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Heavy grazing intensifies antagonistic effects of nitrogen and phosphorus addition on the water use efficiency of grasslands in Inner Mongolia

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Abstract

Background Plant water use efficiency (WUE) is a key indicator of coupling between the carbon and water cycles in grassland ecosystems and is influenced by both grazing and nutrient availability. However, the regulatory effects of grazing intensity on plant WUE under nitrogen (N) and phosphorus (P) addition remain unclear.

Methods This study employed stable carbon isotope techniques in a typical steppe ecosystem in Inner Mongolia, China, to investigate the effects of grazing, N and P addition, and their interactions on the foliar $\delta^{13}\text{C}$ values of four dominant plant species. This research aims to assess the dynamics and underlying mechanisms of grassland WUE under grazing management in the context of global change.

Results Grazing intensity and nutrient addition significantly affected the $\delta^{13}\text{C}_{\text{leaf}}$ values. Heavy grazing increased the $\delta^{13}\text{C}_{\text{leaf}}$ values of *Cleistogenes squarrosa* and *Carex korshinskyi* but decreased those of *Leymus chinensis* and *Stipa grandis*. N addition increased $\delta^{13}\text{C}_{\text{leaf}}$ across all species, whereas P addition significantly increased $\delta^{13}\text{C}_{\text{leaf}}$ in *Cleistogenes squarrosa* but decreased it in the other species. A significant antagonistic interaction between N and P addition was observed, where P inhibited the positive effects of N. This antagonistic effect was amplified under heavy grazing, leading to reduced plant WUE. Structural equation modeling analysis further identified soil temperature and leaf total N as the dominant factors governing plant WUE dynamics, with both factors contributing to an increase in plant WUE.

Conclusions This study highlights that grazing can modulate the effects of N and P addition on WUE in grasslands. Specifically, heavy grazing exacerbated the antagonistic effects of N and P addition on plant WUE, suggesting that overgrazed grasslands under N and P enrichment could face increased water limitation. In light of concurrent environmental changes, future grassland management should consider the interactive effects between grazing intensity and nutrient dynamics on plant water relations, growth and plant community composition.

Keywords Water use efficiency, Stable carbon isotope, Grazing, N addition, P addition

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Introduction

Grasslands, the largest terrestrial biomes, play a pivotal role in soil carbon cycling (Liu et al. 2023). In arid and semiarid grasslands, water is a critical regulator of plant growth, influencing both plant productivity and soil nutrient availability (Liu et al. 2020b; Nguyen et al. 2017). The ability of plants to effectively utilize water is crucial for their competitive advantage (Zhang et al. 2016). Water use efficiency (WUE), which reflects the trade-off between carbon (C) assimilation and water consumption, is a key indicator of a plant's ability to adapt to water stress (Chen et al. 2020; Wang et al. 2023). As climate change and anthropogenic disturbances intensify (Mukherjee et al. 2018; Trenberth et al. 2013), predicting vegetation responses to shifting water regimes becomes increasingly complex. However, many studies have focused on the effects of N and P addition and interactions on grasslands (Lekberg et al. 2021; Li et al. 2022a), whereas few studies have focused on the effects of grazing on carbon and water cycling processes under N and P deposition and its regulatory mechanisms.

Quantifying these interactions requires integrating physiological measurements with isotopic proxies of WUE strategies. Measurements of the relative abundances of the stable isotopes ^{13}C and ^{12}C ($\delta^{13}\text{C}$) in plant organic tissue can provide both long- and short-term indicators of WUE, with higher $\delta^{13}\text{C}$ values correlated with increased WUE (Ehleringer and Cooper 1988; Farquhar et al. 1989). As the dominant land use in grasslands (Gillson and Hoffman 2007), grazing modulates WUE through three primary pathways: herbivory, trampling, and nutrient redistribution via excreta (Liu et al. 2017; Zhou et al. 2020). Mechanistically, grazing-induced physical damage to leaves, particularly reductions in stomatal density, decreases stomatal conductance (g), constraining CO_2 uptake and creating a carbon–water trade-off that suppresses photosynthesis and WUE (Liang et al. 2023; Liu et al. 2016). Concurrently, diminished aboveground biomass reduces the leaf area index (LAI), further limiting photosynthetic capacity (Zhang et al. 2022). Paradoxically, grazing also enhances soil nutrient availability through fecal deposition, which can improve plant N use efficiency and indirectly enhance WUE (Zhou et al. 2020). This dualistic impact is mediated by grazing intensity: heavy grazing exacerbates physical and biomass damage, severely reducing WUE (Cao et al. 2024), whereas light grazing may optimize WUE in certain species by balancing moderate biomass removal with nutrient enrichment (Shen et al. 2019). Therefore, studying the mechanisms affecting plant water utilization strategies across different grazing intensities is essential.

N and P deposition has emerged as an additional critical driver affecting grassland WUE beyond grazing

disturbances. Parallel to grazing impacts, while grazing affects plant–water relationships, atmospheric nutrient deposition introduces additional complexity. As global change intensifies (Estes et al. 2011; Su et al. 2022a), N and P deposition has emerged as an additional critical driver affecting grassland WUE beyond grazing disturbances (Radujković et al. 2021; Vitousek et al. 2010). N addition stimulates nitrogen mineralization, increasing soil N availability. This promotes aboveground biomass production, enhances leaf N content, and improves photosynthetic efficiency, thereby increasing WUE (Grassi and Minotta 2000; Liu et al. 2024). Conversely, P addition enhances root development and water uptake capacity (Ma et al. 2025), while also facilitating nitrogen retention in soils to support photosynthesis (Cheng et al. 2018; Fisk et al. 2013). Synergistic N and P effects are critical: combined N and P additions amplify WUE more than does N alone by alleviating stoichiometric imbalances. For example, P mitigates N-induced nutrient disequilibrium, enabling leaf area expansion while curtailing excessive transpiration. These findings underscore the interdependence of N and P in plant metabolic processes (El-Madany et al. 2021). However, despite these findings, how grazing interacts with N and P enrichment to modulate WUE in dominant grassland species remains unresolved.

The regulatory mechanisms and key drivers of grassland WUE are significantly influenced by both grazing and N and P addition, with substantial overlap in their pathways. Specifically, plant characteristics play a crucial role in modulating WUE. For example, Yang et al. (2022) reported a positive correlation between WUE and both total leaf carbon (structural reinforcement of cell walls reducing water loss) and total leaf N (enhancing photosynthesis-related enzyme activity). N enrichment increases plant WUE by increasing the leaf N content and aboveground biomass (Grassi and Minotta 2000). Moderate grazing may stimulate soil N mineralization via fecal deposition, synergizing with N enrichment to increase leaf N and WUE (Zhou et al. 2020). In contrast, heavy grazing reduces the leaf area index (LAI) and depletes both leaf carbon (via biomass loss) and N (via impaired uptake), even under nutrient addition (Zhang et al. 2022; Su et al. 2022b). These findings highlight that the net effect of grazing on WUE depends on whether nutrient enrichment can offset grazing-induced losses in leaf C and N pools.

Soil properties also mediate plant responses to grazing and nutrient addition, influencing WUE through feedback between belowground and aboveground processes (Zhou et al. 2020). The response of plant WUE to grazing-induced soil moisture reduction is time dependent. Initially, plants may experience reduced WUE due

to a soil moisture deficits, but they can adapt to prolonged soil moisture deficiency by increasing WUE over time (Wang et al. 2020). N and P additions synergistically enhance WUE by increasing aboveground biomass, which reduces soil surface evaporation (Cheng et al. 2018). P addition reduces soil N_2O emissions and NO_3^- leaching, helping maintain the soil N content and enabling more efficient water use by plants for growth. Improved N use efficiency enhances WUE, improving ecosystem productivity and stability (Chen et al. 2017, 2022; Mori et al. 2013). Grazing enhances soil nutrient availability through the deposition of animal excreta, which improves plant WUE (Liu et al. 2017; Zhou et al. 2020). The overlap between grazing and N and P addition on soil properties and vegetation characteristics may enhance or weaken these impacts. However, the interactive effects of grazing and N and P addition on plant WUE regulation remain unclear.

Here, we evaluated the effects of grazing intensity on plant WUE under N and P addition in semiarid grasslands. We conducted a grazing experiment in which sheep were subjected to N and P fertilization either separately or simultaneously over three growing seasons (2019–2021). Specifically, we tested the following hypotheses: (1) Grazing intensity can regulate the effects of N and P addition on plant WUE because both promote WUE. Different grazing intensities affect plant WUE, potentially altering the impact of N and P addition. (2) Soil nutrients are the main driver of WUE because they can influence plant WUE by affecting leaf nutrient content, biomass, and other factors. Addressing these hypotheses will help understand the changes in plant WUE in grasslands under grazing and N and P deposition and the underlying mechanisms, which is crucial for formulating grassland water management strategies.

Materials and methods

Study site

The study was conducted at the Grassland Ecosystem Research Station, 45 km northeast of Xilinhot, Inner Mongolia, China ($44^{\circ}15'N$, $116^{\circ}31'E$). The area has a temperate, semiarid climate with an average annual temperature of 2.8°C and precipitation of 272 mm, mostly during the growing season (May–September). The elevation is 1,146 m. According to the Chinese soil classification, the soil is sandy loam chestnut soil. On the basis of previous work and vegetation surveys of this experimental platform, the main plant species in the plots included *Stipa grandis*, *Leymus chinensis*, *Cleistogenes squarrosa*, and *Carex korshinskyi* (Li et al. 2022b).

Experimental design

The experimental site was established in 2019 using a randomized block design and included three grazing treatments (0, 2, and 4 sheep ha^{-1} , representing no, moderate, and heavy grazing, respectively). The grazing livestock used were 2 year-old Mongolian sheep, with an average weight of 30 kg. Grazing occurred monthly from June–August each year, with each event ending when the sward height of the moderately grazed plots reached ~ 6 cm. Within each grazing block, four nutrient amendment subplots ($6\text{ m} \times 8\text{ m}$) were established: control (no fertilization), N addition (urea, 46% N), P addition (superphosphate, 16% P_2O_5), and combined N and P addition. The experimental matrix yielded 36 independent plots (3 grazing levels $\times$ 4 fertilization regimes $\times$ 3 replicates). A 3-m-wide grazing path was established between two rows of plots, with a 1-m interval between adjacent plots. All the treatments were applied synchronously during the growing season (June–August) from 2019 onward. The fertilizers were surface-broadcast at rates of $10\text{ g N m}^{-2}\text{ yr}^{-1}$ and $5\text{ g P m}^{-2}\text{ yr}^{-1}$, corresponding to local management practices.

Field sampling

The plant samples were collected in mid-August 2021, during the peak growing season. A five-point sampling method was used to collect 5–10 individuals from the dominant plant species in each plot. The plants were placed in paper envelopes and transported to the laboratory. The soil samples were collected using a soil auger (70 mm internal diameter) and divided into three layers: 0–10, 10–20, and 20–30 cm. Five replicate samples were taken from each plot, mixed thoroughly, and placed in a resealable bag. To avoid edge effects, the sampling points for the plants and soil were located at least 30 cm from the edges of the plots.

Laboratory measurements

The plant samples were separated into stems, leaves, and roots, washed with deionized water to remove dust, heated at 105°C for 30 min to inactivate enzymes, and then dried to a constant weight at 65°C . The roots and leaves were first ground with a plant grinder (FW100, TEISITE, China) and then finely ground with a ball mill (MM400, Retsch, Germany) for further analysis. The soil samples were air-dried, passed through a 2 mm sieve, ground into a fine powder, and passed through a 100-mesh sieve. The ground soil and plant samples were then placed in aluminum foil cups, air removed, and sealed. These processing steps ensured sample homogeneity and suitability for subsequent analysis and testing. The $\delta^{13}\text{C}$ values of the plant and soil samples were measured using a vario ISOTOPE select elemental analyzer coupled with

an Isotope 100 stable isotope mass spectrometer (Elementar, Germany). The $\delta^{13}\text{C}$ values of the samples were calculated as follows:

$$\delta^{13}\text{C} = \frac{R_{\text{Sample}}}{R_{\text{Standard}}} \times 1000\text{‰} \quad (1)$$

where R_{Sample} is the $^{13}\text{C}/^{12}\text{C}$ ratio of the sample and R_{Standard} is the $^{13}\text{C}/^{12}\text{C}$ ratio of the standard. The internationally accepted Vienna Pee Dee Belemnite (VPDB) standard was used for $\delta^{13}\text{C}$ measurements.

Soil element monitoring

Soil temperature (ST) and soil moisture (SM) were measured using a needle-type soil moisture meter (HH2, Delta-T Devices, UK). The soil pH was measured in deionized water at a 1:2.5 w/v ratio. Available P (AP) was determined by 0.5 mol/L sodium bicarbonate extraction followed by the molybdenum-antimony colorimetric method. The soil available N (AN) was measured with an AA3 flow analyzer, with ammonium-N and NO_3^- -N used as the main indicators. The total carbon (TC) and total N (TN) contents of the plant and soil samples were determined using an Isotope 100 stable isotope mass spectrometer coupled with an elemental analyzer.

Data analysis

All the statistical analyses were conducted using R statistical software, version 4.1.3, and SPSS ver. 26.0 (IBM Corp., USA). The significance level was set at $p < 0.05$. The differences in the soil properties and vegetation characteristics between the treatments were tested for significance by using a one-way ANOVA with the least significant difference (LSD) multiple comparison (SPSS ver. 26.0). A linear model was used to examine the effects of grazing, N addition, and P addition on plant leaves ($\delta^{13}\text{C}_{\text{leaf}}$) and root $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{root}}$) (SPSS ver. 26.0). We used random forest modeling to identify the major factors that impact $\delta^{13}\text{C}_{\text{leaf}}$ and $\delta^{13}\text{C}_{\text{root}}$ in plants. We then used ordinary least squares (OLS) regression models to demonstrate the relationships between the $\delta^{13}\text{C}_{\text{leaf}}$ and $\delta^{13}\text{C}_{\text{root}}$ values in plants and soil properties and vegetation characteristics. In addition, we used piecewise structural equation modeling (PSEM) to examine the common responses of plant leaves and roots to soil physicochemical properties and plant traits. The PSEM method is widely applied in ecological research to explore causal relationships among multiple variables (Lefcheck 2016). In this study, a model was constructed on the basis of the interactions among soil physicochemical properties, plant traits, and environmental factors, with several pathways defined and the significance of each pathway evaluated. All path

coefficients were estimated using the PSEM package in R software, and model fit was optimized through step-wise adjustments and model comparisons (Grace 2006). This approach allowed us to analyze effectively the mechanisms by which plant leaves and roots respond to soil physicochemical properties and uncover their potential ecological implications. Three-way analysis of variance and a general linear model were performed using SPSS ver. 26.0 (IBM Corp., USA), and figures were generated using Origin 2023 (OriginLab, USA).

Results

Interaction between grazing and N and P addition on $\delta^{13}\text{C}$

The $\delta^{13}\text{C}_{\text{leaf}}$ values of different plant species were significantly influenced by grazing intensity and nutrient addition. Heavy grazing significantly increased the $\delta^{13}\text{C}_{\text{leaf}}$ values of *C. squarrosa* and *C. korshinskyi* (Fig. 1c, d). N addition significantly increased the $\delta^{13}\text{C}_{\text{leaf}}$ values of all four plant species, whereas P addition significantly increased the $\delta^{13}\text{C}_{\text{leaf}}$ values of *C. squarrosa* (Fig. 1c). Additionally, under moderate grazing, simultaneous N and P addition significantly increased the $\delta^{13}\text{C}_{\text{leaf}}$ values of *C. korshinskyi* (Fig. 1d). This suggests that the effects of grazing and nutrient addition on WUE (as indicated by $\delta^{13}\text{C}$) vary notably among species and that grazing intensity amplifies the effects of nutrient addition, particularly under heavy grazing conditions. Three-factor interaction analysis revealed that heavy grazing combined with N addition significantly increased the $\delta^{13}\text{C}_{\text{leaf}}$ values of *L. chinensis* and *S. grandis* ($p < 0.05$). Heavy grazing combined with P addition significantly decreased the $\delta^{13}\text{C}_{\text{leaf}}$ values of *L. chinensis*, *S. grandis*, and *C. squarrosa* but significantly increased the $\delta^{13}\text{C}_{\text{leaf}}$ values of *C. korshinskyi* ($p < 0.05$). N and P addition significantly decreased the $\delta^{13}\text{C}_{\text{leaf}}$ values of *L. chinensis* and *C. korshinskyi* ($p < 0.05$). N and P addition under heavy grazing significantly reduced the $\delta^{13}\text{C}_{\text{leaf}}$ values of all the plant species ($p < 0.05$, Table 1).

The $\delta^{13}\text{C}_{\text{root}}$ values of different plant species were influenced by grazing and nutrient addition, with varying effects observed across treatments. Under the no-grazing control, N addition significantly increased the $\delta^{13}\text{C}_{\text{root}}$ values of *L. chinensis* and *C. korshinskyi*, but simultaneous N and P addition significantly reduced the $\delta^{13}\text{C}_{\text{root}}$ values of *L. chinensis* (Fig. 2a, d). Under moderate grazing, the addition of either N or P alone significantly increased the $\delta^{13}\text{C}_{\text{root}}$ values of *L. chinensis* (Fig. 2a). Three-factor interaction analysis indicated that moderate grazing combined with N increased the $\delta^{13}\text{C}_{\text{root}}$ values of *L. chinensis*, whereas N and P addition significantly reduced the $\delta^{13}\text{C}_{\text{root}}$ values of *L. chinensis*. Under heavy grazing, N and P addition significantly increased the

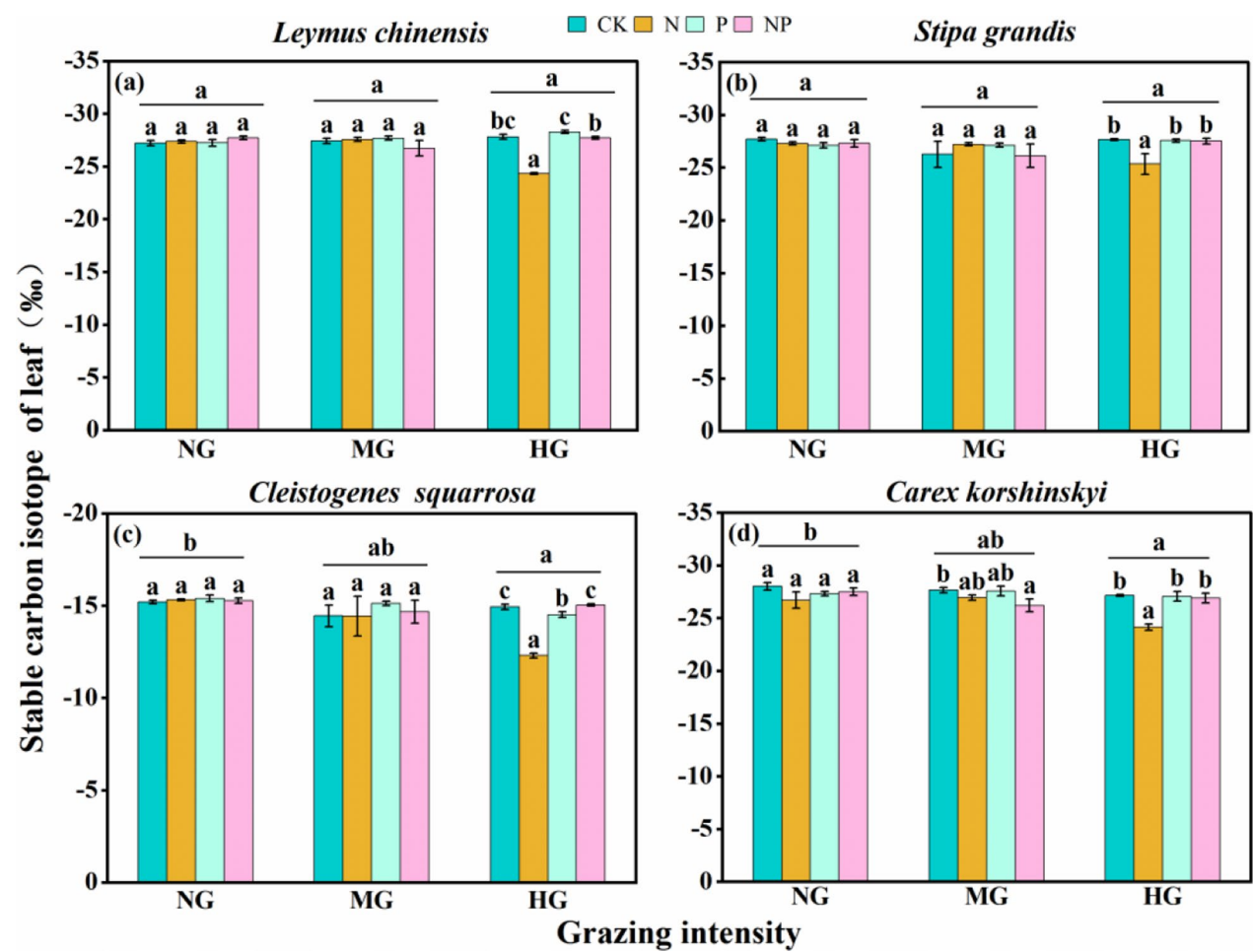


Fig. 1 Responses of the $\delta^{13}C_{leaf}$ values of typical dominant grassland species (*Leymus chinensis*, *Stipa grandis*, *Cleistogenes squarrosa*, and *Carex korshinskyi*) to grazing and N and P addition. Each group of data in the bar chart represents the mean of multiple replicate experiments, and the error bars indicate the standard deviation. Grazing or N and P treatment results labeled with the same lowercase letter are not significantly different (significance level, $p < 0.05$). NG no grazing, MG moderate grazing, HG heavy grazing; columns with different colors represent different fertilization treatments

Table 1 Analysis of the interaction effects of grazing and N and P addition on $\delta^{13}C_{root}$ and $\delta^{13}C_{leaf}$ of major dominant species of plants in typical grasslands (F value)

Treatment	$\delta^{13}C_{leaf}$				$\delta^{13}C_{root}$			
	<i>Leymus chinensis</i>	<i>Stipa grandis</i>	<i>Cleistogenes squarrosa</i>	<i>Carex korshinskyi</i>	<i>Leymus chinensis</i>	<i>Stipa grandis</i>	<i>Cleistogenes squarrosa</i>	<i>Carex korshinskyi</i>
G	1.8	2.02	7.11**	6.89**	1.21	1.42	1.1	2.01
N	17.85***	4.14^	3.23^	19.52***	9.37***	0.2	1.48	7.06*
P	13.03**	1.69	5.59*	1.85	23.94**	0.08	1.18	1.12
G×N	16.83***	3.32^	1.78	1.51	5.63***	0.31	0.81	1.48
G×P	16.09***	3.81*	1.75	4.80*	0.67	0.14	0.6	0.12
N×P	4.59*	1.18	3.00^	6.49*	40.81***	0.49	0.02	1.06
G×N×P	12.16***	6.51**	6.01**	4.46*	12.22***	1.49	1.68	0.94

Three-way ANOVA was performed on the $\delta^{13}C_{root}$ and $\delta^{13}C_{leaf}$ values; ** $p < 0.01$, * $p < 0.05$, ^ $p < 0.1$

The bolded content is to highlight the results

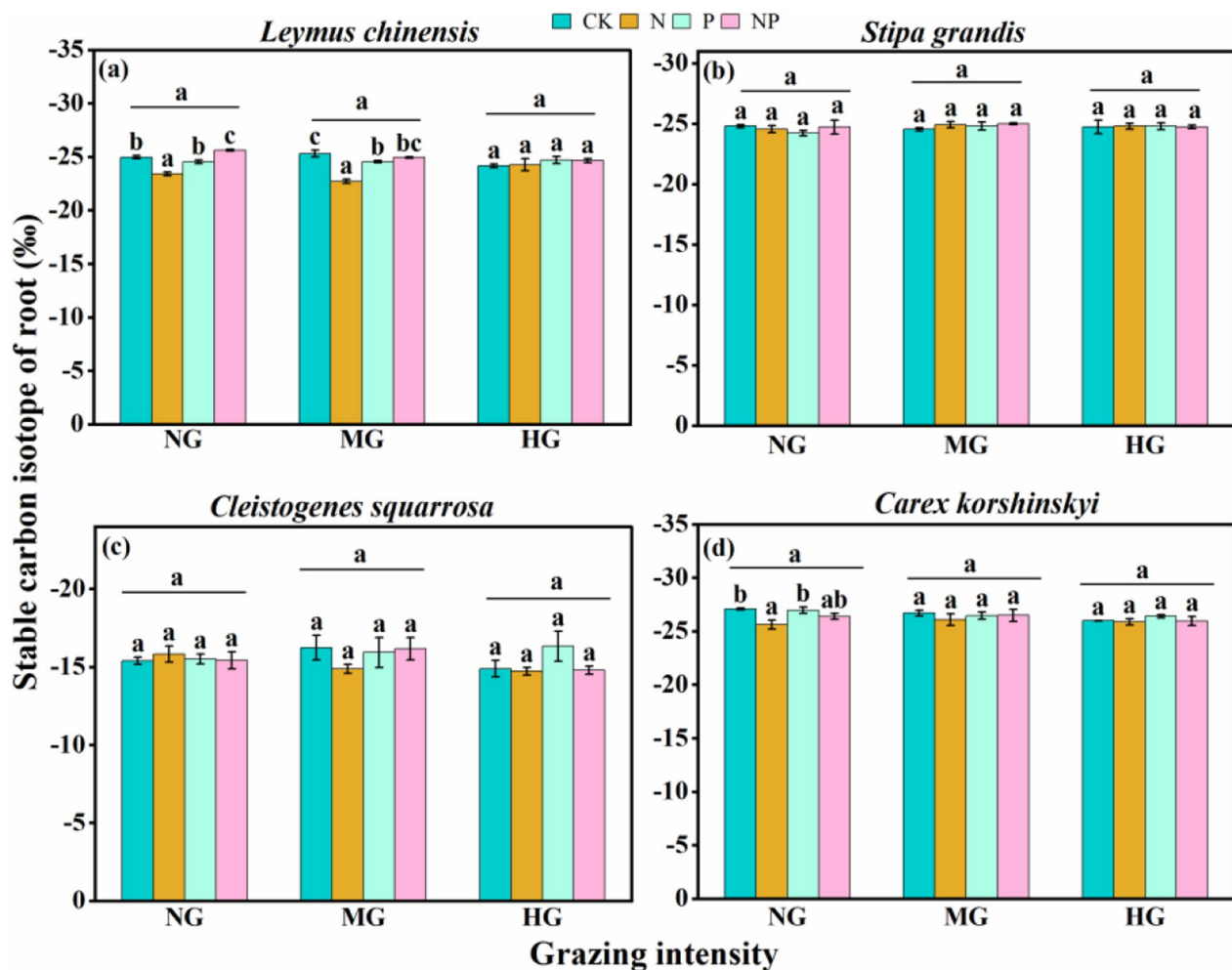


Fig. 2 Effects of grazing and N and P addition on $\delta^{13}C_{root}$ values of the dominant grassland species. Each group of data in the bar chart represents the mean of multiple replicate experiments, and the error bars indicate the standard deviation. Grazing or N and P treatment results labeled with the same lowercase letter do not differ significantly (significance level, $p < 0.05$). NG no grazing; MG moderate grazing, HG heavy grazing; columns with different colors represent different fertilization treatments

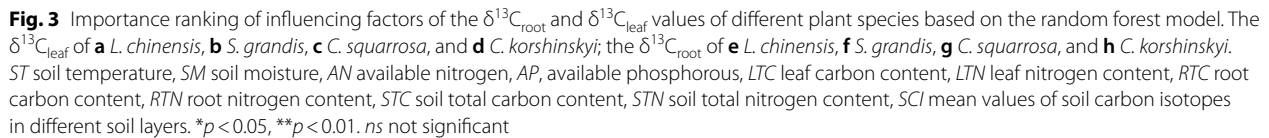
$\delta^{13}C_{root}$ values of *L. chinensis* ($p < 0.001$, Table 1). In general, the interaction effects of grazing and nutrient addition on $\delta^{13}C_{root}$ were relatively weak, but the impact of nutrient addition under moderate grazing was more pronounced in certain species.

Major factors affecting $\delta^{13}C$ in plants

The variations in $\delta^{13}C_{leaf}$ and $\delta^{13}C_{root}$ are driven primarily by the combined effects of plant nutrient content and soil physicochemical properties, with different plants exhibiting sensitivity to specific factors. Random forest model analysis revealed that the $\delta^{13}C_{leaf}$ values of the different plant species were predominantly influenced by leaf nutrient contents (C and N contents) and soil factors. The $\delta^{13}C_{leaf}$ values of *L. chinensis* were significantly affected by the LTC and LTN (Fig. 3a), whereas those of *S. grandis* were driven mainly by AN (Fig. 3b).

In contrast, *C. squarrosa* and *C. korshinskyi* were influenced by the ST and STC, respectively (Fig. 3c, d). Additionally, the $\delta^{13}C_{root}$ values of *S. grandis* were primarily determined by $\delta^{13}C_{soil}$ (Fig. 3f), ST and AN were the major factors affecting the $\delta^{13}C_{leaf}$ values of *C. squarrosa* (Fig. 3g), whereas the $\delta^{13}C_{root}$ values of *C. korshinskyi* were shaped by a combination of root N content, STC, AN, and ST (Fig. 3h). The sensitivity of different plants to specific factors reflects their unique adaptation strategies and functional traits within the ecosystem.

Next, we used linear regression analysis to explore the relationships between the $\delta^{13}C_{leaf}$ and $\delta^{13}C_{root}$ values of various grassland plants and environmental factors. The $\delta^{13}C_{leaf}$ values of *L. chinensis* were significantly positively correlated with the LTC and significantly negatively correlated with the LTN (Fig. 4a), implying that an increase in the LTC increased the WUE of *L. chinensis*, whereas



Grazing intensity and nutrient addition significantly affect key soil and plant traits, and their interactions exhibit complex patterns. ANOVA revealed that

The correlation between the aboveground biomass and $\delta^{13}\text{C}_{\text{leaf}}$ values varied across grassland species. For *L. chinensis* and *S. grandis*, relatively high $\delta^{13}\text{C}_{\text{leaf}}$ values were associated with relatively high aboveground biomass (Fig. 6a, b), suggesting that these species tend to exhibit relatively high WUE values as biomass increases. In contrast, *C. squarrosa* and *C. korshinskyi* were negatively correlated, with $\delta^{13}\text{C}_{\text{leaf}}$ values decreasing as aboveground biomass increased (Fig. 6c, d), indicating that these species may adopt alternative resource

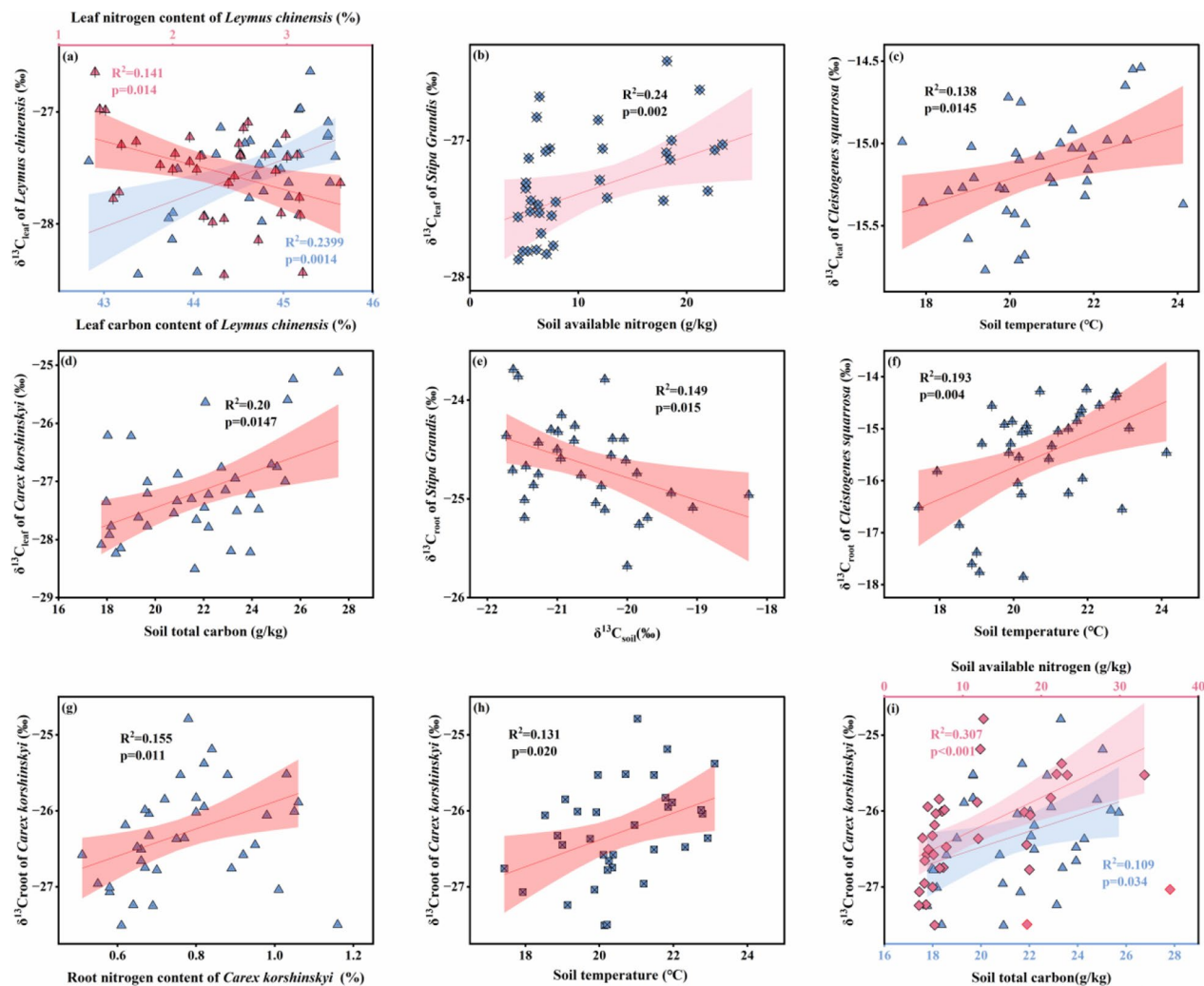


Fig. 4 Correlation analysis of $\delta^{13}\text{C}_{\text{leaf}}$ and $\delta^{13}\text{C}_{\text{root}}$ values with important factors. LTC, leaf carbon content; LTN, leaf nitrogen content; RTN, root nitrogen content; STC, soil total carbon content; STN, soil total nitrogen content; SCL, mean soil carbon isotope values in different soil layers. The shaded area indicates the 95% confidence limit ($p < 0.05$)

allocation strategies or exhibit distinct ecological adaptations under conditions of relatively high biomass. These patterns highlight species-specific responses to biomass accumulation and water use, underscoring the functional diversity within grassland ecosystems.

Discussion

Interactive effects of grazing and N and P addition on plant WUE

Nutrient enrichment and herbivore pressure are key drivers affecting the composition, diversity, and function of plant communities in grassland ecosystems (Borer et al. 2014; Clark and Tilman 2008; Estes et al. 2011). Previous studies have often reported that the interaction between N and P is not significant, or is additive in nature (Jiang et al. 2021; Luo et al. 2020; Sundqvist et al. 2014; Yue

et al. 2017). We found that the simultaneous addition of N and P significantly reduced the plant WUE, whereas P addition alone had no significant effect. This suggests that N addition triggers the negative effects of P and that P addition nullifies the positive effects of N, leading to an unchanged WUE when N and P are added simultaneously. These findings indicate an antagonistic interaction effect between N and P on plant WUE. A possible explanation for this antagonism is that P addition promotes N leaching by releasing DON (Neff et al. 2000) and stimulates N emissions (e.g., N_2O) by providing abundant nitrification or denitrification substrates (Mehnaz et al. 2019). These mechanisms reduce the availability of soil nutrients and counteract the beneficial effects of N addition.

Our results demonstrate that heavy grazing exacerbates the antagonistic effects of N and P on plant WUE. This is

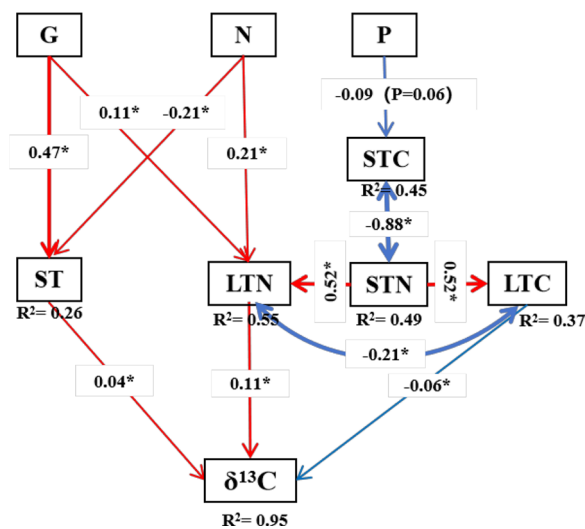


Fig. 5 Effects of grazing and nitrogen and phosphorus addition on plant carbon isotopes detected by piecewiseSEM modeling. Red and blue arrows indicate significant positive and negative relations (*, $P < 0.05$), respectively, with path coefficients shown on the arrows. G is a grazing treatment, N is a nitrogen treatment, P is a phosphorus treatment. LTC, leaf carbon content; LTN, leaf nitrogen content; STC, soil total carbon content; STN, soil total nitrogen content; $\delta^{13}\text{C}$, plant carbon isotope values. See Fig. 3 for the indicators of STC, ST, STN, LTC, and LTN. Fisher's $C = 5.422$ with P -value = 0.942 and on 12 degrees of freedom

likely due to the fact that heavy grazing causes soil compaction, reduces aboveground biomass, increases soil temperature, and promotes N leaching (Li et al. 2021a, 2021b; Neff et al. 2000). Our results indicate that in heavily grazed areas, N addition can increase plant WUE, whereas P addition has no significant effect on WUE. This confirms that temperate grasslands in the Northern Hemisphere are limited primarily by N rather than P (Zhang et al. 2023). Previous studies have shown that N and P addition increases plant WUE, but they overlooked the regulatory role of grazing in this effect (El-Madany et al. 2021; Shen et al. 2022; Wang et al. 2018). A relatively high WUE typically enhances grassland productivity (Cui et al. 2024), which in turn may stabilize grassland productivity. Our study highlights the interactive effects of grazing and nutrient addition on plant ecological adaptability and grassland ecosystem function, providing important insights for understanding plant water use and improving grassland management strategies.

We found that heavy grazing and N and P addition decreased $\delta^{13}\text{C}_{\text{leaf}}$ but increased the $\delta^{13}\text{C}_{\text{root}}$ values of *L. chinensis*. This inconsistent response may be due to differences in the biochemical compositions of leaves and roots, as different compositions have different $\delta^{13}\text{C}$ values (Dungait et al. 2008). The main chemical components of photosynthetic organs (leaves) are lignin and

lipids, while the main components of nonphotosynthetic organs (roots) are cellulose and hemicellulose (Winkler et al. 1978). Carbonaceous compounds with lower $\delta^{13}\text{C}$ values than the initial photosynthetic products are used to synthesize lignin and lipids, whereas the remaining ^{13}C -enriched material is used to synthesize cellulose and is transferred to the outer parts of the leaves (Hobbie and Werner 2003). Under heavy grazing, animal feeding reduces the number of leaves and decreases photosynthesis, exacerbating the antagonistic effects of N and P. This leads to a reduction in the C source for synthesizing lignin and lipids, whereas the ^{13}C -enriched C source increases, ultimately resulting in decreased $\delta^{13}\text{C}_{\text{leaf}}$ values and increased $\delta^{13}\text{C}_{\text{root}}$ values (Leavitt and Long 1986). Our study reveals the mechanisms by which grazing and nutrient addition affect the $\delta^{13}\text{C}$ values of plant roots, providing a more comprehensive and reliable scientific basis for understanding the response of C and water cycling processes in grassland ecosystems.

Key factors affecting plant WUE under grazing and N and P addition

Our study explored how grazing intensity and N and P addition interact to influence plant WUE in semiarid grasslands. We found that grazing and nutrient addition act as key drivers of plant WUE, with significant effects varying across species (Fig. 1). Specifically, N and P addition improved the nutritional status of the plants, increasing their carbon and N contents and thereby enhancing their photosynthetic efficiency and WUE. However, heavy grazing involves complex interactions with nutrient addition, leading to a reduction in WUE. This reduction is likely due to altered soil conditions, such as compaction and decreased nutrient availability, as well as changes in plant morphology, including reduced leaf area and altered root-to-shoot ratios. These findings underscore the importance of considering both grazing and nutrient factors together to understand their collective impact on plant WUE.

In terms of response mechanisms common to plant species, $\delta^{13}\text{C}$ is influenced primarily by leaf traits, especially the TC and TN contents in leaves. Grazing and N addition directly promote the LTN, whereas P addition indirectly enhances the LTN by increasing the STN (Fig. 5). This increase in N improves the plant's carboxylation capacity and photosynthetic rate, ultimately increasing WUE (Farquhar et al. 1982). However, the LTN decreases as the LTC increases, which in turn reduces the photosynthetic rate and suppresses WUE (Fig. 5). This process indicates that N and P addition optimizes the nutritional status of plant carbon and N, increasing the photosynthetic capacity and WUE. Additionally, we determined that the ST is the key soil

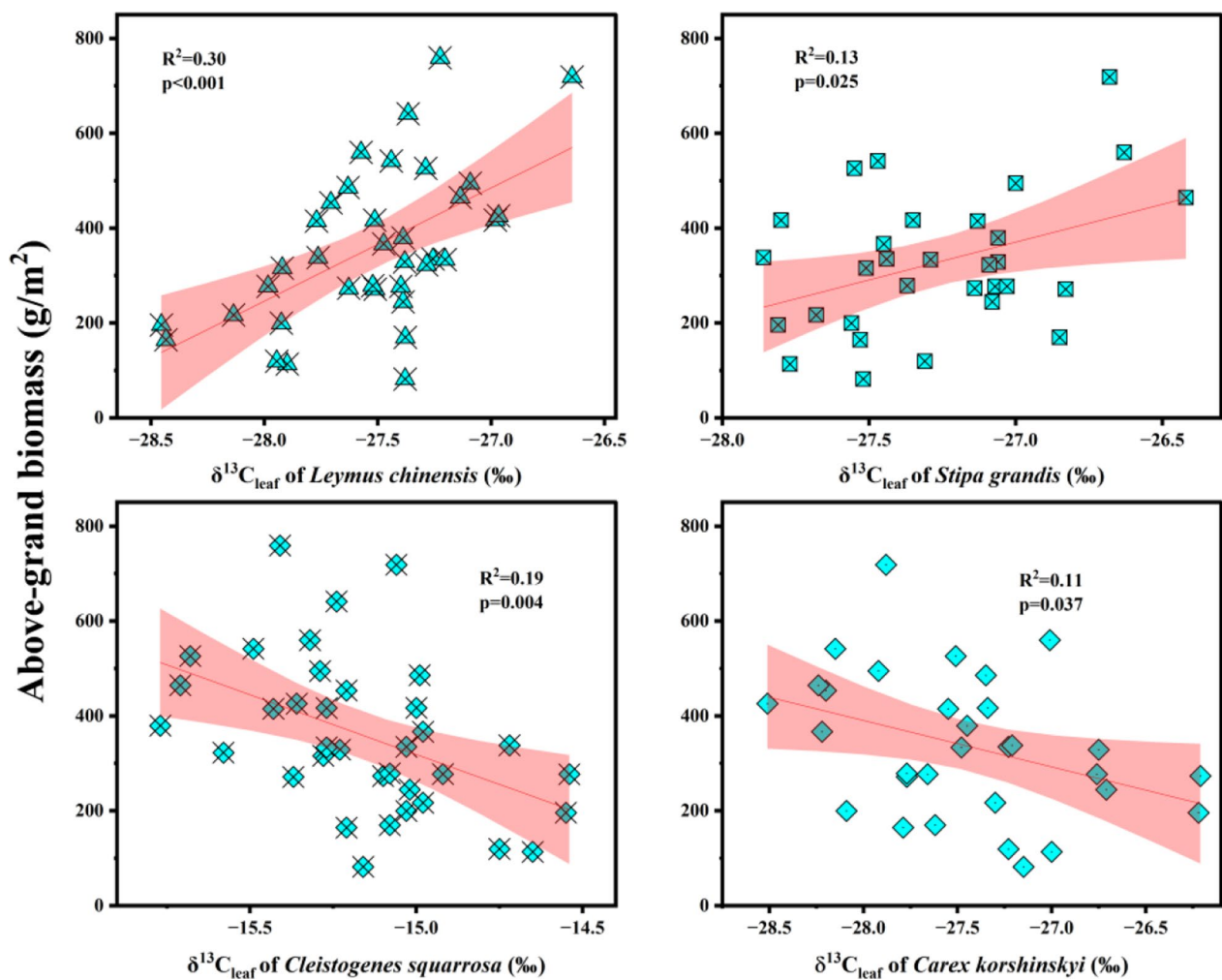


Fig. 6 Relationship between aboveground biomass and $\delta^{13}\text{C}_{\text{leaf}}$. The shaded area indicates the 95% confidence limit ($p < 0.05$). Aboveground biomass in relation to the $\delta^{13}\text{C}_{\text{leaf}}$ of **a** *Leymus chinensis*, **b** *Stipa grandis*, **c** *Cleistogenes squarrosa*, and **d** *Carex korshinskyi*

property influencing plant WUE. N addition reduces the ST by increasing biomass, decreasing solar radiation, and increasing the SM (Guo et al. 2017; Yue et al. 2021). However, grazing mitigates the negative effects of N on the ST by reducing biomass, increasing solar radiation, and accelerating soil water evaporation (Yan et al. 2018). Increased surface temperature promotes carboxylase activity and enhances photosynthesis, leading to increased $\delta^{13}\text{C}$ values and increased WUE (Pankasem et al. 2024).

In terms of response mechanisms common to plant species, we observed a decrease in $\delta^{13}\text{C}_{\text{leaf}}$ in *L. chinensis* with increasing N content. This is likely because N enhances the efficiency of carbon assimilation during photosynthesis, but it cannot fully counterbalance the negative effects of heavy grazing on leaf morphology and physiology (Flexas et al. 2007; Midolo et al. 2019).

Additionally, the increase in soil available N increased the availability of substrates for photosynthesis, not only increasing photosynthetic rates but also supporting plant growth and biomass accumulation, thereby increasing WUE. These findings indicate that grazing and nutrient addition indirectly regulate plant photosynthetic rates and WUE by influencing soil conditions.

Environmental factors are the primary drivers of $\delta^{13}\text{C}_{\text{root}}$ variation among different plant species. Specifically, $\delta^{13}\text{C}_{\text{root}}$ tends to increase with increasing ST, likely because plant organs preferentially utilize ^{12}C during respiration, resulting in an enrichment of ^{13}C in plant tissues (Leavitt and Long 1986). Rising temperatures increase root turnover and enhance drought resistance traits (length, surface area, volume, root length density, specific root length, and specific surface area) in response to reduced SM (Liu et al. 2020a, 2020b; Volder et al. 2007).

Our study revealed that $\delta^{13}\text{C}_{\text{root}}$ increases with AN, possibly because increased soil AN promotes aboveground productivity (Stevens et al. 2015), and aboveground and belowground productivity have a synergistic growth relationship (Yang et al. 2009). Well-developed roots facilitate C accumulation and the enrichment of heavy isotopes ($\delta^{13}\text{C}$), leading to increased isotope values (Leavitt and Long 1986). Our study indicates that grazing and nutrient addition indirectly affect $\delta^{13}\text{C}_{\text{leaf}}$ and $\delta^{13}\text{C}_{\text{root}}$, i.e., plant WUE, by influencing soil nutrients.

The role of leaf $\delta^{13}\text{C}$ in reflecting aboveground biomass

Our findings suggest that $\delta^{13}\text{C}_{\text{leaf}}$ can serve as a potential indicator of aboveground biomass, with the aboveground biomass increasing as the $\delta^{13}\text{C}_{\text{leaf}}$ values of *L. chinensis* and *S. grandis* increase (Fig. 6a, b). The finding is consistent with the results of previous studies (Fan et al. 2011). In the present study, however, the aboveground biomass decreased as the $\delta^{13}\text{C}_{\text{leaf}}$ values of *C. squarrosa* and *C. korshinskyi* increased (Fig. 6c, d). This difference may be due to the selective foraging of *L. chinensis* and *S. grandis* by livestock, leading to reduced aboveground biomass and decreased canopy coverage of these plants (Dungait et al. 2008). A reduction in canopy coverage shifts the plant competition dynamics from belowground nutrient competition to aboveground light competition (Craine et al. 2013). As light intensity increases, the photosynthetic efficiency of shorter plants improves, leading to higher WUE and increased aboveground biomass for shorter plants (i.e., *C. squarrosa* and *C. korshinskyi*). However, community biomass decreases due to the loss of aboveground biomass from *L. chinensis* and *S. grandis*, resulting in a reduction in aboveground biomass as the $\delta^{13}\text{C}_{\text{leaf}}$ values of *C. squarrosa* and *C. korshinskyi* increase. The productivity of grassland ecosystems is a key indicator of their function, with C isotope variation reflecting fluctuations in productivity (Bond-Lamberty et al. 2012). Our results indicate that the $\delta^{13}\text{C}_{\text{leaf}}$ values of tall plants can positively reflect aboveground biomass, whereas the negative correlation between short plants and biomass is attributed to the release of light competition pressure. This study not only helps reveal the internal mechanisms of ecosystem function but also provides valuable scientific evidence for addressing climate change and developing ecosystem conservation strategies.

From a broader ecological and grassland management perspective, this study highlights that the driving mechanisms of WUE in grassland plants are complex and shaped by the combined effects of plant species, environmental factors, and their interactions. By better understanding the response mechanisms of WUE in different plant species, we can more effectively predict and manage the WUE of grassland ecosystems under

various management scenarios. In the context of global challenges, such as climate change and grassland degradation, proper grazing management and nutrient regulation can help maintain the water balance of grassland ecosystems, increasing their ecological restoration capacity and productivity. This research not only offers valuable theoretical insights into grassland ecology but also provides practical guidance for sustainable grassland management.

Although this study effectively reveals the effects of grazing and N and P addition on plant WUE and explores their interactions and key driving factors, certain limitations remain. While $\delta^{13}\text{C}_{\text{leaf}}$ is a valuable tool for indicating WUE, the absence of plant photosynthetic trait measurements (such as photosynthetic rate and stomatal conductance) prevents direct validation of the isotope-based findings from a physiological perspective (Jiang et al. 2024). Future research that integrates data on plant photosynthetic characteristics will help to better validate and complement these findings. This approach not only provides more detailed physiological explanations for the mechanisms driving plant WUE but also strengthens the theoretical foundation for predicting and regulating WUE in grassland ecosystems under various management scenarios. By combining photosynthesis-related parameters with isotope analysis, future studies could more accurately assess the effects of grazing and nutrient addition on the water dynamics of grassland plants, thus offering more comprehensive and effective scientific support for grassland management.

Conclusion

This study elucidated the effects of grazing and nutrient addition on the WUE of four dominant grassland species, based on $\delta^{13}\text{C}_{\text{leaf}}$ measurements. Our findings highlight the importance of considering the role of grazing in modulating nutrient effects on WUE. Specifically, grazing intensified the negative effects of combined N and P enrichment on WUE, suggesting that management strategies should address both grazing intensity and nutrient dynamics. The underlying mechanisms likely involve changes in plant nutritional status and soil properties, which influence photosynthetic efficiency, stomatal conductance, and carbon allocation. Better understanding of interactions between grazing and nutrient addition is crucial for developing effective grassland management strategies, especially given the implications on plant growth and water use. Future research should further examine the temporal dynamics of interactive grazing and nutrient effects and integrate direct physiological measurements with isotope-based methods to deepen the understanding of WUE in changing grassland ecosystems.

Abbreviations

WUE	Water use efficiency
ST	Soil temperature
SM	Soil moisture
AN	Available nitrogen
AP	Available phosphorous
LTC	Leaf carbon content
LTN	Leaf nitrogen content
RTC	Root carbon content
RTN	Root nitrogen content
DON	Dissolved organic nitrogen
STC	Soil total carbon content
STN	Soil total nitrogen content
SCI	Mean values of soil carbon isotopes in different soil layers

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13717-025-00621-0>.

Supplementary material 1. **Fig. S1** Effects of grazing and N and P addition on important factors: (a) lead C content of *Leymus chinensis*, (b) leaf N content of *Leymus chinensis*, (c) root N content of *Carex korshinskyi*, (d) soil temperature, (e) soil TN, (f) soil AN, and (g) soil $\delta^{13}\text{C}$. Each group of data in the bar chart represents the mean of multiple replicate experiments, and the error bars indicate the standard deviation. Grazing or N and P treatment results labeled with the same lowercase letter do not differ significantly (significance level, $p < 0.05$). NG, no grazing; MG, moderate grazing; HG, heavy grazing; columns with different colors represent different fertilization treatments.

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Author contributions

Shiming Tang designed the research. Xuebao Xu, Yanlong Li, Guangyi Lv, Tongrui Zhang, Bin Zhang, Zhaokai Sun, Zhenpeng Liu, Xinning Wang, Hongyun Yao, Cuiping Gao, Xinya Wang, Chongzhi Sun, Fengqin Yuan, and Lei Zhang performed the experiment and collected the data. Xuebao Xu performed the statistical analyses and wrote the manuscript. Chengjie Wang and Shiming Tang jointly served as corresponding authors, responsible for the overall design of the paper and manuscript revisions. All authors agreed to this submission.

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Availability of data and materials

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

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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