

Characteristics of Soil Carbon and Nitrogen Mineralization Following Grassland Enclosure on the Semi-arid Loess Plateau (Postprint)

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Abstract

The distribution and mineralization of soil organic carbon and total nitrogen are key components and indicators in studies on soil ecological effects following the enclosure of degraded grasslands. Through a combination of field investigation and laboratory incubation experiments, we examined the content variations and mineralization characteristics of soil organic carbon and total nitrogen in grasslands with different enclosure durations in a semi-arid loess region. The results demonstrated that the effects of enclosure on soil organic carbon and total nitrogen in degraded grasslands of the semi-arid loess region were primarily manifested in the 0-40 cm soil layer, with the influence of enclosure duration gradually diminishing after exceeding 17 years. Enclosure significantly increased the soil organic carbon mineralization rate and C_{min}/C_0 ; its effect on the organic carbon mineralization rate was independent of enclosure duration and soil layer depth, whereas its effect on C_{min}/C_0 was dependent on both enclosure duration and soil layer depth. Enclosure significantly enhanced the soil nitrogen mineralization rate in the 0-40 cm layer, but decreased it in the 40-80 cm layer, and also reduced N_{min}/N_0 across the 0-80 cm profile. The carbon and nitrogen mineralization rates exhibited significant correlations with organic carbon and total nitrogen, but relatively weak correlations with the C/N ratio. These findings indicate that the transformation of soil carbon and nitrogen elements following degraded grassland enclosure is primarily influenced by soil layer depth, enclosure duration, and soil carbon and nitrogen contents.

Full Text

Preamble

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Influence of Grazing Exclusion on Soil Organic Carbon and Nitrogen Mineralization in Semiarid Grasslands of the Loess Plateau

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Abstract

Grazing exclusion is an important method for rehabilitating degraded ecosystems in grassland-dominated parts of semiarid regions. However, how ecosystem processes respond to this rehabilitation has not been well assessed. To understand how carbon and nitrogen cycling in degraded grasslands responds to grazing exclusion, it is essential to study the distribution and mineralization of soil organic carbon and nitrogen. Field measurements and laboratory incubations of soil (at 0–80 cm depth) from the Loess Plateau's semiarid region were combined to analyze soil organic carbon and nitrogen distribution and mineralization after various years of grazing exclusion. Samples were collected from grasslands where grazing was excluded for 17, 22, and 27 years, respectively. As a control, additional soil samples were collected from continuously grazed grasslands that neighbored the grazing exclusion sites. Organic carbon and nitrogen contents, as well as organic carbon and nitrogen mineralization rates, were measured.

The effects of grazing exclusion on the distribution of organic carbon and nitrogen mainly occurred in the top 0–40 cm of the soil. Grazing exclusion significantly increased soil organic carbon and nitrogen content, with the largest increases observed after 17 years of exclusion. However, beyond 17 years, the effects on organic carbon and nitrogen distribution became weaker with time. Furthermore, although organic carbon mineralization rates decreased with soil depth for all treatments, for grazing-excluded grasslands the organic carbon mineralization ratio (C_{min}/C_0) increased more with soil depth. The grazing exclusion effects on mineralization rate were independent of exclusion years or soil layers, in contrast to the effects on C_{min}/C_0 , which did correlate with both

these factors.

In addition, nitrification and mineralization rates of nitrogen decreased with soil depth, whereas in contrast, the ammonification rate increased. At 0–40 cm soil depth, nitrification rates ranged between 1.5 and 2.5 g g^{-1} , and ammonification rates between -1.6 and 0.1 g g^{-1} , indicating a nitrification-dominated nitrogen mineralization in this soil layer. At 40–80 cm depth, nitrification and ammonification rates ranged between 1.3 and 1.8 g g^{-1} , and between 0.7 and 0.8 g g^{-1} , respectively, suggesting that nitrification and ammonification contributed similarly to nitrogen mineralization in deeper soils. Furthermore, grazing exclusion significantly increased the mineralization rate of nitrogen in the 0–40 cm layer, while it decreased this in the 40–80 cm layer. The overall nitrogen mineralization ratio (N_{min}/N_0) also decreased when grazing was excluded. Finally, the mineralization of organic carbon and nitrogen were significantly correlated with soil organic carbon and nitrogen content, but less so with the soil carbon to nitrogen ratio (C/N). Overall, the results of this study indicate that the turnover of organic carbon and nitrogen in grazing-excluded grassland was determined mainly by soil layer, grazing exclusion duration, and soil organic carbon and nitrogen content.

Keywords: grazing management; soil organic carbon; nitrogen; soil profiles; carbon mineralization; soil properties

Introduction

China's total grassland area is approximately 41.7% of its national land area, with the Loess Plateau alone containing 32.6% of this grassland. Grassland degradation is severe in this region due to long-term overgrazing and human production activities, leading to weakened water and soil conservation functions [3]. Various measures for grassland resource protection and improvement, such as reducing livestock carrying capacity and implementing grazing exclusion, have been widely promoted and have achieved certain results. Degenerated grassland restoration through enclosure is an effective method for protecting and restoring natural grassland vegetation both domestically and internationally [2]. After grazing exclusion, degraded grasslands show significantly increased above-ground and belowground biomass [4-5], enhanced soil seed bank richness and diversity, substantial changes in grassland vegetation community structure, and increased species diversity [6-7], with succession processes tending toward normal stages and the distribution of constructive and companion species becoming increasingly apparent [2].

Soil organic carbon and total nitrogen content and their dynamic equilibrium processes are important indicators reflecting soil quality and grassland health, directly affecting grassland soil fertility and productivity [8]. Soil organic carbon and nitrogen mineralization is the process by which soil organisms decompose and utilize soil organic carbon and nitrogen through their metabolic activities, while releasing inorganic carbon and nitrogen. This process is directly related to

nutrient release and supply in soil and the maintenance of soil quality, and is a key factor affecting plant growth and vegetation productivity [9]. Currently, researchers have conducted numerous studies on soil organic carbon and nitrogen mineralization characteristics under different land use types and soil management measures, laying a foundation for understanding soil carbon and nitrogen dynamics [10-12]. Some studies have been conducted on grassland communities with the longest enclosure times on the plateau [13-16], but research on the effects of different enclosure years on soil organic carbon and nitrogen distribution, as well as the impact on soil carbon and nitrogen mineralization, urgently needs to be carried out. This study focuses on grasslands in the Ningxia Yuwushan Grassland Nature Reserve that have been enclosed since [year], analyzing the relationship between soil organic carbon and nitrogen mineralization and soil organic carbon and nitrogen content.

1 Study Area Overview

This study was conducted in the Ningxia Yuwushan Grassland Nature Reserve, located in the northeastern part of Guyuan City, Ningxia, within the loess hills of the central Loess Plateau (36°13′–36°19′ N; 106°24′–106°28′ E). The reserve has an elevation of 1800–2148 m and covers an area of 6700 hm². The mean annual precipitation is 425 mm, and the mean annual temperature is 6.9°C, with 124 frost-free days. The soil is gray-cinnamon soil developed from loess parent material. The main grassland vegetation includes *Stipa bungeana* (*Stipa bungeana*), *Artemisia sacrorum* (*Artemisia sacrorum*), *Potentilla acaulis* (*Potentilla acaulis*), and *Stipa grandis* (*Stipa grandis*). Among these, the bunchgrass *Stipa bungeana* has the widest distribution in this region.

2 Methods

2.1 Sample Collection

In the Yuwushan Nature Reserve, we selected grasslands with different grazing exclusion durations and adjacent continuously grazed grasslands as study sites. The soil type, vegetation composition, natural conditions, and soil physicochemical properties were similar before enclosure [17]. Study plots were established in 2009 in the Yuwushan Nature Reserve. All plots were unfertilized. The dominant plants were *Stipa bungeana* and *Artemisia sacrorum*. To reduce topographic differences, sampling plots were selected at similar altitudes, with dominant vegetation of *Stipa bungeana* and *Artemisia sacrorum* in each plot.

The grazing carrying capacity of the selected grazed grasslands varied by year, but previous studies showed no significant change in soil organic carbon and nitrogen in adjacent continuously grazed grasslands during the study period [18]. Therefore, the different grazing exclusion year grasslands selected in this study, along with the adjacent continuously grazed grasslands serving as controls, could be used to analyze changes in soil carbon and nitrogen content and mineralization characteristics under different grazing exclusion years.

In August, we established three sampling plots (30 m × 30 m) in both the enclosed grasslands and continuously grazed grasslands, which served as replicates for each enclosure year or grazing treatment. Within each plot, we randomly selected three sampling points and collected soil profile samples at depths of 0-5, 5-10, 10-20, 20-40, 40-60, and 60-80 cm. Soils from the same layer were mixed to form composite samples. After removing plant roots and residues from the soil samples...

2.2 Sample Measurement

A portion of air-dried soil samples was ground and passed through a 0.25 mm sieve for determination of soil organic carbon and total nitrogen content. Soil organic matter and total nitrogen content were measured using a VARIO EL III CHO elemental analyzer (Elementar). Soil organic carbon mineralization was determined using laboratory constant temperature incubation and alkali absorption methods. The specific method involved placing 10.0 g of soil sample at the bottom of a 250 ml glass tissue culture bottle, adjusting soil moisture to 60% of field capacity with distilled water, and adding 5 ml of 0.5 mol/L NaOH solution. The samples were incubated at 25°C for 5 days. The CO₂ released during incubation was calculated by titrating the NaOH solution. Soil nitrate nitrogen and ammonium nitrogen before and after incubation were measured using a flow analyzer to calculate soil nitrogen mineralization.

2.3 Data Calculation and Analysis

SPSS 16.0 software was used for statistical analysis. Two-way ANOVA and Tukey's test were used to analyze differences among different grazing exclusion years and soil depths. Correlation analysis was used to examine relationships between mineralization characteristics and soil organic carbon and total nitrogen content. All figures were created using SigmaPlot 10.0.

3 Results

3.1 Soil Carbon and Nitrogen Profile Distribution

Soil organic carbon and total nitrogen content in the study area gradually decreased with increasing soil depth. Average soil organic carbon across all treatments decreased from 30.6 g/kg (0-5 cm) to 16.2 g/kg (60-80 cm), a reduction of 88.7%. Total nitrogen decreased from 3.1 g/kg (0-5 cm) to 1.7 g/kg (60-80 cm), a reduction of 80.8%. The C/N ratio ranged between 8.0 and 12.0 across all soil profiles.

Grazing exclusion significantly affected soil organic carbon and total nitrogen, and these effects were related to soil depth. In the 0-40 cm layer, soil organic carbon content increased by 19.5%, 32.4%, and 29.8% after 17, 22, and 27 years of exclusion, respectively, while total nitrogen content increased by 29.8%, 34.8%, and 29.3%, respectively. However, there were no significant differences

between different grazing exclusion years. In the 40-80 cm layer, grazing exclusion had no significant effect on soil organic carbon and total nitrogen. These results indicate that the effects of grazing exclusion on degraded grassland soil organic carbon and total nitrogen mainly occurred in the 0-40 cm layer, and the influence of exclusion years became weaker over time.

shows the ANOVA results for the effects of grazing exclusion on soil carbon and nitrogen content and mineralization characteristics. The differences in soil organic matter and nitrogen among different grazing exclusion years at each soil layer reached extremely significant levels ($P < 0.01$).

3.2 Soil Organic Carbon Mineralization Characteristics

Soil organic carbon mineralization rate gradually decreased with increasing soil depth, with average values across all treatments decreasing from 69.8 g g^{-1} (0-5 cm) to 36.8 g g^{-1} (60-80 cm). However, the ratio of mineralized organic carbon to organic carbon content (C_{min}/C_0) showed trends influenced by grazing exclusion. In deep soil layers of grazing-excluded grasslands, C_{min}/C_0 increased more compared to surface soils.

[Figure 2: see original paper] shows the effects of grazing exclusion years on grassland soil organic carbon mineralization rate and ratio. Compared to unenclosed grasslands, soil organic carbon mineralization rates in the 0-80 cm layer significantly increased, but differences among different grazing exclusion years were not significant. The mineralization rate in the 0-80 cm layer increased by 23.3%-57.4% after 17 years and by 33.7%-49.5% after 22 years of exclusion. In the 0-40 cm layer, C_{min}/C_0 increased by 6.8%, 4.5%, and 8.0% after 17, 22, and 27 years, respectively. The increase in C_{min}/C_0 was more pronounced with longer exclusion years. In the 40-80 cm layer, C_{min}/C_0 increased by 15.8%, 35.0%, and 58.9%, respectively, with no significant differences among different exclusion years.

3.3 Soil Nitrogen Mineralization Characteristics

Soil nitrification rate and mineralization rate gradually decreased with increasing soil depth. Average nitrification rates across all treatments decreased from 4.9 g g^{-1} (0-5 cm) to 1.3 g g^{-1} (60-80 cm), while mineralization rates decreased from 1.5 g g^{-1} to 0.5 g g^{-1} . In contrast, ammonification rates increased with soil depth, from -1.6 g g^{-1} (0-5 cm) to 0.8 g g^{-1} (60-80 cm). The proportion of nitrification and ammonification varied by soil layer. In the 0-40 cm layer, nitrification rates ranged from 1.5 to 2.5 g g^{-1} and ammonification rates from -1.6 to 0.1 g g^{-1} , indicating that nitrification dominated nitrogen mineralization. In the 40-80 cm layer, nitrification and ammonification rates ranged from 1.3- 1.8 g g^{-1} and 0.7- 0.8 g g^{-1} , respectively, suggesting that both processes contributed similarly to nitrogen mineralization in deeper soils.

Soil nitrogen mineralization was affected by grazing exclusion, soil depth, and their interaction. Compared to unenclosed grasslands, nitrification rates in the

0–40 cm layer increased under all grazing exclusion years, while in the 40–80 cm layer, nitrification rates increased significantly only after 17 years but decreased after 22 and 27 years. Grazing exclusion had no significant effect on ammonification rates in the 0–20 cm layer, but significantly decreased ammonification rates in the 20–80 cm layer, promoting ammonium fixation by soil microorganisms.

Grazing exclusion significantly increased nitrogen mineralization rates in the 0–40 cm layer but decreased mineralization rates in the 40–80 cm layer. The overall nitrogen mineralization ratio (N_{min}/N_0) also decreased, with reductions of 12.3% and 38.4% after 17 and 22 years, respectively. The average mineralization rate in the 0–40 cm layer increased by 26.5% and 34.3%, respectively, while the average rate in the 40–80 cm layer decreased by 5.6% and 4.0%.

[Figure 3: see original paper] shows the effects of grazing exclusion years on grassland soil nitrogen mineralization.

4 Discussion

4.1 Effects of Grazing Exclusion on Soil Carbon and Nitrogen

Grazing exclusion can significantly increase soil organic carbon and total nitrogen content [19–22]. Li et al. [20] found that enclosed grassland in Inner Mongolia had significantly higher soil organic matter, available nitrogen, and total potassium than adjacent continuously grazed grassland. Mekuria et al. [21] reported that soil organic matter, total nitrogen, and phosphorus content increased significantly compared to unenclosed grassland in Tigray, Ethiopia. Grazing exclusion in semiarid Loess Plateau grasslands also significantly increased soil carbon and nitrogen content, which is beneficial for soil fertility recovery. However, some studies show no effect of grazing exclusion on soil fertility. Najmeh Ghorbani et al. [23] found no difference in soil organic carbon content between grazed and ungrazed grasslands (20–25 years) in southwestern Iran. Wang et al. [24] reported no change in soil nitrogen content compared to unenclosed grassland in Yuzhong County, Gansu Province. This demonstrates that the effects of grazing exclusion cannot be generalized and depend on environmental conditions and land use history.

In this study, soil organic carbon and nitrogen content no longer increased significantly after 17 years of grazing exclusion, possibly because the ecosystem carbon and nitrogen cycle gradually approached equilibrium over time, with inputs and outputs of soil organic carbon and total nitrogen becoming balanced. Qiu et al. [17] showed that after 17 years of grazing exclusion in Yuwushan grasslands, aboveground biomass and ecosystem carbon and nitrogen storage no longer increased with exclusion duration. Other studies in northern Chinese grasslands also indicate that ecosystem productivity and carbon and nitrogen storage stabilize after a certain period of enclosure [25–27]. Werth et al. [28] obtained similar results in enclosed grasslands in southwestern Germany. With prolonged exclusion, ecosystem species richness increases [20,29], and accumulated litter on the surface reduces contact with soil, slowing decomposition and

limiting further increases in soil carbon and nitrogen inputs [17].

The effects of grazing exclusion on soil carbon and nitrogen content in this study were significant only in the 0–40 cm layer, consistent with the findings of Wen et al. [26]. This is because soil carbon and nitrogen after grazing exclusion mainly originate from aboveground litter and belowground plant roots [30], which are concentrated in the 0–40 cm surface layer for most grassland ecosystems [31]. With annual precipitation of 425 mm in the Yuwushan reserve, downward leaching of soil carbon and nitrogen with rainfall is limited, so increases in soil carbon and nitrogen content after grazing exclusion mainly occurred in the 0–40 cm layer and decreased with soil depth.

4.2 Effects of Grazing Exclusion on Soil Carbon and Nitrogen Mineralization

Our results show that grazing exclusion increased soil organic carbon and nitrogen mineralization amounts and rates to varying degrees. Xu et al. [32] found that soil carbon mineralization accumulation in long-term enclosed *Stipa grandis* grassland was higher than in freely grazed grassland. Wang et al. [33] reported that soil carbon mineralization in three enclosed alpine grasslands on the Tibetan Plateau was higher than in freely grazed grassland. Mineralization rates were significantly positively correlated with soil organic carbon and total nitrogen content, consistent with our results.

Soil nitrogen mineralization rates increased after grazing exclusion [10,34–35]. Compared with in situ incubation, laboratory incubation provides optimal temperature and moisture conditions for microorganisms, resulting in higher nitrogen mineralization rates. Our laboratory incubation experiment showed that different treatments exhibited similar trends and profile distribution patterns for both soil organic carbon content and organic carbon mineralization rate, with extremely significant positive correlations. Nitrification rates and nitrogen mineralization rates also showed similar trends and profile distribution patterns with soil total nitrogen content, with extremely significant positive correlations. These results indicate that the increase in soil carbon and nitrogen mineralization rates after grazing exclusion in Loess Plateau grasslands was mainly influenced by soil carbon and nitrogen content.

However, soil ammonification rate was significantly negatively correlated with organic carbon and total nitrogen content, possibly due to increased ammonium fixation by microorganisms and plants during organic matter mineralization. With reduced grazing pressure, the increased plant and microbial biomass intensifies competition for ammonium nitrogen, leading to greater ammonium fixation [36].

C_{min}/C_0 represents the proportion of mineralizable organic carbon to total soil organic carbon. Higher C_{min}/C_0 indicates that soil organic carbon is more easily mineralized and less stable. Our results show that with prolonged grazing exclusion, the proportion of mineralizable organic carbon in soil gradually

increased, and its stability gradually decreased, indicating that newly added organic carbon after grazing exclusion was mainly active organic carbon, consistent with Wang et al. [37]. N_{min}/N_0 was significantly lower in enclosed grasslands than in unenclosed grasslands, indicating that nitrogen accumulated after grazing exclusion was relatively stable and not easily mineralized and lost. However, since soil nitrogen must be mineralized into inorganic nitrogen before it can be utilized by plants and microorganisms, the decrease in N_{min}/N_0 after grazing exclusion may also indicate reduced soil nitrogen availability. This is because the study area received no external nitrogen input, and grazing exclusion promoted biogeochemical cycling of nitrogen, increasing plant uptake and utilization of soil inorganic nitrogen. The nitrogen absorbed by plants was returned to soil as organic nitrogen with lower mineralization potential, thereby reducing N_{min}/N_0 in the soil.

5 Conclusion

After grazing exclusion of degraded grasslands on the Loess Plateau, soil organic carbon and total nitrogen content in the 0-40 cm layer significantly increased, indicating that the effects of grazing exclusion on degraded grassland soil organic carbon and total nitrogen mainly occurred in the 0-40 cm layer. The influence of grazing exclusion years became weaker over time. Soil organic carbon mineralization rate gradually decreased with increasing soil depth. Compared with surface soils, the trend of C_{min}/C_0 with soil depth in unenclosed grasslands was reversed. Grazing exclusion significantly increased C_{min}/C_0 in deep soil layers.

Grazing exclusion significantly increased soil organic carbon mineralization rates and C_{min}/C_0 . The effects on organic carbon mineralization rate were independent of grazing exclusion years or soil layers, while the effects on C_{min}/C_0 were related to both exclusion years and soil layers. Soil nitrification and mineralization rates gradually decreased with increasing soil depth, while ammonification rates increased with depth. Nitrification dominated nitrogen mineralization in the 0-40 cm layer, while both nitrification and ammonification contributed similarly in the 40-80 cm layer. Grazing exclusion significantly increased nitrogen mineralization rates in the 0-40 cm layer but decreased mineralization rates in the 40-80 cm layer, and also decreased N_{min}/N_0 .

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