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Differential Responses of Dominant Plants to Grazing in Typical Temperate Grassland in Inner Mongolia

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Abstract: *Leymus chinensis*, *Stipa grandis*, *Artemisia frigida*, and *Cleistogenes squarrosa* are the dominant plant species in typical temperate grasslands in Xilingol. Intensive studies related to overgrazing, which resulted in a dominant plant shift, have been carried out in recent years, but the ways in which these four species respond to different grazing intensities remain elusive. In this study, the contents of primary metabolites, secondary metabolites, and phytohormones in the leaves of these species under five grazing intensities were assayed and compared. The results showed that *A. frigida* contained higher amounts of lignin, while *C. squarrosa* contained higher amounts of total flavonoids than the other species. *Leymus chinensis* showed a different accumulation of cellulose and tannin in response to grazing, compared with the other three species. *Stipa grandis* and *A. frigida* increased in soluble protein contents in response to different grazing treatments. In particular, the contents of phytohormones, such as abscisic acid, salicylic acid, and gibberellins, were markedly changed under grazing. *Leymus chinensis* exhibited different abscisic acid and gibberellins accumulation patterns compared with the other species, under the different grazing intensities. Patterns of salicylic acid accumulation were similar (except under light and moderate grazing intensities in *A. frigida*) among the four species. The results indicated that the four species differed in adaptive strategies to cope with the different grazing intensities, and phytohormones played important roles in coordinating the regulation of their growth and grazing tolerance. This study provides a foundation for elucidating the mechanisms of overgrazing-induced degradation of the Xilingol grassland.

Keywords: grazing; grassland; degradation; dominant species; phytohormone

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1. Introduction

Grassland is the dominant vegetation type in China, covering 41.7% of the country's total land area, with 20% of this area distributed in Inner Mongolia [1–3]. However, because of unsustainable utilization, 30–50% of Inner Mongolia's grasslands have been degraded and have suffered severe ecological and economic impacts [3].

Overgrazing is a major factor responsible for grassland degradation in Inner Mongolia [3,4]. This degradation is characterized by individual miniaturization, decreased plant productivity and vegetation cover, altered species composition, reduced biodiversity, and soil degradation [3,5–7]. Previous studies have shown that overgrazing results in a shift in the dominant plant taxa in grassland ecosystems [8,9]. The Xilingol grasslands of Inner Mongolia, which are representative of Eurasian steppe, have been degraded, owing to livestock overgrazing [6,10]. Studies of the grasslands of the Xilin River Basin of Inner Mongolia show that the dominant plant species *Leymus chinensis*, *Stipa grandis*, and *Stipa krylovii* have been replaced by *Artemisia frigida* and *Cleistogenes squarrosa*, following long-term heavy (or over) grazing [1,11]. The dominance of *A. frigida* was subsequently replaced by *Potentilla acaulis* following heavy grazing [12]. Although several studies have examined

the characteristics of the changes and constructed theoretical models to explain this response [5,11,13,14], the basic mechanism that has contributed to this shift in dominance remains to be fully elucidated.

Grazing imposes multiple stresses on plants (e.g., wounding, defoliation, and saliva deposition), but the impact on plant morphological and physiological traits differs with grazing intensity [2,15]. It is common for plants to synthesize primary metabolites, such as soluble sugars and starch, and secondary metabolites associated with growth inhibition and palatability, such as phenolic compounds and condensed tannins, as a defense response to herbivory [16]. Lignin and cellulose are the major components of plant cell walls, and provide strong mechanical support and protection to the plant body [17,18]. Lignin and cellulose are involved in tolerance to abiotic and biotic stresses and their production can be triggered by the various stresses [19–24]. Phytohormones, which regulate diverse processes of plant growth and development, perform important functions in plant adaptation to environmental stimuli [25,26]. For instance, the auxin indole-3-acetic acid (IAA) is strongly and rapidly elicited in response to herbivory [27], jasmonate (JA) and abscisic acid (ABA) accumulation, which is triggered following a herbivore attack [28,29], and hormone cross-talk is commonly involved in the plant response to herbivory [30,31]. In *L. chinensis*, plant height is successively reduced with an increase in grazing intensity, and the contents of soluble sugars, protein, tannins, total flavonoids, and total phenols are affected concomitantly with grazing stress [32]. The contents of phytohormones, including ABA, auxin, salicylic acid (SA), and JA, are altered in *L. chinensis* in response to different grazing intensities of sheep [2,32], and phytohormones are involved in the regulation of the trade-off between growth and defense response [32]. These indicated physiological response strategies differed among different grazing intensities in *L. chinensis* [2,32,33]. However, little is known about the physiological responses of *S. grandis*, *A. frigida*, and *C. squarrosa* to different grazing intensities.

In this study, to clarify the differences in response strategies among *L. chinensis*, *S. grandis*, *A. frigida*, and *C. squarrosa*, which are the dominant species present in typical Xilingol grassland, under different grazing intensities, we examined the primary and secondary metabolic responses, especially hormonal responses, among these four species. The present results indicated that the four study species differed in adaptive strategies to cope with the different grazing intensities. The results will lay a foundation for elucidating the mechanism for the shift in dominant plant taxa against the overgrazing-induced degradation of Xilingol grassland.

2. Materials and Methods

2.1. Study Site Description and Sampling

This study was performed at the Inner Mongolia Typical Grassland Ecological Protection and Restoration Research Station of the Chinese Academy of Agricultural Sciences, which is located at Xilinhot, Inner Mongolia, China (116°42′ E, 43°38′ to 44°49′ N). The study area has a temperate semiarid continental climate. The annual mean temperature is 4.6 °C and the annual mean precipitation is 300–360 mm, predominantly falling from June to August [2]. The dominant plant species are *L. chinensis*, *S. grandis*, *S. krylovii*, *A. frigida*, and *C. squarrosa*. Grazing in the study area was prohibited from 2007 to 2013. The present grazing experiment was initiated in 2014 and continued in the following 5 years without interruption (except extremely heavy grazing treatment, which was not conducted in 2017 due to severe drought), with grazing starting in early June and lasting for 3 months in each year. Grazing intensity applied in each plot of 1.33 ha was designed as follows: no grazing (NG, control); light grazing (LG) with four sheep; moderate grazing (MG) with eight sheep; heavy grazing (HG) with twelve sheep; and extremely heavy grazing (EHG) with sixteen sheep. Three replicate plots per grazing intensity (i.e., 15 plots in total) were established. After grazing for 6 years (5 years for EHG), the HG and EHG plots were severely degraded and the dominant plant species had changed from *L. chinensis* and *S. grandis* to *A. frigida* and *C. squarrosa*. The statistical was subject to peseudoreplication [34]. One replicate plot

per grazing intensity was used for plant height measurement and sampling. Each sampling plot comprised three biological replicates. A total of 15 individual plants of each species in the sampling plot for each grazing intensity were randomly chosen for plant height measurement and statistical analysis. The leaves of ten plants (or five bunches) of each species in the sampling plot for each grazing intensity were randomly sampled and respectively pooled as one replicate sample prior to grazing (on 15 June 2020). The collected samples were immediately frozen in liquid nitrogen for subsequent analysis in the laboratory. In addition, for cellulose and lignin measurement, leaf samples were dried at 65 °C for 48 h.

2.2. Lignin and Cellulose Contents Assay

The sampled leaves were oven-dried to a constant weight. Following the method of van Soest [35,36], acid-detergent fiber (ADF) was extracted from the neutral-detergent fiber, then hydrolyzed with 72% sulfuric acid. Cellulose was isolated from the ADF. The remaining residue, which contained lignin, was further dried and dry-ash treated with a furnace (500 °C).

2.3. Soluble Protein Assay

The soluble protein content was measured using a method described previously [37], with some modifications. In brief, 0.5 g frozen samples were extracted with 50 mL boric acid/phosphoric acid buffer (8.9 g sodium tetraborate decahydrate, 12.2 g sodium dihydrogen phosphatemonohydrate, 100 mL tert-Butanol, add distilled water to 1000 mL), to which 1 mL sodium azide was added. The mixture was incubated at room temperature for 3 h. After filtering the mixture with quantitative filter paper, the residue was washed with boric acid/phosphoric acid buffer. The filter paper and residue were dried at 60 °C. The nitrogen content was determined from the filter paper and residue using the Kjeldahl method. Data are expressed as milligrams of soluble protein per gram of sample.

2.4. Tannins and Total Flavonoid Determination

For determination of tannin content, frozen leaf samples (0.5 g) were extracted using a mixture of acetone and distilled water (1:1, v/v). After shaking 160 rpm for 40 min, the mixture was incubated at room temperature and filtered with medium-speed quantitative filter paper. Subsequently, 1 mL filtrate were added to 2.5 mL sodium tungstate-phosphomolybdic acid mixture (0.3 M sodium tungstate, 0.01 M phosphomolybdic acid, and 5% phosphoric acid) and 5 mL sodium carbonate solution (0.7 M) and made up to 50 mL with distilled water, then incubated at room temperature for 30 min. The absorbance at 760 nm was measured using an ultraviolet-visible spectrophotometer (UV2550, Shimadzu). A standard curve for tannic acid was generated and the tannin content was expressed as milligrams of tannic acid equivalents per gram of sample.

For measurement of the total flavonoid content, frozen leaf samples (0.5 g) were extracted using 70% methanol and incubated in a 65 °C water bath, with shaking at 160 rpm for 2 h. After filtering the mixture, the residue was washed with 70% methanol, the filtrates were combined, and made up to a 50 mL constant volume with 70% methanol. To 1 mL of the supernatant, 2 mL of 0.1 mol L^{-1} aluminum trichloride and 3 mL of 1 mol L^{-1} potassium acetate were added, and made up to 10 mL constant volume with 70% methanol, then incubated at room temperature for 30 min. The absorbance at 420 nm was measured using an ultraviolet-visible spectrophotometer (UV2550, Shimadzu). A standard curve for rutin was generated and the total flavonoid content was expressed as milligrams of rutin equivalents per gram of sample.

2.5. Plant Hormone Measurement

For extraction of ABA, gibberellins (GAs), and IAA, fresh leaf samples (0.2 g) were ground to powder in liquid nitrogen, to which 2 mL pre-chilled 80% methanol was added, and the mixture was incubated at 4 °C overnight. After centrifugation at 4000× g and 4 °C for 20 min, the supernatant was collected and concentrated to one-third of the original

volume under reduced pressure at 40 °C. For extraction and decolorization, 6 mL petroleum ether was added three times and the ether phase discarded. The aqueous phase was extracted three times with 4 mL ethyl acetate, then the ester phase was combined and evaporated to dryness under reduced pressure at 40 °C. Acetic acid solution (pH 3.5, 0.4 mL) was added, purified with a Sep-Pak C18 column, and eluted with methanol. After collection, the eluate was concentrated to dryness under reduced pressure at 40 °C. The residue was dissolved with a mixture of methanol and 0.1% glacial acetic acid solution (60:40, v/v) and diluted to 0.4 mL. After filtering with a 0.22 μM millipore filter, the filtrate was used for high-performance liquid chromatography (HPLC) analysis with the following chromatographic conditions: column, Waters ACQUITY UPLC C18 (1.7 μm, 2.1 mm × 50 mm); column temperature 25 °C; detection wavelength 260 nm; solvent flow rate 0.3 mL min^{-1}; mobile phase of methanol:0.1% glacial acetic acid (60:40, v/v); and sample injection volume 10 μL.

For extraction of SA, fresh leaf samples (0.2 g) were ground to powder in liquid nitrogen, and added to 0.4 mL of 5% trichloroacetic acid and 0.4 mL of 60% ether. The mixture was vortexed and extracted for 12 h, and then centrifuged at 4000× g for 5 min. The upper ether phase was collected and the aqueous phase was re-extracted twice with ether. The ether phases were combined and evaporated under vacuum rotation. After dissolving in 0.8 mL of 50% methanol:50% acetic acid buffer (pH 3.2), the mixture was filtered through a 0.22 μM millipore filter and the filtrate was used for HPLC analysis, with the following chromatographic conditions: column, Waters ACQUITY UPLC C18 (1.7 μm, 2.1 mm × 50 mm); column temperature 25 °C; detection wavelength 306 nm; solvent flow rate 0.3 mL min^{-1}; mobile phase of methanol:water:glacial acetic acid (59:40:1, $v/v/v$); and sample injection volume 10 μL.

2.6. Statistical Analysis

Data were expressed as the mean ± 95% confidence interval (CI) of three replicates (except fifteen replicates for plant height). Data analysis was performed with R version 4.1.3 using the R package 'agricolae'. The data for plant height and contents of phytohormones, lignin, cellulose, tannins, total flavonoids, and soluble proteins among five grazing intensities and four species were analyzed for statistical significance using the two-way ANOVA. Differences between the pairs of the grazing intensities and species were identified using the least significant difference (LSD) test and considered to be statistically significant at the 5% significance level.

3. Results and Discussion

3.1. Plant Height

The heights of *L. chinensis*, *S. grandis*, *A. frigida*, and *C. squarrosa* plants were all reduced by the different degrees of grazing disturbance (Figure 1). Except for LG of *S. grandis*, the plant height of all other species in each grazing treatment differed significantly from the control (NG). In particular, the plant height of *L. chinensis* and *S. grandis* decreased concomitantly with the increase in grazing intensity, with significant differences observed among HG and EHG, compared with LG and MG. However, in *A. frigida* and *C. squarrosa*, the minimum plant heights were recorded under LG and MG, respectively. The taller plant height of *A. frigida* and *C. squarrosa* under HG and EHG, compared with that under LG, and LG and MG, respectively, was in accordance with the conclusion that these two species were mainly dominant in degraded typical grassland. Under different grazing intensities (excluding EHG), the tallest plants were *S. grandis*, followed by *L. chinensis*, *A. frigida*, and *C. squarrosa*, with significant differences observed among each species.

3.2. Cellulose and Lignin Contents

The cellulose content of *L. chinensis* was significantly increased in the grazing treatments (LG, MG, HG, and EHG) compared with NG, and evidently higher under LG and EHG than MG and HG (Figure 2A). In contrast, compared with NG, grazing treatments

distinctly decreased the cellulose content of *A. frigida*; EHG obviously reduced the cellulose content compared with LG, MG and HG (Figure 2A). In *S. grandis*, compared with NG, HG and EHG, the cellulose content was significantly increased under LG and MG, but no significant differences among NG, HG and EHG (Figure 2A). In *C. squarrosa*, the cellulose content was significantly decreased under LG and EHG, compared with NG and HG, and no significant difference was observed between NG and HG (Figure 2A).

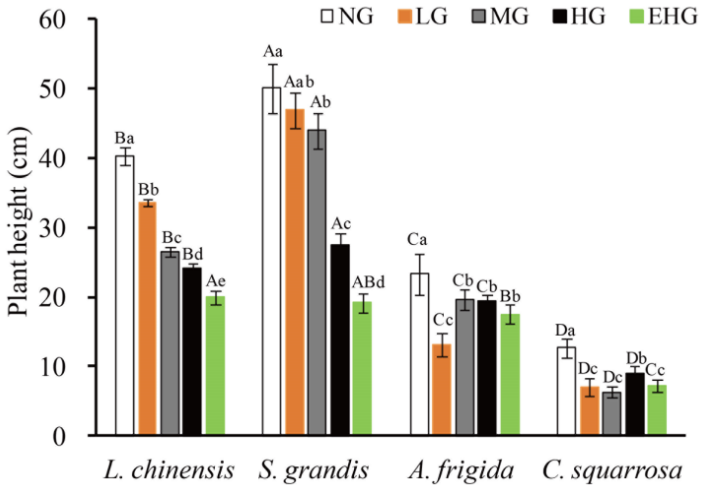

Figure 1. Plant height of *Leymus chinensis*, *Stipa grandis*, *Artemisia frigida*, and *Cleistogenes squarrosa* under different grazing intensities at Xilinhot, Inner Mongolia. NG, no grazing; LG, light grazing; MG, moderate grazing; HG, heavy grazing; EHG, extremely heavy grazing. The bars and error bars represent the mean ± 95% CI of fifteen replicates. Significant differences among grazing intensities within each species are marked with different small letters, significant differences among species within each grazing intensities are marked with different capital letters (LSD test, $p < 0.05$).

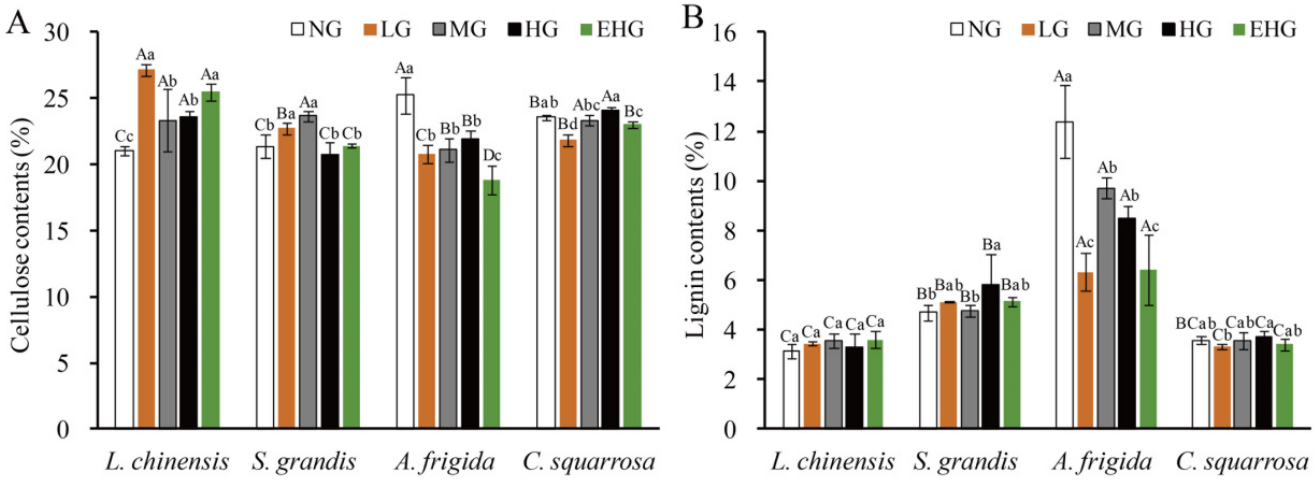

Figure 2. Cellulose (**A**) and lignin (**B**) contents in *Leymus chinensis*, *Stipa grandis*, *Artemisia frigida*, and *Cleistogenes squarrosa* under different grazing intensities at Xilinhot, Inner Mongolia. NG, no grazing; LG, light grazing; MG, moderate grazing; HG, heavy grazing; EHG, extremely heavy grazing. The bars and error bars represent the mean ± 95% CI of three replicates. Significant differences among grazing intensities within each species are marked with different small letters, significant differences among species within each grazing intensities are marked with different capital letters (LSD test, $p < 0.05$).

Overall, among the four species, *A. frigida* had the highest lignin contents followed by *S. grandis*; significant differences were observed between *A. frigida* and *S. grandis*, as well as in *A. frigida* and *S. grandis* compared with *L. chinensis* and *C. squarrosa* (except in

the comparison between *S. grandis* and *C. squarrosa* under NG), under different grazing intensities (Figure 2B). This finding is consistent with a previous report that *A. frigida* is a subshrub with a high lignin content [38–40]. The lignin contents of *A. frigida* were significantly decreased in the grazing treatments compared with NG, with higher contents were observed under MG and HG than LG and EHG. Grazing significantly increased the lignin content in HG compared with NG and MG in *S. grandis*. No significant differences in lignin contents were observed among the different grazing intensities for *L. chinensis* and *C. squarrosa* (except for the significant difference between LG and HG for *C. squarrosa*).

Taken together, compared with NG, the decreased contents of cellulose and lignin in *A. frigida* under the grazing treatment indicated that *A. frigida* showed a distinct strategy compared with the other three species for adaptation to grazing by reducing cell wall-mediated mechanical support. This finding was consistent with the previous observation that overgrazing induces a creeping growth habit in *A. frigida*, which is a grazing-facilitated species [9,12].

3.3. Soluble Protein Contents

Soluble protein is an important plant nutrient and osmoregulatory substance [32,41]. The soluble protein content in leaves of *L. chinensis* were significantly increased under EHG compared with NG, and significantly decreased under HG compared with LG and EHG (Figure 3). In *S. grandis* and *A. frigida*, except under EHG of *A. frigida*, the soluble protein contents were obviously increased under grazing treatments compared with NG; the peak values were observed under HG and LG, respectively (Figure 3). In contrast, in *C. squarrosa*, soluble protein contents were reduced under the grazing treatments compared with NG, and significant difference was observed under MG (Figure 3). Additionally, soluble protein contents were significantly lower in *S. grandis* and *A. frigida* than in *L. chinensis* and *C. squarrosa* under NG. These results indicated that grazing stress enhanced soluble protein-mediated osmotic adjustment in *S. grandis* and *A. frigida*.

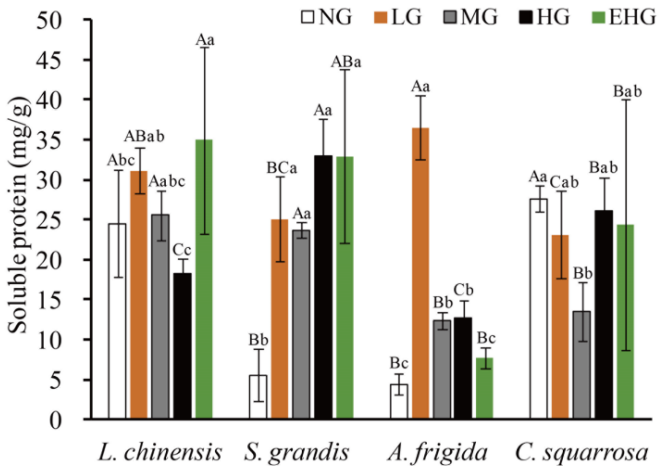

Figure 3. Soluble protein contents in *Leymus chinensis*, *Stipa grandis*, *Artemisia frigida*, and *Cleistogenes squarrosa* under different grazing intensities at Xilinhot, Inner Mongolia. NG, no grazing; LG, light grazing; MG, moderate grazing; HG, heavy grazing; EHG, extremely heavy grazing. The bars and error bars represent the mean ± 95% CI of three replicates. Significant differences among grazing intensities within each species are marked with different small letters, significant differences among species within each grazing intensities are marked with different capital letters (LSD test, $p < 0.05$).

3.4. Tannin and Total Flavonoid Contents

Tannins and total flavonoids are carbon-based secondary metabolites involved in plant defense against herbivory [16,32]. The tannin concentration varies with a variety of factors, such as plant genotype and tissue developmental stage, as well as environmental

stimuli [42]. The tannin content may be increased in response to herbivory and be positively associated with a plant's herbivory defense capability, which is manifested as deterrence and/or toxicity [42].

The tannin content of *L. chinensis* was decreased under MG, HG, and EHG compared with NG, and a significant difference was detected under EHG (Figure 4A). In *S. grandis* and *A. frigida*, grazing promoted tannin accumulation compared with NG, and a significant difference was observed under LG and MG in *S. grandis*, and under MG and EHG in *A. frigida*; while tannin accumulation was obviously decreased under HG and EHG compared with LG and MG in *S. grandis*, and decreased under HG compared with MG and EHG in *A. frigida* (Figure 4A). In *C. squarrosa*, with the increase in grazing intensity, the tannin content was at first slightly elevated and then reduced, and was significantly lower under HG compared with NG (Figure 4A). *C. squarrosa* showed significantly higher tannin contents than that of *A. frigida* under all five grazing intensities, *S. grandis* under NG, HG and EHG, and *L. chinensis* under MG and EHG. Differences in the tannin contents among the study species further illustrated the different response patterns that resulted from grazing. The tannin-associated defense response under grazing was increased in *S. grandis* and *A. frigida*, but was notably decreased under EHG in *L. chinensis* and HG in *C. squarrosa*, and *C. squarrosa* demonstrated predominantly better higher defense than the others under EHG.

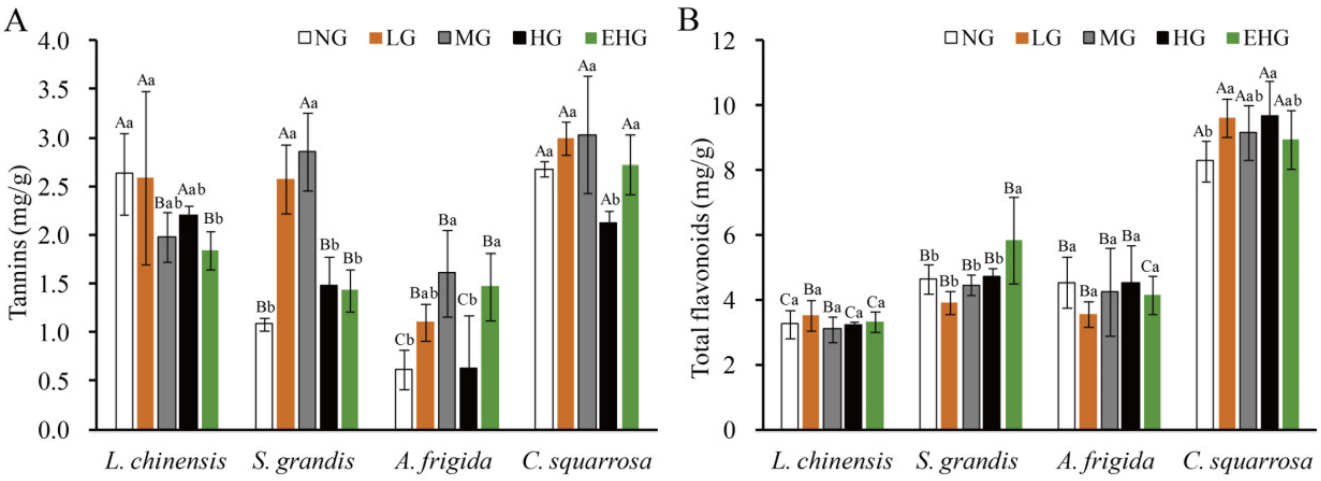

Figure 4. Tannin (**A**) and total flavonoid (**B**) contents in *Leymus chinensis*, *Stipa grandis*, *Artemisia frigida*, and *Cleistogenes squarrosa* under different grazing intensities at Xilinhot, Inner Mongolia. NG, no grazing; LG, light grazing; MG, moderate grazing; HG, heavy grazing; EHG, extremely heavy grazing. The bars and error bars represent the mean ± 95% CI of three replicates. Significant differences among grazing intensities within each species are marked with different small letters, significant differences among species within each grazing intensities are marked with different capital letters (LSD test, $p < 0.05$).

Flavonoids are phenolic compounds that perform diverse roles in plant development and defense, such as functioning as antioxidants in stressed plants to inhibit the production of reactive oxygen species caused by oxidative stresses [43–45]. The total flavonoid contents were slightly changed under the different grazing intensities in each species compared with NG, but significant differences were not observed, except for a significant increase under EHG in *S. grandis* and under LG and HG in *C. squarrosa* (Figure 4B). Notably, the total flavonoid contents in *C. squarrosa* were obviously higher than those of the other three species. This finding suggested that *C. squarrosa* may have a higher antioxidant capability in response to stresses than the other three species, at least with regard to flavonoids.

3.5. Plant Hormone Contents

Abscisic acid is a type of terpenoid that plays a critical regulatory role in a diverse range of plant growth and development processes and in adaptations to environmental stimuli, such as seed germination and dormancy, and drought, salt, and osmotic stress responses [46–48]. Elevation in ABA content in response to environmental stimuli is essential for the stress tolerance of plants [49,50]. In particular, ABA is prominently involved in responses to abiotic stresses [26].

The most outstanding function of ABA is in plant water balance and regulation of osmotic stress tolerance [51]. In the present study, the accumulation of ABA under the different grazing intensities differed among each species (Figure 5A). Compared with NG, grazing (except LG) significantly increased the accumulation of ABA in *L. chinensis*, for which the highest accumulation was observed under EHG. Except NG, significant differences were also demonstrated by comparison pairs among LG, MG, HG, and EHG in *L. chinensis*. In *S. grandis*, ABA accumulation significantly increased under MG and HG, but decreased under LG and EHG, compared with NG; obvious differences were evident under MG and HG compared with LG and EHG. In *A. frigida*, ABA accumulation significantly increased under LG, MG and HG, and decreased under EHG, compared with NG, while ABA accumulation significantly decreased under MG, HG and EHG and the lowest value was recorded under EHG, compared with LG. With regard to *C. squarrosa*, compared with NG, ABA accumulation was distinctly increased under MG, but was reduced under LG, HG, and EHG; compared with LG, ABA accumulation was remarkably increased under MG, HG and EHG, and a significant difference was observed among MG, HG and EHG. Additionally, *C. squarrosa* obviously accumulated higher ABA under NG, MG and HG than the other three species. Overall, the results inferred that MG elevated the abiotic stress tolerance of the four species by increasing the ABA content, whereas EHG decreased the ABA contents of *S. grandis*, *A. frigida*, and *C. squarrosa,* which is consistent with the reduced stress tolerance of those species. The remarkably enhanced ABA content under EHG of *L. chinensis* may be associated with the elevated abiotic stress tolerance of this species.

Salicylic acid is a phenolic compound [52] that plays a variety of roles in plant growth and development, including a central role in the regulation of plant defense signaling [52–54]. Accumulation of SA is commonly induced in plants after pathogen infection, accompanied by the activation of disease resistance responses and growth size inhibition [55,56]. In the present study, a similar trend in SA accumulation was observed in *L. chinensis*, *S. grandis*, and *C. squarrosa* (Figure 5B). In these three species, except under EHG of *L. chinensis*, SA accumulation significantly decreased under the grazing treatments compared with NG, with the lowest SA content being observed under HG; except under MG of *C. squarrosa*, SA accumulation significantly decreased under MG and HG compared with LG. In *A. frigida*, the SA content was significantly increased under LG and MG, but obviously decreased under HG and EHG, compared with NG (Figure 5B); HG and EHG distinctly decreased SA content compared with LG and MG. Among the different grazing treatments (LG, MG, HG, and EHG), except under MG of *A. frigida*, *L. chinensis* significantly accumulated higher SA content than the other species. These results indicated that SA-associated biotic stress tolerance was weakened by the grazing treatments. This may be due to two reasons. First, the plants were analyzed during the early stage of the growing season, when plants are more inclined to be in states of active growth. Second, plants were sampled prior to implementation of the grazing treatments in June 2020; thus, the defense system was inactivated by the decrease in SA contents in the different grazing plots. Taken together, these changes facilitated the dwarfism of plants to restore growth by optimizing the balance between growth and defense. In addition, in *A. frigida,* the enhanced SA contents under LG and MG may be closely associated with its specific growth strategy under competition with other plants. In *L. chinensis* and *S. grandis*, SA content treatment significantly increased under EHG compared with LG, MG and HG, which may positively associated with the plant's growth phenotype under EHG.

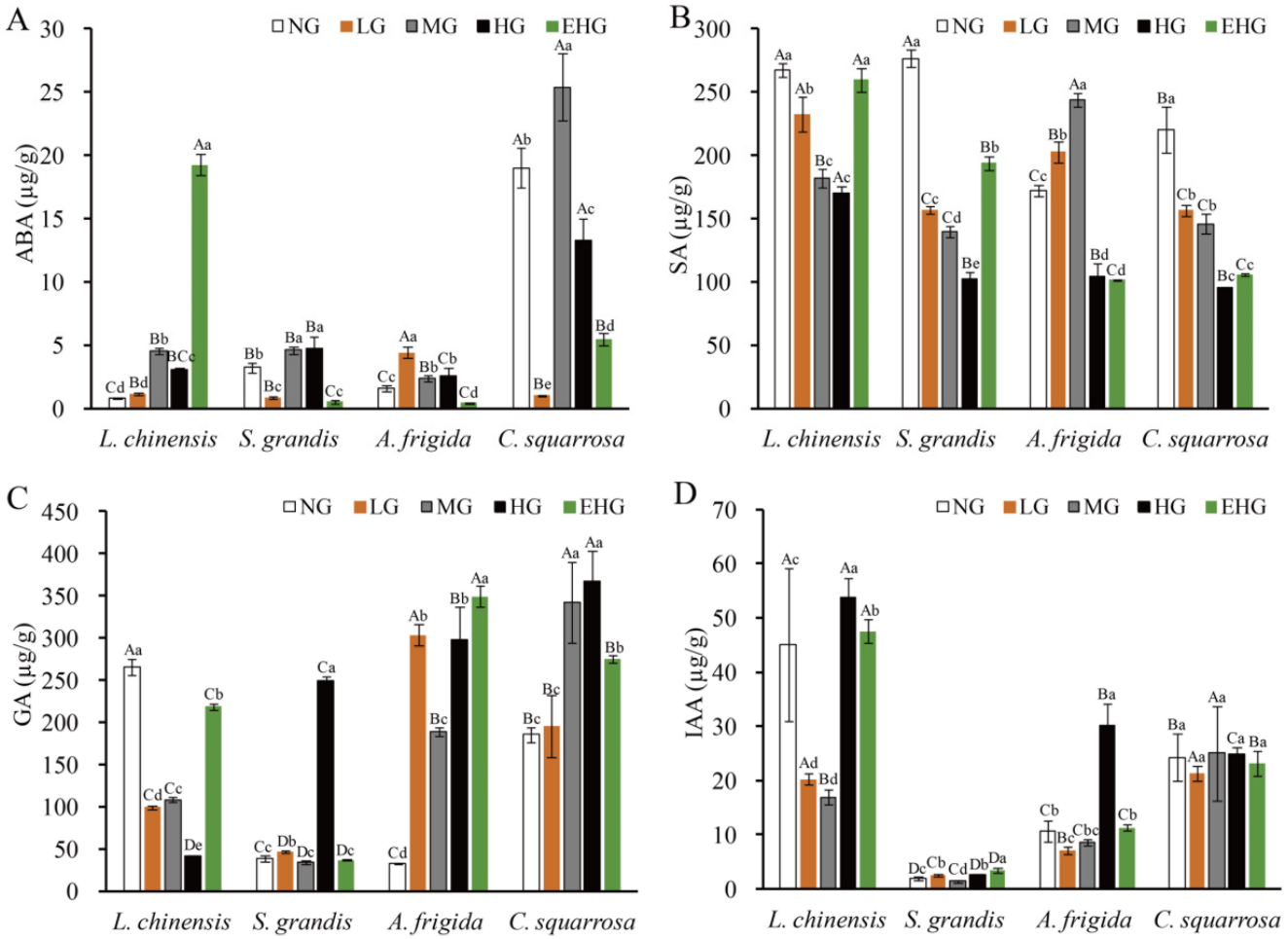

Figure 5. Phytohormone contents in *Leymus chinensis*, *Stipa grandis*, *Artemisia frigida*, and *Cleistogenes squarrosa* under different grazing intensities at Xilinhot, Inner Mongolia. (**A**) Abscisic acid (ABA), (**B**) salicylic acid (SA), (**C**) gibberellins (GA), (**D**) indole-3-acetic acid (IAA). NG, no grazing; LG, light grazing; MG, moderate grazing; HG, heavy grazing; EHG, extremely heavy grazing. The bars and error bars represent the mean ± 95% CI of three replicates. Significant differences among grazing intensities within each species are marked with different small letters, significant differences among species within each grazing intensities are marked with different capital letters (LSD test, $p < 0.05$).

Gibberellins are diterpenoid compounds involved in diverse plant developmental processes and function as a crucial plant growth regulator [57–59]. The contents of GAs are positively associated with plant growth, and increase or decrease GA accumulation, which results in corresponding taller or dwarf plants [59]. The dwarfism phenotype of GA-deficient mutants strongly supports the essential roles of GAs in normal plant growth [59]. In *L. chinensis*, compared with NG, the grazing treatments obviously reduced GA content; compared with LG and MG, HG significantly decreased GA content, while EHG significantly increased GA content (Figure 5C). In *A. frigida* and *C. squarrosa*, the grazing treatments distinctly elevated GA content compared with NG, except under LG for *C. squarrosa*; EHG obviously increased GA content in *A. frigida*, deceased GA content in *C. squarrosa*, compared with MG and HG (Figure 5C). In *S. grandis*, LG and HG significantly enhanced GA content compared with NG (Figure 5C). Under different grazing intensities, except in the comparison between *S. grandis* and *A. frigida* under NG, significant differences were observed among the four species. These results indicated that the GA regulation patterns of *L. chinensis* differed from those of the other three species under the grazing treatments. The dwarfism of plants under different grazing treatments with elevated GA

contents, was beneficial to promote growth at an early growth stage in the absence of grazing stress.

Indole-3-acetic acid is the most common auxin and is a crucial regulator of diverse plant developmental processes, including morphogenesis and adaptive responses [60,61]. In the present study, IAA accumulation did not differ significantly between NG and the different grazing treatments in *C. squarrosa* (Figure 5D). Similar trends in IAA accumulation were exhibited by *L. chinensis*, *A. frigida*, and *S. grandis* among different grazing treatments compared with NG; specifically, IAA accumulation decreased under LG (but not in *S. grandis*) and MG, and increased under HG and EHG, but no significant differences were observed under MG and EHG in *A. frigida*, compared with NG; significant differences were observed under HG and EHG (except under EHG in *A. frigida*), compared with LG (but not in *S. grandis*) and MG (Figure 5D). Under different grazing intensities, the accumulation of IAA was obviously lower in *S. grandis* than the other species. The differences in IAA contents accumulated under different intensities of grazing pressure may be associated with the regulation of plant morphogenesis. The elevated IAA contents under HG and EHG may have positively promoted plant dwarfism to restore growth.

Taken together, *L. chinensis* has a different adaptive strategy to respond to grazing by changing ABA and GA contents. Phytohormones played important roles in coordinating the regulation of its growth and grazing tolerance.

4. Conclusions

In summary, the present results show that the four study species (*L. chinensis*, *S. grandis*, *A. frigida*, and *C. squarrosa*) differ in their adaptive strategies to cope with the different grazing intensities. *Artemisia frigida* contained higher contents of lignin than the other three species, but the lignin content was significantly decreased by the grazing treatment, whereas *C. squarrosa* contained higher total flavonoids than the other three species. *Leymus chinensis* showed a different grazing response, with respect to cellulose and tannin contents, compared with the other three species. *Stipa grandis* and *A. frigida* demonstrated an increased accumulation of soluble protein in response to grazing, while the soluble protein contents significantly reduced under MG in *C. squarrosa*, and significantly increased under EHG in *L. chinensis*, compared with NG. The contents of certain phytohormones (ABA, SA, and GAs) remarkably changed under grazing among the four species. Specifically, compared with NG, the ABA contents were distinctly elevated under the different grazing treatments in *L. chinensis* (except LG), whereas a different ABA response pattern (at first elevated under MG for *S. grandis* and *C. squarrosa* or LG for *A. frigida*, and thereafter reduced under EHG) was displayed by the other three species. The GA contents were significantly decreased by the grazing treatment in *L. chinensis*, but increased in the other three species (except under MG and EHG in *S. grandis* and under LG in *C. squarrosa*). Similar trends in SA (except under LG and MG in *A. frigida*) and IAA contents were observed among the four species. The present results indicate that *L. chinensis* has a different adaptive strategy to respond to grazing by changing ABA and GA contents, and these phytohormones may be involved in coordinating the regulation of its growth and grazing tolerance. We interpret that dwarfism of plants under different grazing treatments may be more inclined to restore growth by promoting GA- (or IAA-) mediated growth and weakening SA-mediated biotic stress tolerance.

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