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Effect of long-term grazing stress on the contents of phenolic compounds in Carex and Aster plants in Mongolia

Purevdorj Erdenetsogt[1#], Nomin Munkhbat[1#], Enkhriimaa Narmandakh[2], Tuvshintogtokh Indree[2], Chuan-Ming Yeh[3], Munkhtsetseg Tsednee[1*]

[1]*Institute of Chemistry and Chemical Technology, Mongolian Academy of Sciences, Ulaanbaatar 13330, Mongolia*
[2]*Botanic Garden and Research Institute, Mongolian Academy of Sciences, Ulaanbaatar 13330, Mongolia*
[3]*Institute of Molecular Biology, National Chung Hsing University, Taichung 40227, Taiwan*

**Corresponding author: mugi@mas.ac.mn; ORCID ID: 0000-0002-2878-0112*

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ABSTRACT

Long-term grazing changes the plant diversity and their growth significantly. At the metabolite level, the contents and compositions of the specialized metabolic compounds in plants could also be altered under long-term grazing conditions. To understand the metabolic changes caused by the grazing stress, in this study, we compared the contents and compositions of major phenolic compounds, together with total flavonoid contents, in two grazing-tolerant plants, *Carex duriuscula* C.A. Mey. and *Aster hispidus* Thumb., and two grazing-non-tolerant plants, *Carex pediformis* C.A. Mey. and *Aster alpinus* L., and a local native plant *Stipa baicalensis* Roshev. under long-term grazing conditions in Mongolia. Our results showed that long-term grazing has altered the contents and compositions of phenolic compounds and flavonoids both in above-ground tissues and roots of the analyzed five plants. Interestingly, such effects could appear to differ depending on plant species. These results provide the first comparative study of metabolite changes in grazing-tolerant and grazing-non-tolerant plants under overgrazing stress in the country.

Keywords: *Carex, Aster, Stipa baicalensis*, grazing, phenol, flavonoid

INTRODUCTION

The structure of the plant community in grassland depends strongly on livestock grazing by livestock and it often reduces significantly under overgrazing conditions [1, 2]. The national reports have shown that about 70% of pasture land in Mongolia has been damaged due to an increase in domestic livestock and climate changes [3]. However, some plant species called grazing-tolerant plants or degradation-indicator plants could still grow under grazing pressure. Previously, the study has reported 37 plant species from 14 families, including Amaranthaceae, Asteraceae, and Poaceae, in Mongolia as grazing-tolerant plants under long-term grazing [2, 4]. The abundances of these identified grazing-tolerant plants could be different in different steppes of Mongolia which is typically divided into six subtypes, including high-cold mountain steppes, mountain steppes, the meadow steppes, dry steppes, decertified steppes and desert steppes [1, 5]. It is also known that under long-term overgrazing in Mongolia, the percentage of dwarf-semi-shrubs and annual plants increases in the plant community compared with that of perennial plants and shrubs [1, 5]. The results thus indicated that the dwarf-semi-shrubs and the annuals are the plants that dominate among the grazing-tolerant plants and appear as the indicators of grassland degradation. Notably, the dominant plant species under grazing pressure in Mongolia are not all unpalatable species, but a mixture of unpalatable and palatable plants [1, 6]. It further suggests that some palatable species might have developed survival strategies against overgrazing stress in the long-term.

The involvements of specialized metabolic compounds in the tolerance or avoidance mechanisms against environmental stresses are often expected in plants [7, 8]. Plant volatile organic compounds, VOCs, are known to be released in increased amounts from the damaged Artemisia adamsii [9]. In addition, the phenolic compounds in plant tissues have been reported to play critical roles in plant adaptation to different abiotic and biotic stresses [10, 11]. Root-released metabolic compounds could also facilitate survival in damaged soil environments for efficient nutrient uptakes [12, 13]. Mongolian plants are rich in

diverse metabolic compounds, especially with various valuable bioactivities in their different tissues, due to habituated harsh environments in the country [14, 15]. Regarding the metabolomic analyses, there are limited, indeed almost no studies, in Mongolian pasture plants under long-term grazing. Understanding how long-term grazing could affect the metabolic composition of the plants would be interesting, yet, important to further improve and restore the damaged vegetation under grazing. In this study, we, therefore, aim to study the metabolic compositions of grazing-tolerant and grazing-non-tolerant plants under long-term grazing conditions in Mongolia.

EXPERIMENTAL

Experimental design: In our study, we used two grazing tolerant plants, *Carex duriuscula* and *Aster hispidus*, and two grazing non-tolerant plants, *Carex pediformis* and *Aster alpinus*, to determine whether metabolic compounds in their tissues have altered under long-term grazing compared with non-grazing condition (Fig. 1).

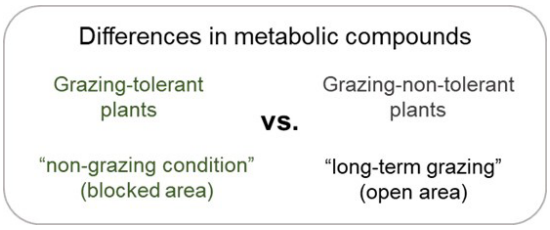

Fig. 1. Experimental design of the study. Grazing-tolerant plants (*Carex duriuscula* and *Aster hispidus*) and grazing non-tolerant plants (*Carex pediformis* and *Aster alpinus*) were selected for the study from both "non-grazing" blocked areas and "long-term grazing" open areas at the same time.

Plant materials: The tissues of five plants *C. duriuscula, C. pediformis, A. hispidus, A. alpinus*, and *Stipa baicalensis* were collected in Mungun-morit plant field station at Mungun-morit soum, Tuv aimag, Mongolia. The field station was established by the Botanic Garden and Research Institute, Mongolian Academy of Sciences, in the 1990s for the long-term plant studies. The blocked pasture area (100 m x 10 m square) was used for a "non-grazing field" as it protects entering livestock into the separated place. The area outside the blocked area is considered as "grazing field". The above-ground tissues and roots of the selected plants were collected in July, August, and September of 2021 and used for the study after drying.

Chemicals and reagents: All organic solvents and chemicals, including methanol, chloroform, pyridine, hydrochloric acid, gallic acid, aluminum chloride, sodium nitrate, catechin, N,O-Bis (trimethylsilyl) -trifluoroacetamide,trimethylchlorosilan methoxyamine, Folin-Ciocalteu reagent, and NP/PEG reagent, used in the study were purchased from Sigma-Aldrich (Sigma-Aldrich Chemie GmbH at Munich, Germany and Sigma Aldrich at St Louis, MO, USA) with analytical grades.

Quantification of total phenols: The extraction and quantification of total phenols in the samples followed the previously standardized method in the lab [16]. Briefly, approximately, 0.1 g of tissue samples were extracted with 60% ethanol at 60 °C for 2 hours and diluted before conducting the quantification procedure by Folin-Ciocalteu reagent. The results are expressed as equivalence to Gallic acid contents after measurement at 750 nm on a spectrophotometer (UV-1600 PC spectrophotometer, VWR International GmbH). The quantification was performed with three replicates of plant samples.

Quantification of total flavonoids: The methods for extraction and quantification of total flavonoids in plant samples were adopted from Dessiee *et al*. [17]. In brief, the flavonoids were determined using an aluminum chloride colorimetric assay. Plant tissue samples were extracted by ethanol at 60 °C for 2 hours and centrifuged. Further, 0.5 mL of extracts taken from the supernatant were used for quantification analysis. The reaction mixture used 5% sodium nitrate and 10% aluminum chloride, as described previously [17], and the absorbance of the solution was measured at 510 nm on a spectrophotometer (UV-1600 PC spectrophotometer, VWR International GmbH). Total flavonoid contents in samples were expressed as equivalence to Catechin contents and all the samples were analyzed in triplicates.

Analysis of phenolic compounds by Gas chromatography-mass spectrometry (GC-MS): For the GC-MS analysis, total metabolites from 100 mg homogenized tissue samples were extracted with 80% methanol (CH_3OH/H_2O v/v, 8:2) under sonication at room temperature for 30 min. The samples were then centrifuged at 13,000 rpm for 15 min to collect the supernatant. The same volume of the supernatant aliquots from different plant samples were further freeze-dried completely after adding the internal standard, Ribitol. The dried extract samples were derivatized with 10 µL methoxyamine (20 mg/mL in pyridine solution) at 30 °C for 90 min and 50 µL N,O-Bis (trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane at 70 °C for 120 min. The metabolite analysis was performed by Gas chromatography/ quadrupole time-of-flight mass spectrometry (Agilent 7250 GC/Q-TOF, Agilent) using Agilent ZORBAX DB5-MS + 10 m Duragard capillary column (30 m x 250 µm x 0.25 µm). The helium flow rate was 1 mL/min and the injector mode used 0.5 mL, split 2:1. The injector and MS transfer line temperatures were 250 °C and 300 °C, respectively. Analytical run temperature program followed 60 °C for 1 min, 10 °C /min to 325 °C, and held constant for 10 min, and the acquisition rate was 5 spectra/second. For the metabolite analysis, three replicates of tissue samples were extracted from different plants.

Detections of metabolic compounds by Thin Layer Chromatography (TLC): The detections of metabolic compounds, including phenolic acids and phenols, in plant tissues were examined by TLC chromatogram using Natural Products-Polyethylene Glycol reagent (NP/PEG reagent) under 366 nm and Vanilin-sulphuric acid reagent following the previously described procedures [18]. The mobile phases were used as shown in the lover panel of the chromatograms in Supplementary data. The band intensities in the samples were estimated using Image J from the chromatograms.

Statistical analysis: The statistical analysis was performed with a Student's *t-test* using three replicates of each measurement data. The significant changes in the compared samples were shown with asterisks, where *, P<0.05, ***, P < 0.001, and ns-not significant.

RESULTS AND DISCUSSION

Contents of total phenols in above-ground parts and roots of plants under long-term grazing: In our study, we used two grazing tolerant plants, *C. duriuscula* and *A. hispidus*, and two grazing non-tolerant plants, *C. pediformis* and *A. alpinus*, to determine whether phenolic compounds in their tissues have altered under long-term grazing. In plants, different phenolic compounds are known to play critical roles against biotic and abiotic stresses [8, 19]. We, therefore, first wanted to determine the contents of total phenols in plants under grazing stress. In plant samples, additionally, we used *S.baicalensis* as a reference plant that is native to the local growing area. The samples were collected from the selected five plants during July, August, and September and the livestock grazing is active during these months. To compare the long-term grazing stress condition to the control non-grazing condition, we collected plant samples from two field growing conditions, the "open-grazing area" and the "closed-non-grazing area". "The closed non-grazing area" in the selected field station of the research institute was established by protecting the area from grazing with blocks over 10 years ago.

We first determined the contents of total phenols in above-ground parts of the plants (Fig. 2). Interestingly, we observed different trends in the alterations of total phenol contents in the analyzed five plants under long-term grazing. In the above-ground part of the local native plant *S. baicalensis,* there was a decrease in the contents of total phenols under long-term grazing in July, whereas the contents were relatively not altered in the following months (Fig. 2A). However, for the grazing-tolerant plant *C. duriuscula*, the contents of total phenols in its above-ground parts showed significant decreases under long-term grazing compared with non-grazing samples in all three months. In contrast, the total phenol contents in above-ground parts of *C. pediformis* plants increased significantly under long-term grazing (Fig. 2A). Of note, the contents of total phenols in *C. pediformis* were relatively higher than that of the other two plants in July and August samples, especially samples collected from long-term grazing conditions. As our interest in comparing two *Carex* plants, we observed that total phenol contents in non-grazing-tolerant *C. pediformis* were significantly higher than those in grazing-tolerant *C. duriuscula*.

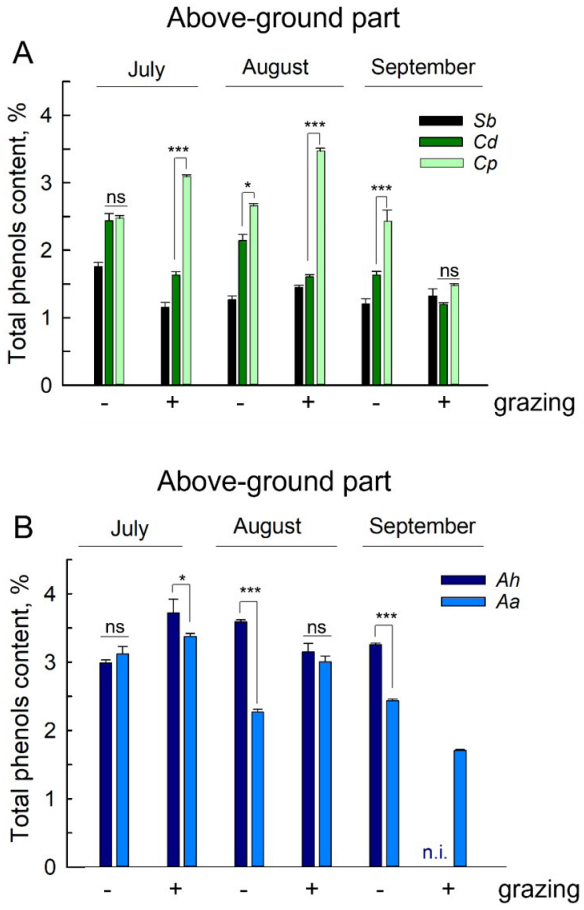

Fig. 2. Time-course analyses of total phenolic compounds in above-ground parts of plants. Total phenols were quantified in above-ground parts of (A) *Stipa baicalensis (Sb), Carex duriuscula (Cd)* and *Carex pediformis (Cp)* and (B) *Aster hispidus (Ah)* and *Aster alpinus (Aa)* plants under indicated months of the year. Asterisks indicate significant changes in the compared samples (Student's t-test; *, P<0.05, ***, P < 0.001). ns-not significant; (-) and (+) symbols correspond to without and with grazing conditions, respectively.

For the case of grazing-tolerant *A. hispidus* plants, the total phenol contents were not significantly affected by the long-term grazing (Fig. 3B). However, the contents of total phenols in above-ground parts of grazing-non-tolerant *A. alpinus* plants tends to increase under grazing stress during summer, in July and August, whereas it decreases in September samples. Furthermore, the comparison of two Aster plants showed that under long-term grazing conditions, these

plants have significant differences in their total phenol contents, higher in the *A. hispidus* tissues than in *A. alpinus* (Fig. 2B). Next, we wanted to determine the total phenol contents in root tissues of the five plants (Fig. 3). In our reference native plant, *S. baicalensis* roots, we obtained a similar trend as observed in above-ground part that its total phenol contents were not significantly altered under long-term grazing stress through the sampling months (Fig. 3A). However, the total phenol contents in both Carex roots were altered under long-term grazing. Besides, their contents of total phenols in both plant roots were higher compared with that of *S. baicalensis* roots (Fig. 3A).

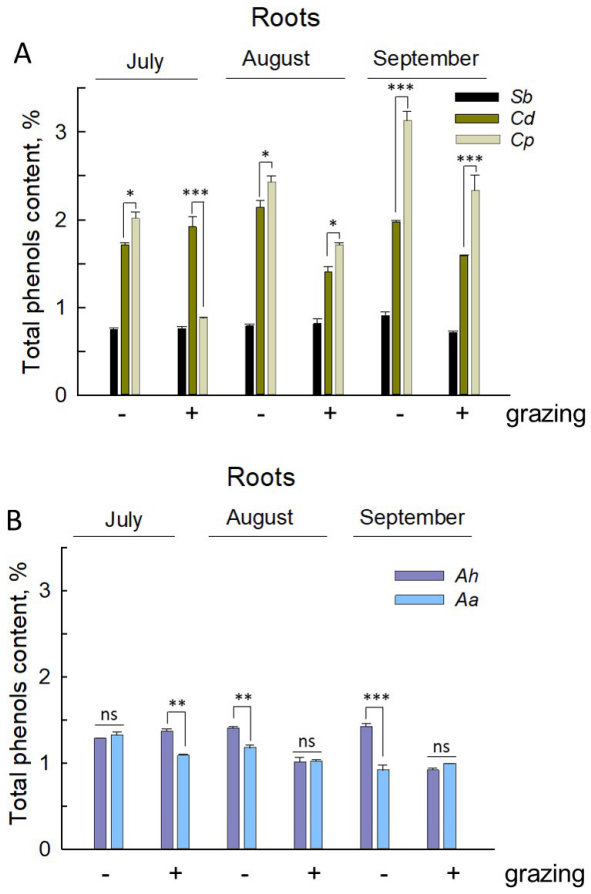

Fig. 3. Time-course analyses of total phenolic compounds in plant roots. Total phenols were quantified in roots tissues of (A) *Stipa baicalensis (Sb), Carex duriuscula (Cd), Carex pediformis (Cp)* and (B) *Aster hispidus (Ah)* and *Aster alpinus (Aa)* plants under indicated months of the year. Asterisks indicate significant changes in the compared samples (Student's t-test; *, P<0.05, ***, P < 0.001). ns-not significant; (-) and (+) symbols correspond to without and with grazing conditions, respectively.

We observed slight or almost no changes in total phenol contents in *C. duriuscula* while the contents of total phenols were decreased, up to half of non-grazing conditions, in *C. pediformis* roots under long-term grazing (Fig. 3A).

For two A*ster* plants, the total phenol contents in *A. hispidus* roots were reduced under grazing in August and September while the contents were not altered in July samples (Fig. 3B). Notably, the total phenols in the roots of *A. alpinus* showed no obvious changes by the long-term grazing stress (Fig. 3B). Overall, these quantification results of total phenols suggest that the changes in total phenols under long-term grazing could be different depending on the plant species. Such alterations of total phenols appeared with a higher degree in two *Carex* plants compared with two Aster plants and *S. baicalensis.*

The relative quantifications of major phenolic compounds in plants under long-term grazing: Since we observed the significant alternations in total phenol contents in *Carex* plant tissues, we next wanted to compare the phenolic compounds in the samples with visual detections using a Thin-layer chromatography (TLC). Our comparative TLC analyses with total methanol extract of the plant samples, including the above-ground parts and root extracts, showed the numbers of interesting bands corresponding to different phenolic compounds affected by long-term grazing stress (Supplementary **Fig. S1** and **Fig. S2**). Therefore, TLC analyses also indicated that the long-term grazing has altered the contents of the phenolic compounds in the studied plants. To further obtain the detailed quantification results of the phenolic compounds in these plants, we chose two *Carex* plant samples and performed GC-MS analysis in their root extracts. The roots samples were collected from non-grazing and long-term grazing conditions. Using GC-MS analysis, we identified 20 major phenolic compounds, including phenolic acids and phenols, in two Carex roots and compared their relative abundances under long-term grazing stress (Table 1). In *C. duriuscula* roots, we observed the increases in relative abundances of only two phenolics, ferulic acid, and benzoic acid ester, under long-term grazing. However, there were six phenolic compounds with decreased abundances in *C. duriuscula* roots under long-term grazing while the other 12 phenolics were not significantly affected by their contents in roots (Table 1). In contrast to *C. duriuscula* samples, we observed more numbers of phenolic compounds, seven phenols, with increased abundances under long-term grazing such as cinnamic acid and 3-O-feruloyl quinic acid, in *C. pediformis* roots. Besides, there were six phenolic compounds with reduced abundances under long-term grazing in *C. pediformis* roots and the remaining seven phenolic compounds showed no significant alterations in their contents by the long-term grazing stress (Table 1). Together, the GC-MS results suggested that long-term grazing affects the relative abundances of major phenolic compounds in two Carex roots differentially. In particular, the grazing stress has resulted in decreased abundances of major phenols in *C. duriuscula* roots compared with that of *C. pediformis* roots.

Table 1. Relative quantifications of the phenolic compounds in *C. duriuscula* and *C. pediformis* roots under non-grazing and long-term grazing stress.

No	Phenolic acids / phenols	*C.duriuscula*			*C.pediformis*		
		non-grazing (NG)	grazing (G)	G/NG ratio	non-razing (NG)	grazing (G)	G/NG ratio
1	Ferulic acid	0.63 ±0.04	0.70 ±0.05	1.1 ↑	0.90±0.04	0.95 ±0.06	1.0 →
2	Caffeic acid	0.16 ±0.02	0.06 ±0.01	0.4 ↓	0.59 ±0.03	0.30 ±0.04	0.5 ↓
3	Benzoic acid	0.56 ±0.06	0.40 ±0.02	0.7 →	0.28 ±0.16	0.16 ±0.02	0.6 →
4	Cinnamic acid	0.89 ±0.12	0.66 ±0.06	0.7 →	0.02 ±0.01	0.26 ±0.10	16.5 ↑
5	Hydroxybenzoic acid	4.66 ±1.05	2.51 ±0.55	0.5 ↓	1.59 ±0.06	1.29 ±0.48	0.8 ↓
6	Chlorogenic acid 2	17.56 ±2.63	3.39 ±0.24	0.2 ↓	2.16 ±0.69	1.28 ±0.18	0.8 ↓
7	Chlorogenic acid 1	1.49 ±0.26	0.74 ±0.39	0.5 ↓	0.23 ±0.04	0.42 ±0.05	1.9 ↑
8	Cis-5-O-Feruloylquinic acid	4.66 ±2.08	1.66 ±0.17	0.4 ↓	0.43 ±0.24	0.52 ±0.30	1.2 →
9	5-O-Feruloylquinic acid	1.71 ±0.61	1.08 ±0.03	0.6 →	0.25 ±0.02	0.42 ±0.05	1.7 ↑
10	3-O-Feruloylquinic acid	0.89 ±0.22	0.66 ±0.15	0.7 →	0.18 ±0.04	2.59 ±0.70	14.2 ↑
11	Benzoic acid ester	0.22 ±0.06	0.30 ±0.01	1.4 ↑	0.34 ±0.04	0.18 ±0.08	0.5 ↓
12	Alpha tocopherol	2.16 ±0.56	1.28 ±0.05	0.5 ↓	1.59 ±0.06	1.73 ±0.01	1.1 →
13	Delta tocopherol	4.26 ±0.52	3.50 ±0.27	0.8 →	2.16 ±0.60	0.14 ±0.03	8.2 ↑
14	Gamma tocopherol	1.41 ±0.06	0.89 ±0.13	0.6 →	0.13 ±0.02	0.12 ±0.01	0.9 →
15	Piceatannol	1.63 ±0.22	1.18 ±0.04	0.7 →	0.58 ±0.24	0.60 ±0.30	1.0 →
16	Resveratrol	23.36 ±3.00	13.83 ±1.85	0.6 →	8.85 ±1.63	9.07 ±0.68	1.0 →
17	Taxifolin	0.86 ±0.22	0.66 ±0.05	0.7 →	1.45 ±0.05	1.07 ±0.08	0.7 →
18	2,4-di-tert-butylphenol	2.16 ±0.53	1.33 ±0.03	0.6 →	1.02 ±0.16	1.35 ±0.19	1.3 ↑
19	Dihydroxybenzoic acid	0.06 ±0.06	0.06 ±0.22	0.9 →	0.09 ±0.02	0.09 ±0.01	1.0 →
20	Sinapyl alcohol	0.09 ±0.01	0.09 ±0.04	1.0 →	0.04 ±0.02	0.10 ±0.01	2.3 ↑

The relative quantifications of the phenolic compounds were obtained using the normalized peak areas of the compounds to that of the internal standard. The arrows indicate the increase (>1.1 ↑), decrease (<0.5 ↓), and no significant change (0.5-1.0 →) in ratios of grazing (G)/non-grazing (NG) conditions, respectively. The relative quantifications are the mean of three biological replicates of root samples.

The contents and detections of flavonoids in the plants under long-term grazing: In addition to phenolic compounds, flavonoids are also known to be involved in plant adaptation to different environmental stresses such as heat and oxidative stress [20, 21]. We, therefore, further wanted to determine the contents of total flavonoids in the five plants. For the flavonoid analysis, we used the above-ground parts of the plants. Interestingly, the contents of total flavonoids in all five plant samples were affected significantly by the long-term grazing (Fig. 4). Relatively, a mild effect was observed in above-ground parts of *S. baicalensis* in August and September whereas its contents were reduced in July (Fig. 4A).

In *C. duriuscula* samples, the contents of total flavonoids showed strong decreases under long term grazing in July and August samples. Of note, we did not detect sufficient amounts of flavonoids in its September samples which needs to be confirmed with further studies in the same months of next few years.

In contrast to *C. duriuscula,* total flavonoid contents were increased significantly in *C. pediformis* samples under long-term grazing (Fig. 4A). Thus, their increased contents made an obvious difference in these two *Carex* plants under grazing stress. For two *Aster* plants, the total flavonoid contents appear not much affected by long-term grazing in *A. hispidus* tissues, whereas the contents of total flavonoids in *A. alpinus* showed increasing trends under grazing (Fig. 4B). Overall, the results of flavonoid compounds suggested that as observed in phenolic compounds, the contents of total flavonoids could be affected differentially for different plant species.

Overview of long-term grazing effects on the contents of total phenols and flavonoids: Our quantification results of total phenols and total flavonoids showed that compared with *Aster* plants, these metabolic compounds in two *Carex* plants have been affected significantly by long-term grazing. To further observe the distinct pattern in *Carex* plants, we generated an illustrative graph using the normalized contents of the phenolic compounds and flavonoid data (Fig. 5). The graph data showed a separate pattern in these plants between non-grazing and long-term grazing stress conditions. Under long-term grazing, the total phenol contents in *C. duriuscula* showed the

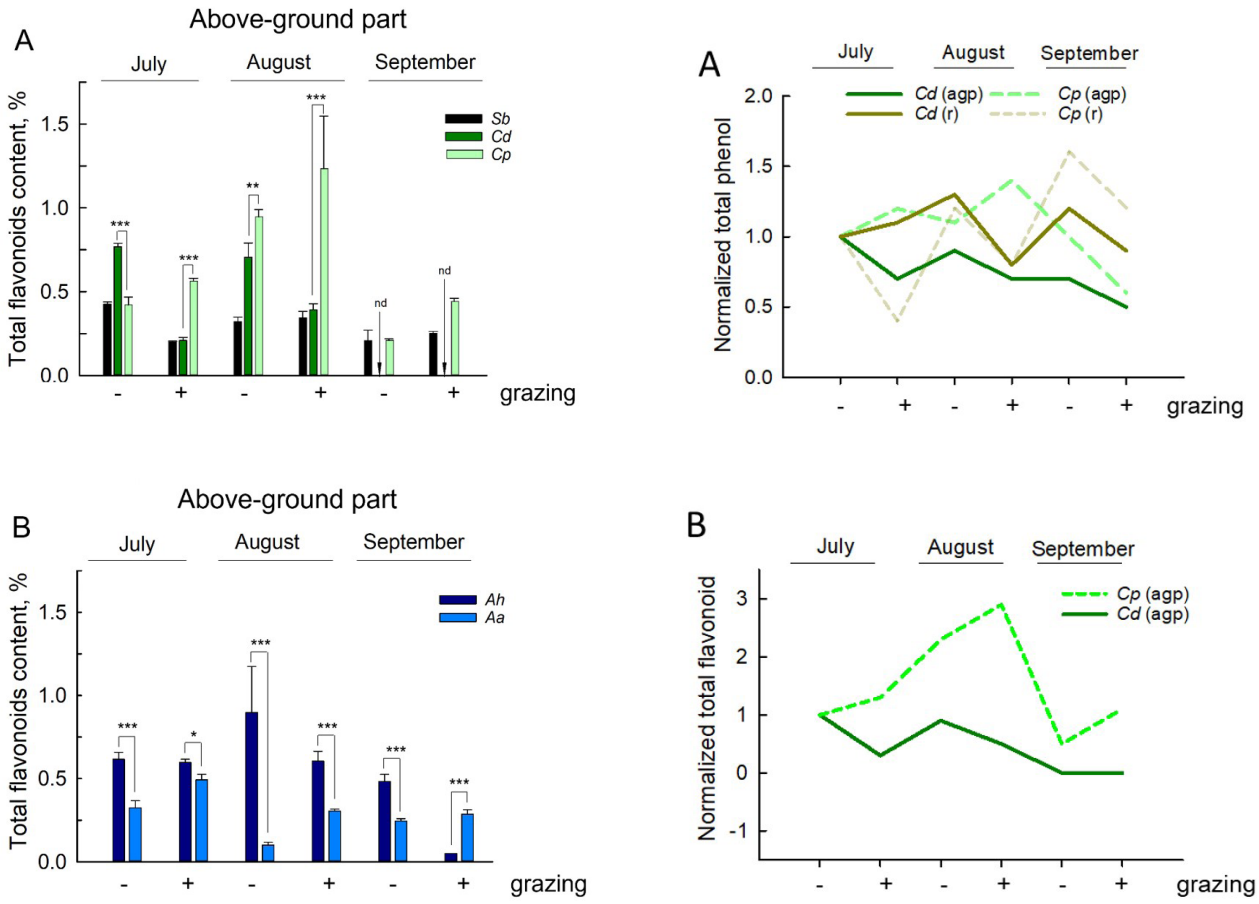

Fig. 4. Time-course analyses of total flavonoids. Total flavonoids were quantified in above-ground parts of (A) *Stipa baicalensis (Sb), Carex duriuscula (Cd)* and *Carex pediformis (Cp)* and (B) *Aster hispidus (Ah)* and *Aster alpinus (Aa)* plants under indicated months of the year. Asterisks indicate significant changes in the compared samples (Student's t-test; *, P<0.05, ***, P < 0.001). ns-not significant; (-) and (+) symbols correspond to without and with grazing conditions, respectively. nd-not detected.

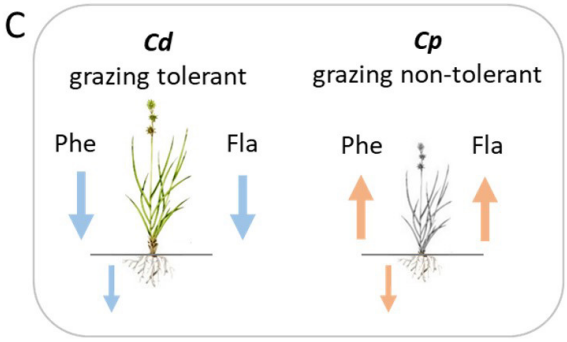

Fig. 5. Summarized overview of total phenolic compounds and flavonoids under grazing. Under grazing stress conditions, the contents of total phenols (Phe) and total flavonoids (Fla) in grazing-tolerant *Carex duriuscula (Cd)* above-ground tissues tended to decrease in contrast to the increased contents of these metabolites in the case of non-tolerant *Carex pediformis (Cp)* plant. Arrows indicate increases and decreases, respectively in the above-ground parts of the plants

tendency in reductions in both above-ground part and root tissues (Fig. 5A). However, in *C. pediformis* tissues, the long-term grazing affects strongly by increasing the contents of total phenols in above-ground tissues while reducing its contents in roots (Fig. 5A). The contents of total flavonoids in above-ground tissues of two plants showed an opposite trend, increases in *C. duriuscula* and decreases in *C. pediformis (*Fig. 5B). In summary, long-term grazing has affected the contents of phenols and flavonoids in different distinct ways in two *Carex* plants (Fig. 5C).

The phenolic compounds in roots are particularly important in regulating root acquisition of essential nutrients from soils and modifying the soil microbial community [22, 23], including under long-term overgrazing conditions [24]. In addition, tannins and flavonoids could also participate in plant stress response and protection from various environmental stresses [24, 25]. Since long-term grazing alters the contents of these metabolic compounds in *Carex* plants, it is interesting to study the overall metabolomic features in different tissues of these plants under long-term grazing in the future. Furthermore, the study of nutrient compositions of the plants under long-

term grazing would also be critical since metabolic compounds contribute to nutrient homeostasis [26] under stress conditions.

CONCLUSION

We have conducted the first comparative metabolomic analysis in grazing tolerant *C. duriuscula* and *A. hispidus,* and grazing non-tolerant *C. pediformis* and *A. alpinus* plants together with *S. baicalensis* plant under long-term grazing conditions in Mongolia. Our analyses on the contents of major phenolic compounds and total flavonoids in these plants showed that long-term grazing has significantly affected the contents of these metabolic compounds in their both above-ground tissues and root parts. Notably, the contents of total phenols and flavonoids in above-ground tissues showed a tendency in reductions in grazing-tolerant *C. duriuscula* while their contents were increased in grazing non-tolerant *C. pediformis*. Besides, the contents of total phenols and flavonoids in the roots of these plants showed decreases under long-term grazing. Taken together, our study provides critical information on the effects of long-term grazing in Mongolian plants. In addition, the study will contribute greatly for further metabolic studies on these plants.

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AUTHOR CONTRIBUTION

#The authors equally contributed in this work.

REFERENCES

1. Munkhzul O., Oyundelger K., Narantuya N., Tuvshintogtokh I., Oyuntsetseg B., *et al*. (2021) Grazing effects on Mongolian steppe vegetation - A systematic review of local literature. *Front. Ecol. Evol.*, **9**, 703220, 1-13. https://doi.org/10.3389/fevo.2021.703220
2. Ahlborn J., von Wehrden H., Lang B., Römermann C., Munkhzul O., *et al*. (2020) Climate - grazing interactions in Mongolian rangelands: Effects of grazing change along a large-scale environmental gradient. *J. Arid. Environ.*, **173**, 104043, 1-10. https://doi.org/10.1016/j.jaridenv.2019.104043
3. Yearly report of Mongolian pasture land (2016 Ministry of Environment and Tourism, Mongolia.
4. Tuvshintogtokh I. (2014) Grassland in Mongolia and their degradation indicator plants. In: *International Symposium on the East Asia Environmental Problems (EAEP2014)*, *Fukuoka*, **8**. https://www.researchgate.net/publication/271509448
5. Tuvshintogtokh I. (2014) The steppe vegetation of Mongolia. Bembi san printing, Ulaanbaatar, 610.
6. Tuvshintogtokh I., Ariungerel D. (2013) Degradation of Mongolian grassland vegetation under overgrazing by livestock and its recovery by protection from livestock grazing. In: Yamamura N., Fujita N., Maekawa A. (eds). *The Mongolian Ecosystem Network: Environmental Issues under Climate and Social Changes*. Ecological research monographs. Springer, Tokyo. 115-130. https: doi.org/10.1007/978-4-431-54052-6_10
7. Shulaev V., Cortes D., Miller G., Mittler R. (2008) Metabolomics for plant stress response. *Physiol. Plant.*, **132**(2), 199-208. https://doi.org/10.1111/j.1399-3054.2007.01025.x
8. Aguirre-Becerra H., Vazquez-Hernandez M.C., Saenz O.D., Alvarado-Mariana A., Guevara-GonzalezR.G.,*etal*.(2021)Roleofstressanddefense in plant secondary metabolites production. In: Pal D., Nayak A.K (eds) *Bioactive Natural Products for Pharmaceutical Applications*. Advanced structured materials, **140**. Springer, Cham. 151-195. https://doi.org/10.1007/978-3-030-54027-2_5
9. Tsubo M., Nishihara E., Nakamatsu K., Cheng Y., Shinoda M. (2011) Plant volatiles inhibit restoration of plant species communities in dry grassland. *Basic App. Ecol.*, **13**(1), 76-84. https://doi.org/10.1016/j.baae.2011.11.005
10. Waśkiewicz A., Muzolf-Panek M., Goliński P. (2013) Phenolic content changes in plants under salt stress. In: Ahmad P., Azooz M., and Prasad M. (eds) *Ecophysiology and Responses of Plants under Salt Stress*. Springer, New York, NY. https://doi.org/10.1007/978-1-4614-4747-4_11
11. Corso M., Perreau F., Mouille G., Lepiniec L. (2020) Specialized phenolic compounds in seeds: Structures, functions, and regulations. *Plant Sci.*, **296**, 10471. https://doi.org/10.1016/j.plantsci.2020.110471
12. Tsednee M., Mak Y.W., Chen Y.R., Yeh K.C. (2012) A sensitive LC-ESI-Q-TOF-MS method reveals novel phytosiderophores and phytosiderophore-iron complexes in barley. *New Phytol.*, **195**(4), 951-961. https://doi.org/10.1111/j.1469-8137.2012.04206.x
13. Tsednee M., Yang S.C., Lee D.C., Yeh K.C. (2014) Root-secreted nicotianamine from *Arabidopsis halleri* facilitates zinc hypertolerance by regulating zinc bioavailability. *Plant Physiol.*, **166**(2), 839-852. https://doi.org/10.1104/pp.114.241224
14. Oidovsambuu S., Tsagaantsooj T., Bekh-Ochir D., Maygmar N., Batkikh I., *et al*. (2024) Cytotoxicity screening of 114 Mongolian plant extracts on liver, colon, breast, and cervix cancer cell lines. *Mong. J. Chem.,* **25**(51), 11-17. https://doi.org/10.5564/mjc.v25i51.2934
15. Selenge D., Jamyansan Ya., Javzan S.,

Delegmaa M., Dumaa M., Gerelt-Od Ya., Solongo A., Nyamsuren E., Mungunshagai B. (2018) Alkaloids from some Mongolian medicinal plants. *Bull. Inst. Chem. Chem. Technol*., (**6**), 10-20. https://doi.org/10.5564/bicct.v0i6.1095

16. Method standard for quantification of phenolic compounds using spectrophotometer (2020) The Institute of Chemistry and Chemical Technology, Mongolian Academy of Sciences, CAS-01-01:2020
17. Ayele D.T., Akele L.M., Melese A.T. (2022) Analysis of total phenolic contents, flavonoids, antioxidant and antibacterial activities of *Croton macrostachyus* root extracts. *BMC Chem*., **16**(30), 1-12. https://doi.org/10.1186/s13065-022-00822-0
18. Gendaram O. (2019) Phytochemicals in leaves of *Cotoneaster mongolica*, their antioxidative, and acetylcholinesterase inhibitory activity. *Mong. J. Chem.,* **20**(46), 1-6. https://doi.org/10.5564/mjc.v20i46.1235
19. Qu K., Cheng Y., Gao K., Ren W., Fry E., *et al*. (2022) Growth-defense trade-offs induced by long-term overgrazing could act as a stress memory. *Front. Plant Science*, **13**, 917354. https://doi.org/10.3389/fpls.2022.917354
20. Csepregi K., Hideg E. (2018) Phenolic compound diversity explored in the context of photo-oxidative stress protection. *Phytoch. Anal*., **29**, 129-136. https://doi.org/10.1002/pca.2720
21. Marchiosi R., dos Santos W.D., Constantin R.P., de Lima R.B., Soares A.R., *et al*. (2020) Biosynthesis and metabolic actions of simple phenolic acids in plants. *Phytochem. Rev*., **19**, 865-906. https://doi.org/10.1007/s11101-020-09689-2
22. Tsednee M., Tanaka M., Giehl R.F., von Wirén N., Fujiwara T. (2022) Involvement of NGATHA-Like 1 transcription factor in boron transport under low and high boron conditions. *Plant Cell Physiol*., **63**(9), 1242-1252. https://doi.org/10.1093/pcp/pcac099
23. Vives-Peris V., de Ollas C., Gómez-Cadenas A., Pérez-Clemente R.M. (2020) Root exudates: from plant to rhizosphere and beyond. *Plant Cell Rep*., **39**, 3-17. https://doi.org/10.1007/s00299-019-02447-5
24. Yu R.P., Zhang W.P., Yu Y.C., Yu S.B., Lambers H., *et al*. (2020) Linking shifts in species composition induced by grazing with root traits for phosphorus acquisition in a typical steppe in Inner Mongolia. *Sci. Total Environ*., **712**, 136495, 1-10. https://doi.org/10.1016/j.scitotenv.2020.136495
25. Li B., Fan R., Sun G., Sun T., Fan Y., *et al*. (2021) Flavonoids improve drought tolerance of maize seedlings by regulating the homeostasis of reactive oxygen species. *Plant Soil*, **461**, 389-405. https://doi.org/10.1007/s11104-020-04814-8
26. Gourlay G., Hawkins B.J., Albert A., Schnitzler J.P., Peter C.C. (2022) Condensed tannins as antioxidants that protect poplar against oxidative stress from drought and UV-B. *Plant Cell Environmen*., **45**(2), 362-377. https://doi.org/10.1111/pce.14242