

Effects of Nitrogen Addition on Soil Nematode Community Characteristics in *Stipa baicalensis* Steppe (Postprint)

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Abstract

This study investigated the *Stipa baicalensis* steppe in Inner Mongolia using a controlled experiment simulating nitrogen deposition via nitrogen addition (0, 15, 30, 50, 100, 150, 200, 300 kg N hm⁻² a⁻¹) to examine the effects of nitrogen deposition on soil nematode community structure and diversity. Results showed that a total of 52 genera were identified across all samples in this experiment, with the dominant taxa being *Helicotylenchus*, *Acrobeles*, and *Aphelenchus*. Soil nematodes exhibited distinct surface aggregation, with no significant changes in relative density. Regarding functional groups, with increasing nitrogen addition levels, both bacterivorous and predator/omnivorous nematodes were negatively correlated with nitrogen addition levels. In terms of ecological indices, there were promoting effects on indices such as SR, diversity (H'), and evenness (J'); the channel index was less than 0.75 across all experimental treatments, indicating that fungal-mediated decomposition was the dominant pathway for organic matter breakdown in the soil; the Wasilewska index indicated that low nitrogen application could improve soil environmental conditions and reduce the impacts of plant-parasitic nematodes on plant communities and productivity. When nitrogen application rates exceeded N100, soil pH decreased significantly, nitrate nitrogen and ammonium nitrogen increased significantly, k-strategist nematodes decreased significantly, r-strategists increased significantly, generational turnover accelerated, and soil nematode community structure changed significantly.

Full Text

Effects of Nitrogen Addition on Soil Nematode Community Characteristics in a *Stipa baicalensis* Steppe

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Abstract

This study investigated the effects of simulated nitrogen deposition on soil nematode community structure and diversity in a *Stipa baicalensis* steppe in Inner Mongolia through a nitrogen addition experiment. The experiment consisted of eight nitrogen application levels: 0, 15, 30, 50, 100, 150, 200, and 300 kg N hm⁻². A total of 52 nematode genera were identified across all samples. The dominant genera were *Helicotylenchus*, *Acrobeles*, and *Aphelenchus*. Soil nematodes exhibited obvious surface aggregation, with no significant change in relative density across nitrogen treatments. From a functional group perspective, predator/omnivore nematodes showed a negative correlation with increasing nitrogen addition levels. Ecological indices such as abundance, diversity, and evenness of soil nematodes increased with nitrogen application, reaching maximum values at N50. The channel index was less than 0.75 across all treatments, indicating that fungi dominated organic matter decomposition pathways. The Wasilewska index suggested that low nitrogen application could improve soil environmental conditions and reduce the impact of plant parasites on plant communities and productivity. When nitrogen application exceeded N100, soil pH decreased significantly while nitrate nitrogen and ammonium nitrogen increased significantly. Nematodes belonging to k-strategists decreased significantly whereas r-strategists increased significantly, accelerating generation turnover and substantially altering soil nematode community structure.

Keywords: *Stipa baicalensis* steppe; nitrogen application; soil nematode; functional index; ecological index

Introduction

Atmospheric nitrogen deposition represents a critical component of global environmental change, with increasingly severe ecological consequences affecting terrestrial ecosystem structure and function [1-3]. Nitrogen is a primary limiting nutrient in grassland ecosystems, and nitrogen addition can enhance above-ground primary productivity. However, the response of soil fauna living in the belowground ecosystem to nitrogen addition is often overlooked. Numerous studies have focused on the direct effects of increased nitrogen deposition on soil animal community structure and composition across different ecosystems [4-5].

Soil nematodes, as one of the most important groups of soil fauna and the most abundant metazoans in soil, occupy a crucial position in the soil detrital food web and respond rapidly to environmental changes. Consequently, soil nematodes are considered sensitive indicator organisms for ecosystem changes [6-7]. While previous nematode research primarily concentrated on plant pathogen control, current ecological focus has shifted toward understanding the role of soil nematodes in ecological environments, their relationships with organisms at other trophic levels, and their interactions with plants, large herbivores, and microorganisms. Studies on the relationships between abiotic factors such as environmental climate factors, fertilization, and soil nematodes have also been hotspots in recent research [8].

Soil nematodes are ubiquitous, and their abundance, nutritional types, and ecological indices vary significantly across different grassland types and management practices [9-10]. Changes in environmental conditions such as temperature and soil nitrogen content caused by global climate change can affect soil nematodes to varying degrees [11-13]. *Stipa baicalensis* is a representative species of temperate meadow steppe in China and provides an ideal experimental platform for studying grassland ecosystem responses to global change. Investigating the effects of nitrogen deposition on soil nematode community changes in Inner Mongolian *Stipa baicalensis* steppe holds important theoretical and practical significance for developing scientific grassland ecosystem management strategies and achieving sustainable development of natural grasslands.

1. Study Site Description

The study area is located in Ewenki Banner on the western slope of the Greater Khingan Mountains in Inner Mongolia (119°42 E, 48°30 N, altitude 760 m), within the typical zone of zonal vegetation of *Stipa baicalensis* steppe. The region experiences a semi-arid continental monsoon climate with mean annual precipitation of 328.7 mm and mean annual temperature of -1.6°C. The soil type is dark chestnut soil. The vegetation type is *Stipa baicalensis* meadow steppe, with the constructive species *Stipa baicalensis* dominating the community. Common companion species include *Leymus chinensis*, *Artemisia commutata*, *Rhaponticum uniflorum*, *Vicia multicaulis*, *Serratula centauroides*, *Thalictrum petaloideum*,

and *Carex duriscula*.

The experimental site was protected by fencing. In May 2010, a long-term nitrogen addition experiment was established. Nitrogen application intensity and frequency followed international standard protocols for similar studies. Eight nitrogen addition levels were established: 0, 15, 30, 50, 100, 150, 200, and 300 kg N hm⁻². Experimental plots (8 m × 8 m = 64 m²) were established on flat terrain in typical *Stipa baicalensis* vegetation, with 8 m buffer zones between plots. Nitrogen was applied in two equal splits in mid-May and mid-June each year, with NH₄NO₃ dissolved in water and uniformly sprayed onto each plot. Control plots received equivalent amounts of water.

2. Sample Collection and Processing

Within each treatment plot, five sampling points were selected using an “S” pattern. Soil samples were collected at two depths: 0-10 cm and 10-20 cm using a soil corer (5 cm diameter). The collected soil samples were placed in ziplock bags and stored in a cooler for transport to the laboratory.

3. Analytical Methods

Nematodes were extracted and counted from fresh samples within 48 hours. A 100 g subsample of fresh soil was taken from each sample, and nematodes were extracted using a modified Baermann funnel method [14]. After extraction, nematodes were fixed and counted, then identified to genus level using morphological identification [15] and taxonomic keys. Nematodes were classified into different functional trophic groups based on head morphology and feeding habitat: bacterivores (*Bacterivores*), fungivores (*Fungivores*), plant parasites (*Plant parasites*), and predators/omnivores (*Predators/Omnivores*) [16]. Soil physico-chemical properties were measured according to standard methods from “Soil Agricultural Chemical Analysis” [17].

4. Data Processing and Analysis

Nematode dominance was classified based on relative abundance: >10% of total individuals as extremely dominant (+++++), 5-10% as dominant (++++), 2-5% as sub-dominant (+++), 1-2% as common (++) , and <1% as rare (+). Ecological indices including the maturity index (MI) for free-living nematodes, Wasilewska index (WI), and channel ratio (NCR) were calculated to characterize nematode community functional structure [16]. Diversity and stability were assessed using richness index, Shannon-Wiener diversity index (H'), and evenness index [16]. Data were analyzed using SPSS 17.0 and Origin 9.1.

1. Effects of Nitrogen Addition on Soil Nematode Relative Density and Dominance

A total of 52 nematode genera were identified across all treatments, with 22,579 individuals collected. The mean nematode density was 225.79 individuals per 100 g dry soil. Dominant genera were *Helicotylenchus*, *Acrobeles*, and *Aphelenchus*. In the 0-10 cm layer, the dominant genus was *Helicotylenchus*, while in the 10-20 cm layer, *Acrobeles* was dominant. The mean nematode density in the 0-10 cm layer was significantly higher than in the 10-20 cm layer, indicating obvious surface aggregation of soil nematodes.

Under nitrogen addition, the relative density of soil nematodes showed a decreasing trend, though the change was not significant. The highest relative density occurred at N15. The number of dominant nematode species increased significantly with nitrogen concentration. Since total nematode density did not change significantly, the increase in dominant species led to a gradual decrease in companion species in the plots.

2. Effects of Nitrogen Addition on Soil Nematode Trophic Group Composition and Spatial Distribution

Bacterivorous nematodes were the most abundant group, accounting for 41.2-54.5% of the total in the 0-10 cm layer. Fungivorous and plant-parasitic nematodes showed similar abundances, comprising 17.6-27% of the total. Predator/omnivore nematodes accounted for 24.6-36.4% in the 10-20 cm layer. The abundance of predator/omnivore nematodes decreased significantly with increasing nitrogen levels, with a more dramatic decline in the 0-10 cm layer. Bacterivorous nematodes increased significantly at low nitrogen levels, peaking at N50, then decreased with further nitrogen addition. Fungivorous nematodes showed peak abundance at N100, while plant-parasitic nematodes showed no significant change with nitrogen addition.

Nitrogen addition had significant effects on bacterivorous nematodes and predator/omnivore nematodes, but not on fungivorous or plant-parasitic nematodes. Significant linear relationships existed between nitrogen application and nematode abundance in the 0-10 cm layer, but not in the 10-20 cm layer.

3. Effects of Nitrogen Addition on Soil Nematode Ecological Indices and Group Distribution

The richness, diversity, and evenness indices of soil nematodes increased with nitrogen concentration up to N50, then gradually decreased, though changes

were not significant. This indicates that low nitrogen addition promoted nematode species diversity and community stability, while excessive nitrogen had inhibitory effects. The maturity index increased with nitrogen gradient up to N100, then decreased suddenly.

The channel index was less than 0.75 in all nitrogen treatments, indicating that fungal decomposition pathways dominated organic matter breakdown. However, the channel index was generally higher in the 0-10 cm layer than in the 10-20 cm layer, suggesting a greater bacterial contribution to organic matter decomposition in surface soil.

The Wasilewska index was greater than 1 in all nitrogen treatments, indicating that mineralization pathways were influenced by microbivorous nematodes. In the experimental plots, c-p2 groups dominated, accounting for 40.1-70.1% of the total, while c-p4 and c-p5 groups combined accounted for 6.2-24.4%. With increasing nitrogen concentration, c-p2 groups increased significantly while c-p4 and c-p5 groups decreased significantly, showing linear relationships with nitrogen gradient. The decline of c-p4 and c-p5 groups was more pronounced in surface soil, indicating that nitrogen addition caused greater environmental changes in upper soil layers, increasing short-generation nematodes while decreasing long-generation nematodes and accelerating generation turnover.

4. Relationship Between Grassland Soil Nematode Communities and Soil Physicochemical Factors

With increasing nitrogen levels, soil pH decreased significantly while soil gradually acidified. Significant linear relationships existed between nematode trophic groups and pH. Bacterivorous nematodes, fungivorous nematodes, and predator/omnivore nematodes decreased linearly with decreasing pH, while plant-parasitic nematodes increased linearly. Nitrogen addition significantly altered nematode community structure by changing soil pH.

Soil nitrate and ammonium nitrogen contents increased significantly with nitrogen addition. Bacterivorous nematodes and predator/omnivore nematodes showed linear decreases with increasing nitrate nitrogen, while c-p2 groups increased linearly. The relationships between nematode groups and soil total phosphorus or organic carbon were not significant. In grassland ecosystems, soil nitrogen had more significant effects on nematode communities than phosphorus.

Discussion

1. Response of Soil Nematode Community Composition and Trophic Groups to Nitrogen Addition Atmospheric nitrogen deposition increasingly affects ecosystem function and biodiversity [18]. Nitrogen addition influ-

ences soil nematode community composition by affecting plant growth, altering soil physicochemical properties, reducing root exudates, and inhibiting microbial extracellular enzyme activity [19-20]. In this experiment, soil nematodes showed obvious surface aggregation, with different nematode groups responding differently to nitrogen levels in surface and subsurface soils.

Long-term nitrogen addition reduces soil microbial biomass carbon and nitrogen and decreases microbial activity and diversity [21]. As bacterivorous nematodes are at the next trophic level, they decrease with reduced food sources. Predator/omnivore nematodes generally feed on microbivorous or plant-parasitic nematodes [16]. Since bacterivorous nematodes accounted for 25.5-54.5% of the community, significant reductions in this group due to nitrogen gradient led to decreased predator/omnivore nematodes as their food source diminished.

Soil nematodes affect aboveground plant growth and community composition [22]. However, in this study, changes in aboveground biomass had minimal effects on nematodes. In water-limited environments, decreased plant biomass reduces fungivorous and plant-parasitic nematode biomass. Nitrogen addition can influence nematode communities by affecting both soil environment and lower trophic level organisms through energy flow.

2. Response of Soil Nematode Ecological Indices and Groups to Nitrogen Addition Soil nematodes are characterized by large populations and rich diversity. Statistical methods are commonly used to calculate relevant ecological indices. In this experiment, richness, diversity, and evenness indices increased with nitrogen gradient but overall changes were not significant. Surface soil showed higher richness and diversity indices than subsurface soil, while evenness showed the opposite pattern, consistent with nematode distribution patterns [26].

Low nitrogen addition promoted nematode community stability and species diversity. Although bacterivorous nematodes were most abundant, the organic matter decomposition pathway was dominated by fungi (channel index < 0.75). In surface soil, the channel index was higher, indicating a greater bacterial contribution. The dominant genus in 0-10 cm soil was *Acrobeles* (Ba2), while in 10-20 cm it was *Aphelenchus* (Fu2). Research shows that fungivorous nematodes can promote fungal nitrogen mineralization [27], but in this experiment, their effect on nitrogen mineralization was less affected by nitrogen addition.

The c-p value characterizes nematode community traits and life history strategies. c-p1 groups have the shortest reproductive cycles, while c-p5 groups have the strongest resistance to external pressure [28]. In this study, c-p2 groups were dominant, accounting for the majority of the community, indicating r-strategist dominance. With increasing nitrogen levels, significant changes in soil habitat led to increased c-p2 groups and decreased c-p4 and c-p5 groups, with significant linear relationships with nitrogen gradient. The more pronounced decline of c-p4 and c-p5 groups in surface soil indicates that nitrogen addition caused

greater environmental changes in upper layers, increasing short-generation nematodes while decreasing long-generation nematodes and accelerating generation turnover.

3. Relationship Between Grassland Soil Nematodes and Physico-chemical Factors Nitrogen fertilizer is a major limiting factor in northern grassland production, but long-term nitrogen application causes significant habitat changes [29]. As nitrogen levels increased, soil pH decreased significantly and soil acidified. Different nematode trophic groups and r-strategists showed negative correlations with pH trends, indicating that soil acidification altered the soil environment and inhibited bacterivorous, fungivorous, and predator/omnivore nematodes through trophic interactions and environmental changes, while having less effect on plant-parasitic nematodes.

Nitrogen addition directly increased soil nitrate and ammonium contents, significantly increasing soil nitrogen availability and food sources for soil organisms. With increasing nitrogen levels, soil nitrate nitrogen increased significantly, but bacterivorous and fungivorous nematodes decreased with increasing nitrate, while c-p2 groups increased. The relationships between nematode groups and soil total phosphorus or organic carbon were not significant. In grassland ecosystems, soil nitrogen has more significant effects on nematode communities than phosphorus, consistent with previous findings. r-strategist groups grow rapidly under nutrient-rich conditions but decrease rapidly when environmental changes occur due to their sensitivity, accelerating belowground community metabolism [31].

Conclusion

Soil nematodes in the surface layer of *Stipa baicalensis* steppe showed obvious surface aggregation. Bacterivorous nematodes and predator/omnivore nematodes were significantly negatively correlated with nitrogen addition levels. Nitrogen application negatively affected soil nematode stability, diversity, and trophic group composition, but had no significant effect on the Wasilewska index or channel index. Nitrogen addition inhibited the growth of bacterivorous nematodes, predator/omnivore nematodes, and k-strategist nematodes by altering soil pH and increasing nitrate and ammonium contents. Nitrogen addition increased short-generation c-p2 groups while decreasing long-generation c-p4 and c-p5 groups, accelerating nematode generation turnover and energy flow but reducing belowground ecosystem stability.

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