Differential Physiological Responses of Dominant Species in Temperate Typical Steppe to Spring and Autumn Frosts

TAN Chaowei, ZHAO Sisi, TIAN Hanwen, HAN Juanjuan

2026-08-12

ChinaRxiv

Abstract

In the temperate typical steppe, the growing season has lengthened and phenology has advanced, with an increasing trend in frost risk in spring and autumn. However, studies on the effects of frost in different seasons on vegetation physiological indicators remain limited. To reveal the response patterns of plant physiological indicators to spring and autumn frost in this region and interspecific differences, this study focused on three dominant species in the temperate typical steppe of Inner Mongolia, namely Potentilla tanacetifolia, Artemisia frigida, and Stipa krylovii, and used an in situ simulation experiment to investigate the effects of spring and autumn frost on leaf chlorophyll and total soluble sugar contents. The results showed that: (1) autumn frost had no significant effect on chlorophyll content but significantly increased the mean total soluble sugar content by 36%; whereas frost in the following spring significantly decreased the mean chlorophyll and total soluble sugar contents by 42% and 54%, respectively, indicating clear seasonal differentiation in the frost responses of chlorophyll and soluble sugar contents. (2) The sensitivity of responses to frost differed among species, ranking from high to low as follows: Potentilla tanacetifolia > Artemisia frigida > Stipa krylovii. (3) There was a certain interaction between the two frost events, mainly reflected in a synergistically intensified effect on total soluble sugars; the interactive effect on chlorophyll was relatively small. (4) The decrease in soil temperature during autumn frost induced an increase in leaf total soluble sugar content; whereas in the following spring, the decline in chlorophyll content was a potential factor leading to the decrease in total soluble sugar content. This study reveals clear seasonal and interspecific differences in the effects of spring and autumn frost on some physiological indicators of dominant species in the temperate typical steppe, providing a reference for understanding vegetation stress resistance under frost events in this region.

Full Text

DOI:10.11931/guihaia.gxzw202603030

Differential Physiological Responses of Dominant Species in Temperate Typical Steppe to Spring and Autumn Frosts

TAN Chaowei, ZHAO Sisi, TIAN Hanwen, HAN Juanjuan*
(School of Geographical Sciences, Southwest University, Chongqing 400715)

Abstract: In temperate typical steppe, the growing season is extending, phenology is advancing, and the risk of spring and autumn frosts is increasing. However, studies on the effects of frosts in different seasons on plant physiological indicators remain relatively lacking. To reveal the response patterns of plant physiological indicators in this region to spring and autumn frosts and their interspecific differences, this study took three dominant species of the temperate typical steppe in Inner Mongolia—*Potentilla tanacetifolia*, *Artemisia frigida*, and *Stipa krylovii*—as the research objects, and used in situ simulation experiments to investigate the effects of spring and autumn frosts on leaf chlorophyll and total soluble sugar contents. The results showed that: (1) autumn frost had no significant effect on leaf chlorophyll content, but caused the mean total soluble sugar content to increase significantly by 36%; whereas spring frost in the following year caused the mean contents of chlorophyll and total soluble sugar to decrease significantly by 42% and 54%, respectively, indicating clear seasonal divergence in the frost responses of chlorophyll content and soluble sugar content. (2) The sensitivity of species to frost responses differed among species, ranking from high to low as: *Potentilla tanacetifolia* > *Artemisia frigida* > *Stipa krylovii*. (3) There was a certain interaction between the two frost events, mainly reflected in a synergistic aggravating effect on total soluble sugars; the interaction had a relatively small effect on chlorophyll. (4) The decrease in soil temperature during autumn frost induced an increase in leaf total soluble sugar content; in the following spring, the decline in chlorophyll content was a potential factor leading to the decrease in total soluble sugar content. This study reveals that the effects of spring and autumn frosts on some physiological indicators of dominant species in temperate typical steppe have pronounced seasonality and interspecific differences, providing a reference for understanding plant resistance under frost events in this region.

Keywords: frost hardening; soluble sugar; chlorophyll; temperate steppe; seasonal difference

CLC number: Q945 **Document code:** A

Differential physiological responses of dominant species in temperate typical steppe to spring and autumn frosts

TAN Chaowei, ZHAO Sisi, TIAN Hanwen, HAN Juanjuan*
(*School of Geographical Sciences, Southwest University, Chongqing 400715, China*)

Abstract: Temperate typical steppe ecosystems are experiencing prolonged growing seasons and advanced phenology, leading to an increased risk of spring and autumn frosts. However, the differential impacts of seasonal frost events on plant physiological indicators remain poorly understood. To clarify the responses of plant physiological traits to spring and autumn frosts as well as interspecific differences, we conducted in situ simulation experiments on three dominant species (*Artemisia frigida*, *Potentilla tanacetifolia*, and *Stipa krylovii*) in the temperate typical steppe of Inner Mongolia, China to investigate the responses of leaf chlorophyll and total soluble sugar contents to frost events in different seasons. The results showed that: (1) Autumn frost significantly induced a 36% increase in total soluble sugar content while chlorophyll remained stable. Conversely, spring frost in the following year caused declines in chlorophyll and total soluble sugar contents by 42% and 54%. This pattern demonstrates a clear seasonal divergence in plant physiological

Funding projects: National Natural Science Foundation of China (32471673); Open Fund Project of the Chongqing Jinfo Mountain Karst Ecosystem National Observation and Research Station (JFS2023A03).
First author: TAN Chaowei (2001–), master' s student, mainly engaged in grassland ecology research, (E-mail) tancwswu@163.com.
***Corresponding author:** HAN Juanjuan, Ph.D., associate professor, mainly engaged in grassland carbon cycling research, (E-mail) jhan20190717@163.com.

responses to frost stress. (2) The three species differed greatly in frost sensitivity, ranked from high to low as: *Potentilla tanacetifolia* > *Artemisia frigida* > *Stipa krylovii*. (3) An interaction existed between the two frost events, which mainly manifested as a synergistic enhancement effect on total soluble sugar. By contrast, the interaction exerted a relatively weak influence on chlorophyll. (4) During autumn frost events, the decrease in soil temperature appeared to induce an increase in leaf total soluble sugar content, whereas in the following spring, the reduction in chlorophyll content was a potential factor contributing to the decline in total soluble sugar content. This study confirm that spring and autumn frosts exert contrasting impacts on physiological indicators of dominant species in temperate steppes, with obvious seasonal variations and interspecific differences. This study provide important insights into the frost resistance mechanisms of steppe vegetation in this region.

Key Words: frost stress, soluble sugar, chlorophyll, temperate grasslands, seasonal variation

Against the background of continuous global climate warming, the risk of vegetation exposure to spring and autumn frost in temperate and cold-temperate regions has increased significantly (Augspurger, 2013; Liu et al., 2018; Tao Zexing and Dai Junhu, 2023). The low-temperature environment formed during frost events can affect plant physiology in many ways. For example, frost suppresses plant photosynthetic capacity (Ploschuk et al., 2014), disrupts osmotic regulation and metabolic balance (Xin et al., 2000; Fürtauer et al., 2019), damages cell-membrane structure, and causes changes in the contents of reactive oxygen species, cellular electrolytes, and abscisic acid, thereby disturbing normal plant physiological metabolism (Ding et al., 2019). However, the degree of frost impact differs markedly among species (Kreyling et al., 2012), photosynthetic types (Liu & Osborne, 2008), and geographical locations (Ma et al., 2019), among other factors; thus, the physiological responses of temperate typical grassland vegetation to frost remain worthy of attention. In addition, spring is the period of plant regreening, when most plants are in the sprouting stage and leaves have relatively weak frost resistance (Vitasse et al., 2014); autumn, by contrast, is the withering period of plants, after a full growing season of material accumulation, when leaves have sufficient reserves of frost-resistant substances and stronger frost resistance. Therefore, changes in plant physiological indicators under frost stress in different seasons may differ. Meanwhile, numerous studies have shown that the effects of frost can persist into subsequent seasons (Muffler et al., 2024; Han et al., 2025; Wang et al., 2025), implying that spring and autumn frosts may interact in their physiological effects on plants. However, relevant research remains limited. Because changes in plant physiological indicators are related to vegetation growth and the carbon-sequestration potential of regional ecosystems, studies of vegetation resistance to spring and autumn frost are of great significance for predicting vegetation growth and dynamic changes under future climatic conditions.

Photosynthesis is the core process by which plants acquire energy and fix carbon. Frost directly reduces photosynthetic efficiency by damaging leaf structure and inducing oxidative stress responses (Ploschuk et al., 2014). Among photosynthetic traits, leaf chlorophyll content is a key parameter characterizing plant photosynthetic capacity and is often used as a major indicator of the degree of frost-stress injury in plants (Li et al., 2020). Frost usually causes thylakoid damage and freezing of water inside and outside cells (Charrier et al., 2013), resulting in chloroplast injury and decreased chlorophyll content, and the magnitude of the decrease is closely related to frost intensity and frequency (Dietrich et al., 2018). However, in some cold-tolerant species (such as reticulate tea and snow lotus), under mild frost stress, metabolites related to chlorophyll synthesis and gene expression may be upregulated, allowing chlorophyll content to remain stable or even increase slightly (Wang et al., 2025; Yu et al., 2025). Therefore, the direction and magnitude of frost effects on chlorophyll content depend on frost intensity and interspecific regulation.

Nonstructural carbohydrates are the main products of plant photosynthesis, and their accumulation in plants is beneficial for survival under unfavorable environments (Carbone et al., 2013). Soluble sugars are their main components (Song Lin et al., 2020). Soluble sugars play key roles in plant frost resistance by lowering cellular freezing points, maintaining osmotic-pressure balance, and scavenging reactive oxygen species (Tarkowski et al., 2015; Duan Wei et al., 2026). Although most studies have found that frost stimulates an increase in plant soluble sugar content (Wang Haimei et al., 2015; Yang Yong' e et al., 2023; Ling et al., 2025), severe frost may damage plant photosynthetic organs, thereby inhibiting soluble-sugar synthesis (Thomas et al., 2004; D' Andrea et al., 2019). In summary, existing studies remain controversial regarding the effects of frost on plant chlorophyll and soluble sugar contents. An in-depth analysis of the coordinated changes between the two under frost stress can not only deepen understanding of plant physiological adaptation, but also

improved the ability to predict plant frost-resistance risk.

Grassland ecosystems account for about 40% of the land surface and play a major role in regulating climate change and vegetation carbon sequestration (Bai & Cotrufo, 2022; Zhang Yingjun et al., 2025). Previous frost-related studies have mainly focused on crops and model plants; these research systems have improved understanding of the molecular mechanisms of plant frost resistance and have enhanced crop frost resistance through genetic breeding (Hosseinpour et al., 2018; Yao Yongwei et al., 2020). However, natural grasslands are subject to the integrated regulation of complex field environments, species interactions, and phenological rhythms, and still require validation through in situ field experiments. Therefore, a field frost-simulation experiment was conducted in a typical steppe of the Mongolian Plateau. This grassland ecosystem is an area with frequent spring and autumn frosts, providing an ideal experimental platform for studying low-temperature response mechanisms across seasons. In this study, we mainly examined the effects of spring and autumn frost on leaf chlorophyll and soluble sugar contents in three dominant species of typical temperate steppe: *Potentilla tanacetifolia*, *Artemisia frigida*, and *Stipa krylovii*, and explored whether there are certain regularities in plant physiological responses to frost. Accordingly, this study addressed the following scientific questions: (1) whether significant differences exist in the physiological responses of dominant species in typical temperate steppe to spring and autumn frost; and (2) whether responses of physiological indicators of dominant grassland species to frost differ among species. This study can provide a theoretical reference for further understanding plant physiological adaptation processes after frost in grasslands.

1 Materials and Methods

1.1 Overview of the experimental site

The experiment was located in Duolun County, Inner Mongolia Autonomous Region, northern China (116°17′E, 42°02′N, 1 324 m). The region is a typical temperate steppe with a semi-arid continental climate. The mean annual air temperature is about 2.4 °C, with air temperatures ranging from -17.5 °C to 18.9 °C. The long-term (1961–2017) mean annual precipitation is about 380 mm, 80% of which is concentrated mainly from June to September. According to the Chinese soil classification, the regional soil type is chestnut soil (Zhang Cuijing et al., 2017). The vegetation is dominated by perennial herbaceous plants, including *Leymus chinensis*, *Artemisia frigida*, *Agropyron cristatum*, *Stipa krylovii*, and *Potentilla tanacetifolia*.

The green-up period of vegetation in this region is mainly concentrated from late April to mid-May, and the senescence period is mainly concentrated from mid-September to early October; these two key growth stages coincide with periods of frequent frost. In spring, frost generally occurs from April 15 to May 15. By analyzing daily minimum temperature data for the past 60 years (1954–2014) during this period, it was found that the mean cumulative frost time in spring was (10.85 ± 0.39) d • a^-1 (Fig. 1: A), and the mean duration of a single frost event per year was (2.89 ± 0.11) d (Fig. 1: B). In autumn, frost generally occurs from September 15 to October 15, with a mean cumulative frost time of (10.23 ± 0.53) d • a^-1 (Fig. 1: A), and the mean duration of a single frost event per year was (3.02 ± 0.13) d (Fig. 1: B). There were no significant differences between the two periods in either cumulative frost time or frost duration ($P > 0.05$). Therefore, in terms of both cumulative frost time and frost duration, spring and autumn frosts have become important environmental limiting factors in this region.

A. Average cumulative frost time; B. Average duration of a single frost event. Spring is defined as April 15 to May 15 of each year, and autumn as September 15 to October 15 of each year. NS indicates no significant differences between treatments ($P > 0.05$); data are presented as mean ± standard error. The same below.

A. Average cumulative frost time; B. Average frost duration of a single frost event. Spring is defined as April 15 to May 15, and autumn as September 15 to October 15 of each year. NS indicate significant differences among

treatments ($P > 0.05$). Data are expressed as mean ± standard error. The same below.

Fig. 1 Average cumulative frost days and duration in spring and autumn from 1954 to 2014

1.2 Experimental design

To simulate natural frost events occurring in spring and autumn, this experiment used a self-developed field cooling device to conduct continuous 7-d frost-simulation experiments from September 24 to September 30, 2017, and from May 10 to May 16, 2018. The two periods are denoted in this study as autumn and spring, respectively. The experiment included four different frost-treatment levels: control (C), autumn frost treatment (A), frost treatment in the following spring (S), and autumn plus following-spring frost treatment (SA). Each treatment included six replicate plots, for a total of 24 plots. All plots were located in the same flat grassland, with basically consistent soil texture, vegetation, and microclimatic conditions, and no obvious block effect; therefore, the plots were assigned using a completely randomized design. Each plot was equipped with one hexagonal open-top gas chamber (OTC) of nearly 4 m^2. During the frost-treatment period, the average internal cooling amplitude could reach 8 °C, and the average frost temperature was -4.99 °C; in spring and autumn it was -5.54 °C and -4.45 °C, respectively. At night, lower frost temperatures occurred, with the minimum reaching approximately -13 °C. During most daytime periods, the air temperature inside the OTC was lower than the ambient temperature, but the air temperature did not reach 0 °C. Therefore, cooling and frost phenomena appeared alternately during the experiment (Han et al., 2025), but this feature was highly consistent with the occurrence pattern of frost in nature: before the arrival of natural frost, air temperature decreases significantly.

The cooling method was implemented by refrigerating an ethylene glycol solution with a refrigeration compressor system and allowing the ethylene glycol solution to circulate repeatedly in a closed pipeline between the OTC and the refrigeration compressor, thereby achieving sustained cooling. At the same time, HOBO temperature probes (S-THB-M008, ONSET Inc, Massachusetts, USA) were installed in plots under different treatments. These probes measured air temperature once every 10 min, helping the cooling device control the cooling amplitude according to real-time temperature changes, thereby reducing differences in frost effects caused by diurnal fluctuations in air temperature. In addition, plastic panels were installed after the cooling device was started and removed before it ended, so as to avoid, to the greatest extent possible, the warming effect produced by the OTC. More detailed frost-simulation methods can be found in Han et al. (2025).

1.3 Measurement indicators and methods

During the 7 d before frost treatment and the 7 d after completion of treatment, soil temperature and volumetric water content at a depth of 10 cm were measured, with 2–3 measurements within each 7-d period. Soil temperature was measured using the temperature probe attached to a portable infrared gas analyzer Li-8100 (LI-COR Inc., Lincoln, NE, USA), and soil volumetric water content was measured synchronously using a soil moisture meter TDR350 (Spectrum Technologies Inc., Aurora, IL, USA). Measurements were conducted between 9:00 and 11:00 in the morning under clear and windless weather conditions.

This study comprehensively considered field sampling conditions, sample preservation, and indicator representativeness, and selected leaf soluble sugar and chlorophyll contents as indicators reflecting plant frost resistance and frost injury. Leaf soluble sugars are the main osmoregulatory substances through which plants respond to low-temperature stress, and are a core indicator reflecting the strength of frost resistance and the degree of response to frost stress; leaf chlorophyll content is sensitive to frost response and can directly reflect the degree of damage to the photosynthetic system. Therefore, the above two indicators were the most suitable choices for evaluating frost effects in this study. Meanwhile, because the measurement of soluble sugar and chlorophyll contents requires relatively large sample masses, in order to reduce destructive sampling of the plots, representative dominant species (the forb *Chrysanthemum chanetii*, the sedge *Carex duriuscula*, and the graminoid *Stipa krylovii*) were selected as the study objects. These three dominant species showed significant advantages in community dominance, vegetation coverage, and vegetation height. Sampling was conducted 1 day before the start of the autumn and following-spring frost treatments and on the 3rd day after the end of the treatments, respectively. During sampling, leaves of the above three species were randomly collected from the control plots and the frost-treatment plots of the corresponding season. The collected leaf samples were packed in plastic bags, vacuum-preserved, and transported to the laboratory. Total soluble sugar content in plant leaves was measured using the anthrone colorimetric method, and chlorophyll content was measured using the spectrophotometric method (Hu Chengfen et al., 2018).

1.4 Statistical analysis

R language (version 4.3.1) was used for all statistical analyses. Before all analyses, the data were subjected to the Shapiro-Wilk normality test and Levene's test for homogeneity of variance. Data that did not meet normality or homogeneity of variance were subjected to normalizing transformations.

state transformation. A linear mixed-effects model was used to test the effect of frost on each index. Frost treatment (Treatment) was included as a fixed effect to test the influence of frost. In the random-effects part, for environmental data, plot was selected as a random effect to account for differences among plots ($Y \sim$ Treatment + (1|plot)); for physiological indices, species was additionally included as a random effect ($Y \sim$ Treatment + (1|plot) + (1|Species)), to simultaneously account for differences among species and among plots. In addition, one-way analysis of variance was used separately to test the effects of frost on the physiological indices of the three species. The constructed models used the F test to verify the significance of main effects. Multiple comparisons were corrected using Tukey' s method.

In addition, this study analyzed the interaction between frosts in the two seasons. Because this experiment did not include a complete 2×2 factorial combination—that is, the four treatment combinations (C, A, S, SA) were not all established in both frost periods—it was not possible to conduct a conventional statistical interaction test by analysis of variance. This study therefore adopted an additive-effects assumption to analyze the interaction between the two frost events: the sum of the relative changes in indices after a single autumn frost (plot A) and after a single spring frost (plot S) was used as the theoretical expected value under the condition of additive frost effects (Expected.SA), and this was compared with the actual observed relative change after the spring frost treatment in the following year (plot SA). The type of interaction was judged from the numerical relationship between the two: if the observed value was lower than the expected value, the effect was a synergistically intensifying effect; otherwise, it was an antagonistic effect. The relative change was calculated as (treatment − control)/control × 100. In addition, because of the limitations of the completely randomized experimental design, the expected values lacked paired replicates among plots for different treatments (for example, the six replicates of plot S and plot A could not be paired with one another), so a significance test could not be performed; only the overall mean was compared with the observed value.

Partial correlation analysis was used to separately explore the relationships between environmental factors and physiological indices under each treatment level before and after the autumn frost and the spring frost of the following year. The

Frost simulation experiment and air temperature changes in frost plots during frost treatments in two seasons.

Figure 1: Frost simulation experiment and air temperature changes in frost plots during frost treatments in two seasons.

Spearman correlation method was used, and all data were standardized to eliminate the influence of magnitude. In addition, a linear mixed-effects model was used to explore the linear relationship between chlorophyll and soluble sugars. Plot was included in the model as a random effect, and the goodness of fit of the overall model (marginal coefficient of determination, R^2_{m}) and the significance results were calculated to evaluate the fitting effect.

2 Results and Analysis

2.1 Environmental changes caused by the treatments

Whether in autumn or in the spring of the following year, the simulated frost treatments led to a significant decrease in air temperature within 7 d. In most cases, the difference in air temperature between the treatment plots and the control plots could reach more than 5 °C (Fig. 2). Therefore, when the ambient air temperature was around 5 °C, a frost effect could be achieved. Judging from the daily dynamics of air temperature in the autumn and spring in this region, during the frost period, the daily duration of frost was about 8 h and occurred mainly at night. In addition, both before the start of the frost treatment and after the end of the frost treatment, no significant differences were found among treatments in soil temperature or soil volumetric water content (Table 1, $P > 0.05$).

Frost simulation experiment

Absolute change (treatment-control).

Fig. 2 Frost simulation experiment and air temperature changes in frost plots during frost treatments in two seasons.

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Table 1 Main effect analysis of environmental factors and physiological indicators under different frost treatments

Season	Index	Before frost F	Before frost P	After frost F	After frost P
Autumn	T_s	1.092	0.360	3.367	0.061
Autumn	VWC	1.182	0.334	1.033	0.379
Autumn	TSS	0.333	0.725	5.076	**0.024**
Autumn	Chl	0.006	0.994	1.028	0.385

Season	Index	Before frost F	Before frost P	After frost F	After frost P
Spring	T_s	0.810	0.463	0.535	0.596
Spring	VWC	2.181	0.147	0.657	0.533
Spring	TSS	0.536	0.595	4.323	**0.042**
Spring	Chl	1.185	0.329	4.332	**0.042**

Note: $\mathbf{T}_s$. Soil temperature at 10 cm depth; **VWC**. Volumetric water content at 10 cm soil depth; **TSS**. Total soluble sugar content; **Chl**. Chlorophyll content. Bold values indicate significant main effects. The same below.

2.2 Changes in frost-driven total soluble sugar and chlorophyll contents

Autumn frost induced a significant increase in leaf total soluble sugar content, whereas frost in the following spring led to significant decreases in total soluble sugar and chlorophyll contents (Table 1, Figure 3, $P < 0.05$). Specifically, before frost treatment in the two seasons, there were no significant differences among different treatments in total soluble sugar or chlorophyll content (Table 1, Figure 3, $P > 0.05$). However, after frost treatment in autumn and in the following spring, significant differences occurred among different treatments in total soluble sugar and chlorophyll (Table 1, $P < 0.05$). Among them, only under autumn frost treatment (A) did the total soluble sugar content increase significantly, by 36% (Figure 3: A, $P < 0.05$), while chlorophyll content showed no significant change (Figure 3: B, $P > 0.05$). After frost in the following spring, total soluble sugar content after autumn plus following-spring frost treatment (SA) decreased significantly, by approximately 54% compared with the control (Figure 3: C, $P < 0.05$); in addition, chlorophyll content decreased significantly by approximately 42% (Figure 3: D, $P < 0.05$), whereas neither indicator changed significantly under following-spring frost treatment alone (S) ($P > 0.05$).

A. Changes in total soluble sugar before and after autumn frost; **B.** Changes in chlorophyll before and after autumn frost; **C.** Changes in total soluble sugar before and after the following spring frost; **D.** Changes in chlorophyll before and after the following spring frost. C denotes the control treatment; A denotes the autumn frost treatment; S denotes the following spring frost treatment; SA denotes the autumn + following spring frost treatment. * indicates $P < 0.05$. The same below.

A. Changes in total soluble sugar before and after autumn frost; B. Changes in chlorophyll before and after autumn frost; C. Changes in total soluble sugar before and after following spring frost; D. Changes in chlorophyll before and after following spring frost. C denotes Control; A denotes Autumn frost; S denotes Following spring frost; SA denotes Autumn + Following spring frost. *

indicates $P < 0.05$. The same below.

Fig. 3 Changes in physiological-indicator contents before and after autumn and following spring frost treatments

Fig. 3 Differences in physiological indicators before and after autumn and following spring frost treatments

Main-effect analysis showed that frost significantly affected some physiological indicators of the dominant plants, and that there were clear interspecific differences. Among them, *Potentilla tanacetifolia* was most strongly affected by frost, followed by *Artemisia frigida*, whereas *Stipa krylovii* exhibited relatively strong frost tolerance. Specifically, after autumn frost, the soluble sugar content of *Potentilla tanacetifolia* showed a significant response to the frost treatment (Table 2, $P = 0.026$), while chlorophyll content was not significantly affected (Table 2, $P = 0.189$); after spring frost, both total soluble sugar and chlorophyll in *Potentilla tanacetifolia* were significantly affected by frost (Table 2, $P < 0.05$). *Artemisia frigida* showed only an extremely significant effect of the following spring frost on chlorophyll content (Table 2, $P < 0.001$). Frost in both periods had no significant effect on the physiological indicators of *Stipa krylovii* (Table 2, $P > 0.05$).

Table 2 Statistical analysis results of main effects of physiological indicators in three dominant species under different frost treatments

Table 2 Statistical analysis results of main effects of physiological indicators in three dominant species under different frost treatments

Season	Index	Species	Before frost F	Before frost P	After frost F	After frost P
Autumn	TSS	*Artemisia frigida*	1.153	0.354	1.930	0.185
Autumn	TSS	*Potentilla tanacetifolia*	0.082	0.922	4.993	**0.026**
Autumn	TSS	*Stipa krylovii*	0.827	0.490	3.329	0.074
Autumn	Chl	*Artemisia frigida*	0.633	0.550	0.162	0.852
Autumn	Chl	*Potentilla tanacetifolia*	0.524	0.606	1.920	0.189
Autumn	Chl	*Stipa krylovii*	1.147	0.389	0.027	0.973
Spring	TSS	*Artemisia frigida*	0.052	0.950	1.285	0.323

Season	Index	Species	Before frost F	Before frost P	After frost F	After frost P
Spring	TSS	*Potentilla tanacetifolia*	0.119	0.889	13.41	**0.001**
Spring	TSS	*Stipa krylovii*	0.472	0.634	1.500	0.265
Spring	Chl	*Artemisia frigida*	1.307	0.300	17.22	**<0.001**
Spring	Chl	*Potentilla tanacetifolia*	1.360	0.286	5.344	**0.026**
Spring	Chl	*Stipa krylovii*	1.128	0.354	0.505	0.617

Multiple-comparison results showed that, after autumn frost, the total soluble sugar content under treatment A in *Potentilla tanacetifolia* increased significantly (Fig. 4: A, $P < 0.05$), whereas chlorophyll content did not change significantly (Fig. 4: B, $P > 0.05$); by comparison, both total soluble sugar and chlorophyll contents in *Artemisia frigida* and *Stipa krylovii* showed no significant changes ($P > 0.05$).

A. Changes in total soluble sugar after autumn frost; **B.** Changes in chlorophyll after autumn frost.

Fig. 4 Differences in physiological indicators of three dominant species after autumn frost

After the frost in the following spring, the total soluble sugar in *Potentilla tanacetifolia* under the S and SA treatments decreased significantly, and the decrease under SA (60.9%) was slightly greater than that under S (45.2%) (Fig. 5: A, $P < 0.05$), whereas the chlorophyll content decreased significantly only under S (Fig. 5: B, $P < 0.05$). For *Artemisia frigida*, the total soluble sugar content did not change significantly ($P > 0.05$), while the chlorophyll content decreased significantly under the SA treatment (Fig. 5: B, $P < 0.05$). The contents of all indicators in *Stipa krylovii* were not significantly affected by frost treatment ($P > 0.05$).

A. Changes in total soluble sugar after following spring frost; **B.** Changes in chlorophyll after following spring frost. ** indicates $P < 0.01$; *** indicates $P < 0.001$. The same below.

Fig. 5 Differences in physiological indicators after following spring frost

2.3 Interaction Between Autumn Frost and Frost in the Following Spring

To investigate the interactive effects of cross-season continuous frost on the physiological indicators of dominant species in typical grasslands, this study compared the differences between the theoretical expected values and the observed values for the relative changes in total soluble sugar and chlorophyll content (Fig. 6).

For total soluble sugar, the overall relative change in the observed value (-54.3%) was far lower than the theoretical expected value (-3.7%), and all species conformed to this pattern (Fig. 6A), indicating that the two frost events had a synergistically intensifying effect: autumn frost aggravated the negative effect of frost in the following spring on this indicator. For chlorophyll, the overall relative change in the observed value (-41.6%) was roughly the same as the expected value (-39.7%), indicating that, overall, the effects of the two frost events on chlorophyll content were mutually independent and that there was no obvious interaction (Fig. 6B). However, at the species level, the interaction patterns for chlorophyll showed relatively large differences (Fig. 6B). The observed value for *Potentilla tanacetifolia* (-41.7%) was much higher than the expected value (-84.6%), indicating an antagonistic effect, whereas *Artemisia frigida* and *Stipa krylovii* showed synergistically intensifying effects.

Expected. SA. Relative change of the theoretical expected value under combined two-season frost treatment; **Observed. SA.** Relative change of observed values after spring frost. Significance tests could not be performed due to the lack of independent replicates for expected values under the completely randomized design.

Expected SA. Relative change of the theoretical expected value under combined two-season frost treatment; **Observed SA.** Relative change of observed values after spring frost. Significance tests could not be performed due to lack of independent replicates for expected values under the completely randomized design.

Fig. 6 Differences of expected and observed values for physiological indicators under combined autumn and spring frosts

2.4 Relationships Between Environmental Factors and Physiological Indicators in Autumn and the Following Spring

The partial correlation analysis results (Fig. 7) showed that in autumn, total soluble sugar was significantly negatively correlated with soil temperature ($P < 0.05$), and was not significantly correlated with soil volumetric water content ($P > 0.05$); chlorophyll was significantly positively correlated with soil volumetric water content ($P < 0.05$). In the following spring, total soluble sugar was not significantly correlated with either soil temperature or volumetric water content ($P > 0.05$); chlorophyll was significantly positively correlated with soil

Fig. 7 Partial correlation analysis between environmental factors and physiological indicators

Figure 2: Fig. 7 Partial correlation analysis between environmental factors and physiological indicators

Fig. 8 Linear relationship between total soluble sugar and chlorophyll

Figure 3: Fig. 8 Linear relationship between total soluble sugar and chlorophyll

volumetric water content ($P < 0.05$).

Fig. 7 Partial correlation analysis between environmental factors and physiological indicators

Total soluble sugar is a product of plant photosynthesis, and its content may have a potential relationship with chlorophyll level. Linear mixed-effects model analysis showed that in autumn, only chlorophyll under the C treatment was significantly negatively correlated with total soluble sugar ($P < 0.05$), and chlorophyll explained 21% of its variation (Fig. 8: A). In the following spring, chlorophyll at all treatment levels was significantly positively correlated with total soluble sugar ($P < 0.05$); chlorophyll under the C treatment explained 55% of the variation in total soluble sugar, that under the S treatment explained 39%, and that under the SA treatment explained 35% (Fig. 8: B).

A. Relationship between total soluble sugar and chlorophyll in autumn; B. Relationship between total soluble sugar and chlorophyll in the following spring. R_m^2 is the marginal coefficient of determination in linear mixed-effects models, representing the proportion of variance explained only by fixed effects.

Fig. 8 Linear relationship between total soluble sugar and chlorophyll

3 Discussion and Conclusions

3.1 Effects of Autumn Frost on Total Soluble Sugar and Chlorophyll Content in Plant Leaves

The phenomenon that frost causes a decline in plant leaf chlorophyll content has been widely reported (Ploschuk et al., 2014; Dietrich et al., 2018; Li et al., 2020). However, this study found that in the typical temperate grassland of Inner Mongolia, autumn frost did not have a significant effect on leaf chlorophyll content. This difference may stem from differences in plant phenological stages. For example, the frost study by Ploschuk et al. (2014) was conducted when the fourth leaf of the plant had fully unfolded, whereas in the present study, autumn frost occurred during the yellowing stage of the plants, when photosynthetic products were preferentially allocated to various organs for the accumulation and regulation of stress-resistant substances (Fermaniuk et al., 2021), leading to a blunted chlorophyll response to frost.

For total soluble sugar, autumn frost produced a significant promoting effect, and such response changes have been confirmed across many species or ecosystems worldwide (Wang Haimei et al., 2015; Yang Yong' e et al., 2023; Ling et al., 2025), indicating

indicates its universality on a global scale. What differs, however, is that the average increase in total soluble sugar content in this study (36%) was slightly higher than that reported by Ling et al. (2025) for the tropical multi-branched cycad (*Cycas multipinnata*, 21%). This may result from the highly efficient stress-response mechanism evolved by temperate grassland plants during their long-term adaptation to cold climates. Frost events induce the conversion of starch in plants into soluble sugars (Fermaniuk et al., 2021), which can reduce freezing of intracellular solution, maintain cell-membrane stability, and protect protein function (Morin et al., 2007; Tarkowski et al., 2015). In addition, this experiment controlled only air temperature; the buffering and heat-preservation effect of soil may form a temperature gradient between plant leaves and root systems. Under cold conditions, the plant crown layer may block the export of newly produced photosynthates to the roots, thereby causing soluble sugars to remain in the leaves (Sperling et al., 2017).

3.2 Effects of Frost in the Following Spring on Total Soluble Sugar and Chlorophyll Contents in Plant Leaves

The effects of frost in the following spring on plant physiological indicators were completely opposite to those in autumn: it significantly reduced leaf chlorophyll and total soluble sugar contents, reflecting the regulatory role of phenological stage in plant frost responses. Because spring is the period when plants are re-greening, newly formed leaves have relatively weak freezing resistance (Vitasse et al., 2014), and frost may cause severe damage to leaves or even leaf abscission, thereby leading to changes in physiological indicators. For leaf chlorophyll, the results of this study were highly similar to those of Kreyling et al. (2012) on three common herbaceous plants in Europe under late-spring frost. Although chlorophyll content was significantly positively correlated with soil moisture ($P < 0.05$), soil moisture did not change significantly during the experiment; thus, water interference can be excluded, and the effect is more likely related to direct frost injury to chloroplasts. During freezing, the most severe freezing phenomena are usually found in chloroplasts (Prozherina et al., 2003), and frost-induced damage to thylakoids, as well as freezing of intra- and extracellular water, can damage chloroplasts (Charrier et al., 2013), causing chlorophyll degradation and loss.

As an important component of plant photosynthates, soluble sugars have accumulation levels closely related to photosynthetic function (Thomas et al., 2004). Previous studies have confirmed that changes in leaf chlorophyll in some species can lead to changes in soluble sugar content (Sun et al., 2017; Yuan et al., 2019). In this study, after spring frost, leaf chlorophyll was significantly positively correlated with total soluble sugar content. Therefore, we infer that photosynthetic decline caused by chlorophyll degradation is a potential reason for the decrease in soluble sugar content. Meanwhile, processes caused by frost, such as intensified plant respiration, involvement of stress-resistant substances, and damage repair, may further consume soluble sugars in leaves (Sperling et al., 2015).

3.3 Differential Responses of Different Species to Frost

This study found that the physiological responses of three dominant species in typical temperate grassland of Inner Mongolia to frost stress showed significant interspecific differentiation, with frost sensitivity ranked as *Potentilla tanacetifolia* > *Leymus chinensis* > *Stipa krylovii*. During evolution, plants form certain morphological and structural traits to cope with environmental changes (Wang Hailing et al., 2021). Leaves with small area and high density can effectively reduce the effects of frost (Suonan et al., 2026). The leaves of *Potentilla tanacetifolia* are broad and flat, relatively thin, and its young tissues are directly exposed to the environment, lacking effective physical protection; therefore, it is relatively sensitive to frost. The leaves of *Leymus chinensis* are pinnately divided, with a smaller single-leaf area, and especially the leaf epidermis is covered with silvery-white hairs, which can reduce the impact of frost (Cai Ping et al., 2007; Jiang et al., 2018). The leaves of *Stipa krylovii* are narrow, long, strongly involute (Huang Juying et al., 2012), effectively reducing the contact area between leaves and the external low-temperature air and giving it stronger frost tolerance. In addition to morphological traits, internal antifreeze signaling pathways, antioxidant systems, and endogenous hormones in plants are also important influencing factors (Wang Fang et al., 2019; Ding et al., 2019), and future studies still need to integrate multi-omics and physiological-biochemical experiments for in-depth analysis.

3.4 Interactive Effects of Autumn Frost and Frost in the Following Spring

By comparing the theoretical expected values and actual observed values of physiological indicators under the superimposed frost conditions in the two seasons, we found that frost in the two seasons had a certain interactive effect. Total soluble sugars showed a synergistically intensified effect, with the actual magnitude of decrease far greater than the expected magnitude. This suggests that the effects of autumn frost may be retained and may intensify the response to frost in the following spring. Although current research on the legacy effects of frost has focused on the carbon cycle (Han et al., 2025), phenological stages (Wang et al., 2025), and frost adaptability (Muffler et al., 2024), studies on osmoregulatory substances remain lacking. In addition, the expected and observed values for chlorophyll changes were basically consistent, indicating that the interaction between frost in the two seasons had a relatively weak effect on it. On the one hand, this may be because the single autumn-frost effect on chlorophyll itself was relatively weak, making it difficult for its frost effect to carry over into the following spring; on the other hand, opposite responses among species may have caused the summed values to cancel each other out, resulting in relatively small overall changes. For example, *Potentilla tanacetifolia* showed [[unclear: sentence continues on next page]]

antagonistic effects, while *L. chinensis* and *S. krylovii* both showed synergistic intensification effects. The above results indicate that the interactive effects of frost differ among indicators and species, and that further investigation of the responses of physiological indicators to interactions among species remains worthwhile.

In summary, this study systematically analyzed the effects of spring and autumn frost on leaf chlorophyll and total soluble sugar contents in three dominant species of the typical temperate grasslands of Inner Mongolia, emphasizing that changes in physiological indicators exhibit both seasonality and interspecific differences, and providing certain support for understanding changes in vegetation frost resistance in this region. However, this study still has certain limitations. First, only three typical species in this region were selected; although they cover the major dominant functional groups of the community, more rare species and other life-form plants were not included, which limits the generality of the conclusions to some extent. Second, the frost experiments in this study included only one spring frost event and one autumn frost event, and lacked high-frequency, long-term observations, making it difficult to reflect the sustained response levels of plants. Subsequent studies may carry out broader long-term monitoring and combine molecular ecological techniques to reveal in greater depth the long-term patterns and intrinsic mechanisms of grassland plant physiological responses to frost.

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