

Soil nematode abundances were increased by an incremental nutrient input in a paddy-upland rotation system

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Article info

Received January 12, 2018
 Accepted June 14, 2018

Summary

To study the effect of fertilization on soil nematode communities in a paddy-upland rotation system, an ongoing thirty-three years long-term fertilizer experiment is conducted which includes seven treatments; an unfertilized treatment (control), nitrogen (N), phosphorus (P), potassium (K) fertilizer treatments (N, NP, NPK) and organic manure (M) combined with chemical fertilizer treatments (MN, MNP, and MNPK). The soil nematode community structure and crop yields were determined in 2012 and 2013. Overall total nematode abundance was increased by an incremental nutrient input both in the rice and wheat fields. Total number of nematode was 1.25 – 2.37 times greater in the rice field and was 1.08 – 2.97 times greater in wheat field in the fertilization treatments than in the unfertilized treatment. Soil free-living nematode abundances was significantly ($P < 0.001$) increased in organic manure combined with chemical fertilizer treatments in rice field. Fungi-feeders and plant-feeding nematodes abundances were not significantly different among treatments in rice and wheat fields. Omnivorous and predatory nematodes were the most dominant groups in the present study. Omnivores, predators and *Prodorylaimus* abundances were significantly ($P < 0.05$) higher in organic manure combined with NPK fertilizer treatments than in chemical fertilizer alone and unfertilized treatments both in rice and wheat fields. Stepwise regressions revealed that soil free-living nematodes were significant predictors of rice grain yields ($R^2 = 0.56$, $P < 0.001$) and omnivorous and predatory nematodes were significant predictors of the wheat grain yield ($R^2 = 0.89$, $P < 0.001$). Therefore, long-term application of organic manure combined with chemical fertilizer could increase nematode abundances and crop yields. Organic manure combined with chemical fertilizer application was recommended in agricultural ecosystem.

Keywords: soil nematode community; trophic group; *prodorylaimus*; crop yields; long-term fertilizer experiment

Introduction

Maintaining sustainable soil utilization in developing countries is essential to supply the demand for food grain production. However, steady crop yields cannot be maintained without the application of some soil amendments (Gruzdeva *et al.*, 2007). Long-term

and large numbers of chemical fertilizer applications have led to the loss of soil fertility, a reduction in soil biodiversity, and polluted farm products (Singh *et al.*, 2016; Ding *et al.*, 2017). Therefore, the application of organic fertilizer has come under consideration. The benefits of long-term manure application to soil include better tilth, improved water-holding capacity and cation exchange capa-

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city, moderated soil temperature, enhanced crop performance, and increased soil organic matter and biological fertility (McSorley & Gallaher, 1997). Accordingly, soil fertility and crop yields are increased and good physical or chemical soil properties and biological communities are maintained. However, the long-term application of chemical fertilizers leads to soil hardening, weak aeration, and weak biological activity (Hopkins & Shiel, 1996).

Paddy rice-upland wheat crop rotation systems are one of the largest agricultural production systems in the world, mainly distributed in southern and eastern Asia (Brar *et al.*, 2013). The 13.5 million hectares were in South Asia, and the other 10.5 million hectares are in South China, widely disseminated throughout the provinces of Jiangsu, Zhejiang, Hubei, Guizhou, Yunnan, Sichuan and Anhui, etc. (Hu *et al.*, 2015). This area accounts for roughly half of the total rice cultivated area in China. Therefore, the protection of the soil environment in the rice-wheat crop system is crucial for the maintenance of sustainable crop yields and to ensure China's food security (Zhang *et al.*, 2016).

Long-term field experiments are indispensable sources of knowledge and provide a tool for examining soil environment changes (Debreczeni & Körschens, 2003). Furthermore, long-term fertilizer experiments could reflect the influence of fertilizer benefit, crop growth and soil environment due to annual changes in climate (Miao *et al.*, 2011; Wei *et al.*, 2016). Some information could be gained regarding sustainable agriculture and humanity's influence on the soil fertility, landscape and agricultural environment (Diacono & Montemurro, 2010; Miles & Brown, 2011; Peterson *et al.*, 2012; Geisseler & Scow, 2014).

Soil free-living nematode communities are known to be sensitive to agricultural management practices such as fertilization, irrigation, and tillage (Porazinska *et al.*, 1999; Zhang *et al.*, 2017a). Furthermore, soil nematodes can be placed into at least five functional

or trophic groups. They occupy a central position in the soil detrital food web and play a crucial role in soil organic-matter decomposition, plant-nutrient mineralization, and nutrient cycling (Neher, 2001). Therefore, soil nematode diversity, species composition, trophic group, and biomass may be useful bioindicators for monitoring soil environmental changes (Gutiérrez *et al.*, 2016; Scharroba *et al.*, 2016; Zhao *et al.*, 2016; Čerevková *et al.*, 2017). Liang *et al.* (2009) reported that total nematode abundance responded positively to manure treatments in monoculture maize fields. Hu & Qi (2013) found that long-term application of micro-organic compost could increase nematode abundance in wheat fields. So far, all studies about the effects of fertilization on the nematode community have mainly focused on the upland cropping systems. However, few investigations have reported the effects of long-term application of inorganic and organic fertilizer on soil nematode communities in the paddy-upland rotation system. Therefore, the aims of this study are to (1) determine the impacts of long-term fertilization on the soil nematode community, (2) compare the differences of the nematode communities under organic manure and inorganic fertilizer treatments in the paddy rice-upland wheat rotation systems.

Materials and Methods

Study site

An ongoing long-term (thirty-three years) fertilizer experiment in a rice-wheat rotation system was initiated from rice cultivation in 1981, belonging to the National Fertilizer Experiment Monitoring Network at Nanhu Experimental Station, Hubei Academy of Agricultural Sciences in Wuchang China, located at latitude 30°28'N, longitude 114°25'E and at an altitude of 20 meters in central China. The experimental site lies in a subtropical monsoon zone, which

Table 1. Soil properties (0–20 cm) in different fertilization treatments in October 2012.

Treatment	Organic Matter (g kg ⁻¹)	Total N (g kg ⁻¹)	Alkaline N (mg kg ⁻¹)	Available P (mg kg ⁻¹)	Available K (mg kg ⁻¹)	pH
Control	21.69c	1.64c	112.22b	8.21c	105.63bc	7.17ab
N	22.02c	1.79bc	113.88b	8.71c	94.77bc	7.37ab
NP	26.02b	1.86bc	129.51b	18.71c	93.12c	7.57a
NPK	26.54b	1.88b	134.35b	18.33c	117.66bc	7.22ab
MN	36.74a	2.71a	188.98a	130.69b	139.14bc	7.22ab
MNP	34.72a	2.61a	173.51a	136.73b	141.27b	7.30ab
MNPK	34.04a	2.70a	189.01a	151.32a	205.94a	7.03b

Control, the unfertilized treatment; N, the inorganic nitrogen fertilizer treatment; NP, the inorganic nitrogen and phosphorus fertilizer treatment; NPK, the inorganic nitrogen, phosphorus and potassium fertilizer treatment; MN, the manure plus inorganic nitrogen fertilizer treatment; MNP, the manure plus inorganic nitrogen and phosphorus fertilizer treatment; MNPK, the manure plus inorganic nitrogen, phosphorus and potassium fertilizer treatment.

Different letters (a, b, c) in the same column indicate significant differences ($P < 0.05$) among treatments according to LSD multiple comparison.

is characterized by hot summers and severe winters, and occasional snowfall during winter. The mean annual temperature is 13 °C, ranging from a minimum of 3.7 °C in January to a maximum of 28.8 °C in July. The mean annual precipitation is 1300 mm, and the annual non-frost period is 240 days from 1981 to 2013. The soil at the experimental site is yellow-brown soil, belonging to the Albic Luvisol in the FAO classification, which has a clay-loam texture with 15 % sand, 36 % silt, and 49 % clay. Soil physical-chemical properties in October 2012 at an 0 – 20 cm depth were shown in Table 1.

Experimental design

The field experiment was constituted of seven treatments and three replicates in a randomized complete block design. Each plot was 40 m² (5 m width × 8 m length). The seven treatments were as follows: (1) the unfertilized treatment, control; (2) the inorganic N fertilizer treatment, N; (3) the inorganic N and P fertilizer treatment, NP; (4) the inorganic N, P and K fertilizer treatment, NPK; (5) the manure plus inorganic N fertilizer treatment, MN; (6) the manure plus inorganic N and P fertilizer treatment, MNP; and (7) the manure plus inorganic N, P and K fertilizer treatment, MNPK. The chemical fertilizers were applied at an annual rate of 150 kg N ha⁻¹, 75 kg P₂O₅ ha⁻¹, and 150 kg K₂O ha⁻¹ (Zhang et al., 2017b). The N,

P, and K fertilizers were applied as urea, ammonium phosphate, and potassium chloride, respectively. 22,500 kg ha⁻¹ of organic fertilizers from pig dung compost was applied to the MN, MNP and MNPK treatments. The pig dung compost contained an average of 282.05 g kg⁻¹ organic C, 15.08 g kg⁻¹ total N, 20.84 g kg⁻¹ P₂O₅, 13.56 g kg⁻¹ K₂O, and 69 % water. 60 % of inorganic fertilizers were applied during the rice growth season and the other 40 % during the wheat growth season, while manure was applied equally (50:50) to the two crops. The N fertilizer was applied three times in the rice growth season. Namely, 40 % of the N fertilizer as basal fertilizer was applied before the cultivating rice, 40 % was applied during the rice tillering stage and 20 % was applied during the rice booting stage. Similarly, the N fertilizer was also applied three times in the wheat growth season. Namely, 50 % of N fertilizer as basal fertilizer was applied before the planting wheat, 25 % during the wheat seedling stage and 25 % was applied during the jointing stage in the wheat growth season. Every year, the P, K fertilizers and manure were applied as basal fertilizer, prior to plough. All basal fertilizers and manure were evenly sprinkled on the soil surface by hand and were incorporated into the plough layer by tillage as soon as possible. Tillage was performed to a depth of 20 cm by a plough, followed by a harrow. The fertilized and unfertilized plots were tilled similarly.

Table 2. The number of soil nematodes (individuals 100 g⁻¹ dry soil) after different fertilization treatments in rice and wheat fields.

	Treatment	FN	BF	FF	PF	OP	<i>Prodorylaimus</i>
Rice field	Control	262.8d	71.9a	6.9a	110.7a	184.1d	82.3d
	N	270.2d	75.2a	8.4a	195.2a	186.6d	93.6d
	NP	339.0d	104.9a	11.6a	181.4a	222.5d	128.7cd
	NPK	477.2c	141.2a	6.0a	173.7a	330.0c	197.6c
	MN	619.1b	113.2a	7.2a	151.7a	498.7b	446.8b
	MNP	704.1ab	66.0a	5.2a	117.0a	633.0a	561.0a
	MNPK	772.2a	129.5a	5.9a	111.7a	636.7a	588.7a
Wheat field	Control	470.2c	65.9bc	10.0a	210.8a	394.3c	178.6d
	N	562.2c	89.0bc	2.5a	173.9a	470.7c	170.0d
	NP	578.6c	134.0ab	0.0a	213.0a	444.7c	176.5d
	NPK	942.8bc	195.2a	11.2a	202.2a	736.3bc	499.7cd
	MN	1238.2b	162.1a	0.0a	232.4a	1076.1b	971.3bc
	MNP	1293.0b	58.8c	3.9a	195.2a	1230.2ab	1103.3ab
	MNPK	1907.2a	195.6a	4.0a	115.9a	1707.6a	1591.2a

FN: the number of free-living nematodes in soil; BF: bacterial feeders; FF: fungal feeders; PF: plant feeders; OP: omnivores and predators. Control, the unfertilized treatment; N, the inorganic nitrogen fertilizer treatment; NP, the inorganic nitrogen and phosphorus fertilizer treatment; NPK, the inorganic nitrogen, phosphorus and potassium fertilizer treatment; MN, the manure plus inorganic nitrogen fertilizer treatment; MNP, the manure plus inorganic nitrogen and phosphorus fertilizer treatment; MNPK, the manure plus inorganic nitrogen, phosphorus and potassium fertilizer treatment.

Different letters (a, b, c) in the same column indicate significant differences ($P < 0.05$) among treatments at the same sampling times according to LSD multiple comparison.

All plots were transplanted with rice seedlings (*Oryza sativa* L.) in summer and were sown with wheat seeds (*Triticum aestivum* L.) in winter, which has been done annually since 1981. The rice was transplanted in June and harvested in October, and the wheat was directly sown in November and harvested in May of the next year. The above-ground crops were harvested with a sickle and removed; thus, no straw returned into the soil in any plot. Nevertheless, rice or wheat stubble and roots were incorporated into the soil with a plow before the subsequent rice or wheat planting. In addition to the fertilizer treatments, all other agronomic managements were identical in the fertilized and unfertilized plots. Rice and wheat grains were separated from straws using a plot

thresher. Grains and straw were weighed after sun-drying and recorded from the whole plot.

Soil sampling

Soil samples from the upper (0 – 20 cm) soil layer were collected in the rice and wheat maturity stages in October 2012 and in May 2013, respectively. Composite soil samples consisting of ten cores (2.5 cm diameter × 20 cm depth) were collected from each plot. Soil samples were stored in tied and insulated plastic bags to prevent moisture loss. They were transferred to 4 °C storage as soon as possible until they were used to analyze the soil nematode community structure.

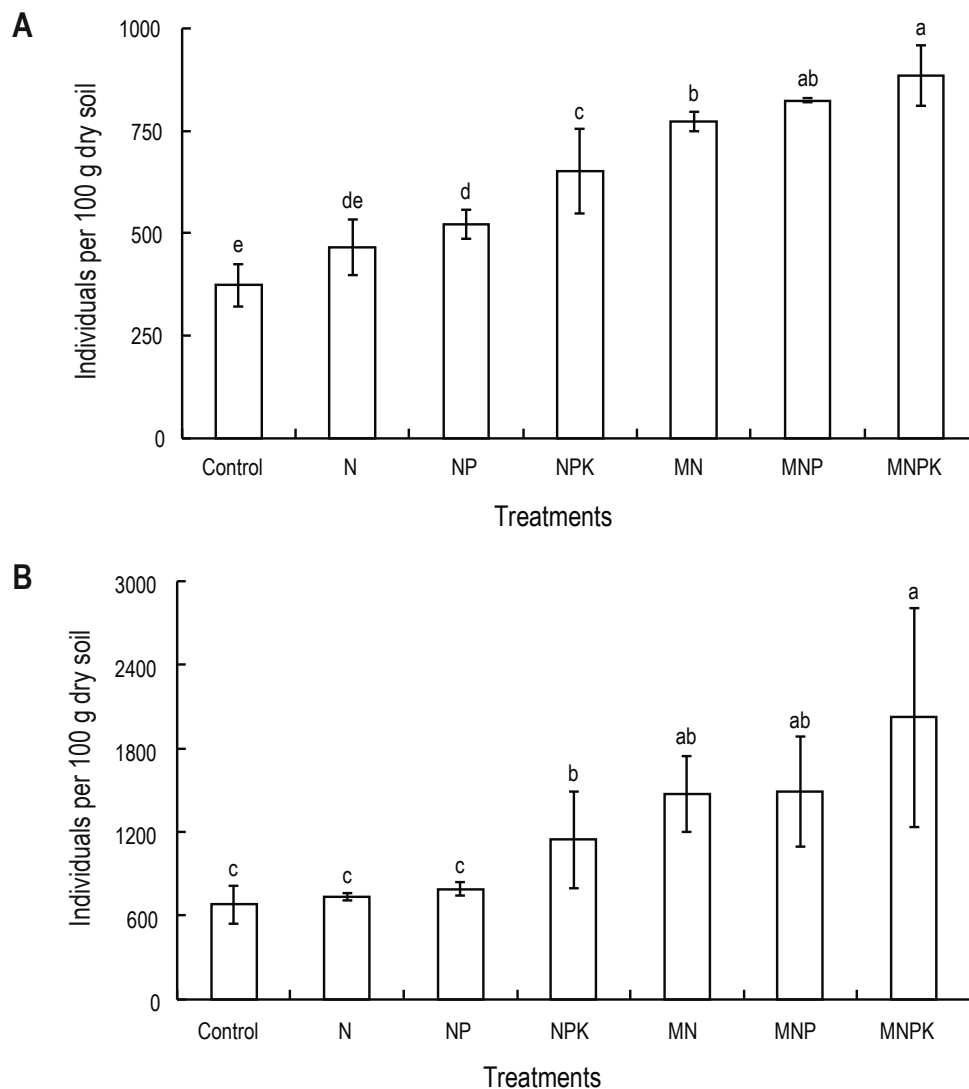


Fig. 1. Total nematode abundance after different fertilization treatments in the rice field (A) and wheat field (B). Control, the unfertilized treatment; N, the inorganic nitrogen fertilizer treatment; NP, the inorganic nitrogen and phosphorus fertilizer treatment; NPK, the inorganic nitrogen, phosphorus and potassium fertilizer treatment; MN, the manure plus inorganic nitrogen fertilizer treatment; MNP, the manure plus inorganic nitrogen and phosphorus fertilizer treatment; MNPK, the manure plus inorganic nitrogen, phosphorus and potassium fertilizer treatment. Data are means \pm SD ($n = 3$). Different letters (a, b, c) above the column indicate significant differences ($P < 0.05$) among treatments according to LSD multiple comparison.

Soil physical-chemical properties analysis

Soil samples collected from the 0 – 20 cm were air-dried at room temperature, mixed, sieved through a 1-mm screen, and sub-samples were used to analyze soil physical-chemical properties. The air-dried sub-samples were ground to pass through a 0.25-mm sieve to determine soil organic matter and total N contents. The potassium dichromate external heating method was used to determine soil organic matter content (Blakemore *et al.*, 1972). The Kjeldahl method and the alkaline-hydrolysable diffusion method were used to determine total N and alkaline-hydrolysable N content (Bremner, 1996). Soil available P was extracted with 0.5 mol L⁻¹ NaHCO₃ (soil: solution=1:20) and measured with the Olsen method (Olsen *et al.*, 1954). Soil available K was extracted with 1 mol L⁻¹ NH₄Ac (soil: solution=1:10) and measured with the flame photometry method (Carson, 1980). Soil pH was measured with 0.01 mol L⁻¹ CaCl₂ slurry (soil: solution=1:2.5) using a glass electrode (Lu, 1999).

Nematode analysis

Field moist subsamples (100 g) of each sample were used to extract nematodes with the sugar flotation and centrifugation method (Barker *et al.*, 1985). The nematode populations were expressed as 100 g⁻¹ dry soil. After counting the total number of nematodes using an anatomical lens, two hundred nematodes per sample were randomly selected and identified to genus according to esophageal and morphology characteristics using an inverted compound microscope (Mal & Lyon, 1975; Ying, 1998).

The characteristics of the nematode communities were described using the following approaches: (1) total number of nematodes per 100 g dry soil; (2) the number of soil free-living nematodes per 100 g dry soil; (3) trophic groups: (a) bacterial feeders; (b) fungal feeders; (c) plant feeders; and (d) omnivore-predators (Yeates *et al.* 1993).

Statistical analysis

One-way variance analysis (one-way ANOVA) was used to detect differences between different treatments. Differences at the $P < 0.05$ level were considered statistically significant under the least significant difference (LSD) test. All statistical analyses and the stepwise regression were performed using SPSS 18.0 version software package.

Ethical Approval and/or Informed Consent

This article does not contain any studies with human participants or animals by any of the authors.

Results

Soil physical-chemical properties

Soil organic matter, total N, alkaline-hydrolysable N and available P content were significantly ($F = 82.892$, $P < 0.001$ in soil organic

matter; $F = 39.068$, $P < 0.001$ in soil total N; $F = 18.383$, $P < 0.001$ in alkaline-hydrolysable N; $F = 224.353$, $P < 0.001$ in available P) higher in the manure combined with chemical fertilizer treatment than in the chemical fertilizer alone and unfertilized treatment. Soil available K content were significantly ($F = 6.443$, $P = 0.002$) higher in the manure combined with NPK fertilizer treatment than in the other fertilization treatments and unfertilized control (Table 1).

Total nematode abundance

Total number of nematodes had an increasing tendency with soil nutrient input increments in rice and wheat fields (Fig. 1). Total number of nematode was 1.25 – 2.37 times greater in rice field and was 1.08 – 2.97 times greater in wheat field in the fertilization treatments than in the unfertilized treatment. In particular, the increase was significant in the NPK and manure combined with the chemical fertilizer treatments ($F = 31.452$, $P < 0.001$ in rice field; $F = 5.253$, $P = 0.005$ in wheat field).

Nematode trophic group abundance

Like the total number of nematodes, the number of soil free-living nematodes (bacterial-feeders + fungal-feeders + omnivores + predators) had an increasing tendency with soil nutrient input increments in rice and wheat fields. In particular, the increase was significant ($F = 41.455$, $P < 0.001$) in the manure combined with chemical fertilizer treatments in rice field. Moreover, the number of soil free-living nematodes were significantly ($F = 9.890$, $P = 0.005$) higher in the NPK treatment than in the NP, N and unfertilized treatments under the rice field (Table 2).

The bacteria-feeding nematode abundance was not significantly different among treatments in rice field; however, the bacteria-feeding nematode abundances were significantly ($F = 6.000$, $P = 0.003$) higher in the MNPK, MN, NPK treatments than in the MNP, N and unfertilized treatments in wheat field (Table 2). The fungi-feeding nematode were the least abundant trophic group in this study. Plant-feeding nematodes were the second-most dominant trophic groups. The number of fungi-feeding and plant-feeding nematodes were not significantly different among treatments in rice and wheat fields. The omnivorous and predatory nematodes were the most dominant groups in the present study (Table 2). The number of omnivore-predators was 1.01 – 3.46 times greater in rice field and was 1.19 – 4.33 times greater in wheat field in the fertilization treatments than in the unfertilized treatment. In particular, omnivore and predator abundances were significantly ($F = 58.636$, $P < 0.001$) higher in the manure combined with the chemical fertilizer treatments than in the chemical fertilizer alone and the unfertilized treatments in rice field. Omnivore and predator abundances were significantly ($F = 5.553$, $P = 0.013$) higher in the MNPK treatment than in the chemical fertilizer alone and unfertilized treatments in wheat field. Omnivore and predator abundances were significantly ($F = 9.640$, $P = 0.005$) higher in the NPK treatment than in the NP, N and unfertilized treatments in rice field. A total of 39 nematode taxa were identified in the present study, of which 11 were bacterial feeders, 5 were fungal feeders, 6 were plant feeders, 12 were omnivores and 5 were predators

Table 3. The number of soil nematode taxa (individuals 100 g⁻¹ dry soil) for each treatment in rice field.

Genus	Guild	Control	N	NP	NPK	MN	MNP	MNPK
<i>Protorhabditis</i>	Ba1	0.0	0.0	1.9	0.0	0.0	0.0	0.0
<i>Rhabditis</i>	Ba1	0.0	0.0	0.0	0.0	0.0	0.0	3.1
<i>Panagrolaimus</i>	Ba1	1.3	3.3	3.7	4.0	7.3	2.6	42.4
<i>Cephalobus</i>	Ba2	4.2	6.6	1.9	0.0	0.0	2.6	0.0
<i>Eucephalobus</i>	Ba2	3.7	1.7	3.4	4.8	4.8	2.6	6.3
<i>Acrobelloides</i>	Ba2	3.3	3.8	0.0	0.0	9.6	0.0	3.1
<i>Monhystera</i>	Ba2	0.0	0.0	0.0	1.9	2.4	0.0	0.0
<i>Plectus</i>	Ba2	3.8	6.7	8.7	17.5	24.8	26.6	13.7
<i>Prismatolaimus</i>	Ba3	4.6	4.6	10.0	30.6	14.7	10.6	30.8
<i>Pseudoaulolaimus</i>	Ba3	48.9	44.2	73.5	80.3	39.8	18.2	24.7
<i>Amphidelus</i>	Ba4	2.0	4.2	1.9	2.0	9.8	2.7	5.2
<i>Aphelenchoides</i>	Fu2	0.0	0.0	1.9	0.0	7.2	5.2	5.9
<i>Tylencholaimus</i>	Fu4	2.5	0.0	1.9	0.0	0.0	0.0	0.0
<i>Doryllium</i>	Fu4	3.1	8.4	7.8	6.0	0.0	0.0	0.0
<i>Leptonchus</i>	Fu4	1.3	0.0	0.0	0.0	0.0	0.0	0.0
<i>Malenchus</i>	H2	20.8	14.9	20.1	4.3	12.5	5.4	0.0
<i>Basiria</i>	H2	43.5	126.4	96.5	98.9	35.3	48.0	39.2
<i>Psilenchus</i>	H2	22.9	22.5	22.3	4.8	4.8	0.0	0.0
<i>Tylenchorhynchus</i>	H3	0.0	0.0	0.0	0.0	32.5	13.4	11.1
<i>Hirschmanniella</i>	H3	22.1	24.8	33.3	56.0	66.6	50.2	61.4
<i>Belondira</i>	H5	1.4	6.6	9.3	9.7	0.0	0.0	0.0
<i>Pristionchus</i>	Om1	0.0	0.0	0.0	0.0	4.8	2.6	5.6
<i>Lordellonema</i>	Om4	0.0	0.0	0.0	0.0	0.0	5.4	0.0
<i>Prodorylaimus</i>	Om4	82.3	93.6	128.7	197.6	446.8	561.0	588.7
<i>Mesodorylaimus</i>	Om4	26.5	18.4	21.1	45.6	2.4	5.3	6.3
<i>Eudorylaimus</i>	Om4	13.9	12.1	0.0	14.2	15.0	15.9	3.1
<i>Thorneella</i>	Om4	1.4	0.0	0.0	0.0	0.0	0.0	0.0
<i>Pungentus</i>	Om4	2.5	1.3	0.0	0.0	0.0	2.7	0.0
<i>Aporcelaimus</i>	Om5	7.1	4.7	3.4	2.0	22.4	13.5	5.4
<i>Sectonema</i>	Om5	1.3	0.0	0.0	2.4	0.0	0.0	0.0
<i>Tripyla</i>	Ca3	0.0	0.0	0.0	2.4	0.0	0.0	0.0
<i>Iotonchus</i>	Ca4	0.0	0.0	0.0	0.0	0.0	2.7	5.4
<i>Mylonchulus</i>	Ca4	0.0	1.3	3.1	2.0	2.6	8.0	10.9
<i>Laevides</i>	Ca5	49.1	55.2	66.1	63.7	4.8	15.9	11.3

Functional guild classifications are made based on the feeding habits and life-cycle characteristics (Ba: bacterivores, Fu: fungivores, Ca: Carnivores, Om: omnivores, H: herbivores) (Yeates, 1993). Suffix numbers are c-p values for the taxon (Bongers & Bongers, 1998). Control, the unfertilized treatment; N, the inorganic nitrogen fertilizer treatment; NP, the inorganic nitrogen and phosphorus fertilizer treatment; NPK, the inorganic nitrogen, phosphorus and potassium fertilizer treatment; MN, the manure plus inorganic nitrogen fertilizer treatment; MNP, the manure plus inorganic nitrogen and phosphorus fertilizer treatment; MNPK, the manure plus inorganic nitrogen, phosphorus and potassium fertilizer treatment.

Table 4. The number of soil nematode taxa (individuals 100 g⁻¹ dry soil) for each treatment in wheat field.

Genus	Guild	Control	N	NP	NPK	MN	MNP	MNPK
<i>Protorhabditis</i>	Ba1	0.0	4.8	0.0	0.0	0.0	0.0	0.0
<i>Rhabditis</i>	Ba1	0.0	0.0	2.6	0.0	0.0	0.0	0.0
<i>Panagrolaimus</i>	Ba1	0.0	11.8	10.5	7.8	23.3	4.0	4.0
<i>Cephalobus</i>	Ba2	2.7	2.3	2.7	6.2	4.0	0.0	0.0
<i>Eucephalobus</i>	Ba2	0.0	0.0	0.0	0.0	4.0	4.0	0.0
<i>Acroboloides</i>	Ba2	0.0	4.8	2.6	0.0	4.0	0.0	0.0
<i>Monhystera</i>	Ba2	4.7	0.0	2.5	3.2	0.0	0.0	13.9
<i>Plectus</i>	Ba2	0.0	14.7	28.3	69.8	30.7	22.2	93.9
<i>Prismatolaimus</i>	Ba3	9.7	21.1	56.5	32.6	47.3	15.7	54.9
<i>Pseudoaulolaimus</i>	Ba3	48.9	29.4	28.3	72.5	49.1	13.0	28.8
<i>Amphidelus</i>	Ba4	0.0	0.0	0.0	3.2	0.0	0.0	0.0
<i>Aphelenchoides</i>	Fu2	2.3	0.0	0.0	0.0	0.0	0.0	0.0
<i>Ditylenchus</i>	Fu2	0.0	0.0	0.0	0.0	0.0	3.9	0.0
<i>Tylencholaimus</i>	Fu4	0.0	0.0	0.0	4.8	0.0	0.0	0.0
<i>Doryllium</i>	Fu4	7.7	2.5	0.0	6.4	0.0	0.0	4.0
<i>Malenchus</i>	H2	0.0	2.3	5.1	14.7	0.0	0.0	0.0
<i>Basiria</i>	H2	136.4	115.7	105.2	75.4	106.1	76.6	36.9
<i>Psilenchus</i>	H2	5.2	7.3	23.0	12.6	5.7	0.0	0.0
<i>Tylenchorhynchus</i>	H3	2.7	0.0	0.0	0.0	34.2	58.3	18.1
<i>Hirschmanniella</i>	H3	66.5	43.8	77.0	88.8	86.4	60.3	60.9
<i>Belondira</i>	H5	0.0	4.9	2.7	10.7	0.0	0.0	0.0
<i>Pristionchus</i>	Om1	6.4	16.9	12.8	3.2	7.9	11.8	13.9
<i>Coomansinema</i>	Om4	0.0	0.0	2.7	0.0	4.0	4.0	0.0
<i>Ecumenicus</i>	Om4	2.3	0.0	0.0	0.0	0.0	0.0	0.0
<i>Prodorylaimus</i>	Om4	178.6	170.0	176.5	499.7	971.3	1103.3	1591.2
<i>Mesodorylaimus</i>	Om4	57.8	79.7	65.0	63.4	13.6	23.4	18.1
<i>Eudorylaimus</i>	Om4	6.4	0.0	2.5	0.0	0.0	7.9	26.7
<i>Thorneella</i>	Om4	7.3	0.0	0.0	0.0	0.0	0.0	0.0
<i>Pungentus</i>	Om4	13.5	12.3	15.3	6.4	0.0	7.9	0.0
<i>Aporcelaimus</i>	Om5	10.5	7.4	2.7	34.5	24.1	18.3	33.9
<i>Labronema</i>	Om4	0.0	7.3	0.0	0.0	0.0	7.9	0.0
<i>Sectonema</i>	Om5	5.0	4.8	5.1	0.0	0.0	0.0	0.0
<i>Tripyla</i>	Ca3	2.3	0.0	10.5	9.6	8.8	3.9	5.9
<i>Miconchus</i>	Ca4	8.0	9.7	12.6	24.1	9.7	16.9	4.0
<i>Iotonchus</i>	Ca4	2.3	9.7	0.0	0.0	9.6	10.4	0.0
<i>Laevides</i>	Ca5	93.7	152.9	139.1	95.5	27.2	14.3	13.9

Functional guild classifications are made based on the feeding habits and life-cycle characteristics (Ba: bacterivores, Fu: fungivores, Ca: carnivores (predators), Om: omnivores, H, plant feeders (herbivores) (Yeates, 1993). Suffix numbers are c-p values for the taxon (Bongers & Bongers, 1998). Control, the unfertilized treatment; N, the inorganic nitrogen fertilizer treatment; NP, the inorganic nitrogen and phosphorus fertilizer treatment; NPK, the inorganic nitrogen, phosphorus and potassium fertilizer treatment; MN, the manure plus inorganic nitrogen fertilizer treatment; MNP, the manure plus inorganic nitrogen and phosphorus fertilizer treatment; MNPK, the manure plus inorganic nitrogen, phosphorus and potassium fertilizer treatment.

Table 5. Crop grain yields after different fertilization treatments.

Treatment	Rice grain yield (t/ha)	Wheat grain yield (t/ha)
Control	3.79d	2.24c
N	4.66cd	2.32c
NP	5.13bc	3.13b
NPK	5.17bc	3.23b
MN	6.04ab	4.56a
MNP	6.10ab	4.65a
MNPK	6.55a	4.74a

Control, the unfertilized treatment; N, the inorganic nitrogen fertilizer treatment; NP, the inorganic nitrogen and phosphorus fertilizer treatment; NPK, the inorganic nitrogen, phosphorus and potassium fertilizer treatment; MN, the manure plus inorganic nitrogen fertilizer treatment; MNP, the manure plus inorganic nitrogen and phosphorus fertilizer treatment; MNPK, the manure plus inorganic nitrogen, phosphorus and potassium fertilizer treatment.

Different letters (a, b, c) in the same column indicate significant differences ($P < 0.05$) among treatments according to LSD multiple comparison.

(Table 3, 4). The number of *Prodorylamus* were significantly ($F = 46.0201$, $P < 0.05$) higher in the manure combined with the chemical fertilizer treatment than in the chemical fertilizer alone and the unfertilized treatments in rice field (Table 2).

Crop yield

Rice and wheat yields were significantly ($F = 3.764$, $P = 0.019$ in rice; $F = 91.648$, $P < 0.001$ in wheat) influenced by the different fertilization treatments (Table 5). Rice grain yields were 1.23 – 1.73 times greater in the fertilization treatments than in the unfertilized treatment. In particular, rice grain yields were significantly ($F = 5.265$, $P = 0.015$) higher in the MNPK treatment than in the chemical fertilizer alone and the unfertilized treatments. Rice grain yields were significantly ($F = 5.415$, $P = 0.014$) higher in the MNPK, MNP, MN treatments than in the N and unfertilized treatments; nevertheless, there were no significant differences between the N and unfertilized treatments.

Wheat grain yields were 1.04 – 2.12 times greater in the fertilization treatments than in the unfertilized treatment. Wheat grain yields were significantly ($F = 91.648$, $P < 0.001$) higher in the manure combined with the chemical fertilizer treatments than in the chemical fertilizer alone and in the unfertilized treatments. Moreover, wheat grain were significantly ($F = 19.641$, $P < 0.001$) higher in the NPK, NP treatments than in the N alone and in the unfertilized treatments; nevertheless, there was no significant difference between the N alone and the unfertilized treatments.

Regression analysis

Stepwise regression equations were conducted to evaluate soil nematode community parameters in rice and wheat fields. These parameters were significantly associated with rice and wheat grain yields. The number of soil free-living nematode was found to be a significant ($F = 23.968$, $P < 0.001$) predictor of rice grain yields. The number of omnivorous and predatory nematodes was found to be a significant ($F = 150.214$, $P < 0.001$) predictor of wheat grain yields (Table 6).

Discussion

Compared to the unfertilized treatment, the total number of nematode was increased in rice and wheat fields due to fertilizer application. In particular, there was a significant increase in the NPK and manure combined with the chemical fertilizer treatments. Similarly, increasing total nematode abundances after application of manure were also reported by Villenave *et al.* (2003), Forge *et al.* (2005) and Hu & Qi (2013). The increase of nematode populations could possibly be linked directly to higher food resources associated with the input of organic matter (Villenave *et al.*, 2003). Increasing total number of nematodes due to application of chemical fertilizer was also reported by other studies (Li *et al.*, 2016; Hu *et al.*, 2017). Villenave *et al.* (2010) also observed that soil free-living nematodes were increased due to organic and mineral fertilizer use.

Table 6. Stepwise regression of grain yields of rice and wheat with the soil nematode community.

	Predictor variable	Constant	Coefficient	R ²	F	P
Rice	FN	3.368	0.004	0.558	23.968	< 0.001
Wheat	OP	1.613	0.005	0.888	150.214	< 0.001

FN: the number of soil free-living nematodes; OP: the number of omnivorous and predatory nematodes.

The values are fitted in the equation: $y = a + bx_1 + cx_2$; y = grain yield; x_1 and x_2 are the independent variables; and a , b and c are coefficients.

Fungi-feeding and plant-feeding nematode abundances were not significantly different among treatments in the present study. This result is not consistent with previous studies showing that organic materials application resulted in an increase of fungal feeders abundances (Ferris & Matute, 2003; Wang *et al.*, 2006; Villenave *et al.*, 2010) and that plant-feeding nematodes were increased due to compost and chemical fertilizer application in a maize field (Hu & Qi, 2010).

The omnivorous and predatory nematodes were the most dominant groups in the present study. In contrast, bacterial feeders were the most abundant group in paddy field (Ou *et al.*, 2005), in yellow squash field (Wang *et al.*, 2006), and in Horqin sandy land (Jiang *et al.*, 2007; Guan *et al.*, 2015). Plant-feeding nematodes were prevalent in maize and wheat fields (Hu & Qi, 2010, 2013; Zhang *et al.*, 2012), in sorghum field (Villenave *et al.*, 2010), and in millet field (Diakhaté *et al.*, 2013). Compared to the unfertilized treatment, the abundance omnivore-predators was increased in rice and wheat fields due to fertilizer use. A similar study also showed that the application of manure to an agricultural field could increase the abundance of omnivorous nematodes (Villenave *et al.*, 2004). Li *et al.* (2010) reported that organic fertilizer application increased the relative abundance of omnivore-carnivores, but different levels of applied nitrogen fertilizer decreased their relative abundance under greenhouse conditions. The composition and abundance of omnivores and carnivores were not affected by the soil amendments (Villenave *et al.*, 2010) and the effect of organic amendment on omnivorous and predatory nematodes was minimal (Wang *et al.*, 2006). However, Forge *et al.* (2005) reported that high amount manure and chemical fertilizer application reduced omnivorous nematode abundance, but predacious nematodes were more abundant in manure-treated soil than in fertilized soil. Abundant omnivorous and predatory nematodes in the manure-treated soils suggested that more complex soil food webs are expected to result in greater biodiversity of the belowground organisms mediating multiple soil ecosystem functions (Ferris *et al.*, 2012). For example, carnivores, through preying on plant-parasitic nematodes, were regarded as having potential for the biological regulation of pest species (Steel & Ferris, 2016). The increase of omnivorous and predacious nematodes were beneficial for soil health because omnivores and predators play an important role in further mineralizing soil nutrients tied up in bacteria-feeders or fungi-feeders, as well as preying on other nematodes including plant-parasitic nematodes (Wang *et al.*, 2006; Khan & Kim, 2007). *Prodorylaimus* were the dominant genera in the present study. However, *Helicotylenchus* and *Scutellonema* were predominant in millet field (Diakhaté *et al.*, 2013), *Pratylenchus* and *Tylenchorhynchus* were dominant in sorghum field (Villenave *et al.*, 2010) and *Tylenchorhynchus*, *Pratylenchus*, and *Rotylenchus* were the dominant genera in wheat field (Hu & Qi, 2013). The number of nematode genera (39) in the present study was lower than in paddy field (45) (Lü *et al.*, 2017), in maize field (48) (Liang *et al.*, 2009), and in banana field (56) (Zhong *et al.*, 2017), but similar to the result in a

continuous sorghum field (38) (Villenave *et al.*, 2010), in soybean field (37) (Pan *et al.*, 2016), and in Tibetan plateau grassland (41) (Hu *et al.*, 2017).

The number of soil free-living nematode was a significant predictor of rice grain yields and the number of omnivorous and predatory nematode was a significant predictor of wheat grain yields. Similarly, Mandal *et al.* (2007) reported that alkaline phosphatase activity was significant ($P < 0.001$) for predicting the grain yields of wheat at the dough stages of wheat growth. Hu & Qi (2013) observed that soil free-living nematode was a significant ($P < 0.05$) predictor of wheat grain yields during the jointing stage of wheat growth.

Conclusions

Based on these results, we deduced that long-term application of organic manure and chemical fertilizer modified the soil nematode community structure, increased crop yields. Total nematode abundance had an increasing tendency with soil nutrient input increments in rice and wheat fields. The number of total soil nematode was significantly increased due to organic manure combined with the chemical fertilizer application in rice field. Omnivores and predators and *Prodorylaimus* were significantly higher in the organic manure combined with NPK fertilizer treatments than in the chemical fertilizer alone and in the unfertilized treatments in rice and wheat fields. Stepwise regressions revealed that the number of soil free-living nematode was a significant predictor of rice grain yields ($R^2 = 0.56$) and the number of omnivorous and predatory nematode was a significant predictor of wheat grain yield ($R^2 = 0.89$). In conclusion, long-term application of organic manure combined with chemical fertilizer could increase nematode abundances and raise crop yields. Organic manure combined with chemical fertilizer application was recommended in agricultural ecosystem.

Conflict of interest

The authors declare there is no conflict of interest.

Acknowledgements

This Project Sponsored by the National Key Research and Development Program of China (2018YFD0200500); the Special Fund for Agro-scientific Research in the Public Interest of China (201203030); the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry (2012-940); the Scientific and Technological Key Achievements Cultivation Project of Hubei Academy of Agricultural Sciences (2017CGPY01) and the National Natural Science Foundation of China (31300442).

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