

## NEMATODE COMMUNITY STRUCTURE UNDER COMPOST AND CHEMICAL FERTILIZER MANAGEMENT PRACTICE, IN THE NORTH CHINA PLAIN

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### SUMMARY

A long-term field experiment was conducted at the Qu-Zhou experimental station, China Agricultural University to study how the nematode community structure was influenced by compost and chemical fertilizer, using no amendment as the control. Soil samples were collected from 0–10 cm and 10–20 cm depths. The different treatments led to a significant difference in the total number of nematodes, bacterivores, plant parasites, omnivores-predators and nematode richness. The total number of nematodes, bacterivores, omnivores-predators and nematode richness were found to decrease in chemical fertilizer-treated plots. Although plant parasites were more abundant under compost treatment than under chemical fertilization, the relative abundance of plant parasites in the compost-treated plots was smaller. The application of chemical fertilizer decreased the number of genera of bacterivores and omnivores-predators. The numbers of total nematodes, bacterivores, plant parasites and omnivores-predators were significantly positively correlated with the contents of total organic carbon, total N, alkali-hydrolysable N, available P and available K. The compost-treated plots tended to have a greater diversity of nematodes than chemical fertilizer treated plots, so there was a healthy soil ecosystem under compost treatment.

### INTRODUCTION

Because of the economic value of crop production in agro-ecosystems, most attention has focused on changes in the abundance and diversity of the plant-parasitic nematode community. In contrast, there has been little research on the free-living soil nematode community. However, soil nematodes play an important role in decomposition and nutrient cycling in soil food webs (Bulluck *et al.*, 2002) and nematodes occupy a central position in the soil food web (Neher, 2001). Although nematodes form only a relatively small biomass in soil, their presence at many trophic levels is vitally important in soil environments and ecosystem processes. So, the nematode community provided an insight into the structure and function of soil food webs (Bongers and Bongers, 1998).

In recent years, sustainable agriculture has received increasing attention. Research on relationships between land management and soil organisms is essential for better understanding of soil ecosystems and sustainable development of agro-ecosystems (Ou *et al.*, 2005). Long-term application of chemical fertilizer has been associated with

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many environmental ills, such as loss of soil fertility, soil erosion, reduction of soil biodiversity and ground water pollution. This may affect soil ecosystem health and sustainable development of agriculture. A key to the success of sustainable agriculture will be conservation of natural resources and greater dependence on natural ecosystem processes (Porazinska *et al.*, 1999).

Agriculture field managers may play an important role in monitoring and assessing soil quality in agro-ecosystems and could modify their agricultural field management strategies according to their findings. Analysis of nematode community structure may reveal the effect of agricultural management practices on the soil environment. Soil nematodes are a good candidate for a bioindicator of the status and processes of an ecosystem.

There has been little research on the effects of soil amendment (organic and inorganic) on nematode community structure in the North China Plain. The objectives of this study were to examine the nematode community structure under long-term organic and inorganic fertilizer application in the North China Plain.

#### MATERIALS AND METHODS

##### *Experimental site and design*

A long-term field experiment was initiated in 1997 at Qu-Zhou experimental station, China Agricultural University. The station is in a continental temperate monsoon zone and the climate in the region is warm and sub-humid with summer rainfall and dry, cold winters. The mean annual temperature is 13.2 °C and ranges from a minimum of -2.9 °C in January to a maximum of 26.8 °C in July, mean annual precipitation is 542.7 mm, of which 60% occurs from July to September, and the annual non-frost period is 201 days. The spring drought is very severe. Light, heat and water resources are abundant and shallow surface groundwater has a high mineral content. The soil at the study site is an improved silt fluvo-aquic soil.

The experiment was designed with three treatments and three replications, and nine plots laid out in a randomized complete block design. Plots, 3 m × 10.5 m each, were planted with winter wheat (*Triticum aestivum*) and summer maize (*Zea mays*) every year from the beginning of 1997. The three treatments were: compost (CM) (15 T ha<sup>-1</sup>), chemical fertilizer (CF) (265.5 kg N ha<sup>-1</sup> and 90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) and no amendment (control, CN). Every 50 kg compost was 60% straw (wheat or maize straw), 30% livestock dung, 5% cottonseed-pressed waste and 5% bran. The compost contained 22.8% C, 0.67% N, 96.5 mg kg<sup>-1</sup> NH<sub>4</sub>-N and 215.6 mg kg<sup>-1</sup> NO<sub>3</sub>-N (dry weight basis).

##### *Soil sampling and physico-chemical analysis*

The soil samples were taken from soil depths of 0–10 cm and 10–20 cm on 10 June 2005. Composite soil samples consisting of 10 cores (2.5-cm diameter × 10-cm deep) were collected from each plot. The soil samples were stored in insulated, closed plastic bags to prevent moisture loss and transported to the laboratory where they were kept at 4 °C until biological analyses were conducted. Soil samples were prepared by removing

root fragments and other organic debris, and thoroughly mixed. Soil moisture in each sample was determined by weight loss at 105 °C for 24 h and expressed as percent dry weight.

Soil subsamples were air-dried for 14 d at room temperature, sieved through a 1-mm screen, mixed, and subsamples were assayed for alkaline hydrolysable N, available P, available K and soil pH. The other air-dried subsamples were ground to pass through a 0.25-mm sieve to determine organic matter content and total N. The potassium dichromate external heating method (Blakemore *et al.*, 1972), the semi-micro Kjeldahl method and the alkaline-hydrolysable diffusion method were used to determine organic matter, total N and alkaline-hydrolysable N. Available P was extracted with 0.5 mol l<sup>-1</sup> NaHCO<sub>3</sub> (soil:solution = 1:20) and measured with the Olsen method (Blakemore *et al.*, 1972). Available K was extracted with 1 mol l<sup>-1</sup> NH<sub>4</sub>Ac (soil:solution = 1:10) and measured by flame photometry. Soil pH was measured in 0.01 mol l<sup>-1</sup> CaCl<sub>2</sub> slurry (soil:solution = 1:2.5) using a glass electrode. All the data are expressed on dry mass basis.

### *Biological analysis*

Nematodes were extracted from 100 g composite fresh soil samples using sugar flotation and centrifugation (Bulluck *et al.*, 2002). The nematodes recovered were counted and preserved in 4% formalin. The nematode populations were expressed per 100 g dry soil (soil bulk density was 1.24, 1.39 and 1.37 g cm<sup>-3</sup> in CM, CF and CN at 0–20 cm, respectively) (Pen-Mouratov and Steinberger, 2005). A randomly selected 100 specimens per sample were identified, mainly to genus level if possible, using an inverted compound microscope (Forge *et al.*, 2005). The abundance of each taxonomic group was estimated from its relative abundance and total nematode abundance, and adjusted to the number of nematodes per 100 g dry soil. The nematodes identified were assigned to four trophic groups: (i) bacterivores (BF); (ii) fungivores (FF); (iii) plant parasites (PP); (iv) omnivores-predators (OP), based on known feeding habitats or stoma and oesophageal morphology (Yeates *et al.*, 1993).

### *Statistical analysis*

Two-way variance analysis (ANOVA) was used to detect overall differences between different treatments and between sampling depth. Difference at  $p < 0.05$  level was considered as statistically significant using the LSD (least significant difference) test. All statistical analyses were performed by SPSS software package.

## RESULTS

### *Total number of nematodes*

The total number of nematodes is shown in Figure 1. Significant differences in the total number of nematode were found between treatments ( $p < 0.01$ ) and between depths ( $p < 0.01$ ; Table 1). The total number of nematodes was significantly greater under CM treatment than under CF treatment and in the CN plot, but no difference was found between CF treatment and the CN plot at 0–10 cm depth. Similar results

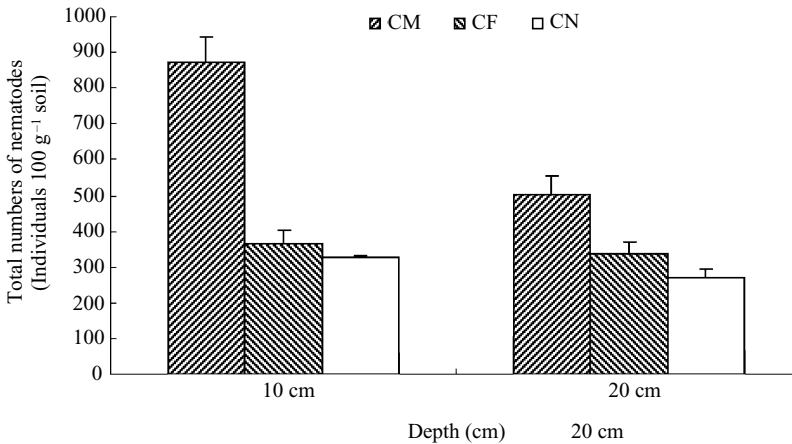


Figure 1. The total number of nematodes in different treatments and depths.

Table 1. Univariate analysis of variance (ANOVA) for number of nematodes and richness.

Index	Treatment		Depth	
	<i>F</i> -test	<i>P</i> value	<i>F</i> -test	<i>P</i> value
Total number of nematodes	52.02	< 0.001	20.30	< 0.01
Trophic group				
BF	45.92	< 0.001	35.22	< 0.001
FF	3.14	0.080	0.038	0.850
PP	16.50	< 0.001	4.09	0.066
OP	22.68	< 0.001	15.36	< 0.01
Nematode richness	21.21	< 0.001	18.94	< 0.01

BF: bacterivores; FF, fungivores; PP: plant parasites; OP: omnivores-predators (absolute numbers).

were obtained for the 10–20 cm depth samples (Figure 1). Under each treatment, the total number of nematodes at 0–10 cm depth was greater than at 10–20 cm (Figure 1). The number of total nematodes was significantly positively correlated with the contents of total organic carbon, total N, alkali-hydrolysable N, available P and available K ( $p < 0.01$ ; Table 2).

### *Trophic groups*

Bacterivores are shown in Figure 2. Significant differences in bacterivores was found between treatments ( $p < 0.01$ ) and between depths ( $p < 0.01$ ; Table 1). Bacterivores were significantly greater under CM treatment than that under CF treatment and the control, but no difference was found between CF treatment and the CN plot at 0–10 cm depth. Bacterivores were significantly greater under CM treatment and in the CN plot than under CF treatment, but there was no difference between the CM treatment and CN plot at 10–20 cm depth (Figure 2). Under CM and CF treatments,

Table 2. Pearson correlation coefficients between soil nematodes and soil chemical properties and wheat biomass.

Indicator	TOC	Total N	Alkali N	Available P	Available K	PB
TN	0.924**	0.919**	0.938**	0.953**	0.946**	0.724*
BF	0.936**	0.892**	0.909**	0.833**	0.960**	0.737*
FF	0.651	0.673*	0.723*	0.653	0.672*	0.437
PP	0.823**	0.826**	0.844**	0.927**	0.845**	0.651
OP	0.914**	0.938**	0.934**	0.899**	0.916**	0.727*

TOC, total organic carbon; TN, total number of nematodes; BF, bacterivores; FF, fungivores; PP, plant parasites; OP, omnivores-predators, PB, 10 wheat plant biomass.

\*\*\*significant at  $p < 0.05$  and  $p < 0.01$ , respectively.

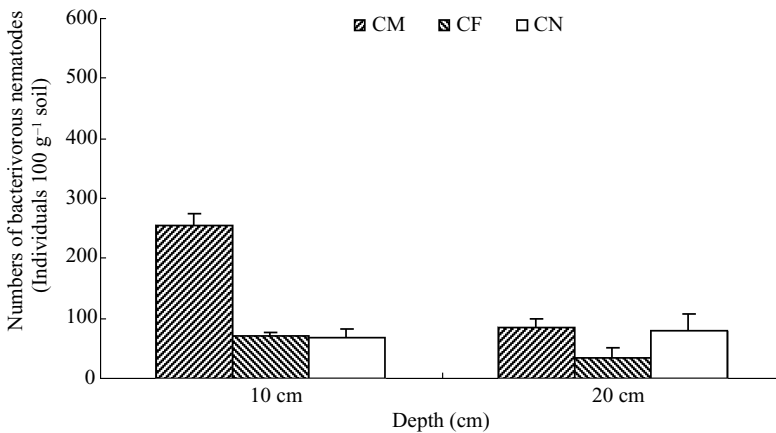


Figure 2. The number of bacterivores in different treatments and depths.

the bacterivores at 0–10 cm depth were higher than that at 10–20 cm (Figure 2). The relative abundance of bacterivores under CM treatment was greater than under CF treatment at 0–10 cm and 10–20 cm depths (Table 3). The bacterivores were significantly positively correlated with the contents of total organic carbon, total N, alkali-hydrolysable N, available P and available K ( $p < 0.01$ , Table 2).

The fungivorous nematodes were the least abundant trophic group in our study (Figure 3). They were significantly greater in CM plots than CF and CN plots, but no difference was found between CF and CN plots in 0–10 cm depth. No difference was found between treatments at 10–20 cm depth (Figure 3). In CF treatment and CN plots, the fungivores at 10–20 cm depth were greater than at 0–10 cm depth, but the fungivores under CM treatment at 10–20 cm depth were less than at 0–10 cm depth (Figure 3). The fungivores was significantly positively correlated with the contents of total N, alkali-hydrolysable N and available K ( $p < 0.05$ , Table 2).

Plant-parasitic nematodes were the dominant trophic group in all treatments (Figure 4). Significant difference in plant parasites was found between treatments

Table 3. The relative abundance (%) of soil nematode in different treatments and depths.

Treatment	0–10 cm			10–20 cm		
	CM	CF	CN	CM	CF	CN
Bacterivores	29.78	19.16	20.76	17.61	10.24	29.52
Cephalobus	10.76	7.25	6.55	1.13	2.84	1.45
<i>Eucephalobus</i>	2.42	1.56	0.88	0.00	1.09	1.46
<i>Acrobeloides</i>	1.20	0.00	1.33	0.00	0.58	0.00
<i>Cervidellus</i>	0.00	0.00	0.00	0.62	0.00	0.00
<i>Acrobeles</i>	0.42	1.03	0.00	1.09	1.72	0.00
<i>Panagrolaimus</i>	3.63	0.00	6.04	1.41	0.00	15.15
<i>Protorhabditis</i>	7.30	5.17	3.00	8.42	2.29	8.40
<i>Poikilolaimus</i>	0.41	0.00	0.00	0.00	0.00	0.00
<i>Rhabditis</i>	3.65	4.16	2.97	4.95	1.72	3.06
Plant parasites	53.72	68.85	70.13	69.42	76.56	56.65
<i>Tylenchus</i>	3.29	4.70	11.27	4.68	4.60	23.01
<i>Filenchus</i>	9.14	6.80	4.76	4.14	0.00	2.49
<i>Psilenchus</i>	0.39	0.51	0.00	0.47	0.00	1.46
<i>Tetylenchus</i>	0.83	0.52	1.33	3.63	1.13	0.47
<i>Brachydorus</i>	1.23	1.06	2.15	0.00	0.00	0.00
<i>Belonolaimus</i>	0.41	0.53	0.00	0.00	0.00	0.00
<i>Tylenchorhynchus</i>	6.41	12.51	5.75	10.95	22.19	11.46
<i>Pratylenchus</i>	13.89	19.50	8.70	26.57	37.48	7.10
<i>Hoplolaimus</i>	0.00	0.00	0.90	0.00	0.00	0.00
<i>Helicotylenchus</i>	9.25	8.75	18.55	9.29	3.88	3.07
<i>Rotylenchus</i>	5.26	9.31	14.54	7.01	3.89	2.01
<i>Paratylenchus</i>	1.97	3.12	0.00	0.62	1.67	0.51
<i>Longidorus</i>	1.23	0.00	1.36	0.47	0.00	0.99
<i>Longidorella</i>	0.41	0.00	0.00	0.00	0.00	0.51
<i>Xiphinema</i>	0.00	1.55	0.83	1.60	1.15	3.58
<i>Trichodorus</i>	0.00	0.00	0.00	0.00	0.57	0.00
Fungivores	6.00	3.16	1.75	4.69	8.03	8.74
<i>Nothotylenchus</i>	2.71	1.06	0.00	0.98	0.57	0.00
<i>Aphelenchus</i>	1.25	0.00	0.90	0.62	0.00	1.55
<i>Aphelenchoides</i>	0.81	2.10	0.42	0.51	6.87	4.59
<i>Tylencholaimus</i>	1.22	0.00	0.43	2.58	0.58	2.60
Omnivores-Predators	10.51	8.82	7.37	8.28	5.17	5.09
<i>Dorylaimus</i>	2.42	0.51	1.25	1.41	2.31	0.52
<i>Mesodorylaimus</i>	1.20	0.52	1.31	0.47	0.58	0.98
<i>Enchodelus</i>	1.25	1.05	1.28	1.60	0.00	0.52
<i>Eudorylaimus</i>	3.59	6.22	1.75	2.26	1.12	0.51
<i>Aporcelaimus</i>	1.62	0.53	0.88	2.07	1.16	2.55
<i>Nyngolaimus</i>	0.00	0.00	0.46	0.00	0.00	0.00
<i>Labronema</i>	0.42	0.00	0.43	0.47	0.00	0.00

CM: compost treatment; CF: chemical fertilizer treatment; CN: control.

( $p < 0.01$ ; Table 1), but there was no difference between depths. The plant parasites were significantly greater under CM treatment than CF treatment and in the control CN plot, but no significant difference was found between the CF treatment and CN plots at 0–10 cm depth. The plant parasites were significantly greater under CM treatment than in the CN plot, but no significant difference was found between the

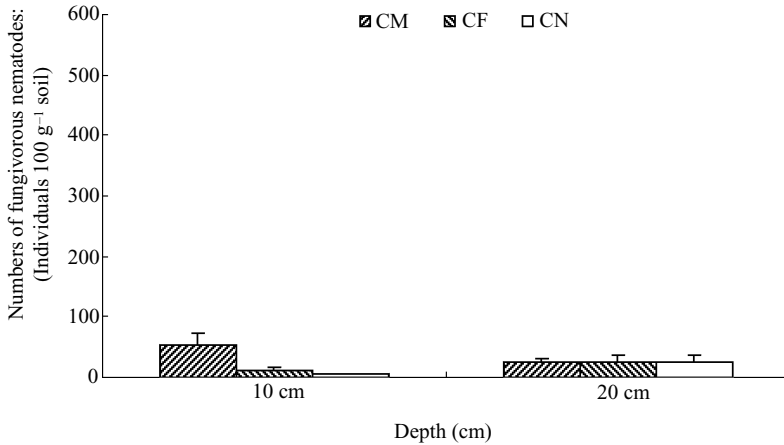


Figure 3. The number of fungivores in different treatments and depths.

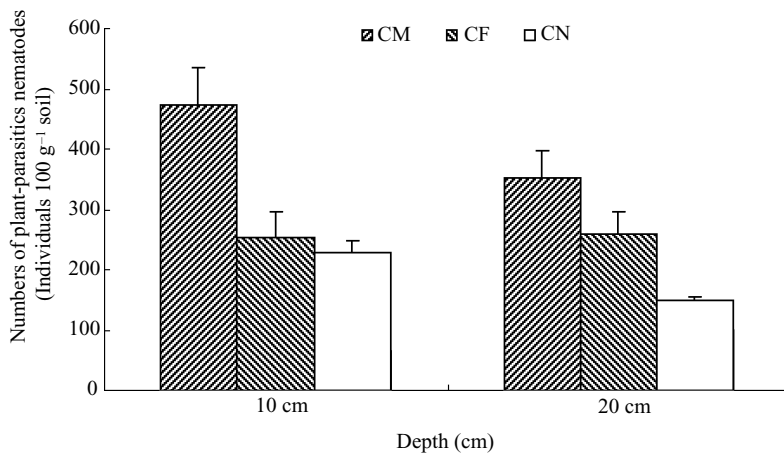


Figure 4. The number of plant parasites in different treatments and depths.

CM treatment and CF plots at 10–20 cm depth (Figure 4). In the CM treatment and CN plots, the plant parasites at 0–10 cm depth were greater than at 10–20 cm depth, but under CF treatment the plant parasites at 10–20 cm depth were greater than at 0–10 cm depth (Figure 4). The relative abundance of plant parasites under CM treatment was less than under CF treatment at 0–10 cm and 10–20 cm depths (Table 3). The plant parasites were significantly positively correlated with the contents of total organic carbon, total N, alkali-hydrolysable N, available P and available K ( $p < 0.01$ , Table 2).

The omnivores-predators are shown in Figure 5. Significant differences in omnivores-predators were found between treatments ( $p < 0.01$ ) and between depths

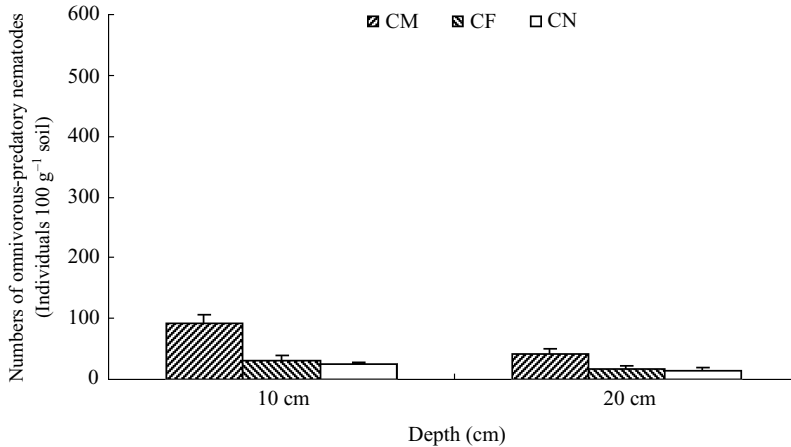


Figure 5. The number of omnivores-predators in different treatments and depths.

( $p < 0.01$ ; Table 1). The omnivores-predators were significantly greater under CM treatment than in the CF treatment and CN plots, but no difference was found between the CF treatment and CN plots at 0–10 cm and 10–20 cm depths (Figure 5). Under each treatment, the omnivores-predators at 0–10 cm depth were greater than at 10–20 cm (Figure 5). The relative abundance of omnivores-predators under CM treatment was greater than in the CF treatment and CN plots at 0–10 cm and 10–20 cm depths (Table 3). The omnivores-predators were significantly positively correlated with the contents of total N, alkali-hydrolysable N, available P and available K ( $p < 0.01$ , Table 2).

#### *Nematode richness*

Thirty-six genera were recorded in our study. There were 33, 27, 30 genera, respectively, under CM, CF treatment and CN plot. In the CM treatment, there were nine genera of bacterivores, 14 genera of plant parasites, four genera of fungivores and six genera of omnivores-predators; in the CF treatment, there were six genera of bacterivores, 13 genera of plant parasites, three genera of fungivores and five genera of omnivores-predators; in the CN plot there were six genera of bacterivores, 14 genera of plant parasites, three genera of fungivores and seven genera of omnivores-predators (Table 3). A significant difference in nematode richness was found between treatments ( $p < 0.01$ ) and depths ( $p < 0.01$ , Table 1). Nematode richness was significantly greater under CM treatment than under CF treatment, but no difference was found between the CF treatment and CN plots at 0–10 cm depth. Nematode richness were significantly greater in the CF treatment and CN plots than that under CF treatment, but no difference was found between the CM treatment and CN plots at 10–20 cm depth (Figure 6). Under each treatment, the nematode richness at 0–10 cm depth was greater than that at 10–20 cm depth (Figure 6).



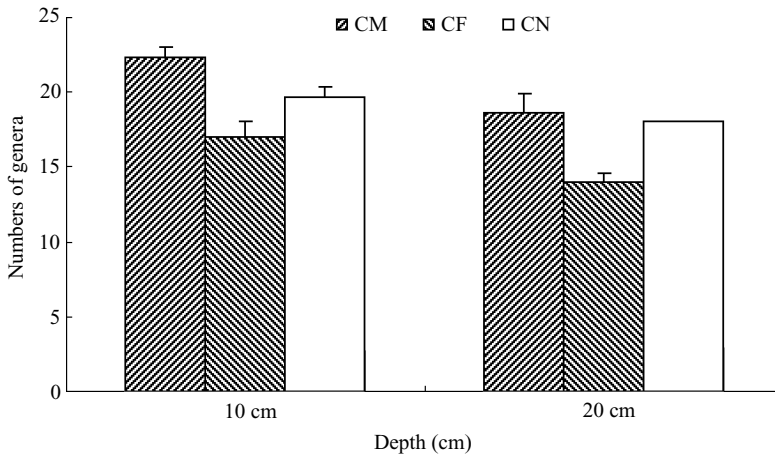


Figure 6. The nematode richness in different treatments and depths.

Table 4. Wheat biomass and yield in soils different treatments in 2005.

Treatment	Plant biomass (g/10 plants)	Wheat yield (t ha <sup>-1</sup> )
CM	36.88 ± 3.65	5.86 ± 0.01
CF	24.98 ± 2.71	5.34 ± 0.01
CN	13.56 ± 0.39	1.67 ± 0.06

CM: traditional compost, CF: chemical fertilizer, CN: control. Values are means ± *s.e.*

#### *Wheat biomass and yield*

Wheat biomass and yield are shown in Table 4. Wheat biomass and yield were significantly greater under CM than in the CF treatment and CN plots, and significantly greater under CF treatment than in the CN plot. Wheat biomass was positively correlated with total numbers of nematodes, bacterivores and omnivores-predators ( $p < 0.05$ ).

#### DISCUSSION

The mean number of nematodes at this experimental site was 269–874 individuals 100 g<sup>-1</sup> dry soil, which is greater than that obtained by Liang *et al.* (2002) in the black soil region of Northeast China (45–260), and by Zolda (2006) in semi-natural steppe grasslands (185–590). The total number of nematodes in the compost treatment was significantly greater than that in the chemical fertilizer treatment and control plot. This may be due to more abundant food in the compost-treated plots. The total number of nematodes in the chemical fertilizer treatment was greater than that in the control plot, because greater residual plant biomass was produced following chemical fertilizer treatment.

The number of bacterivorous nematodes in the compost treatment was greater than that in the chemical fertilizer treatment and control plots. This increase could be linked directly to higher bacterial populations that were associated with the input of compost in these treatments (Bulluck *et al.*, 2002). This is consistent with previous findings that bacterivorous nematodes were more prevalent under compost than chemical fertilizer treatment (Ferris and Matute, 2003).

Plant-parasitic nematodes were the dominant trophic group in our experiment. This was consistent with the result reported by Ou *et al.* (2005) for maize fields, and by Neher (1999). The plant-parasitic nematodes were more abundant in the compost treatment than the chemical fertilizer treatment at both depths, because there was more food for plant-parasitic nematodes under the compost treatment (Wang *et al.*, 2006), although the relative abundance of plant-parasitic nematodes was greater in the chemical fertilizer treatment. There was a greater nematode population in chemical fertilizer treatment than the control because more root biomass was produced under chemical fertilizer treatment, thus providing more feeding sites for plant-parasitic nematodes. One major negative impact of chemical fertilizer on soil health was the increase in the relative abundance of plant-parasitic nematodes compared to the compost treatment.

The omnivorous-predatory and fungivorous nematodes were the least abundant trophic group in our study. Compost also stimulated the abundance and percentage of fungivores compared to chemical fertilizer at 10 cm depth. This result is consistent with previous findings (Wang *et al.*, 2004), as is our observation of increased omnivorous-predatory nematode abundance in compost treatment (Wang *et al.*, 2006). The predatory nematodes may suppress plant-parasitic nematodes through predation. The enhancement of predatory nematodes also may contribute to increased nutrient mineralization as nutrients from microbes consumed by bacterivorous nematodes are released at the predatory trophic levels (Yeates and Wardle, 1996). This is an important process in maintaining sustainable soil utilization because availability of nutrients from the soil organic matter to plants relies on the mineralization of nutrients from their immobilized forms. Significant differences were found between treatments for total number of nematodes, bacterivorous and omnivorous-predatory nematodes. These showed that the total number of nematodes, bacterivorous and omnivorous-predatory nematodes were sensitive to soil amendments. Ou *et al.* (2005) reported that the number of total nematodes, bacterivorous and plant-parasitic nematodes were positively correlated with the contents of total organic carbon, and alkali-hydrolysable N. This result was in agreement with our study. This indicated that nematodes could enhance soil mineral nutrients.

The number of nematode genera (36) in our experimental site was less than that in alpine habitats (Hoschitz and Kaufmann, 2004) and at an intertidal marsh (Wu *et al.*, 2005), but similar to that observed by Thornton and Matlack (2002). Nematode richness, as indicated by the number of genera (Ekschmitt *et al.*, 2001), reflects biodiversity of soil habitat. There were more nematode genera in the compost treatment than that in the chemical fertilizer treatment, so this reflected a greater biodiversity in compost treatment. The other negative impact

of chemical fertilizer on soil health was the decrease soil biodiversity. In particular, application of chemical fertilizer decreased genera of bacterivorous and omnivorous-predatory nematodes, which were tightly correlated with nutrient cycling. Accordingly, sustainable development of agriculture was influenced.

In conclusion, a negative impact of chemical fertilizer on the soil nematode community and soil ecosystem health was found in our study. This experiment was only conducted in the summer. Therefore, further study of the seasonal dynamics of soil nematode community is needed in order to understand better the effect of different fertilizers on soil nematode community, nutrient cycling and the soil ecosystem.

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