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Effects of nitrogen and phosphorus additions on soil nematode community of soybean farmland

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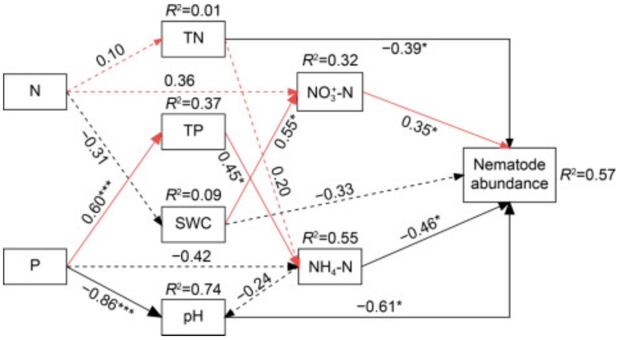
ABSTRACT



- The nitrogen (N) and phosphorus (P) addition promotes the abundance of soybean soil nematodes.
- The addition of nitrogen can alleviate the suppression of phosphorus on nematodes.
- Phosphorus addition affects nematode abundance by ammonium nitrogen.

With global warming, the increasing of industrial and agricultural activities and demand for fossil fuels, large amounts of nitrogen and phosphorus compounds are released into the atmosphere, resulting in an annual increase in nitrogen and phosphorus deposition. The nematodes, as one of the main functional groups of soil organisms, occupy multiple trophic levels in soil detritus food networks. However, few studies on the effects of nitrogen and phosphorus on soil nematodes, and the results are uncertain. This experiment was conducted in soybean farmland and four treatments included control, nitrogen addition, phosphorus addition, and N + P addition. The results showed that both phosphorus and N + P addition significantly increased the abundance of soil nematodes, but that had no effects on soil nematodes richness. Redundancy analysis showed that nitrate nitrogen, soil moisture content, and pH were environmental factors driving different dietary changes in soil nematode communities. The effect of phosphorus addition on the abundance of nematode communities mainly affects ammonium nitrogen. Our findings revealed that nitrogen addition when phosphate fertilizer is added to soybean farmland will have a certain positive effect on the soil underground food web, which provides a basis for better explaining the effect of nitrogen and phosphorus addition on soybean farmland.

Keywords soil nematodes, nitrogen addition, phosphorus addition, soil food web, soybean

1 Introduction

With global warming, the increasing of industrial and agricultural activities and demand for fossil fuels, large amounts of nitrogen and phosphorus compounds are released into the atmosphere, resulting in an annual increase in nitrogen and phosphorus deposition fluxes (Galloway et al., 2008; He et al., 2011). First, as an imperative source of materials for the ecosystem, atmospheric nitrogen and phosphorus deposition plays a significant role in maintaining plant productivity in terrestrial ecosystems. Second, excessive nitrogen and phosphorus deposition can cause many ecological and environmental problems (Quinn-Thomas et al., 2010; Zhang et al., 2019). Continued atmospheric nitrogen and phosphorus deposition worldwide has led to the loss of nutrients, continued soil acidification, increased mortality of some plants, and reduced species diversity (Gilliam, 2006; Bobbink et al., 2010; Liu et al., 2015). Study results have indicated that since the 1980s, Chinese agriculture has been concentrated on limited land and invested in many fertilizers and other resources, and with the intensification of nitrogen and phosphorus deposition, eventually leading to soil acidification, especially in the North China Plain (Guo et al., 2010). The addition of nitrogen and phosphorus can changes soil physicochemical properties, microbial biomass and microbial community structure, thereby lead to a positive or negative effect on aboveground plants (Xia et al., 2020; Tian and Niu, 2015). Therefore, exploring how nitrogen and phosphorus addition affects the aboveground and underground

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processes of farmland ecosystem is of great importance for understanding and evaluating the ecological effects of regional ecosystems.

The functions of soil organisms in terrestrial ecosystems include degradation of organic matter, driving nutrient cycling, conversion of various pollutants, and production and consumption of greenhouse gases. As one of the main functional groups of soil organisms, nematodes occupy multiple trophic levels in the soil detrital food network, which is of great significance for the decomposition of soil organic matter, the maintenance of soil ecosystem stability, and the promotion of material circulation and energy flow (Zhao et al., 2012; Bardgett and van der Putten, 2014). As a predator of soil microorganisms, nematodes respond rapidly to changes in soil environment, which can reflect climate conditions, ecosystem succession status, nutrient cycling and soil ecosystem health (Yeates, 2003; Ferris and Bongers, 2006; Zhao et al., 2021). Therefore, studying the effect of nitrogen and phosphorus addition on soil nematode communities is helpful to understand how nitrogen and phosphorus addition affects the growth and development of crops in farmland ecosystems.

At present, many scholars have carried out a great deal of studies on the interaction of nitrogen or phosphorus addition on soil nematode communities, but less attention has been paid to the effects of nitrogen and phosphorus addition on soil nematode communities. Previous studies have shown that nitrogen and phosphorus additions do not have a direct effect on soil nematodes, but rather indirectly affect nematodes by changing soil physicochemical properties and plant growth (Boot et al., 2015; Chen et al., 2019). For example, previous researchers have found that increased availability of nitrogen and phosphorus increases ammonium and aluminum toxicity and soil acidification, which negatively affected nematode communities, particularly phytophagous nematodes (Chen et al., 2015; Karbin et al., 2015). The possible mechanism of influence of nitrogen addition significantly increasing the relative abundance of plant parasites was that nitrogen fertilizer addition promotes plant growth and significantly increased aboveground plant biomass (Liang et al., 2009), thereby providing more resources for plant parasite nematodes (Bongers T. and Bongers M. et al., 1998). It has also been shown that phosphorus addition affected soil nematodes mainly by changing soil pH and total phosphorus content (Wei et al., 2012; Yang et al., 2019). In farmland ecosystems, the application of nitrogen and phosphorus fertilizers increased soil nematode abundance, genus richness, maturity index (MI), and structural index (SI) (Niu et al., 2022). Besides, the nitrogen application significantly increased the nematode abundance of bacterivores and plant-parasites and reduced the nematode abundance of omnivores/predators and fungivores in a farm in Hamilton (Sarathchandra et al., 2001). Adding nitrogen and phosphorus reduced the abundance of bacterivores in the soybean neotropical rotation period in northeast China (Pan et al., 2010). The addition of nitrogen and phosphorus significantly reduced the channel index (*CI*) of nematodes, but significantly increased the enrichment index (*EI*), and the abundance of bacterivores, fungivores and omnivores/predators fluctuates with the sampling time (Pan et al., 2015). Based on the above research, we found that even in the same ecosystem, different conclusions have been drawn, so the impact of nitrogen and phosphorus addition on farmland ecosystems is still uncertain, especially when nitrogen and phosphorus are added at the same time, whether there is an interaction between them. Therefore, our study aims to reveal whether nitrogen and phosphorus interact in soybean farmland ecosystems through a pot control experiment.

In this study, the impact of nitrogen and/or phosphorus addition on soil nematode communities in farmland ecosystems were investigated. Before the start of the study, we measured the physical and chemical properties of the soil in the experimental plot and found that the soil nutrient content of the plot was at a low level. Nitrogen and phosphorus addition can be counted as input to exogenous nutrients, which may have a positive effect on soil nematode communities. We put forward the following assumptions: 1) Nitrogen addition will change the structure of soil nematode community, which may contribute positively to the abundance, diversity and/or community complexity of soil nematode; 2) The application of phosphorus will change the trophic groups of nematodes and may have a positive impact on soil nematodes; 3) There will be an interaction between nitrogen addition and phosphorus addition. And they promote each other, which will have a more positive effect on soil nematodes.

2 Materials and methods

2.1 Study site and experimental design

The study site was located at the Global Change Ecological Experiment Station of Henan University in Jinming Campus, Kaifeng City, Henan Province (114°30′ E, 34°81′ N, about 50 m above sea level). This region has a continental temperate monsoon climate. According to the data recorded by the National Meteorological Information Center of China, the annual average temperature is 14°C and the annual average precipitation is 625.6 mm. This area is an agricultural planting area, mainly consisting of wheat, soybean and corn crops. This experiment adopted a completely randomized block design, which is a pot experiment. Soybean seeds with full seeds and the same size were selected for sowing, and 3 plants were fixed in each pot after emergence. The experiment can be divided into control group and treatment group, with a total of 4 treatments, including control group (CK), nitrogen addition (N), phosphorus addition (P), nitrogen and phosphorus addition (N + P). Each treatment was set with 5 replicates, a total of 20 plots. The total amount of fertilizer applied was urea 10 g m^{-2} and superphosphate 112.5 g m^{-2} (superphosphate contains 12% P_2O_5, equivalent to adding P_2O_5 13.5 g m^{-2}), where CK (no nutrient added), N added (urea 10 g m^{-2}), P added (superphosphate 112.5 g m^{-2}), N + P added (urea 10 g m^{-2} and superphosphate 112.5 g m^{-2}). According to the pot surface area, urea was calculated by applying 0.7 g per pot and superphosphate was 7.95 g per pot, and fertilization was averaged applied at the branching stage and the first flowering stage of soybean. Water the potted plants once a week, with the same amount of water per pot, each time the amount of water is 600−1000 mL. The positions of the pot were randomly arranged and regularly changed.

2.2 Nematodes sampling and soil chemical analysis

During this study, the portable soil moisture tester was applied to regularly detect the soil temperature and soil volume moisture at the depth of 0−10 cm. These soil samples were collected during the maturity of soybean. Soil samples of 0−10 cm soil layers were collected at 5 random points within each plot using a soil drill (6 cm diameter) and mixed to make a sample, and a total of 20 samples were collected. After the sample is collected, it passed through a 2 mm soil sieve. Some of the screened soil samples were dried to analyze the soil's physical and chemical properties, and some were stored in ice pack and brought back to the laboratory for soil microbial index analysis. After natural air-drying, soil samples were passed through a 100-mesh soil sieve, and the soil pH value was determined by the potential method, and the soil carbon and nitrogen content were determined by the semi-constant element analyzer (vario EL cube, Germany). The total phosphorus in soil was decomposed by H_2SO_4-$HClO_4$ digestion method and determined by phosphomolybdate blue method. The available nitrogen in soil was determined by automatic chemical analyzer (SmartChem200, AMS, France). Three soybean aboveground plants were randomly selected and taken from each plot, treated at 105°C for 48 h, and then baked to a constant weight at 60°C to measure the aboveground biomass.

2.3 Soil nematode extraction and identification

The principle of separating soil nematodes by using the improved Baermann funnel method was to use the water taxis and weight of nematodes to separate from the soil and collect them at the bottom of the wet funnel (Ruess, 1995). Select a suitable glass funnel, connect a rubber tube at the end, clamp the rear end of the rubber tube with a spring clip, and add water to the funnel to drive out all bubbles in the rubber tube. Put 100 g of fresh soil into a small non-woven cotton net and put it into a funnel and add water until the soil is immersed. After 48 h, open the clamp and collect it with plastic centrifuge tube. Put the centrifuge tube in the centrifuge for 2500 r min^{-1} and centrifuge for 5 min, draw the upper liquid of the centrifuge tube close to the water surface, and keep 10 mL in the centrifuge tube. The 60−65°C water bath centrifuge tube for 3 min and use the same amount of triethanolamine-formalin fixed solution for short-term storage. 100 nematodes were randomly selected from each soil (less than 100 nematodes were all identified) and counted and observed under the light microscope, and the 100 individuals encountered were identified to trophic group (bacterivores, fungivores, plant-parasites, omnivores/predators) and functional guild (Bongers, 1990; Yeates et al., 1993). The functional guild was defined by the nematode's trophic behavior and by its ecological life strategy as a colonizer or persister (Bongers, 1990; Ferris et al., 2001).

The ecological index of nematodes is calculated by the following formula:

Maturity index $MI = \sum_{i=1}^{n} v_i \times f_i$

Plant parasite index $PPI = \sum_{i=1}^{n} v'_i \times f'_i$

with v_i (v'_i) represented the c-p value of non-plant parasites (plant-parasites) taxa i, n is the number of non-plant parasitic (plant parasitic) soil nematode taxa; f_i (f'_i) represented the proportion of individuals in non-plant parasitic (plant parasitic) soil nematode group i in soil nematode communities to the total number of individuals in the community.

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

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

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

with s (structure food web component) calculated as the weighted frequencies of Ba3–Ba5, Fu3-Fu5, Pr3–Pr5, and Om3-Om5, and b (basal food web component) as the weighted frequencies of Ba2 and Fu2, and e (enrichment component) calculated as the weighted frequencies of Ba1 and Fu2 (Ferris et al., 2001).

Channel index $CI = 100 \times [0.8Fu2/(3.2Ba1 + 0.8Fu2)]$

2.4 Statistical analysis

Two-way ANOVA was used to analyze the individual and interaction effects of nitrogen and phosphorus addition on soil physicochemical properties, average aboveground biomass, nematode abundance, nematode richness and nematode ecological index, and LSD test was used to test the differences between the above variables for different treatments Redundancy analysis was used to verify the correlation and variability interpretation of different environmental factors and soil nematode communities. The analysis of variance was performed using SPSS25.0, and the redundancy analysis was performed using CANOCO5.0. The significance level is set to $\alpha = 0.05$. IBM SPSS Amos was used to draw structural equation models (SEM). How nitrogen and phosphorus additions directly or indirectly affect soil nematode abundance through environmental factors is explored through structural equation modeling (SEM).

3 Results

3.1 Variation in the environmental parameters

Compared with nitrogen-phosphorus addition and control, nitrogen addition significantly increased nitrate nitrogen (NO_3^--N) by 51% and 85%, respectively ($P < 0.05$). In addition, nitrogen addition significantly increased the aboveground biomass of soybean ($P < 0.05$). Phosphorus addition significantly increased soil moisture content (SWC), soil total phosphorus (TP), ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) ($P < 0.05$), which increased by 15%, 29%, 214% and 68%, respectively, compared with the control. Compared with the control, all treatments significantly reduced soil pH, and there were significant differences between treatments, with the largest reduction in phosphorus addition (Table 1).

3.2 Nematode abundance and community structure

A total of 6757 nematodes were obtained in this sample, 1883 nematodes were identified, and a total of 30 nematode genera were found, among which *Chiloplacus*, *Apelenchus*, *Tylenchorhynchus*, and *Cephalobus* were the dominant genus. In CK, N + P, N, P, P, the genera of *Chiloplacus* accounted for 33.3%, 26.2%, 32% and 25.7%; *Cephalobus* accounted for 9.5%, 19.8%, 5.8% and 13%; *Apelenchus* accounted for 12.3%, 9.7%, 12.3% and 11.4%; *Tylenchorhynchus* accounted for 10.5%, 12.6%, 20.2% and 2.4% (Fig. 1). Nitrogen addition and phosphorus addition increased the abundance of nematode communities by 20% and 44%, Nitrogen and phosphorus additions increased nematode abundance by 20% and 44%, respectively, but both produced significant differences. Nitrogen and phosphorus addition significantly increased the abundance of nematode communities by 139%. Among them, the relative abundance of bacterivores and plant-parasites were the most affected, nitrogen addition had a significant negative effect on bacterivores, reducing its number by 14%, while phosphorus addition had a significant positive effect on the relative abundance of bacterivores, increasing its number by 14%, and nitrogen-phosphorus addition had little effect on the relative abundance of bacterivores. For plant-parasites, nitrogen addition significantly increased its number by 44%, phosphorus addition significantly reduced its number by 50%, and nitrogen-phosphorus addition had little effect on its number, Phosphorus addition has increased significantly the relative abundance of omnivores/predators (Figs. 2 and 3).

3.3 Nematode ecological index

There were no significant differences in plant parasitic nematode maturity index (*PPI*), maturity index (*MI*), nematode between the treatments. Phosphorus addition significantly increased the Wasleska index (*WI*) compared to the control ($P < 0.05$). All treatments reduced the channel index (*CI*), but only phosphorus addition was significantly different

Table 1 Effects of control (CK), phosphorus addition (P), nitrogen addition (N), and nitrogen addition combined with phosphorus addition (NP) on environmental parameters. The data are presented as mean ± SE. Different letters indicate significant differences between different treatments ($P < 0.05$).

Parameters	Treatment				Two-way ANOVA		
	CK	P	N	NP	N	P	NP
SWC (v/v)	0.13 ± 0.01b	0.15 ± 0.00a	0.14 ± 0.00ab	0.13 ± 0.00b	0.653	0.653	<0.01
pH	7.68 ± 0.01a	7.39 ± 0.03d	7.58 ± 0.01b	7.47 ± 0.02c	0.570	<0.001	<0.001
TC (g kg^{-1})	2.44 ± 0.73a	2.22 ± 0.44a	2.63 ± 0.40a	2.33 ± 0.66a	0.802	0.654	0.941
TN (g kg^{-1})	0.30 ± 0.04a	0.30 ± 0.02a	0.33 ± 0.03a	0.30 ± 0.02a	0.660	0.714	0.558
TP (mg g^{-1})	0.31 ± 0.02b	0.38 ± 0.03a	0.32 ± 0.01b	0.37 ± 0.01ab	0.919	<0.01	0.614
NH_4^+-N (mg kg^{-1})	0.41 ± 0.07b	1.29 ± 0.08a	0.81 ± 0.26ab	0.73 ± 0.08ab	0.601	0.013	<0.01
NO_3^--N (mg kg^{-1})	2.57 ± 0.14c	4.34 ± 0.50ab	4.76 ± 0.69a	3.14 ± 0.38bc	0.307	0.870	<0.01
AGB	2.99 ± 0.36b	3.46 ± 0.21ab	4.18 ± 0.09a	3.41 ± 0.29ab	0.044	0.568	0.037

SWC: soil water content; TC: total soil carbon; TN: total soil nitrogen; TP: total soil phosphorus; NH_4^+: ammonium nitrogen; NO_3^-: nitrate nitrogen; AGB: above ground biomass.

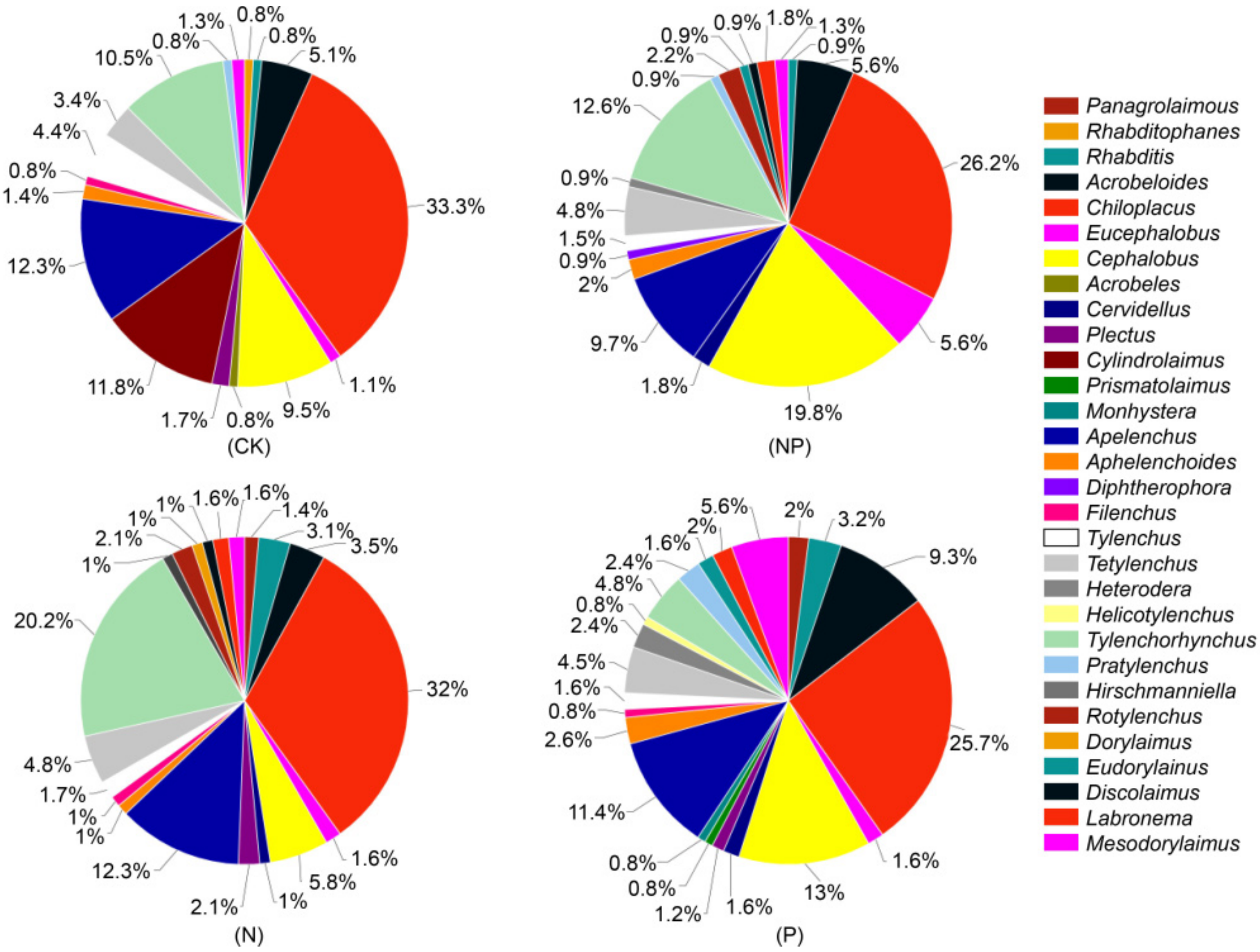

Fig. 1 Genus-level nematode community composition under different treatments. Experimental treatments included CK, control; P, phosphorus addition; N, nitrogen addition; NP, nitrogen addition and phosphorus addition.

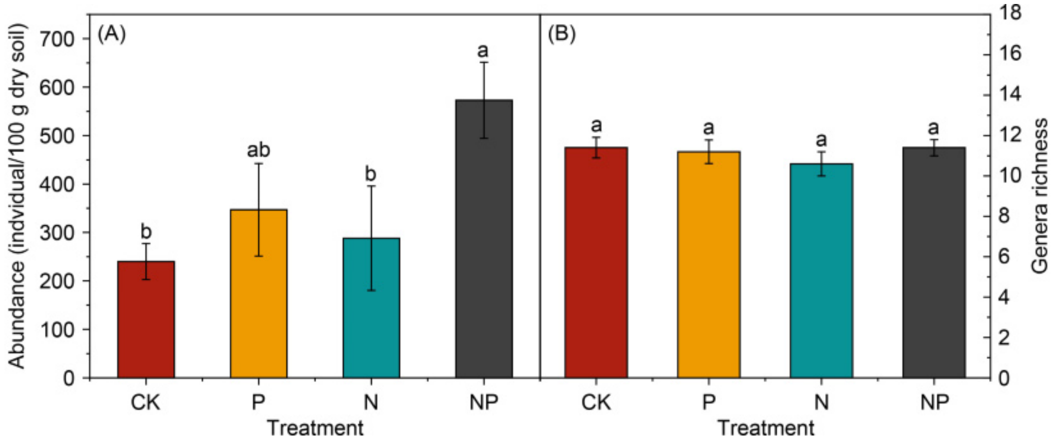

Fig. 2 Effects of control (CK), phosphorus addition (P), nitrogen addition (N), and nitrogen addition combined with phosphorus addition (NP) treatment on the abundance, genera richness of soil nematode community. The data are presented as mean ± S.E. Different letters indicate significant differences among different treatments ($P < 0.05$).

($P < 0.05$). Compared with CK, nitrogen addition significantly reduced the base index BI ($P < 0.05$) (Table 2). None of the treatments resulted in significant changes in enrichment index (*EI*), structural index (*SI*) compared to CK, but nitrogen-phosphorus mixing significantly reduced *EI* compared to phosphorus addition alone (Table 2).

3.4 Soil properties and correlation with nematode communities

Redundancy analysis (RDA) showed that environmental parameters explained 52.65% of the changes in the relative abundance of nematodes with different feeding habits, 36.71%

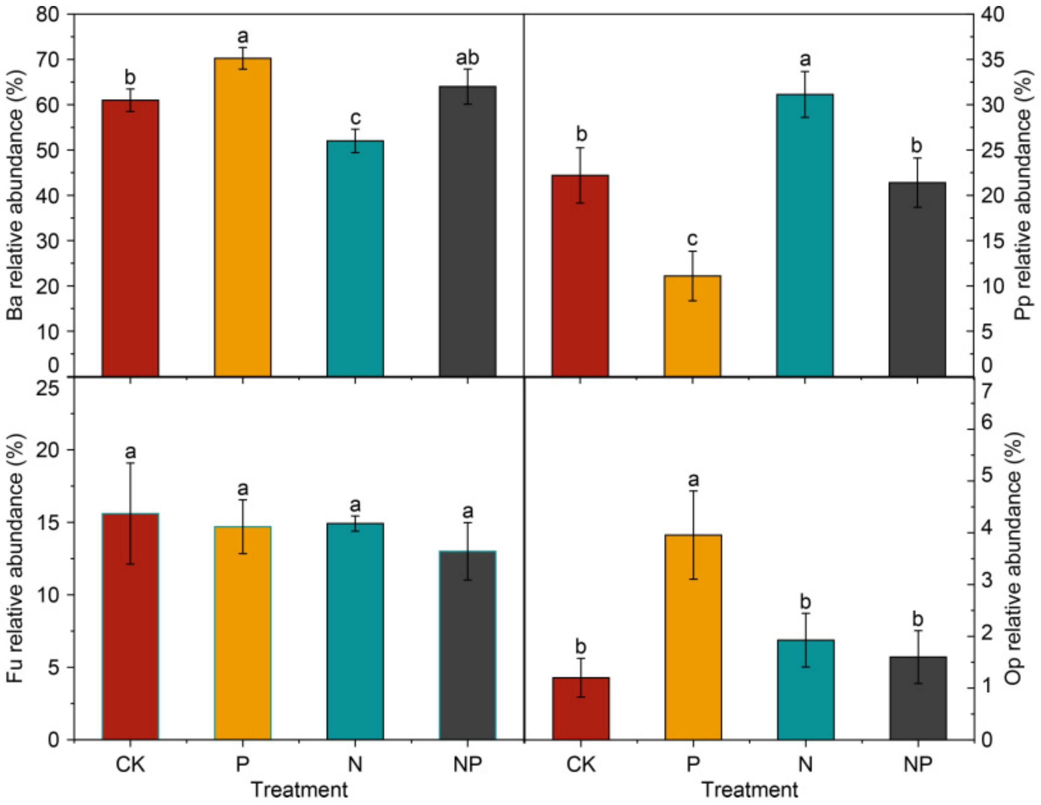

Fig. 3 Effects of control (CK), phosphorus addition (P), nitrogen addition (N), and nitrogen addition combined with phosphorus addition (NP) treatment on the relative abundance of different feeding nematodes in soil nematode communities. The data are presented as mean ± S.E. Different letters indicate significant differences among different treatments ($P < 0.05$).

Table 2 Values of soil nematode indices in control (CK), phosphorus addition (P), nitrogen addition (N), and nitrogen addition combined with phosphorus addition (NP) treatment. The data are presented as mean ± SE. Different letters indicate significant differences among different treatments ($P < 0.05$).

Parameters	Treatment				Two-way ANOVA		
	CK	P	N	NP	N	P	NP
PPI	2.75 ± 0.04a	2.87 ± 0.08a	2.96 ± 0.02a	2.95 ± 0.02a	<0.01	0.244	0.218
MI	2.14 ± 0.11a	2.08 ± 0.05a	2.09 ± 0.01a	2.03 ± 0.02a	0.455	0.328	0.944
CI	77.51 ± 3.05a	49.67 ± 7.06b	70.27 ± 8.26a	74.23 ± 3.95a	0.167	0.063	0.017
EI	20.68 ± 2.11ab	27.88 ± 3.17a	25.38 ± 2.28ab	18.09 ± 2.15b	0.318	0.985	<0.01
SI	42.96 ± 4.97ab	36.71 ± 4.86b	54.8 ± 2.38a	42.16 ± 3.35ab	<0.01	<0.01	0.440
BI	49.13 ± 3.24a	50.05 ± 2.38a	39.32 ± 2.45b	51.36 ± 3.11a	0.151	0.035	0.066

of nematode community structure changes on the RDA-axis1, and 15.94% of the nematode community structure changes on the RDA-axis2 (Fig. 4). From the structural equation model, it can be found that the model fits the nitrogen addition and phosphorus addition well for the richness of nematode communities Impact, the model directly and indirectly explained 58% of the changes in soil nematode community abundance. Through the model, it can be found that phosphorus addition has a significant positive effect on total phosphorus (TP) and a significant negative effect on pH, ammonium nitrogen (NH_4^+-N) and pH are significantly negatively correlated with the richness of nematode communities, and total phosphorus (TP) has a significant positive effect on ammonium nitrogen (NH_4^+-N). From the structural equation model, it can be seen that the effect of phosphorus addition on the abundance of nematode communities mainly affects pH by affecting total phosphorus (TP) and ammonium nitrogen (NH_4^+-N), and finally pH will negatively affect the abundance of nematodes (Fig. 5).

4 Discussion

4.1 Effects of nitrogen addition on soil nematode communities

Contrary to the first hypothesis, the results of this research indicate that nitrogen addition had no significant effect on nematode species richness and abundance. Previous partial studies had shown that nitrogen fertilizer had significant effect on soil nematode abundance, but many studies had also reported an insignificant effect of nitrogen addition on soil nematode abundance (Lokupitiya et al., 2000; Ruan et al., 2012; Wei et al., 2012; Li et al., 2013). In our study, nitrogen addition had a significant effect on soil nematode community structure compared to the control group (Fig. 3). Nitrogen addition significantly increased the relative abundance of plant-parasites, possibly because the addition of nitrogen fertilizer promoted plant growth and significantly increased above-ground plant biomass (Liang et al., 2009), promoted root cell division and proliferation, facilitates root growth and development, and provided more resources for plant-parasites nematodes (Bongers T. and Bongers M., 1998). Nitrogen addition significantly reduced the relative abundance of bacterivores, which was inconsistent with previous studies (Fig. 2). It had been reported that nitrogen deposition can promote the development of soil nematode community structure toward the bacterivores, thereby enhancing the role of bacterial decomposition channels in organic matter decomposition (Song et al., 2016; Liu et al., 2020), leading to simplification of nematode communities (Cesarz et al., 2015).

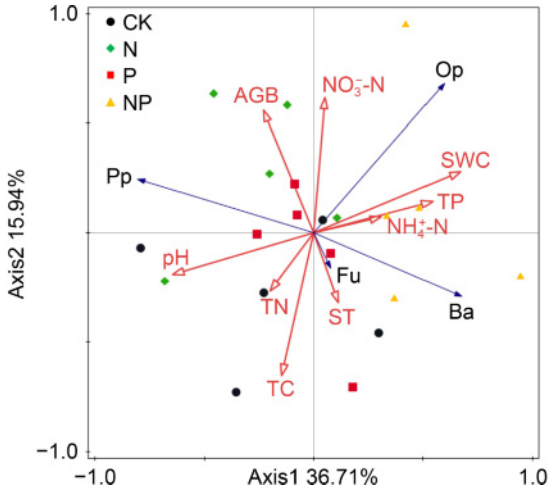

Fig. 4 Redundancy analysis (RDA) revealed the relationship between different feeding soil nematode and environmental factors. SWC: soil water content; ST: soil temperature; TC: total soil carbon; TN: total soil nitrogen; TP: total soil phosphorus; NH_4^+: ammonium nitrogen; NO_3^-: nitrate nitrogen; AGB: above ground biomass.

We found that nitrogen addition significantly reduces soil pH, the decrease in soil pH indicates soil acidification (Table 1). Previous studies had shown that soil acidification will change the environment for optimal growth and reproduction of nematodes, leading to degradation of nematode communities (Li et al., 2010, 2013). Structural equation model showed that Nitrogen addition can change the nitrate nitrogen content, leading to a positive effect on nematode abundance, which was contrary to previous studies (Chen et al., 2021), because the soil was very poor and had fewer nutrients, while the increase of nitrate nitrogen will provide more nutrients for soil nematodes and improve soil nutrients, thereby positively affecting soil nematode communities (Table 1, Fig. 5). At the same time, nitrogen addition also increased the soil nematode community enrichment index (*EI*), indicating that nitrogen addition increased the resource richness of underground food webs and provided more food resources for nematode communities (Table 2). Our research showed that nitrogen addition had no significant effect on soil carbon and nitrogen content (Table 1). This

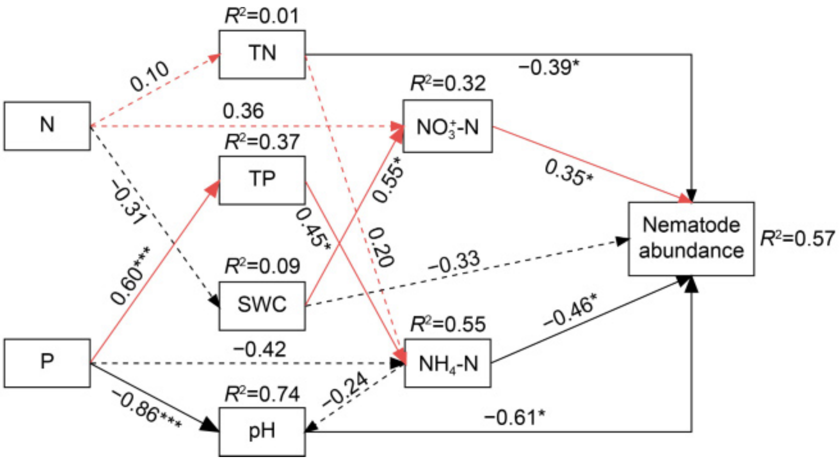

Fig. 5 Results of structural equation models (χ^2 = 16.429, df = 21, P = 0.745, TLI = 1.157, CFI = 1.000, P (RMSEA) = 0.0001) for the direct and indirect effects of nitrogen addition and/or phosphorus addition on soil nematode abundance. The solid red lines with arrows indicate significant positive effects, and the solid black lines indicate significant negative effects ($P < 0.05$). The dashed red lines with arrows indicate insignificant positive effects, and the dashed black lines indicate insignificant negative effects ($P > 0.05$). The number on the arrows is the standardized path coefficient. The R^2 value indicates the proportion of variation explained by the relationship with other variables.

result is consistent with the findings of previous studies (Cheng et al., 2021; Zhou et al., 2016). The possible reason was that the low carbon and nitrogen content in the region did not meet the optimal requirements for plant growth, therefore nitrogen addition can only increase the nitrogen uptake by plants in the short-term, but cannot significantly change the soil carbon and nitrogen content (Zhang and Han., 2012; Cheng et al., 2021).

Although nitrogen addition did not significantly change soil nematode abundance and species richness, overall nitrogen addition had a small positive effect on soil food webs. This is the same as the second half of our first hypothesis. Nitrogen application improved the soil environment, increased soil food web resource richness, and significantly increased the mean aboveground biomass (Gebremikael et al., 2016; Martinez et al., 2023). However, because the amount of nitrogen may have been applied too little, the richness of soil nematodes and species richness was not changed.

4.2 Effects of phosphorus addition on soil nematode communities

Previous studies had shown that soil nematode activity was affected by changes in physicochemical properties (Jiang et al., 2015; Zhang et al., 2016). The addition of phosphorus was found to significantly reduce soil pH (Table 1). The decrease in soil pH indicated a tendency for soil acidification, causing the loss of cations in the soil and the rise of exchangeable acid ions such as Al^{3+} and H^+, eventually leading to increased soil acidification and no longer suitable for nematode survival (Shao et al., 2017). Phosphorus addition increases soil NH_4^+ concentration and is toxic to soil nematode communities, especially plant-parasites. The probable reason is that the acceleration of microbial decomposition in the soil under the addition of phosphorus consumes more organic matter and produces many active substances (Karbin et al., 2015). In this study, phosphorus input significantly reduced the abundance of plant-parasites, which may be related to the specific feeding habits of plant-parasites. When the concentration of ammonium ions in the soil is too high, ammonium ions in soil can be directly absorbed by plants and gathered in root tissues, while plant-parasites usually directly pierce plant root cells with the special structure of mouth needles, sucking cell fluids and root secretions containing ammonium ions, a process that may be toxic to plant-parasites and reduce their abundance (Yeates et al., 1993; Wei et al., 2012). Phosphorus addition significantly increased the relative abundance of bacterivores, the possible reason is phosphorus input promoted the decomposition of microorganisms in the soil and improved microbial activity, so that bacterivores had more food sources, while the increase in the abundance of bacterivores and the increase of soil microbial activity could bring more food resources to omnivores/predators, resulting in a significant increase in the relative abundance of omnivores/predators (Zhang et al., 2009; Zhang et al. 2019).

In this study, the addition of phosphorus significantly decreased the nematode channel index (*CI*) (Table 2), which was due to the increase in bacterivores (Table 2, Fig. 3). Zhao et al. (2014) also obtained the same results in tropical secondary forests. Phosphorus input significantly increased *WI*, which means improved soil health, and the inhibitory effect on plant parasitic nematodes may be the main reason.

Contrary to our second hypothesis, we found that the addition of phosphorus had a negative effect on soil nematode communities. The addition of phosphorus in the soil leaded to salt toxicity, which has a negative effect on nematodes. In this study, the phosphorus application rate was 112.5 g m^{-1}, which is enough to cause damage to nematodes. Research evidence suggests that phosphorus addition affects soil nematodes primarily by changing soil pH and total phosphorus content (Wei et al., 2012; Yang et al., 2019). In summary, the results suggest that the effect of phosphorus on soil nematode communities in soybean farmland ecosystems is mediated by changes in soil pH, total phosphorus, and ammonium nitrogen.

4.3 Interaction effects of nitrogen and phosphorus addition on soil nematode communities

In agroecosystems, nitrogen and phosphate fertilizers are often used together. In this study, neither nitrogen nor phosphorus alone significantly changed nematode abundance, but nitrogen and phosphorus addition significantly increased nematode abundance, mainly because the addition of nitrogen and phosphorus increases soil nutrients and improves the soil environment, while the ammonium nitrogen (NH_4^+-N) content is kept in a suitable range, it will not produce serious ammonium toxicity on nematodes (Wei et al., 2012; Chen et al., 2021). The addition of nitrogen and phosphorus had a significant interaction on nematode abundance, it shows that phosphorus application has a positive effect on nematode abundance under ordinary nitrogen application conditions (Zhang et al., 2022). Previous studies had shown that nitrogen and phosphorus addition had no significant effect on soil nematode abundance and richness in temperate grassland systems, but it has significant positive effects on nematode community structure, trophic taxa and ecological index (Zhang et al., 2022). Therefore, the response of nematode communities to nitrogen and phosphorus addition varies significantly between different types of ecosystems and soils with different nutrient status (Pan et al., 2010; Pan et al., 2015).

Furthermore, changes in nitrogen and phosphorus availability have an interactive effect on carbon use in nematode communities, as phosphorus-induced effects are stronger under nitrogen-rich ecosystems (Zhao et al., 2014). Therefore, our third hypothesis is correct, nitrogen and phosphorus additions have an interactive effect on soil nematodes, which will have a more positive effect on nematodes, significantly increasing the richness of soil nematodes. The results showed that nitrogen and phosphorus addition increased soil nutrients, improved soil environment, and enriched soil food web resources (Zhao et al., 2014).

5 Conclusion

In summary, our experiments found that nitrogen and phosphorus mixing had a significant positive effect on the richness of nematode communities compared with nitrogen or phosphorus alone but had no significant effect on the number of nematode species. Compared with nitrogen alone or phosphorus, nitrogen and phosphorus additions have a significant interaction on ammonium nitrogen, nitrate nitrogen and PH in the soil, in which the addition of phosphorus will cause severe acidification of the soil, and the addition of nitrogen and phosphorus will alleviate this situation, that is, the addition of nitrogen will reduce the acidification of phosphorus on the soil and maintain soil health. Therefore, add nitrogen fertilizer when phosphate fertilizer is added to soybean farmland will have a certain positive effect on the soil underground food web, which provides a basis for better explaining the effect of nitrogen and phosphorus addition on soybean farmland.

Author contributions

Huiying Zhang: Conceptualization, Methodology, Data measurement, Formal analysis, Validation, Writing – original draft. Mengyang Tian: Conceptualization, Writing – review and editing. Meiguang Jiang: Data measurement, Validation. Jingyuan Yang: Data measurement. Qi Xu: Data measurement. Ying Zhang: Data measurement. Minglu Ji: Methodology. Yuteng Yao: Methodology. Cancan Zhao: Writing – review and editing. Yuan Miao: Conceptualization, Funding acquisition, Writing – review and editing.

Data availability

Data will be made available on request.

Conflict of interest

The authors declare no competing interests.

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