Grazing Intensity Changed The Activities of Nitrogen Assimilation Related Enzymes In Desert Steppe Plants

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Abstract

Background: The study on nitrogen assimilation mechanism of grazing grassland plants is of great significance to reveal the law of nutrient absorption and utilization of grassland vegetation.

Methods: This study took *Stipa breviflora* desert steppe which was grazed for 17 years as the research object, and sampled the root system, leaf and rhizosphere soil of constructive species *Stipa breviflora* under the treatments of no grazing, light grazing, moderate grazing and heavy grazing during the peak growing season. The activities of enzymes related to nitrogen assimilation in roots and leaves were measured, and the related factors affecting nitrogen content were analyzed.

Results: The results showed that heavy grazing significantly increased the total nitrogen content in the root system of *Stipa breviflora*, but decreased the total nitrogen content in the leaves, and the performance of grazing prohibition was consistent with that of heavy grazing; The activities of Nitrate reductase (NR), glutamine synthetase (GS), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvate transaminase (GPT) were stronger under light or moderate grazing. Under grazing prohibition and heavy grazing, the content of proline in roots and leaves of *Stipa breviflora* increased significantly, especially in leaves; NR, GS, GOT and GPT were significantly correlated with total nitrogen content in roots and leaves of *Stipa breviflora*.

Conclusions: Grazing prohibition and heavy grazing were not conducive to the nitrogen absorption and utilization of *Stipa breviflora*, which was closely related to the reduction of nitrate and ammonium nitrogen contents in the rhizosphere soil of *Stipa breviflora* by grazing. Grazing prohibition and heavy grazing affected the nitrogen content of *Stipa breviflora* by affecting the activities of related enzymes in the process of nitrogen assimilation of roots and leaves.

Introduction

Grazing is considered to be an important measure of grassland management. However, with the intensification of human activities, the disturbance of grazing on grassland has been widely concerned (Klein et al., 2007; Zheng et al., 2011), and livestock grazing management is very important for future grassland protection (Tóth et al., 2018). Previous studies have shown that grazing affects grassland ecosystem mainly by changing plant and soil properties (Wang et al., 2012b; Zheng et al., 2012; Travers et al., 2019). Desert grassland accounts for 39% of the grassland in Inner Mongolia (Xiong et al., 2021), in recent years, grassland degradation has become more and more serious under long-term overgrazing, with the decrease of vegetation coverage and biodiversity and productivity (Han et al., 2017). Therefore, it is very important to understand how better grazing management will affect grassland ecosystem, especially desert grassland. Kotanen et al. (2000) studies have shown that grazing can lead to the decrease of nutrient content and leaf area of plants, thus damaging photosynthesis of plants. There are many studies on the effects of grazing on nitrogen uptake and utilization of plants (Wang et al., 2012a;

sun et al., 2018b; Shen et al., 2019), but they are mainly focused on the level of nitrogen stoichiometry, while there are relatively few studies on the mechanism of grazing on nitrogen assimilation of plants.

Nitrogen is one of the essential elements for plant growth. Nitrogen assimilation refers to the process in which plants absorb nitrogen from the environment and synthesize nitrogen-containing organic compounds such as amino acids and proteins, in which many enzymes participate. The inorganic nitrogen sources (ammonium salt and nitrate) in soil are the main nitrogen sources of plants. Although most plants can absorb NH_4^+, NO_3^- is the main nitrogen source of plants. The plants mainly rely on the cortex cells of the root to absorb nitrate from the rhizosphere soil. Of course, the leaves of plants can also absorb nitrate, but the amount of nitrate absorption is less. In the process of nitrate assimilation, NO_3^- is reduced to NH_4^+ by nitrate reductase and nitrite reductase (Meyer and Stitt, 2001), and then assimilated to glutamic acid and glutamine by glutamic acid synthase (Lea and Miflin, 1974; Lea and Forde, 2001), After that, other amino acids or nitrogenous compounds were formed under the action of transaminase. Glutamine synthetase and glutamic acid synthetase are two important enzymes involved in the catalysis during the glutamic acid synthetase cycle. The two important enzymes involved in the catalysis in the process of ammonia transfer are pyruvate aminotransferase and glutamic oxaloacetate transaminase (Matsumoto et al., 2000; Xu et al., 2004). The research on the mechanism of plant nitrogen assimilation is relatively mature, mainly on wheat, rice, corn, soybean and other major crops (ward et al., 1989; Corsetti et al., 2003; Kaur et al., 2015).

The soil of *Stipa breviflora* desert steppe is poor and the natural conditions are poor. The species competition caused by grazing is also reflected in the competition of nutrient resources (Xiong et al., 2021; sun et al., 2018a). In order to adapt to the environment, species gradually form the external morphological characteristics and internal physiological adaptation strategies (Bai et al., 2012; An et al., 2018). The plant material selected in this study is *Stipa breviflora*, which is a constructive species in desert steppe. The population characteristics and morphological characteristics of *Stipa breviflora* have changed greatly under different grazing intensities for a long time. For example, with the increase of grazing intensity, the individuals of *Stipa breviflora* grew dwarfing, the leaves became shorter, the plant clusters became smaller, and the aboveground biomass decreased, but its important value increased with the increase of grazing intensity, and the relative coverage and density also increased significantly (Li et al., 2017). Smith and Li et al. Have similar conclusions in other grazing grassland studies (Smith, 1998; Li et al., 2014). Previous studies showed that the total nitrogen content in plant roots and leaves changed significantly under different grazing treatments (Wang et al., 2011; Niu et al., 2016; Xiong et al., 2021).

However, it is not clear whether the changes of total nitrogen content in roots and leaves of *Stipa breviflora* under different grazing intensities are related to the changes of enzyme activities involved in nitrogen assimilation. The scientific problems to be solved in this study are: 1. How does grazing intensity affect the activities of nitrogen assimilation related enzymes and nitrogen compounds in the roots and leaves of *Stipa breviflora*? 2. Are the contents of total nitrogen and nitrogen compounds in the roots and leaves of *Stipa breviflora* related to the activities of nitrogen assimilation related enzymes under grazing treatment? It is important to study the mechanism of plant nitrogen assimilation in grazing grassland for revealing the succession cause of grassland community and the change law of plant nutrient absorption and utilization. This study will provide technical and theoretical guidance for the rational and sustainable utilization of grazing grassland.

Methods

Plant materials and sources

Stipa breviflora samples were collected in long-term grazing experimental plots, which is located in Siziwang base (41 ° 47'17 "N, 111 ° 53'46" E, altitude 1 450 m) of comprehensive experiment and demonstration center of Inner Mongolia Academy of agriculture and animal husbandry sciences, China. *Stipa breviflora* is a wild constructive species of desert grassland in the western part of Inner Mongolia Autonomous Region, and it is not an endangered plant species. The manager of Inner Mongolia Academy of agriculture and animal husbandry sciences allowed us to collect samples of *Stipa breviflora*, and to identify and supervise our samples. The annual average precipitation of desert steppe is 280 mm, more than 80% of which is concentrated in May to September, and the average annual temperature was 3.4 °C. The soil type was light chestnut soil.

Experimental design

The experiment was a randomized block experiment. The experimental plots were about 50 hm^2 of natural grassland, and were enclosed for 17 years (2004-2020). The plots were divided into three blocks. Each block had four treatment plots (no grazing, light grazing, moderate grazing and heavy grazing). Each treatment was repeated three times, and the area of each experimental plot was 4.4 hm^2. The stocking rate of each treatment was 0 (no grading, CK), 0.91 (light grading, LG), 1.82 (model grading, Mg) and 2.71 (heavy grading, Hg) sheep unit · $(hm^2 \cdot A^{-1})^{-1}$, respectively. The stocking rate was set according to the results of Wei et al. (2000). In this experiment, 2-year-old adult goats in Siziwang Banner were selected and grazed from June to November every year. During the experiment, the management measures of the grazing area were the same. The sheep were driven into the grazing area at 6 a.m. and back to the shed at 6 p.m. every day, during which the sheep were free to feed. Drink water twice a day in the morning and evening, and supplement salt regularly with salt brick.

Sampling and measurements

Sampling of the roots and leaves of *Stipa breviflora*: sampling was conducted on August 10, 2020. A transect passing through the center of the study plot was selected, and six sampling points with a spacing of 50 m were arranged on the transect. Five clumps of plants were randomly selected around each sampling point to dig the plant roots 20 cm deep underground to remove the loose soil on the surface of the roots, and the rhizosphere soil was obtained by shaking method. After the above ground green leaves and roots were separated, they were immediately put into the ice box, and every three sampling points were combined into one sample. The root and leaf of each treatment were 6 replicates, 12 plots, a total of 48 samples. Half of them were used to determine the activities of nitrogen assimilation related enzymes and nitrogen compounds in roots and leaves. Half of them were killed at 105 ℃ for 30 minutes and then dried at 65 ℃ for 24 hours to determine the total nitrogen content. Five replicates of each treatment sample were selected for determination of nitrogen assimilation related enzyme activities and nitrogen compounds. The remaining samples and the remaining set of replicates were stored in - 80 ℃ refrigerator (backup). The contents of total nitrogen in rhizosphere soil, plant roots and leaves were determined by element analyzer.

Nitrate reductase (NR) activity, proline content, nitrate nitrogen content, soluble protein content and free amino acid content were determined according to Zou's method (2000); glutamine synthetase (GS) activity was determined according to Jin's method (2007); glutamic acid synthetase (GOGAT) was determined according to Zhao's method (2010), Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvate transaminase (GPT) were determined according to Wu's method (1998).

Statistical analyses

Using Microsoft Excel software was used to process data, make charts and tables, and SPSS17.0 software was used to analyze the significant differences of nitrogen metabolism related enzyme activities, nitrogen compounds and nitrate nitrogen and ammonium nitrogen content in rhizosphere soil of *Stipa breviflora* under different grazing intensities, and the correlation analysis of total nitrogen content, nitrogen assimilation content and nitrogen assimilation related enzyme activities in root system and leaf weight of *Stipa breviflora* (Person detection). PowerPoint software, Excel software and sigma plot 14.0 software were used for drawing.

Results

Grazing intensity had a significant effect on the total nitrogen content in roots and leaves of *Stipa breviflora* (Fig. 1). The total nitrogen content of the root system of *Stipa breviflora* was significantly higher than that of light grazing and moderate grazing, while the heavy grazing treatment of total nitrogen content in leaves was significantly lower than that of light grazing and moderate grazing, but there was no significant difference between the two. The total nitrogen content in the root system of *Stipa breviflora* under grazing prohibition was higher than that under light grazing and moderate grazing, while the total nitrogen content in the leaves was lower than that under light grazing and moderate grazing, but there was no significant difference among grazing prohibition, light grazing and moderate grazing. The results showed that grazing prohibition and heavy grazing increased the total nitrogen content in the roots and decreased the total nitrogen content in the leaves of *Stipa breviflora* to a certain extent, especially under heavy grazing.

As shown in Fig. 2, grazing intensity had different effects on soluble protein and free amino acid contents in roots and leaves of *Stipa breviflora*. The content of soluble protein in the roots of *Stipa breviflora* under grazing prohibition was significantly higher than that under light, medium and heavy grazing, and the content of soluble protein under light grazing was significantly higher than that under medium and heavy grazing. There was no significant difference in soluble protein content of *Stipa breviflora* leaves under different grazing treatments. With the increase of grazing intensity, the content of free amino acids in the roots of *Stipa breviflora* increased at first and then decreased, which was the highest under moderate grazing level and the lowest under no grazing treatment. But in leaves, it decreased first and then increased, which was the lowest under light grazing and the highest under heavy grazing.

The effect of grazing intensity on the proline content in roots and leaves of *Stipa breviflora* was the same, which decreased first and then increased with the increase of grazing intensity. The proline content was the lowest under moderate grazing level, and the highest under heavy grazing treatment, and the proline content in heavy grazing treatment was significantly higher than that in no grazing, light and moderate grazing treatment.

According to Table 1, the activities of glutamine synthetase, glutamic oxaloacetic transaminase and glutamic pyruvate transaminase were significantly negatively correlated with the total nitrogen content in roots and leaves, while the activities of nitrate reductase, glutamic oxaloacetic transaminase and glutamic pyruvate transaminase were significantly positively correlated with the total nitrogen content in roots and leaves. The content of soluble protein in root and leaves was positively correlated with the activities of glutamine synthetase, nitrate reductase, glutamic oxaloacetate transaminase and pyruvate transaminase. Nitrate nitrogen content in roots and leaves was significantly positively correlated with glutamate synthetase activity, while nitrate nitrogen and free amino acid content were significantly negatively correlated with glutamine synthetase activity. The contents of nitrate nitrogen and free amino acids in roots and leaves were positively correlated with the activities of nitrate reductase, glutamic oxaloacetic transaminase and glutamic pyruvate transaminase.

Table 1
Correlation Analysis of nitrogen content, nitrogen compound content and nitrogen assimilation related enzyme activities in roots and leaves of *Stipa breviflora* under different grazing treatments

□□	**TN**	**SP**	**Pro**	**NN**	**FAA**
GOGAT	0.665	0.674	0.656	0.789*	0.652
GS	-0.922**	− .937**	− .744*	-0.816*	-0.891**
GOT	0.996**	0.994**	0.688	0.871**	0.961**
GPT	0.978**	0.975**	0.66	0.856**	0.945**
NR	0.992**	0.984**	0.629	0.893**	0.933**

Note: GOGAT, GS, got, GPT and NR represent glutamate synthetase, glutamine synthetase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and nitrate reductase respectively. TN, SP, Pro, NN and FAA represent total nitrogen content, soluble protein, proline, nitrate nitrogen and free amino acid respectively. "* *" means significant correlation at 0.01 level and "*" means significant correlation at 0.05 level. "-" means significant negative correlation at 0.01 or 0.05 level.

Grazing intensity significantly affected the activities of glutamic acid synthase and glutamine synthetase in roots and leaves of *Stipa breviflora* (Fig. 4). The activity of glutamate synthase in roots and leaves of *Stipa breviflora* under moderate grazing was significantly lower than that under no grazing, light grazing and heavy grazing, and the activity of glutamate synthase in leaves under light and heavy grazing was significantly lower than that under no grazing. With the increase of grazing intensity, the activity of glutamine synthetase in roots and leaves of *Stipa breviflora* first increased and then decreased. The activity of glutamine synthetase in roots was the highest under heavy grazing level, and that in leaves was the highest under light grazing level, which was significantly higher than other grazing levels.
The effects of grazing intensity on the activity of glutamic oxaloacetic transaminase in the roots and leaves of *Stipa breviflora* were different (Fig. 5). The activity of glutamic oxaloacetic transaminase in the roots of *Stipa breviflora* under heavy grazing was significantly higher than that under no grazing, while the activity of glutamic oxaloacetic transaminase in the leaves of *Stipa breviflora* under mild and moderate grazing was significantly higher than that under no grazing and heavy grazing. Grazing had the same effect on the activity of glutamate pyruvate transaminase in the roots and leaves of *Stipa breviflora*. There was no significant difference among light, moderate and heavy grazing, but they were significantly higher than that of no grazing.

The effects of grazing intensity on nitrate reductase activity in roots and leaves of *Stipa breviflora* were different (Fig. 6). The activity of nitrate reductase in roots under heavy grazing was the lowest, which was significantly lower than that under no grazing, while there was no significant difference between light and moderate grazing. The activity of nitrate reductase in leaves of *Stipa breviflora* was significantly decreased under grazing prohibition and heavy grazing, but there was no significant difference between light and moderate grazing. Grazing had a significant effect on the content of nitrate nitrogen in the roots and leaves of *Stipa breviflora*. The highest content of nitrate nitrogen in the roots and leaves of *Stipa breviflora* was under light grazing. The content of nitrate nitrogen in the roots and leaves of *Stipa breviflora* under moderate and heavy grazing was significantly lower than that under light grazing.

The nitrogen absorbed and assimilated by plants mainly came from ammonium and nitrate in rhizosphere soil. As shown in Fig. 7, with the increase of grazing intensity, the content of ammonium nitrogen in the rhizosphere soil of *Stipa breviflora* decreased gradually, and the content of ammonium nitrogen in the forbidden grazing and light grazing treatments was significantly higher than that in the heavy grazing treatment. The nitrate content in the rhizosphere soil of *Stipa breviflora* under moderate and heavy grazing treatments was significantly lower than that under no grazing and light grazing treatments.

The activities of enzymes related to nitrogen assimilation in roots and leaves of *Stipa breviflora* changed in different degrees under grazing prohibition and heavy grazing (Fig. 8). Although the content of nitrate and ammonium nitrogen in the rhizosphere soil of *Stipa breviflora* was high under grazing prohibition, the content of nitrate nitrogen absorbed by the root system of *Stipa breviflora* was low. Although the nitrate reductase activity was high, the activities of glutamine synthetase, glutamic oxaloacetic transaminase and glutamic pyruvate transaminase in the root system were weak, and the content of free amino acids in the root system was still low. The activities of nitrate reductase, glutamine synthetase, glutamic oxaloacetic transaminase and glutamic pyruvate transaminase in leaves of *Stipa breviflora* under grazing prohibition were weak, and the content of synthetic amino acids in leaves was also low.

Under heavy grazing, the contents of nitrate nitrogen and ammonium nitrogen in the rhizosphere soil of *Stipa breviflora* were low, and the activities of nitrate reductase and glutamine synthetase in the roots were weak. Although the activities of glutamic oxaloacetic transaminase and glutamic pyruvate transaminase were high, the content of free amino acid in the roots was low. The content of nitrate nitrogen in leaves was also lower under heavy grazing, except the activity of glutamic pyruvate transaminase was higher, and the activities of other enzymes such as nitrate reductase, glutamine synthetase, glutamate synthetase and glutamate oxaloacetate transaminase were weak.

Discuss

Grazing is one of the most important utilization methods of desert steppe, but its fragile ecological characteristics make soil and vegetation extremely sensitive to the disturbance of grazing livestock (Han et al., 2017; Li et al., 2018). In low productivity ecosystems, grazing results in a decrease in soil organic carbon and nitrogen pools (Holst et al., 2007; Hafner et al., 2012). Grazing makes more and more material and energy of grassland be taken away, and the absorption and utilization of plant nutrients can well reflect the growth state and internal characteristics of plants. Grassland degradation under heavy grazing will lead to the decrease of plant biomass and nutrients (Belsky 1986; Acker 1990).

The results showed that the contents of NH_4^+ and NO_3^- in the rhizosphere soil of *Stipa breviflora* under heavy grazing were significantly lower than those under no grazing and light grazing, and the contents of NH_4^+ and NO_3^- were the highest under no grazing. This is different from many studies, previous studies have shown that with the increase of grazing intensity, the nitrogen content in soil increases gradually due to the increase of feces and urine emissions of livestock (Whitehead 2000; Zaman et al., 2009). The reason for this difference is due to the different grazing forms. In this experiment, the livestock were fed in the experimental plot during the day, and were driven back to the cage at night to rest, and most of the feces and urine of livestock were left in the cage (Sun et al., 2018a). The experimental design of other studies is that livestock feed and rest from morning to night in the experimental area, and livestock excreta are distributed in the experimental area, so the soil nitrogen content is relatively high.

We found that the total nitrogen content in roots and leaves of *Stipa breviflora* under grazing prohibition treatment was similar to that under heavy grazing treatment, with high nitrogen content in roots and low nitrogen content in leaves. Based on the analysis of nitrogen assimilation related enzyme activities and nitrogen compound contents in the roots and leaves of *Stipa breviflora* under different grazing intensities, we concluded that high nitrogen (no grazing) and low nitrogen (heavy grazing) environments were not conducive to nitrogen absorption and utilization in the roots of *Stipa breviflora*. In this study, the nitrate nitrogen content in the root system of *Stipa breviflora* under grazing prohibition, moderate grazing and heavy grazing was significantly lower than that under light grazing, and the nitrate nitrogen content in the leaves of *Stipa breviflora* under moderate grazing and heavy grazing was also lower, which indicated that the NO_3^+ absorption capacity of *Stipa breviflora* was weakened by grazing prohibition, heavy grazing and even moderate grazing.

Nitrogen assimilation plays a crucial role in plant life activities, which directly affects the growth and development of plants (Ge et al., 2014). Nitrate reductase (NR), glutamine synthetase (GS), glutamic acid synthetase (GOGAT), glutamic oxaloacetate transaminase (got) and glutamic pyruvate transaminase (GPT) activities directly affect the nitrogen assimilation process of plants, and then affect the assimilation efficiency of nitrogen (Yu et al., 2018). The results showed that the total nitrogen content in roots and leaves of *Stipa breviflora* was significantly correlated with the activities of nitrate reductase, glutamate synthase, glutamate oxaloacetate transaminase and glutamate pyruvate transaminase. NR is a key enzyme in the process of nitrogen metabolism, and its activity is closely related to nitrogen assimilation capacity (Zhang et al., 2008). Part of NO_3^- absorbed by plant roots generates NH_4^+ under the catalysis of NR and NIR, and the other part is transferred to leaves. In this study, NR in the leaves of *Stipa breviflora* was significantly reduced under the medium grazing prohibition and heavy grazing treatment, while the NR in the root system was stronger under grazing prohibition and weak under severe grazing. This indicated that the heavy grazing treatment significantly reduced the transformation of NO_3^- to NH_4^+ in the root system of *Stipa breviflora*, and the conversion of NO_3^- to NH_4^+ in leaves was significantly reduced by grazing prohibition and heavy grazing, which affected the efficiency of nitrogen assimilation.

Under the catalysis of GS and GOGAT, NH_4^+ forms glutamic acid and glutamine, and GS and GOGAT process are carried out simultaneously. Li et al. (2018) studied the process of nitrogen assimilation in maize, and showed that the higher the activity of GS and GOGAT, the stronger the ability of nitrogen assimilation. Our research is slightly different from its results, there was a significant negative correlation between GS and total nitrogen content in roots and leaves of *Stipa breviflora*, that is, the higher GS activity in roots and leaves, the lower total nitrogen content in roots and leaves of *Stipa breviflora*. However, this is similar to the results of Fei et al. (2003), which showed that GS activity did not show the same trend with biomass and nutrient content. The reason may be related to the content of NH_4^+ in plant roots and leaves, because studies have shown that GS is more sensitive to low concentration of NH_4^{+1}, and GS activity will be significantly reduced under high NH_4^+ (Zhang & Xu, 2011). There was a significant positive correlation between GOGAT activity and total nitrogen content in roots and leaves, indicating that grazing treatment with strong GOGAT activity in roots and leaves of *Stipa breviflora* had higher total nitrogen content in roots and leaves, which was the same as previous studies (Wang et al., 2020; Li et al., 2018).

GOT and GPT are important transaminases in the process of nitrogen assimilation in plant roots and leaves. Wang et al. (2020) showed that with the increase of nitrogen application rate, the activities of GOT and GPT in oat leaves increased or increased first and then decreased, indicating that the suitable nitrogen addition was beneficial to the increase of GOT and GPT activities in plant leaves to a certain extent, but the GOT and GPT activities would be weakened when the nitrogen content in soil was too low or too high. This study also showed that GOT and GPT activities were significantly correlated with total nitrogen content in roots and leaves of *Stipa breviflora*, indicating that the higher GOT and GPT activities in roots and leaves of *Stipa breviflora*, the higher total nitrogen content. In this study, GOT and GPT in leaves of *Stipa breviflora* were relatively weak under grazing prohibition and heavy grazing, which was closely related to the low total nitrogen content in leaves of *Stipa breviflora*.

The process of nitrogen assimilation in plants is complex, not only involving many enzymes, but also the amount of substrate produced affects the assimilation efficiency (Zhang & Xu, 2011). Our results showed that heavy grazing significantly reduced the content of soluble protein and amino acids in roots, and significantly increased the content of amino acids in leaves, and correlation analysis showed that this was related to the changes of GS, GOT, GPT and NR activities in roots and leaves. Therefore, we believe that grazing prohibition and heavy grazing significantly affect the formation of substrate in the process of nitrogen assimilation. In our study, the proline content in roots and leaves of *Stipa breviflora* was higher than that of light and moderate grazing, especially in heavy grazing. Grazing can destroy the growth state and adaptability of plants, so it has been considered as the biological stress of most plant species (Shen et al., 2019). The results showed that the content of proline could reflect the stress intensity of plants to a certain extent (Ibarra Caballero et al., 1988; Rasluni et al., 2004). Therefore, we believe that grazing prohibition and heavy grazing are not conducive to the nitrogen absorption, utilization and assimilation of *Stipa breviflora*.

Conclusion

There were significant differences in the activities of nitrogen metabolism enzymes in the roots and leaves of *Stipa breviflora* under grazing prohibition and heavy grazing, which was significantly related to the higher total nitrogen content in the roots and lower total nitrogen content in the leaves of *Stipa breviflora* under grazing prohibition and heavy grazing. The changes of the enzyme activity were mainly manifested in the decrease of NR and GS activity in the root of *Stipa breviflora* under heavy grazing, and the activities of NR, GS, GOGAT and GOT in leaves were decreased. The activities of GS, GOT and GPT in the root system of *Stipa breviflora* decreased, while the activities of NR, GS, GOT and GPT in the leaves decreased under grazing prohibition, which may be the result of the adaptation of plants to grazing. It is suggested that the change of the efficiency of nitrogen assimilation related enzymes in *Stipa breviflora* is related to the decrease of nitrate and ammonium nitrogen content in rhizosphere soil of *Stipa breviflora* under grazing pressure.

Abbreviations

CK: no grazing; LG: light grazing; MG: moderate grazing; HG: heavy grazing; NR: nitrate reductase; GS: glutamine synthetase; GOGAT: glutamic acid synthetase; GOT: Glutamic oxaloacetic transaminase; GPT: glutamic pyruvate transaminase; TN: total nitrogen content; SP: soluble protein; Pro: proline, NN: nitrate nitrogen; FAA: free amino acid.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors declare that they have no competing interests.

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Authors' contributions

AMZ was responsible for writing the manuscript; GDH were responsible for the design and management of the experiment. HLL, YHW, assisted AMZ in sampling and determination of physiological and biochemical indexes. All authors have read and approved the manuscript.

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Figures

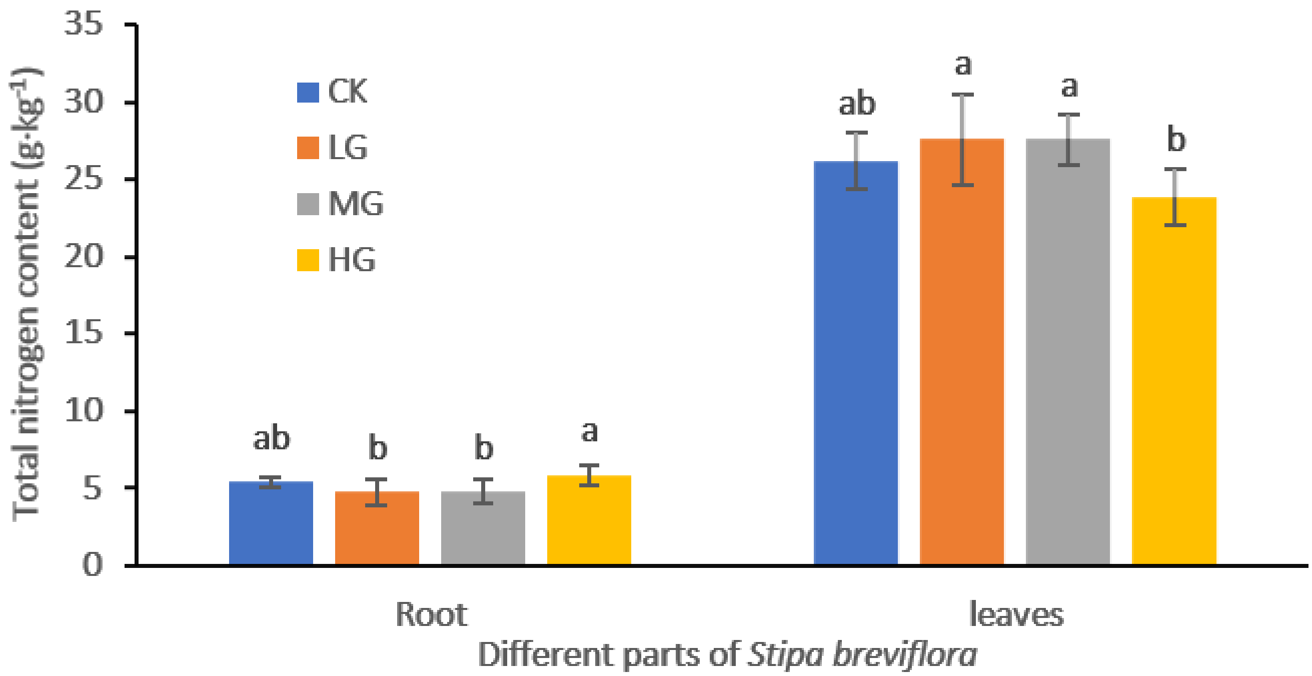

Figure 1

Effect of grazing intensity on total nitrogen content in roots and leaves of Stipa breviflora. Different lowercase letters indicate that the total nitrogen content in roots or leaves of Stipa breviflora is significantly different at 0.05 level between different grazing treatments. CK, LG, MG and HG refer to no grazing, light grazing, moderate grazing and heavy grazing respectively.

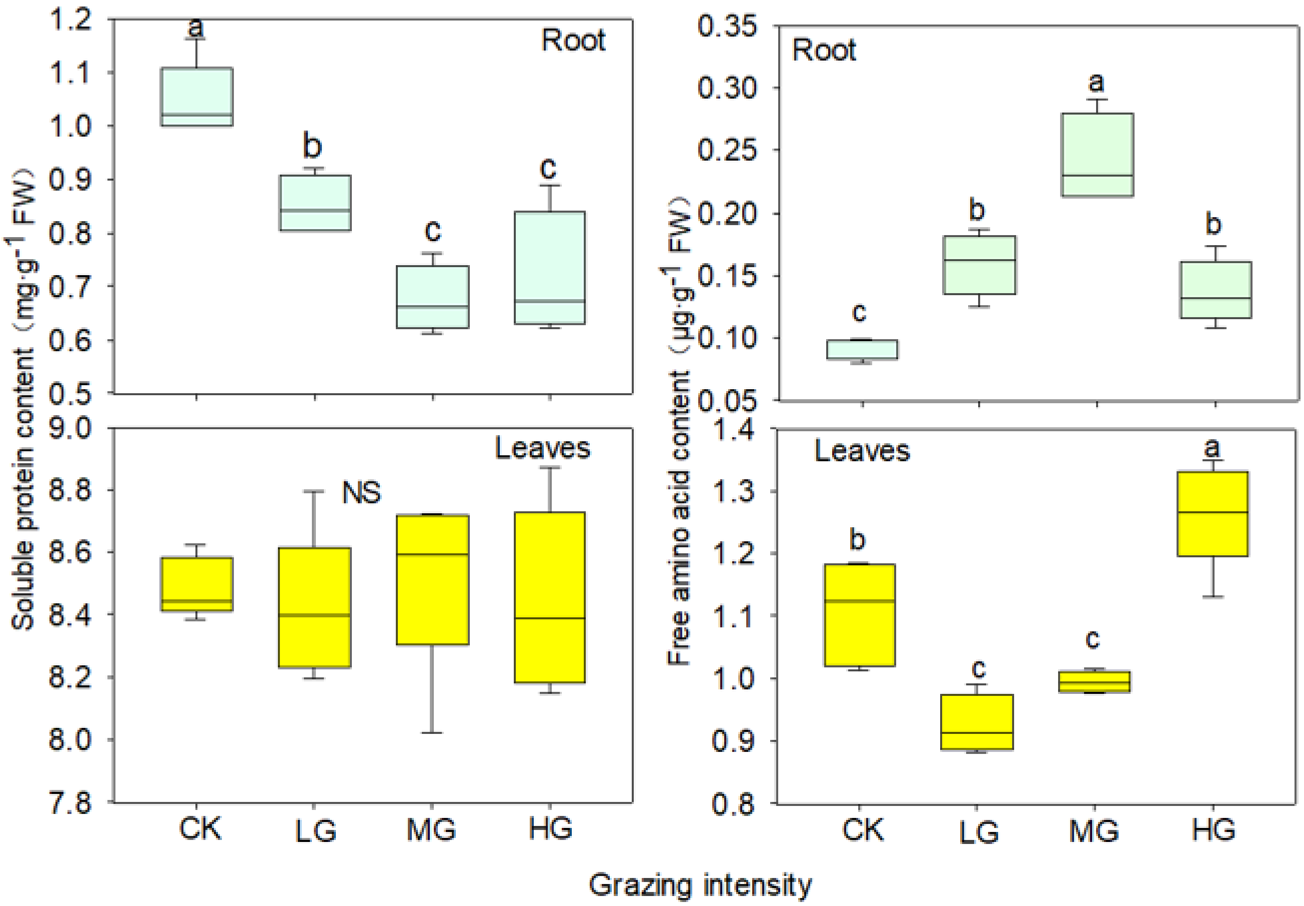

Figure 2

Effects of grazing on soluble protein and free amino acid contents in roots and leaves of Stipa breviflora. Different lowercase letters indicate that the total nitrogen content in roots or leaves of Stipa breviflora is significantly different at 0.05 level between different grazing treatments. "Ns" in the figure means that there is no significant difference among grazing treatments. CK, LG, MG and HG refer to no grazing, light grazing, moderate grazing and heavy grazing respectively. The same as below

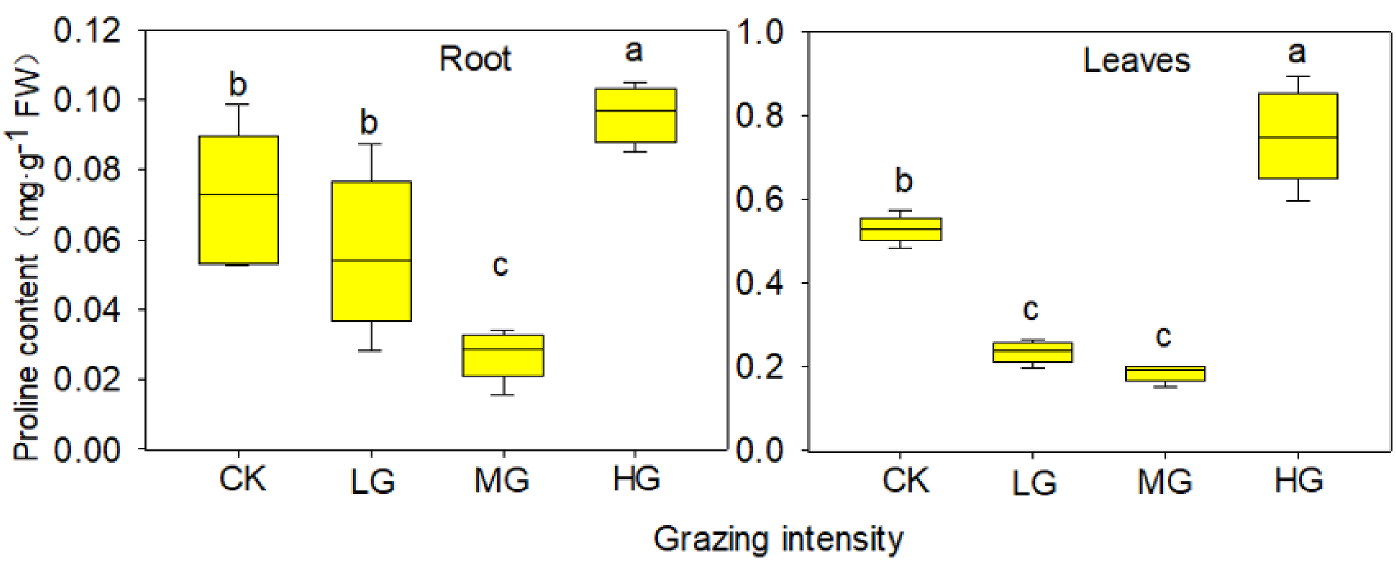

Figure 3

Effect of grazing on proline content in roots and leaves of Stipa breviflora

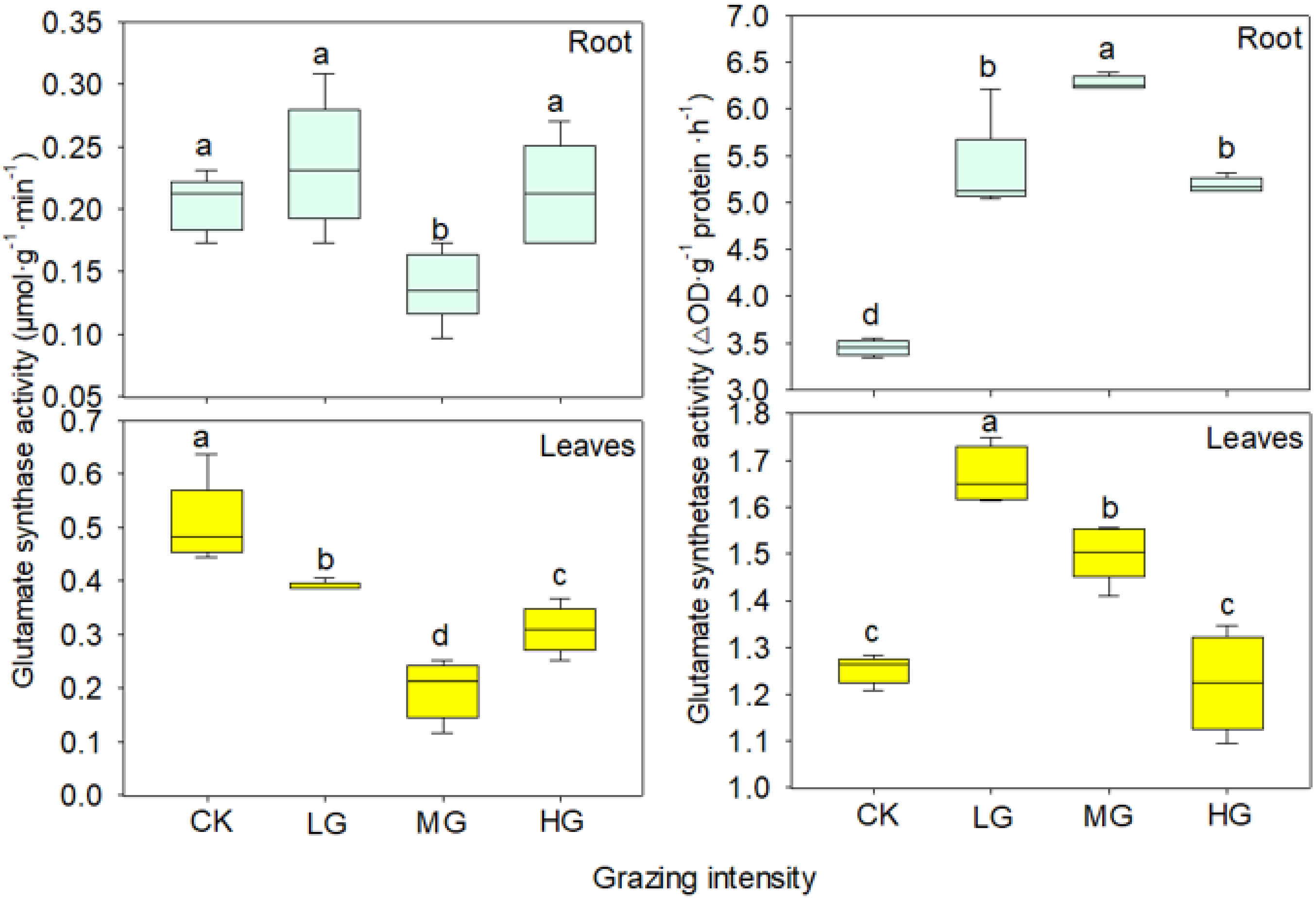

Figure 4

Effect of grazing on glutamate synthetase and glutamine synthetase activity in roots and leaves of Stipa breviflora

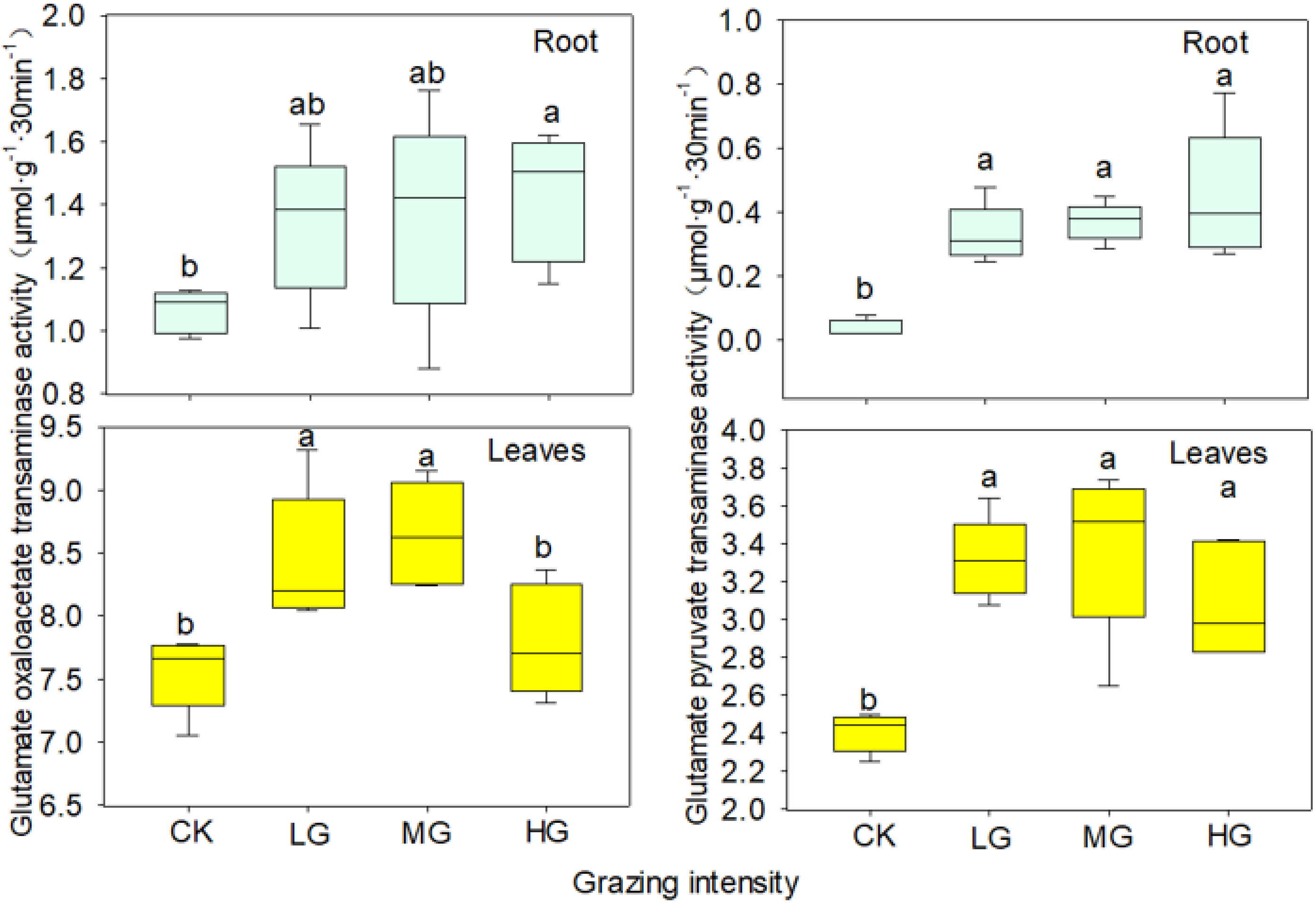

Figure 5

Effects of grazing on activities of glutamic oxaloacetic transaminase and glutamic pyruvate transaminase in roots and leaves of Stipa breviflora

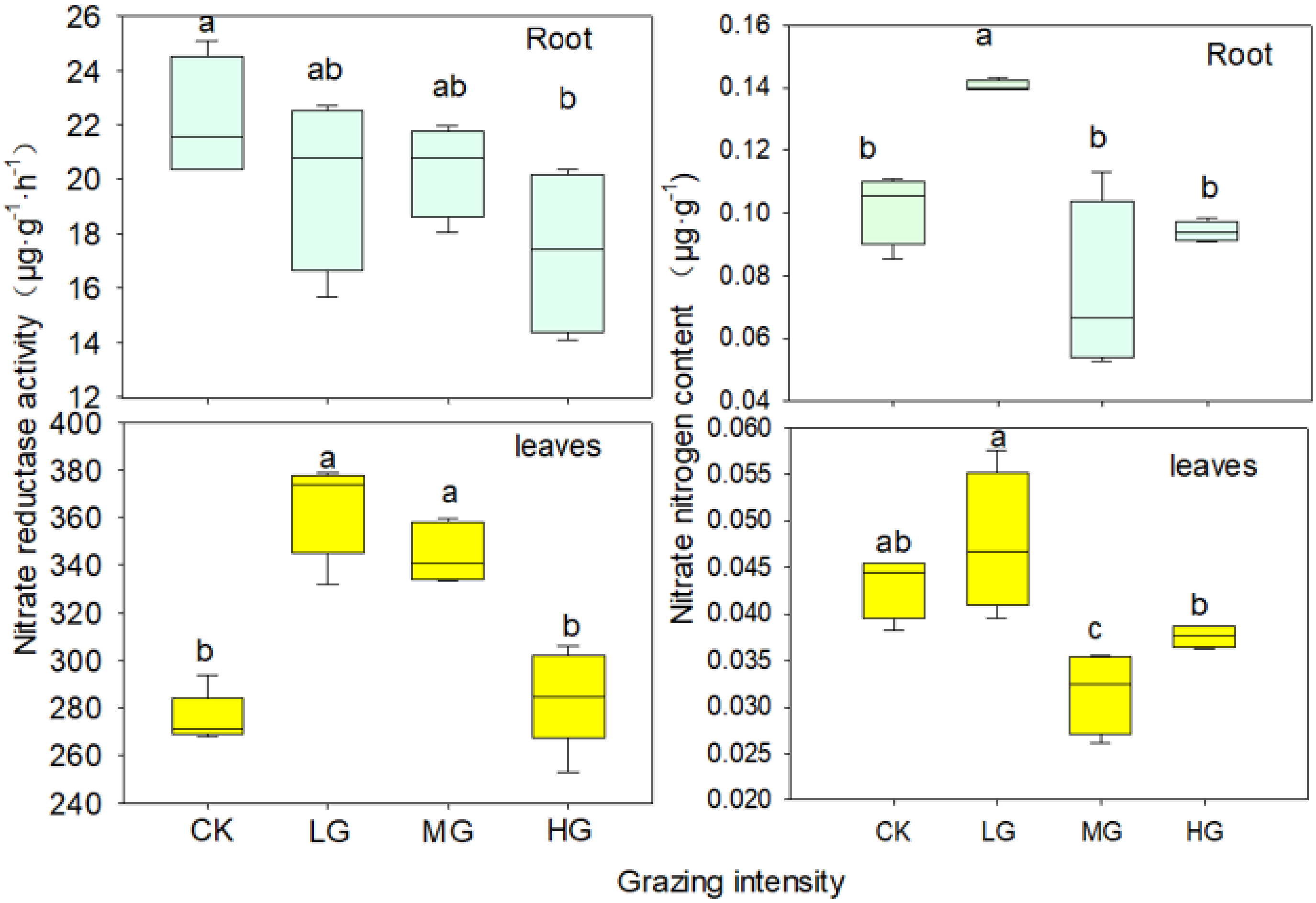

Figure 6

Effects of grazing on nitrate reductase activity and nitrate content in roots and leaves of Stipa breviflora

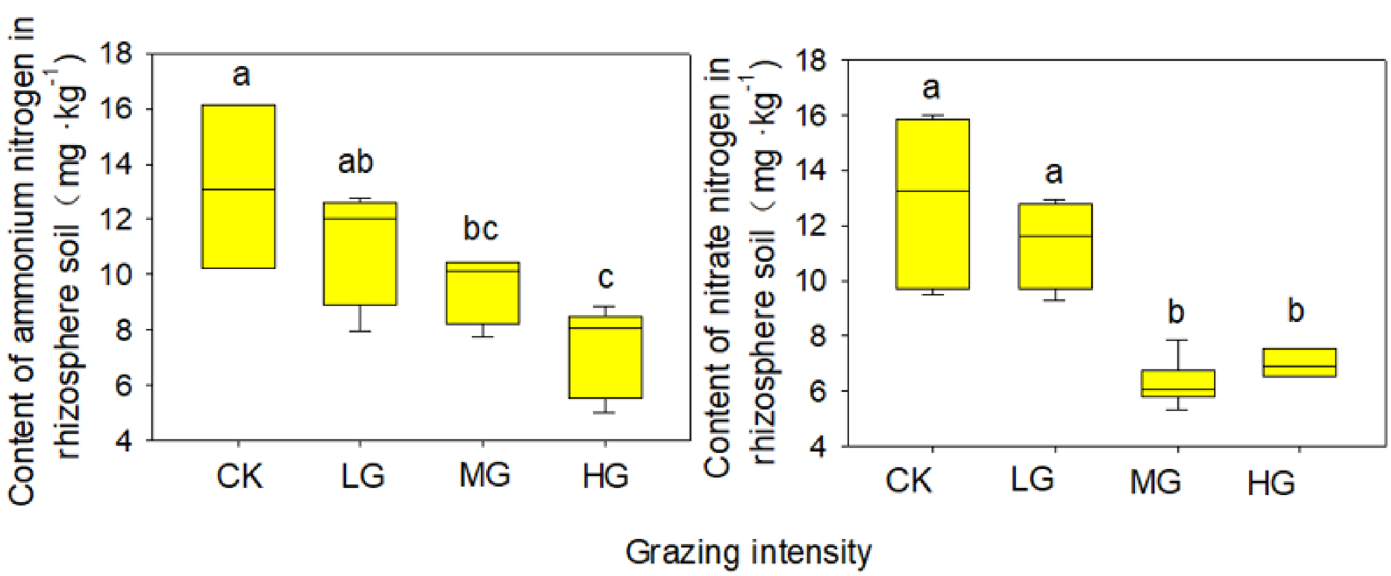

Figure 7

Effects of grazing on the contents of ammonium and nitrate in the rhizosphere soil of Stipa breviflora

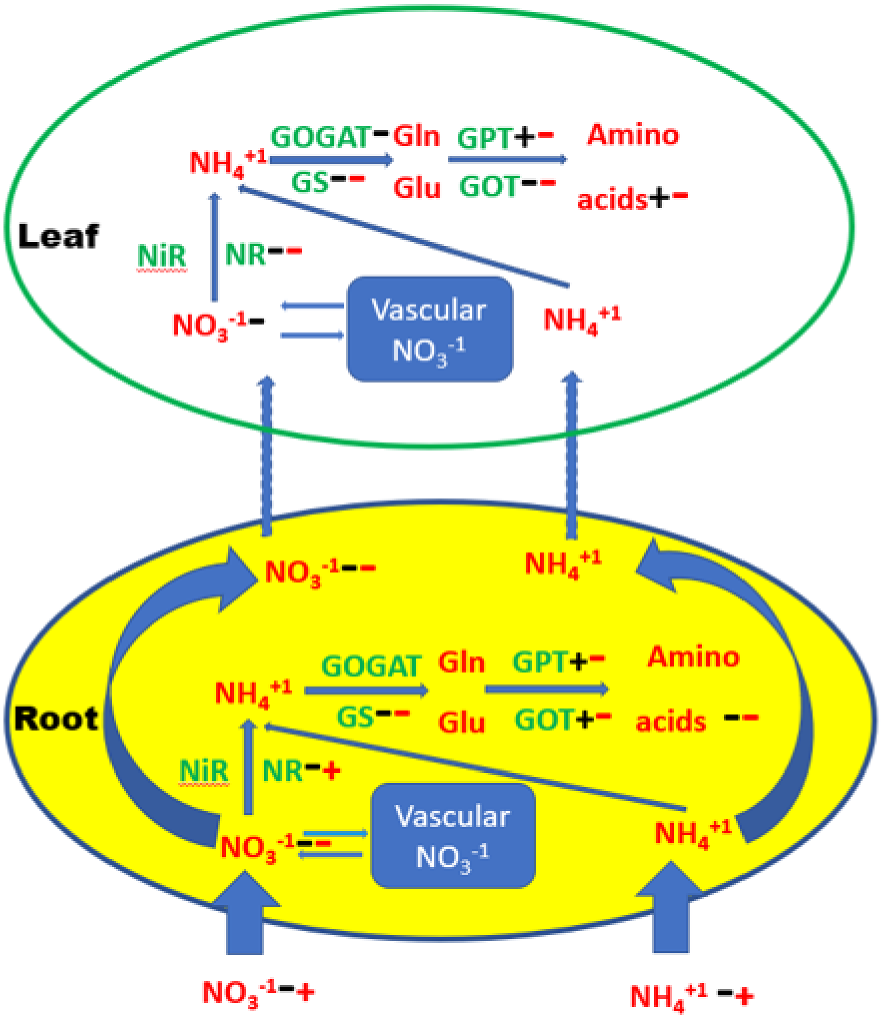

Figure 8

Effect of grazing on nitrogen absorption and assimilation pathway of Stipa breviflora. The black "+" and "-" in the figure represent the positive and negative effects of heavy grazing on the index, and the red "+" and "-" represent the positive and negative effects of grazing prohibition on the index.