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Moderate grazing significantly increases water use efficiency in alpine meadow on the Tibetan Plateau

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April 05, 2024

Abstract

Water use efficiency is an important indicator of drought tolerance of plants. The response was still unclear of water use efficiency to different grazing intensity and its adaptive mechanisms in alpine meadow. Here, we analyzed water use efficiency of plant dominant species, coexisting species and functional group level plants to grazing intensity by δ13C index in alpine meadow. Furthermore, this study revealed the driven factors of water use efficiency combined available nitrogen, soil bulk density, soil organic carbon, soil water content and evapotranspiration. Grazing increased plants δ13C by 3.37%. Moderate grazing significantly increased the δ13C of Gramineae by 4.84% (P<0.05), and increased δ13C of Cyperaceae, Leguminosae, and Forb by 3.45%, 0.81%, and 1.40% respectively. Some dominant species and coexisting species have the highest δ13C value under moderate grazing. This study indicated that moderate grazing significantly improved the water use efficiency of alpine meadows. Path analysis resulted showed that the water use efficiency was negatively correlated with evapotranspiration (P<0.05), soil water content, soil organic carbon, and soil bulk density. It was positively correlated with available nitrogen. Our study provides new insights that moderate grazing help to increase water use efficiency in alpine meadows. In addition, evapotranspiration may be the main driving factor affecting the water use efficiency of alpine meadow.

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Abstract

Water use efficiency is an important indicator of drought tolerance of plants. The response was still unclear of water use efficiency to different grazing intensity and its adaptive mechanisms in alpine meadow. Here, we analyzed water use efficiency of plant dominant species, coexisting species and functional group level plants to grazing intensity by $\delta^{13}C$ index in alpine meadow. Furthermore, this study revealed the driven factors of water use efficiency combined available nitrogen, soil bulk density, soil organic carbon, soil water content and evapotranspiration. Grazing increased plants $\delta^{13}C$ by 3.37%. Moderate grazing significantly increased the $\delta^{13}C$ of Gramineae by 4.84% (P<0.05), and increased $\delta^{13}C$ of Cyperaceae, Leguminosae, and Forb by

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3.45%, 0.81%, and 1.40% respectively. Some dominant species and coexisting species have the highest $\delta^{13}C$ value under moderate grazing. This study indicated that moderate grazing significantly improved the water use efficiency of alpine meadows. Path analysis resulted showed that the water use efficiency was negatively correlated with evapotranspiration ($P<0.05$), soil water content, soil organic carbon, and soil bulk density. It was positively correlated with available nitrogen. Our study provides new insights that moderate grazing help to increase water use efficiency in alpine meadows. In addition, evapotranspiration may be the main driving factor affecting the water use efficiency of alpine meadow.

KEYWORDS: alpine meadow, grazing activity, water use efficiency, $\delta^{13}C$

1 | INTRODUCTION

Plant leaf carbon isotope composition ($\delta^{13}C$) is the integration of photosynthetic activities during plant leaf tissue synthesis, which can reflect the relative relationship between plant water loss and carbon harvest (Shen et al., 2019). It is often used to indirectly indicate the long-term water-use efficiency (WUE) of plants (Liu et al., 2016; Stewart et al., 1995; Farquhar et al., 1982). A large number of studies have shown that the $\delta^{13}C$ value of plant leaves has a robust positive correlation with water use efficiency. Larger of $\delta^{13}C$ indicated higher plants WUE (Su et al., 2005; Qu et al., 2001; Farquhar et al., Shen et al., 2017; Pan et al., 2011). Grazing is the main utilization mode of grassland ecosystems. The species richness and coverage of grassland will decrease significantly during grassland degradation processes (Su et al., 2005).

More than 60% of alpine meadows were degraded seriously than moderate degradation, and grazing is the leading factor in the degradation of alpine meadows (Cao et al., 2018). Previous studies focused on impacts of grazing intensities on grassland multi-functions (Du et al., 2019; Shen et al., 2019; Zhang et al., 2019). The grazing activities played a role in balancing and stabilizing the plant community (Wang, 2018). Grazing activities decreased the biomass and diversity of grassland ecosystems (Lin et al., 2017; Xu et al., 2015; Gang et al., 2019), soil water content (Zhang et al., 2019; Lin et al., 2011), and evapotranspiration process (Zhang et al., 2019; He et al., 2014; Shu et al., 2019). The stable carbon isotope technology is fast and accurate due to its labeling, integration and indication functions (Jiang et al., 1996). Some studies based on the $\delta^{13}C$ stable isotope technology have found that the long-term WUE of plants is studied through the monthly and annual changes of the WUE in desert plants (Su et al., 2005). Grazing has a significant impact on plants stable isotope indicators (Chen, 2013). Grazing pastures in Guinea, Brazil, Tanzania, plant WUE shows an increasing trend under moderate grazing (Cavalcante et al., 2016; Wang, 2016). The $\delta^{13}C$ of *Stipa breviflora* plants showed a downward trend with the grazing activities increasing (Wang, 2016). The WUE of typical plants showed a downward trend under grazing conditions, but the soil shows an upward trend in the desert steppe of Inner Mongolia (Zhu et al., 2015).

The $\delta^{13}C$ value is affected by environmental factors such as evapotranspiration, soil water content, soil organic carbon, and soil bulk density, which in turn affects plant WUE. Zhang (2003) studied the changes in the $\delta^{13}C$ values of terrestrial C_3 plants in 72 regions of China's Xinjiang, Gansu, Inner Mongolia, Qaidam Basin and the Qinghai-Tibet Plateau and found that precipitation and $\delta^{13}C$ values are negatively correlated. With an increase of 100 mm, the $\delta^{13}C$ value of the plant is negative 0.33content has a weak negative correlation with WUE (Wu et al., 2019). The WUE of Haibei alpine meadow in Qinghai is positively correlated with the change of evapotranspiration (Shi et al., 2020). Zhu (2020) et al studied the correlation between the $\delta^{13}C$ value and soil organic carbon under different grazing treatments in the Xilamuren desert steppe in central and western Inner Mongolia, and found that they are negatively correlated. The content of soil organic carbon in Panyang Lake is different from $\delta^{13}C$. The relationship between the two was also found to be negative (Wang et al., 2016).

Although, the responses of WUE to grazing at the species level and community level were vastly researched. However, few studies focused on the response of WUE of alpine meadows to grazing intensity and adaptation mechanisms. This study aimed to reveal the long-term WUE variation characteristics under different grazing intensities and its driven factors. Thus, we study different species, function groups and community level WUE under long-term grazing. Furthermore, main driven factors were discussed including soil available nitrogen,

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organic carbon, evapotranspiration, soil water content, and bulk density. The following scientific questions were answered: (1) How did the WUE of the dominant species and coexisting species respond to different grazing intensities? (2) How did plant functional group species WUE respond to different grazing intensities? (3) How did the coupling factors affect WUE in different grazing intensities?

2 | MATERIALS AND METHOD

2.1 | Site description

The research plots were carried out in Qinghai Haibei alpine grassland ecosystem national observation and research station (Haibei Station, 37°39.876' N, 101deg10.748' E), with an altitude of 3227.6 m. The sample plot is a winter pasture. The grazing period is from the beginning of October to the end of April of the following year, so the plant growth season is not disturbed by grazing. It belongs to typical plateau continental climate, the annual average precipitation and temperature are 560 mm and -1.7 degC, respectively. The precipitation from May to September during the growing season accounts for about 80% of the total annual precipitation.

The plant growing season has rain and heat at the same time, and soil type is alpine meadow soil. Since the 1980s, the three pastures and grazing channel grasslands have carried relatively stable grazing intensity, and formed obvious grazing gradients in terms of surface characteristics and species composition (Table 1, Fig. 1).

The native vegetation of the alpine meadow in this area is cold mesophytic, wet mesophytic and dry mesophytic perennial Kobresia as a constructive vegetation type. The community structure is relatively complex and the species composition is rich. There are about 30 species m^{-2}, and the dominant species are mainly including*Kobresia humilis, K. pygmaea, Elymus nutants, Festuca rubra, Poa annua, Oxytripis ochrocephala, Gentiana straminea, Potentilla nivea, Saussurea superba, Thermopsis lanceolate* . Coexisting species including*Stipa aliena, Poa annua, Kobresia humilis, Kobresia pygmaea, Oxytropis ochrocephala, Thermopsis lanceolata, Saussurea superba, Leontopodium nanum, Gentiana straminea, Potentilla nivea, Anaphalis lactea Maxim.* Among them, *Stipa aliena* and *Poa annua*belong to Gramineae, *Kobresia humilis* and *Kobresia pygmaea*belong to Cyperaceae, *Oxytropis ochrocephala* and *Thermopsis lanceolata* belong to Leguminous, *Saussurea superba, Leontopodium nanum, Gentiana straminea, Potentilla nivea* and *Anaphalis lactea Maxim* belong to Forb group.

2.1 | Experimental design

The dominant species and coexisting species plants in the three grazing treatment samples were collected, and the top healthy, complete, mature leaves (try to pick the same part of each plant) were picked and bagged, and each plant was collected 15-20 leaves (at least 10 individual plants growing independently), mixed and packed into cowhide envelopes. The standard harvesting method was used to collect plant species at the functional group level (25 cm x 25 cm).

The total biomass of the community was investigated using a sample method (25 cm x 25 cm). The aboveground biomass was collected by standard harvesting method, and the soil and below-ground biomass were collected by root drilling method, sampling from 0 to 20 cm, using a 2 mm soil sieve, washing with water to obtain root samples, drying at 65degC to constant weight, weighing. Soil samples are used to determine soil physical and chemical properties. The soil water content is measured by the ring knife method, and the soil evapotranspiration is measured by a small lysimeter, three replicates are set for each plot in the experiment.

Washing the plant leaves with ultrapure water, these were dried at 65 to constant weight. Then, leaves were smashed through a 100-mesh sieve. Approximately 2 mg of the plant powder sample were burnt in the TOC/TNb analyzer to collect CO_2, and injected into a stable carbon isotope mass spectrometer (Thermo, MAT-253) for stable carbon isotope value analysis. This analysis accuracy is +- 0.1

The expression formula of carbon isotope composition is:

$\delta^{13}C = (R_{sam} / R_{sta} - 1) \times 1000$

In this formula, R_{sam} and R_{sta} are the$^{13}C/^{12}C$ values of test plants and standard samples, respectively.

Data Analysis

Mean, standard error, and variance analysis are processed by R and Excel 2010. Path analysis is processed by SPSS (Versiton 21.0). Originpro 9.1 software were used to complete relative maps.

3 | RESULT

3.1 | WUE variation characteristics of dominant species in different grazing intensity

The $\delta^{13}C$ value of each species differed greatly both in enclosure plots and grazed treatments. The average $\delta^{13}C$ values of dominant species in three grazing treatments were -27.61, -26.16 and -27.20$\delta^{13}C$ value of dominant species increased firstly and then decreased with grazing intensity increasing. The overall range of 9 species is from -24.92 ± 0.07 to -28.08 ± 0.013.16highest and lowest values are Gentiana *straminea* and Leontopodium nanum respectively. The $\delta^{13}C$ value of *Stipa aliena, Gentiana straminea, Oxytropis ochrocephala* are the highest under enclosure, moderate grazing, and heavy grazing gradients (Table 2) Moderate grazing and heavy grazing can increase the $\delta^{13}C$ value of dominant species by 5.25% and 1.48% respectively. In other words, both moderate and heavy grazing activities can improve the WUE in alpine meadows.

3.2 | WUE variation characteristics of 11 coexisting species indifferent grazing intensity

The $\delta^{13}C$ value of *Gentiana straminea* and*Anaphalis lactea Maxim* were highest and lowest respectively. The average $\delta^{13}C$ value of functional group plants under moderate grazing were the largest, and the average $\delta^{13}C$ value of the functional group plants in three experimental treatments were -27.09, -27.00 and -27.04The $\delta^{13}C$ value of *Poa annua, Kobresia humilis, K. pygmaea,* and *Potentilla nivea* increased significantly under moderate grazing ($P<0.05$, Fig. 2). *Leontopodium nanum, Oxytripis ochrocephala, and Anaphalis lactea Maxim* had the highest $\delta^{13}C$ value under heavy grazing. *Stipa aliena, Thermopsis lanceolata, Saussurea superba* and *Gentiana straminea*had the highest $\delta^{13}C$ value under enclosure. In other words, moderate grazing can increase coexisting species plant WUE by 3.32

3.3 | Variation characteristics of functional group WUE in different grazing intensity

The $\delta^{13}C$ values of the two functional groups of Gramineae and Cyperaceae were the highest in moderate grazing. Both Gramineae and Cyperaceae are regrading as fine forages. Moderate grazing help to increase forages WUE. The $\delta^{13}C$ values of the two functional groups of Leguminosae and Forb were the highest in heavy grazing. These drought tolerances increased with grazing intensity increasing. Except for Gramineae plants, the WUE of the other three functional groups Cyperaceae, Leguminosae, and Forb under the three treatments didn't reach a significant difference. WUE of Gramineae plants is more sensitive and adaptive. Moderate grazing significantly increased Gramineae plants $\delta^{13}C$ and its WUE approximately 4.84% comparing with control ($P<0.05$, Fig. 3). The $\delta^{13}C$ value of Gramineae and Cyperaceae plants increased first and then decreased with the increase of grazing intensity. Leguminosae and Forb showed an upward trend.

Overall, grazing activities increased the $\delta^{13}C$ value of plants. The average $\delta^{13}C$ values of each functional group in the three treatments of enclosure, moderate grazing and heavy grazing were -27.47Moderate grazing and heavy grazing increased WUE by 2.07% and 1.82% respectively compared with enclosure.

3.4 | Effect of soil physical and chemical properties on WUE variation

Soil evapotranspiration, soil organic carbon, water content and soil bulk density were negative correlate with the $\delta^{13}C$ value, and evapotranspiration and soil organic carbon have reached a significant level ($P<0.05$). However, available nitrogen was significant positive correlate with WUE ($P<0.01$, Table 3). Grazing intensity is positively correlated with WUE. Grasslands WUE was higher under tolerant environment.

3.5 | Path analysis of direct and indirect effects on WUE in alpine meadow

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Path analysis indicated that evapotranspiration has a direct and significant negative correlation effect on $\delta^{13}C$ value ($P<0.05$). Furthermore, available nitrogen, soil organ carbon, soil bulk density and soil water content have indirect effects on the $\delta^{13}C$ value (Fig. 4). Meanwhile, the indirect effects of soil available nitrogen, evapotranspiration, and soil bulk density were relative higher through soil organ carbon from -0.85 to 0.51, respectively. Soil organ carbon and available nitrogen were showed positive effect by evapotranspiration approximately 0.27 and 0.22, respectively. Evapotranspiration and available nitrogen were showed effect by soil water content approximately 0.22 and -0.15, respectively. Furthermore, available nitrogen was showed positive effect on WUE by soil water content was 0.38.

4 | DISCUSSION

Previous studies have shown that grazing activities reduced WUE of plant species (Zhu et al., 2015; Huang et al., 2017; Wang, 2017; An et al., 2015). However, our experimental results show that all the dominant species, coexisting species, functional group and community level species, the WUE of plants is the highest under moderate grazing treatment, and grazing activities increase the WUE of plants. Similar results were revealed that moderate grazing WUE increased by 37.72% compared to traditional grazing (Cavalcante et al., 2016). In Inner Mongolia typical grassland community WUE was highest under moderate grazing (Lv et al., 2016), and WUE under grazing treatment increased by 62.86 % Compared to no grazing. In Inner Mongolia Stipa*breviflora* grassland WUE increased by 0.56% under moderate grazing (Wang, 2018). There may be two reasons: On the one hand, the feeding of plants by livestock causes damage to the leaves of plants under moderate grazing, the canopy structure of plants will also change during the grazing process. These changes may directly limit the transportation and fixation of carbon in plant leaves. The reduction of plant leaf area will weaken the photosynthetic capacity of plants. Plants will increase their net photosynthetic rate to enhance photosynthesis capacity, increase grazing tolerance of grassland plants, and increase the compensatory growth ability of plants. Therefore, the WUE of plants increases (Wang, 2018; Lv, 2016). On the other hand, grazing can increase soil fertility through livestock excrement and urine, improve the microenvironment of plant growth, and enable plants to gain stronger living ability. After plants undergo grazing, their resource utilization capacity increases, so the WUE of plants increases (Pia et al., 1995). Meanwhile, studies have also shown that the saliva remaining on the section of the gnawed plant during the gnawing process of animals can promote the growth of plants, thereby improving WUE of plants (Johnston et al., 2008).

Grazing plays an important role in balancing ecosystem plants (Zhu, 2017). Moderate grazing could improve grasslands biomasses and richness, but heavy grazing significantly decreased biomasses and richness (Du et al., 2019). The WUE of plants is the highest under the moderate grazing in this study. This indicated that the plants are highly tolerant to drought environment under moderate grazing. When suffering from water stress, they can improve their WUE to ensure water supply (Sun et al., 2005). Severe grazing state WUE shows a downward trend compared to moderate grazing. This may be because grassland degradation is more serious and biomass is reduced (Du et al., 2019), the bare grassland area is larger, and vegetation is scarce under severe grazing treatment. Therefore, plant WUE showed a downward trend. But the WUE of Forb was the highest in the state of heavy grazing. Due to the large amount of grazing by livestock, the growth space of Forb increased, which led to the dominant position of Forb in the sample plot. Therefore, Forb has the highest WUE and strong ability to adapt to the environment at the level of functional groups. Gramineae, which are fond of livestock, have the lowest WUE.

Some environmental factors that affect the process of gas exchange metabolism also affect the WUE of many plants, including soil water content (Ehleringer, 1994; Stewart et al., 1995; Korol et al., 1999; Wu et al., 2019), precipitation (Anderson et al., 2000; Stewart et al., 1995), evapotranspiration (Xu et al., 1998), nitrogen availability (Hogberg et al., 1993; Guehl et al., 1995). Among them, soil water content, precipitation and evapotranspiration have a negative correlation with WUE. This is consistent with the results of this experiment. It is also because when the water content decreases, plants will better adapt to the environment by increasing their own WUE (Sun et al., 2005; Chen, 2003). Studies have shown that evapotranspiration will decrease with the increase of grazing intensity (Shu et al., 2019), and the increase of WUE is related to

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lower evapotranspiration and the coverage and biomass of dominant species in the community. This research result has also been verified by many scholars (Xu et al., 1998; Cavalcante et al., 2016; Varnamkhasti et al., 1995). There is a negative correlation between the WUE and the soil bulk density. It may be speculated that after the soil bulk density increases, the soil water content shows a downward trend (Shu et al., 2019). When plants are subjected to water stress, they will increase their water use efficiency to survive better. There is a negative correlation with soil organic carbon content, which may be due to the increase of soil organic matter content in the enclosure. Microorganisms preferentially use^{12}C to enrich the residual organic matter with^{13}C in the process of soil organic carbon decomposition. Because enclosure slows down this decomposition process, the WUE in the enclosed plot is lower than that in the free grazing plot (Zhu et al., 2020). Nitrogen plays a vital role in the growth and development of plants, and the increase in soil nitrogen can improve plant WUE (Chen, 2003).

5 | CONCLUSION

Considering that there are certain differences in the WUE of different plant species under different grazing intensity. Through the study of the changes in the WUE of plants at the level of dominant species, coexisting species and functional groups of alpine meadows under the three treatments of enclosure, moderate grazing and heavy grazing in Haibei, Qinghai, and the adaptation mechanism of WUE of alpine meadow the study. Draws the following conclusions:

1) The order of the WUE is Gramineae > Cyperaceae > Forb > Leguminous analysis from the perspective of functional groups.

2) The WUE of the dominant species, coexisting species and functional group level species are the highest under moderate grazing, followed by heavy grazing, and the lowest under enclosure. Grazing activities increased the WUE of plants.

3) There is a negative correlation between soil water content, evapotranspiration, soil bulk density, soil organic carbon and WUE, and the evapotranspiration will significantly affect WUE. There is a positive correlation between available nitrogen and grazing intensity.

This research shows that evapotranspiration was perhaps the main driven factor in the WUE of alpine meadow in Qinghai. Our results provide new insights that Moderate grazing help to increase forages WUE. These drought tolerances of Leguminosae and Forb increased with grazing intensity increasing.

ACKNOWLEDGEMENTS

This work is supported by Qinghai innovation platform construction project (2020-KF-12), western light of Chinese Academy of Science for talents, and the national natural science foundation of China (31770532).

DATA AVAILABILITY STATEMENT

Grassland plant leaves isotopte data in this paper would be upload in dryad when this manuscript could be accepted.

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