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RESEARCH ARTICLE

Long-term fertilization enriches soil food web mainly through bottom-up regulation in a rice–wheat cropping system

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Highlights

- Long-term fertilization enhances most functional groups.
- Manure and manure+NPK surpass NPK in enriching food web components.
- High-fertility soils diminish manure *vs*. manure+NPK effect differentiation.
- Fertilization alters the food web primarily *via* bottom-up regulatory pathways.

Abstract

Long-term fertilization shapes the size and community composition of the soil food web by altering resource quantity and quality. Previous studies have examined specific components; however, comprehensive effects on the entire food web and underlying mechanisms remain unclear. In this study, a 30-year experiment in a rice–wheat cropping system was conducted to assess the effects of chemical fertilizer (NPK), manure (M), and their combination (NPKM), with unfertilized soil as the control. Biomass or abundance across key taxonomic and functional groups — including microorganisms, protozoa, nematodes, mites, collembolans, enchytraeids, and earthworms — was measured, and nematode ecological indices were calculated to evaluate bottom-up and top-down regulatory influences. Results showed that long-term fertilization increased resource inputs, thereby enhancing most functional groups. M and NPKM outperformed NPK across most groups, with relative increases compared to NPK ranging from 20.69 to 972.52% under M (average: 241.62%) and from 26.55 to 792.30% under NPKM (average: 189.02%). However, differences between M and NPKM were not significant, as high soil fertility diminished the divergence in their effects. Strong evidence for bottom-up control was observed, supported by positive correlations among functional groups and by increases in the enrichment index (51.27 and 28.49%) and enrichment footprint (11.80 and 47.17%) under M and NPKM relative to NPK. Partial least squares path modeling further confirmed that bottom-up forces predominantly determined food web structure and biomass. These findings indicate that sustained organic inputs, particularly when integrated with mineral fertilizers, effectively enhance soil food web complexity and size primarily through bottom-up regulatory mechanisms.

Keywords: long-term fertilization, soil food web, nematode-based indices, bottom-up effect, top-down effect, rice–wheat system

1. Introduction

The biotic communities in soil consist of roots, microorganisms (e.g., bacteria and fungi), microfauna (e.g., protozoa and nematodes), mesofauna (e.g., mites, Collembola, and Enchytraeidae), and macrofauna (e.g., earthworms) (Islam *et al.* 2023). These organisms interact through feeding relationships to form a complex network known as the soil food web. They deliver provisioning, supporting, and regulating ecosystem services in agroecosystems by recycling nutrients, modifying soil structure, and suppressing pests and diseases (Mulder *et al.* 2011). Specifically, soil microorganisms govern nutrient

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supply through the decomposition of organic matter (OM) and subsequent processes of nutrient mineralization and immobilization. Protozoa enhance nutrient availability by grazing on bacteria. Nematodes occupy diverse and central trophic positions within the soil food web and are linked to nitrogen cycling and plant growth. Mites, Collembola, Enchytraeidae, and earthworms contribute to plant litter fragmentation, modify soil architecture at the microscale, and mix soil particles with organic matter (Stockdale *et al.* 2006; D'Hose *et al.* 2018). Certain functional groups — defined as organisms sharing similar feeding preferences, reproductive rates, and predators (Holtkamp *et al.* 2008), such as predatory nematodes and mites, regulate pest populations *via* predation, thereby providing regulating services (Ferris *et al.* 2001; Steel and Ferris 2016). The soil food web is typically characterized by its size, referring to the total abundance or biomass of major trophic groups (de Vries *et al.* 2013), and its structure, which reflects the composition and trophic interactions among these groups (Ferris *et al.* 2001).

Chemical fertilizer, manure, and their combination are common nutrient management practices that have been critical for enhancing agricultural productivity and soil health. Chemical fertilizer supplies high concentrations of nitrogen (N), phosphorus (P), and potassium (K) directly to crops without adding OM, resulting in approximately 50% higher yields compared to unfertilized fields (Yang *et al.* 2025). Increased above-ground crop biomass under fertilization leads to greater below-ground root biomass and OM input (Geisseler and Scow 2014), which provides energy substrates for the soil food web. However, excessive use of inorganic fertilizers causes significant adverse effects on soil and the environment, including soil acidification and elevated greenhouse gas emissions (Yu *et al.* 2025). The impacts of chemical fertilizers on soil organisms are variable and depend on application rates and the type of nitrogen fertilizer applied. Over-application generally exerts negative effects on soil microorganisms (Geisseler and Scow 2014). In contrast, organic fertilizers primarily supply exogenous OM and limited available nutrients, thereby stimulating the entire soil food web. A limitation of organic fertilizers is their inability to rapidly increase crop yield due to slow nutrient release and poor synchronization with crop nutrient demands. Combinations of inorganic and organic fertilizers offer both high OM content and substantial N, P, and K inputs, representing a promising direction for sustainable fertilizer development (Cao *et al*. 2024; Wang *et al*. 2025). To date, long-term fertilization effects on soil food web components have mostly been studied in isolation — for example, focusing on soil microbes (Geisseler and Scow 2014; Hu *et al.* 2024), protozoa (Hu *et al.* 2024), nematodes (Liang *et al.* 2009), mites (Cao *et al.* 2011), earthworms (Bertrand *et al.* 2015), or small subsets such as micro-food webs (Zhang *et al.* 2016). Nevertheless, comprehensive studies examining changes across the entire soil food web remain scarce. Generally, organic or combined inorganic-organic amendments positively affect lower trophic levels. However, for higher trophic groups such as predatory mites, negative responses have also been reported (Cao *et al.* 2011).

Understanding the factors that regulate soil food web structure is essential for elucidating ecosystem processes and services. Exogenous organic substrates can shape upper trophic level communities through metabolic activity, representing a bottom-up effect, whereas predators that control lower-level organisms *via* consumption representing a top-down effect (Dyer and Letourneau 2003; Asiloglu *et al.* 2021). Additionally, predator effects on non-adjacent trophic levels constitute indirect species interactions, known as trophic cascades (Liu *et al.* 2016; Ripple *et al.* 2016). In simple soil food chains, such as the nutrient–bacteria–protist axis, these effects can be inferred from shifts in community composition in response to variations in bottom-up drivers (e.g., nutrient availability) and top-down regulators (e.g., protist abundance/diversity) (Asiloglu *et al.* 2021; Bodur *et al.* 2024). However, in complex food webs, the presence of intermediate and omnivorous consumers complicates such inference (Holtkamp *et al.* 2008; Chen *et al.* 2019). Consequently, ecological indices — such as those based on nematode communities — have been developed to infer bottom-up and top-down influences (Dou *et al*. 2025). Ferris and colleagues integrated nematode colonizer-persister (cp) scaling, food sources, and carbon utilization efficiency to develop indices including the enrichment index (EI), enrichment footprint (efoot), maturity index (MI), structure index (SI), and structure footprint (sfoot), which collectively assess food web dynamics (Du Preez *et al.* 2022). Using these indices, researchers have evaluated how fertilization regulates the soil food web and quantified the relative contributions of bottom-up *vs*. top-down forces. For instance, Chen *et al.* (2021) demonstrated that long-term straw incorporation combined with chemical fertilizer primarily regulated the soil food web through top-down mechanisms. Conversely, Kou *et al.* (2020) found that stover mulching drove soil micro-food web structure and activity *via* bottom-up effects.

The rice–wheat rotation system is a dominant agricultural practice in the middle-lower reaches of the Yangtze River in Central China. To investigate soil fertility dynamics under long-term fertilization, a field experiment has been conducted since 1981. Previous research indicated that organic fertilizer, either alone or in combination with chemical fertilizer, significantly improved soil productivity, physicochemical properties, and fertility, while rational application of compound chemical fertilizer also enhanced soil quality (Xu *et al.* 2015). However, the responses of the entire soil food web to prolonged fertilization and the underlying regulatory mechanisms remain poorly understood. Therefore, this study aimed to: (i) examine the effects of long-term application of chemical fertilizer, manure, and manure combined with chemical fertilizer on the abundance and biomass of functional groups; and (ii) explore the mechanisms by which long-term fertilization regulates soil food web size. We hypothesized that: (i) long-term fertilization would positively influence soil food web components, with greater enhancements under manure and manure-chemical fertilizer treatments than under chemical fertilizer alone, due to increased organic substrate inputs; and (ii) both bottom-up (resource-driven) and top-down (predation-driven) forces jointly shape soil food web size, as

fertilization alters basal resource availability and predator-prey interactions at higher trophic levels.

2. Material and methods

2.1. Study site and experiment design

The study site and experimental design were described in detail in our previous work (Hu *et al.* 2019). Briefly, the long-term trial was conducted at the Nanhu Experimental Station, Hubei Academy of Agricultural Sciences, Wuhan, China (30°29′42′′N, 114°18′54′′E), located in a subtropical monsoon climate zone with soil classified as yellow-brown earth. The experiment included nine treatments with three replicates; however, only four treatments were selected for this study: (1) no fertilizer input (CK); (2) mineral N-P-K fertilizer (NPK); (3) pig manure compost (M); (4) NPK+manure (NPKM). In the NPK treatment, 60 kg N ha^{-1}, 30 kg P_2O_5 ha^{-1}, and 60 kg K_2O ha^{-1} were applied during the wheat growing season: 50% of N plus full P_2O_5/K_2O as basal fertilizer before sowing, 25% N at tillering, and 25% N at jointing. For rice, 90 kg N ha^{-1}, 45 kg P_2O_5 ha^{-1}, and 90 kg K_2O ha^{-1} were applied, 40% N plus full P_2O_5/K_2O as basal fertilizer before sowing, 40% N at tillering, and 20% N at booting. Urea, ammonium phosphate, and potassium chloride were used as fertilizers in both seasons. The M treatment received 11.25 t ha^{-1} of pig manure compost per season, with an average composition of organic carbon (OC), 282.1 g kg^{-1}; total N, 15.1 g kg^{-1}; total P, 9.1 g kg^{-1}; total K, 11.3 g kg^{-1}; and moisture content, 69.0%. In the NPKM treatment, the chemical fertilizer application matched that of the NPK treatment, and the manure application was identical to that in the M treatment.

2.2. Soil sampling

Samples for analyzing soil physicochemical properties, roots, and biota (microorganisms, protozoa, nematodes, microarthropods, and earthworms) were collected on April 24 (wheat maturity) and October 18 (after rice harvest) in 2010, and on June 2 (after wheat harvest) and October 19 (after rice harvest) in 2011. Enchytraeid samples were taken on May 31, 2013, and May 29, 2014. Soil samples consisted of 10 cores (4.6 cm diameter) from the 0–20 cm depth layer, excluding those for earthworms, roots, and bulk density. Earthworms were manually collected from two 50 cm×50 cm×20 cm subplots per plot. Wheat roots were sampled from two randomly selected 20 cm×20 cm subplots, whereas rice roots were collected from five plants per plot. Bulk density was determined using a 100 cm^3 soil core sampler, with triplicate measurements per plot.

The soil was homogenized and divided into three subsamples, except for bulk density samples. Air-dried subsamples (200 g) were sieved (<2 mm) for analysis of pH, AN, available P and K (AP and AK), soil organic carbon (SOC), labile organic carbon (LOC), and recalcitrant organic carbon (ROC). Freeze-dried subsamples (50 g) were sieved (<2 mm) and stored at –20°C for microbial community analysis. Approximately 1,000 g of fresh subsamples were stored at 4°C for analysis of microbial biomass carbon (MBC), protozoa, nematodes, and microarthropods.

2.3. Soil physicochemical properties and soil food web components

Soil pH was measured in a 1:1 soil-to-water suspension using a pH electrode. AN was determined using the alkaline hydrolysable diffusion method. AP was extracted with 0.5 mol L^{-1} $NaHCO_3$ and quantified *via* the Olsen method, while AK was extracted with 1 mol L^{-1} NH_4OAc and measured using a flame photometer. SOC was analyzed using the potassium bichromate-dilution heat colorimetric method following Bao (2000). ROC was defined as the acid-hydrolysis-resistant fraction (6 mol L^{-1} HCl), including lignin, and was quantified using the dichromate redox titration method. LOC, comprising cellulose, hemicellulose, and water-soluble sugars, was calculated as SOC minus ROC (Plante *et al.* 2006). Soil MBC was determined using the $CHCl_3$ fumigation-extraction method (Wu *et al.* 2006). Roots separated from soil were manually washed, oven-dried at 65°C, and expressed as root biomass (kg ha^{-1}).

Soil microbial abundances were assessed *via* phospholipid fatty acids (PLFAs), following Dunfield (2007). Bacterial biomass was indicated by 12 fatty acids: 15:0, i15:0, a15:0, i16:0, 16:1ω9, 16:1ω7t, i17:0, a17:0, 17:0, cy17:0, cy19:0 (Frostegård and Bååth 1996), while fungal biomass was represented by 18:2ω6 (Joergensen and Wichern 2008). PLFA concentrations were expressed as nmol g^{-1} dry soil. Bacteria and fungi were assumed to utilize LOC and ROC, respectively (Fig. 1) (Holtkamp *et al.* 2008).

Protozoan abundance was determined using the most probable number method (Yin 1992). Protozoa were categorized into three functional groups: flagellates, ciliates, and amoebae. All groups were presumed to graze on bacteria, with flagellates serving as prey for ciliates and amoebae (Fig. 1) (Moore and De Ruiter 2012). Abundance was expressed as individuals per gram of dry soil.

Nematodes were extracted from 100 g of fresh soil using the elutriation-sugar flotation method (Ingham 1994). After counting the total number, 100 individuals were randomly selected and identified to genus level. Nematodes were assigned to five functional groups: bacterivores, fungivores, plant parasites, omnivores, and predators. Abundance was reported as individuals per 100 g of dry soil. Bacterivores and fungivores consumed bacteria and fungi, respectively, while plant-parasitic nematodes fed on root tissues. Omnivorous nematodes consumed bacteria, flagellates, amoebae, bacterivores, fungivores, and plant-parasitic nematodes. Predatory nematodes preyed upon all aforementioned nematode groups (Fig. 1) (Holtkamp *et al.* 2008).

Microarthropods, including mites (Acari) and collembolans, were extracted from 300 g of fresh soil using a Tullgren funnel under 25 W lighting for 48 h (Cao *et al.* 2011). Mites were classified to family level and grouped as fungivorous cryptostigmatic, omnivorous non-cryptostigmatic, and predaceous mites. Fungivorous cryptostigmatic mites consumed fungi; omnivorous non-cryptostigmatic mites fed on fungi and fungivorous nematodes; predaceous mites preyed on all nematode groups, fungivorous cryptostigmatic mites, and omnivorous non-cryptostigmatic mites (Fig. 1) (Vreeken-Buijs *et al.* 1994; Holtkamp *et al.* 2008). Collembolans

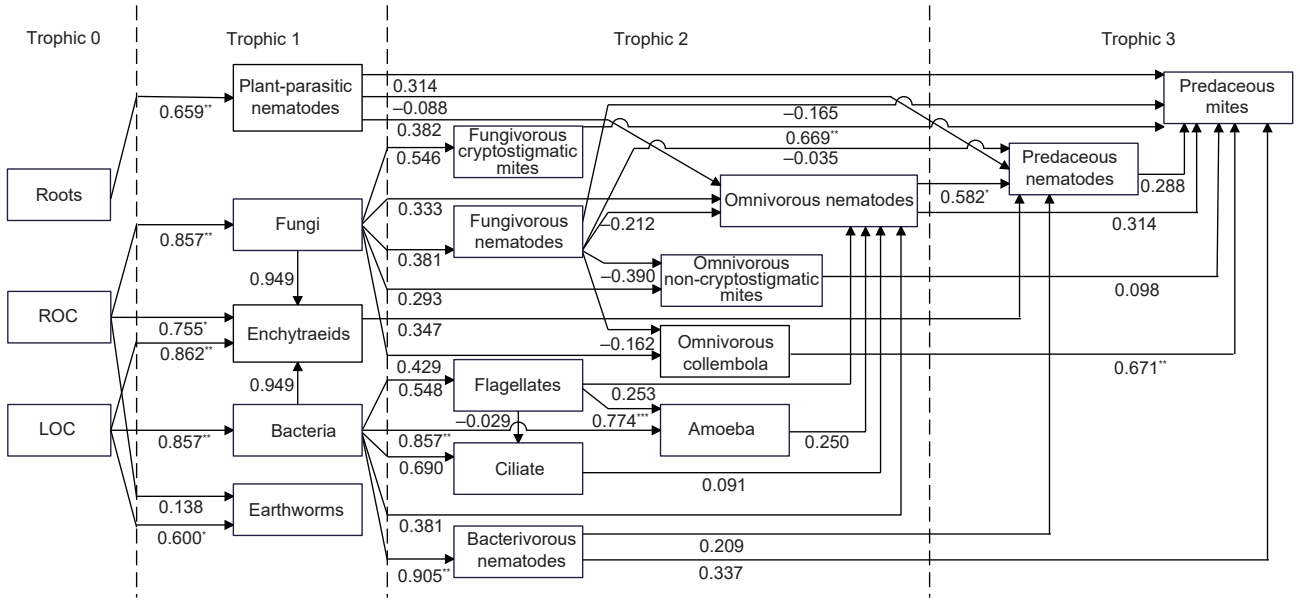


Fig. 1 Soil food web diagram. Arrows represent trophic interactions. Numbers below horizontal arrows and beside vertical arrows indicate Spearman's correlation coefficients between corresponding soil components in the food web. ROC, recalcitrant organic carbon; LOC, labile organic carbon. *, P<0.05; **, P<0.01; ***, P<0.001.

were identified to family level and classified as omnivorous. Omnivorous collembolans consumed fungi and fungivorous nematodes and served as prey for predaceous mites (Fig. 1) (Vreeken-Buijs *et al.* 1994).

Enchytraeids were extracted using the silica gel extraction method and counted under a stereoscopic microscope (Li *et al.* 2021). They were assumed to feed on bacteria, fungi, LOC, and ROC, and were preyed upon by predatory nematodes (Fig. 1) (Holtkamp *et al.* 2008; Moore and De Ruiter 2012). Earthworms were hand-sorted and weighed in the laboratory, and were presumed to consume ROC and LOC (Moore and De Ruiter 2012).

2.4. Nematode-based indices

MI is computed as a weighted mean cp value:

$$MI=\sum(v_i \times p_i)$$

where v_i is the cp value and p_i is the proportion of genus "i" among free-living nematodes (Bongers 1990).

EI and SI are calculated from the weighted faunal components (Ferris *et al.* 2001):

$$EI=100\times[e/(e+b)]$$

$$SI=100\times[s/(b+s)]$$

where b, e, s are weighted basal (b), enrichment (e), and structural (s) components of the nematode assemblage. $b=(Ba_2+Fu_2)\times W_2$, where $W_2=0.8$; $e=(Ba_1\times W_1)+(Fu_2\times W_2)$, where $W_1=3.2$; $s=(Ba_n\times W_n+Fu_n\times W_n+Om_n\times W_n+Pr_n\times W_n)$, where n=3–5, $W_3=0.8$, $W_4=3.2$, $W_5=5.0$. Ba, Fu, Om, and Pr denote the abundances of bacterivores, fungivores, omnivores, and predatory nematodes, respectively, and n indicates the cp value of nematode taxa.

NMF is the sum of C utilization of the production and respiration components (Ferris 2010):

$$NMF=\sum[N_t\times(0.1\times(W_t/m_t)+0.273\times W_t^{0.75})]$$

where N_t is abundance, W_t is body mass, and m_t is the cp value of genus "t"; W_t data are available at http://nemaplex.ucdavis.edu/. The efoot and sfoot represent the metabolic footprints of low (cp 1–2) and high (cp 3–5) trophic groups, respectively. Functional metabolic footprints (FMF) of nematodes were calculated following Ferris (2010). The FMF was geometrically defined by sequentially connecting the following points: (SI–0.5F_s/k, EI), (SI, EI+F_e/k), (SI+0.5F_s/k, EI), (SI, EI–0.5F_e/k), and (SI, EI), where k is the scaling constant, SI and EI represent the structure index and enrichment index, respectively. The structure footprint (F_s) represents the sum of standardized carbon source utilization by structural indicator taxa, whereas the enrichment footprint (F_e) represents the corresponding sum for enrichment indicator taxa.

2.5. Data analysis

Three potential factors of fertilization, sampling year, and crop type were evaluated, with fertilization effects as the primary focus. One-way analysis of variance (ANOVA) was used to assess fertilization effects within individual seasons. Two-way ANOVA was used to test fertilization effects with year included as a factor, and three-way ANOVA was used to assess the effects of fertilization, year, crop type, and their interactions. Least significant difference (LSD) post hoc tests were applied to compare treatment means. $Log_{10}(x+1)$, $1/x$, and square root transformations were applied to data with heterogeneous variances. Spearman's correlation analysis was performed to examine prey-predator relationships among functional groups, using the mean biomass or abundance across four sampling times as input. ANOVA and correlation analyses were conducted using SPSS 26.0 (IBM Corp., Armonk, USA), with significance levels set at P<0.05, 0.01, or 0.001.

Partial least squares path modeling (PLS-PM) was

employed to evaluate fertilization-driven regulation of soil food webs *via* bottom-up and top-down pathways. A theoretically informed conceptual model guided PLS-PM construction. The initial model included paths: (1) from fertilization to bottom-up and top-down effects; and (2) from these effects to the soil food web. Fertilization was represented by three observed variables: roots, ROC, and LOC. Bottom-up control was indicated by EI and efoot, while top-down control was represented by SI, MI, and sfoot. The soil food web structure was characterized by the sum of standardized biomass (0–1 normalized) of all functional groups in the food web, with bacterial and fungal biomass represented by MBC. Model fit was assessed using R^2 (coefficient of determination) and GoF (goodness-of-fit) (Wang *et al.* 2023). PLS-PM analyses were performed using the "plspm" package in R (version 4.4.1).

3. Results

3.1. Effects of long-term fertilization on soil physico-chemical properties

Long-term fertilization significantly increased soil AN, AP, SOC, and MBC (excluding pH), with values following the order: CK<NPK<M<NPKM (Fig. 2; Appendices A and B). Relative to CK, fertilization elevated AN, AP, SOC, and MBC by 9.24–32.42, 55.10–759.93, 18.26–52.53, and 45.10–118.72%, respectively. Compared to NPK, both NPKM and M treatments exhibited significantly higher levels: AN increased by 21.20% (P<0.001) and 17.54% (P<0.001); AP rose by 454.43% (P<0.001) and 315.16% (P<0.001); SOC improved by 28.73% (P<0.001) and 21.24% (P<0.001); and MBC increased by 50.73% (P<0.001) and 40.16% (P<0.001), respectively. Bulk density showed an inverse trend, with M and NPKM treatments being 4.18% (P<0.05) and 9.89% (P<0.05) lower than NPK. Soil AK followed CK<M<NPK<NPKM, with NPKM exceeding NPK by 21.32% (P<0.05). Overall, NPKM consistently yielded the most substantial improvements.

3.2. Effects of long-term fertilization on soil food web component organisms

Nineteen functional groups were identified and classified into four trophic levels (Fig. 1). Trophic level 0 included roots, ROC, and LOC, with biomass following CK<NPK<M<NPKM (Table 1; Appendix B). Compared to CK, fertilization increased root biomass, ROC, and LOC by 37.01–76.85, 15.65–40.41, and 27.24–92.94%, respectively. Relative to NPK, root biomass increased by 40.07% (P<0.001) in NPKM and by 14.41% (P >0.05) in M; ROC was significantly higher by 21.41% (P<0.001) and 20.29% (P<0.001); and LOC increased by 51.63% (P<0.001) and 24.24% (P<0.05). No significant differences were observed between M and NPKM treatments.

Trophic level 1 encompassed bacteria, fungi, plant — parasitic nematodes, enchytraeids, and earthworms — all enhanced by long-term fertilization (Table 1; Appendices C–E). Although bacterial and fungal abundance followed CK<NPK<M<NPKM, differences among CK, NPK, and M were not statistically significant. Plant-parasitic nematodes, enchytraeids, and earthworms followed CK<NPK<NPKM<M, with no significant difference between NPKM and M. However, plant-parasitic nematodes were significantly more

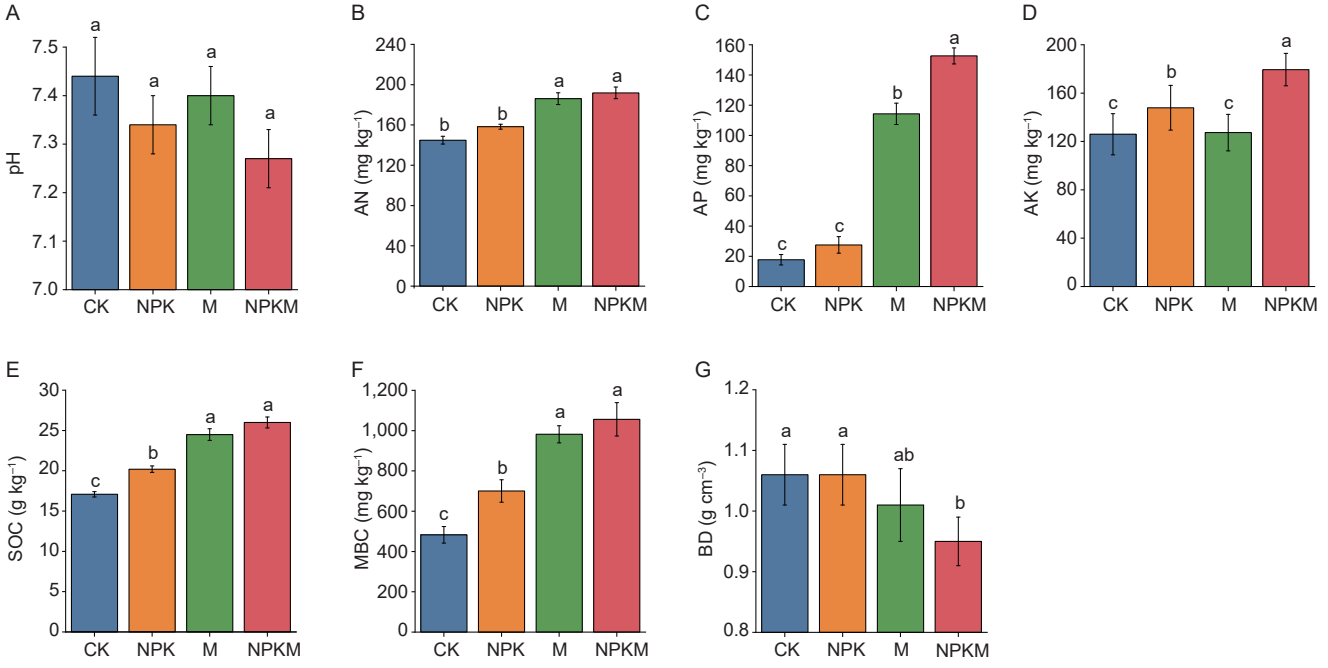


Fig. 2 Selected soil physicochemical properties across different fertilization treatments. CK, non-fertilized control; NPK, chemical fertilizer; M, manure; NPKM, chemical fertilizer combined with manure; AN, alkali-hydrolyzable nitrogen; AP, available phosphorus; AK, available potassium; SOC, soil organic carbon; MBC, microbial biomass carbon; BD, bulk density. Data are mean±standard error (n=3). Different letters indicate significant differences at P<0.05. Two-way ANOVA was used to analyze differences in pH, AN, AP, AK, and BD; three-way ANOVA was applied for SOC and MBC.

Table 1 Abundance or biomass of functional groups under different fertilization treatments[1)]

Taxa[2)]	CK	NPK	M	NPKM	Year (Y)	Crops (C)	Fertilizers (F)	Y×C	Y×F	C×F	Y×C×F
Trophic level 0											
Roots (kg ha^{-1})	436.50±74.54 c	598.04±86.29 b	684.23±83.47 ab	771.94±160.89 a	<0.01	<0.001	<0.001	ns	ns	<0.01	<0.05
Recalcitrant organic carbon (g kg^{-1})	13.24±0.38 c	15.31±0.38 b	18.42±0.61 a	18.59±0.37 a	ns	ns	<0.001	ns	ns	ns	ns
Labile organic carbon (g kg^{-1})	3.84±0.43 c	4.89±0.53 bc	6.07±0.67 ab	7.41±0.52 a	<0.05	ns	<0.001	ns	ns	ns	ns
Trophic level 1											
Bacteria PLFAs (nmol g^{-1})	17.37±0.51 c	20.79±1.08 bc	25.59±2.89 ab	29.12±1.92 a	–	ns	<0.01	–	–	ns	–
Fungi PLFAs (nmol g^{-1})	0.84±0.07 b	0.87±0.07 b	1.05±0.11 b	1.33±0.05 a	–	ns	<0.01	–	–	ns	–
Herbivorous nematodes (individual/100 g)	46.67±11.5 b	61.51±8.17 b	103.14±9.53 a	90.34±13.61 a	ns	<0.01	=0.001	ns	ns	ns	ns
Enchytraeids (individual/100 g)	0.45±0.45 b	0.92±0.58 b	8.44±4.26 a	4.22±1.88 a	<0.01	–	<0.05	<0.05	–	–	–
Earthworm (g m^{-2})	3.92±1.45 b	5.38±2.37 b	15.89±5.69 a	14.49±4.06 a	ns	<0.001	<0.01	ns	ns	<0.05	ns
Trophic level 2											
Flagellate (individual g^{-1})	2,694.54±1,264.18 b	979.40±323.36 b	10,504.30±3,762.37 a	8,739.22±2563.12 a	ns	ns	<0.05	<0.001	ns	ns	<0.05
Ciliate (individual g^{-1})	204.32±198.63 a	34.25±31.59 a	319.68±292.15 a	220.09±147.87 a	ns	ns	ns	ns	ns	ns	ns
Amoebae (individual g^{-1})	385.79±242.10 b	699.67±392.70 b	918.19±490.42 a	1,157.86±645.12 a	<0.001	<0.001	<0.001	<0.001	<0.05	<0.05	ns
Bacterivorous nematodes (individual/100 g)	89.092±9.07 c	157.12±22.78 b	298.70±42.82 a	304.74±35.54 a	ns	ns	<0.001	<0.05	ns	ns	ns
Fungivorous nematodes (individual/100 g)	272.73±58.31 a	184.77±18.34 a	243.51±31.30 a	233.83±52.28 a	ns	ns	ns	ns	ns	ns	ns
Omnivorous nematodes (individual/100 g)	199.92±46.43 c	316.91±71.74 bc	384.73±116.36 b	694.07±203.11 a	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05
Fungivorous cryptostigmatic mites (individual kg^{-1})	6.12±2.43 a	7.70±4.22 a	11.55±3.60 a	14.90±6.37 a	<0.001	ns	ns	ns	ns	ns	ns
Ominvorous non-cryptostigmatic mites (individual kg^{-1})	0.75±0.51 b	1.85±1.03 ab	1.81±1.12 ab	5.47±2.73 a	ns	ns	<0.05	ns	<0.05	ns	ns
Omnivorous collembola (individual kg^{-1})	3.65±1.30 a	10.73±30.00 a	13.14±5.71 a	9.85±2.51 a	<0.01	ns	ns	ns	ns	ns	ns
Trophic level 3											
Predaceous nematodes (individual/100 g)	58.43±13.16 b	115.87±23.63 a	58.13±11.90 b	52.6±8.15 b	<0.01	ns	<0.05	<0.05	ns	ns	ns
Predaceous mites (individual kg^{-1})	0.34±0.34 b	0.71±0.48 b	2.17±0.84 a	1.06±0.55 ab	<0.01	ns	<0.05	ns	ns	ns	<0.05

[1)] CK, non-fertilized control; NPK, chemical fertilizer; M, manure; NPKM, chemical fertilizer combined with manure.
[2)] PLFAs, phospholipid fatty acids.
Data are presented as mean±standard error (n=3). Different lowercase letters within the same row indicate significant differences at P≤0.05. Two-way ANOVA was used to analyze differences among bacteria, fungi, and enchytraeids, while three-way ANOVA was applied to other soil functional groups. ns indicates no significant difference, and – indicates that no statistical analysis was performed.

abundant in NPKM and M than in NPK, increasing by 48.68% (P=0.001) and 67.68% (P=0.001), respectively. Enchytraeid abundance increased by 358.24% (P<0.05) and 815.53% (P<0.05), while earthworms increased by 169.41% (P<0.01) and 195.42% (P<0.01).

Trophic level 2 consisted of nine functional groups, including microbivorous consumers and omnivorous organisms (Table 1; Appendices D–G). No significant differences were detected in ciliates, fungivorous nematodes, cryptostigmatic mites, or omnivorous collembolans. Amoebae, bacterivorous nematodes, and omnivorous nematodes followed CK<NPK<M<NPKM. Abundance of amoebae in NPKM and M was significantly greater than in NPK, increasing by 65.49% (P<0.001) and 31.23% (P<0.001), respectively; bacterivorous nematodes rose by 93.96% (P<0.001) and 90.11% (P<0.001); and omnivorous nematodes increased by 110.01% (P<0.001) and 21.40% (P<0.001). Flagellate abundance followed NPK<CK<NPKM<M, with NPKM and M exceeding CK by 224.33 and 289.84%, respectively. Omnivorous non-cryptostigmatic mite abundance followed CK<M<NPK<NPKM, with NPKM exceeding CK by 629.63%.

Trophic level 3 included two functional groups: predaceous nematodes and predaceous mites (Table 1; Appendices D and G). Predaceous nematode abundance was significantly higher in NPK than in CK, M, and NPKM across all sampling years and crops. In contrast, predaceous mite abundance was the highest under M and the lowest under CK, with NPKM and M exceeding NPK by 48.25% (P<0.05) and 204.35% (P<0.05), respectively.

3.3. Bottom-up and top-down effects derived from long-term fertilization

Based on feeding relationships, correlations between functional groups at lower and adjacent or higher trophic levels are shown in Fig. 1. Functional groups in trophic level 0 exhibited significant positive correlations with those in trophic level 1, except for ROC and earthworms. Correlations between trophic levels 0 and 2 were predominantly positive, with significant associations observed between roots and omnivorous nematodes (P<0.01), LOC and amoebae (P<0.01), and LOC and bacterivorous nematodes (P<0.01). No significant correlations existed between trophic levels 0 and 3, except for the relationship between LOC and predaceous mites. Between trophic levels 1 and 2/3, only bacteria showed significant positive correlations with amoebae (P<0.01) and bacterivorous nematodes (P<0.01). Between trophic levels 2 and 3, omnivorous nematodes were positively correlated with predaceous nematodes (P<0.05), and collembolans with predaceous mites (P<0.01). These findings indicate dominant bottom-up control within the soil food web.

Nematode ecological indices are presented in Table 2 and Appendix H. Fertilization consistently increased EI and efoot across years and crop types. EI followed the order CK<NPK<NPKM<M, with M and NPKM exceeding NPK by 51.27% (P<0.05) and 28.49% (P<0.05), respectively. The efoot followed the order CK<NPK<M<NPKM, with NPKM and M surpassing NPK by 47.17% (P<0.001) and 11.80% (P<0.001). MI did not differ between NPK and CK, but NPK was 8.55% higher than NPKM and 10.17% higher than M (P<0.001 for both comparisons). SI decreased under fertilization, with NPKM and M being 3.24% (P<0.05) and 3.84% (P<0.05) lower than NPK, respectively. Sfoot was significantly enhanced by fertilization, following the order CK<NPK<M<NPKM, with NPKM and M exceeding NPK by 42.04% (P<0.001) and 11.80% (P<0.05). In the EI–SI plot (Fig. 3), M and NPKM were located in Quadrant B (EI>50 and SI>50), whereas NPK and CK were in Quadrant C (EI<50 and SI>50). FMF followed the order CK<NPK<M<NPKM (Fig. 3), with M and NPKM exceeding NPK by 66.75% (P<0.001) and 384.16% (P<0.001), respectively.

PLS-PM analysis revealed that fertilization strengthened bottom-up effects, thereby promoting soil food web size (Fig. 4). While top-down effects also increased, they exerted negative impacts on food web structure. Bottom-up effects dominated, as indicated by higher path coefficients linking

Table 2 Ecological indices under different fertilization treatments

Treatments[1)]	Bottom-up effects[2)]		Top-down effects[3)]		
	EI	efoot	MI	SI	sfoot
CK	31.70±9.14 b	319.64±67.58 c	3.39±0.08 a	96.71±0.69 a	258.35±54.40 c
NPK	36.66±7.21 ab	540.2±109.32 b	3.43±0.10 a	95.05±1.03 ab	432.78±84.73 b
M	55.46±6.12 a	603.96±157.91 b	3.08±0.12 b	91.41±1.83 b	442.86±119.91 b
NPKM	51.27±7.37 a	1,022.61±272.45 a	3.16±0.14 b	92.06±1.63 b	746.67±202.23 a
ANOVA effects					
Year (Y)	<0.01	<0.001	<0.001	<0.05	<0.001
Crops (C)	ns	<0.001	<0.001	<0.001	<0.001
Fertilizers (F)	<0.05	<0.001	0.001	<0.01	<0.001
Y×C	ns	<0.01	ns	ns	ns
Y×F	ns	<0.001	ns	ns	<0.001
C×F	<0.05	<0.01	<0.05	ns	<0.01
Y×C×F	<0.05	<0.05	ns	ns	ns

[1)] CK, non-fertilized control; NPK, chemical fertilizer; M, manure; NPKM, chemical fertilizer combined with manure.
[2)] EI, enrichment index; efoot, enrichment footprint.
[3)] MI, maturity index; SI, structural index; sfoot, structure footprint.
Data are presented as mean±standard error (n=3). Different letters within a column indicate significant differences at P<0.05. Values marked as ns are not statistically significant.

these forces to food web organization.

4. Discussion

In the present study, soil resources — including roots, SOC, ROC, LOC, and soil nutrients — followed the order CK<NPK<M<NPKM, consistent with our previous findings (Xu *et al.* 2015) and other studies (Gao *et al.* 2023; Kumari *et al.* 2024). Most functional groups across trophic levels 1–3 exhibited the same trend, with significant differences observed between NPK and M or NPKM treatments in multiple cases. These results indicate that long-term application of manure, either alone or combined with chemical fertilizer, enriches the soil food web more effectively than chemical fertilizer alone. This enhancement occurred because M and NPKM treatments supplied greater quantities of substrates (Fig. 1) and energy, which cascaded upward to functional groups at higher trophic levels. Birkhofer *et al.* (2008) reported elevated bacterial and fungal PLFA biomarkers, as well as nematode guilds (bacterivores, herbivores, and omnivores), under NPKM (manure combined with fertilizer) and M (composted manure) compared to NPK. Forge *et al.* (2005) observed significantly higher abundances of protozoa, bacterivorous, and fungivorous nematodes in M (dairy manure slurry) relative to NPK. Kautz *et al.* (2006) documented increased microarthropod abundance (e.g., mites and collembolans) in NPKM (straw+sorghum leaf green manure+chemical fertilizer) and M (straw+sorghum green manure) compared to NPK. Wang *et al.* (2015) similarly found higher microarthropod abundance in M (soybean cake) than in NPK. Pelosi and Römbke (2016) reviewed the positive effects of organic fertilizers on enchytraeids. Cao *et al.* (2006) demonstrated that organic fertilizer (farmyard manure) enhanced earthworm abundance in both low- and high-fertility soils compared to NPK.

Notably, the nonsignificant increases from M to NPKM are likely attributable to long-term manure applications elevating soil fertility to near-maximum levels, beyond which additional inputs of chemical fertilizers exert limited effects. SOC content in M treatments exceeded 20.0 g kg^{-1}, an exceptionally high value for rice–wheat systems in the middle-lower reaches of the Yangtze River plains (Xu *et al.* 2015). EI-SI profiling (Fig. 3) revealed that both M and NPKM treatments fell within the maturing quadrant, indicating low-to-moderate disturbance levels (Ferris and Benavides 2024), which further explains their nonsignificant differences. Despite the lack of statistical significance, NPKM showed higher abundances of small-bodied groups (e.g., bacteria, fungi) but lower abundances of large-bodied trophic level 3 groups compared to M. These patterns suggest that chemical fertilizers may promote certain soil biota while inhibiting others. Indeed, high concentrations of chemical fertilizers can be toxic to sensitive, large-bodied species (Ferris *et al.* 2012; Pelosi and Römbke 2016). Li *et al.* (2021) reported reduced biomass of collembolans, enchytraeids, and earthworms under conventional management (organic+chemical fertilizers) compared to organic-only management. Similarly, Cao *et al.* (2011) documented decreased mite abundance and diversity under long-term chemical fertilizer application.

Biomass generally declined with increasing trophic level. Holtkamp *et al.* (2008) reported that biomass at trophic level 0 was hundreds-fold greater than at level 1 and thousands-fold greater than at level 2. Although some functional groups in this study were quantified by abundance rather than biomass, it can be inferred that M and NPKM treatments enriched the total biomass of the soil food web more than NPK, given the higher biomass values at trophic levels 0–1 in these treatments. Additionally, a higher functional metabolic footprint (FMF) reflects greater carbon allocation to biomass production and elevated CO_2 respiration rates (Zhong *et al.* 2017). In this study, the FMF followed the order CK<NPK<M<NPKM, further confirming nutrient-driven expansion of the food web.

EI and efoot — derived from nematode abundance, carbon allocation patterns, and metabolic rates — reflect fertilization-

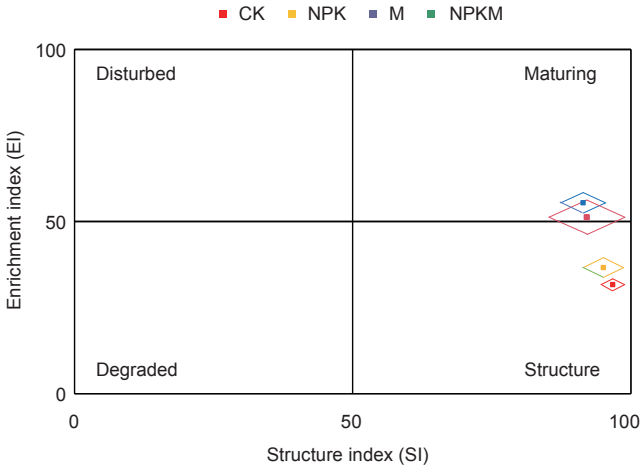


Fig. 3 Functional metabolic footprint of nematodes under different fertilization treatments. CK, non-fertilized control; NPK, chemical fertilizer; M, manure; NPKM, chemical fertilizer combined with manure. The area of rhombus represents functional metabolic footprints.

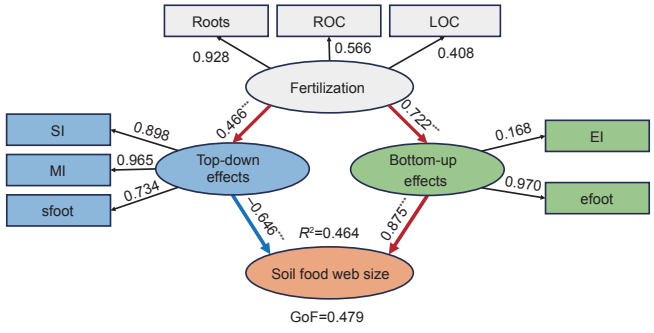


Fig. 4 Partial least squares path modeling (PLS-PM) illustrating the effects of fertilization on the soil food web size. Red and blue lines indicate positive and negative correlations, respectively. ROC, recalcitrant organic carbon; LOC, labile organic carbon; SI, structural index; MI, maturity index; sfoot, structure footprint; EI, enrichment index; efoot, enrichment footprint. Numbers adjacent to the arrows represent standardized path coefficients, indicating the strength and direction of relationships. Values associated with thin black arrows denote outer loadings, reflecting the reliability of latent variable indicators. ***, P<0.001.

induced resource availability (e.g., labile carbon) and nutrient mineralization capacity (Chen *et al.* 2021; Du Preez *et al.* 2022). Both indices increased progressively from CK to NPK and more markedly under M and NPKM, indicating enhanced food web functionality with increasing fertility and reinforcing strong substrate-driven bottom-up effects. Furthermore, substrate enrichment under fertilization increased biomass or abundance across most functional groups, demonstrating upward propagation to higher trophic levels. Significant positive Spearman correlations between adjacent and higher trophic groups (Fig. 1) further support dominant bottom-up control. Several studies have documented such nutrient-driven bottom-up effects. For example, Li *et al.* (2021) found greater biomass across functional groups under organic management compared to conventional management, attributed to improved soil nutrient availability. Moore and de Ruiter (2012) synthesized data from six agricultural food webs, showing resource-driven increases in the biomass of most functional groups.

MI and SI reflect environmental disturbances (e.g., fertilization), with lower values indicating higher disturbance intensity due to the loss of high-trophic-level nematodes (Du Preez *et al.* 2022). In this study, MI and SI followed an opposite trend to EI and efoot, confirming the presence of top-down effects. Sfoot quantifies the strength of regulatory functions (Ferris 2010), and the sfoot order CK<NPK<M<NPKM indicates greater carbon and energy allocation to higher trophic levels, thereby reinforcing top-down regulation. Zhang *et al.* (2016) demonstrated strong top-down regulation *via* predation by omnivorous-predators on bacterivores and fungivores. Moore and de Ruiter (2012) synthesized evidence that strong top-down effects at lower trophic levels coexist with strong bottom-up effects at higher levels. Nevertheless, PLS-PM analysis in this study prioritized bottom-up over top-down regulation of the soil food web (Fig. 4).

The underlying mechanism for the dominance of bottom-up regulation likely stems from the quality of exogenous organic substrates. Low C/N ratio (<30:1) fertilizers (e.g., pig manure) tend to increase the abundance of organisms within the bacterial decomposition channel, including bacteria, protozoa, and bacterial-feeding nematodes. Because bacterivorous fauna typically exhibit higher biomass and faster turnover rates than fungivorous fauna, they channel more carbon and energy toward opportunistic feeders and less toward omnivores and predators (Bernal *et al.* 2009). In this study, a higher efoot relative to sfoot further supports this pattern. In contrast, inputs with higher C/N ratios (>30:1) tend to favor organisms in the fungal decomposition channel, such as fungi and non-plant-parasitic stylet-bearing nematodes. Given that fungivorous fauna generally have lower biomass and slower turnover rates, they transfer more carbon and energy to omnivores and predators. For instance, Chen *et al.* (2021) found that straw incorporation (C/N ratio>30:1) increased sfoot and the abundance of omnivorous and predaceous nematodes without altering EI or efoot, indicating top-down-driven regulation.

5. Conclusion

Based on a long-term fertilization trial lasting over 30 years, this study highlights the effects of chemical fertilizer, manure, and their combination on the size and structure of soil food web components, and explores how long-term fertilization regulates the soil food web. The results suggest that long-term fertilization increases the biomass or abundance of most functional groups primarily through bottom-up effects, although top-down effects were also evident. Manure and manure plus chemical fertilizer enriched the soil food web more than chemical fertilizer alone by providing additional nutrients and substrates. However, the difference between manure and manure plus fertilizer treatments was not statistically significant, likely due to the already high soil fertility achieved under long-term manure application. By integrating quantitative biomass profiling with nematode-based ecological indicators, this study offers a comprehensive framework to disentangle food web regulatory mechanisms in agricultural soils. Our findings provide new insights into multi-trophic responses to fertilization and underscore the importance of organic amendments in promoting soil ecological resilience. Further research is needed to understand how long-term fertilization affects soil food web structure (e.g., link properties, chain length, omnivory, and decomposition pathways) and function (e.g., carbon and nutrient mineralization) to improve nutrient management strategies.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

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