

Nutrient enrichment reshapes soil nematode communities across multiple scales through trait-based and abundance-based pathways

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Abstract

Additions of nitrogen (N) and phosphorus (P) in grasslands alters aboveground and belowground resources, triggering bottom-up effects throughout the soil food web. However, these nutrient-driven effects on soil fauna composition and functional traits remain poorly understood. We investigated how 7-year N, P, and combined N and P (NP) additions influenced soil nematode communities in an alpine meadow. We investigated the effects of 7-year N, P, and NP additions on alpine meadow nematode communities using trait-based (body size) and abundance metrics at the genus, trophic-group, and community levels. At genus level, among the seven dominant genera, five showed no body size change across treatments, except *Acrobeloides* increased under P and *Aphelenchoides* decreased under NP, while abundances of all seven were suppressed, especially under single N or P addition. At trophic level, P addition increased body size of bacterivores, fungivores, and plant parasites, while NP extended this effect to omnivore–predators. Single N or P addition reduced the abundances and biomass of the first three groups, but not omnivore–predators. At community level, N or P addition decreased biomass, abundance, and diversity, whereas NP mitigated abundance and diversity losses and stabilized community structure. Only NP transmit positive effects through the entire food web. Soil organic carbon was the main driver of community composition, while total phosphorus governed trophic-specific traits. Our findings reveal that nutrient enrichment restructured nematode communities through trait-based and abundance-based pathways, and balanced stoichiometry is essential for maintaining soil food web integrity in alpine ecosystems.

Introduction

Soil nematodes are abundant, functionally diverse, and occupy multiple trophic positions, making them ideal indicators of soil food web responses to environmental changes (Ferris et al. 2001; van den Hoogen et al. 2019). Nematode community dynamics are tightly linked to the quantity and quality of basal resources, especially under nutrient enrichment (Bardgett et al. 2005). Consequently, soil nematodes can be affected directly or indirectly by changes induced by nutrient additions (Hou et al. 2023; Li et al. 2024). However, traditional assessments relying on abundance and taxonomic diversity on community level may overlook rapid phenotypic adjustments that precede population-level shifts. Trait-based approaches, particularly those centered on body size, which is a master trait governing metabolism, feeding rates, and energy flow, promise to enable earlier and more mechanistic detection of disturbance effects (Woodward et al. 2005; Andriuzzi and Wall 2018). Nevertheless, how different fertilization regimes regulate soil nematode communities, particularly through trait-based pathways across multiple ecological scales, remains inadequately understood.

Two contrasting hypotheses predict the distribution of body size responses across trophic levels. The high-trophic-level sensitivity hypothesis argues that large-bodied, K-selected organisms such as omnivore–predators are more vulnerable to environmental change due to their long generation times, low reproductive rates and high energetic demands (Bongers 1999; Vasseur and McCann 2005). By contrast, the trophic attenuation hypothesis posits that effects of basal resource pulses weaken at

higher trophic levels due to inherent thermodynamic inefficiencies and energy loss during transfer (Lindeman 1942; Hessen et al. 2013). In this view, lower trophic levels (e.g., plant parasites, fungivores and bacterivores) should exhibit stronger and faster trait responses to resource enrichment, while top consumers remain energetically constrained unless nutrient supply is balanced. Despite these theoretical expectations, empirical tests of these competing hypotheses in soil food webs are scarce, particularly concerning morphological traits such as body size.

Alpine grasslands on the Qinghai–Tibetan Plateau (QTP) cover $1.78 \times 10^6 \text{ km}^2$, accounting for approximately 64% of the region area, and play a critical role in maintaining regional ecological security (QTP) (Zhao et al. 2025b). Widespread degradation driven by climate change and overgrazing led to the extensive use of nitrogen (N) and phosphorus (P) fertilizers to restore plant productivity (Bai et al. 2010; Sheng et al. 2022). However, the addition of a single nutrient may create stoichiometric imbalances in microbial prey, such as elevated N:P or C:P ratios, thereby reducing food quality for consumers and dampening potential positive effects on growth (Elser et al. 2000). Consequently, single-nutrient additions can disrupt belowground trophic interactions and reduce the diversity of soil food webs (Ineichen et al. 2020; Cui et al. 2022). In contrast, combined N and P (NP) fertilization can alleviate such stoichiometric constraints, more effectively alleviate nutrient co-limitation, and enhance soil microbial activity (Zeng and Wang 2015; Shaw et al. 2019). This combined approach may improve the efficiency of carbon and energy transfer through the food web and promote a more stable community structure (Harpole et al. 2011; Xing et al. 2023). Nevertheless, the role of nutrient stoichiometry in mediating trait-based responses remains unexplored in most empirical systems. Here, we used a 7-year factorial N, P, and NP fertilization experiment in a QTP alpine meadow to investigate how nutrient enrichment reshapes soil nematode communities through trait-based (body width, body mass) and abundance-based metrics at the genus, trophic group, and community levels. We hypothesized that: 1) Trait-based metrics (body size) are more sensitive to fertilization than abundance-based metrics, because resource alterations first manifest at the individual level before translating into population changes; 2) Consistent with the trophic attenuation hypothesis, lower trophic levels will exhibit more sensitive body size increases than higher trophic levels, rather than the reverse pattern predicted by the high-trophic-level sensitivity hypothesis; 3) Balanced NP addition will have more positive and stabilizing effects on nematode communities than N or P alone, by maintaining stoichiometric balance and energy transfer efficiency.

Materials and methods

Site description

The experiment was established in Hongyuan County (102°35'–102°58' E, 32° 5'–32° 83' N), located on the eastern QTP, Sichuan Province. The region has a mean elevation of approximately 3,500 m above sea level and is characterized by a cold temperate continental monsoon climate. The mean annual temperature is 0.96°C, and annual precipitation ranges from 600 to 800 mm, with most rainfall occurring during the growing season (May–September). The vegetation is dominated by alpine meadow species, particularly *Kobresia pygmaea* and *Kobresia setchwanensis*, with 70%–90% vegetation coverage. The soil

is classified as Mat Cry-gelic Cambisol according to the FAO system, corresponding to Inceptisol in the USDA taxonomy (Zi et al. 2018).

Experimental design

A long-term fertilization experiment was established in 2014 in a lightly degraded alpine meadow. The study area (100 m × 100 m) was fenced to exclude grazing. Four fertilization treatments were applied: control (CK, no fertilization), nitrogen (N) addition ($\text{CO}(\text{NH}_2)_2$, 46%, 30 g N m⁻² yr⁻¹), phosphorus (P) addition ($\text{Ca}(\text{H}_2\text{PO}_4)_2$, 16% P₂O₅, 30 g P m⁻² yr⁻¹), and combined nitrogen and phosphorus (NP) addition (15 g N + 15 g P m⁻² yr⁻¹). Fertilization rates were determined based on available research and local fertilization practices (Fan et al. 2010; Yu et al. 2010). Each plot measured 2 m × 2 m, with six replicates per treatment arranged in a completely randomized design. Plot corners were marked with permanent stakes, and a 2 m buffer zone separated plots to prevent cross-contamination. Fertilizers were evenly broadcast once annually in May at the onset of plant growth.

Soil sampling, nematode extraction, and identification

In July 2021, soil samples (0–10 cm) were collected from each plot via a five-point sampling method with a 5-cm-diameter soil auger. In the laboratory, soil nematodes were extracted from 50 g fresh soil using Baermann funnel method (48 h) (Barker 1985), and fixed in 5% formaldehyde solution. Total nematodes were counted under a stereomicroscope, and at least 100 individuals per sample were randomly selected and identified to the genus under a compound microscope (40×) (Yeates et al. 1993; Bongers 1994; Yin 2000). Maximum body width (body diameter, μm) was measured for each identified individual using a digital imaging system. Nematodes were assigned to four trophic groups: bacterivores (Ba), fungivores (Fu), plant parasites (Pl), and omnivore–predators (OP) (Yeates et al. 1993; Okada et al. 2005; Neher et al. 2012). Abundances were expressed as individuals per 100 g dry soil based on gravimetrically determined soil moisture content.

Plant and soil measurements

Plant and soil environmental characteristics of the four grasslands were investigated in July 2021. Within each plot, a 0.5 m × 0.5 m subplot was used to assess plant community characteristics, including species richness (number of species, PS), coverage (visual estimation, CO), and aboveground biomass (harvested, oven-dried at 65°C to constant weight, AB). Soil samples were air-dried and sieved (2 mm and 0.25 mm) prior to analysis. Soil chemical properties were determined according to well-established methods (Bao 2000). The following properties were measured: soil pH (soil:water = 1:2.5) using a glass electrode, soil organic carbon (SOC) by dichromate oxidation, total nitrogen (TN) using the Kjeldahl method, total phosphorus (TP) using acid digestion followed by molybdenum blue colorimetry, and stoichiometric ratios (C:N, C:P, N:P) were calculated accordingly.

Nematode traits and community indices

Body size at genus and trophic levels

Nematode body mass (μg fresh weight) was estimated based on body width using trophic-group-specific allometric equations (Andrássy 1956; Zhao et al. 2019), listed as follows.

$$W_{Ba} = \frac{(D_{Ba} \times 15.4 + 71.8) \times D_{Ba}^2}{1.6 \times 10^6}$$

1

$$W_{Fu} = \frac{(D_{Fu} \times 22.3 + 16.4) \times D_{Fu}^2}{1.6 \times 10^6}$$

2

$$W_{Pl} = \frac{(D_{Pl} \times 13.6 + 205.4) \times D_{Pl}^2}{1.6 \times 10^6}$$

3

$$W_{Om} = \frac{(D_{Om} \times 35.7 - 288.2) \times D_{Om}^2}{1.6 \times 10^6}$$

4

$$W_{Pr} = \frac{(D_{Pr} \times 20.2 + 53.3) \times D_{Pr}^2}{1.6 \times 10^6}$$

5

where 'W' is the fresh body mass (μg) per individual, 'D' is the greatest body diameter (μm). Omnivores (Om) and predators (Pr) were combined into a single omnivore-predator (OP) group for statistical analyses due to their similar trophic positions.

Seven dominant genera (*Tylenchus*, *Aphelenchoides*, *Prismatolaimus*, *Acrobeloides*, *Rotylenchus*, *Filenchus*, *Aphelenchus*) were selected based on a frequency of more than six individuals per treatment in every replicate. For each of the seven dominant genera, genus-level mean body width and mass were computed. Trophic-level mean size was calculated by pooling individuals within each group.

Biomass, abundance and diversity indices

Total biomass (μg per 100 g dry soil) and abundances (individuals per 100 g dry soil), taxonomic richness (mean number of genera per sample, S), Shannon index (H') and Simpson index (D) were calculated at trophic and community levels. The Shannon index was calculated as follows (Shannon 1948):

$$H' = - \sum_{i=1}^S (p_i \times \ln(p_i))$$

6

Ecological indices

The enrich index (EI), structure index (SI), basal index (BI), channel index (CI), the free-living maturity index (MI) and the plant parasite maturity index (PPI) of nematode communities were calculated for each treatment to evaluate soil food web condition (Bongers 1990; Ferris et al. 2001). The formulae were as follows:

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

7

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

8

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

9

$$CI = 100 \times Fu / (Ba + Fu)$$

10

$$MI/PPI = \sum_{i=1}^S (v_i \times f_i)$$

11

where e represents the weighted proportion of enrichment opportunist nematodes (Ba_1 and Fu_2 guilds); b represents the weighted proportion of basal opportunist nematodes (Ba_2 and Fu_2 guilds); s represents the weighted proportion of structure indicator nematodes (Ba_3 – Ba_5 , Fu_3 – Fu_5 , OP_3 – OP_5 guilds); Fu and Ba denote the abundances of fungivores and bacterivores, respectively; v_i is the c-p (colonizer-persister) value of taxon i ; and f_i is the relative frequency of taxon i .

Trophic structure

Both absolute abundance (individuals per 100 g dry soil) and relative abundance (percentage of total individuals) of each trophic group were calculated for each plot to evaluate changes in community structure. Relative abundance was emphasized because it better reflects each group's community role and changes in trophic structure than absolute abundance.

Statistical analysis

One-way ANOVA with Tukey's HSD test was used to test treatment effects. Non-metric multidimensional scaling (NMDS) based on Bray–Curtis distance was performed using CANOCO for Windows 5.0 to assess community composition. For clarity of visualization, only the top 15 genera with the greatest contributions were shown on ordination plots.

Redundancy analysis (RDA) was performed using CANOCO for Windows 5.0 to evaluate relationships between the taxonomic composition of nematode community and environmental variables (plant and soil variables). Prior to ordination, species data were Hellinger-transformed to reduce the influence of rare taxa (Legendre and Gallagher 2001). Variation partitioning analysis (VPA) was then applied to quantify the relative contributions of plant versus soil variables, and forward selection with Monte Carlo permutation tests (499 permutations) was used to identify significant predictors.

Random forest regression was employed to evaluate the relative importance of environmental factors in predicting nematode parameters at the genus, trophic-group, and overall community levels. All random forest analyses were conducted in R version 4.3.3 (Team 2024), using the *randomForest* package (Liaw and Wiener 2002) with 1000 trees. For each response variable, explained variation (%) was extracted, and predictor importance was quantified using the permutation-based increase in out-of-bag mean squared error (%IncMSE). Spearman's rank correlation analysis was used to determine the direction of bivariate relationships, and statistical significance was assessed using two-tailed tests, with significance threshold of $P < 0.05$. Finally, all graphical outputs were visualized using the *ggplot2* package (Villanueva and Chen 2019).

Results

Genus-level responses

Body size changes were highly genus-specific (Fig. 1A, B). Among the seven dominant genera, *Acrobelloides* increased significantly in body width and mass under P addition ($P < 0.01$), whereas *Aphelenchoides* showed significant decreases in these traits, particularly under NP treatment ($P < 0.05$). In contrast, the remaining genera (*Tylenchus*, *Prismatolaimus*, *Rotylenchus*, *Filenchus*, and *Aphelenchus*) showed no significant changes in body size across treatments.

The abundances of all seven genera were significantly affected by the nutrient additions (Fig. 1C). Specifically, the abundances of *Aphelenchoides*, *Prismatolaimus*, and *Acrobelloides* significantly decreased under individual N and P treatments but showed non-significant declines under the NP

treatment. The abundances of *Tylenchus* and *Aphelenchus* decreased only under the P treatment ($P < 0.05$), while those of *Rotylenchus* and *Filenchus* decreased significantly only under the NP treatment.

Trophic-group-level responses

Body size

N addition alone significantly increased the body mass of PI only. In contrast, P addition alone significantly enhanced both body width and mass of Ba, Fu, and PI (Fig. 2). The NP addition significantly increased body width and mass of Ba and OP, as well as the body mass of PI. The P-driven effects were therefore dominant at lower trophic levels, whereas NP effects extended to the higher trophic levels.

Biomass, abundance and diversity

Fertilization generally reduced nematode biomass, abundance and diversity across trophic groups (Fig. 3). For Ba, biomass, abundance, and diversity (richness and Shannon index) declined significantly under N and P, but partially recovered under NP. For Fu, biomass and abundance decreased across treatments, though significantly only for abundance under P and NP; Shannon index increased under N and P but decreased under NP, while richness remained unchanged. PI showed a significant decline in abundance under all treatments, but biomass decreased only under P ($P < 0.05$), with richness and Shannon index unchanged across treatments. OP exhibited non-significant decreasing trends in biomass and abundance across treatments, yet diversity increased under P ($P < 0.01$).

Regarding changes in the relative abundances of the four trophic groups, different fertilizations shifted the trophic structure of the nematode community (Fig. A1). Notably, additions of N and P alone reduced Ba relative abundance and increased those of Fu and OP ($P < 0.05$ and 0.01), thereby shifting the trophic structure from a Ba-PI-dominated community to a PI-Fu-dominated one. In contrast, NP addition increased only Ba relative abundance ($P < 0.01$), maintaining a stable trophic structure.

Community-level responses

The results of non-metric multidimensional scaling (NMDS) showed that the taxonomic composition of nematode communities under the four treatments was clearly separated along Axis 1 and Axis 2 (Fig. 4A). Overall, the genera of *Chiloplectus*, *Pararotylenchus*, *Odontopharynx*, *Plectonchus* and *Filenchus* were the main groups associated with the separation along the first two axes across the treatments.

Regarding biomass and diversity, Fertilization reduced total biomass, though not significantly, and significantly decreased abundance, particularly under P (Fig. 4B). N addition reduced richness and Shannon index ($P < 0.01$). In contrast, NP addition moderated these negative effects, while P addition had no significant effect on richness or the Shannon index.

As for the soil food web condition (Fig. 4C), N addition significantly reduced the enrichment index and structure index, and increased basal index and channel index ($P < 0.05$ or 0.01). In contrast, NP maintained an enrichment index above 50 and a stable structure index (Fig. A2).

Environmental drivers

SOC decreased under both N and P additions, whereas TP increased under P and NP additions ($P < 0.05$ or 0.01 ; Fig. 5). The C/N and C/P ratios declined across treatments, while the N/P ratio increased under N addition but decreased under P and NP additions ($P < 0.01$). At the genus level, nematode-environment associations were genus-specific (Fig. 6A). Specifically, *Aphelenchus* body width and mass were negatively affected by plant richness (PR), while *Tylenchus* body size responded positively to pH, and *Filenchus* body size was negatively influenced by C/P but positively by TP. Regarding abundances, SOC, C/N, and C/P exerted positive effects on *Prismatolaimus*, *Acrobelloides*, *Aphelenchoides*, and *Filenchus*, while TP negatively affected *Tylenchus* and *Filenchus*. Overall, environmental factors had weaker effects on body size than on abundance.

At the trophic level, plant biomass (PB) correlated positively with Fu biomass, richness, and abundance, as well as with OP abundance, while PR correlated positively only with Ba richness (Fig. 6B). SOC and the C/N were positively associated with Ba biomass, diversity, abundance and relative abundance, but SOC alone negatively correlated with Ba and PI body width, and with PI body mass and relative abundance. Meanwhile, SOC also exerted a positive effect on Fu abundance. TP correlated positively with OP diversity but negatively with PI abundance and biomass. The C/P was positively associated with Fu and PI abundances yet negatively associated with OP diversity and relative abundance, and Fu body width.

At the community level, total abundance was positively correlated with SOC, C/N, and C/P. Both richness and the Shannon index were positively correlated with both PR and PB. Additionally, the Shannon index was positively correlated with SOC and C/N but negatively correlated with TN (Fig. 6C). The EI was positively, and the CI negatively, correlated with PR. Redundancy analysis (RDA) identified SOC as the primary driver of community taxonomic composition, explaining 22.0% of the total variance, followed by C/N and C/P (Fig. 7A, B). Variation partitioning analysis (VPA) further indicated that nematode community composition was also regulated by soil stoichiometry in combination with other soil factors (Fig. 7C).

Discussion

Genus-specific responses of body size and abundance to nutrient enrichment

Our finding that body size remained stable for five of the seven dominant genera under fertilization, whereas *Acrobelloides* (Ba) increased significantly under P and *Aphelenchoides* (Fu) decreased significantly under NP (Fig. 1A, B), reveals pronounced genus-specificity in trait-based responses. This

pattern offers an opportunity to explore the ecological mechanisms that determine when body size serves as a sensitive indicator of nutrient enrichment.

The positive response of *Acrobeloides* to P addition is likely driven by P-induced enhancement of bacterial biomass and shifts toward copiotrophic bacterial dominance. In P-limited alpine meadows, P enrichment typically stimulates fast-growing bacterial taxa and increases microbial resource quantity, directly promoting somatic growth of bacterivorous nematodes (Zi et al. 2022; Liu et al. 2023). The body size increase of *Acrobeloides* under P thus reflects improved prey quality (copiotrophic bacteria with higher nutrient content) and quantity (greater bacterial biomass), which together alleviate the stoichiometric constraints on consumer growth (Leff et al. 2015; Zhou et al. 2023). In contrast, the reduced body size of *Aphelenchoides* under NP addition is consistent with nutrient-induced suppression of saprotrophic basidiomycetes. NP fertilization often shifts fungal communities toward arbuscular mycorrhizal fungi and reduces saprotrophic fungal biomass, lowering food quality for specialized fungivores (Liu et al. 2023; Yang et al. 2024). This interpretation is supported by the observation that *Aphelenchoides* abundance also declined significantly under NP, indicating that both trait and demographic responses converge when resource quality deteriorates (Ruess et al. 2000).

The stability of body size in the remaining five genera (*Filenchus* (Fu) and *Rotylenchus* (PI), *Tylenchus* (PI), *Prismatolaimus* (Ba) and *Aphelenchus* (Fu)) points to several buffering mechanisms. First, these genera may possess broader physiological tolerances or greater dietary flexibility, which buffer them against relatively short-term nutrient fluctuations. *Aphelenchus*, for instance, is known to feed on a diverse range of fungal taxa, which may confer resistance to shifts in fungal community composition (Okada and Kadota 2003; Okada et al. 2005). Second, the generation times of these taxa may be sufficiently long that phenotypic adjustments lag behind resource pulses, such that body size changes require longer experimental durations to manifest (Bongers 1999). Third, the stoichiometric imbalance created by single-nutrient additions may be partially offset by internal nutrient storage or flexible C:N:P homeostasis in these genera, a mechanism that remains poorly explored in soil nematode ecophysiology (Xue et al. 2023).

In contrast to the trait responses, the abundances of all seven dominant genera were reduced by fertilization, with treatment-specific patterns (Fig. 1C). This strong decoupling between trait- and abundance-based metrics partially rejects our first hypothesis that trait-based metrics are more sensitive to fertilization than abundance-based metrics, and contrasts with meta-analyses showing that trait shifts often precede demographic changes (Hou et al. 2023; Xing et al. 2023). Several interrelated mechanisms explain this decoupling.

First, soil acidification is a primary driver of the decline in nematode abundance. N enrichment significantly reduces soil pH, which directly affects nematode survival and reproduction, as many species have narrow pH tolerances (Hu et al. 2024; Liu et al. 2025; Wu et al. 2026). Moreover, high N addition exerts direct toxic effects on nematodes, further suppressing abundance beyond the stress caused by pH reduction (Xu et al. 2026). Second, resource quantity reduction, as reflected by the decline

in SOC under N and P additions, limits the basal energy available to the soil food web. SOC is a primary driver of microbial biomass in alpine soils, and its depletion directly constrains the carrying capacity for microbivorous nematodes (Wardle 2002; Bardgett et al. 2005). Third, stoichiometric imbalance between nematode bodies and their resources intensifies bottom-up limitation on population regulation. The positive correlations between nematode abundance and C:N or N:P ratios indicate that both resource quality and stoichiometric balance serve as critical limiting factors (Ping et al. 2019; Xue et al. 2023). Collectively, these genus-level patterns demonstrate that abundance-based metrics are more broadly sensitive to fertilization than body size traits in this alpine meadow system.

Trophic- specific responses to nutrient enrichment

Generally, PI feed on plant roots and are primary consumer; Fu and Ba feed on fungi and bacteria, making them secondary consumer; and OP feed on other smaller nematodes and protozoa and are therefore tertiary consumers (Yeates et al. 1993; Mikola 1998). Analyses of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values within nematode bodies further indicate that the order of trophic levels from low to high is: PI < Fu < Ba < OP (Kudrin et al. 2015; Melody et al. 2016). Our finding that body size increases were strongest at lower trophic levels (PI under N, P, and NP; Fu and Ba under P; OP only under NP) and attenuated progressively up the food web (Fig. 2) provides strong empirical support for our second hypothesis that lower trophic levels will exhibit more sensitive body size increases than higher trophic levels. This finding is consistent with the notion that the effects of basal resource pulses weaken at higher trophic levels due to inherent thermodynamic inefficiencies and energy loss during transfer (Lindeman 1942; Hessen et al. 2013).

This trophic gradient in sensitivity can be mechanistically understood through three interacting factors. First, energetic transfer efficiency in soil food webs is inherently low: only a small fraction of energy is transferred between trophic levels, limiting the propagation of bottom-up effects to top predators (Lindeman 1942; Barnes et al. 2014). The pronounced body size increase in PI under all fertilization treatments reflects direct access to nutrient enhanced root resources (Qi et al. 2023; Guan et al. 2024), and PI are primary consumers that experience the full force of bottom-up resource pulses (Andriuzzi and Wall 2018). Ba and Fu, as secondary consumers, receive attenuated signals through microbial intermediates, explaining why their body size responses were confined to P addition, which most effectively stimulated microbial biomass in the P-limited system (Zi et al. 2022; Liu et al. 2023; Song et al. 2023). OP, as tertiary consumers, require the stoichiometrically balanced resource base provided only by NP addition to overcome the cumulative energy losses across multiple trophic transfers (Hessen et al. 2013; Liu et al. 2025). Second, generation time and metabolic rate scale with trophic position. Higher-trophic nematodes have longer generation times and lower reproductive rates, reducing their phenotypic responsiveness to short-term nutrient enrichment (Bongers 1999). The body size increase in OP under NP, but not under N or P alone, suggests that balanced stoichiometry is required to overcome the energetic threshold that limits somatic growth in these large-bodied, K-selected organisms (Vasseur and McCann 2005). Third, stoichiometric constraints intensify at higher trophic levels. Consumers at higher trophic positions face greater elemental imbalances between their own biomass and their prey, because the C:N:P stoichiometry of prey becomes increasingly variable and potentially mismatched (Hessen et al.

2013). Only NP addition, by alleviating both N and P co-limitation, generated sufficient resource stoichiometry to transmit positive effects through the entire food web (Harpole et al. 2011). This interpretation is consistent with ecological stoichiometry theory, which predicts that consumer growth is constrained by the most relatively scarce element in its resources (Sterner and Elser 2002).

Contrary to the increasing body size, the biomass and abundance of PI, Fu, and Ba were significantly reduced by single-nutrient additions, whereas the NP addition alleviated this decline (Fig. 3). This trait–demography decoupling at the trophic level mirrors the pattern observed at the genus level but reveals an additional layer of complexity: body size increases can coexist with population declines when per-capita resource availability improves but total resource quantity diminishes. Specifically, P addition enhanced per-capita food quality for Ba and Fu (driving body size increases) while simultaneously reducing total microbial biomass through P-induced shifts in community composition (driving abundance declines) (Liu et al. 2023; Qi et al. 2023).

The PI pattern merits particular attention. PI abundance declined under all treatments, but biomass decreased only under P, with diversity unchanged across treatments. With no concurrent decline in body mass, the decrease in abundance was the primary cause of the significant reduction in PI biomass. This is probably attributable to P-induced changes in root morphology, defense chemistry, or dilution effects from enhanced root biomass, a mechanism that may reduce PI colonization or feeding efficiency without affecting the body size of those individuals that successfully establish (Hu et al. 2017; Zhao et al. 2025a).

The observed trophic gradient in demographic responses (Ba > PI > Fu > OP in terms of sensitivity) further supports the trophic attenuation framework: lower trophic levels, which are more directly coupled to basal resources, exhibited stronger abundance declines under nutrient imbalance. These findings are contrary to the classic “high-trophic-level sensitivity hypothesis”, which predicts that large-bodied, K-selected organisms are more vulnerable to environmental change due to their long generation times and high energetic demands (Bongers 1999). Instead, our results are consistent with the trophic attenuation hypothesis, underscoring that energy attenuation, rather than trophic-level sensitivity, governs the propagation of nutrient enrichment effects through soil food webs (Lindeman 1942; Hessen et al. 2013).

Community-level responses indicating balanced NP fertilization stabilize soil food webs

Our community-level results confirm that balanced NP fertilization outperforms single N or P addition in maintaining soil nematode diversity and soil food web stability, supporting our third hypothesis. N addition exerted the strongest negative effects, reducing nematode abundance, diversity, and EI, while increasing BI and CI, indicating a stressed, fungal-dominated soil food web (Ferris et al. 2001). P addition had moderate negative effects, while NP addition mitigated diversity loss, maintained high EI (> 50), and preserved structural complexity (SI > 50).

The mechanistic basis for these community-level patterns can be traced to resource stoichiometry. Single-nutrient additions create stoichiometric imbalances in microbial prey, elevated N:P or C:P ratios under N addition, and reduced N:P ratios under P addition, that reduce food quality for consumers and dampen energy transfer efficiency. Combined NP fertilization alleviates such imbalances, improving the efficiency of carbon and energy transfer through the food web and promoting more stable community structure (Harpole et al. 2011; Hessen et al. 2013). This interpretation is consistent with the resource stoichiometry theory, which posits that consumer growth and community stability are maximized when resource C:N:P ratios match consumer demands (Sternner and Elser 2002).

RDA identified SOC as the primary driver of nematode community composition, explaining 22.0% of variance, while TP mediated trophic-level trait dynamics. These findings align with global studies showing that SOC shapes soil biodiversity by determining basal energy availability, while P availability regulates trophic interactions in P-limited ecosystems (Wardle 2002; Liu et al. 2023). The SOC effect is particularly noteworthy: SOC declined under both N and P additions, and its positive correlation with nematode abundance and diversity across treatments indicates that carbon availability is the ultimate constraint on soil food web structure in this alpine ecosystem. VPA further revealed that soil stoichiometry (C:N:P ratios) plays a critical role in structuring nematode communities, emphasizing that nutrient balance, rather than single-nutrient supply, governs soil biodiversity and food web stability (Xing et al. 2023; Liu et al. 2025).

From a global change perspective, our results have important implications for alpine grassland restoration. Climate change and overgrazing have led to widespread degradation of QTP grasslands, and fertilization is widely used to restore productivity (Zhao et al. 2025b). However, single-nutrient (N or P) fertilization disrupts soil food webs and reduces biodiversity, while balanced NP fertilization maintains soil biodiversity and food web stability, enhancing ecosystem resilience (Xing et al. 2023; Liu et al. 2025). Our results clearly confirm that combined NP fertilization alleviates the negative impacts on soil biodiversity and maintains a more complex and enriched soil food web. This fertilization strategy offers a sustainable solution to promote grassland productivity while avoiding ecological risks caused by nutrient imbalance. Therefore, balanced NP fertilization should be prioritized over single-nutrient additions in alpine grassland restoration.

Synthesizing our findings across genus, trophic, and community levels, we propose a dual-pathway framework to explain how nutrient enrichment effects on alpine meadows nematodes. First, trait-based plasticity, driven by resource quality (e.g., body size increased in *Acroboloides* (Ba) under P addition and in *Pl* across all treatments). This pathway operates most strongly at lower trophic levels and exhibits genus-specificity. Second, abundance suppression, driven by reduced resource quantity (e.g., abundance declines across all seven genera and trophic groups). This pathway operates across all trophic levels but exerts strongest effects at lower levels. The net community outcome depends on the combined effects of these pathways. This dual-pathway framework advances our understanding of soil food web responses to fertilization and stresses that both trait-based and abundance-based metrics must be considered in ecological assessments.

Conclusions

This multiscale study demonstrates that N, P, and combined NP fertilization regulate soil nematode communities through both trait-based and abundance-based pathways in an alpine meadow. Fertilization induced contrasting genus-specific and trophic-level responses, with body size increasing and abundance declining. Contrary to high-trophic-level, lower trophic levels exhibited more sensitive body size increases and stronger abundance decrease, indicating that energetic inefficiencies limit the propagation of resource pulses to top consumers. Single P fertilization, especially N fertilization, decreases the abundance and diversity of soil nematodes, but balanced NP fertilization mitigates diversity loss and stabilizes soil food webs, underscoring that nutrient stoichiometry, rather than the supply of any single nutrient, governs soil biodiversity and food web stability in alpine ecosystems. Soil organic carbon emerges as the primary driver of community composition, while total phosphorus mediates trophic-specific trait dynamics. Collectively, our findings support the dual-pathway framework proposed here, which integrates trait-based plasticity and abundance suppression, offering a conceptual foundation for predicting community responses to nutrient enrichment and highlighting the need for integrated trait- and abundance-based monitoring in soil ecological assessments.

Declarations

Funding

Declaration of competing interest

Authors declare that they have no known competing financial interests.

Author Contribution

Hui Wang: Writing – original draft, investigation, identification, formal analysis, data curation, formal analysis, original draft. Linxi Meng: investigation, identification. Jinhao Ma: investigation, writing – review and editing, validation. Xiao Ren: writing – review and editing. Yuying Wang: writing – review and editing. Yanjiang Cai: conceptualization, writing– review and editing. Lei Zhou: Writing – formal analysis, data curation and formal analysis. Pengfei Wu: Writing – original draft, Writing – review & editing, Methodology, Conceptualization, Funding acquisition. Xue Wei: investigation, writing – review and editing, validation.

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Data availability

The data will be made available upon request.

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Figures

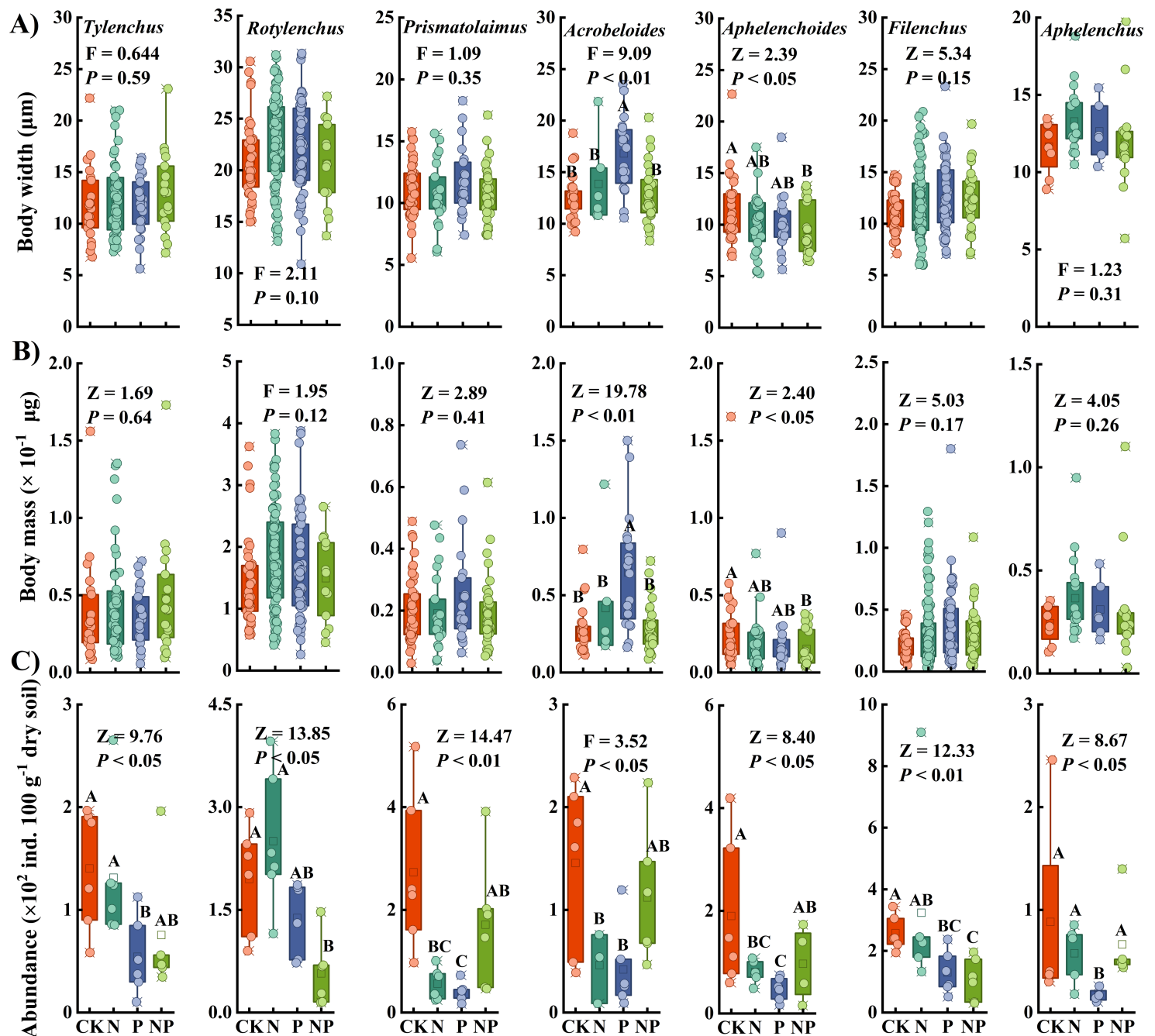


Figure 1

Body width (A), body mass (B) and abundances (C) of dominant nematode genera under different fertilization treatments. Each dot represents the body width or body mass of an individual nematode. F represents the result of a parametric test, and Z represents the result of a non-parametric test. Different uppercase letters on the bars indicate significant differences among treatments at the $P < 0.05$ level.

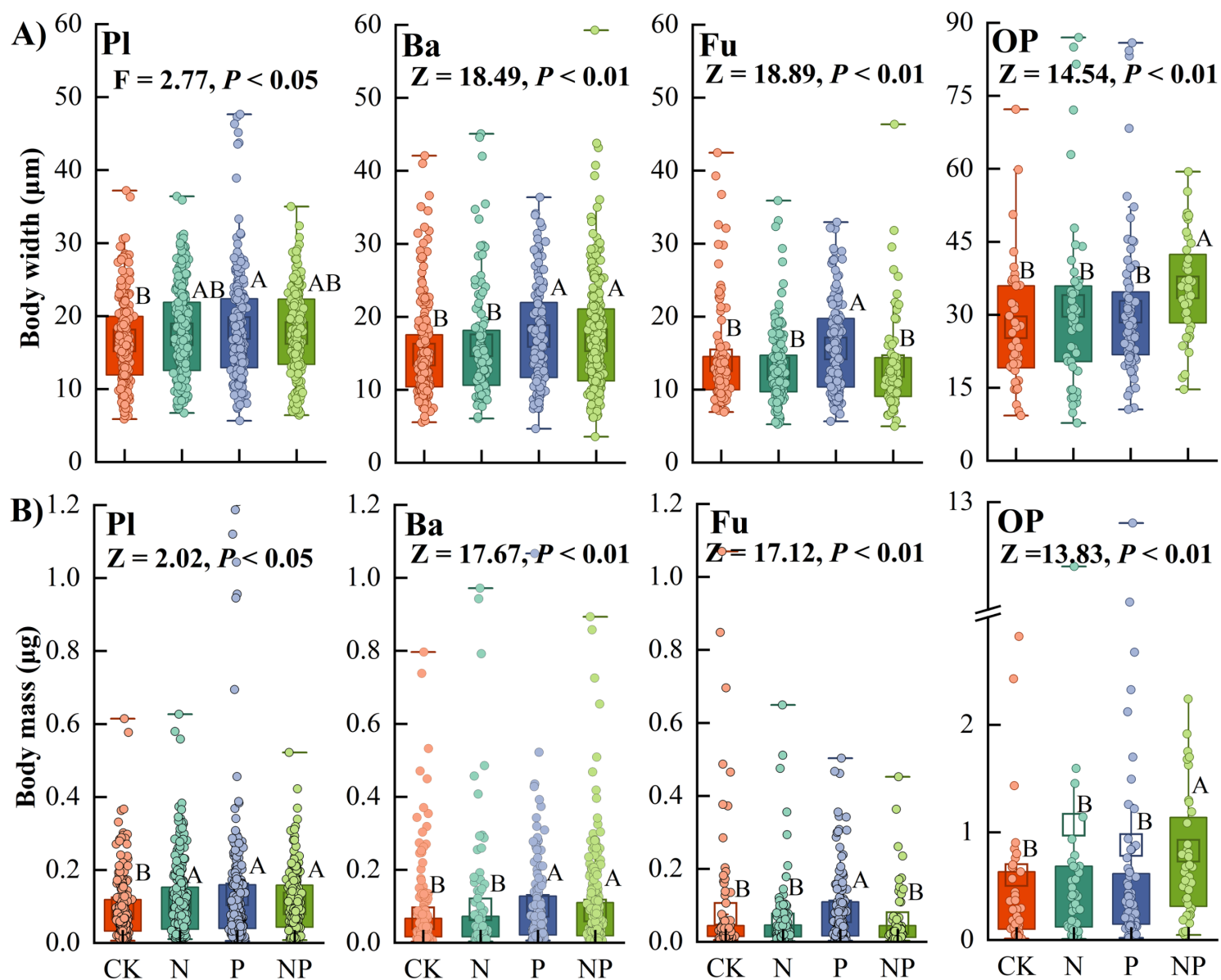


Figure 2

Body width and mass of bacterivores (Ba), fungivores (Fu), plant-parasites (PI) and omnivore-predators (OP) under different fertilization treatments. Each dot represents the body width or body mass of an individual nematode. F denotes parametric tests, and Z denotes non-parametric tests. Different uppercase letters on the bars indicate significant differences among treatments at $P < 0.05$ level.

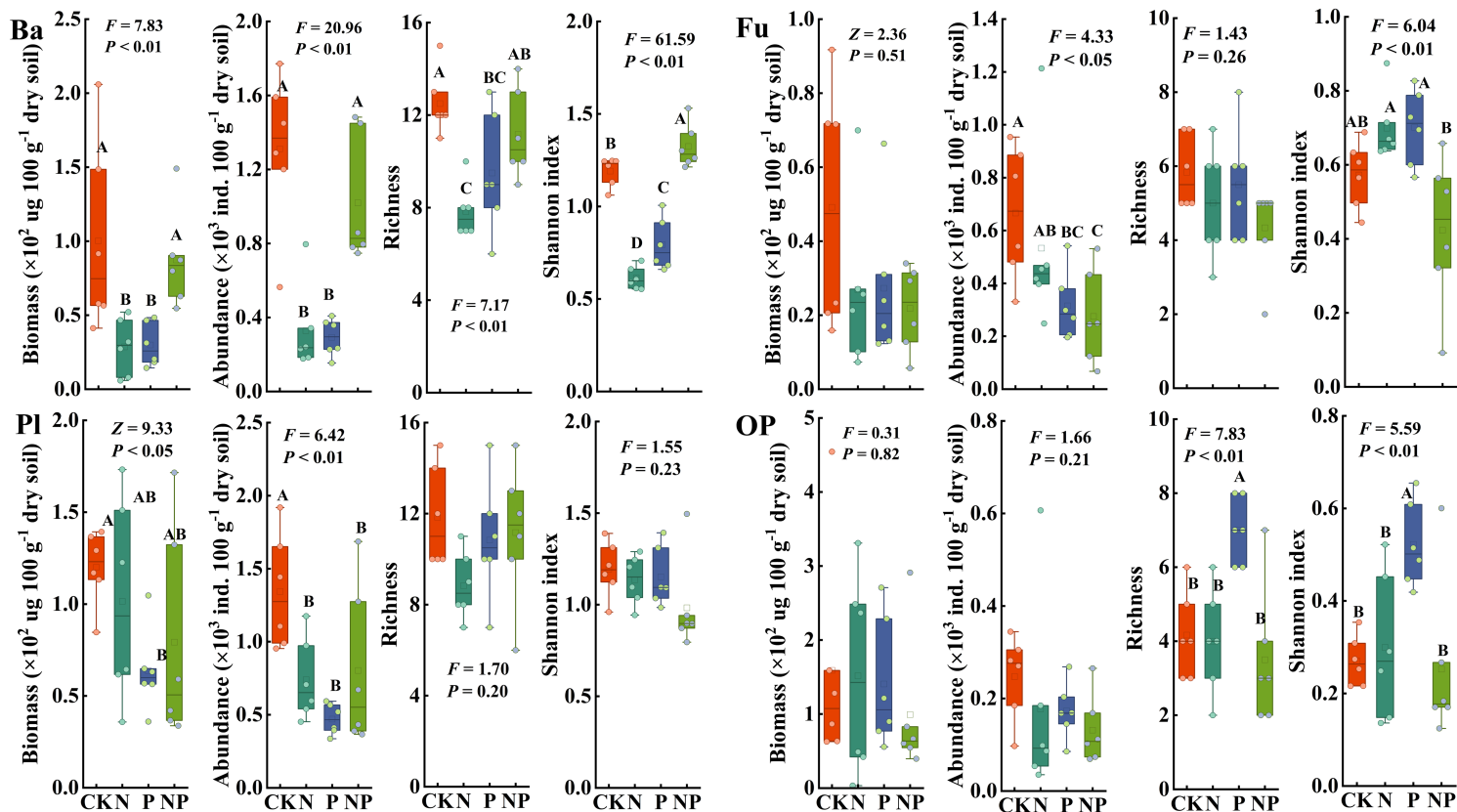


Figure 3

Effects of different fertilization treatments on the biomass, abundance, richness and Shannon index of different trophic groups of the soil nematodes (mean $\pm$ SE). F denotes parametric tests, while Z denotes non-parametric tests. Different uppercase letters on the bars indicate significant differences among treatments at $P < 0.05$ level.

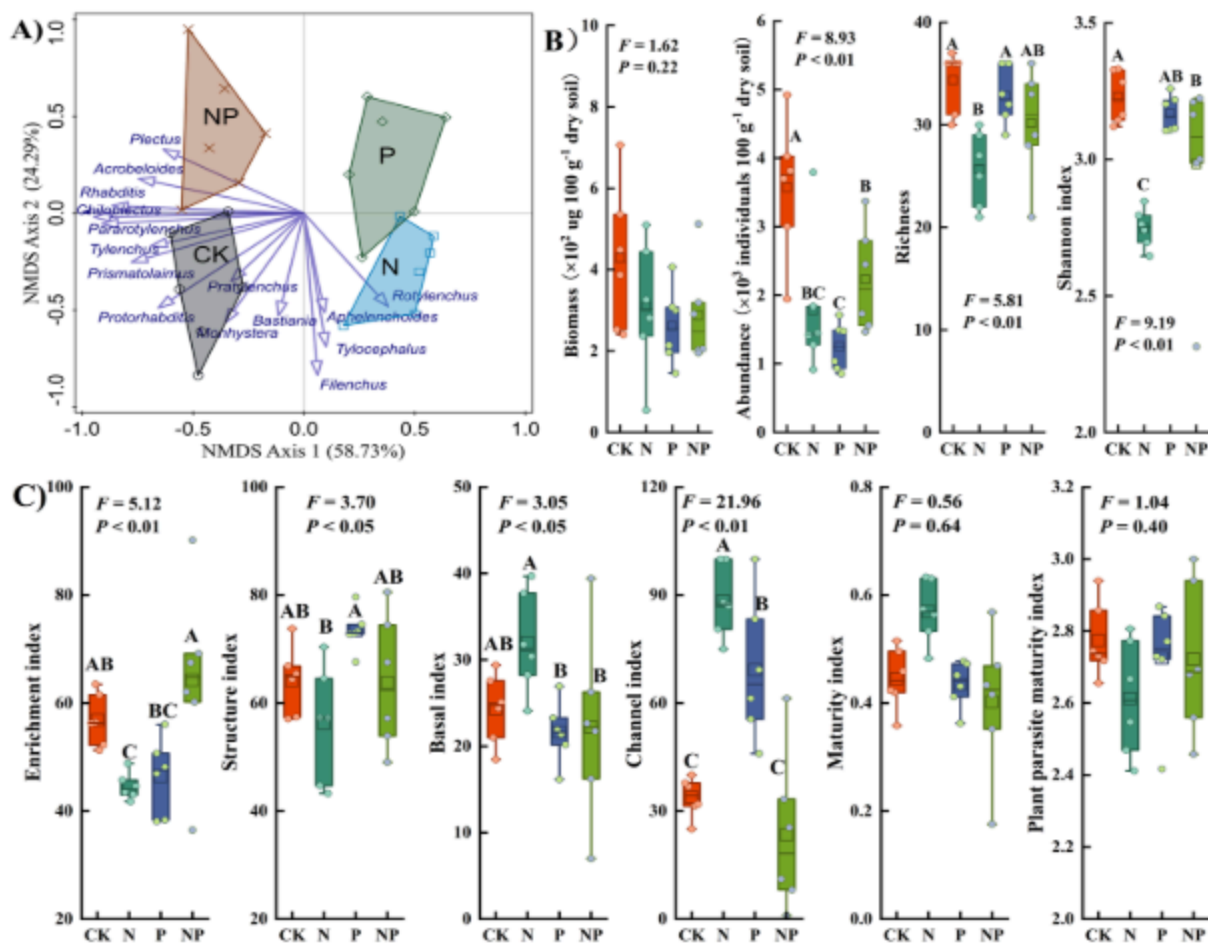


Figure 4

Effects of different fertilization treatments on (A) taxonomic composition (NMDS), (B) biomass, abundance, richness, and Shannon index, and (C) enrichment index, structure index, basal index, channel index, maturity index, and plant parasite index of soil nematode communities. In the NMDS plot, samples are grouped by treatment with convex hulls, and only the top 15 genera with the highest relative contributions are displayed. Different uppercase letters on the bars indicate significant differences among treatments at the $P < 0.05$ level.

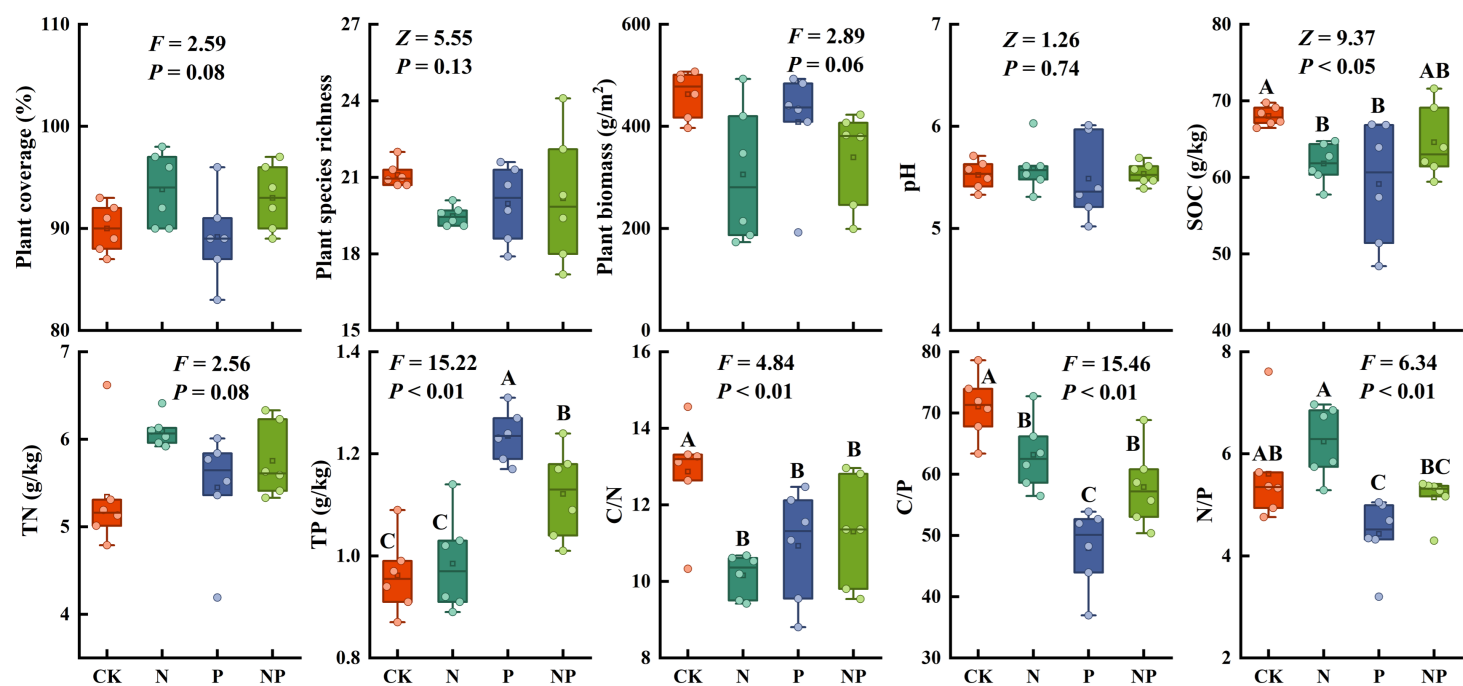


Figure 5

Effects of different fertilization treatments on plant community parameters and soil properties. SOC: soil organic carbon; TN: soil total nitrogen; TP: soil total phosphorus. Different uppercase letters on the bars indicate significant differences among treatments at $P < 0.05$ level.

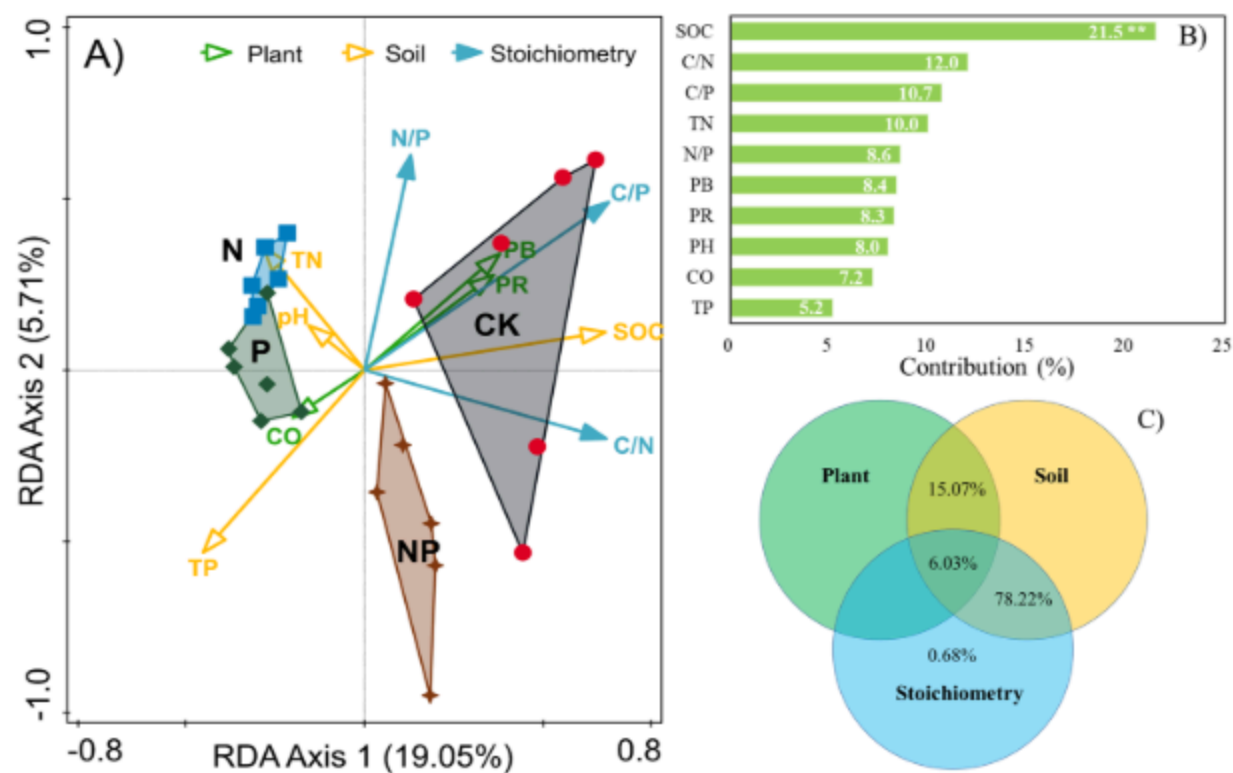


Figure 6

(A) Redundancy analysis (RDA) showing relationships between soil nematode communities and environmental factors, (B) results of interactive forward selection, and (C) variation partitioning analysis (VPA) quantifying the relative contributions of plant and soil factors to nematode community variation.

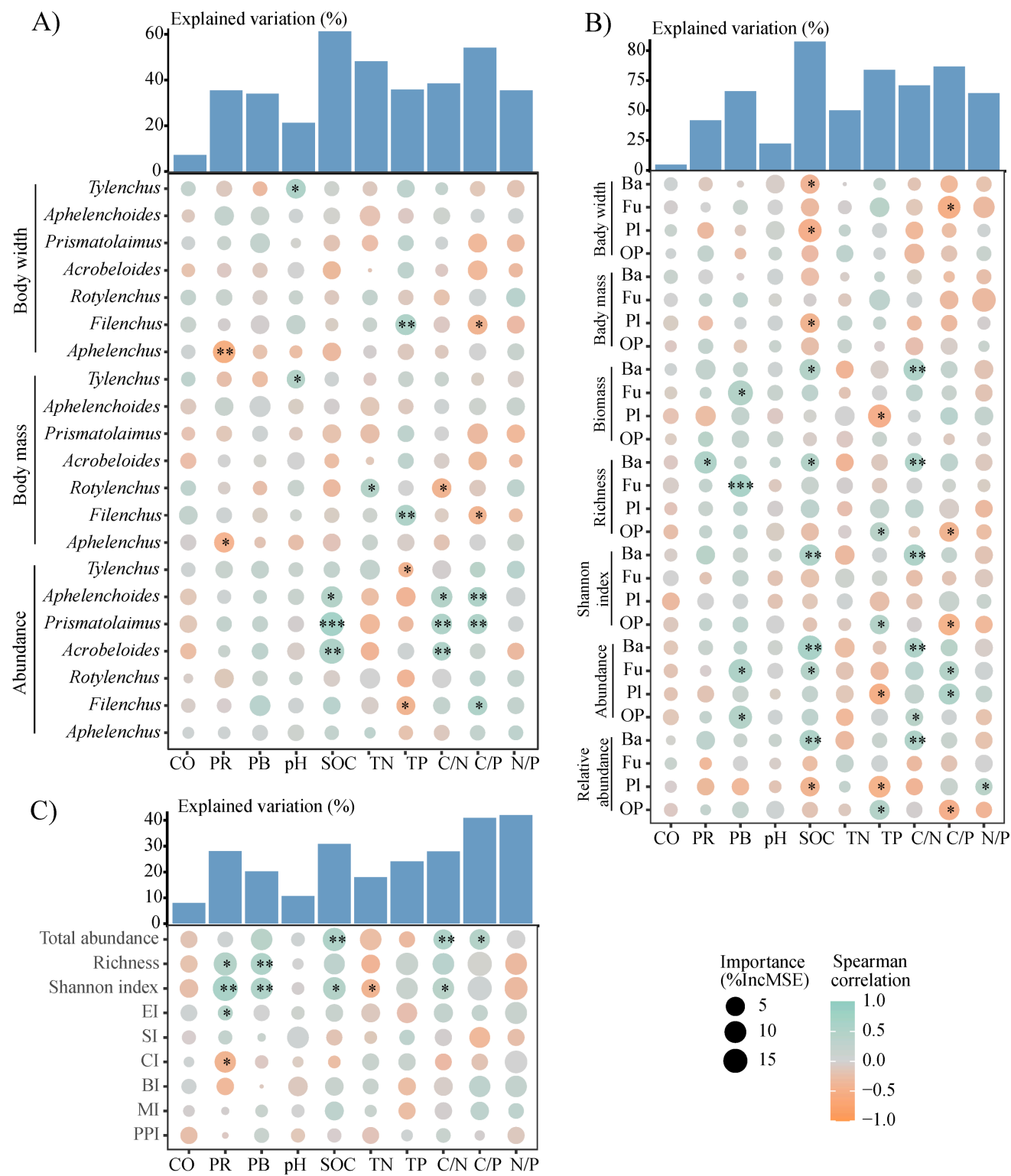


Figure 7

Random forest regression heatmaps depicting the relationships between environmental factors and (A) body width and mass of seven dominant genera, (B) diversity and body size of four trophic groups, and (C) soil nematode community metrics. Co: Coverage; PR: Plant richness; PB: Plant biomass. Asterisks indicate significance at $P < 0.05$ or $P < 0.01$ levels.

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