

Responses of Soil Nematode Communities to Different Fertilizer Measures in a Peach Orchard

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Abstract

Soil nematodes constitute a vital component of the soil food web, playing a crucial role in ecosystem energy flow and material cycling. They serve as important bioindicators for assessing soil health and ecosystem recovery. However, research exploring the effects of selenium and organic fertilizers on soil nematode community and structure remains limited. In this study, we selected locally used bio-organic fertilizer as a control (CK) and established various fertilization strategies in a newly established peach orchard in southwestern China. These strategies included rapeseed meal cake fertilizer (RMC), green manure (*Euphorbia helioscopia*, GM), selenium fertilizer (SF), a combination of green manure + rapeseed meal cake fertilizer (GM + RMC), and a combination of selenium fertilizer + rapeseed meal cake fertilizer (SF + RMC). High-throughput sequencing and q-PCR methods were employed to determine the nematode genus composition and abundances. The results revealed GM + RMC significantly increased the total abundance of soil nematodes, while SF enhanced nematode diversity. Furthermore, GM + RMC notably promoted the metabolic activity of bacterivorous nematodes, and SF boosted the metabolic activity of fungivorous nematodes. However, most ecological indices of soil nematode communities did not exhibit significant differences among the six fertilization treatments. This may be attributed to the relatively short duration of the fertilization treatments. The soil nutrient level, particularly total nitrogen, emerged as the primary factor shaping the soil nematode community and its functional groups. Our findings provide a deeper understanding

of how nematode communities respond to fertilization measures in orchard ecosystems and offer valuable insights into sustainable development management.

Keywords

Soil Nematode, Community Structure, Fertilization, High-Throughput Sequencing, Peach Orchard

1. Introduction

As the most abundant metazoan, soil nematodes exhibit high diversity and play crucial roles in major ecosystem processes such as nutrient cycling [1]. Specifically, they function as a predator of soil organisms and respond swiftly to soil disturbances [2], making them widely used as bioindicators for assessing soil health [3] [4]. Furthermore, soil nematodes occupy various trophic levels within the soil food network [5], reflecting the condition in agricultural ecosystems [6]. For instance, long-term incorporation of agricultural residues enhances the complexity of the soil microbe-nematode network and ecosystem multifunctionality [7]. Therefore, a comprehensive understanding of how soil nematode communities respond to agricultural management practices, such as fertilization, is essential for guiding healthy soil management and promoting agricultural sustainable development.

Since the 1980s, Chinese agriculture has heavily relied on extensive use of fertilizers to boost productivity [8], resulting in reduced species diversity [9] and ultimately leading to soil acidification [10] [11]. N fertilization, for instance, can have mixed effects on soil nematode communities [12], increasing aboveground productivity while altering soil biotic communities and changing the food web structure and ecological function [9]. High mineral fertilization rates generally result in lower richness and diversity of nematodes [13], with excessive N fertilization simplifying their community structure and functions [14]. However, combining organic fertilization with site-specific N fertilization regimes has been suggested as an effective strategy for protecting and enhancing soil fauna (e.g., nematode) functional communities globally [15]. Despite this, the specific impacts of fertilization on soil nematode communities and their roles in ecosystem processes, particularly in specific regions or agroecosystems, remain unclear. Moreover, limited information is available on the effects of fertilization on soil food webs, where soil nematodes play a crucial role in biodiversity and ecosystem services [16].

While chemical fertilizers effectively enhance crop and fruit yields [17], their overapplication poses risks to soil health [18], leading to soil acidification [10] [11], biodiversity loss [9], soil degradation, and other numerous environmental concerns. Achieving a balance between fertilizer usage and soil health preservation is crucial. Replacing chemical fertilizers with organic fertilizers, green ma-

nure, Se fertilizer, or integrating their use are considered as feasible solutions [19]–[23]. Organic fertilizer, derived from natural sources, enriches the soil with essential nutrients, improves soil structure, enhances water retention, and fosters beneficial microbial activity [19] [20]. Green manure and rapeseed cake fertilizers are examples of organic fertilizers that provide a rich source of organic matter and nutrients [21] [22]. Selenium (Se), a crucial trace element, can enhance plant growth, improve crop quality, and mitigate heavy metal contamination in soils when applied as a fertilizer [24]. However, the specific impacts of selenium on soil nematode communities remain largely unexplored. Optimized fertilization practices are key to sustainable agricultural production, fostering nutrient abundance, improving soil properties, and enhancing the vitality of biological communities [25].

The current research for soil nematodes includes both traditional morphological identification and modern molecular biological techniques. Traditional identification, conducted under an optical microscope, is challenging due to time consumption and specialized knowledge requirements. Consequently, ecological studies are often confined to the family or genus level, which limits the depth of our understanding of soil nematode diversity [26]. However, recent advancements in molecular biology have transformed the field, enabling more rapid nematode ecological studies [27]. Techniques such as high-throughput sequencing and real-time quantitative fluorescence PCR (q-PCR) offer profound insights into nematode relative abundance and quantification [28], effectively surmounting the limitations of morphological identification in terms of time and expertise [29].

In this study, we utilized the combined power of high-throughput sequencing and q-PCR to explore the impact of diverse fertilization measures on soil nematode communities in a peach orchard in southwest China. The fertilization treatments included RMC (rapeseed meal cake fertilizer), GM (green manure), SF (selenium fertilizer), GM + RMC (a blend of green manure and rapeseed meal cake fertilizer), and SF + RMC (a blend of selenium and rapeseed meal cake fertilizer), with locally applied organic fertilization (CK) serving as a reference. Our hypotheses were as follows: 1) Compared to conventional local fertilization practices or the use of single fertilizer, combinations of green manure/selenium fertilizer and rapeseed meal cake fertilizer will enhance the abundance and diversity, maturity and structural indices of soil nematodes, 2) Given the complex effects of N fertilization on the soil nematode community [9] [12], we expected soil N level to be the primary driver of soil nematode community dynamics under these various fertilization measures.

2. Materials and Methods

2.1. Description of the Studied Area

The study area is situated in Maojiashan (formerly known as Liyuan Village, located at 104°26'N, 30°38'E), within Shanquan Town, Longquanyi District, Chengdu City. It is nestled in the core of the Longquan Mountain range. The climate in this

region is temperate, featuring four distinct seasons, and falls under the category of a subtropical monsoon humid climate. Annual rainfall varies between 800 to 1100 mm, with the rainy season predominantly occurring from June to September, contributing to 85% of the annual rainfall. January and December constitute the dry seasons, characterized by lower precipitation, dry winters, and spring droughts. The frost-free period lasts spans 280 days. The soil comprises neutral purple soil derived from slate and shale, possessing a depth exceeding 60 cm.

2.2. Experimental Design

The newly established peach orchard was previously a forested area. In the middle and late January of 2022, soil improvement measures were taken in the newly established peach orchard by means of deep soil tillage and the application of bio-organic fertilizer at a rate of 45 tons per hectare. Golden Honey Peach No. 1, provided by Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences, was selected as the experimental material, and fruit seedlings were cultivated in late February. In February 2023, six fertilization treatments were established in the peach orchard, including 1) conventional management with bio-organic fertilizer (CK) from Sichuan Tianbao Biotechnology Co., Ltd, China, 2) Application of rapeseed meal cake fertilizer (RMC) with an organic content of 80% (9375 kg·ha⁻¹). 3) Planting glabrous hairy vetch (*Vicia villosa* var. *glabrescens*) as green manure (GM) at s sowing rate of 45 kg·ha⁻¹. 4) Spraying selenium fertilizer (SF) provided by Agricultural Resources and Environment Research Institute, Guangxi Academy of Agricultural Sciences. The selenium-rich biological nano-grade amino acid-based nutrient solution containing 0.2% selenium was mixed with 70-100 times of water and sprayed through the spraying system 1 - 2 times during the young fruit stage, with a 5 - 7 day interval if sprayed twice [30]. 5) A combination of planting glabrous hairy vetch and rapeseed meal cake fertilizer (GM + RMC), wit half the application amounts of GM and RMC compared to their individual treatments. 6) A combination of selenium fertilizer and rapeseed meal cake fertilizer (SF + RMC), with half the application amounts of SF and RMC compared to their individual treatments. Each treatment has three replicates. The fertilization and management methods were as follows: conventional management with bio-organic fertilizer at 500 kg·ha⁻¹ served as the control (CK). For the GM + RMC and SF + RMC treatments, the application rates of RMC, GM, and SF were reduced to the half of those used in their respective single treatments. All other management and site conditions were consistent across treatments.

2.3. Determination of Soil Properties

In mid-June 2023, soil samples were collected using a soil auger with a 5 cm diameter, reaching a depth of 15 cm. An S-shaped sampling pattern, encompassing 8 - 10 points, was employed, and the collected soil samples were subsequently combined into a single composite sample. Each treatment involved 3 replicates. To prepare the samples for analysis, they were then sieved through a 10-mesh

sieve to eliminate roots and stones. One portion of the sieved soil was transported back to the laboratory in an ice box for soil nematode testing, while another portion was retained for measuring the soil's chemical properties. The soil bulk density was determined using the cutting ring method, and soil moisture content (%) at the sampling points was measured using a soil parameter meter (TDR200). The assessment of soil chemical properties includes the determination of soil pH, soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), total potassium (TK), available phosphorus (AP), available potassium (AK), $\text{NH}_4^+\text{-N}$, and $\text{NO}_3^-\text{-N}$. The specific methodologies employed for these measurements are referenced in Bao [31].

2.4. 18S rRNA Amplification and High-Throughput Sequencing

A total of 100 g fresh soil underwent processing via the Baermann funnel method [32], and after 48 hours, a separation experiment was conducted to isolate soil nematodes. Approximately 10 ml nematode solution was collected, and the total DNA of the nematode community was extracted following the instructions provided by the soil DNA kit manufactured by Omega Bio-tek (Norcross, GA, USA). The quality of the extracted DNA was verified using 1% agarose gel electrophoresis, while its concentration and purity of the DNA were measured using a NanoDrop 2000 spectrophotometer. Subsequently, the soil DNA extract was preamplified using primers NeMF (5'-GGGGAAGTATGGTT GCAAA-3') and 18Sr2b (5'-TACAAAGGGCAGGGACGTAAT-3'). This was followed by PCR amplification of the nematode 18S rDNA sequence using primers NF1 F (5'-GGTGGTGCATGGCCGTTT TTATT-3') and 18Sr2bR (5'-TACAAAGGGCAGGGACGTAAT-3') [33]. Sequencing was carried out using the Miseq PE300 platform by Shanghai Majorbio Pharmaceutical Technology Co., Ltd. The raw sequencing data underwent quality control using fastp software version 0.20.0 [34], and assembly was performed using FLASH software version 1.2.7 [35]. These sequences were then clustered into Operational Taxonomic Units (OTUs) based on 97% similarity using UPARSE software version 7.1 [36]. After quality control and assembly, the optimized sequences were further processed using the Delber plugin in the QIIME II pipeline [37] to remove sequences annotated to chloroplasts and mitochondria. To reduce the influence of sequencing depth on subsequent diversity analysis, the sequence count in all samples was normalized to the minimum sample sequence count. Taxonomic information was obtained by referencing the PR² sequence database.

2.5. Quantitative Fluorescence PCR Analysis of Soil Nematodes

The Reaction Mixture consisted of 2X ChamQ SYBR Color qPCR Master Mix (5 µl) from Vazyme Biotech Co., Ltd. (Nanjing, China), 0.4 µl of each primer (5 µM), 0.2 µl of 50 X ROX Reference Dye, 1 µl of template DNA, and ddH₂O to adjust the total volume to 10 µl. The PCR program was set as follows: an initial denaturation step at 95°C for 3 minutes, followed by cycles of denaturation at 95°C for 5 sec-

onds, annealing at 58°C for 30 seconds, and extension at 72°C for 1 minute. After completing these steps, the prepared samples in a 96-well plate were placed in an ABI 7300 Real-Time Fluorescent Quantitative PCR Instrument (Applied Biosystems, USA) to initiate the reaction. Once the constructed plasmid was confirmed to be correct through sequencing, the OD260 value of the plasmid was measured using an ultraviolet spectrophotometer (NanoDrop2000, Thermo Fisher Scientific, USA), and the copy number (copies/μl) was subsequently calculated employing a designated formula. Based on the genera from OTU taxon, combined with the database <http://Nemaplex.ucdavis.edu>, a total of 15 nematodes were identified at the genus level in the peach orchards (**Table S1**). The fresh weight of these nematodes was estimated by multiplying their respective OTU abundances, determined through qPCR, by their fresh body mass. The body mass data were sourced from Zhao *et al.* [38] and a publicly accessible database located at <http://Nemaplex.ucdavis.edu>.

2.6. Soil Nematode Indices Calculations

The Shannon-Wiener (H') index was calculated as described in Gao *et al.* [39] to assess nematode diversity. Furthermore, based on the feeding habits, nematodes are categorized into four trophic groups: bacterivores, fungivores, omnivores-predators, and plant parasites [40]. Additionally, they were grouped into five c-p categories, spanning from r-strategists to k-strategists, labeled c-p1 through c-p5 [41]. In the study, key ecological nematode indices, including the nematode maturity index (MI), plant parasite index (PPI), enrichment index (EI), structural index (SI), basic index (BI), and channel index (CI), were calculated [41]-[44]. The nematode maturity index (MI) and plant parasite index (PPI) are used to effectively gauge environmental perturbations and are calculated as follows:

$$MI = \sum v(i) \times f(i)$$

$$PPI = \sum v(i) \times f(i')$$

where $v(i)$ is the cp value of taxon i , and $f(i)$ is the frequency of each taxon and $f(i')$ is the frequency of plant parasites [41] [42]. The EI measures how opportunistic bacterial feeders (Ba1 and Ba2) and fungal feeders (Fu2) react to changes in food availability and nutrient enrichment [43]. The SI offers a way to assess the complexity of the soil food web, with higher values indicating a well-structured and stable ecosystem [43]. The BI, which focuses on the abundance of nematodes that feed on bacteria and fungi within the cp grouping, can indicate poor soil health when values are high [43] [45]. Finally, the CI provides insight into the decomposition pathways within the soil food web, with high values suggesting that fungal-feeding nematodes are the primary drivers of organic matter decomposition, while low values indicate a greater dominance of bacterial-driven decomposition [43]. The four indices were calculated as follows [43]:

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

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

$$BI = 100 \times b / (s + e + b)$$

$$CI = 0.8 \times Fu2 / (3.2 \times Ba1 + 0.8 \times Fu2) \times 100$$

where e represents the weighted frequencies of enrichment component, specifically Ba1 and Fu2, b denotes the weighted frequencies of basal component, comprising Ba2 and Fu2, and s corresponds to the weighted frequencies of structural component, which encompass Ba₃-Ba₄, Fu₃-Fu₄, Om₃-Om₅, and Pr₂-Pr₅ nematodes. The trophic groups are labeled as Ba for bacterivores, Fu for fungivores, Pr for predators, and Om for omnivores, with each group further differentiated by specific numbers indicating their respective cp values [44].

The nematode metabolic footprint (NMF) analysis served as a tool to assess the nematode-mediated carbon and energy flows, ultimately evaluating the nematode community based on the combined area of the enrichment and structural footprints [44]. The NMF results were presented through rhombuses, specifically at the following coordinates: (SI – 0.5*Fs/k, EI), (SI, EI + 0.5*Fe/k), (SI + 0.5*Fs/k, EI), and (SI, EI – 0.5*Fe/k) [44]. In this context, Fs and Fe represent the structure and enrichment footprints, respectively, with k being a constant value of 3 [43]. The Fs, Fe, and other nematode indices were computed using the NINJA website (<https://sieriebriennikov.shinyapps.io/ninja/>) [46]. Furthermore, faunal analysis entails constructing a four-quadrant diagram (illustrated in **Figure 3**) by plotting the enrichment index (EI) against the structural index (SI). This diagram visually represents key attributes that reflect the stability of the soil food web and the availability of resources [44].

2.7. Statistical Analysis

Before conducting the statistical analysis, all data were tested for normality and homogeneity of variances, and those that did not meet the criteria underwent natural logarithmic transformation, square root transformation, or rank transformation. The effects of different fertilization treatments on soil physico-chemical properties, nematode abundance, diversity, and ecological indices were analyzed using a one-way ANOVA. To determine significant differences among variables across various treatments, the Least Significant Different (LSD) test was employed, with a significance level set at 0.05. To further explore relationships, Canonical Correspondence Analysis (CCA) was conducted to evaluate the associations between the proportion of each genus-level taxon and environmental variables. Additionally, Redundancy Analysis (RDA) was employed to examine the correlations between the abundance of functional groups and environmental factors. The ANOVA was carried out using SPSS 20.0, while the CCA and RDA analyses were conducted with Canoco 5.0.

3. Results

3.1. Soil Physicochemical Properties

Fertilization measures have diverse effects on soil properties (**Table 1**). Specifi-

cally, soil bulk density was notably lower in the green manure (GM) treatment compared to the other five treatments. Furthermore, the available phosphorus (AP) content in GM was significantly higher than in other treatments. Soil pH values were significantly elevated in the rapeseed meal cake fertilizer (RMC) and selenium fertilizer + rapeseed meal cake fertilizer (SF + RMC) treatments than compared to the other four treatments. The total nitrogen (TK) content in the control (CK) and RMC treatments was significantly higher than in the SF + RMC treatment. Ammonium nitrogen ($\text{NH}_4^+\text{-N}$) levels in CK were significantly higher than in the other five treatments, whereas nitrate nitrogen ($\text{NO}_3^-\text{-N}$) levels in selenium fertilizer (SF) were significantly lower than in the other five treatments. Available potassium (AK) in CK, RMC, GM, and SF was significantly higher than in the GM + RMC and SF + RMC treatments. However, soil organic carbon content under different fertilization treatments did not reach a significant level ($p > 0.05$).

Table 1. Soil properties under different fertilization measures in the peach orchard (mean $\pm$ SE).

Properties	CK	RMC	GM	SF	GM + RMC	SF + RMC
Bulk density ($\text{g}\cdot\text{cm}^{-3}$)	1.73 $\pm$ 0.11a	1.59 $\pm$ 0.10a	1.40 $\pm$ 0.08b	1.63 $\pm$ 0.18a	1.59 $\pm$ 0.01a	1.62 $\pm$ 0.12a
pH	8.17 $\pm$ 0.11b	8.38 $\pm$ 0.05a	8.16 $\pm$ 0.03b	8.23 $\pm$ 0.19b	8.11 $\pm$ 0.03b	8.42 $\pm$ 0.08a
SOC ($\text{g}\cdot\text{kg}^{-1}$)	7.93 $\pm$ 0.13a	5.87 $\pm$ 0.76a	7.84 $\pm$ 3.79a	6.48 $\pm$ 1.14a	5.53 $\pm$ 2.05a	5.93 $\pm$ 0.08a
TN ($\text{g}\cdot\text{kg}^{-1}$)	1.01 $\pm$ 0.03a	0.92 $\pm$ 0.14a	0.91 $\pm$ 0.33ab	0.90 $\pm$ 0.07ab	0.87 $\pm$ 0.17ab	0.65 $\pm$ 0.25b
TP ($\text{g}\cdot\text{kg}^{-1}$)	1.39 $\pm$ 0.09ab	0.80 $\pm$ 0.06b	1.42 $\pm$ 0.81a	0.97 $\pm$ 0.07ab	1.10 $\pm$ 0.19ab	1.11 $\pm$ 0.03ab
TK ($\text{g}\cdot\text{kg}^{-1}$)	13.71 $\pm$ 0.35ab	11.94 $\pm$ 1.34bc	11.34 $\pm$ 1.96c	14.81 $\pm$ 0.31a	12.94 $\pm$ 0.22bc	12.53 $\pm$ 7.36bc
AP ($\text{g}\cdot\text{kg}^{-1}$)	20.73 $\pm$ 2.45b	35.33 $\pm$ 10.96b	92.57 $\pm$ 84.12a	8.65 $\pm$ 8.07b	18.83 $\pm$ 6.85b	19.12 $\pm$ 7.36b
AK ($\text{g}\cdot\text{kg}^{-1}$)	74.72 $\pm$ 2.35a	79.68 $\pm$ 12.26a	69.58 $\pm$ 20.80a	73.75 $\pm$ 20.74a	44.24 $\pm$ 3.84b	43.24 $\pm$ 7.69b
$\text{NH}_4^+\text{-N}$ ($\text{mg}\cdot\text{kg}^{-1}$)	73.62 $\pm$ 69.30a	2.68 $\pm$ 0.23b	27.82 $\pm$ 22.83b	3.14 $\pm$ 0.71b	2.53 $\pm$ 0.53b	4.23 $\pm$ 2.23b
$\text{NO}_3^-\text{-N}$ ($\text{mg}\cdot\text{kg}^{-1}$)	90.77 $\pm$ 1.79a	85.75 $\pm$ 5.31a	81.75 $\pm$ 16.79a	37.61 $\pm$ 43.18b	12.37 $\pm$ 4.22b	77.12 $\pm$ 24.15a

Note: CK, control; RMC, rapeseed meal cake fertilization; GM, interplanting a green manure *Vicia villosa*; SF, selenium fertilization; GM + RMC, a measure combined with green manure and rapeseed meal cake fertilizer; SF + RMC, a measure combined selenium fertilizer and rapeseed meal cake fertilizer. Bulk density, soil bulk density; SOC, soil organic matter; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AP, available phosphorus; AK, available potassium; $\text{NH}_4^+\text{-N}$, ammonium nitrogen; $\text{NO}_3^-\text{-N}$, nitrate nitrogen. Different letters in the same row indicate significant differences between treatments at levels of $p < 0.05$.

3.2. Soil Nematode Community Composition and Diversity

A total of 15 genera were recorded across all the peach orchard (Table S1), with *Acrobeloides* and *Aphelenchus* being the dominant genera. The abundance of soil nematode varied considerably among the different fertilization treatments (Table S1). Specifically, the highest total nematode abundance was observed in the GM + RMC treatment, reaching 3.58×10^8 copies per 100 g of dry soil. Compared to the CK treatment, the total nematode abundance in the GM + RMC treatment

increased by 399.11%, while it was 1.74% - 58.91% lower in the other four treatments (**Figure 1(a)**). In terms of soil nematode diversity, the SF treatment exhibited a significantly higher diversity compared to other fertilization measures ($p < 0.05$, **Figure 1(b)**). The abundance of trophic groups also varied across the peach orchards, with bacterivores ranging from 1.45×10^6 to 2.62×10^8 copies, fungivores from 1.97×10^6 to 6.35×10^7 copies, plant parasites from 0 to 3.24×10^7 copies, and omnivores-predators from 0 to 1.71×10^7 copies per 100 g of dry soil (**Table 2**). Furthermore, the GM + RMC treatment showed a notably greater abundance of bacterivores compared to the other five treatments ($p < 0.05$). However, no significant differences in the abundance of fungivores, herbivores and omnivores-predators were observed among these fertilization treatments (**Table 2**).

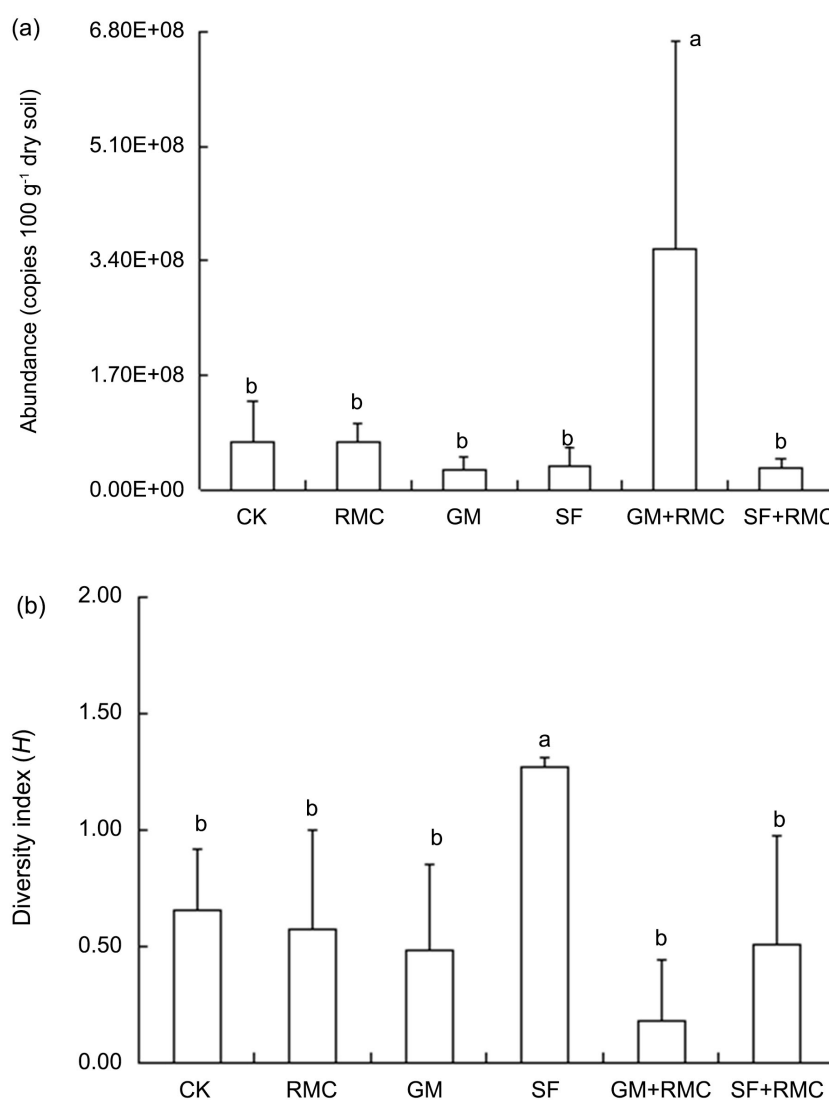


Figure 1. Total nematode abundance (copies 100 g⁻¹ dry soil) (a) and diversity (b) among different fertilization measures in peach orchards. The same letter indicates no significant differences between the treatments at $p > 0.05$.

Table 2. The abundance of trophic groups of soil nematodes (copies 100 g⁻¹ dry soil) among different fertilization measures (mean $\pm$ SE).

Treatments	Bacterivores	Fungivores	Herbivores	Omnivores-predators
CK	2.40E+07 $\pm$ 2.57E+07b	3.14E+07 $\pm$ 4.12E+07a	8.82E+08 $\pm$ 1.53E+09a	1.37E+08 $\pm$ 2.37E+08a
RMC	4.17E+07 $\pm$ 4.69E+07b	2.14E+07 $\pm$ 3.42E+07a	1.34E+05 $\pm$ 2.33E+05b	2.50E+07 $\pm$ 4.33E+07ab
GM	2.28E+07 $\pm$ 1.15E+07b	8.73E+06 $\pm$ 8.54E+06a	1.32E+06 $\pm$ 2.28E+06b	3.23E+04 $\pm$ 4.65E+04b
SF	1.45E+06 $\pm$ 1.25E+06b	2.27E+07 $\pm$ 2.63E+07a	5.22E+06 $\pm$ 9.05E+06b	2.98E+06 $\pm$ 4.23E+06b
GM + RMC	2.62E+08 $\pm$ 1.53E+08a	2.25E+08 $\pm$ 3.37E+08a	1.26E+07 $\pm$ 1.74E+07b	1.99E+07 $\pm$ 3.42E+07ab
SF + RMC	1.47E+07 $\pm$ 2.01E+07b	9.28E+06 $\pm$ 1.48E+07a	0.00E+00 $\pm$ 0.00E+00b	2.70E+05 $\pm$ 4.68E+05b

Note: CK, control; RMC, rapeseed meal cake fertilization; GM, a green manure *Vicia villosa*; SF, selenium fertilization; GM + RMC, a measure combined with green manure and rapeseed meal cake fertilizer; SF + RMC, a measure combined selenium fertilizer and rapeseed meal cake fertilizer. Different letters indicate significant differences among fertilization measures based on the LSD's test ($p < 0.05$).

3.3. Soil Nematode Indices and Metabolic Footprint

The SI index in the SF treatment was significantly higher than in the CK treatment ($p < 0.05$, **Table 3**). Additionally, the CI index in both the GM and SF treatments was significantly higher than in the SF + RMC treatment ($p < 0.05$, **Table 3**). When comparing the NMF values, the soil bacterivorous nematode groups showed a significant increase in the GM + RMC treatment compared to the SF and SF + RMC treatments ($p < 0.05$, **Table 4**). Conversely, the NMF values of soil fungivorous nematode groups were notably higher in the SF treatment compared to the GM and GM + RMC treatments ($p < 0.05$, **Table 4**). The total soil nematodes metabolic footprint was significantly elevated in the treatment that combined green manure and organic fertilizer (GM + RMC), as compared to the high-carbon control (CK) and selenium fertilization (SF) treatments ($p < 0.05$, **Figure 2**). Furthermore, the metabolic footprint of total soil nematodes was also notably higher in the organic fertilizer treatment (RMC) compared to SF ($p < 0.05$, **Figure 2**). However, no significant differences were observed among the six fertilization treatments in the peach orchard for the other nematode indices (**Table 3**), the NMF values of plant-parasitic and, predatory nematodes, as well as the enrichment

Table 3. Soil nematode indices under different fertilization measures (mean $\pm$ SE).

	CK	RMC	GM	SF	GM + RMC	SF + RMC
MI	1.88 $\pm$ 0.20a	2.21 $\pm$ 0.29a	2.08 $\pm$ 0.14a	2.41 $\pm$ 0.23a	1.97 $\pm$ 0.08a	2.08 $\pm$ 0.12a
PPI	1.00 $\pm$ 1.73a	0.00 $\pm$ 0.00a	1.00 $\pm$ 1.73a	1.87 $\pm$ 1.63a	0.67 $\pm$ 1.16a	1.00 $\pm$ 1.73a
EI	41.89 $\pm$ 30.66a	34.63 $\pm$ 18.91a	11.67 $\pm$ 1.00a	22.39 $\pm$ 8.37a	27.85 $\pm$ 17.58a	29.00 $\pm$ 33.63a
SI	0.00 $\pm$ 0.00b	42.09 $\pm$ 36.69ab	9.58 $\pm$ 16.59ab	52.30 $\pm$ 23.35a	8.06 $\pm$ 13.96ab	28.45 $\pm$ 9.83ab
BI	58.11 $\pm$ 30.66a	38.99 $\pm$ 14.37a	80.51 $\pm$ 13.08a	40.34 $\pm$ 16.30a	67.15 $\pm$ 17.87a	53.75 $\pm$ 21.20a
CI	44.74 $\pm$ 47.86ab	56.62 $\pm$ 38.65ab	100.00 $\pm$.00a	100.00 $\pm$.00a	79.72 $\pm$ 35.12ab	35.88 $\pm$ 55.66b

Note: CK, control; RMC, rapeseed meal cake fertilization; GM, a green manure *Vicia villosa*; SF, selenium fertilization; GM + RMC, a measure combined with green manure and rapeseed meal cake fertilizer; SF + RMC, a measure combined selenium fertilizer and rapeseed meal cake fertilizer. Different letters indicate significant differences among fertilization measures based on the LSD's test ($p < 0.05$).

Table 4. Soil nematode metabolic footprints (NMF) values under different fertilization measures (mean $\pm$ SE).

	CK	RMC	GM	SF	GM + RMC	SF + RMC
Bacterivore	6.69 $\pm$ 4.70ab	3.35 $\pm$ 1.94ab	9.54 $\pm$ 4.29a	0.60 $\pm$ 0.63b	4.11 $\pm$ 2.66ab	2.25 $\pm$ 2.09b
Fungivore	2.26 $\pm$ 2.25ab	1.20 $\pm$ 1.90ab	0.46 $\pm$.50b	2.95 $\pm$ 1.00a	0.22 $\pm$ 0.23b	1.41 $\pm$ 1.58ab
Plant parasite	0.04 $\pm$ 0.08a	0.71 $\pm$ 1.24a	1.22 $\pm$ 2.12a	3.46 $\pm$ 5.25a	0.33 $\pm$ 0.58a	5.34 $\pm$ 9.02a
Predator	231.78 $\pm$ 401.45a	528.28 $\pm$ 216.97a	465.37 $\pm$ 251.44a	447.79 $\pm$ 227.19a	240.35 $\pm$ 242.19a	395.51 $\pm$ 242.90a
Fe	3.88 $\pm$ 2.15a	4.51 $\pm$ 2.20a	4.37 $\pm$ 2.45a	3.11 $\pm$ 1.02a	4.28 $\pm$ 2.62a	3.06 $\pm$ 1.51a
Fs	232.14 $\pm$ 401.14a	528.28 $\pm$ 216.97a	465.41 $\pm$ 251.50a	447.85 $\pm$ 227.10a	240.72 $\pm$ 241.81a	395.51 $\pm$ 242.90a

Note: CK, control; RMC, rapeseed meal cake fertilization; GM, a green manure *Vicia villosa*; SF, selenium fertilization; GM + RMC, a measure combined with green manure and rapeseed meal cake fertilizer; SF + RMC, a measure combined selenium fertilizer and rapeseed meal cake fertilizer. Different letters indicate significant differences among fertilization measures based on the LSD's test ($p < 0.05$).

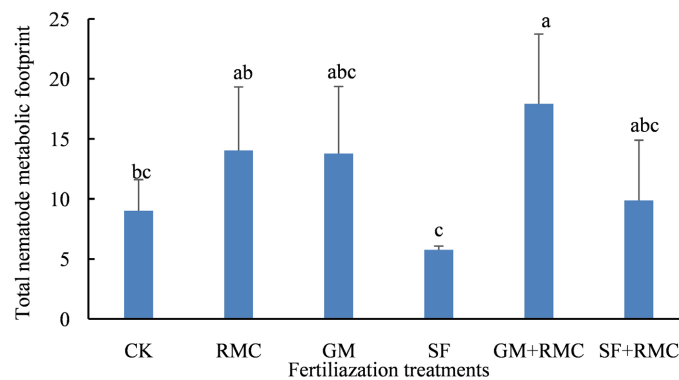


Figure 2. Total soil nematode metabolic footprint under different fertilization measures.

nematode footprint (Fe) and structural nematode footprint (Fs) (Table 4). Furthermore, the metabolic footprints under the six fertilization measures are concentrated in the region where the enrichment index is less than 50 and the structure index is also less than 50 (*i.e.*, Quadrant D, Figure 3). Only the functional footprint of selenium fertilization treatment (SF) lies in the region where the enrichment index is less than 50 and the functional index is greater than 50 (*i.e.*, Quadrant C, Figure 3).

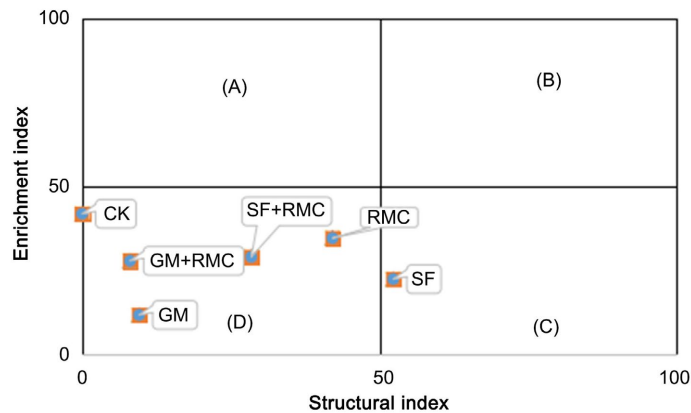


Figure 3. Metabolic footprint of soil nematodes under different fertilization measures in the peach orchards. A, Quadrant A; B, Quadrant B; C, Quadrant C; D, Quadrant D.

3.4. Relationships between Soil Nematodes and Environment Factors

The CCA results showed that the first axis and the second axis explained 15.40% and 7.78% of the community variation at the genus level of soil nematodes, respectively. Among the environmental variables, soil total nitrogen (TN) and total potassium (TK) were the significant factors influencing the community variation of soil nematode genera in the peach orchard (**Figure 4(a)**). The results of redundancy analysis (RDA) revealed that soil organic carbon, total nitrogen (TN), and total phosphorus (TP), were the most factors influencing the variation of soil nematode functional groups (**Figure 4(b)**).

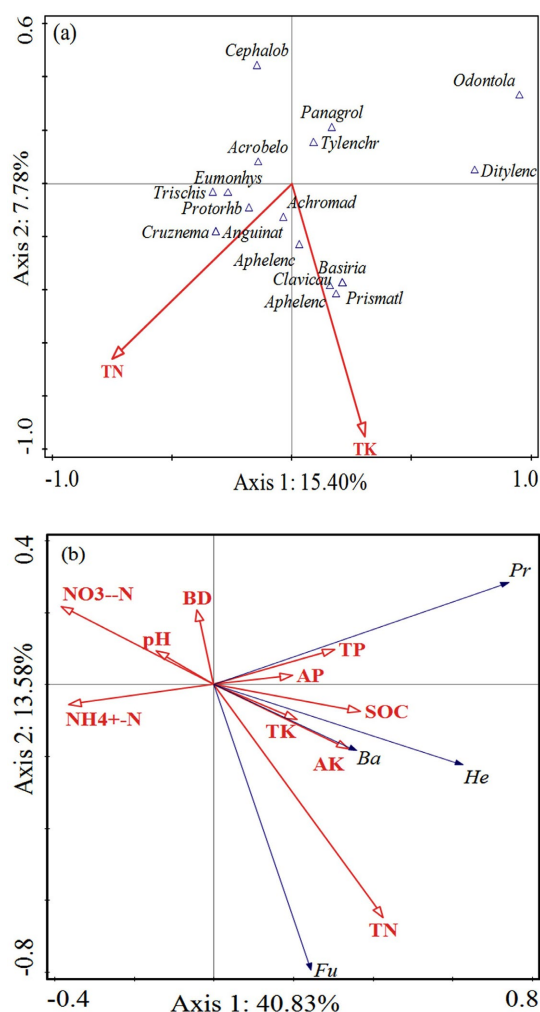


Figure 4. Relationships between edaphic factors and soil nematodes communities at genus level (a), and individual abundance of each functional group (b). BD, soil bulk density; pH, soil pH value; SOC, soil organic matter; TN, total nitrogen; AP, available phosphorus; AK, available potassium; $\text{NH}_4^+ \text{-N}$, Ammonium nitrogen; $\text{NO}_3^- \text{-N}$, nitrate nitrogen. Ba, bacterivores; Fu, fungivores; He, herbivores; Pr, omnivores-predators. The red arrow represents environmental factors, while the blue arrow stands for the species number of soil nematodes and the abundance of each functional group. The smaller the angle between the two colored arrows is, the greater the mutual influence is.

4. Discussion

4.1. Effects of Fertilization Treatments on the Abundance, Diversity, Structural and Enrichment Indices of Soil Nematode Communities

In our study, a combination of green manure and rapeseed meal cake fertilizer (GM + RMC) significantly increased the total abundance of soil nematodes in the peach orchard (**Figure 1(a)**), indicating both of green manure and input of organic fertilizer (e.g., rapeseed meal cake fertilizer) could significantly increase the number and diversity of nematodes [47] [48], through green manure improving soil structure (lower soil bulk density, **Table 1**) and rapeseed meal cake fertilizer increasing nutrient content [8], such as total nitrogen and available potassium (**Table 1**). Selenium fertilizer treatment (SF) notably elevated the diversity of soil nematodes compared to other treatments (**Figure 1(b)**). This result does not support the first hypothesis, which predicted that a combination of two fertilizers would have better effects on soil nematode abundance and diversity than a single fertilizer, indicating that fertilizer measures have complex effects on soil nematode communities. This phenomenon may be caused by two reasons. On one hand, selenium fertilizer can enhance the antioxidant enzyme activity and photosynthesis of crops, thereby promoting their growth [24] [30]. On the other hand, we applied selenium through foliar fertilization, which minimizes disturbance and impacts on the soil's structure and inherent properties. Consequently, this approach may have provided nematodes with a more conducive environment (e.g., higher total potassium content, as shown in **Table 1**), allowing for greater restoration of their populations over time and space [7].

Notable exceptions were observed in the higher structure index (SI) of the SF treatment compared to the CK (**Table 4**), indicating minimal disturbance to the soil [49] and the maintenance of soil environment stability [50]. This finding contradicts the first hypothesis, which suggested that a combination of two fertilizers would enhance both the structural index and maturity index. The magnitude of the structure index reflects the connectivity of the soil food web, with higher values indicating higher relative connectivity and longer food chains [51]. The index is primarily influenced by omnivorous/predatory nematodes, which are more sensitive to soil disturbances and require longer recovery times [51]. No significant differences in SI were observed among the other five fertilization treatments (**Table 4**), implying that restoring nematode community structure and maturity indices in peach orchards may require a prolonged period, potentially influenced by soil organic carbon content [52] [53]. Higher levels of soil organic carbon facilitate faster nematode settlement and maturity [52] [53]. However, our study found no significant changes in soil organic carbon content across the applied fertilization treatments (**Table 1**), which explains the lack of profound effects on nematode structural and ecological indices (**Table 4**). This result may also be attributed to the short duration of fertilization. Therefore, this underscores the importance of long-term monitoring to comprehensively assess the impacts of fertilization on

soil properties and nematode communities.

4.2. Effects of Fertilization Treatments on Soil Nematode Metabolic Activity

The application of green manure treatment significantly boosted the metabolic activity of bacterivorous nematodes compared to selenium fertilization treatments (namely, SF and SF + RMC treatments). Conversely, selenium fertilization significantly enhanced the metabolic footprint of fungivorous nematodes when compared to green manure measures (specifically, GM and GM + RMC treatments) (**Table 3**). The stimulatory effect of green manure on the microbial system is primarily attributed to the introduction of fresh organic material or easily assimilable rhizodeposits above- or below-ground [23], which also helps buffer microclimatic conditions [54]. This, in turn, boosts microbial populations and positively influences bacterivorous nematodes [55]. On the other hand, selenium fertilization causes less disturbances to the soil and introduces organic matter into the soil in a way that may promote the fungal community compared to other fertilization measures. This, in turn, exerts a significant bottom-up control over the nematode community within the soil microbial food web, leading to an increase in the abundance of fungivorous nematodes in the soil [51] [56].

The functional footprint of nematodes, which reflects the ability of soil food webs to regulate and maintain metabolic balance, is the combined area enclosed by the enrichment footprint and structural footprint [43] [57]. Precipitation has been proven to be the main regulator for soil nematode community and footprint among the large gradient of agricultural ecosystems [45]. Our study revealed that the total soil nematodes metabolic footprint was significantly elevated in treatments combining green manure and rapeseed meal cake fertilizer (GM + RMC) and the rapeseed meal cake fertilizer treatment (RMC), compared to the control (CK) and selenium fertilization (SF) treatments, respectively (**Figure 2**). These changes in nematode community composition and stability ultimately affect the functioning and nutrient cycling of soil ecosystems through biological interactions within the soil food web [58]. The enrichment index (EI) and structural index (SI) can be illustrated on a two-dimensional graph (*i.e.*, faunal analysis), providing an indication of the status of the soil food web [51]. Notably, the metabolic footprints observed under selenium fertilization treatment (SF) fell within Quadrant C ($SI > 50$, $EI < 50$) (**Figure 3**), suggesting a state of undisturbed recycling of endogenous resources indicative of favorable environmental conditions [43] [57]. Conversely, metabolic footprints associated with other fertilization treatments predominantly clustered in Quadrant D ($SI < 50$, $EI < 50$) (**Figure 3**), highlighting resource-constrained systems operating under stressful environmental conditions [43] [57].

4.3. Factors Influencing Soil Nematode Communities

Based on numerous fertilization experiments, a decline in soil pH has been shown

to significantly reduce the diversity of soil bacteria and fungi, subsequently impacting the abundance and diversity of soil nematodes [59]. However, in our study, soil pH was not a significant factor influencing soil nematodes abundance (Figure 4), possibly due to the relative minor pH variances (ranging from 0.01 to 0.31) observed among these fertilization treatments (Table 1). Soil organic matter serves as an energy and carbon source for soil organisms, influencing the abundance of soil nematodes [52] [53]. For instance, as soil organic matter increases, the overall abundance of soil nematodes and the number of nematodes functional groups also significantly rise [60]. Nevertheless, in our study, soil organic carbon was not a crucial factor affecting soil nematode communities (Figure 4), likely because the convergence of soil organic carbon levels under all the fertilization measures (Table 1) resulted in similar soil nematode community [61]. However, green manure stood out as an exception, significantly reducing soil bulk density compared to other treatments ($p < 0.05$, Table 1). This underscores the advantages of incorporating green manure into orchard management. Green manure plants, through their root systems growth, promote soil loosening, enhancing porosity, and consequently lower soil bulk density [62]. This improvement in soil structure facilitates better root penetration, water infiltration, and air circulation, all essential for healthy plant growth [63]. Furthermore, our results showed a significantly higher level of available phosphorus (AP, Table 1) in the soil under green manure fertilization. This suggests that planting green manure in orchards does not compete with fruit trees for nutrients but rather contributes to increasing soil AP availability, benefiting fruit tree growth. The decomposition of green manure releases nutrients, including phosphorus, into the soil, enriching it and creating a more conducive environment for plant growth and root development [64]. Our study emphasizes the importance of adopting optimized fertilization practices, particularly the use of green manure, to achieve sustainable agricultural production while safeguarding soil health and promoting ecosystem services. It is worth noting that the effect of organic fertilizer, such as rapeseed meal cake fertilizer treatment (RMC), may not be fully evident within a short duration of just 1.5 years. In the early stages of orchard development, green manure exerts beneficial effects not only through its roots on soil physical properties but also through the favored microclimate created by its coverage of the orchard surface.

Among the environmental variables examined, soil total nitrogen (TN) and total potassium (TK) emerge as significant factors influencing the variability of soil nematode genera in peach orchards (Figure 4(a)). Meanwhile, soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP) were identified as the primary drivers influencing the variation of soil nematode functional groups (Figure 4(b)). This underscores the importance of soil nutritional status, particularly the levels of key nutrients like carbon, nitrogen, and phosphorus, in determining the structure and functional diversity of soil nematode communities [65]. Specifically, soil total nitrogen content, which is influenced by fertilization measures, was found to be the most influential factor driving both the soil nematode community and its functional groups. This aligns with our second hypothesis and the research

conducted by Zhang *et al.* [66]. Additionally, these findings emphasize the importance of considering the intricate interplay among multiple environmental factors when studying soil nematode communities and their implications for soil ecosystem functioning and nutrient cycling. Future research could delve deeper into the mechanisms linking soil nematode community changes to ecosystem functions and explore the potential impacts of other environmental variables that may have been overlooked in this study.

5. Conclusion

In our study, we investigated the responses of the soil nematode community to various fertilization treatments in a newly established peach orchard in China. Although there were no significant differences in soil organic carbon among the selected fertilization treatments, the green manure (GM) treatment successfully decreased soil bulk density and increased soil available phosphorus levels. Furthermore, only the combined treatment of green manure and rapeseed meal cake fertilizer (GM + RMC) significantly boosted nematode abundance in the peach orchard without altering their diversity. Furthermore, GM enriched the presence of bacterivorous nematodes, while selenium fertilization (SF) treatment favored fungivorous nematodes. Notably, metabolic footprint significantly increased in GM + RMC and RMC treatments compared to the control (CK) and SF treatments, suggesting that the GM + RMC treatment would be the preferred fertilization treatment in the early stage of orchard development. Our study emphasizes that soil pH and organic carbon had minimal influence on nematode communities, potentially due to their small variations. Conversely, nutrient levels, particularly total nitrogen, emerged as crucial factors shaping nematode community structure and functional diversity. Our findings highlight the intricate interplay of environmental factors influencing nematode communities and their implications for soil ecosystem functioning. Future research endeavors should delve deeper into the mechanisms linking nematode dynamics to ecosystem functions and investigate a broader range of environmental variables, providing invaluable insights for optimizing soil management practices to support sustainable agriculture and ecosystem health.

Author Contribution

Conceptualization, W.X. Peng, X.W. Li, and H. Wang, Data curation, Z.L. Zhou and X.D. Wu, Formal analysis, X.D. Wu, Funding acquisition, X.W. Li, Investigation, Q. Liu, W.J. Yang, Z.L. Zhou and G.J. Dai, Methodology, Z.L. Zhou, Project administration, L.L. Ma and W.J. Yang, Supervision, Q. Liu, Validation, G.J. Dai, Writing original draft, X.W. Li and Z.L. Zhou, Writing review & editing, W.X. Peng. All authors have read and agreed to the published version of the manuscript.

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No applicable.

Data Availability Statement

Data will be made available on request.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Supplement

Table S1. Soil nematode abundance (copies per 100 g dry soil) under different fertilization measures (means $\pm$ SE, n = 3).

Genus	Guild	HCK	HB	HG	HX	HGB	HXB
<i>Cruznema</i>	ba1	0.000 $\pm$ 0.000b	0.000 $\pm$ 0.000b	0.000 $\pm$ 0.000b	0.000 $\pm$ 0.000b	0.001 $\pm$ 0.001a	0.000 $\pm$ 0.000b
<i>Panagrolaimus</i>	ba1	0.000 $\pm$ 0.000a	0.000 $\pm$ 0.000a	0.000 $\pm$ 0.000a	0.000 $\pm$ 0.000a	0.000 $\pm$ 0.000a	0.002 $\pm$ 0.003a
<i>Protorhabditis</i>	ba1	0.231 $\pm$ 0.392a	0.024 $\pm$ 0.036b	0.000 $\pm$ 0.000b	0.000 $\pm$ 0.000b	0.002 $\pm$ 0.003b	0.000 $\pm$ 0.000b
<i>Acrobeloides</i>	ba2	0.295 $\pm$ 0.282bc	0.478 $\pm$ 0.368abc	0.575 $\pm$ 0.303ab	0.028 $\pm$ 0.017c	0.846 $\pm$ 0.225a	0.328 $\pm$ 0.530bc
<i>Cephalobus</i>	ba2	0.016 $\pm$ 0.012a	0.000 $\pm$ 0.000a	0.239 $\pm$ 0.209a	0.000 $\pm$ 0.000a	0.005 $\pm$ 0.009a	0.000 $\pm$ 0.000a
<i>Odontolaimus</i>	ba3	0.000 $\pm$ 0.000b	0.000 $\pm$ 0.000b	0.000 $\pm$ 0.000b	0.000 $\pm$ 0.000b	0.000 $\pm$ 0.000b	0.106 $\pm$ 0.183a
<i>Prismatolaimus</i>	ba3	0.000 $\pm$ 0.000b	0.000 $\pm$ 0.000b	0.000 $\pm$ 0.000b	0.011 $\pm$ 0.020a	0.000 $\pm$ 0.000b	0.000 $\pm$ 0.000b
<i>Aphelenchoides</i>	fu2	0.000 $\pm$ 0.000b	0.003 $\pm$ 0.004b	0.000 $\pm$ 0.000b	0.043 $\pm$ 0.074a	0.000 $\pm$ 0.000b	0.000 $\pm$ 0.000b
<i>Aphelenchus</i>	fu2	0.457 $\pm$ 0.406ab	0.242 $\pm$ 0.377ab	0.091 $\pm$ 0.090b	0.566 $\pm$ 0.125a	0.096 $\pm$ 0.130b	0.070 $\pm$ 0.121b
<i>Ditylenchus</i>	fu2	0.000 $\pm$ 0.000b	0.000 $\pm$ 0.001b	0.000 $\pm$ 0.000b	0.115 $\pm$ 0.174ab	0.005 $\pm$ 0.009b	0.228 $\pm$ 0.394a
<i>Basiria</i>	he1	0.000 $\pm$ 0.000a	0.000 $\pm$ 0.000a	0.000 $\pm$ 0.000a	0.008 $\pm$ 0.013a	0.000 $\pm$ 0.000a	0.000 $\pm$ 0.000a
<i>Tylenchorhynchus</i>	he3	0.002 $\pm$ 0.003a	0.000 $\pm$ 0.000a	0.095 $\pm$ 0.165a	0.147 $\pm$ 0.249a	0.046 $\pm$ 0.079a	0.244 $\pm$ 0.423a
<i>Achromadora</i>	pr3	0.000 $\pm$ 0.000b	0.096 $\pm$ 0.167a	0.000 $\pm$ 0.000b	0.052 $\pm$ 0.067ab	0.000 $\pm$ 0.001b	0.022 $\pm$ 0.033ab
<i>Trischistoma</i>	pr3	0.000 $\pm$ 0.000b	0.157 $\pm$ 0.268a	0.000 $\pm$ 0.000b	0.002 $\pm$ 0.004b	0.000 $\pm$ 0.000b	0.000 $\pm$ 0.000b
<i>Clavicaudoides</i>	pr5	0.000 $\pm$ 0.000b	0.000 $\pm$ 0.000b	0.000 $\pm$ 0.000b	0.027 $\pm$ 0.047a	0.000 $\pm$ 0.000b	0.000 $\pm$ 0.000b

Note: Guild represents trophic group and cp value. Different lowercase letters indicate significant differences at $p < 0.05$ level.