ).

The compositions of the nematode communities in the sites were compared. The number of families/genera at 0–10 cm was higher than at 10–20 cm in every site. Additionally, the nearer to the sea line the site was, the higher was the number of families/genera in both depths. The total number of families/genera in the four sites correlated negatively with soil salt contents and the correlation coefficient was  $-0.8477$  ( $P < 0.01$ ). Significant differences occurred in the numbers between site 4 and site 1 in both 0–10 cm ( $\alpha = 0.05$ ;  $F = 11.50$ ;  $df = 3.11$ ;  $P = 0.0028$ ) and 10–20 cm ( $\alpha = 0.05$ ;  $F = 14.66$ ;  $df = 3.11$ ;  $P = 0.0013$ ) depths. No significant differences were found for the number of families/genera between site 4 and site 2 and between site 2 and site 3 in both soil depths. The number of families/genera in 0–10 cm depth was significantly higher than in 10–20 cm depth. No significant differences were found between two depths in same site ( $F = 0.64$ ;  $df = 1.23$ ;  $P = 0.6015$ ). However, the total number of families/genera in the four stations were apparently different ( $F = 106.15$ ;  $df = 3.11$ ;  $P < 0.0001$ ).

In this study, as the distance from the coastline increased, nematode population and community increased, soil salt content decreased and organic matter content and total nitrogen content increased. The nematode abundance correlated closely with the soil organic matter content ( $r = 0.9748$ ;  $n = 4$ ;  $P = 0.025$ ). From the Table 1, the salt content intercorrelated with organic matter content and total nitrogen content. These results suggest that nutrient level and salt content affect the abundance of nematode. The results were in agreement with the findings of Li et al. (2007b) that the abundance of soil nematodes correlates well with changes in soil organic matter, total nitrogen

Table 2. Nematode families/genera identified in the study

| Trophic group         | Family                  | Genera                  | Individuals     | Dominance | c-p <sup>1</sup> |
|-----------------------|-------------------------|-------------------------|-----------------|-----------|------------------|
| Bacterivores          | <i>Cephalobidae</i>     | <i>Eucephalobus</i>     | 12 <sup>2</sup> | +         | 2                |
|                       |                         | <i>Chiloplacus</i>      | 32              | +         | 2                |
|                       |                         | <i>Acrobeloides</i>     | 22              | +         | 2                |
|                       |                         | <i>Cephalobus</i>       | 7               | +         | 2                |
|                       |                         | <i>Acrobeles</i>        | 1               | +         | 2                |
|                       | <i>Plectidae</i>        | <i>Plectus</i>          | 1               | +         | 2                |
|                       |                         | –                       | 18              | +         | 1                |
|                       | <i>Rhabditidae</i>      | <i>Rhabditis</i>        | 5               | +         | 1                |
|                       |                         | <i>Caenorhabditis</i>   | 2               | +         | 1                |
|                       | <i>Diplogastridae</i>   | –                       | 13              | +         | 1                |
|                       | <i>Brevibuccidae</i>    | <i>Brevibucca</i>       | 26              | +         | 1                |
| Fungivores            | <i>Aphelenchidae</i>    | <i>Aphelenchus</i>      | 11              | +         | 2                |
|                       | <i>Paraphelenchidae</i> | <i>Paraphelenchus</i>   | 6               | +         | 2                |
|                       | <i>Aphelenchoididae</i> | <i>Aphelenchoides</i>   | 2               | +         | 2                |
| Plant-parasites       | <i>Tylenchidae</i>      | <i>Malenchus</i>        | 55              | ++        | 2                |
|                       |                         | <i>Tylenchus</i>        | 24              | +         | 2                |
|                       |                         | <i>Boleodorus</i>       | 55              | ++        | 2                |
|                       |                         | <i>Filenchus</i>        | 10              | +         | 2                |
|                       |                         | <i>Lelenchus</i>        | 3               | +         | 2                |
|                       |                         | <i>Miculenchus</i>      | 2               | +         | 2                |
|                       |                         | <i>Psilenchus</i>       | 3               | +         | 2                |
|                       | <i>Nothotylenchidae</i> | <i>Cephalenchus</i>     | 13              | +         | 2                |
|                       |                         | <i>Helicotylenchus</i>  | 3456            | +++       | 3                |
|                       | <i>Hoplolaimidae</i>    | <i>Rotylenchus</i>      | 3               | +         | 3                |
|                       |                         | <i>Pararotylenchus</i>  | 13              | +         | 3                |
|                       | <i>Belonolaimidae</i>   | <i>Tylenchorhynchus</i> | 29              | +         | 3                |
|                       |                         | <i>Merlinius</i>        | 4               | +         | 3                |
| <i>Pratylenchidae</i> | <i>Pratylenchus</i>     | 2                       | +               | 2         |                  |
| <i>Dolichodoridae</i> | <i>Dolichodorus</i>     | 3                       | +               | 3         |                  |
| Omnivores-predators   | <i>Dorylaimidae</i>     | –                       | 149             | ++        | 4                |
|                       |                         | <i>Aporcelaimus</i>     | 18              | +         | 4                |
|                       | <i>Longidoridae</i>     | –                       | 19              | +         | 5                |
|                       |                         | <i>Longidorus</i>       | 1               | +         | 5                |
|                       | <i>Mononchidae</i>      | <i>Mononchus</i>        | 2               | +         | 4                |

<sup>1</sup>c-p (colonizer-persister) values for nematode genera or families were based on Bongers (1990, 1999)

<sup>2</sup>absolute value per 100 g fresh soil

Table 3. The number of individuals and families/genera of soil nematodes at 0–10 cm and 10–20 cm soil depths in the sites

| Site | Number of individual per 100 g dry soil |                            |                             | Number of families/genera  |                           |                         |
|------|---|----------------------------|-----------------------------|----------------------------|---------------------------|-------------------------|
|      | 0–10 cm                                 | 10–20 cm                   | total                       | 0–10 cm                    | 10–20 cm                  | total                   |
| 1    | 17.9* ± 2.0 <sup>b</sup>                | 13.5 ± 2.0 <sup>c</sup>    | 31.3 ± 12.4 <sup>b</sup>    | 2.0 ± 0.8 <sup>c</sup>     | 1.0 ± 0.0 <sup>c</sup>    | 3.0 ± 1.0 <sup>d</sup>  |
| 2    | 267.8 ± 32.6 <sup>b</sup>               | 38.6 ± 1.2 <sup>b</sup>    | 306.4 ± 18.5 <sup>b</sup>   | 11.0 ± 2.9 <sup>a, b</sup> | 6.7 ± 2.0 <sup>a, b</sup> | 17.7 ± 1.2 <sup>b</sup> |
| 3    | 113.6 ± 19.7 <sup>b</sup>               | 33.5 ± 7.1 <sup>b, c</sup> | 147.2 ± 11.3 <sup>b</sup>   | 8.0 ± 0.8 <sup>b</sup>     | 4.3 ± 0.5 <sup>b</sup>    | 12.3 ± 1.2 <sup>c</sup> |
| 4    | 4318.9 ± 395.8 <sup>a</sup>             | 333.3 ± 17.1 <sup>a</sup>  | 4659.9 ± 465.1 <sup>a</sup> | 13.0 ± 2.4 <sup>a</sup>    | 9.7 ± 1.7 <sup>a</sup>    | 22.7 ± 2.1 <sup>a</sup> |

\*within each column, values followed by different letters are significantly different at  $\alpha = 0.05$  (one-way ANOVA) Values shown are the means of three replications,  $\pm$  standard error of mean

and phosphorus concentrations. The results were of much significance in studying the soil nematode community and the entire soil biota. It would be necessary to take much more samples in this area for further studies on other soil variables.

### Trophic groups

In this study, plant parasites were found to be the most abundant trophic group of nematodes in the four sites and in both depths; the relative abundance averaged 91.3%. Within each site, plant parasites were the dominant trophic group, except for both depths of site 3. The relative abundances of plant parasite in 0–10 cm depth of site 4 and in

10–20 cm of site 1 were significantly higher than those in the other sites ( $F = 54.02$ ;  $P < 0.0001$  for 0–10 cm;  $F = 125.46$ ;  $df = 3.11$ ;  $P < 0.0001$  for 10–20 cm). The total populations at 0–10 cm of different sites were higher than those of 10–20 cm (two-way ANOVA,  $F = 219.40$ ;  $df = 1.23$ ;  $P < 0.0001$ ;  $F = 261.31$ ;  $df = 3.11$ ;  $P < 0.0001$ ;  $F = 196.53$ ;  $df = 3.23$ ;  $P < 0.0001$ ) (Figure 2).

Bacterivores were not found in either depth in site 1. As for the number of bacterivores, there was a significant interaction between soil depths and different sites ( $F = 18.50$ ;  $df = 1.3$ ;  $P < 0.0001$ ). The total number of bacterivores in two depths of soils was different in four sites (one-way ANOVA,  $F = 49.73$ ;  $df = 3.11$ ;  $P < 0.0001$  at 0–10 cm;  $F = 26.96$ ;  $df = 3.11$ ;  $P = 0.0002$  at 10–20 cm). In

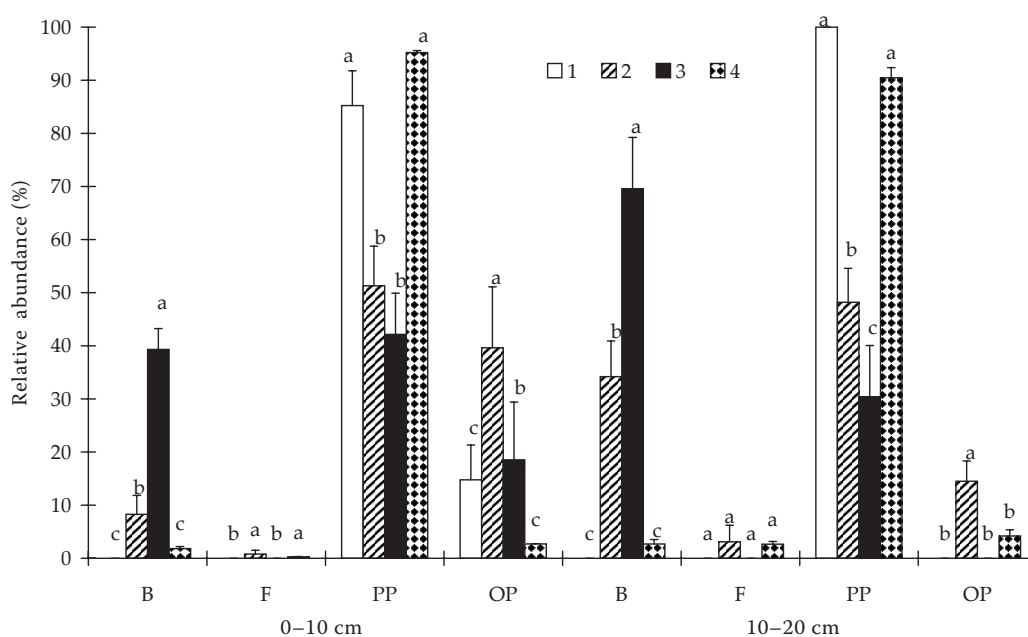


Figure 2. Absolute abundance of nematode trophic groups in different sites B – bacterivores; F – fungivores; PP – plant parasites; OP – omnivore-predators; data are means ( $n = 3$ ); 1, 2, 3 and 4 stand for different distance of sites from coastline – site 1: 6 km, site 2: 8 km, site 4: 11.5 km, site 5: 16 km

both depths, the relative abundance of bacterivores showed significant differences in different sites ( $F = 1715.48$ ;  $df = 3.11$ ;  $P < 0.0001$  at 0–10 cm;  $F = 91.66$ ;  $df = 3.11$ ;  $P < 0.0001$  at 10–20 cm), and their relative abundances in 0–10 cm in four sites were higher than those of 10–20 cm ( $F = 18.50$ ;  $df = 1.23$ ;  $P < 0.0001$ ).

Fungivores were the least abundant trophic group in this study. The average of relative abundance in four sites in both depths was 0.45%. Fungivores were not found in either depth in sites 1 and 3. No differences were found in fungivores abundance in both depths of four sites ( $F = 3.37$ ;  $df = 1.11$ ;  $P = 0.0752$  for 0–10 cm;  $F = 1.15$ ;  $df = 1.11$ ;  $P = 0.3872$  for 10–20 cm).

Abundances of omnivore-predators in all sites in 10–20 cm depth were lower than those of the 0–10 cm depth ( $F = 31.54$ ;  $df = 1.23$ ;  $P < 0.0001$ ). The individual was not observed in sites 1 and 3 in the 10–20 cm depth. In both depths, relative abundance of OP in site 2 was higher than those in other sites (one-way ANOVA,  $F = 9.69$ ;  $df = 3.11$ ;  $P = 0.0049$  for 0–10 cm;  $F = 18.29$ ;  $df = 3.11$ ;  $P = 0.0014$  for 10–20 cm).

A previous report showed that the community of plant parasites is controlled by plant species

(McSorley 1997). Plant root distribution affects the distribution and the relative abundance of various taxa in the nematode community (Wasilewska 1997, Manlay et al. 2000). In our study, plant parasitic nematodes were the most dominant trophic group. The absolute abundance in 0–10 cm depth was lower than in 10–20 cm. Plant-parasitic nematodes of site 4 were the most abundant. This abundance may be related to the vegetation coverage, since plant roots are nematodes' main nutrition resource. The soil of site 1 had been eroded and always leached by seawater so that nutrients were undoubtedly minimal and soil was infertile. Less soil organic matter in site 1 inhibited the reproduction of soil microorganisms, which serve as food for omnivore-predators, bacterivores and fungivores. Thus, there were no bacterivores and fungivores, and only few omnivore-predators in site 1.

### Ecological indices of the nematode community

The Shannon-Wiener index ( $H'$ ) fluctuated in the four sites and in both depths. Differences of Shannon-Wiener indexes were found between

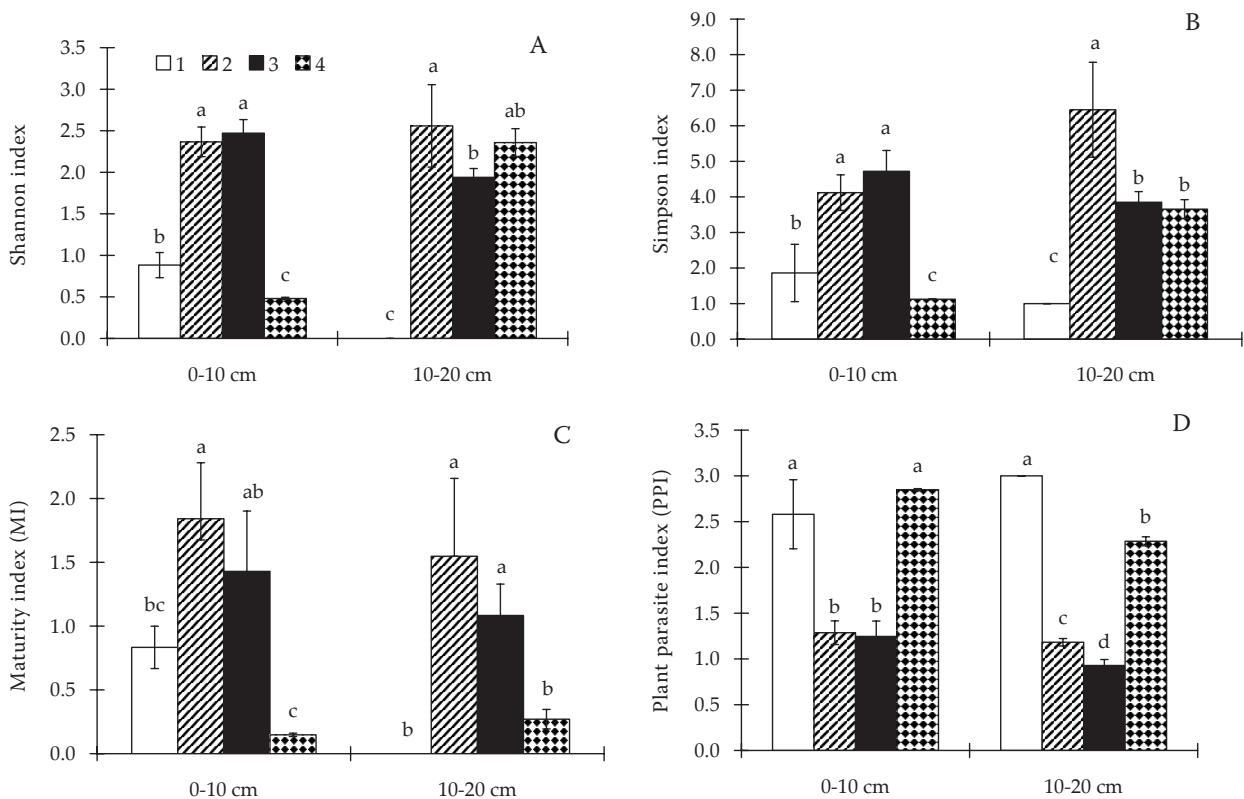


Figure 3. Ecological indices of soil nematodes in different sites at 0–10 cm and 10–20 cm soil depths. Data are means ( $n = 3$ ); 1, 2, 3 and 4 stand for different distance of sites from coastline – site 1: 6 km, site 2: 8 km, site 4: 11.5 km, site 5: 16 km

sites and depths. The order of Shannon indexes were: site 1 < site 4 < site 3 < site 2. All differences except that between sites 3 and 2 were significant. In 0–10 cm depth, the maximum and the minimum value of the index were found in sites 3 and 4, respectively. The values in site 3 and site 2 were significantly higher than those in sites 1 and site 4 (one-way ANOVA,  $F = 151.62$ ;  $df = 1.11$ ;  $P < 0.0001$ ). No differences in the Shannon index were found in 0–10 cm soil between site 2 and site 3. In 10–20 cm depth, the minimum Shannon index was found in site 1. No significant difference was showed in two depths of site 1 ( $F = 3.55$ ;  $df = 1.23$ ;  $P = 0.0804$ ) (Figure 3A).

The Simpson indexes in two depths of different sites showed significant difference as well ( $F = 55.63$ ;  $df = 1.23$ ;  $P < 0.0001$ ). In 0–10 cm depth, the Simpson indexes in site 3 and site 2 were significantly higher than those in site 1 and site 4 ( $\alpha = 0.05$ ;  $F = 36.25$ ;  $df = 1.11$ ;  $P = 0.0003$ ). In 10–20 cm depth, the Simpson index in site 2 was significantly higher than those in the other sites ( $F = 1455.83$ ;  $df = 1.11$ ;  $P < 0.001$ ); the value in site 1 was the lowest. With respect to the Simpson index, there was no significant difference between sites 3 and 4 ( $\alpha = 0.05$ ) (Figure 3B). The value of the maturity index in 10–20 cm depth of site 1 was the lowest. In same site, there were no significant differences found for MI in both depths of sites 2–4 ( $F = 6.56$ ;  $df = 1.23$ ;  $P = 0.0227$ ). The value of the MI in 10–20 cm soil depth in site 1 was 0.0, as the only plant parasitic nematode was observed in the soil layer (Figure 3C). The values of the plant parasite index in the different sites were in the order: site 1 > site 4 > site 2 > site 3. The higher values in 0–10 cm and 10–20 cm depths were observed in site 4 and site 1 and they were significantly higher than those in the same depth at other sites ( $\alpha = 0.05$ ) (Figure 3D).

All ecological indices, except for PPI indices, show the same trend in different sites. A higher Shannon index means greater diversity. In our study, the index was 1.63, which was lower than that in the agroecosystems reported by Freckman and Ettema (1993). The maturity index, based on the composition of the nematode community, can reflect the degree of disturbance of the soil ecosystem (Bongers 1990). The MI and PPI in sites 1 and 4 were statistically similar, which was a consistent trend. Difference of vegetation or soil functions (decomposition and mineralization) may be a preferable explanation. The MI and PPI exhibited an inverse relationship in four sites. This result supported the results of Bongers

(1990). On the other hand, Bongers et al. (1997) reported that the value of MI decreases with increasing nutrient status. However, in our study, the MI in site 1 was the lowest. The result that the MI in site 1 was lower and PPI in site 1 was higher than those in the other sites seems also different from that reported by Bongers et al. (1997). The reason for this difference was probably the poor nutrient status in site 1 (soil salt content was high, organic matter and total nitrogen content were lower). In fact, the environmental factors that can affect the nematode population are very complicated. For example, soil physical chemistry characteristics or up-ground vegetation have the effects on nematode population. Therefore, further studies on soil nematode and soil environment of different succession stage in Yellow River Delta will be necessary.

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