

RESEARCH ARTICLE

Effects of fertilizer and weed species richness on soil nematode community in a microcosm field experiment

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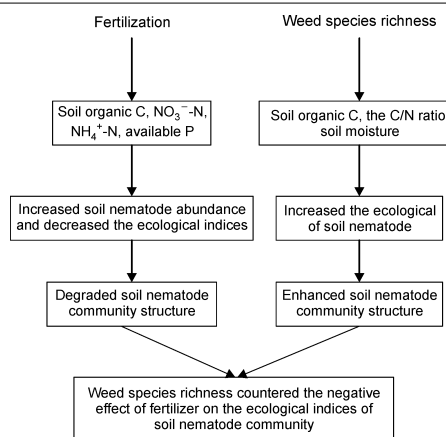
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HIGHLIGHTS

- Fertilizer increased soil nematode abundance and decreased the ecological index of soil nematode community.
- Except for bacterivore density, weed species richness had no effect on soil nematode abundance and increased the ecological index of soil nematode community.
- Fertilizer degraded, whereas weed species richness enhanced soil nematode community structure. Weed species richness may relieve the negative effect of fertilizer on the diversity of nematode community.

GRAPHICAL ABSTRACT



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ABSTRACT

Both fertilizer and plant species richness may affect the soil nematode community. However, the influence of fertilizer and weed species richness interaction is unclear. Nematode abundance and biodiversity in wheat and weed plots soil treated with nitrogen, phosphate and potassium fertilizer addition and weed species richness (0, 1, 2 and 4 weed levels) were investigated in a long-term microcosm experiment established in 2010 at Kaifeng, China. The results demonstrated that fertilizer treatment increased the abundance of total nematode, bacterivores, and plant parasites whereas it decreased the total genera number, the Shannon–Wiener diversity index (H'), Margalef richness index (SR), the total maturity index (ΣMI), and structure index (SI), and degraded the structure of the nematode community. In contrast, weed species richness increased these ecological indices and enhanced the structure of the nematode community. Principal component analysis (PCA) indicated that the fertilizer's effect was more significant than weed species richness. Redundancy analysis (RDA) demonstrated that fertilizer affected soil nematode mainly through increasing soil available phosphorus by 4.71 times and ammonium nitrogen content by 74.03%; weed species richness increased the diversity indices of soil nematode mainly through enhancing soil moisture by 2.07%. Our results suggest weed species richness may relieve the negative effect of fertilizer on the diversity of soil nematode community.

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1 Introduction

Soil nematodes are the most abundant mesofauna and occupy important positions at most trophic levels in the soil food web (Yeates, 2003). Soil nematodes comprise a wide

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range of trophic groups, including bacteria, fungi, and plant feeders, as well as predators and omnivores (Yeates et al., 1993). Previous studies have reported that soil nematodes are sensitive to various disturbances (e.g., agricultural management) and can be used as ecological indicators (Todd, 1996; Neher, 2001; Zhao and Neher, 2013; Zhao et al., 2013).

From 1996 to 2005, Chinese cereal grain yields increased by 10%, while use of chemical fertilizers increased the amount by 51% (Chen et al., 2011). Nitrogen, phosphorus, and potassium (NPK) are the most common elements in limiting primary productivity in most terrestrial ecosystems. Previous field studies focused on the effects of NPK fertilizer on soil nematode abundance and the diversity index of soil nematode community, and have revealed different results. Compared with the control treatment, addition of NPK fertilizer had no effects on the abundance of nematode trophic groups (Todd, 1996; Forge, et al., 2005; Zhu and Zhu, 2015; Hu et al., 2018). Studies also found NPK fertilizer significantly increased total nematode density (Okada and Harada, 2007; Hu et al., 2018), and bacterivorous nematode density in agricultural ecosystems (Pan et al., 2010; Hu and Qi, 2010; Sun, et al., 2016), relative to no-fertilizer treatments. However, addition of NPK fertilizer significantly decreased the abundance of total nematode (Zhang et al., 2009; Zhang et al., 2014), fungivores (Zhang et al., 2016), and plant parasites (Zhang et al., 2009; Pan et al., 2010) compared to the control soils. In addition to its impacts on nematode abundance, NPK addition has different results on soil nematode diversity indices. Relative to the control, NPK fertilizer decreased the nematode maturity index (Forge et al., 2005) and had no impact on the nematode diversity (Zhang et al., 2009). These results indicated that the effect of fertilizer on soil nematode community depends on the specific environment. A recent study indicated that fertilization patterns (control, inorganic fertilizers, and mixed fertilizers) had a greater influence on the nematode gut microbiome than fertilization duration (Zheng et al., 2020).

Also, soil nematode abundance and community diversity may be influenced indirectly by shifts in plant species diversity and plant identity (De Deyn et al., 2004). Studies that focused on the effects of plant species richness on soil nematode communities are abundant. Many studies have reported positive or neutral effects of plant species richness on the soil nematode community structure (Lu et al., 2016; Dietrich et al., 2021). Nematode genera number tended to be positively related to plant species richness (Viketoft et al., 2009). Plant species diversity may increase soil nematode density by changing soil physical and chemical characters (Scherber et al., 2010; Eisenhauer, et al., 2013). Study also demonstrated that plant species diversity increased the Simpson evenness diversity index of the soil nematode community (Deyn et al., 2004). However, other work presented evidence that demonstrated that plant species diversity did not affect nematode abundance (De Deyn et al., 2004) and had no effect on nematode diversity (Sohlenius et al., 2011). Farmland weeds are always considered harmful plants and to be among the biggest threat to agriculture (Blackshaw et al., 2004; Ross and

Van Acker, 2009). However, weeds are an essential component of agroecosystems (Marshall et al., 2003), and play a vital role in supporting biological diversity (Marshall et al., 2003; Gibbons et al., 2006); they affect both the microbe-feeding nematode and the herbivorous nematode (Loreau et al., 2001; Wardle, et al., 2004). Weeds serve as reservoirs for nematodes (Anwar et al., 2009). In a typical winter wheat field, there are more than 30 kinds of weeds, mainly consisting of Gramineae, and broadleaf weeds. Gramineae weed *Avena fatua*, broadleaf weeds *Medicago sativa*, *Cichorium intybus*, and *Descurainia sophia* are the most frequent agricultural weeds which geminate, grow and occur with winter wheat as accompanying species at the same time. Previous studies focused on the effect of weeds on plant parasite nematodes (Anwar et al., 2009; Thomas et al., 2009). The effect of the combination of winter wheat and different weed species richness on soil nematode community in farmland ecosystems is still unknown.

Studies have demonstrated that fertilizer, especially NPK fertilization treatment, promotes crops' growth, and affects weed species richness (Wan et al., 2012). NPK fertilizer management help to maintain soil weed seed bank diversity (Feng et al., 2008) and weed species richness (Jiang et al., 2018). Competition for nutrient and solar radiation between crops and weeds was found to be the main indirect effect of fertilization on weed species diversity changes (Tang et al., 2014). If the interaction of NPK fertilizer and weed species richness affect soil nematode community is still not known. A review study has demonstrated that plant species richness had no or weak effects on the abundance and the ecological indices of soil nematode community in a short time duration (Anderson, 2011). The effect of plant species richness on the soil nematode community depended on experimental duration (Eisenhauer et al., 2012). Soil nematodes contribute to nutrient cycling and have important roles in the soil food web as consumers at various levels (Lu et al., 2020). Compared to weed species richness, NPK fertilizer may have more effects on abundance and community structure of soil nematodes, which are ascribed to the high nutrient content in the soil resulting from use of fertilizer.

The Huang-Huai-Hai plain has become the main grain production area in China. To increase grain yield, chemical fertilizer is used extensively, which has caused a series of issues including production costs and environmental problems such as eutrophication of groundwater and soil acidification (Zhao et al., 2006). In addition, the extensive use of herbicides significantly decreased weed species diversity in cropland (Schmitz et al., 2014). Soil nematode responses to weed species richness combined with NPK fertilizer are poorly understood in the Huang-Huai-Hai plain. In this study, we designed a plastic box experiment to explore the impacts of NPK fertilization, weed species richness, and the interaction of NPK fertilizer and weed species richness on the soil nematode community in the winter wheat field. We hypothesize that: 1) NPK fertilizer may decrease but weed species richness may increase the abundance and diversity of

soil nematode; 2) the interaction of NPK fertilizer and weed species richness may affect the soil nematode community; 3) the effect of NPK fertilizer on soil nematode community may be more remarkable than that of weed species richness.

2 Materials and methods

2.1 Site description

This study was conducted at the crop field station of Henan University (34°48'N, 114°18'E), Kaifeng City, China. The temperate monsoon climate has four distinct seasons, with an average annual precipitation of 634.20 mm, a mean annual temperature of 14°C, and an average annual solar illumination of 2267.60 h. The soil originates from the Yellow River's alluvial sediments and is classified as fluvisol (IUSS Working Group W.R.B.2007). Prior to use of fertilizer, the soil contained approximately 0.30 g kg⁻¹ total nitrogen, 0.02 g kg⁻¹ ammonium nitrogen, 0.61 g kg⁻¹ total phosphate, 0.01 g kg⁻¹ available phosphate and 3.2 g kg⁻¹ organic carbon and had a pH of 7.41.

2.2 Weed seedlings

The weed species were legume (*Medicago sativa*), Compositae (*Cichorium intybus* L.), forb (*Avena fatua*), and Cruciferae (*Descurainia sophia*). On September 10th each year, from 2010 to 2013, seeds of weeds were sown on wet paper in Petri dishes and placed in a climate chamber (14 h light, 60% moisture, 20°C). Then germinated seeds of each species were transplanted to seedling trays filled with soil (2:1, compost:soil) 14 days after sowing.

2.3 Experimental design and treatments

The experimental design is shown in Table 1. A fertilizer addition and weed species richness experiment was arranged in blocks as a complete randomized design and established in October 2010. Treatments consisted of two factors, including fertilizer (control; nitrogen, phosphorus, and potassium fertilizer) and weed species richness (0, 1, 2, and 4) (Table 1). There were 72 plots including 36 control treatments and 36 NPK fertilizer treatments. These were both composed of 6 plots with 0 weed species richness, 12 plots with 1 weed species richness, 12 plots with 2 weed species richness, and 6 plots with 4 weed species richness. We had 4 weed species in our experiment. Under the 1, and 2 weed species richness treatments, we adopted fake replicates, and every 2 plots were chosen as 1 replicate to avoid the differences in results from weed species selection. Each of the 72 experimental plots consisted of 72 plastic boxes of 75 cm × 50 cm × 50 cm arranged randomly in 6 rows and 12 columns in the experiment field and surrounded by a 1-m wide buffer strip. 72 experimental plots were redistributed randomly every two weeks in order to eliminate a possible source of experimental

error. There were two holes (3 cm × 3 cm) in the bottom of the boxes. Fluvisol soil from the alluvial sediments of the Yellow River mentioned above was mixed well, and 50 kg of soil was placed in each plastic box, and the depth of soil in each plastic box was 45 cm, and made the same rotation a winter wheat (*Triticum aestivum*) and 4 species weeds – maize (*Zea mays*).

Weeds and wheat were sown in October and harvested in early June the following year. The planting distribution of weeds and wheat is shown in Fig. 1. Eight wheat plants were uniformly planted in each box's center, and either 0, 1, 2, or 4 species of weed plants (each with approximately five leaves) from the seedling trays were planted around the eight wheat plants. Maize was sown in June and harvested in September. Four individuals of maize were planted in per plot. Urea was used as N fertilizer, calcium superphosphate and potassium sulfate were used as P, and K sources, respectively, and both were mixed uniformly with soils.

For the winter wheat and weed plots, N application consisted of basal fertilization (urea 90 kg ha⁻¹) and topdressing (urea 60 kg ha⁻¹), whereas P and K applied as basal fertilizer (calcium superphosphate 75 kg ha⁻¹, potassium sulfate 150 kg ha⁻¹). Application time of basal fertilization and topdressing was October and March of the following year, respectively. For maize plots, basal fertilization (urea 60 kg ha⁻¹) and topdressing (urea 90 kg ha⁻¹) were used for the application of N, and basal fertilization (calcium superphosphate 60 kg ha⁻¹, potassium sulfate 150 kg ha⁻¹) was used for the application of P and K, respectively. The application time of basal fertilization and topdressing was June and August, respectively. Fertilizer level references were according to the local household fertilizer management.

2.4 Nematodes sampling

At the ripening stages of winter wheat on May 20th, 2013, and 2014, the plants, including winter wheat and 1, 2, 4 species of weed in each box, were harvested. Plants (biomass) were cut at ground level and dried in an oven at 70°C to a constant weight. Five cores were collected randomly inside each box with a soil auger (2 cm in diameter and 10 cm in depth) and combined carefully to obtain a total of 72 composite samples. Each composite soil sample was divided into two aliquots. The first was used to analyze soil physicochemical properties, including soil organic carbon (soil organic C), total nitrogen (total N), available phosphorus (available P), nitrate nitrogen (NO₃⁻-N), ammonium nitrogen (NH₄⁺-N), soil C/N ratio (soil organic C/total N), soil moisture, and soil pH. The other was used to extract and identify soil nematodes.

Nematodes were extracted from 50 g of fresh soil from each composite sample following the modified Baermann funnel method (Barker, 1985). Another 50 g of fresh soil was analyzed for soil moisture content. Soil water content was measured gravimetrically using a soil sample from each box dried at 105°C for 24 h. Extracted nematodes were preserved by TAF fixation (40% formaldehyde 7 mL, triethanolamine 2 mL, and distilled water 91 mL) in a refrigerator. According to

Table 1 The experimental design under fertilizer and weed species richness treatments.

Factor	Level of factor	Explanation	Plots	Replicates
Fertilizer	Control	No fertilizer addition	36	24
	NPK fertilizer	N: urea 60 kg ha ⁻¹ (basal fertilization), urea 60 kg ha ⁻¹ (topdressing) P: calcium superphosphate 60 kg ha ⁻¹ (basal fertilizer) K: potassium sulfate 150 kg ha ⁻¹ (basal fertilizer)	36	24
Weed species richness	0 weed species richness	8 wheats + 8 wheats	6	6
	1 weed species richness	8 wheats + 8 <i>Medicago sativa</i> ;	3	6
		8 wheats + 8 <i>Cichorium intybus</i> ;	3	Every 2 plots as 1 replicate
	8 wheats + 8 <i>Avena fatua</i> ;	3		
	2 weed species richness	8 wheats + 8 <i>Descurainia sophia</i>	3	3
		8 wheats + 4 <i>Medicago sativa</i> + 4 <i>Cichorium intybus</i> ;	2	6
		8 wheats + 4 <i>Medicago sativa</i> + 4 <i>Avena fatua</i> ;	2	Every 2 plots as 1 replicate
		8 wheats + 4 <i>Medicago sativa</i> + 4 <i>Descurainia sophia</i> ;	2	
		8 wheats + 4 <i>Cichorium intybus</i> + 4 <i>Avena fatua</i> ;	2	
		8 wheats + 4 <i>Cichorium intybus</i> + 4 <i>Descurainia sophia</i> ;	2	
4 weed species richness	8 wheats + 4 <i>Avena fatua</i> + 4 <i>Descurainia sophia</i>	2	2	
	8 wheats + 2 <i>Medicago sativa</i> + 2 <i>Cichorium intybus</i> + 2 <i>Avena fatua</i> + 2 <i>Descurainia sophia</i>	6	6	

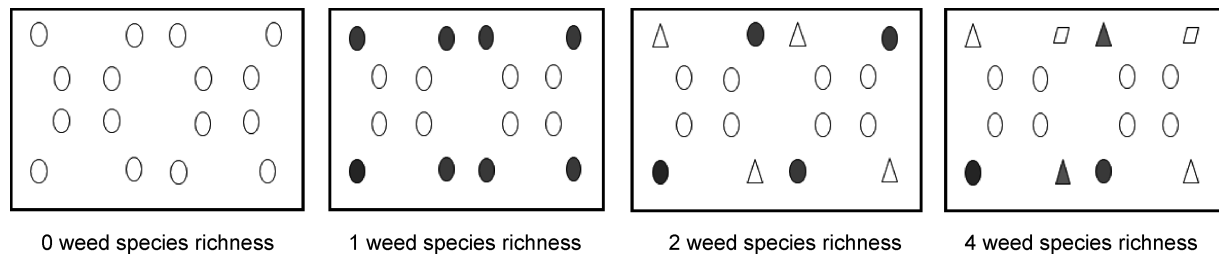


Fig. 1 Layout graph of wheats and weed plants in the experimental microcosm. ○ indicates winter wheat; ● indicates 1 weed species; ●△ indicates 2 weed species; △ ● ▲ □ indicates 4 weed species richness.

soil moisture content, 50 g fresh soil was converted into the weight dry soil, and nematode abundance was determined as the number of individuals per 100 g dry soil. Subsequently, 100 nematodes from each sample were identified by genus using an optical microscope (Motic, BA210, Motic Corporation). For samples with fewer than 100 nematodes, all specimens were identified. Soil nematodes were assigned to four trophic groups: plant parasites (Pp), bacterivores (Ba), fungivores (Fu) and predators-omnivores (Po) (Yeates et al., 1993) with corresponding colonizer-persister (cp) groups (Bongers, 1999). Nematodes in cp-1 have short generation duration, high fecundity, and are regarded as enrichment opportunists and tolerant to disturbance and can be described as r-strategists. In contrast, cp-5 nematodes produce few large eggs, have a long-life cycle combined with a long generation time, and are generally intolerant of disturbance and inhabit stable, mature ecosystems (Ferris et al., 2001; Sieriebriennikov et al., 2014).

2.5 Soil nematode community analysis

The nematode communities were characterized by the Shannon–Wiener diversity index (H'), Margalef richness index (SR), the Pielou evenness index (E), the total maturity index ($\sum MI$), the plant-parasitic maturity index (PPI), enrichment index (EI), structure index (SI), and channel index (CI). $\sum MI$, PPI, EI, SI, and CI are calculated by assigning a colonizer-persister (cp) weight to nematode genera.

The $\sum MI$ and PPI values were computed for each sample, as described by Bongers (1990). Small and large $\sum MI$ and PPI values represent disturbed and stable soil ecosystems, respectively. Also, EI, SI, and CI values were computed for each sample (Ferris et al., 2001). EI and SI were used to characterize the soil food webs. A high EI value indicates an enriched environment, and a high SI value indicates a complex and stable food web. CI was calculated for each sample to evaluate soil food web decomposition pathways (Ferris and Matute, 2003).

2.6 Soil physicochemical properties and plant determination

Soil organic C was determined by the potassium dichromate external heating method. Soil total N was determined by the

Kjeldahl digestion method. Soil available P was determined by molybdenum antimony colorimetry, NaOH melting, and 0.5 mol L⁻¹ NaHCO₃ extraction. The concentrations of NO₃⁻-N and NH₄⁺-N were measured using a Smart Chem 200 Discrete Auto Analyzer (AMS System Italy). Soil pH was measured in a 1:2.5 soil/CaCl₂ (0.01 mol L⁻¹) suspension (Bao, 2000).

2.7 Data analysis

The average data of every 2 plots under the 1, and 2 weed species richness treatments was as 1 replicate data, and variance analysis used the average data. Data were used to determine natural log, square root, or they were rank, transformed when required to achieve normality and homogeneity of variance. Three-way ANOVA was performed to evaluate the effect of the fertilizer, weed species richness, and sample treatment time on the total nematode genera number, the abundance of total nematode and each trophic group, and the ecological indices of soil nematode community including H' , SR, E, $\sum MI$, PPI, EI, SI, and CI. If the effects were significant, the least significant difference (LSD) was used to test the effects of the fertilizer, weed species richness, and sample treatment time on soil nematodes' abundance and ecological indices. ANOVAs were performed with SPSS version 19.0 statistical software package (SPSS Inc, Chicago, IL, USA). The purpose of PCA in CANOCO version 4.5 (Ithaca NY, USA) was to examine the effects of NPK fertilizer and weed species richness on the composition of the soil nematode community. Redundancy analysis (RDA) was used to determine the relationship between soil physical-chemical properties, plant biomass including wheat and weed, and soil nematode community composition using CANOCO 4.5 software (Ithaca NY, USA). Monte Carlo permutation tests were applied to compute statistical significance ($n = 499$) (Lepš, 2003). Figures were plotted with GraphPad Prism 5.

3 Results

3.1 Biomass of wheat and weeds

The biomasses of wheat and four weed species are shown in Table 2. Compared with the control plots, fertilizer significantly

Table 2 Biomass of wheat and four species weeds under NPK fertilizer and weed species richness treatments.

Sample time	Factor	Factor	Wheat biomass	<i>Medicago sativa</i> biomass	<i>Cichorium intybus</i> biomass	<i>Avena fatua</i> biomass	<i>Descurainia sophia</i> biomass	
2013 year	Control	0 weed species richness	29.15±3.66	0.00	0.00	0.00	0.00	
		1 weed species richness	29.97±4.52	37.57±1.32	25.91±5.97a	36.19±9.67a	57.09±7.41a	
		2 weed species richness	28.06±4.08	31.00±6.00	3.30±1.09b	25.50±2.49ab	3.52±1.13b	
		4 weed species richness	30.56±4.84	8.86±4.59	2.89±1.14b	16.91±3.37b	1.44±0.34b	
	NPK fertilizer	0 weed species richness	107.77±18.47	0.00	0.00	0.00	0.00	
		1 weed species richness	122.70±17.67	57.19±19.48a	52.56±24.07a	98.46±34.83	94.05±10.10a	
		2 weed species richness	127.77±11.25	26.51±9.12ab	25.52±9.88ab	73.42±7.54	59.16±8.82b	
		4 weed species richness	97.57±17.77	9.54±3.59b	4.19±2.17b	61.46±11.89	39.24±3.85b	
	2014 year	Control	0 weed species richness	44.14±6.73b	0.00	0.00	0.00	0.00
			1 weed species richness	80.21±7.12a	2.99±0.46ab	6.90±2.07a	58.20±11.34a	3.35±0.22
			2 weed species richness	87.69±8.63a	3.40±0.63a	3.94±0.56b	17.65±1.83b	2.31±0.45
			4 weed species richness	62.45±11.10ab	1.59±0.59b	1.80±0.26b	21.39±7.95b	2.31±0.50
NPK fertilizer		0 weed species richness	178.18±29.12b	0.00	0.00	0.00	0.00	
		1 weed species richness	274.00±11.51a	5.75±2.40	1.46±0.55b	127.73±19.55a	22.66±6.52a	
		2 weed species richness	259.72±16.81a	3.19±0.58	5.57±1.08a	74.79±18.91ab	21.29±4.83ab	
		4 weed species richness	252.35±18.00a	3.01±1.82	2.58±0.56b	57.52±10.81b	7.98±1.11b	

increased wheat biomass by 2.55 times ($F = 147.45$, $P < 0.001$), weed *Avena fatua* biomass 1.96 times ($F = 41.93$, $P < 0.001$), and *Descurainia Sophia* biomass 3.68 times ($F = 25.58$, $P < 0.001$) (Table 2). Fertilizer did not affect the biomasses of weed *Medicago sativa*, and *Cichorium intybus*. Weed species richness significantly impacted primary production and wheat biomass (Table 3). Fertilizer and weed species richness both significantly affected primary production. Compared with the 0 weed species richness treatments, wheat biomass was increased 45.81% ($F = 2.75$, $P = 0.102$), 45.51% ($F = 2.94$, $P = 0.091$), and 64.40% ($F = 5.57$, $P = 0.023$) under the 1, 2, and 4 weed species richness treatments, respectively (Table 2, Table 3). Primary production, and wheat biomasses in 2014 year were increased by 0.32 times, and 1.23 times than in 2013 year, respectively. The interaction of fertilizer and weed species richness significantly affected primary production. The interaction of fertilizer and sample time significantly affected primary production and wheat biomasses (Table 3).

3.2 Soil physical and chemical properties

Compared with the control plots, fertilizer increased organic C by 4.63% ($F = 6.67$, $P = 0.011$), total N 13.73% ($F = 43.08$, $P < 0.000$), NO_3^- -N 2.02 times ($F = 334.91$, $P < 0.000$), NH_4^+ -N 74.03% ($F = 1076.31$, $P < 0.000$), and available P 4.71 times ($F = 1567.34$, $P < 0.000$), but decreased pH by 0.28% ($F = 3.50$, $P = 0.06$) (Table 3, Table 4). Relative to the 0 weed species richness, soil organic C were increased by 3.03% ($F = 5.52$, $P = 0.022$), 7.28% ($F = 26.52$, $P < 0.0001$) and 63.54% ($F = 63.54$, $P < 0.0001$) under the 1, 2, and 4 weed species richness treatments, respectively. Meanwhile, soil moisture was enhanced by 3.91% ($F = 4.00$, $P = 0.049$), and 7.45% ($F = 10.16$, $P = 0.003$) under the 2 and 4 weed species richness treatments, respectively (Table 4). Soil organic C, and soil moisture were found the highest values under the 4 weed species richness (Table 4). The sample time significantly affected soil organic C, the C/N ratio, NO_3^- -N, available P, and pH. The interaction of factors significantly affected soil's physical and chemical properties (Table 3).

3.3 Nematode abundance

The total genera number was increased by 1.40% ($P > 0.1$), 5.39% ($F = 3.22$, $P < 0.1$), 8.25% ($F = 11.39$, $P < 0.01$) under the 1, 2 and 4 weed species richness plots, respectively, in comparison with 0 weed species richness plots (Table 5 and Fig. 2A). The abundance of total nematodes, bacterivorous, and plant parasites were increased by 59.74% ($F = 44.95$, $P < 0.001$), 59.03% ($F = 71.57$, $P < 0.001$), and 50.38% ($F = 21.56$, $P < 0.001$) under the fertilizer plots, respectively, compared with the control plots (Table 5, Fig. 2B, 2C, and 2E). Weed species richness significantly affected the abundance of bacterivores. The total genera number was higher ($F = 25.06$, $P < 0.001$) in 2013 than in 2014. The interaction of

fertilizer and sample time significantly affected the abundance of bacterivores. The total genera number was affected significantly by the interaction of weed species richness and sample time (Table 5).

3.4 Nematode composition

A total of 32 and 30 soil nematode genera were identified during the study in 2013 (Supplementary Table 1) and 2014 (Supplementary Table 2), respectively. Bacterivores *Rhabditis* and *Cephalobus*, plant parasites *Pratylenchus* were dominant genus (all proportions $> 10\%$). *Tylenchorhynchus* was the dominant genus in the fertilizer treatments. The abundance of bacterivores (38.33%–53.08%) and plant parasites (30.25%–43.83%) was highest, followed by omnivore-predators (5.17%–14.33%); fungivores (3.33%–9.25%) had the lowest abundance in the control and NPK fertilizer treatments (Fig. 3).

3.5 The ecological indices

H' , SR, E, $\sum MI$, SI, and CI were significantly decreased by 4.29% ($F = 11.03$, $P = 0.001$), 7.82% ($F = 15.66$, $P = 0.0001$), 3.69% ($F = 14.94$, $P = 0.0002$), 5.23% ($F = 10.76$, $P = 0.0013$), 16.11% ($F = 34.40$, $P < 0.0001$) and 60.46% ($F = 23.58$, $P < 0.0001$) (Table 5, Fig. 4A, 4B, 4C, 4D, 4G and 4H) whereas EI was increased by 18.97% ($F = 49.16$, $P < 0.0001$) (Table 5, Fig. 4F) when the fertilizer was added, relative to the control treatments. Weed species richness affected the indices $\sum MI$ and SI (Fig. 4D, 4G). $\sum MI$ was decreased by 5.09% ($F = 3.35$, $P = 0.072$) under the 1 weed species richness, compared with the 0 weed species richness (Fig. 4D). SI was decreased by 14.81% ($F = 11.66$, $P = 0.0011$), 7.57% ($F = 4.78$, $P = 0.032$), and 1.62% ($F = 0.11$, $P = 0.743$) under the 1, 2, and 4 weed species richness, respectively, compared with the 0 weed species richness (Fig. 4G). H' and SR increased with the increasing weed species richness were enhanced by 4.28% ($F = 4.29$, $P = 0.044$), 7.53% ($F = 5.15$, $P = 0.028$) under 4 species richness plots, respectively, compared with the 0 weed species richness plots (Table 5, Fig. 4A and 4B). Compared with in 2013, H' , SR, and E index was decreased by 7.22% ($F = 37.81$, $P < 0.000$), 10.19% ($F = 29.74$, $P < 0.000$), and 3.69% ($F = 14.94$, $P < 0.000$) respectively, whereas $\sum MI$ index were increased by 4.19% ($F = 3.80$, $P = 0.053$) in 2014, respectively. The interaction of fertilizer and weed species richness clearly affected E and EI. The interaction of fertilizer and sample time substantially affected SR and $\sum MI$. EI and CI were affected by weed species richness and sample time interaction. The interaction of three factors affected EI and CI (Table 5, Fig. 4F and 4H).

3.6 Relationships between soil nematode community and fertilizer, weed species richness, sample time, plant biomass, or soil properties

Soil organic C was positively correlated with the nematode

Table 3 Three-way ANOVA for effects of NPK fertilizer (FE), weed species richness (WSR), and sample time (T), and their interactions on soil physical and chemical characters.

Soil parameters	FE			WSR			T			FE × WSR			FE × T			WSR × T			FE × WSR × T		
	F	P		F	P		F	P		F	P		F	P		F	P		F	P	
Soil organic C (g kg ⁻¹)	15.61	0.000		22.63	0.000		21.02	0.000		1.13	0.338		0.32	0.573		3.39	0.020		0.62	0.602	
Total N (g kg ⁻¹)	273.34	0.000		6.38	0.000		0.08	0.783		1.82	0.148		0.00	1.000		2.45	0.066		0.27	0.829	
C/N ratio	69.81	0.000		7.30	0.000		14.39	0.000		3.37	0.021		0.04	0.841		4.96	0.003		1.12	0.344	
NO ₃ ⁻ -N	491.27	0.000		0.72	0.543		43.63	0.000		0.37	0.777		40.65	0.000		0.74	0.532		1.52	0.212	
NH ₄ ⁺ -N	943.08	0.000		2.30	0.081		0.15	0.700		1.94	0.126		1.13	0.290		0.45	0.718		0.88	0.455	
Available P (mg kg ⁻¹)	3741.75	0.000		1.22	0.306		127.67	0.000		0.63	0.598		105.46	0.000		3.75	0.013		4.37	0.006	
Soil moisture	2.27	0.134		6.85	0.000		0.02	0.897		0.77	0.511		5.44	0.021		2.23	0.088		0.50	0.685	
pH	4.82	0.030		1.47	0.227		57.58	0.000		0.55	0.650		9.84	0.002		1.69	0.173		0.10	0.963	
Primary production	531.93	0.000		20.62	0.000		99.57	0.000		11.67	0.000		14.40	0.000		0.07	0.975		1.55	0.206	
Wheat biomass	346.47	0.000		5.88	0.001		145.11	0.000		1.25	0.294		40.56	0.000		3.32	0.022		1.02	0.386	

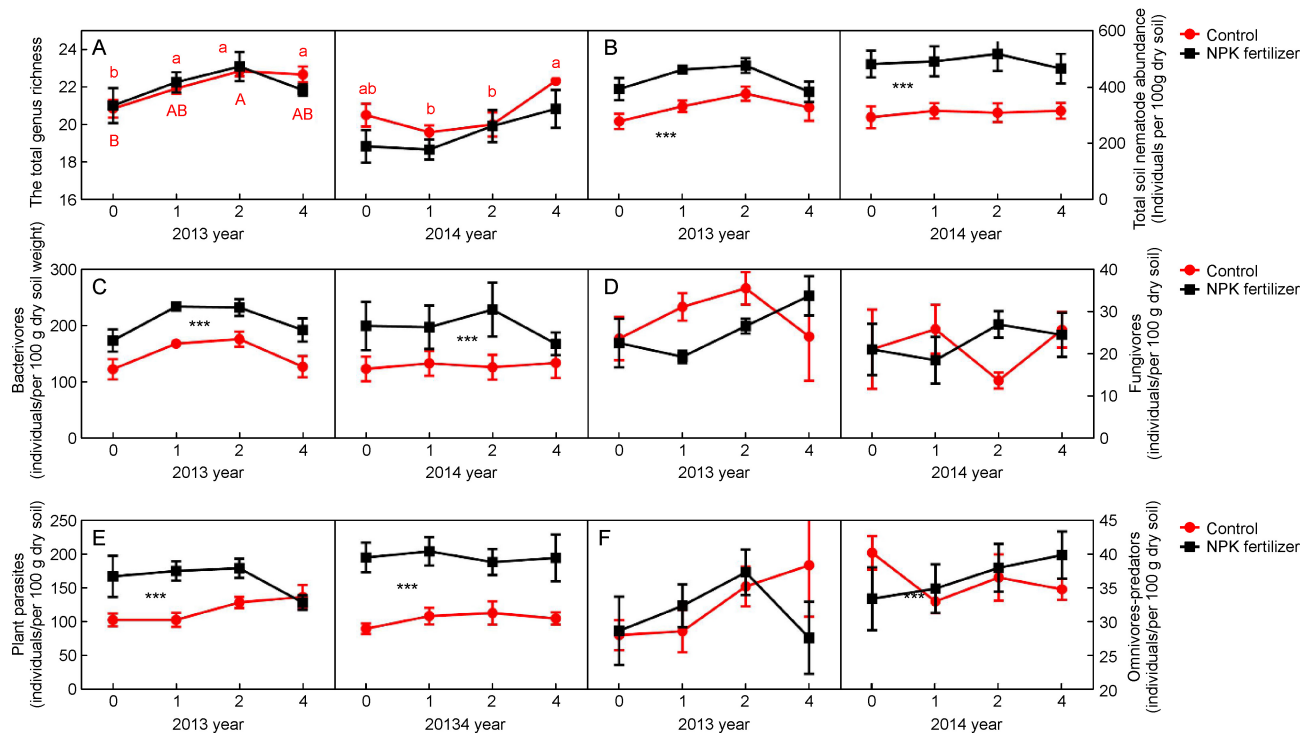


Fig. 2 The total genus richness of soil nematode (Fig. 2A); total soil nematodes (Fig. 2B), bacterivores (Fig. 2C), fungivores (Fig. 2D), plant parasites (Fig. 2E) and omnivore-predator individuals (Fig. 2F) per 100 g dry soil weight under the NPK fertilizer and weed species richness treatments in 2013 and 2014. All values are means \pm SE ($n = 6$). 0, 1, 2, and 4 represents 0, 1, 2, and 4 weed species richness, respectively. *** indicates the significant difference between the control and NPK fertilizer treatments. Lowercase, and capital letters represent the significant differences under the different weed species richness in the control and NPK fertilizer treatments, respectively.

total genera number and nematode density except for plant parasites and H' , SR, E (Table 6). Evident negative correlations were observed between E, $\sum MI$, and soil total N (Table 6). The soil C/N ratio was correlated positively with the nematode total genera number, total nematode density, plant parasites density, omnivore-predator density, and H' , E, $\sum MI$. NO_3^- -N was positively correlated with total nematode density, bacterivore density, plant parasites density, and EI and negatively correlated with E, $\sum MI$, SI, and CI. NH_4^+ -N was negatively correlated with total nematode density, bacterivore density, plant parasites density, and EI and positively correlated with H' , SR, E, $\sum MI$, SI, and CI. Soil available P content was positively correlated with total nematode density, bacterivore density, plant parasites density, and EI and negatively correlated with H' , SR, E, $\sum MI$, SI, and CI. Soil moisture was positively correlated with the nematode total genera number, omnivore-predator density, H' , SR, E, $\sum MI$, and SI. Soil pH negatively correlated with bacterivores density and positively correlated with $\sum MI$ (Table 6).

PCA indicated that fertilizer not weed species richness affected the composition of soil nematode community, because the distance between control and fertilizer quadrats was farther than that between weed species richness treatments (Fig. 5). The first and second axes explained 15.70%, and 10.00% of the variance, respectively. The third and fourth axes explained altogether the 14.00% of the

variance (Fig. 5). Redundancy analysis (RDA) showed that up to 54.55% of the total variance in soil nematode community composition was explained by the content of available P ($F = 15.18$, $P = 0.002$) and NH_4^+ -N ($F = 15.01$, $P = 0.002$). The first ($F = 15.71$, $P = 0.002$) and second axes ($F = 2.49$, $P = 0.002$) of RDA analysis explained 10.80% and 2.80% variance, respectively (Fig. 6). Soil C/N ratio ($F = 6.19$, $P = 0.002$), Soil organic C ($F = 1.83$, $P = 0.014$), soil moisture ($F = 2.84$, $P = 0.004$), and total N ($F = 10.25$, $P = 0.002$) significantly affected soil nematode community composition and together explained 22.22% of the variance. Weed biomass explained 11.82% of the variance. Mainly weed *Avena fatua* ($F = 2.595$, $P = 0.002$) and weed *Descurainia sophia* ($F = 1.783$, $P = 0.030$) significantly affected nematode community composition and explained 3.76%, 3.78% of the variance, respectively (Fig. 6).

4 Discussion

4.1 Effect of NPK fertilizer on the soil nematode community

Our experiment on the farmland conditions indicated that addition of NPK fertilizer clearly affects the nematode community abundance. In the present study, NPK fertilizer remarkably increased the abundance of total nematode, bacterivores, and plant parasites. The increasing effect of

Table 4 Comparisons of mean soil physical and chemical variations under NPK fertilization and weed species richness treatments during the winter wheat mature stage in 2013 and 2014.

Sample time	Treatments	Soil organic C (g kg ⁻¹)	Total N (g kg ⁻¹)	NO ₃ ⁻ -N	NH ₄ ⁺ -N	C/N ratio	Available P (mg kg ⁻¹)	Soil moisture	pH
2013 year	Control + 0weed species richness	3.14±0.03b	0.44±0.01b	2.31±0.81	8.61±0.60	7.14±0.10b	3.04±0.23	20.62±0.43b	8.29±0.03
	Control + 1weed species richness	3.21±0.02ab	0.46±0.01a	2.33±0.45	8.91±1.40	6.95±0.07b	3.29±0.23	21.10±0.34ab	8.30±0.01
	Control + 2weed species richness	3.49±0.04a	0.44±0.05b	2.92±0.65	8.50±1.01	7.92±0.12a	3.06±0.13	22.15±0.49a	8.28±0.02
	Control + 4weed species richness	3.63±0.12a	0.47±0.00a	2.29±0.62	8.93±1.05	7.94±0.25a	3.45±0.41	22.26±0.58a	8.31±0.00
2014 year	NPK fertilizer + 0weed species richness	3.27±0.05b	0.51±0.01	8.47±1.61	14.17±0.91	6.43±0.16	20.85±0.06	22.82±0.36	8.31±0.01
	NPK fertilizer + 1weed species richness	3.44±0.06b	0.51±0.01	8.39±1.41	15.36±1.48	6.78±0.16b	20.10±0.30	22.10±0.41	8.32±0.02
	NPK fertilizer + 2weed species richness	3.56±0.07a	0.51±0.01	9.04±1.87	14.76±1.07	6.96±0.17ab	19.26±0.57	22.95±0.30	8.29±0.03
	NPK fertilizer + 4weed species richness	3.73±0.09a	0.53±0.01	9.29±1.62	14.68±0.81	7.07±0.09a	19.75±0.20	22.48±0.60	8.30±0.03
2014 year	Control + 0weed species richness	3.12±0.03b	0.44±0.00b	2.11±0.47	8.44±0.77	7.16±0.12	2.94±0.15	21.26±1.12b	8.36±0.02
	Control + 1weed species richness	3.16±0.02b	0.46±0.01a	2.38±0.64	8.85±1.43	6.88±0.09	2.94±0.23	21.27±0.37b	8.40±0.01
	Control + 2weed species richness	3.24±0.03b	0.46±0.00a	2.03±0.56	8.12±0.84	7.11±0.10	2.81±0.07	21.90±0.55ab	8.42±0.02
	Control + 4weed species richness	3.38±0.04a	0.47±0.01a	3.10±2.13	8.36±1.16	7.23±0.17	3.18±0.43	24.12±0.67a	8.45±0.02
	NPK fertilizer + 0 weed species richness	3.20±0.04b	0.50±0.01b	7.43±1.91	14.12±1.05	6.42±0.14b	12.80±0.74	20.98±0.92b	8.34±0.01b
	NPK fertilizer + 1 weed species richness	3.32±0.06ab	0.52±0.01ab	5.67±1.57	15.13±1.34	6.45±0.12b	15.57±0.76	21.42±0.58b	8.35±0.00b
	NPK fertilizer + 2 weed species richness	3.39±0.05a	0.53±0.01a	6.05±1.26	15.67±1.23	6.43±0.14b	15.72±0.51	22.04±0.35ab	8.35±0.00ab
	NPK fertilizer + 4 weed species richness	3.48±0.10a	0.52±0.01ab	5.56±1.60	14.60±0.57	7.06±0.15a	15.62±0.66	23.21±0.27a	8.37±0.01a

Data are means±standard error ($n = 6$). Significant differences of variable mean among different weed species richness treatments under each fertilizer treatment are indicated by different letter superscripts ($P < 0.05$).

Table 5 Three-way ANOVA for effects of NPK fertilizer (FE), weed species richness (WSR), and sample time (T), and their interactions on the total nematode genus richness, the abundance of total soil and all trophic group nematodes, and the ecological index.

Soil nematode index	FE			WSR			T			FE × WSR			FE × T			WSR × T			FE × WSR × T		
	F	P		F	P		F	P		F	P		F	P		F	P		F	P	
The total genus richness	2.53	0.114		4.60	0.004		34.68	0.000		0.67	0.571		2.33	0.129		3.14	0.028		0.25	0.860	
Total and trophic group nematode density																					
The total soil nematode	45.47	0.000		1.67	0.176		1.05	0.308		0.30	0.822		3.77	0.055		0.49	0.687		0.15	0.927	
Bacterivores	67.55	0.000		2.73	0.047		0.02	0.881		0.20	0.894		5.95	0.016		1.30	0.278		0.18	0.913	
Fungivores	0.14	0.705		0.66	0.580		3.82	0.053		1.94	0.127		0.73	0.396		0.83	0.480		2.10	0.103	
Plant parasites	15.58	0.000		0.56	0.641		2.76	0.099		0.90	0.444		0.82	0.397		0.02	0.998		0.31	0.820	
Omnivores-predators	0.00	0.965		1.24	0.299		0.05	0.819		0.56	0.639		0.10	0.751		0.36	0.782		1.14	0.334	
Nematode community index																					
H'	19.83	0.000		3.46	0.018		29.87	0.000		2.02	0.115		0.81	0.371		2.54	0.060		1.06	0.368	
SR	21.02	0.000		3.19	0.026		25.08	0.000		0.45	0.716		3.95	0.049		1.08	0.360		0.14	0.935	
E	22.23	0.000		1.61	0.190		11.47	0.001		3.06	0.030		0.41	0.525		1.18	0.319		1.97	0.122	
ΣMI	16.94	0.000		3.09	0.030		4.40	0.038		2.39	0.072		3.96	0.049		0.27	0.845		1.45	0.232	
PPI	0.45	0.502		0.79	0.500		2.89	0.091		0.82	0.483		1.07	0.304		0.37	0.773		0.73	0.534	
EI	55.94	0.000		1.69	0.173		2.31	0.131		4.36	0.006		0.13	0.720		4.87	0.003		5.25	0.002	
SI	35.87	0.000		4.05	0.009		0.76	0.387		1.32	0.272		0.54	0.465		0.47	0.702		1.37	0.254	
CI	48.28	0.000		1.93	0.128		1.82	0.180		1.32	0.271		0.11	0.744		4.19	0.007		6.31	0.001	

H', the Shannon–Wiener diversity index; SR, the Margalef species richness index; E, the Pielou evenness index; ΣMI, the total maturity index; PPI: the plant-parasitic maturity index; EI, enrichment index; SI, structure index; CI, channel index.

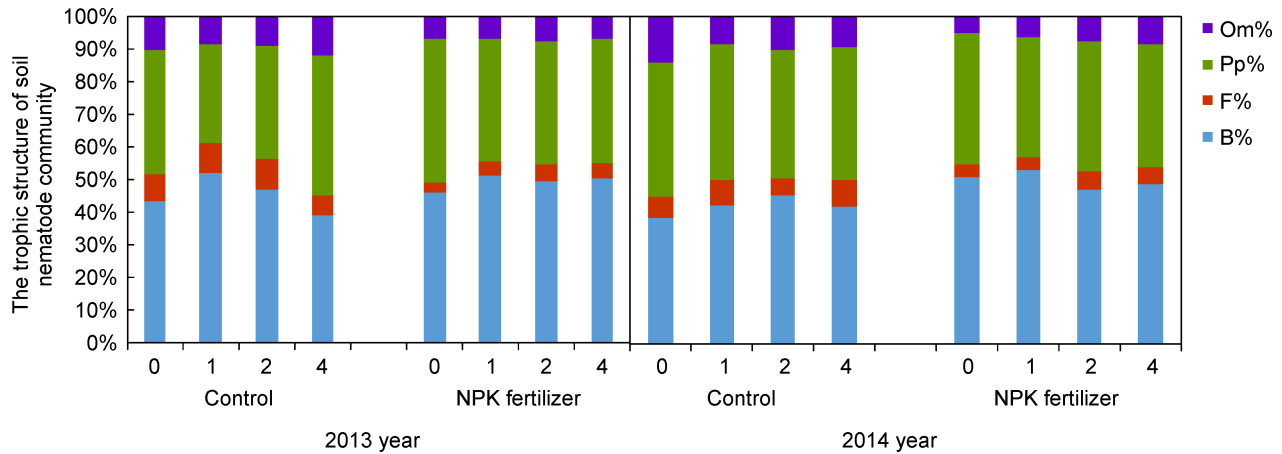


Fig. 3 The trophic structure of the soil nematode community under NPK fertilizer and weed species richness treatments in 2013, and 2014 year. 0, 1, 2, and 4 represents 0, 1, 2, and 4 weed species richness, respectively. Replicates are 6.

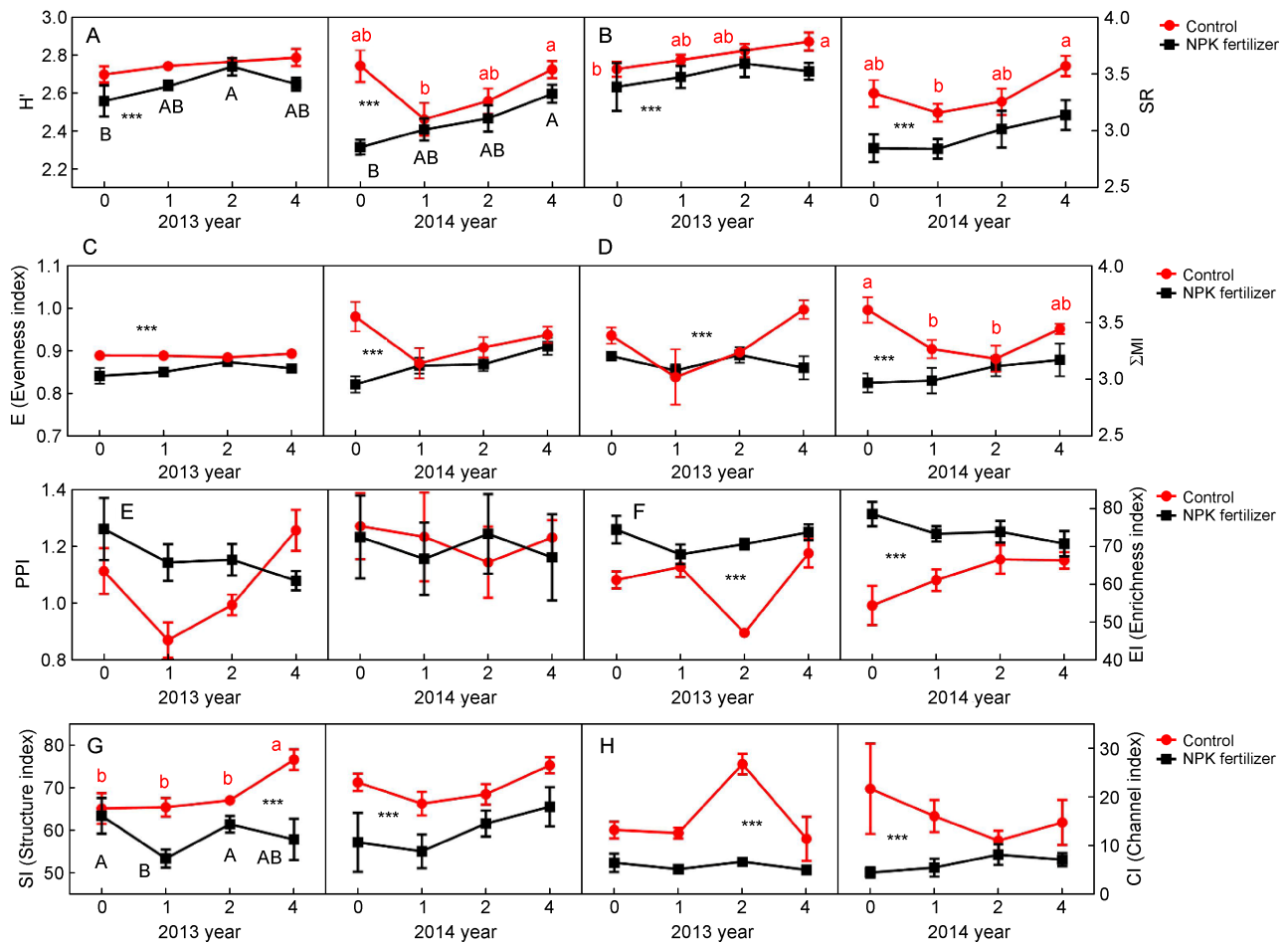


Fig. 4 The Shannon–Wiener diversity index (H') (Fig. 4A), the Margalef species richness index (SR) (Fig. 4B), the Pielou evenness index (E) (Fig. 4C), the total maturity index (ΣMI) (Fig. 4D), plant-parasitic index (PPI) (Fig. 4E), enrichment index (EI) (Fig. 4F), structure index (SI) (Fig. 4G) and channel index (CI) (Fig. 4H) under NPK fertilizer and weed species richness treatments in 2013 and 2014. All values are means \pm SE ($n = 6$). 0, 1, 2, and 4 represents 0, 1, 2, and 4 weed species richness, respectively. *** indicates the significant difference between the control and NPK fertilizer treatments. Lowercase and capital letters represent the significant differences under the different weed species richness in control and NPK fertilizer treatments, respectively.

Table 6 Pearson' correlation between soil physical-chemical properties and soil nematode variables.

Soil nematode variables	Soil organic C		Total N		C/N		NO ₃ ⁻ -N		NH ₄ ⁺ -N		Available P		Soil moisture		pH	
	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P
The total genus richness	0.463	0.000	0.024	0.772	0.277	0.001	0.054	0.524	0.088	0.294	0.013	0.874	0.316	0.000	-0.033	0.695
Total nematode	0.235	0.005	-0.078	0.353	0.238	0.004	0.399	0.000	-0.488	0.000	0.468	0.000	-0.048	0.564	-0.110	0.189
Bacterivores	0.204	0.014	0.001	0.986	0.162	0.052	0.447	0.000	-0.535	0.000	0.504	0.000	-0.010	0.903	-0.201	0.015
Fungivores	0.199	0.017	0.012	0.888	0.120	0.150	-0.026	0.755	0.053	0.530	-0.069	0.414	0.050	0.549	-0.102	0.224
Plant parasites	0.126	0.131	-0.098	0.244	0.173	0.039	0.266	0.001	-0.332	0.000	0.329	0.000	-0.119	0.157	0.024	0.779
Omnivore-predators	0.231	0.005	-0.160	0.055	0.274	0.001	0.005	0.949	-0.035	0.675	0.032	0.706	0.216	0.009	-0.066	0.430
H'	0.370	0.000	-0.052	0.536	0.272	0.001	-0.105	0.209	0.250	0.003	-0.172	0.040	0.435	0.000	-0.037	0.660
SR	0.280	0.001	0.055	0.514	0.123	0.142	-0.154	0.066	0.299	0.000	-0.212	0.011	0.312	0.000	0.049	0.556
E	0.215	0.010	-0.266	0.001	0.400	0.000	-0.176	0.035	0.273	0.001	-0.234	0.005	0.421	0.000	-0.022	0.790
ΣMI	0.064	0.445	-0.270	0.001	0.287	0.000	-0.205	0.014	0.230	0.005	-0.235	0.005	0.196	0.019	0.194	0.020
PPI	0.050	0.550	-0.067	0.423	0.086	0.306	0.081	0.335	-0.085	0.311	0.109	0.195	-0.112	0.180	0.163	0.051
EI	-0.026	0.754	0.118	0.158	-0.083	0.325	0.373	0.000	-0.471	0.000	0.461	0.000	-0.004	0.967	0.057	0.495
SI	0.017	0.841	-0.073	0.385	0.058	0.490	-0.358	0.000	0.390	0.000	-0.393	0.000	0.237	0.004	0.136	0.104
CI	0.067	0.424	-0.028	0.737	0.045	0.589	-0.385	0.000	0.463	0.000	-0.483	0.000	-0.017	0.841	-0.047	0.573

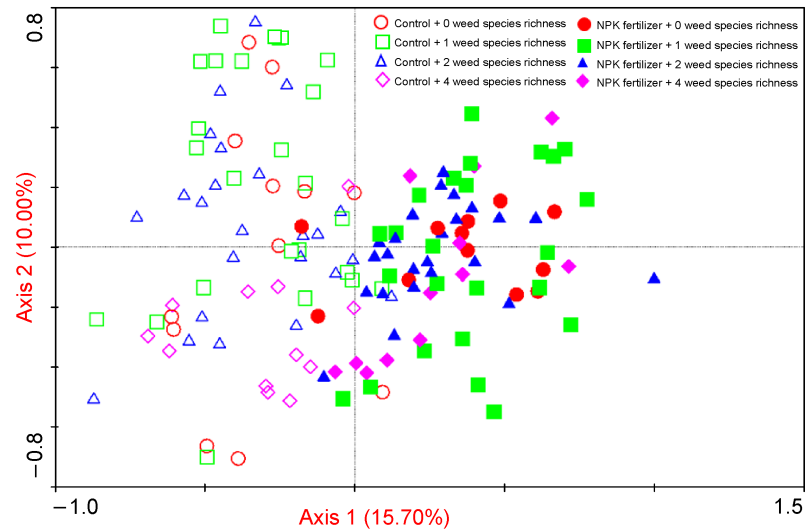


Fig. 5 PCA of the effect of NPK fertilizer and weed species richness on soil nematode community composition. Axis 1, and 2 represents PC1, and PC2, respectively. 15.70%, and 10.00% represents the contribution rate of PC1, and PC2 to the overall variance, respectively.

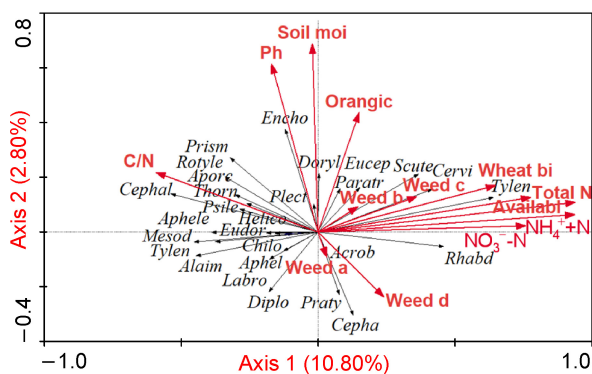


Fig. 6 Biplot of Redundancy analysis (RDA) of the relationship between soil physical-chemical properties, wheat biomass, weed biomass and soil nematode community composition. Axis 1, and 2 represents RDA1, and RDA2, respectively. 10.80%, and 2.80% represents the explain contribution of environmental factors to soil nematode community variation, respectively. C/N: the ratio of carbon to nitrogen; Soil moi: soil moisture; Organic: organic carbon; Weed a, Weed b, Weed c, and Weed d represent the biomass of *Medicago sativa*, *Cichorium intybus*, *Avena fatua*, and *Descurainia Sophia*, respectively. Total N: total nitrogen; Availabl: available phosphorus; NO_3^- -N: nitrate nitrogen; NH_4^+ -N: ammonium nitrogen; Ph: pH; Wheat bi: wheat biomass.

total nematode abundance might be that soil in the long term NPK fertilizer addition had more soil organic C, total N, NO_3^- -N, and available P. The abundance of bacterivores was higher in the NPK fertilizer than in control treatments, which indicated that the enhancing contents of organic C and available P in the soil treated with NPK fertilizer promoted microbial activity and biomass, providing more food for bacterivores (Sarathchandra et al., 2001). NPK fertilizer significantly increased the abundance of plant parasites, which was consistent with previous study findings, which demonstrated that chemical

fertilizer treatment could increase plant-parasitic nematodes relative to control (Wang et al., 2006). The reason may be ascribed to the higher plant biomass, growth, and excretion of root (Vestergård, 2004; Majdi and Nylund, 1996) in the NPK fertilizer treatments than in control treatments (Table 2). These results indicated that fertilizer increased the abundance of total nematodes, bacterivores, and plant-parasites, which was contrary to our first hypothesis.

The ecological indices of H' are linked to the diversity of soil nematodes. H' was higher in the control than in the NPK fertilizer treatments in this study, which implies a trend of more extraordinary soil nematode biodiversity and a relatively stable environment in the control treatments. Our result indicated that the control treatments have higher nematode diversities in agreement with a previous study (Lenz and Eisenbeis, 2000). The lower SR in the NPK fertilizer treatments indicates a less complicated community structure, and the lower E showed the uneven distribution of soil nematode. In the present study, NPK fertilizer significantly increased the contents of NO_3^- -N, NH_4^+ -N, and available P, which potentially had negative impacts on the total genus richness and E index of soil nematodes. Therefore, NPK fertilizer decreased the soil nematode community's diversity, which was supportive of our first hypothesis.

Nematode abundance disagreed, but diversity agreed, with our first hypothesis. The reason may be that *Rhibditiis*, *Tylenchorhynchus* had higher relative abundance in NPK fertilizer (Supplement Table 1, and Supplement Table 2) than in the control treatments, which resulted in decreasing nematode diversity.

The lower Σ MI showed highly disturbed soil ecosystems in the fertilizer treatments. EI reflects the availability of resources to the soil food web and primary decomposers' response to the resources (Ferris et al., 2001). In our study, EI values were higher in the case of NPK fertilizer treatment than for the control treatments under all weed species richness

treatments. The higher SI values in the control plot indicated a complex and stable food web. The lower SI values in the NPK fertilizer treatments implied that the soil food web had fewer trophic links compared with the control treatments. The relative abundance of omnivore-predators in the control treatments was partially responsible for the difference in SI values. The nematode channel index CI was developed to evaluate decomposition pathways (Ferris et al., 2001). The CI values were lower in the NPK fertilizer than those in the control treatments, reflecting the lower abundance of fungivores in the fertilizer treatments, and the bacteria-dominated decomposition pathway is characteristic of the fertilizer treatments.

4.2 Effect of weed species richness on the soil nematode community

Except for bacterivore abundance, weed species richness had no impact on soil nematode abundance, which was not in agreement with the supposition that weed species richness may increase the abundance of soil nematode. The reason may be the single weed species had different effects on nematode abundance. For example, the abundance of bacterivores, plant parasites, and omnivore-predators was the highest under the single weed *Descurainia sophia*, *Avena fatua*, and *Medicago sativa* treatments, respectively. However, the lowest abundance of bacterivores, fungivores, plant parasites, and omnivore-predators was found under the single weed *Avena fatua*, *Cichorium intybus*, *Descurainia sophia*, and *Cichorium intybus*, treatments, respectively. Effects of 2 or 4 weed species richness on soil nematode abundance may be mutually counteracted, and the reason may be that root exudation of different weed species has negative or positive effect on the abundance of soil nematode. Our study indicated weed species richness significantly increased the total nematode genera number in accordance with a previous study (De Deyn et al., 2004; Dietrich et al., 2021). In the present study, the positive correlations between the total nematode genera number and soil organic carbon, the C/N ratio, soil moisture confirmed that weed species richness could positively affect the total nematode genera number. One reason may be that high weed species diversity released more organic material from living roots by sloughing-off border cells, secretion of mucilage, root exudation, and senescence of root epidermis (Nguyen, 2003) and promoted the accumulation of soil organic matter (Fornara and Tilman, 2008; Eisenhauer et al., 2013). High weed species richness increased the C/N ratio and soil moisture (Table 3), which may be one reason. Another reason may be that enrichment of resource availability by increasing the weed species richness could alleviate the intensity of interspecific competition and increase the total nematode genera number (Sohlenius et al., 2011).

In terms of nematode ecological indices, weed species richness increased H' , and because the E index was similar, it was mainly the total nematode genera number that caused this difference in H' . Another reason may be the increasing

weed species richness which led to the increase of plant functional groups. Because in our study the highest weed species richness contained the most plant functional groups including Legume (*Medicago sativa*), Composite (*Cichorium intybus*), Forb (*Avena fatua*), and Cruciferae (*Descurainia sophia*). Furthermore, the increased plant functional groups resulted in a more diversified environment, which supported more diversity of soil nematode genera. The increase of soil nematode genera led to an increase in H' . Previous study demonstrated that increasing plant species richness significantly increased diversity of collembolan (Sabais et al., 2011). However, another study demonstrated that a higher diversity of plant species did not increase the diversity of nematodes (Viketoft et al., 2009). The increase of SR was also ascribed to the increase of the total nematode genera number. These results partly demonstrated weed species richness increased the diversity index of soil nematode community.

Weed species richness significantly increased ΣMI , and SI, which was attributed to the complementarity in resource quality of the component plant species (Deyn et al., 2004) and the increasing quantity and diversity of resource in the soil food web but did not influence EI and CI. The high ΣMI and SI values indicated weed species richness enhanced soil food web structure (Ferris et al., 2001).

4.3 The effect of the sample time

The increase of total genus richness was ascribed to high total weed biomasses in the year 2013. Total weed biomasses were increased by 44.56% in 2013, compared with in 2014. The increase of weed biomasses may provide the food resource of soil nematode and support a more abundant nematode population through "bottom-up" control (Keith et al., 2009). More abundant nematode population promotes the genus richness of soil nematode.

The higher E and high total genus richness resulted in the increase of H' in 2013, relative to that in 2014. The increase of total genus richness resulted in the increase of SR. The lower ΣMI in 2013 was due to the enrichment effect of soil nematode. The enrichment-opportunists *Rhabditis*, *Diplogastrellus*, and *Caenorhabditis* relative abundance and total nematode abundance in 2014 was higher than that in 2013, which resulted in the higher ΣMI .

4.4 The interactive effect of fertilizer and weed species richness on the soil nematode community

The interactive effects of fertilizer and weed species richness on soil nematodes were found only in E and EI, which supported our second hypothesis. The NPK fertilizer inhibited the growth of weeds and decreased weed species richness (Tang et al., 2014), which resulted in the interaction effect on the E index of the soil nematode community. The presumable reason may be the NPK fertilizer treatment had the lowest light transmittance, and weeds are sensitive to light. Therefore the lower light transmittance on the ground may be one of the

main reasons weed species richness was reduced (Tang et al., 2014). Fertilizer significantly increased soil nutrients' content, which improved winter wheat and weed biomass growth (Table 2). Weed *Medicago sativa*, and weed *Cichorium intybus* produces perennial roots, maintaining high soil organic carbon (Dupont et al., 2014) and a rich environment. Therefore, the interaction of fertilizer and weed species richness affected EI.

Application of fertilizer decreased the total genera number, H', SR, $\sum MI$, and SI. In contrast, weed species richness increased these indices, which indicated that weed species richness counteracted the negative effect of fertilizer on the soil nematode community. Fertilizer and weed species richness affected soil nematode community mainly by changing soil physical-chemical properties (Fig. 6). PCA analysis indicated that fertilizer's effect on soil nematode was more significant than the effect of weed species richness on soil nematode community, which demonstrated our third hypothesis. The results suggest that in the long term species-rich plant communities experience higher levels of nutrient cycling and N availability than species-poor communities (Reich et al., 2012). We suggest that weed conservation seems a promising step in fertilized farmland, which improves soil nematode diversity and enhances the soil nematode community.

5 Conclusion

In summary, our results demonstrated that long-term fertilizer addition in agroecosystem increased soil nematode abundance, whereas it decreased the ecological indices of soil nematode community and degraded the soil food web. In contrast, except for bacterivore density, weed species richness had no effects on the abundance of soil nematode but increased the soil nematode community's ecological indices and enhanced the structure of the soil nematode community. Weed species richness weakened the negative effect of the fertilizer treatments on the soil nematode community. Our findings also suggest that maintaining appropriate weed species richness in the fertilizer treatments may improve ecosystems' stability and provide a better understanding of weed integrated management in farmland ecosystems.

Author contributions

Conceptualization, X.N., and Y.G.; investigation, X.N., X.F., S.C., and F.S.; writing—original draft preparation, X.N.; writing—review and editing, X.N., F.S., and Y. G. All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

The authors declare no conflict of interest.

Electronic supplementary material

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