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Experimental warming alleviates leaf-level physiological stress of *Kobresia pygmaea* in alpine meadows on the Northeastern Qinghai-Tibet Plateau

Lei Zheng^{1*}, Xiaodong Wu², Genghao Ji³ and Guimin Liu³

Abstract

Background Climate warming can strongly influence alpine plant physiology, but the leaf-level responses of dominant alpine meadow species to warming may vary across elevation and growing-season stages. These spatial and seasonal patterns remain insufficiently known.

Results We examined the physiological responses of *Kobresia pygmaea* on the northeastern Qinghai-Tibet Plateau to long-term passive open-top chamber (OTC) warming across four elevations (3691, 3786, 3981, and 4131 m a.s.l.) from July to September. Across all sites and months, OTC warming increased mean near-surface air temperature by 1.34 °C and surface soil temperature by 1.32 °C, while reducing relative humidity by 5.16% and surface soil moisture by 5.17%. Warming reduced leaf malondialdehyde by 20.2% and proline by 45.3%, suggesting lower membrane lipid peroxidation and reduced osmotic adjustment. Superoxide dismutase activity increased slightly by 2.4%, whereas peroxidase activity increased by 26.3%, indicating enzyme-specific antioxidant regulation. Chlorophyll a, chlorophyll b, and total chlorophyll increased by 72.0%, 66.2%, and 70.2%, respectively. Three-way ANOVA showed significant effects of warming, month, elevation, and their interactions on most physiological indicators.

Conclusion Moderate OTC warming improved the leaf-level physiological status and photosynthetic pigment capacity of *K. pygmaea* during the growing season. These benefits were season- and elevation-dependent and occurred under a concurrent drying microclimate. The results provided evidence of a leaf-level physiological response to warming by the dominant species in alpine meadows.

Keywords Alpine meadow, *Kobresia pygmaea*, Open-top chamber, Chlorophyll, Oxidative stress, Tibetan Plateau

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Introduction

High-elevation ecosystems are highly sensitive to climate warming because low temperature constrains plant metabolism, nutrient cycling, growing-season length, and soil thermal regimes [1–3]. Alpine ecosystems above the treeline also support high biodiversity and store substantial soil carbon and nitrogen pools [4–6]. The Qinghai-Tibet Plateau is the largest alpine ecosystem in the world, and its warming rate has exceeded the global average over recent decades [7, 8]. Understanding plant responses to this warming is therefore important for predicting the stability and functioning of alpine grasslands.

Evidence from remote sensing and field observations indicates that warming can promote alpine vegetation greening, as reflected by increases in NDVI, leaf area index, and biomass in many cold-limited regions [9–13]. However, contrasting trends have also been reported, especially where warming enhances evapotranspiration and reduces soil water availability [14–16]. In permafrost-affected meadows, changes in active-layer hydrology and soil moisture can further modify vegetation responses [17]. These findings indicate that plant responses to warming depend on the balance between thermal release from cold limitation and moisture-mediated stress.

Experimental warming, especially through open-top chambers (OTCs), provides a widely used approach for evaluating plant responses to warming under field conditions [18]. Previous warming experiments have shown changes in plant growth, photosynthetic activity, chlorophyll content, and oxidative stress indicators [19–25]. At the physiological level, malondialdehyde (MDA) indicates membrane lipid peroxidation [26], proline reflects osmotic adjustment under cold and drought stress [27], superoxide dismutase (SOD) and peroxidase (POD) are important antioxidant enzymes [28], and chlorophyll pigments are closely linked to photosynthetic capacity [29]. However, many studies have focused on single sites, end-of-season measurements, or community-level responses, leaving uncertainty about how physiological responses vary across both elevation and time within the growing season [19, 30].

Kobresia pygmaea is a dominant mat-forming sedge in alpine meadows of the Qinghai-Tibet Plateau and contributes to plant productivity, soil organic matter accumulation, and topsoil stability [31–34]. Its shallow root system and low stature make it sensitive to both temperature and soil moisture fluctuations, making it a useful species for examining physiological responses to warming. Nevertheless, the combined influence of warming, elevation, and seasonal progression on *K. pygmaea* leaf physiology remains poorly understood.

In this study, we used a long-term passive OTC warming experiment established along an elevational gradient in the northeastern Qinghai-Tibet Plateau (Fig. 1). We

collected leaf samples in July, August, and September to capture within-season physiological dynamics. We quantified MDA, proline, SOD activity, POD activity, chlorophyll a, chlorophyll b, and total chlorophyll, alongside concurrent measurements of air temperature, relative humidity, soil temperature, and soil moisture inside and outside the OTCs. We aimed to (1) quantify how OTC warming modifies near-surface microclimate; (2) test whether warming reduces oxidative and osmotic stress while altering antioxidant enzyme activities and chlorophyll pools in *K. pygmaea*; and (3) evaluate whether these responses vary among elevations and months. By focusing on leaf-level physiological traits, this study provides mechanistic insight into the response of a dominant alpine meadow sedge to coupled warming and drying.

Results

Effects of warming on microclimate

OTC warming produced a warmer and drier near-surface microclimate (Table 1). Across all sites and months, mean near-surface air temperature increased from 23.09 °C in control plots to 24.43 °C in warmed plots, an increase of 1.34 °C. Mean surface soil temperature increased from 22.56 °C to 23.88 °C, an increase of 1.32 °C. In contrast, relative humidity declined from 49.14% to 43.98%, equivalent to a 5.16%-point decrease, and surface soil moisture declined from 18.52% to 13.35%. There was also a seasonal pattern of microclimate conditions, i.e., the air and soil temperatures generally decreased from July to September. The humidity and soil moisture showed stronger site-dependent variation (Figs. 2 and 3).

Effects of warming on stress indicators and antioxidant enzymes

Leaf MDA content was lower in warmed plots than in controls at most site-month combinations (Fig. 4). Mean MDA concentration declined from 8.18 to 6.53 $\mu\text{mol g}^{-1}$, equivalent to a 20.2% reduction. Proline declined more strongly, from 51.18 to 28.02 $\mu\text{g g}^{-1}$, a 45.3% reduction. These simultaneous declines indicate that moderate warming reduced membrane lipid peroxidation and lowered the need for osmotic adjustment in *K. pygmaea* leaves. Antioxidant enzyme responses were enzyme-specific. Mean SOD activity increased only slightly, from 136.52 to 139.78 U g^{-1} (+2.4%), whereas POD activity increased from 171.40 to 216.48 U g^{-1} (+26.3%). The pattern of SOD and POD responses varied among months and elevations, indicating that warming modified antioxidant regulation rather than uniformly stimulating all enzymes.

Effects of warming on chlorophyll pigments

Experimental warming substantially increased chlorophyll pools in *K. pygmaea* leaves (Fig. 5). Chlorophyll

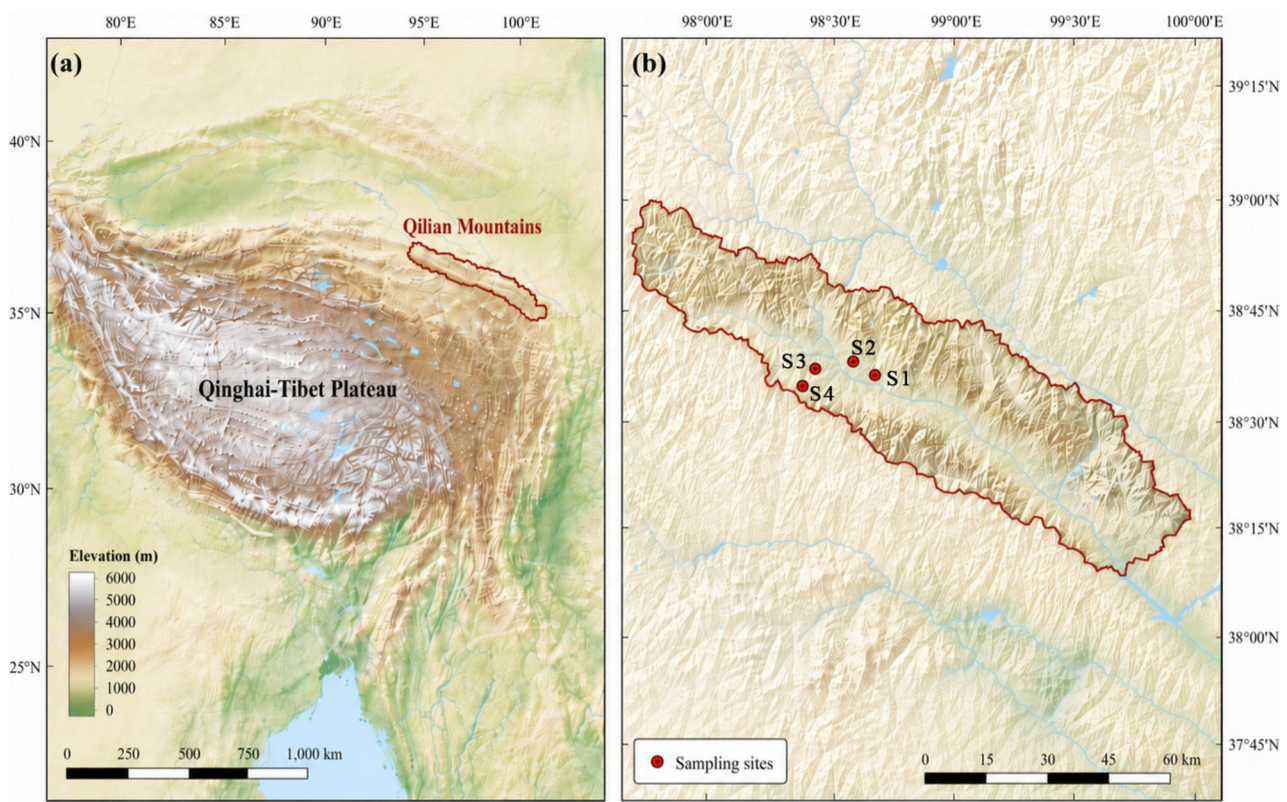


Fig. 1 Experimental warming sites on the northeastern Qinghai-Tibet Plateau. Panel (a) shows the location of the Qilian Mountains region on the Qinghai-Tibet Plateau; panel (b) shows the enlarged study area and sampling sites

Table 1 Overall treatment means and warming-induced changes across all elevations and months

Variable	Control mean	Warming mean	Difference	Relative change (%)
Air temperature (°C)	23.09	24.43	1.34	5.8
Surface soil temperature (°C)	22.56	23.88	1.32	5.8
Relative humidity (%)	49.14	43.98	-5.16	-10.5
Surface soil moisture (%)	18.52	13.35	-5.17	-27.9
MDA (μmol g ⁻¹)	8.18	6.53	-1.66	-20.2
Proline (μg g ⁻¹)	51.18	28.02	-23.16	-45.3
SOD (U g ⁻¹)	136.52	139.78	3.26	2.4
POD (U g ⁻¹)	171.40	216.48	45.08	26.3
Chl <i>a</i> (mg g ⁻¹)	1.35	2.33	0.97	72.0
Chl <i>b</i> (mg g ⁻¹)	0.59	0.98	0.39	66.2
Total Chl (mg g ⁻¹)	1.94	3.30	1.36	70.2
Chl <i>a/b</i>	2.44	2.58	0.14	5.7

a increased from 1.35 to 2.33 mg g⁻¹ (+72.0%), chlorophyll *b* increased from 0.59 to 0.98 mg g⁻¹ (+66.2%), and total chlorophyll increased from 1.94 to 3.30 mg g⁻¹ (+70.2%). The Chl *a/b* ratio increased slightly from 2.44 to 2.58 (+5.7%). Thus, warming increased the overall photosynthetic pigment pool, whereas the relative allocation between chlorophyll *a* and chlorophyll *b* changed only weakly. Several control plots showed lower pigment contents in September, while warmed plots generally maintained higher pigment concentrations, although

phenological observations were not available to directly test delayed senescence.

Monthly treatment means and factorial ANOVA

Averaged across the four elevations, treatment differences varied among months (Table 2). The three-way factorial ANOVA confirmed significant effects of month, elevation, experiment, and several interaction terms on most physiological indicators (Table 3). Warming significantly reduced MDA and proline and significantly increased POD, chlorophyll *a*, chlorophyll *b*, and total

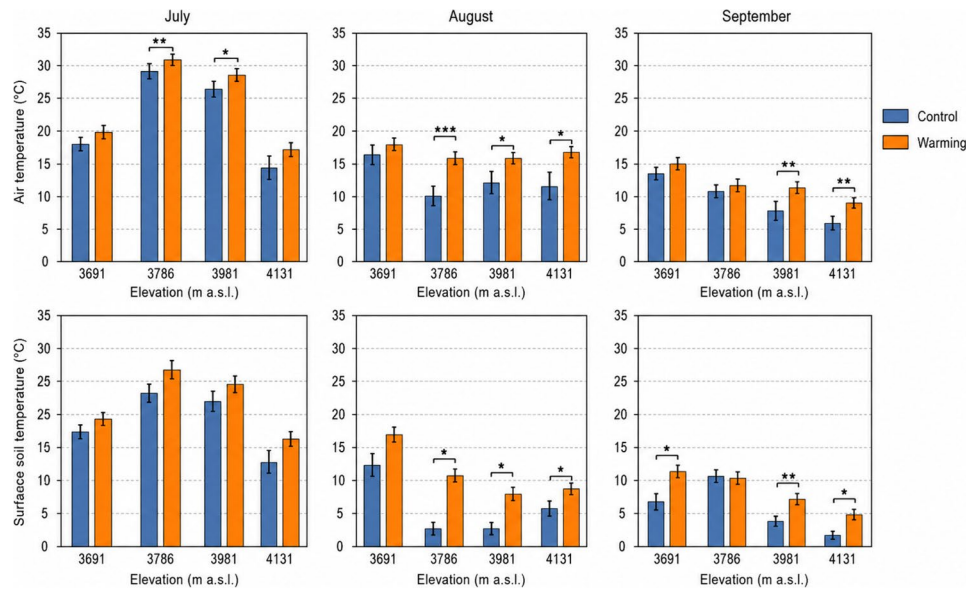


Fig. 2 Effects of OTC warming on near-surface air temperature and surface soil temperature across months and elevations. Bars indicate mean $\pm$ SD ($n=3$ plot-level replicates). Values are shown as mean $\pm$ SD. Pairwise comparisons between control and warming treatments were conducted at the same elevation and month using Welch's two-sample t-test, and P values were adjusted using the false discovery rate method. Asterisks indicate significant differences: *, **, and ***; no asterisk indicates

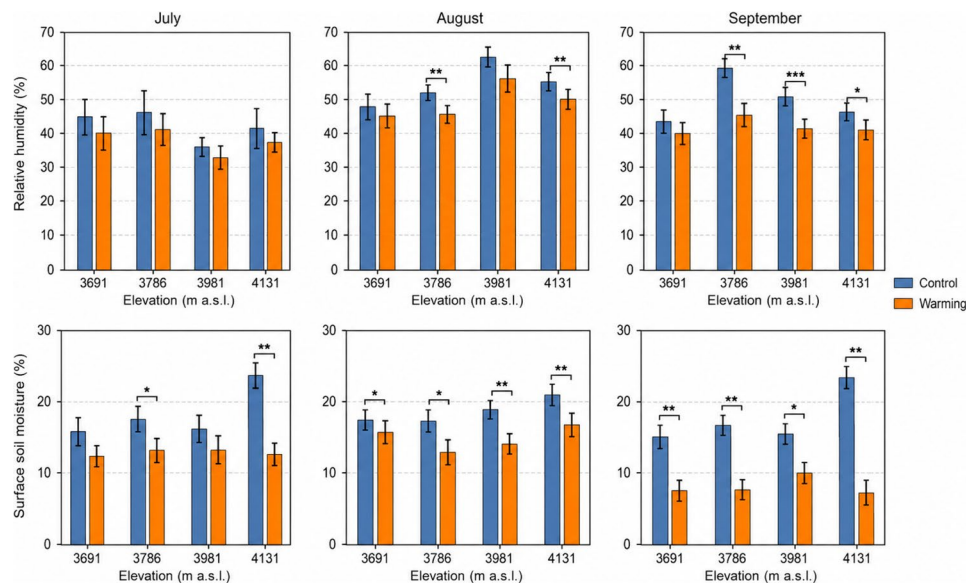


Fig. 3 Effects of OTC warming on relative humidity and surface soil moisture across months and elevations. Bars indicate mean $\pm$ SD ($n=3$ plot-level replicates). Pairwise comparisons between control and warming treatments were conducted at the same elevation and month using Welch's two-sample t-test, and P values were adjusted using the false discovery rate method. Asterisks indicate significant differences: *, **, and ***; no asterisk indicates

chlorophyll. The warming effect on SOD was statistically weak relative to the other traits, and the Chl a/b ratio was not significantly affected by warming. Significant Month $\times$ Experiment and Elevation $\times$ Experiment interactions for several variables indicate that warming responses were season- and elevation-dependent.

Discussion

The microclimatic effects observed in this study were generally consistent with international OTC warming experiments. The 1.34 °C increase in near-surface air temperature falls within the range commonly reported for ITEX-style passive warming experiments, where OTCs typically increase plant-level air temperature by approximately 1–3 °C depending on chamber design, irradiance, wind exposure, and vegetation structure [18,

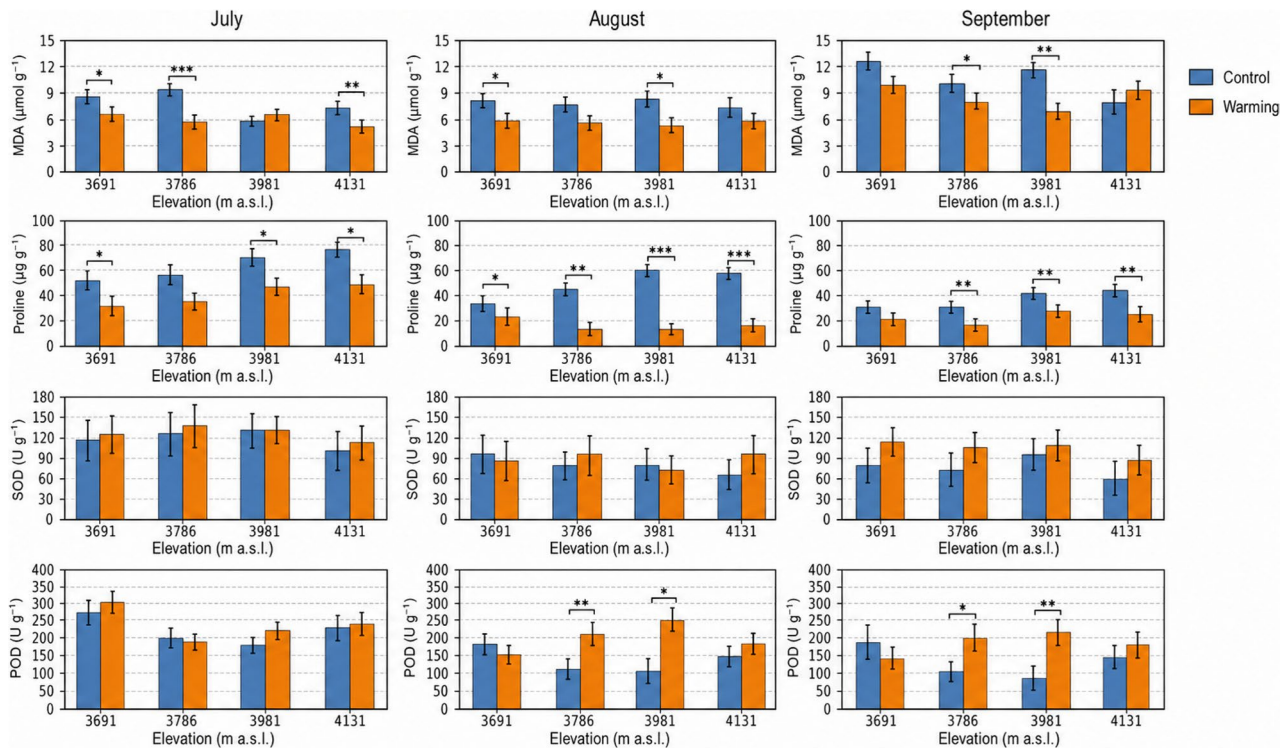


Fig. 4 Effects of OTC warming on Malondialdehyde (MDA), proline, Superoxide Dismutase (SOD), and Peroxidase (POD) in *K. pygmaea* leaves across months and elevations. Bars indicate mean $\pm$ SD ($n=3$ plot-level replicates). Values are shown as mean $\pm$ SD. Pairwise comparisons between control and warming treatments were conducted at the same elevation and month using Welch's two-sample t-test, and P values were adjusted using the false discovery rate method. Asterisks indicate significant differences: *, **, and ***; no asterisk indicates

35, 36]. The 1.32 °C increase in surface soil temperature was also within a reasonable range for passive warming experiments on the Qinghai-Tibet Plateau [37–40]. The decreases in relative humidity and surface soil moisture were expected side effects of OTC warming, because reduced air exchange and higher temperature can increase vapor pressure deficit and evapotranspiration [18, 41–44]. The relatively strong reduction in surface soil moisture may reflect the semi-arid alpine climate of the northeastern Qinghai-Tibet Plateau, strong daytime radiation, shallow active-layer hydrology, and the low canopy height of *K. pygmaea* meadows.

The decline in MDA and proline suggests that moderate warming relieved part of the cold-induced physiological stress experienced by *K. pygmaea*. MDA is widely used as an index of membrane lipid peroxidation, whereas proline accumulation reflects osmotic adjustment under cold, drought, salinity, and other stresses [26, 27, 45]. Similar reductions in stress indicators under warming have been reported in cold-limited alpine plants where moderate temperature increases improve metabolic conditions [30, 46]. However, the direction of MDA and proline responses is not universal. In warmer or drier systems, warming can intensify drought stress and increase oxidative damage [24, 25, 47, 48]. In our study, the concurrent drying was substantial but did not reverse

the overall reduction in MDA and proline, indicating that thermal release from cold limitation was still the dominant leaf-level response during most of the sampling period.

The antioxidant enzyme results further show that warming did not uniformly stimulate the entire defense system. SOD changed only slightly, whereas POD increased strongly. This enzyme-specific pattern is biologically plausible because SOD acts early in reactive oxygen species detoxification, while POD is involved in hydrogen peroxide scavenging and can also be associated with cell-wall modification and seasonal stress responses [28, 49–52]. The stronger POD response at some elevations and months may indicate that warming enhanced metabolic activity while simultaneously creating mild secondary stress through drying or seasonal transition. Therefore, the antioxidant response should be interpreted as a reorganization of defense regulation rather than a simple improvement or deterioration of plant stress status.

The large increases in chlorophyll a, chlorophyll b, and total chlorophyll indicate that OTC warming enhanced photosynthetic pigment capacity. This finding agrees with warming studies showing that moderate temperature increases can stimulate photosynthetic traits and leaf physiological activity in cold-limited alpine plants

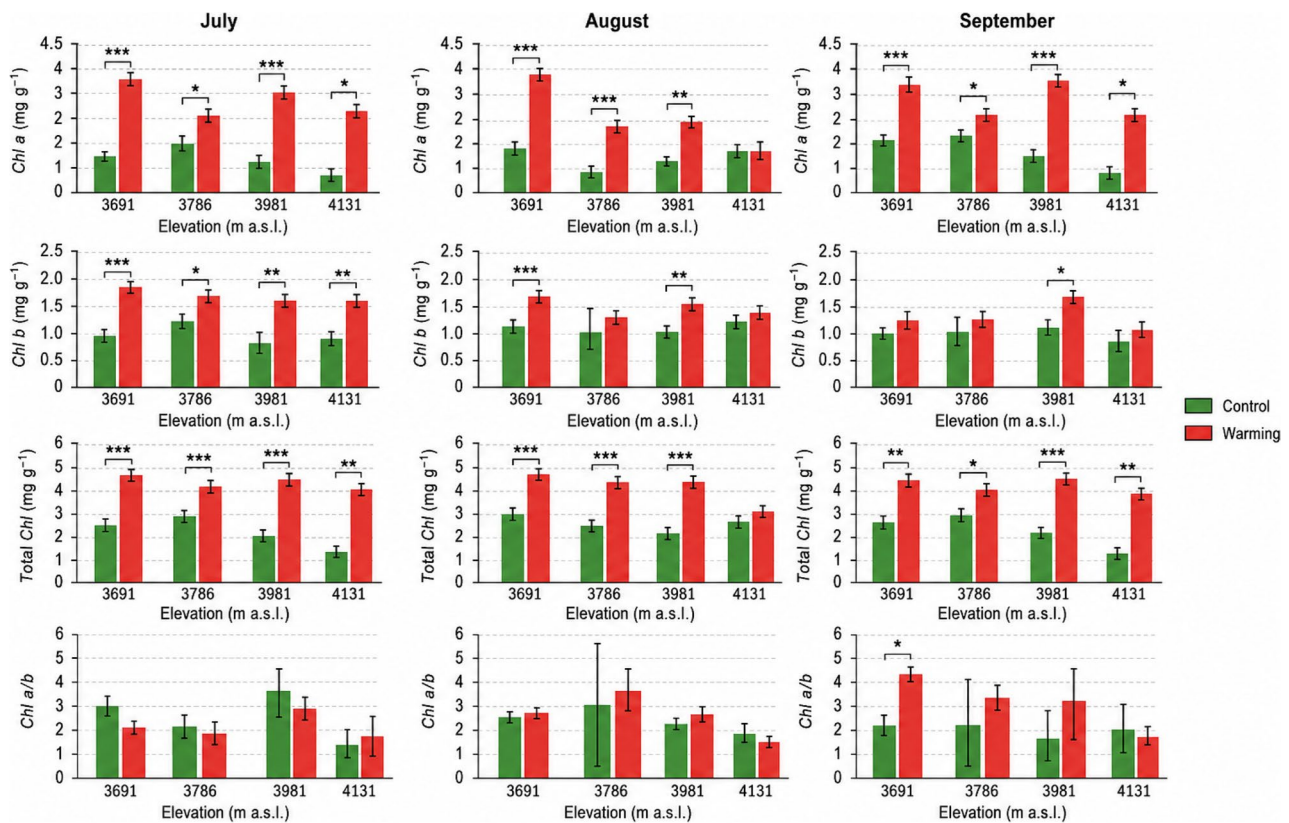


Fig. 5 Effects of OTC warming on chlorophyll *a*, chlorophyll *b*, total chlorophyll, and the chlorophyll *a/b* ratio across months and elevations. Bars indicate mean $\pm$ SD ($n = 3$ plot-level replicates). Values are shown as mean $\pm$ SD. Pairwise comparisons between control and warming treatments were conducted at the same elevation and month using Welch's two-sample *t*-test, and *P* values were adjusted using the false discovery rate method. Asterisks indicate significant differences: *, **, and ***; no asterisk indicates

[19, 30, 46, 53]. The non-significant response of the Chl *a/b* ratio indicates that warming mainly expanded the total pigment pool rather than strongly altering pigment composition. At the same time, the seasonal and elevational interactions caution against a simple generalization. The apparent maintenance of higher pigments in warmed plots later in the growing season may be consistent with delayed chlorophyll degradation, but phenology was not directly measured; therefore, this should be treated as an interpretation rather than a confirmed phenological effect [54].

The significant interactions among warming, month, and elevation demonstrate that the physiological effects of warming were context-dependent. This finding is consistent with broader warming experiments showing that plant responses vary among sites, seasons, and species [24, 25, 36, 55]. In our study, warming benefits were not equally expressed across all elevations or sampling months. Such variability may arise from differences in baseline temperature, soil moisture, microtopography, vegetation structure, and long-term acclimation to the chambers. Because the OTCs were established in 2014 and measurements were taken in 2022, the observed responses should be interpreted as the outcome of

long-term warming exposure rather than purely acute short-term plasticity. Long-term warming may modify soil nutrients, microbial processes, rooting environment, and plant acclimation, all of which could affect the physiological responses measured here [22, 23, 37, 43, 44].

This study has several limitations. First, the analysis is based on leaf-level physiological traits of a single dominant species during one growing season. Therefore, the results cannot directly demonstrate changes in biomass, net primary productivity, community composition, ecosystem carbon exchange, or pasture yield. Second, phenological stage was inferred from sampling month rather than directly recorded. Third, although each treatment-elevation-month combination included three plot-level replicates, the sample size was small, and the study should be interpreted as a field experiment with limited replication. Despite these limitations, the study provides a valuable physiological dataset for a dominant alpine meadow sedge along an elevation gradient.

The main innovation of this study is the integration of OTC-induced microclimate change with multiple leaf physiological indicators across both elevation and growing-season time. Compared with many warming studies that emphasize biomass, phenology, or community

Table 2 Monthly treatment means (mean ± SD) averaged across the four elevations

Month	Treatment	Air temperature (°C)	Surface soil temperature (°C)	Relative humidity (%)	Surface soil moisture (%)	MDA (μmol g ⁻¹)	Proline (μg g ⁻¹)	SOD (U g ⁻¹)	POD (U g ⁻¹)	Chl a (mg g ⁻¹)	Chl b (mg g ⁻¹)	Total Chl (mg g ⁻¹)	Chl a/b
Jul.	Control	27.93 ± 2.45	27.38 ± 2.20	42.51 ± 5.43	18.22 ± 3.38	6.17 ± 0.82	65.09 ± 14.32	146.82 ± 4.70	228.69 ± 59.18	1.47 ± 0.21	0.77 ± 0.20	2.24 ± 0.30	2.05 ± 0.67
Jul.	Warming	29.53 ± 2.34	28.57 ± 1.68	38.77 ± 3.42	13.54 ± 1.51	5.37 ± 0.56	41.86 ± 10.12	148.59 ± 5.84	248.85 ± 54.12	2.82 ± 0.39	1.53 ± 0.25	4.35 ± 0.50	1.88 ± 0.37
Aug.	Control	22.58 ± 1.23	21.77 ± 1.29	54.38 ± 4.87	19.12 ± 1.74	7.20 ± 0.91	50.37 ± 12.92	134.15 ± 9.92	136.44 ± 37.37	1.78 ± 0.43	0.66 ± 0.18	2.44 ± 0.55	2.88 ± 0.82
Aug.	Warming	24.03 ± 0.92	23.43 ± 1.25	50.06 ± 4.30	15.64 ± 1.63	5.10 ± 0.98	17.55 ± 4.86	136.45 ± 5.58	203.10 ± 45.18	2.81 ± 0.76	0.94 ± 0.21	3.74 ± 0.93	3.01 ± 0.53
Sep.	Control	18.76 ± 2.38	18.54 ± 2.14	50.52 ± 6.27	18.22 ± 3.38	11.17 ± 1.89	38.08 ± 7.69	128.59 ± 4.04	149.06 ± 44.55	0.80 ± 0.26	0.34 ± 0.12	1.14 ± 0.37	2.39 ± 0.44
Sep.	Warming	19.73 ± 2.06	19.64 ± 2.10	43.12 ± 2.30	10.87 ± 2.01	9.11 ± 1.36	24.65 ± 5.41	134.31 ± 5.94	197.48 ± 24.91	1.35 ± 0.42	0.47 ± 0.13	1.82 ± 0.54	2.85 ± 0.54

Table 3 Three-way ANOVA for *K. pygmaea* physiological indicators. Values are F statistics with exact P value and partial eta squared in parentheses: F (P; η²p). Residual df = 48 for all models

Effect	df	MDA (μmol g ⁻¹)	Proline (μg g ⁻¹)	SOD (U g ⁻¹)	POD (U g ⁻¹)	Chl a (mg g ⁻¹)	Chl b (mg g ⁻¹)	Total Chl (mg g ⁻¹)	Chl a/b
Month	2	175.91 (< 0.001; 0.88)	114.35 (< 0.001; 0.83)	41.92 (< 0.001; 0.64)	29.06 (< 0.001; 0.55)	791.98 (< 0.001; 0.97)	241.49 (< 0.001; 0.91)	1259.50 (< 0.001; 0.98)	23.15 (< 0.001; 0.49)
Elevation	3	3.13 (0.034; 0.16)	33.12 (< 0.001; 0.67)	1.90 (0.142; 0.11)	4.83 (0.005; 0.23)	137.57 (< 0.001; 0.90)	7.65 (< 0.001; 0.32)	132.02 (< 0.001; 0.89)	6.18 (0.001; 0.28)
Experiment	1	61.82 (< 0.001; 0.56)	314.97 (< 0.001; 0.87)	4.63 (0.036; 0.09)	29.32 (< 0.001; 0.38)	1266.56 (< 0.001; 0.96)	199.54 (< 0.001; 0.81)	1773.63 (< 0.001; 0.97)	1.37 (0.247; 0.03)
Month × Elevation	6	3.08 (0.012; 0.28)	2.31 (0.048; 0.22)	0.72 (0.638; 0.08)	2.42 (0.040; 0.23)	41.70 (< 0.001; 0.84)	10.56 (< 0.001; 0.57)	58.22 (< 0.001; 0.88)	2.47 (0.037; 0.24)
Month × Experiment	2	4.10 (0.023; 0.15)	18.40 (< 0.001; 0.43)	0.67 (0.516; 0.03)	2.64 (0.082; 0.10)	73.09 (< 0.001; 0.75)	48.17 (< 0.001; 0.67)	166.00 (< 0.001; 0.87)	2.34 (0.107; 0.09)
Elevation × Experiment	3	3.14 (0.034; 0.16)	9.64 (< 0.001; 0.38)	0.77 (0.514; 0.05)	7.78 (< 0.001; 0.33)	21.23 (< 0.001; 0.57)	4.36 (0.009; 0.21)	28.05 (< 0.001; 0.64)	0.28 (0.841; 0.02)
Month × Elevation × Experiment	6	4.58 (< 0.001; 0.36)	3.38 (0.007; 0.30)	0.48 (0.823; 0.06)	1.61 (0.166; 0.17)	33.93 (< 0.001; 0.81)	3.53 (0.006; 0.31)	41.05 (< 0.001; 0.84)	0.84 (0.545; 0.10)

structure, this study links warming and drying to oxidative damage, osmotic adjustment, antioxidant enzyme activity, and photosynthetic pigment pools in *K. pygmaea*. The results provide evidence of the leaf-level physiological response of a dominant alpine meadow species to experimental warming and show that warming effects depend jointly on thermal release, moisture limitation, elevation, and season.

Conclusions

Long-term passive OTC warming produced a moderate warming and drying microclimate in alpine meadows of the northeastern Qinghai-Tibet Plateau. Under this treatment, *K. pygmaea* leaves showed lower MDA and proline concentrations, higher POD activity, and substantially larger chlorophyll pools, indicating alleviation of leaf-level oxidative and osmotic stress and enhancement of photosynthetic pigment capacity. The response of SOD was weak, and the Chl *a/b* ratio was not significantly affected, suggesting that warming altered specific physiological processes rather than uniformly improving all traits. The magnitude of warming effects varied across months and elevations, highlighting the importance of seasonal and altitudinal context when evaluating alpine plant responses to climate warming. These findings support the interpretation that moderate warming can improve the physiological status of *K. pygmaea* during the growing season, but the concurrent decline in surface soil moisture and the absence of biomass, carbon flux, and phenological measurements limit broader ecosystem-level conclusions. Future studies should combine leaf physiology with growth, reproductive output, community composition, soil hydrology, and carbon-exchange measurements to determine how warming affects alpine meadow function over longer timescales.

Methods

Study area

The study was conducted in alpine meadow habitats on the northeastern Qinghai-Tibet Plateau. The region has a cold plateau continental climate, a short growing season, strong solar radiation, and pronounced vertical gradients in vegetation and soil thermal conditions. Meteorological records from 1961 to 2022 indicate that extreme high temperature ranges from 28.5 to 32.4 °C, extreme low temperature ranges from −27.8 to −29.0 °C, annual precipitation ranges from 401.9 to 632.3 mm, annual evaporation ranges from 1041.2 to 1234.2 mm, and mean relative humidity is approximately 58%.

Experimental design and sampling

Four experimental sites were established along an elevational gradient: S1 at 3691 m a.s.l., S2 at 3786 m a.s.l., S3 at 3981 m a.s.l., and S4 at 4131 m a.s.l. These sites

represent increasing elevation and associated habitat variation from alpine meadow to alpine swamp meadow and alpine cold desert meadow. Passive warming was achieved using OTCs following the general ITEX approach [18]. The chambers were constructed from 3-mm transparent organic glass material, with a height of 52 cm and an upper side length of 45 cm. The warming experiment was established in July 2014.

At each elevation, warmed and adjacent ambient control plots were established in relatively flat and homogeneous vegetation. The dataset used for statistical analysis included three plot-level replicates per treatment at each elevation and sampling month. Plot-level samples were used as the experimental units. In 2022, leaves of *K. pygmaea* were collected during the middle growing season (12–15 July), late growing season (12–15 August), and yellowing period (12–15 September). Healthy fully expanded leaves were collected from each plot, pooled at the plot level, placed in sealed bags, preserved in liquid nitrogen in the field, and transported to the laboratory for physiological analyses. No direct phenological scores were recorded; therefore, seasonal interpretations are based on sampling month and leaf physiological status rather than direct phenological observations.

Field sampling was conducted with the permission of the Cryosphere Research Station on the Qinghai-Tibet Plateau, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, under its long-term ecological research authorization for the study area. No protected or endangered species were involved. Voucher specimens of *K. pygmaea* were deposited at the Cold Regions Ecology Laboratory, Lanzhou Jiaotong University, under deposition number 2022-QL-Warming and were identified by Xiaodong Wu.

Microclimate and physiological measurements

Near-surface air temperature, relative humidity, surface soil temperature, and surface soil moisture were measured in warmed and control plots during the sampling periods. Air temperature and relative humidity were recorded with a temperature-humidity sensor connected to the EGM-4 environmental monitoring system (PP Systems, USA), and soil temperature and surface soil moisture were measured in the surface soil layer using matched field sensors. Measurements were paired between warmed and control plots to characterize the OTC-induced microclimatic differences.

Physiological measurements included MDA content, proline content, SOD activity, POD activity, chlorophyll *a*, chlorophyll *b*, total chlorophyll, and the chlorophyll *a/b* ratio. MDA was determined using a thiobarbituric acid method and expressed on a fresh-weight basis as $\mu\text{mol g}^{-1}$. Proline was measured using the acid-ninhydrin colorimetric method and expressed as $\mu\text{g g}^{-1}$. SOD

activity was assayed based on the inhibition of nitro blue tetrazolium photoreduction and expressed as U g^{-1} . POD activity was determined using the guaiacol oxidation method and expressed as U g^{-1} . Chlorophyll pigments were extracted with organic solvent and quantified spectrophotometrically, and concentrations were expressed as mg g^{-1} . All biochemical variables were calculated on the same mass basis as the original dataset.

Statistical analysis

All statistical analyses were conducted using the original plot-level dataset. For each variable, treatment means were calculated across all elevations and months and expressed as mean changes between control and warmed plots. Error bars in figures represent standard deviation (SD), and $n=3$ indicates three plot-level replicates within each treatment-elevation-month combination.

The effects of Month, Elevation, Experiment, and their interactions on physiological variables were tested using three-way factorial ANOVA. Month, elevation, and experiment were treated as categorical factors, and the full interaction model was fitted for each physiological indicator. The ANOVA table reports degrees of freedom, F statistics, exact P values, and partial eta squared as an effect-size measure. Because the reviewers noted concerns about multiple uncorrected pairwise tests, the revised manuscript focuses on the factorial ANOVA for statistical inference. Pairwise comparisons were not used as the primary basis for interpreting treatment effects.

Abbreviations

QTP	Qinghai-Tibet Plateau
OTC	Open-top chamber
MDA	Malondialdehyde
Pro	Proline
SOD	Superoxide dismutase
POD	Peroxidase
Chl a	Chlorophyll a
Chl b	Chlorophyll b
Chlt	Total chlorophyll

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12870-026-09905-9>.

Supplementary Material 1.

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Authors' contributions

All authors contributed to the study conception and design. performed the Laboratory experiments were performed by LZ, GJ and GL. Computational data analyses were performed by XW. All authors contributed in writing the first draft of the manuscript. All authors revised and approved the final version of the manuscript.

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Data availability

All data generated or analysed during this study are included in this published article and supplementary file.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

- Pepin N, Lundquist J. Temperature trends at high elevations: patterns across the globe. *Geophys Res Lett*. 2008;35(14):L14701.
- Byrne MP, Boos WR, Hu S. Elevation-dependent warming: observations, models, and energetic mechanisms. *Weather Clim Dynamics*. 2024;5(2):763–77.
- Broadbent AA, Newbold LK, Pritchard WJ, Michas A, Goodall T, Cordero I, Giunta A, Snell HS, Pepper WV, Grant HK. Climate change disrupts the seasonal coupling of plant and soil microbial nutrient cycling in an alpine ecosystem. *Glob Change Biol*. 2024;30(3):e17245.
- Praeg N, Steinwandter M, Urbach D, Snethlage MA, Alves RP, Apple ME, Bilovitz P, Britton AJ, Bruni EP, Chen Tw. Biodiversity in mountain soils above the treeline. *Biol Rev*. 2025;100(5):1877–949.
- Williams MW, Brooks PD, Seastedt T. Nitrogen and carbon soil dynamics in response to climate change in a high-elevation ecosystem in the Rocky Mountains, USA. *Arct Alp Res*. 1998;30(1):26–30.
- Zhao L, Wu X, Wang Z, Sheng Y, Fang H, Zhao Y, Hu G, Li W, Pang Q, Shi J, et al. Soil organic carbon and total nitrogen pools in permafrost zones of the Qinghai-Tibetan Plateau. *Sci Rep*. 2018;8(1):3656.
- Mu C, Abbott BW, Norris AJ, Mu M, Fan C, Chen X, Jia L, Yang R, Zhang T, Wang K, et al. The status and stability of permafrost carbon on the Tibetan Plateau. *Earth Sci Rev*. 2020;211:103433.
- Xu X-M, Zhang Z-Q, Tai B-W, Gao S-R, Yang Y-Z, Wu Q-B. Climate warming and wetting poses a severe threat to permafrost engineering stability on the Qinghai-Xizang Plateau. *Adv Clim Change Res*. 2025;16(1):93–108.
- Carlson BZ, Corona MC, Dentant C, Bonet R, Thuiller W, Choler P. Observed long-term greening of alpine vegetation—a case study in the French Alps. *Environ Res Lett*. 2017;12(11):114006.
- Wang Z, Zhang X, Niu B, Zheng Y, He Y, Cao Y, Feng Y, Wu J. Divergent climate sensitivities of the alpine grasslands to early growing season precipitation on the Tibetan Plateau. *Remote Sens*. 2022;14(10):2484.
- Lou P, Wu T, Yang S, Wu X, Chen J, Zhu X, Chen J, Lin X, Li R, Shang C et al. Deep learning reveals rapid vegetation greening in changing climate from 1988 to 2018 on the Qinghai-Tibet Plateau. *Ecol Ind*. 2023;148:110020.
- Zhang Y, Yang Z, Qu-zong C-r, Gyal S, Mazhang W, Yang Y, Zhang G, Se C, Quzhen D, Wang S. Spatial variations in carbon dioxide fertilization effect on vegetation greening across the Tibetan Plateau. *Glob Planet Change*. 2025;252:104839.
- Shen M, Wang S, Jiang N, Sun J, Cao R, Ling X, Fang B, Zhang L, Zhang L, Xu X. Plant phenology changes and drivers on the Qinghai-Tibetan Plateau. *Nat Reviews Earth Environ*. 2022;3(10):633–51.
- Kashyap A, Kumar Pandey V, Bera D, Parashar D, Kumar A, Pandey A, Ullah S, Alkhuraji WS, Zhran M. Vegetation loss and climate-driven fragmentation in the Himalayan alpine ecosystem: an AI/ML-based LULC and carbon stock analysis. *Theoret Appl Climatol*. 2026;157(6):340.
- Duan C, Shi P, Zong N, Zhang X, Yu C. Assessing rangeland sensitivity to degradation in North Tibet. *Rangel Ecol Manage*. 2022;84:86–97.

16. Qiao Y, Duan Z. Understanding alpine meadow ecosystems. Landscape and ecosystem diversity, dynamics and management in the Yellow River Source Zone. Springer; 2016. pp. 117–35.
17. Li C, Sun H, Liu L, Dou T, Zhou M, Li W, Wu X. The importance of permafrost in the steady and fast increase in net primary production of the grassland on the Qinghai–Tibet Plateau. *CATENA*. 2022;211:105964.
18. Hollister RD, Elphinstone C, Henry GH, Björkman AD, Klanderud K, Björk RG, Björkman MP, Bokhorst S, Carbognani M, Cooper EJ. A review of open top chamber (OTC) performance across the ITEX Network. *Arct Sci*. 2022;9(2):331–44.
19. Fu G, Shen Z-X, Sun W, Zhong Z-M, Zhang X-Z, Zhou Y-T. A meta-analysis of the effects of experimental warming on plant physiology and growth on the Tibetan Plateau. *J Plant Growth Regul*. 2015;34(1):57–65.
20. Xu Z, Shimizu H, Ito S, Yagasaki Y, Zou C, Zhou G, Zheng Y. Effects of elevated CO₂, warming and precipitation change on plant growth, photosynthesis and peroxidation in dominant species from North China grassland. *Planta*. 2014;239(2):421–35.
21. Wang J, Defrenne C, McCormack ML, Yang L, Tian D, Luo Y, Hou E, Yan T, Li Z, Bu W. Fine-root functional trait responses to experimental warming: a global meta-analysis. *New Phytol*. 2021;230(5):1856–67.
22. Zhou X, Chen X, Qi X, Zeng Y, Guo X, Zhuang G, Ma A. Soil bacterial communities associated with multi-nutrient cycling under long-term warming in the alpine meadow. *Front Microbiol*. 2023;14:1136187.
23. Wang Z, Han I, Lee J, Li G, He P, Baldwin MT, Kao-Kniffin J, Wu L, Zhou J, Gu AZ. Climate warming enhances biodiversity and stability of grassland soil phosphorus-cycling microbial communities. *ISME J*. 2025;19(1):wraf118.
24. Penuelas J, Gordon C, Llorens L, Nielsen T, Tietema A, Beier C, Bruna P, Emmett B, Estiarte M, Gorissen A. Nonintrusive field experiments show different plant responses to warming and drought among sites, seasons, and species in a north-south European gradient. *Ecosystems*. 2004;7:598–612.
25. Hoepfner SS, Dukes JS. Interactive responses of old-field plant growth and composition to warming and precipitation. *Glob Change Biol*. 2012;18(5):1754–68.
26. Morales M, Munné-Bosch S. Malondialdehyde: facts and artifacts. *Plant Physiol*. 2019;180(3):1246–50.
27. Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A. Role of proline under changing environments: a review. *Plant Signal Behav*. 2012;7(11):1456–66.
28. Saed-Moucheshi A, Sohrabi F, Fasihfar E, Baniasadi F, Riasat M, Mozafari AA. Superoxide dismutase (SOD) as a selection criterion for triticale grain yield under drought stress: a comprehensive study on genomics and expression profiling, bioinformatics, heritability, and phenotypic variability. *BMC Plant Biol*. 2021;21(1):148.
29. Yuan T, Ren W, Zhang J, Mahmood M, Jia Z, Zhang S, Wang M, Liang S, Yuan F, Liu Y. Synergistic effect of grassland plants and beneficial rhizosphere bacteria helps plants cope with overgrazing stress. *BMC Plant Biol*. 2025;25(1):614.
30. Shi F, Wu Y, Wu N, Luo P. Different growth and physiological responses to experimental warming of two dominant plant species *Elymus nutans* and *Potentilla anserina* in an alpine meadow of the eastern Tibetan Plateau. *Photosynthetica*. 2010;48(3):437–45.
31. Lan Y, Fan B, Guo X, Si M, Li B, Qian D, Lin L. Effect of grazing management strategies on the vegetation parameters and soil nutrients in alpine Kobresia pygmaea meadow on the northeastern Qinghai–Tibetan Plateau. *Global Ecol Conserv*. 2023;48:e02680.
32. Lv Y, Sun Y, Yi S, Meng B. Human activities dominant the distribution of Kobresia pygmaea community in alpine meadow grassland in the east source region of Yellow River, China. *Front Ecol Evol*. 2023;11:1127973.
33. Li Y, Xia Z, Sun J, Yang T, Yu Z. Peat hydraulic structure maintains the stability of permafrost slope peatlands in the central Qinghai–Tibet Plateau. *Water Resour Res*. 2025;61(12):e2025WR041170.
34. Lin L, Cao G, Xu X, Zhang F, Huang J, Fan B, Li B, Li Y. Kobresia pygmaea meadows as disclimax communities in the same geographic and climatic environments in Qinghai–Tibet Plateau, China. *J Plant Ecol*. 2023;16(5):rtad010.
35. Klein JA, Harte J, Zhao XQ. Dynamic and complex microclimate responses to warming and grazing manipulations. *Glob Change Biol*. 2005;11(9):1440–51.
36. Bokhorst S, Huiskes A, Aerts R, Convey P, Cooper EJ, Dalen L, Erschbamer B, Gudmundsson J, Hofgaard A, Hollister RD. Variable temperature effects of Open Top Chambers at polar and alpine sites explained by irradiance and snow depth. *Glob Change Biol*. 2013;19(1):64–74.
37. Chen Y, Han M, Yuan X, Hou Y, Qin W, Zhou H, Zhao X, Klein JA, Zhu B. Warming has a minor effect on surface soil organic carbon in alpine meadow ecosystems on the Qinghai–Tibetan Plateau. *Glob Change Biol*. 2022;28(4):1618–29.
38. Mu C, Zhang T, Zhao Q, Su H, Wang S, Cao B, Peng X, Wu Q, Wu X. Permafrost affects carbon exchange and its response to experimental warming on the northern Qinghai–Tibetan Plateau. *Agric For Meteorol*. 2017;247:252–9.
39. Bai W, Wang G, Liu G. Effects of elevated air temperatures on soil thermal and hydrologic processes in the active layer in an alpine meadow ecosystem of the Qinghai–Tibet Plateau. *J Mt Sci*. 2012;9(2):243–55.
40. Wang J, Wu Q. Impact of experimental warming on soil temperature and moisture of the shallow active layer of wet meadows on the Qinghai–Tibet Plateau. *Cold Reg Sci Technol*. 2013;90–91:1–8.
41. Wang Y, Xu Y, Jia C, Hu X. Reconsidering warming effects on seedling recruitment in Tibetan Plateau's alpine meadows via open-top chamber experiments. *J Appl Ecol*. 2025;62(4):884–98.
42. Ren F, Zhou H-k, Zhao X-Q, Han F, Shi L-N, Duan J-C, Zhao J-Z. Influence of simulated warming using OTC on physiological–biochemical characteristics of *Elymus nutans* in alpine meadow on Qinghai–Tibetan plateau. *Acta Ecol Sin*. 2010;30(3):166–71.
43. Wen J, Qin R, Zhang S, Yang X, Xu M. Effects of long-term warming on the aboveground biomass and species diversity in an alpine meadow on the Qinghai–Tibetan Plateau of China. *J Arid Land*. 2020;12(2):252–66.
44. Zhu Y, Tang S, Xue W, Ma J, Luo J, Hu L, Ren X, Wang Y, Wu P. Responses of microarthropods and nematodes to long-term warming in alpine meadows are precipitation-dependent effects. *Eur J Soil Biol*. 2025;127:103776.
45. Jiang J, Guo Z, Sun X, Jiang Y, Xie F, Chen Y. Role of proline in regulating turfgrass tolerance to abiotic stress. *Grass Res*. 2023;3(1):1–7.
46. Shen H, Dong S, Li S, Wang W, Xiao J, Yang M, Zhang J, Gao X, Xu Y, Zhi Y. Effects of warming and N deposition on the physiological performances of *Leymus secalinus* in alpine meadow of Qinghai–Tibetan Plateau. *Front Plant Sci*. 2020;10:1804.
47. Li J, Bai X, Ran F, Zhang C, Yan Y, Li P, Chen H. Effects of combined extreme cold and drought stress on growth, photosynthesis, and physiological characteristics of cool-season grasses. *Sci Rep*. 2024;14(1):116.
48. Bibi S, Ahmad MSA, Bahadur A, Naeem Z, Hameed M, Li Y. Resilience in aridity: from biomass reduction to antioxidant accumulation in sewan grass. *BMC Plant Biol*. 2025;25(1):1622.
49. Rizhsky L, Hallak-Herr E, Van Breusegem F, Rachmilevitch S, Barr JE, Rodermeil S, Inzé D, Mittler R. Double antisense plants lacking ascorbate peroxidase and catalase are less sensitive to oxidative stress than single antisense plants lacking ascorbate peroxidase or catalase. *Plant J*. 2002;32(3):329–42.
50. Xu L, Liu H, Mittler R, Shabala S. Useful or merely convenient: can enzymatic antioxidant activity be used as a proxy for abiotic stress tolerance? *J Exp Bot*. 2025;76(6):1524–33.
51. Nawaz M, Shabbir S, Nazir MM, Anas M, Farooq M, Xu H, Yang W, Ahmad P, Li L, Wang Z. Adaptive strategies of carpet grass to drought: Insights from physiological and antioxidant responses. *J Plant Growth Regul*. 2026;45(2):945–59.
52. Kumar P, Singh B, Rajak S, Pandey S, Pati P. Dynamics of reactive oxygen species and lignin biosynthesis during leaf spot disease of *Withania somnifera* (L.) Dunal. *Plant Biol*. 2023;25(5):757–70.
53. Zhou Z, Su P, Yang J, Shi R, Ding X. Warming affects leaf light use efficiency and functional traits in alpine plants: evidence from a 4-year in-situ field experiment. *Front Plant Sci*. 2024;15:1353762.
54. Collins CG, Elmendorf SC, Hollister RD, Henry GH, Clark K, Björkman AD, Myers-Smith IH, Prevéy JS, Ashton IW, Assmann JJ. Experimental warming differentially affects vegetative and reproductive phenology of tundra plants. *Nat Commun*. 2021;12(1):3442.
55. Fazlioglu F, Wan JS. Warming matters: alpine plant responses to experimental warming. *Clim Change*. 2021;164(3):56.

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