Postprint: Legacy Effects of Long-term Nitrogen Addition on Plant Community Structure and Function in Tianshan Grasslands

Authors: Tao Xinran, Li Haining, Gong Yanming, Liu Yanyan, Liu Xuejun, Kaihui Li(1,3)

Date: 2025-07-14T11:39:24+00:00

Abstract

Against the backdrop of gradually decreasing global atmospheric nitrogen deposition, the legacy effects of long-term nitrogen addition on alpine grassland ecosystems remain unclear. Therefore, this study conducted a 16-year controlled experiment in the Bayanbulak alpine grassland in the central Tianshan Mountains to investigate the legacy effects of long-term nitrogen addition on grassland plant communities. The results showed that: (1) In terms of functional traits of dominant species, four years after fertilization cessation, the high nitrogen treatment (N15, 15 g N·m-2·a-1) still exhibited positive legacy effects on plant height (+20%), leaf area (+16%), and specific leaf area (+5%) of Leymus tianschanicus, and negative legacy effects on plant height (-23%) and specific leaf area (-1.5%) of Festuca kryloviana, with the legacy effects on Festuca kryloviana gradually weakening with recovery time. (2) At the community level, long-term nitrogen addition had positive legacy effects on rhizomatous grasses and negative legacy effects on bunch grasses, consequently exerting significant positive legacy effects on community coverage and aboveground net primary productivity, with the legacy effects showing a decreasing trend year by year. Under the N15 treatment, the increase in community coverage decreased from 32% to 18%, and the increase in community aboveground net primary productivity decreased from 64% to 44%. (3) Regarding soil chemical properties, nitrogen addition had significant positive legacy effects on soil total nitrogen content, while its legacy effects on soil total phosphorus and organic carbon content were not significant, and the negative legacy effect on soil pH weakened year by year. Under the N15 treatment, the inhibitory effect on soil pH in 2024 (-1.4%) was significantly alleviated compared to 2023 (-3.4%). Soil total phosphorus and organic carbon content showed low correlation with vegetation characteristics, and the combined explanatory power of the four soil factors for vegetation variation was low. This study demonstrates that against the backdrop of decreasing or ceased

atmospheric nitrogen deposition, historical nitrogen deposition continues to exert persistent legacy effects on grassland ecosystems, and the legacy effects on some indicators gradually weaken with recovery time.

Full Text

Legacy Effects of Long-Term Nitrogen Addition on Plant Community Structure and Function in the Tianshan Grassland

TAO Xinran[1,2,3], LI Haining[3,4], GONG Yanming[1,3], LIU Yanyan[1,3], LIU Xuejun[1,5], LI Kaihui[1,3]

[1]Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi, Xinjiang 830011, China
[2]University of Chinese Academy of Sciences, Beijing 100049, China
[3]Bayinbuluk Alpine Grassland Observation and Research Station of Xinjiang, Bayinbuluk, Xinjiang 841314, China
[4]College of Grassland Science, Xinjiang Agricultural University, Urumqi, Xinjiang 830052, China
[5]College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China

Abstract

Against the backdrop of gradually declining global atmospheric nitrogen deposition, the legacy effects of long-term nitrogen addition on alpine grassland ecosystems remain unclear. This study investigated these legacy effects through a controlled experiment conducted in the Bayinbuluk alpine grassland in the central Tianshan Mountains. The results revealed that: (1) Four years after cessation of fertilization, high nitrogen treatment (15 g $N \cdot m^{-1}$) exhibited persistent positive legacy effects on the functional traits of *Leymus tianschanicus*, significantly increasing plant height (+20%), leaf area (+16%), and specific leaf area (+5%). Conversely, it showed negative legacy effects on *Festuca kryloviana*, reducing plant height (-23%) and specific leaf area (-1.5%), with these effects gradually diminishing over recovery time. (2) At the community level, long-term nitrogen addition demonstrated positive legacy effects on rhizomatous grasses but negative effects on bunchgrasses, significantly enhancing community cover and aboveground net primary productivity (ANPP). However, these legacy effects showed a decreasing trend over time. Under N15 treatment, the increase in community cover declined from 32% to 18%, while the ANPP enhancement decreased from 64% to 44%. (3) Regarding soil chemical properties, nitrogen addition had significant positive legacy effects on total soil nitrogen content but no significant effects on total phosphorus or organic carbon content. The negative legacy effect on soil pH gradually weakened, with the inhibitory effect under N15 treatment decreasing from -3.4% in 2023 to -1.4% in 2024. Soil total phosphorus and organic carbon content showed low correlations with vegetation characteristics, and the four soil factors collectively explained only a small proportion of vegetation variation. This study demonstrates that under scenarios of reduced or ceased atmospheric nitrogen deposition, historical nitrogen deposition continues to exert persistent legacy effects on grassland ecosystems, with some of these effects gradually weakening over recovery time.

Keywords: nitrogen deposition; legacy effect; response ratio; functional group composition; leaf functional traits

1 Introduction

Atmospheric nitrogen deposition represents a critical factor of global change that has significantly impacted terrestrial ecosystems. Numerous studies have shown that excessive nitrogen deposition alters plant community structure, reduces species diversity, and affects ecosystem functions and services. In terms of soil properties, global-scale analyses indicate that nitrogen addition leads to soil acidification in grasslands and significantly increases total soil nitrogen content. However, whether these changes are reversible after nitrogen input ceases remains a subject of debate in the scientific community. Some research suggests that the impacts of long-term nitrogen addition are nearly irreversible, with limited vegetation and soil responses and slow ecosystem recovery even after atmospheric nitrogen deposition decreases. For instance, plant community structure has not recovered significantly, and species diversity remains suppressed. Other studies indicate that the effects of long-term nitrogen addition recover relatively quickly, with the promotion of rhizomatous plants and inhibition of bunchgrasses diminishing after nitrogen addition stops, and community productivity gradually returning to control levels.

Long-term nitrogen inputs exert profound multifaceted impacts on grassland ecosystems. Many studies have demonstrated that nitrogen addition significantly alters plant functional traits (e.g., plant height, leaf area, and specific leaf area), drives changes in plant community species composition, and enhances ecosystem net primary productivity. In soil, nitrogen addition-induced acidification persists, and soil organic nitrogen and organic carbon content continue to show significant differences along historical nitrogen addition gradients. These results indicate that the legacy effects of long-term nitrogen addition are extremely persistent. However, some researchers argue that recovery is rapid, with the positive legacy effects on soil ammonium nitrogen disappearing after cessation of nitrogen addition, and the legacy effects on total soil nitrogen and phosphorus becoming non-significant after 1-2 years. Soil properties often control the legacy effects of nitrogen addition on plants, and changes in soil environment directly or indirectly affect plant community biomass. The recovery of soil pH under medium-low nitrogen addition levels can influence plant community productivity. Moreover, differences in recovery dynamics may stem from heterogeneity in ecosystem types, environmental conditions, and nitrogen addition duration. Given the continuously changing global nitrogen deposition patterns, investigating the legacy effects of long-term nitrogen deposition is scientifically important for assessing the long-term potential impacts of nitrogen deposition on grassland ecosystems.

This study focused on the Bayinbuluk alpine grassland in the central Tianshan Mountains of Xinjiang. We analyzed multiple indicators including leaf functional traits, functional group composition, community cover, and aboveground net primary productivity at species, functional group, and community levels, combined with soil chemical properties. Our objective was to explore the legacy effects of long-term nitrogen addition on plant community structure and function in the Tianshan grassland, providing a scientific basis for grassland ecosystem restoration management.

1.1 Study Area Description This study utilized a nitrogen addition experimental platform established in 2013 at the Bayinbuluk Alpine Grassland Ecosystem Observation and Research Station of Xinjiang. The station is located in Bayinbuluk Grassland, northwestern Hejing County, Bayingolin Mongol Autonomous Prefecture, Xinjiang Uygur Autonomous Region (42°18 ~43°34 N, 82°27 ~86°17 E). The area features a typical alpine climate with an average altitude of 2500 m, mean annual temperature of -4.7°C, annual precipitation of 276.2 mm, annual evaporation of 1022.9–1247.5 mm, and sunshine duration of 2466–2616 h. Dominant plant species include *Leymus tianschanicus*, *Festuca kryloviana*, and *Koeleria cristata*.

1.2 Experimental Design The experimental platform initiated nitrogen addition treatments in 2013, with four nitrogen addition levels (0, 5, 10, and 15 $g\ N \cdot m^{-2} \cdot yr^{-1}$), each replicated six times. Each plot measured 5 m × 5 m, with 1 m wide buffer zones between plots. Nitrogen was applied twice annually during the growing season (late May and late June) by dissolving NH_4NO_3 in water and uniformly spraying it onto treatment plots; control plots received equal amounts of water without nitrogen. In 2020, each plot was divided into three subplots: one subplot ceased nitrogen addition for natural recovery, one continued nitrogen addition, and another was subjected to mowing. This study only used the natural recovery subplots to investigate legacy effects of long-term nitrogen addition on grassland plant community structure and function.

1.3 Measurements 1.3.1 Plant Community Survey and Productivity Measurement

In early August each year, plant species cover, height, and richness were recorded in a 1 m × 1 m quadrat within each plot. Species were classified into three functional groups: rhizomatous grasses, bunchgrasses, and forbs. Aboveground plant parts were clipped at ground level, sorted by species, and placed in envelopes for laboratory analysis. Plant samples were oven-dried at 105°C for

30 minutes, then at 65°C to constant weight before weighing. Community aboveground net primary productivity (ANPP) was calculated as the sum of aboveground biomass of all species in each quadrat.

1.3.2 Leaf Functional Trait Measurement

In early August, leaves of dominant species (*Leymus tianschanicus* and *Festuca kryloviana*) were collected for scanning and weighing. Mean leaf area was obtained using a leaf area analyzer (EPSON Perfection V19 Flatbed Scanner). Leaf surfaces were blotted dry, and fresh weight was measured using an analytical balance (precision 0.0001 g). Leaves were then oven-dried at 65°C to constant weight before measuring dry weight. Specific leaf area (SLA) and leaf dry matter content (LDMC) were calculated as:

- SLA = leaf area / leaf dry weight
- LDMC = leaf dry weight / leaf fresh weight

1.3.3 Soil Chemical Property Measurement

Soil pH was measured using a mixed indicator colorimetric method with a soil-to-water ratio of 1:2.5. Total soil nitrogen content was determined using the semi-micro Kjeldahl method. Total phosphorus content was measured using the molybdenum-antimony colorimetric method. Soil organic carbon content was determined using the potassium dichromate oxidation method.

1.4 Data Analysis Changes in community structure were characterized using the relative values of different functional groups across historical nitrogen addition gradients and years. The response ratio (RR) was used to test how legacy effects of historical nitrogen addition on plant communities changed over time:

RR = ln(treatment mean / control mean)

Linear mixed-effects models were used to test the legacy effects of historical nitrogen addition and year on communities, functional groups, and dominant species, with historical nitrogen addition and year as fixed factors and plot as a random factor. Tukey's HSD method was used for multiple comparisons among nitrogen addition gradients ($P < 0.05$). Pearson correlation analysis revealed relationships between vegetation and soil chemical characteristics. Redundancy analysis (RDA) quantified the contribution of soil chemical properties to vegetation characteristics. Data were log-transformed when normality and homogeneity assumptions were violated. All analyses and plotting were performed using R software (v4.4.1) with packages including metafor, lmerTest, Hmisc, and rdacca.hp.

2 Results

2.1 Legacy Effects of Nitrogen Addition on Functional Traits of Dominant Species Linear mixed-effects models showed that historical nitrogen addition and year significantly affected the functional traits of both dominant species, except for LDMC of *Leymus tianschanicus* (Table 1), indicating clear legacy effects after nitrogen addition ceased. However, the two species showed different response patterns (Figures 1 and 2). For *L. tianschanicus*, historical nitrogen addition generally promoted plant height, leaf area, and SLA while inhibiting LDMC. The N15 treatment showed significant positive legacy effects on plant height, leaf area, and SLA, with relatively stable interannual variation. In contrast, the negative legacy effect on LDMC showed more pronounced interannual variation, with -28.98% in 2023 and -22.78% in 2024, though the effect was no longer significant by 2024.

Festuca kryloviana showed contrasting responses, with historical nitrogen addition inhibiting plant height and SLA while promoting LDMC and leaf area. The inhibitory effect on plant height weakened over time, with the negative effect under N15 treatment decreasing from -30.99% in 2023 to -22.78% in 2024. Additionally, legacy effects on leaf area and SLA decreased significantly in 2024 and were no longer significant, suggesting these trait legacy effects may disappear with recovery time.

2.2 Legacy Effects of Nitrogen Addition on Plant Community Cover Linear mixed-effects models indicated that historical nitrogen addition and year significantly affected community cover and relative cover of functional groups (Table 2), showing clear legacy effects after nitrogen addition stopped. Historical nitrogen addition generally increased community cover, but this positive legacy effect showed an obvious declining trend over years (Figure 3). Specifically, the promoting effect of low nitrogen treatment (N5) was no longer significant by 2024, while the effect of high nitrogen treatment (N15) remained significant but with response ratios decreasing annually.

Different functional groups showed distinct responses to historical nitrogen addition with clear interannual variation (Figure 4). Rhizomatous grass relative cover increased significantly along the nitrogen addition gradient, while bunchgrass relative cover decreased significantly, and forbs showed an initial increase followed by a decrease. These response changes weakened with recovery time, particularly evident in the N15 treatment. Under N15, rhizomatous grass relative cover increased by 32% in 2023, but the promoting effect weakened annually, dropping to 18% by 2024. Conversely, bunchgrasses showed a continuous decreasing trend. Forb relative cover showed no significant interannual trend. These results indicate that the positive legacy effects of nitrogen addition on relative cover of the two grass functional groups gradually weakened over time.

2.3 Legacy Effects of Nitrogen Addition on Community Aboveground Net Primary Productivity Similar to community cover results, historical nitrogen addition and year significantly affected community ANPP and relative values of functional groups (Table 2), indicating significant legacy effects after nitrogen addition ceased. Response ratios showed that high nitrogen treatment (N15) had more significant legacy effects than low nitrogen treatment (N5), with response ratios changing gradually over years (Figure 3). This suggests that higher levels of historical nitrogen addition had significant positive legacy effects on community ANPP, promoting productivity even four years after nitrogen addition stopped. However, this promoting effect decreased over time, declining from 64% to 44%.

Different functional groups showed varying responses (Figure 5). Relative ANPP of rhizomatous grasses increased significantly along the historical nitrogen addition gradient, with N15 treatment increasing it by 70% in 2023, though the promoting effect weakened annually. In contrast, relative ANPP of bunchgrasses showed a significant decreasing trend, with N15 treatment reducing it by 23% in 2023 and 22% in 2024. Forb relative ANPP accounted for a small proportion (4.6%–15%) and fluctuated without significant changes. These results demonstrate that the positive legacy effect of nitrogen addition on rhizomatous grass relative ANPP gradually weakened over time.

2.4 Legacy Effects of Nitrogen Addition on Soil Chemical Properties Linear mixed-effects models showed that historical nitrogen addition significantly affected soil pH and total nitrogen content but not total phosphorus or organic carbon content (Table 3). Different soil chemical properties showed distinct response patterns (Figure 6). Overall, historical nitrogen addition reduced soil pH, but this negative effect weakened annually, with the inhibitory effect under N15 treatment decreasing from -3.4% in 2023 to -1.4% in 2024 (no longer significant). In contrast, nitrogen effects on total nitrogen content showed stronger persistence: the positive effect under N15 treatment remained significant across years, indicating the soil nitrogen pool remained saturated, continuously providing growth advantages for nitrogen-loving plants like *L. tianschanicus* and thus maintaining community productivity legacy effects.

The legacy effect on total phosphorus content was not significant, consistent with studies on alpine meadows in the Qinghai-Tibet Plateau. Regarding carbon-nitrogen coupling, soil organic carbon showed higher response ratios under low nitrogen (N5) conditions. This may occur because moderate nitrogen addition promotes soil carbon accumulation through increased litter input, while excessive nitrogen may enhance organic matter decomposition more than accumulation, reducing soil carbon storage—similar to findings from European temperate grasslands. Correlation analysis showed that soil total phosphorus and organic carbon content had no significant correlations with vegetation indicators, possibly because plant uptake depends on available phosphorus rather than total phosphorus (which includes occluded forms), and soil organic carbon may indirectly regulate vegetation through soil microorganisms (not quantified in this study).

Redundancy analysis showed that the four soil factors explained only 18.78% of total vegetation variation, with the first two axes explaining 90.46% of constrained variation (63.96% and 26.50%, respectively). Soil pH and total nitrogen content played dominant roles among measured environmental factors. However, unexplained variation accounted for 81.22%, suggesting other unconsidered factors (such as soil trace elements and microbial activities) may importantly regulate vegetation changes.

---

3 Discussion

3.1 Effects of Nitrogen Addition Cessation on Plant Functional Traits Changes in plant functional traits directly reflect plant resource acquisition strategies and adaptation to external environments. Our results showed that four years after nitrogen addition stopped, the two dominant species responded differently to historical nitrogen addition. High nitrogen levels (N15) still significantly promoted plant height, leaf area, and SLA of *L. tianschanicus* while reducing LDMC. This aligns with previous studies on plant resource acquisition strategies, indicating that in resource-rich environments, plants increase height and leaf area to enhance light competition and improve photosynthetic efficiency through higher SLA. LDMC reflects plant resource acquisition capacity and leaf tissue construction. Under sufficient nitrogen, *L. tianschanicus* may allocate more photosynthate to stem growth rather than accumulating dry matter in leaves.

In contrast, *F. kryloviana* showed different responses, with high nitrogen levels still promoting LDMC and leaf area four years after nitrogen addition stopped. This may reflect differences in resource competition strategies among functional groups. As a rhizomatous grass, *L. tianschanicus* tends to increase height and leaf area to expand niche space and improve photosynthetic efficiency. As a bunchgrass in the lower canopy, *F. kryloviana* has weaker light competition and tends to adjust leaf traits to accumulate photosynthates. Additionally, while leaf area and SLA typically correlate positively, *F. kryloviana*'s SLA was suppressed under high nitrogen, possibly due to increased leaf thickness from long-term nitrogen addition.

3.2 Effects of Nitrogen Addition Cessation on Key Community Characteristics Nitrogen addition changes environmental conditions and resource availability (including soil nitrogen and light), with different functional groups showing varying resource use capacities. Competitive species may occupy space of other species and become dominant. This study found that long-term nitrogen addition increased rhizomatous grass relative cover and ANPP while decreasing bunchgrass proportions, altering plant community structure. This change involves two mechanisms: (1) rhizomatous grasses are taller and more competitive for light, and (2) their well-developed rhizomes efficiently use enriched soil nitrogen, showing clear competitive advantages especially under high nitrogen. Bunchgrass proportions decreased along nitrogen addition gradients, showing compensatory effects between functional groups.

After nitrogen addition stopped, resource conditions changed again, potentially creating new competitive advantages. Previous studies showed that after nitrogen addition cessation, relative abundance of perennial grasses increased while perennial forbs and annuals decreased. In our study area, perennial forbs accounted for a small proportion with non-significant changes, while perennial rhizomatous and bunchgrasses were the main functional groups. Over recovery time, the positive legacy effect of nitrogen addition on rhizomatous grass proportions gradually decreased, as did its promoting effect on community cover and ANPP. This suggests that under future scenarios of reduced atmospheric nitrogen deposition, nitrogen deposition will still have positive legacy effects on community ANPP, maintaining high material production capacity. However, as plant-available nitrogen decreases, the fertilization-induced productivity enhancement will gradually weaken. Future research should incorporate longer-term observations to accurately assess recovery dynamics.

3.3 Effects of Nitrogen Addition Cessation on Soil Physicochemical Properties Soil carbon, nitrogen, and phosphorus contents are closely related to grassland ecosystem function and stability. Our results showed that four years after nitrogen addition stopped, high nitrogen conditions still had significant positive legacy effects on total nitrogen content, indicating the soil nitrogen pool remained saturated and continued providing growth advantages for nitrogen-loving plants like *L. tianschanicus*, thus maintaining community productivity legacy effects. The non-significant legacy effect on total phosphorus content aligns with studies on alpine meadows in the Qinghai-Tibet Plateau. Regarding carbon-nitrogen coupling, soil organic carbon showed higher response ratios under low nitrogen (N5), possibly because moderate nitrogen addition promotes soil carbon accumulation through increased litter input, while excessive nitrogen may enhance organic matter decomposition more than accumulation, reducing soil carbon storage—similar to findings from European temperate grasslands.

Correlation analysis showed that soil total phosphorus and organic carbon content had no significant correlations with vegetation indicators, possibly because plant uptake depends on available phosphorus rather than total phosphorus (which includes occluded forms), and soil organic carbon may indirectly regulate vegetation through soil microorganisms (not quantified in this study). Redundancy analysis showed that the four soil factors explained only 18.78% of total vegetation variation, suggesting other unconsidered factors (such as soil trace elements and microbial activities) may importantly regulate vegetation changes. Future research should incorporate multi-dimensional indicators including soil microorganisms, trace elements, and enzyme activities to more comprehensively understand ecosystem responses to nitrogen deposition.

4 Conclusion

Through a 16-year nitrogen addition experiment combined with four years of natural recovery, this study analyzed the legacy effects of long-term nitrogen addition on plant community structure and function in the Tianshan grassland. The main conclusions are:

1) Nitrogen addition had significant legacy effects on functional traits of the two dominant species. Historical nitrogen addition maintained positive legacy effects on plant height, leaf area, and SLA of *Leymus tianschanicus*. In contrast, *Festuca kryloviana* showed negative legacy effects on plant height and SLA, which weakened annually and may disappear with extended recovery time.

2) Nitrogen addition had legacy effects on relative cover and ANPP of different functional groups, consequently affecting community structure and function. These legacy effects gradually decreased over time, particularly under high nitrogen treatment (N15), where the promoting effects on community cover and ANPP showed clear attenuation.

3) Four years after nitrogen addition stopped, total soil nitrogen content still showed significant positive legacy effects, while soil pH, total phosphorus, and organic carbon content showed no significant changes. Soil total phosphorus and organic carbon content had low correlations with vegetation characteristics, and the four soil factors collectively explained only a small proportion of vegetation variation. Future research should explore other factors affecting plant community structure and function.

This study demonstrates that under scenarios of reduced or ceased atmospheric nitrogen deposition, historical nitrogen deposition continues to exert persistent legacy effects on grassland ecosystems, with some effects gradually diminishing over recovery time.

References

[?]
[?]
[?]
[?]
[?]
[?]
[?]
[?]
[?]
[?]
[?]
[?]

[?]
[?]
[?]
[?]
[?]
[?]
[?]
[?]
[?]
[?]
[?]
[?]
[?]
[?]
[?]
[?]
[?]
[?]
[?]
[?]
[?]

Note: Figure translations are in progress. See original paper for figures.