Root System Pattern Changes in Stipa Plants Under Long-term Grazing Exclusion in Semi-arid Grasslands: Postprint

Authors: Su Jishuai, Zhao Jie, Jing Guanghua, Wei Lin, Liu Jian, Cheng Jimin, Zhang Jin’e

Date: 2017-10-30T00:00:00+00:00

Abstract

Taking the roots and soil of Stipa plants in Yunwu Mountain grasslands under different enclosure durations as the research object, this study investigated root characteristics, soil properties, and their relationships to explore and analyze the effects of enclosure on Stipa root patterns. The results showed that: (1) Root biomass, root length density, root surface area, and root volume of Stipa plants decreased slightly in the early stage of enclosure, then increased slowly, and were significantly enhanced in grasslands enclosed for 30 a. (2) With increasing enclosure duration, the compositional patterns of various root indices among the three Stipa species exhibited similar trends: Stipa bungeana accounted for the highest proportion in grazed grasslands, gradually decreased thereafter, and disappeared in grasslands enclosed for 30 a; the proportion of Stipa grandis first increased and then decreased, reaching its maximum in grasslands enclosed for 22 a; Stipa przewalskyi only appeared in grasslands enclosed for 30 a and occupied a dominant position. (3) The proportion of 0-0.6 mm diameter class roots in Stipa grandis and Stipa przewalskyi was higher than that in Stipa grandis, making their root diameter significantly lower than that of Stipa grandis, and their specific root length and specific root area significantly higher than those of Stipa grandis; additionally, the root tissue density of Stipa bungeana was significantly higher than that of Stipa bungeana and Stipa przewalskyi. (4) Long-term enclosure significantly increased soil moisture, nutrient content, and soil N:P ratio while significantly decreasing soil C:N ratio, but had no significant effect on microbial biomass carbon and nitrogen. (5) Correlation analysis between Stipa root characteristics and soil indices revealed that Stipa roots were significantly influenced by soil nitrogen resources.

Full Text

Root Pattern of Stipa Plants in Semiarid Grassland After Long-Term Grazing Exclusion

**Su Jishuai[1], Zhao Jie[1], Jing Guanghua[2], Wei Lin[3], Liu Jian[1], Cheng Jimin[1],[3],*, Zhang Jin' e **

[1]College of Animal Science and Technology, Northwest A&F University, Yangling 712100, China
[2]Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, Yangling 712100, China
[3]Institute of Soil and Water Conservation, Northwest A&F University, Yangling 712100, China
Landscape Administration Bureau of Guyuan City, Guyuan 756000, China

*Corresponding author. E-mail: gyzcjm@ms.iswc.ac.cn

Abstract

We selected five grassland sites with different grazing exclusion durations (0, 5, 9, 22, and 30 years) on Yunwu Mountain to investigate root systems of Stipa plants and surrounding soils in the 0–30 cm soil layer. Roots were washed and identified to species level based on attached aboveground parts and root color, texture, and branching patterns. Root samples of the same species were scanned using an Epson Scanner to obtain images for analysis of root morphological traits using WinRhizoPro software. Root length, surface area, and volume were analyzed at 0.1 mm intervals in root diameter, enabling calculations of specific root length (SRL), specific root surface area (SRS), and root tissue density (RTD). Soil samples were analyzed to determine soil moisture, bulk density, pH, organic carbon, total nitrogen, total phosphorus, available nitrogen, available phosphorus, microbial biomass carbon, and microbial biomass nitrogen. One-way analysis of variance was conducted to determine the effect of grazing exclusion time on root traits and differences among Stipa species. Redundancy analysis was performed to identify soil variables that best explained variations in Stipa root traits.

The main results were as follows: (1) Root biomass, root length density, root surface area, and root volume of Stipa plants showed a slight decrease at the beginning of grazing exclusion, but thereafter gradually increased and showed significant increases in grassland with 30 years of grazing exclusion. (2) All root trait indexes showed similar species compositional changes with grazing exclusion time. Specifically, the proportion of *Stipa bungeana* was highest in grazed grassland, then gradually declined with grazing exclusion time and disappeared after 30 years; the proportion of *Stipa grandis* initially increased, peaked in grasslands after 20 years of grazing exclusion, then sharply decreased to initial status; and *Stipa przewalskyi* appeared only in grassland with 30 years of grazing exclusion and became the dominant species. (3) Compared with *S. grandis*, the roots of *S. bungeana* and *S. przewalskyi* were thinner and had higher SRL and SRS, mainly resulting from a larger proportion of root traits in the 0–0.6 mm root diameter class. Besides, root tissue density of *S. bungeana* was higher than that of *S. grandis* and *S. przewalskyi*. (4) Long-term grazing exclusion significantly increased soil water content, total nitrogen, total phosphorus, nitrate nitrogen, and available phosphorus, while significantly decreasing the soil carbon-to-nitrogen ratio and increasing the soil nitrogen-to-phosphorus ratio, but showed limited impacts on soil bulk density, pH, ammonium nitrogen, microbial biomass carbon, and microbial biomass nitrogen. (5) Redundancy analysis showed positive correlations among root biomass, root length density, root surface area, and root volume, and total soil nitrogen and ammonium nitrogen were significant factors explaining variations in Stipa root traits after grazing exclusion. Furthermore, root traits were positively affected by soil water content and available phosphorus, and negatively affected by soil pH. In conclusion, inherent differences in root traits among the three species and changes in soil properties collectively drove the root trait patterns of Stipa plants in semiarid grassland after long-term grazing exclusion in this area.

Keywords: grazing exclusion; Stipa plants; root morphological trait; species compositional pattern

1. Introduction

Overgrazing and unreasonable cultivation have caused 50%–90% of grasslands nationwide to be in various stages of degradation. Fencing and grazing exclusion are widely used in degraded grassland restoration management due to their economic feasibility and operational simplicity. After grazing exclusion, grassland vegetation coverage and aboveground and root biomass increase significantly. The increased vegetation biomass enhances organic matter input to soil, accompanied by changes in plant community structure. Soil physicochemical properties improve, specifically through increases in soil carbon and nitrogen content and storage, while grazing exclusion also affects soil biological community structure characteristics. However, studies indicate that long-term grazing exclusion is not conducive to maintaining grassland diversity and plant community self-renewal processes.

Grassland ecosystems store plant roots, rhizomes, and aboveground stem-leaf bases in soil, with root biomass concentrated in the 0–30 cm soil layer and decreasing exponentially with depth. Unlike the single light resource aboveground, underground soil nutrient resources exhibit distinct spatial and temporal heterogeneity. Plant root morphological characteristics and their plasticity in response to soil nutrient heterogeneity determine aboveground growth performance. Specific root length and specific root surface area affect a plant' s competitive advantage in communities. Root morphological plasticity is influenced by soil nutrient levels.

Stipa communities are the dominant vegetation in the Yunwu Mountain grassland of Guyuan, Ningxia. Vegetation surveys show that *Stipa bungeana* is the dominant species during the initial stage of grassland grazing exclusion, gradually replaced by congeneric plants *Stipa grandis* and *Stipa przewalskyi* in later stages. Previous studies have focused on aboveground biological characteristics of the three Stipa species, with root research mostly remaining at the community level and rarely investigating vegetation roots at the species level. The role of plant root responses to soil property fluctuations induced by grazing exclusion in the process of dominant species replacement remains unclear. This study investigates Stipa plants in grasslands with different grazing exclusion durations on Yunwu Mountain, examining changes in underground root characteristics and surrounding soil properties with exclusion duration at the species level to explore the causes of Stipa species replacement from a root perspective, providing scientific basis for grassland restoration and utilization.

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2. Study Area

The study area is located in Yunwu Mountain National Nature Reserve in northeastern Guyuan City, Ningxia Hui Autonomous Region. The reserve implemented fencing measures in 1982, with exclusion durations up to 35 years. The area has an elevation of 1800–2100 m, mean annual temperature of 7.01°C, mean annual rainfall of 425.42 mm, and annual evaporation of 1017–1739 mm. The frost-free period lasts 137 days. Soil type is mainly mountain loess. The vegetation community comprises 66 plant species, with dominant plants including *Stipa bungeana*, *Artemisia sacrorum*, and *Thymus mongolicus*.

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3. Experimental Design and Field Sampling

We selected grazed grassland and grasslands with 5, 9, 22, and 30 years of grazing exclusion as study sites using a space-for-time substitution approach, with grazed grassland as the control. All study sites had similar site conditions (slope $> 50°$) and were under extremely heavy grazing before exclusion. Moderately grazed grassland was achieved through wire fencing to control plot area and sheep numbers.

In each grassland type, three 30 m × 30 m plots were established, with five 0.5 m × 0.5 m quadrats randomly set in each plot. Root systems of Stipa plants were collected using excavation methods to a depth of 30 cm. Large soil clumps were gently shaken off, and the root-soil mixtures were numbered and brought back to the laboratory. Soil samples were collected from 0–20 cm depth around each quadrat using stainless steel soil augers and mixed into one composite sample per plot. Part of the soil was sieved (0.25 mm) to remove plant roots for microbial biomass carbon and nitrogen determination. The remaining soil was air-dried and ground (0.15 mm sieve) for determination of soil organic carbon, available nitrogen, and other properties. Soil cores (0.5 m × 0.3 m × 0.3 m) were excavated for soil bulk density and water content measurements.

General information of study sites

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4. Root Trait Measurements

Stipa root-soil mixtures were soaked in water for 20 minutes to remove large soil aggregates, then slowly rinsed under tap water to remove adhering soil particles and plant residues. The three Stipa species were distinguished based on connected aboveground parts and root color, texture, and other characteristics. Roots of the same species from the same quadrat were treated as one sample. Roots were placed in transparent plastic trays, scanned at 300 dpi resolution (Epson Scanner 10000XL Pro, Canada) to obtain root images, then analyzed using WinRhizoPro V2012b software at 0.1 mm diameter intervals to obtain root length, surface area, and volume. Scanned roots were oven-dried at 65°C to constant weight.

Calculated root traits included: - Root biomass (g/m^2) = root dry weight / sampling volume - Root length density (m/m^2) = total root length / sampling volume - Specific root length (m/g) = total root length / root dry weight - Specific root surface area (cm^2/g) = total root surface area / root dry weight - Root tissue density (g/cm^3) = root dry weight / root volume - Sampling volume = 0.5 m × 0.5 m × 0.3 m = 0.075 m^3

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5. Soil Property Measurements

Soil water content and bulk density were measured using the oven-drying method. Soil pH was determined potentiometrically. Soil organic carbon was measured by potassium dichromate oxidation. Total nitrogen was determined by Kjeldahl digestion. Total and available phosphorus were measured by molybdenum-antimony colorimetry. Ammonium and nitrate nitrogen were measured by flow analysis. Soil microbial carbon and nitrogen were determined by chloroform fumigation. Soil C:N ratio = soil organic carbon content / total nitrogen content. Soil N:P ratio = total nitrogen content / total phosphorus content. Microbial biomass C:N ratio = microbial biomass carbon / microbial biomass nitrogen.

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6. Statistical Analysis

One-way ANOVA in SPSS 18.0 was used to compare Stipa root traits and soil properties among different grazing exclusion durations, with Duncan' s multiple range test for significance ($P < 0.05$). Redundancy analysis was performed using Canoco 4.5 to examine relationships between root traits and soil properties, with Monte Carlo tests to assess significance of soil variables in explaining root trait variation ($P < 0.05$).

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7. Results

7.1 Root Traits of Stipa Plants Under Different Grazing Exclusion Durations With increasing grazing exclusion duration, root biomass, root surface area, and root volume of Stipa plants showed similar patterns: a slight decrease at the beginning of exclusion, followed by a gradual increase, with significant increases in the 30-year exclusion grassland. Root biomass increased from 91.37 g/m^2 in grazed grassland to 208.50 g/m^2 in the 30-year exclusion grassland ($P < 0.05$). Root length density increased from 13.53 (10^3 m/m^2) to 29.09 (10^3 m/m^2) ($P > 0.05$). Root surface area increased from 425.91 (10^3 cm^2/m^2) to 656.52 (10^3 cm^2/m^2) ($P < 0.05$). Root volume increased from 48.79 (10^3 cm^3/m^2) to 56.52 (10^3 cm^3/m^2) ($P < 0.05$).

Lower root trait values in 5-year exclusion grassland may result from plants increasing allocation to aboveground parts to compete for light and occupy space after grazing removal. In grazed grassland, herbivory causes Stipa plants to produce more roots for soil nutrient acquisition to support compensatory aboveground growth. Stipa' s extensive, dispersed root system ensures stronger competition for soil water and nutrients than non-grass plants, enabling it to replace other species during succession. This may also explain why root traits significantly increased in long-term exclusion grasslands.

[Figure 1: see original paper] Root traits of Stipa plants in grasslands with different grazing exclusion time. Different lowercase letters indicate significant differences ($P < 0.05$).

7.2 Species Composition Patterns of Stipa Root Systems Species composition in the Stipa root layer varied significantly among grasslands with different exclusion durations. Grasslands with 22 years exclusion consisted of *S. bungeana* and *S. grandis*, while the 30-year exclusion grassland comprised *S. grandis* and *S. przewalskyi*. *S. przewalskyi* appeared only in the 30-year exclusion grassland.

Changes in species composition of root biomass, surface area, and volume showed similar patterns with exclusion duration: *S. bungeana* proportion was highest in grazed grassland, continuously decreasing with exclusion time and disappearing after 30 years; *S. grandis* proportion initially increased, peaked in 22-year exclusion grassland, then rapidly decreased to grazed grassland levels; *S. przewalskyi* rapidly expanded in 30-year exclusion grassland and became dominant.

This species replacement may be related to shifting resource competition types during exclusion and the biological characteristics of the three Stipa species.

[Figure 2: see original paper] Species compositional pattern of Stipa root traits in grasslands with different grazing exclusion time.

7.3 Root Diameter Distribution Patterns Among Three Stipa Species

S. bungeana and *S. przewalskyi* showed similar root diameter distribution patterns, differing from *S. grandis*. Specifically, *S. bungeana* and *S. przewalskyi* had higher proportions of root length, surface area, and volume in the 0.6 mm diameter class than *S. grandis*, while *S. grandis* had higher proportions in the >0.7 mm diameter class.

Higher proportions of fine roots in *S. bungeana* and *S. przewalskyi* suggest greater ability to explore and acquire soil resources. Except for root tissue density, root traits between *S. bungeana* and *S. przewalskyi* showed no significant differences, but both had significantly lower root diameter and higher specific root length and surface area than *S. grandis* ($P < 0.05$). Root tissue density was significantly higher in *S. bungeana* than in *S. grandis* and *S. przewalskyi* ($P < 0.05$), with no significant difference between the latter two.

Root traits of three Stipa plants

[Figure 3: see original paper] Distribution pattern of root traits in root diameter classes in three Stipa plants.

7.4 Soil Properties in the 0–20 cm Layer Under Different Grazing Exclusion Durations

Different soil properties showed varying patterns with exclusion duration. Grazing exclusion significantly increased soil water content, total nitrogen, nitrate nitrogen, available phosphorus, and soil N:P ratio, while decreasing soil C:N ratio and microbial C:N ratio. Effects became significant after 9 years of exclusion. Soil bulk density, pH, microbial biomass carbon, microbial biomass nitrogen, and ammonium nitrogen showed no significant differences ($P > 0.05$).

Compared with grazed grassland, exclusion increased soil water content from 16.57% to 21.37–22.48% ($P < 0.05$). Soil organic carbon increased from 30.03 g/kg to 34.14 g/kg in 30-year exclusion grassland ($P < 0.05$). Total nitrogen increased from 1.44 g/kg to 2.71 g/kg ($P < 0.05$). Total phosphorus increased from 0.90 g/kg to 1.18 g/kg ($P < 0.05$). Available phosphorus increased from 19.85 mg/kg to 48.41 mg/kg ($P < 0.05$). Soil C:N ratio decreased from 21.78 to 14.23 ($P < 0.05$), while soil N:P ratio increased from 1.60 to 2.40 ($P < 0.05$).

Soil properties of 0–20 cm soil layer in grasslands with different grazing exclusion time.

8. Discussion

8.1 Effects of Grazing Exclusion on Stipa Root Traits and Soil Properties Fencing excludes herbivory, increasing aboveground productivity and thus organic matter allocation to roots, leading to increased root biomass. In this study, Stipa root biomass showed a slight initial decrease, likely because Stipa, as a palatable forage, rapidly increased aboveground allocation after grazing exclusion to compete for light and space. In grazed grassland, herbivory stimulates Stipa to produce more roots for nutrient acquisition to support compensatory growth.

As a bunchgrass, Stipa has an extensive, dispersed root system with higher specific root length and surface area than non-grass plants, and high plasticity to produce more roots in nutrient-rich patches. This enables stronger competition for soil resources. Combined with significantly improved soil properties under long-term exclusion, Stipa root biomass and morphological traits increased substantially.

Redundancy analysis showed significant positive associations between Stipa root traits and soil nitrogen resources. The significant increase in soil nitrogen under long-term exclusion contributed to increased root traits, which were also closely related to the root characteristics of the dominant species *S. przewalskyi.*

8.2 Mechanisms of Species Replacement Among Three Stipa Species Vegetation surveys show distinct aboveground ecological niches among the three Stipa species: *S. bungeana* has a small, short-statured bunch; *S. grandis* is tall and compact; and *S. przewalskyi* is large and dense with the most tillers. With increasing exclusion duration, *S. bungeana* niche width decreases and disappears after 30 years; *S. grandis* niche width first increases then decreases; and *S. przewalskyi* appears only in 30-year exclusion grassland with the smallest niche width.

Root-based species composition matches aboveground patterns, but root trait fluctuations are smaller than aboveground variations. Unlike aboveground patterns, *S. przewalskyi* root proportion exceeds *S. grandis* in 30-year exclusion grassland, likely related to its biological characteristics.

Consistent with previous studies, long-term exclusion significantly improved soil water and nutrient content. Increased soil water content results from enhanced vegetation cover and surface litter reducing evaporation. Litter decomposition releases nutrients, with soil biota playing dominant roles initially. Soil moisture and microbial activity promote nutrient availability. Because samples were from the surface (0–20 cm) with abundant resources, microbial biomass carbon and nitrogen did not change significantly with exclusion duration, though other studies show plant inputs affect microbial communities.

The species replacement can be explained as follows: In the semi-arid Yunwu Mountain region, long-term grazed grasslands have extensive bare ground with strong surface evaporation, making water the primary limiting factor. *S. bungeana*' s smaller size requires less water and nutrients, while its high specific root length and surface area ensure sufficient nutrient acquisition. Its high root tissue density suggests longer root lifespan, reducing carbon costs. Additionally, *S. bungeana* seedlings are more drought-tolerant than *S. grandis*, and can produce bulbils at leaf sheath bases for temporary dormancy, enabling successful establishment during early exclusion.

As exclusion continues, soil water and fertility improve, and competition shifts from belowground to aboveground. *S. grandis*' s height advantage enables it to intercept light first, reducing photosynthesis in shorter *S. bungeana*, leading to *S. grandis* dominance and *S. bungeana* decline. Finally, *S. przewalskyi* may increase its bunch size to reduce shading by *S. grandis* while its high root biomass, specific root length, and surface area enable it to preempt soil resources, inhibiting *S. grandis* growth. Other plants widely distributed in exclusion grasslands, such as *Artemisia sacrorum* with strong allelopathic effects, may also influence Stipa species replacement.

[Figure 4: see original paper] Redundancy analysis between root traits of Stipa plant and soil properties. Only the top 5 explanatory soil factors are shown. $^{**}P < 0.01$, $^{*}P < 0.05$.

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9. Conclusion

During early grazing exclusion, Stipa plants primarily allocated resources to aboveground growth, showing no significant changes in root biomass or morphological traits. With increasing exclusion duration, improved soil water and fertility significantly enhanced Stipa root biomass and morphological traits. *Stipa bungeana* became dominant in grazed and early exclusion grasslands due to its strong drought tolerance and low water/nutrient requirements. As soil conditions improved during exclusion, *Stipa grandis* benefited from aboveground competition to become dominant. Finally, *Stipa przewalskyi* utilized its high productivity and root competition to replace *S. grandis*, showing potential to become the dominant species in long-term exclusion grasslands.

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