



Reduction of PSII photosynthetic performance by low temperature is the reason for the growing inhibition of *Kobresia pygmaea* Clarke

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Abstract

Kobresia pygmaea Clarke is mainly distributed in the alpine region from 3800 to 4500 m elevation on the Qinghai-Tibet Plateau. It is excellent in low-temperature tolerance and trampling resistance and plays an important role in maintaining the stability of regional ecological environments. Mainly based on the chlorophyll fluorescence imaging technique, using grass sods blocks with a size of 20 × 20 cm² collected from *K. pygmaea* forb meadow, simulated a low-temperature treatment in cultural room with 8 °C/4 °C (day/night) at diurnal photoperiod, we analyzed the effects of low temperature on PSII photochemical efficiency and non-photochemical quenching, as well as the interaction between low temperature and light intensity. The results showed that low temperature could significantly decrease the maximum quantum efficiency of PSII photochemistry (F_v/F_m) and inhibit the leaf growth of *K. pygmaea*. The light response curve of PSII relative linear electron transport rate was reduced by low temperature, while it recovered to almost normal level after two days of removing adversity. Further experiments under steady-state actinic light confirmed that light intensity was the main factor affecting the PSII photochemical efficiency, and its reduction caused by the low temperature showed a certain degree of reversibility. The non-photochemical quenching (NPQ) was significantly affected by light intensity, and its fast and slow components (NPQ_f and NPQ_s) in NPQ accounted for 36% and 64% of NPQ, respectively. The decrease in NPQ_f and corresponding increase in NPQ_s slowly recovered after relieving from low-temperature condition. The relative inhibition of photosynthesis (P_{inh}) was significantly enhanced by low temperature and reduced after removal of adversity. Strong light intensity could intensify the adverse effect of low temperature on PSII operating efficiency F_q'/F_m' and efficiency factor F_q'/F_v' , as well as NPQ. In conclusion, low temperature was one of the main reasons limiting the photosynthetic performance of *K. pygmaea* on the Qinghai-Tibet Plateau and could aggravate photosynthetic photoinhibition. The adversity of low temperature frequently occurring in the plant growing season on the Qinghai-Tibet Plateau was constituted of the main factor of the low productivity level in alpine meadow.

Keywords Alpine plant · Non-photochemical quenching · Photosynthetic photoinhibition · Qinghai-Tibet Plateau

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1 Introduction

The Qinghai-Tibet Plateau is the highest plateau in the world with an average altitude of more than 4000 m and is known as "the roof of the world" and "the third pole" (Chen et al. 2019). The region of plateau frequently presents a harsh and extreme climate with low temperature, strong winds and high solar radiation during the plant growing season. For this reason, alpine plants are often exposed to abiotic stress (Miehe et al. 2019). *Kobresia pygmaea* C. B. Clarke, an herbaceous perennial of Cyperaceae with dense rhizomes and strong vitality, is a constructive species in the alpine meadow belt of the Qinghai-Tibet Plateau and Himalayas (Zhou 2001). As an important dominant species in the alpine

meadow, the alpine species of *K. pygmaea* has high nutritional value and occupies a large proportion in the available grassland in the region of the Qinghai-Tibet Plateau (Li et al. 2010). Due to its excellent characteristics of resistance to low temperature, drought, trampling and soil erosion (Wang et al. 2008), it is of great importance to maintain regional ecological environment stability.

Alpine *K. pygmaea* meadow is mainly distributed at an altitude of 3800 to 4500 m, and its distribution ranges from a low slope of 3000 m to an upland of 5960 m, indicating its successful adaptation to alpine habitats (Zhou 2001). Low temperature is a typical abiotic stress on the Qinghai-Tibet Plateau, especially in early spring and late autumn. Cold stress has influenced plant growth and geographical distribution, which can not only limit the growth and development of plants but also affect their yield and quality of forage (Tesso 2013; Valizadeh-Kamran et al. 2017). Therefore, the productivity level or primary productivity of meadows is very low, restricting the sustainable development of animal husbandry on the plateau. Moreover, natural disasters such as strong winds and snowfall are frequent happened. Because of extreme weather and overgrazing, the degradation of grassland is serious and the secondary bare land (named the black soil beach by local people) is often formed. However, due to the limitations of the unique habitat of the plateau and the configuration of dwarf plants, early much of the work is only focused on its distribution pattern, breeding strategies, phenological characteristics, and so on (Li et al. 2003; Sun et al. 2007), there is little work on the photosynthetic physiological processes and their adaptability to harsh climates.

Stronger solar radiation reaches the Earth's surface due to the lower air pressure and higher atmospheric transparency in the region of the Qinghai-Tibet Plateau (Peng et al. 2015). Generally, except for driving photosynthesis, most of the solar light energy absorbed by the photosynthetic apparatus is in surplus (Xu 2002); in this case, the extra light energy must be dissipated safely through an effective approach (Tikkanen et al. 2013; Yamori et al. 2014), avoiding the potential damage of excess excitation energy to the photosynthetic apparatus. Savitch and his coworker (2009) researched the effects of low-temperature stress on excitation energy partitioning and photoprotection using C_4 plant maize sensitized to cold stress and found that the fraction utilized by PSII photochemistry was twofold lower, whereas the proportion of thermally dissipated energy was only 30% higher in cold-stressed plants than in control plants. However, it is not clear whether the photoinhibition degree of the photosynthetic apparatus of alpine plants will be enhanced (synergistic effects), or whether resistance to another stress will be increased (cross-tolerance) after a period of exposure to low temperature. The hypotheses of this paper are as follows: (1) low temperature can inhibit the growth of *K. pygmaea* and reduce the PSII photochemical quantum

efficiency in leaves; (2) after relieving low-temperature condition, plant growth can recover to its normal status; and (3) low-temperature adversity can accelerate the degree of photoinhibition induced by strong solar light.

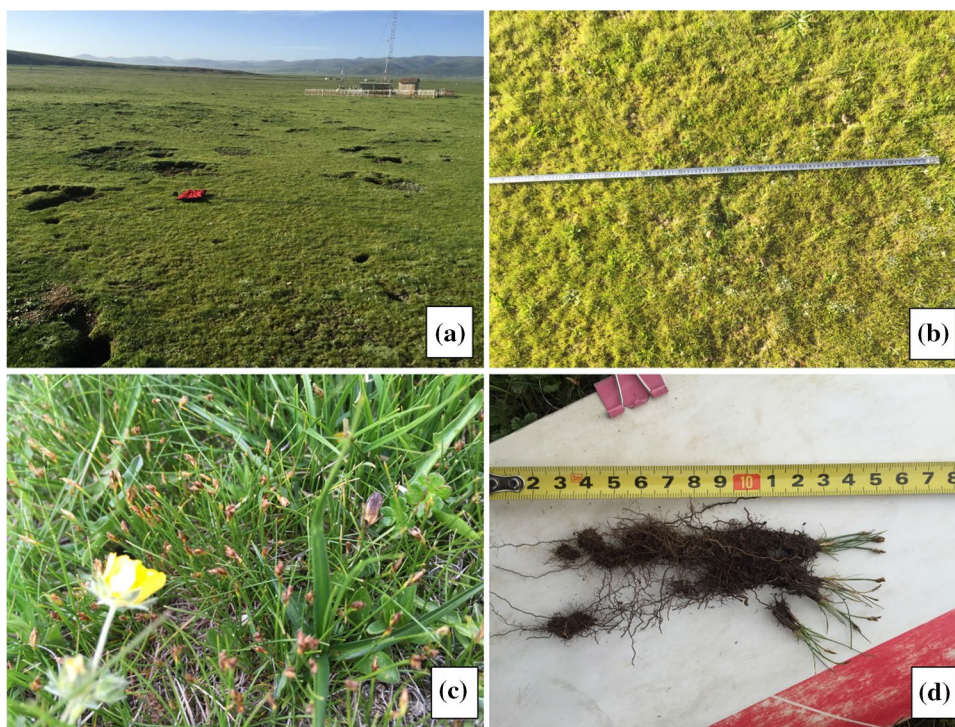
The Qinghai-Tibet Plateau, as a sensitive and driving region of global change (Shrestha et al. 2012), is extremely prominent in its ecosystem degradation, species extinction and deterioration of natural environments (Yu and Lu 2011). Therefore, it is particularly important to reveal the physiological and ecological adaptation mechanisms of native species of *K. pygmaea* to extreme adversity and the potential degradation crisis of the alpine meadow ecosystem. Based on a chlorophyll fluorescence image analysis system (CF imager), we studied the photosystem II (PSII) photochemical efficiency and non-photochemical energy distribution characteristics impacted by simulating low-temperature adversity and discussed the interaction effect between low temperature and light intensity on alpine species of *K. pygmaea*.

2 Materials and methods

Plant material and sample plots – Plant material was taken from a typical *K. pygmaea* forb meadow near the Alpine Grassland Ecosystem Research Station of the Source Region of Three Rivers, Qinghai University and Tsinghua University. The station is located in Zhenqin Town, Chengduo County, Yushu Prefecture, Qinghai Province, China. Its geographical coordinates are 33°N and 97°E, with an altitude of 4270 m. The average annual temperature is -5.6 to 3.8 °C, and the average annual precipitation is 562.2 mm. Precipitation is mainly distributed from June to September during the plant growing season, accounting for approximately 75% of the annual precipitation. In July and August 2019, the average temperature at station is 7.4 °C; the average maximum and minimum temperatures are 14 °C and 1.8 °C, respectively. There is no absolute frost-free period during plant growing season. The climate is a typical plateau continental climate with a longer cold season and shorter warm season, and there are almost no frost-free periods. The soil is alpine meadow soil (Mat Crygelic Cambisols) with rich humus content but poor decomposition, which has low effective fertility. The soil pH was 6.92, organic matter content was 2.36%, available nitrogen was 14.0 mg L⁻¹, ammonia nitrogen was 5.1 mg L⁻¹, nitrate nitrogen was 8.9 mg L⁻¹, available phosphorus was 7.0 mg L⁻¹, and available potassium was 76.5 mg L⁻¹.

K. pygmaea C. B. Clarke is the dominant herbage in the *K. pygmaea* forb meadow (Fig. 1); the subdominant species are: *Kobresia humilis* (C. A. Mey.) Serg., *Stipa aliena* Keng, *Poa poiphagorum* Bor, *Polygonum viviparum* L., *Potentilla nivea* L., *Anaphalis lactea* Maxim., *Thalictrum alpinum* L., etc.; associated plants include *Festuca ovina*

Fig. 1 The landscape of alpine meadow and dominant species of *K. pygmaea*



L., *Elymus nutans* Griseb., *Aster tsarungensis* (Griers.) Ling, *Saussurea superba* Anthony, *Taraxacum mongolicum* Hand. –Mazz; miscellaneous and poisonous weeds include *Oxytropis kansuensis* Bunge, *Gentiana sino-ornata* Balf., *Stellera chamaejasme* L., *Euphorbia sieboldiana* Morr. et Decne., *Pedicularis kansuensis* Maxim., *Lamiophlomis rotata* (Benth.) Kudo, etc. There was no grazing utilization by sheep and yaks in the alpine *K. pygmaea* forb meadow near the site of the station during the period of field experiments in the warm season.

Experimental design – The experiments were conducted from June 6 to 15, 2019 (forage green up period), and August 5 to 15, 2019 (exuberance period). This paper mainly analyzed the results from the experiment conducted in the exuberance period. We chose 3 flat fields in *K. pygmaea* forb meadow and dug up 2 blocks of grass sods with a size of $20 \times 20 \text{ cm}^2$ and depth of approximately 15 cm in each field. After sealing 4 edges of grass block with plastic bandages,

all turfs were set outdoors in natural light for 1 day; during this period, we carefully removed other plant species, withered grass and dopants from the grass cortex and kept the *K. pygmaea* in its natural growth state. On August 7, the aboveground biomass was mowed near above the yellow leaf sheath to ensure that the stubble height was consistent; a piece of turf block was picked from each field site and placed in an artificial low-temperature climate chamber, setting a low-temperature group (Low Temp). The control group (Control) was placed under an artificial light source in the cultivation rack of a semi-automatic illumination culture room, adjusting to a light intensity similar to that in an artificial climate chamber (Table 1). The light intensity in the climate chamber was $600 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, the air temperature was $8 \text{ }^\circ\text{C}$ during the day and $4 \text{ }^\circ\text{C}$ at night with a 12-h photoperiod. The intensity of the artificial light source in the culture room was $600 \pm 20 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, and the air temperature was $23 \pm 1 \text{ }^\circ\text{C}$ during the day and $18 \pm 2 \text{ }^\circ\text{C}$ at night with a 12-h photoperiod.

Table 1 Schematic diagram of the experimental design, treatment and processes (August 5 to 15, 2019)

Groups	Field adaptation		Low-temperature treatment $600 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$; 12 h photoperiod				Recovery		
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Control	Sealing and removing	Mowing	23/18 $^\circ\text{C}$ (day/night)				23/18 $^\circ\text{C}$ (day/night)		
Low Temp			8/4 $^\circ\text{C}$ (day/night)				23/18 $^\circ\text{C}$ (day/night)		

The measurements of chlorophyll fluorescence parameters were conducted at Day 6 and Day 9

Sufficient water was sprayed in the morning and evening to keep the meadow moist in the control group; a small amount of water was sprayed on the turf block in the low-temperature group every day to maintain a moisture level similar to that of the control group. Chlorophyll fluorescence parameters and leaf growth status were measured during the low-temperature treatment and the recovery phase. On the 7th day, the low-temperature group was transferred to a culture room irradiated with artificial light for 2 days of restoration. After completing all tests on August 15, the soil water contents were measured, and soil samples were taken from the inside of turf blocks using a cutting ring. The relative water contents of the soil in the low-temperature group and control group were $25.20 \pm 1.44\%$ and $24.75 \pm 1.25\%$, respectively, showing no significant difference.

Determination of chlorophyll fluorescence parameters. Chlorophyll fluorescence images were determined nearly every two days from the 3rd day. The chlorophyll fluorescence parameters were measured by using a chlorophyll fluorescence image analyzer (chlorophyll fluorescence imager, CF imager: Technological Ltd, UK) with a light-emitting diode (LED) red and blue light source. The turf block was put in the sample chamber, and the focal length of the charge-coupled device camera (CCD camera) was adjusted. The indoor light was turned off, the built-in protocol was run for experimental purposes, and fluorescence images of plants were measured chlorophyll after 15 min of dark adaptation. During measurement, the indoor temperature was maintained at $20 \pm 1.5^\circ\text{C}$, and the air humidity was kept constant.

Measurement protocol.

Rapid light response curve: The minimum chlorophyll fluorescence yield (F_o) in the open state of the PSII reaction center and the maximum chlorophyll fluorescence yield (F_m) in the closed state were determined after accurate dark adaptation for 15 min. The steady-state actinic light was turned on, and fluorescence parameters were measured with 25, 50, 100, 150, 200, 300, 500, 800, 1000, 1200, 1500, 1800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ successively. To determine every fluorescence parameter at light intensities of 25, 50, 100, 150 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, stable illumination lasted for 2 min. After light intensities higher than 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, each light intensity was stabilized for 1 min. The maximum fluorescence yield (F_m') and the steady-state fluorescence yield (F_s) were measured for each point of light intensity. The saturation pulse light in the determination of F_m and F_m' was 6840 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and lasted for 60 ms.

Steady state chlorophyll fluorescence parameterssec: After dark adaptation for 15 min, the F_o and the F_m were determined. Successively turning on 400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ low steady-state actinic light and 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ high steady-state actinic light, the F_m' and the F_s were determined for 5, 10 and 15 min of continuous

illumination with each light intensity. The actinic light was turned off, and the F_o and F_m' were measured at accurate dark recovery times of 5, 10 and 15 min. The saturation pulse light intensity in the determination of F_m , F_m' and F_m'' was 6840 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and lasted for 60 ms.

Leaf localization and image analysis. The paired measurement data of the 6th day and 8th day (2 days after moving to the culture room) were used for analysis in this paper. In the graphic edit frame of the fluorescent image, the linear small leaves of *K. pygmaea* were located and segmented and then resolved into data values. Chlorophyll fluorescence parameter data of 30 leaves were obtained as one group for each tested turf block. More details on the specific operational steps can be found in the CF imager manual (<http://www.technologica.co.uk/>).

PSII photochemical efficiency and non-photochemical dissipation: The PSII maximum photochemical quantum efficiency (F_v/F_m) of the dark-adapted leaves was calculated with the minimum chlorophyll fluorescence yield (F_o) and maximum chlorophyll fluorescence yield (F_m) after accurate dark adaptation for 15 min, where $F_v = F_m - F_o$. PSII maximum efficiency (F_v'/F_m') and PSII operational efficiency (F_q'/F_m') were calculated by the following equations (Genty et al. 1989): $F_v'/F_m' = (F_m' - F_o')/F_m'$; $F_q'/F_m' = (F_m' - F_s)/F_m'$. The PSII efficiency factor (F_q'/F_v') and the non-photochemical quenching (NPQ) were calculated according to the formulas of Bilger and Björkman (1990): $F_q'/F_v' = (F_m' - F_s)/(F_m' - F_o')$; $\text{NPQ} = F_m/F_m' - 1$. F_o' in the above formulas was estimated by the empirical formulas of Oxborough and Baker (1997): $F_o' = F_o/(F_v/F_m + F_o/F_m')$; among them, F_o and F_m were the chlorophyll fluorescence yield after accurate dark-adaptation for 15 min.

Photosynthetic photoinhibition and components of non-photochemical quenching: The components of fast and slow relaxation of non-photochemical quenching (NPQ_f and NPQ_s) were calculated by referring to the formulas of Maxwell and Johnson (2000): $\text{NPQ}_s = (F_m - F_m^r)/F_m^r$; $\text{NPQ}_f = (F_m/F_m') - (F_m^r/F_m^r)$. In the above formula, F_m is the fluorescence yield of accurate dark adaptation for 15 min; F_m^r selected the fluorescence yield from accurate dark recovery for 5, 10 and 15 min. The relative photoinhibition of photosynthesis was estimated by the method of Dodd and coworkers (Dodd et al. 1998): $P_{\text{inh}} = 100 \times (1 - (F_v^r/F_m^r)/(F_v/F_m))$, of which $F_v = F_m - F_o$, $F_v^r = F_m^r - F_o^r$.

Leaf growth and relative growth rate On the 4th and 7th days after low-temperature treatment and the 9th day after normal temperature was restored for 2 days, the leaf length of the plants was measured by a steel ruler. The longest leaves were selected from each plant for determination, and 10 plants were measured from each sod block with a total of 30 samples. The average relative growth rate of leaf length

was calculated as: $R = (\ln L_m - \ln L_n) / (m - n)$, where L_m and L_n represent leaf length at day m and day n , respectively. \ln , natural logarithm.

Statistical analysis SPSS 16.0 software was used for statistical analysis. The independent sample t test was used to compare the significance of differences between the low-temperature and control groups. The differences between different treatments and different recovery times were analyzed by using multivariate ANOVA; the minimum significant difference method (LSD) was used for multiple comparisons, and the significance level was set as $\alpha = 0.05$. The interaction effects between light intensity and low temperature were judged by general linear model univariate variance analysis (GLM univariate). Using Microsoft Excel software to collate data and make graphs, the data in the graph were represented by the mean, and the vertical bar represents the standard deviation (SD).

3 Results

The inhibition of low-temperature adversity on the leaf growth of *K. pygmaea* – After mowing, the leaf growth of the control group was significantly greater than that of the plants treated with low temperature in each corresponding period ($p < 0.001$), of which leaf length had distinct differences on day 4, day 7 and day 9 ($p < 0.05$) (Fig. 2). There was no significant difference between day 4 and day

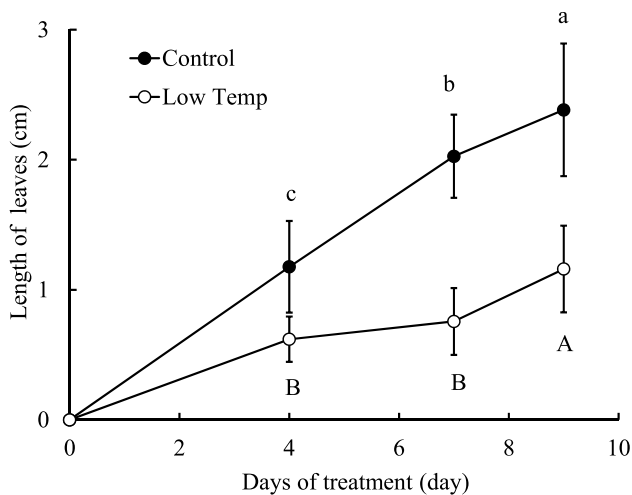


Fig. 2 Effects of low-temperature stress on leaf growth of *K. pygmaea* after leaves clipping. Different lower-case and capital letters in the figure indicate significant differences in leaf length among the 9 days of growing in the control and low-temperature groups, respectively ($\alpha = 0.05$). There were highly significant differences in leaf length between the control and low-temperature groups on the 4th, 7th and 9th days ($p < 0.001$), and significant differences are not marked in the figure

7 ($p > 0.05$); however, after 2 days of normal temperature growth resumption (day 9), the leaf length was significantly higher than that on the day 4 and day 7 ($p < 0.05$).

Figure 3 shows the changes in the average relative growth rate (R) of leaf length in different time periods after mowing. The results indicated that compared with the control group, low temperature caused the R value to be significantly reduced during days 0 to 3 and days 3 to 6 ($p < 0.001$). As it grew, the R of leaves decreased significantly in turn ($p < 0.05$). After moving the low-temperature group to the normal temperature culture room, its R increased significantly, showing no significant difference from the R value on days 0 to 3 ($p > 0.05$).

Response of the PSII photochemical efficiency to low-temperature adversity – The relative linear electron transport rate (rETR) of the PSII reaction center increased with increasing actinic light intensity (Fig. 4). The rETR increased rapidly when the light intensity was less than $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ but only slightly increased when the light intensity was more than $800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. There were obvious differences in the light response curve of rETR between groups or treatments; in the middle to high light intensity range, Control 8 Days $>$ Control 6 Days $>$ Low Temp and Recovery 6 + 2 Days $>$ Low Temp 6 Days.

In view of the short balance time (1 and 2 min) at each light intensity in measuring the light response curve, the PSII photochemical efficiency of *K. pygmaea* leaves at steady-state light intensities of 400 and $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$

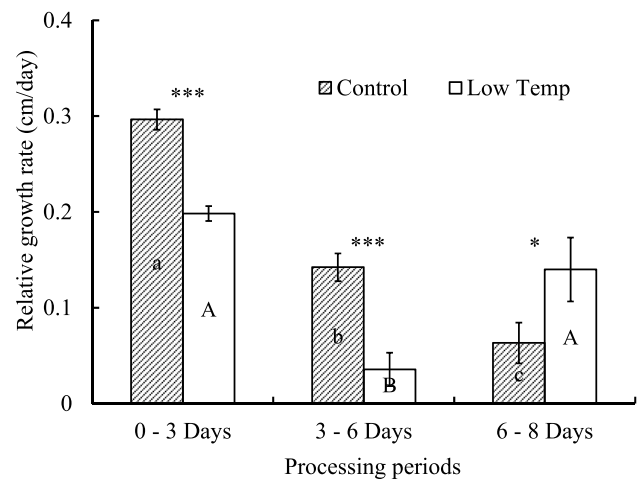


Fig. 3 Variations in the relative growth rate of leaves of *K. pygmaea* after mowing and its response to low temperature. Different lower-case and capital letters in the figure indicate significant differences in the relative growth rate of leaves among different growth times in the control and low-temperature groups, respectively ($\alpha = 0.05$). “***” and “*”, indicate highly significant differences ($p < 0.001$) and significant differences ($p < 0.05$) between the control and low-temperature groups, respectively

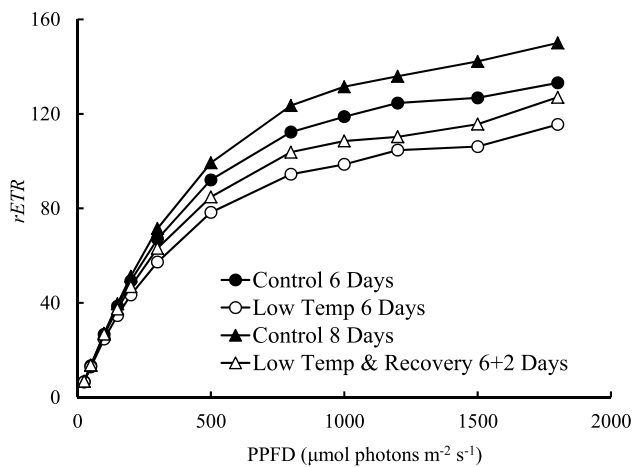


Fig. 4 Comparison of light response curves of PSII relative electron transfer efficiency (rETR) in leaves of *K. pygmaea* under control and low-temperature conditions and after two days of recuperation

was further determined. Figure 5 shows that under continuous irradiation with $400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ low light intensity for 10 min, the PSII operating efficiency (F_q'/F_m'), the maximum efficiency (F_v'/F_m') and the efficiency factor (F_q'/F_v') were all significantly higher than those under high light intensity ($p < 0.01$ and $p < 0.001$). Except for F_q'/F_v' , the values of F_q'/F_m' and F_v'/F_m' under different treatments showed a similar variation trend; that is, the Control 8 Days group was higher, while the Low Temp 6 Days group was lower; meanwhile, the variation trend from the two steady-state light intensities was relatively consistent.

PSII non-photochemical quenching and component analysis – The light response curve of PSII non-photochemical quenching (NPQ) also showed an increasing process of rapid to slow (Fig. 6). In the control group (Control 6 Days and Control 8 Days), the NPQ increased rapidly when the light intensity was less than $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, but only slightly increased when the light intensity was more than $800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. The low-temperature group increased rapidly before $200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and then increased slowly, while it was the demarcation point of the light response curve for recuperated plants (Low Temp and Recovery 6+2 Days) near approximately $300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Within the range of medium to high light intensity, the difference of NPQ between different treatments was more obvious, that was: Control 8 Days > Control 6 Days > Low Temp and Recovery 6+2 Days > Low Temp 6 Days.

The determination of NPQ under steady-state light intensity showed that there was an extremely significant difference between low and high light intensities ($p < 0.001$). At $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ high light intensity, the relative changes of each treated group were consistent with the order of the light response curves and showed significant

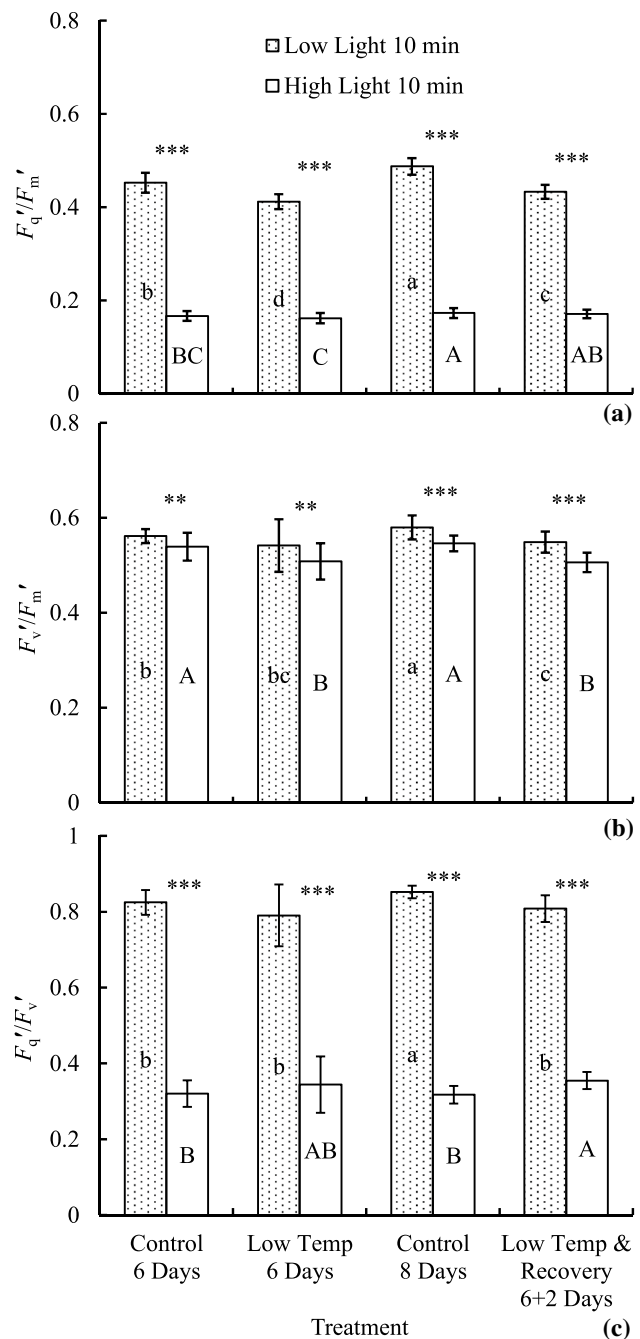


Fig. 5 Variations in PSII operating efficiency (F_q'/F_m') (a), PSII maximum efficiency (F_v'/F_m') (b), and PSII efficiency factor (F_q'/F_v') (c) at different actinic light intensities and their response to low temperature and recovery. Different lower-case and capital letters in the figures indicate significant differences in F_q'/F_m' , F_v'/F_m' and F_q'/F_v' among different treatments after irradiation at low and high light for 10 min, respectively ($\alpha = 0.05$). “***” and “***”, indicate highly significant differences between low and high light irradiation ($p < 0.01$ and $p < 0.001$)

differences ($p < 0.05$) except for Control 6 Days and Control 8 Days. At two actinic lights, the recuperated plants (Low

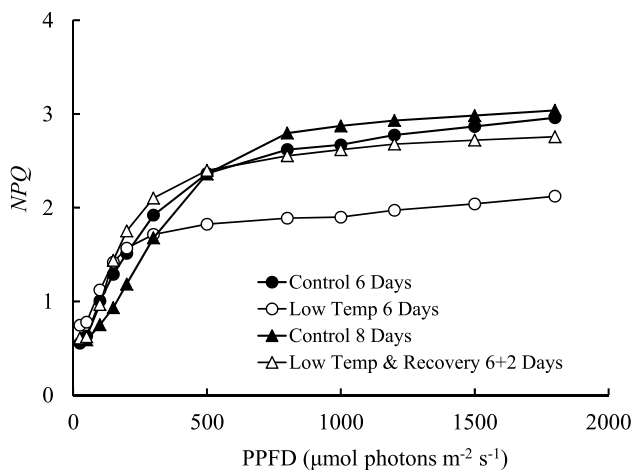


Fig. 6 Comparison of light response curves of PSII non-photochemical quenching (NPQ) in leaves of *K. pygmaea* under control and low-temperature conditions and after two days of recuperation

Temp and Recovery 6+2 Days) were significantly higher than those of the low-temperature group ($p < 0.05$) (Fig. 7a).

After continuous low and high steady-state irradiation for a total of 30 min and then accurate dark relaxation restoration for 15 min, the fast component (NPQ_f) and the slow component (NPQ_s) of PSII non-photochemical quenching were further analyzed. The results showed that NPQ_f decreased slightly and NPQ_s increased slightly with increasing dark relaxation time; however, there was no significant difference among the three time points of dark relaxation for low-temperature-treated and recovered plants (Fig. 7b, c). On average, NPQ_f and NPQ_s accounted for 36% and 64% of PSII non-photochemical quenching, respectively; among them, NPQ_f and NPQ_s accounted for 30% and 70% of NPQ in the low-temperature group, 39% and 61% for recuperated plants; 36% and 64% for control group on 6th day and 38% and 62% on 8th day.

Influence of low-temperature adversity on photoinhibition of photosynthesis – Figure 8a shows that the maximum quantum efficiency of PSII photochemistry F_v/F_m was higher in the control group (Control 6 Days and Control 8 Days) and significantly lower in the low-temperature group; however, after the plants were restored in the normal temperature culture room for 2 days, the F_v/F_m value (Low Temp and Recovery 6+2 Days) was significantly higher than that of the low-temperature group and significantly lower in the control group ($p < 0.05$). The relative inhibition of photosynthesis (P_{inh}) was higher in the low-temperature group; with removal of low-temperature adversity, the P_{inh} decreased and was significantly lower than that of the control at 6 days and in the low-temperature group ($p < 0.05$) (Fig. 8b).

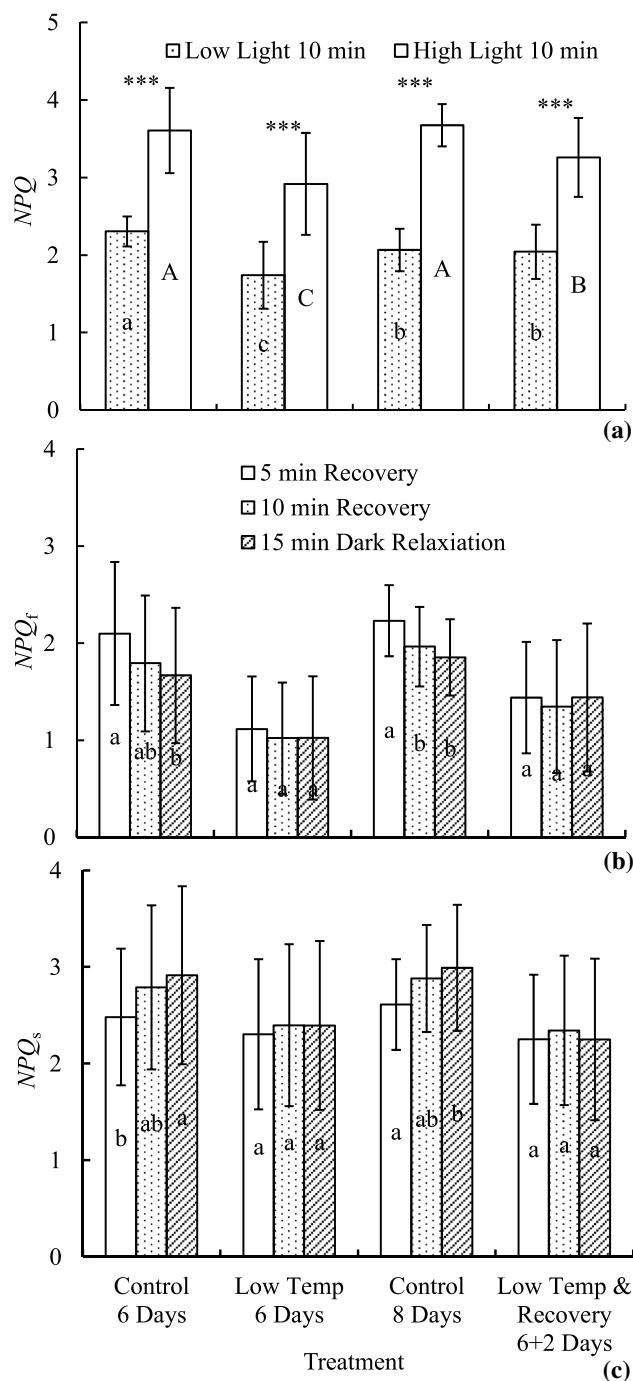


Fig. 7 Effects of low temperature on PSII non-photochemical quenching (NPQ) (a) and its fast and slow components (NPQ_f and NPQ_s) (b, c) in leaves of *K. pygmaea*. Different lower-case and capital letters in **a** indicate significant differences in NPQ among different treatments after irradiation at low and high light for 10 min, respectively ($\alpha = 0.05$), and “***” indicates a highly significant difference between low and high light irradiation ($p < 0.001$). Different lower-case letters in **b, c** indicate significant differences in NPQ_f and NPQ_s within different treatments during the period of dark relaxation times among 5, 10 and 15 min ($\alpha = 0.05$)

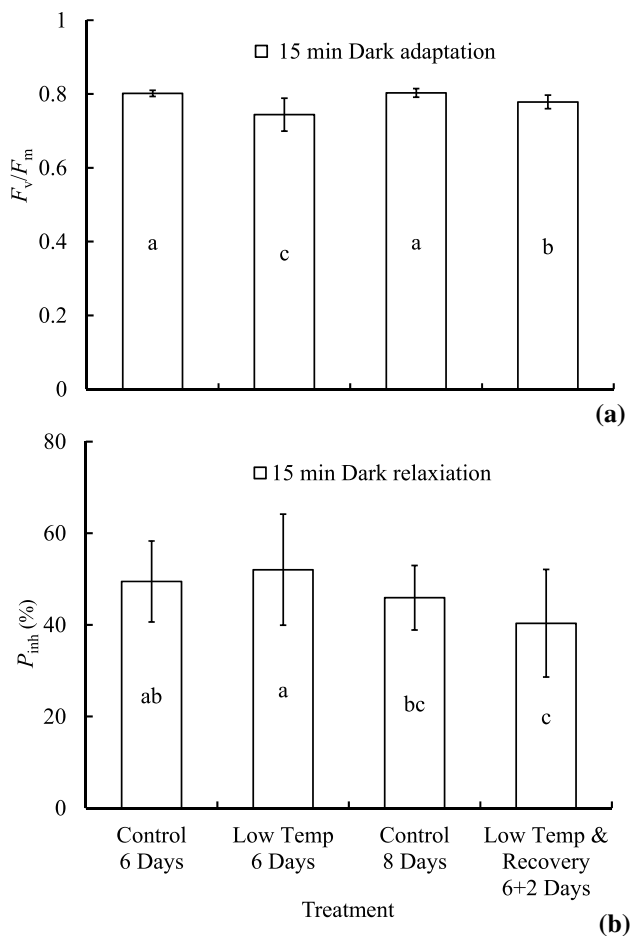


Fig. 8 Effects of low temperature on PSII maximum quantum efficiency of PSII photochemistry (F_v/F_m) (a) and relative photoinhibition of photosynthesis (P_{inh}) (b) in leaves of *K. pygmaea*. Different lower-case letters in the figures indicate significant differences in F_v/F_m and P_{inh} among the control, low temperature and two days of recuperation ($\alpha=0.05$)

4 Discussion

***K. pygmaea* is a kind of hardy plant in alpine meadows on the Qinghai-Tibet Plateau** – When environmental temperature is below the lower limit of the optimal growth temperature of plants, it can cause an adversity of low temperature, which varies with plant species and growth period (Larcher 1980; Kuss 1986). Low-temperature stress can result in a series of changes from molecules to cells, tissues to phenotypes in plants (Moellering et al. 2010; Liu et al. 2019), which are often expressed as acute or chronic abiotic environmental stresses. Physiological responses mainly include changes in cell membrane structure (Lu et al. 2019), enzyme function (Pearce 2001; Theocharis et al. 2012), soluble sugars and proteins, osmotic regulatory substances such as proline (Meena et al. 2019), stomatal conductance and photosynthesis (Foyer et al. 2002; Shi et al. 2016). It is generally

recognized that the adversity of low temperature is one of the important environmental limiting factors affecting plant growth, development and geographical distribution; the effects on leaves are usually expressed as a decrease in growth rate and photosynthetic rate.

The alpine species of *K. pygmaea* is a typical cold mesophyte with a dense rhizome tufted. As an herbaceous perennial, the aboveground is lower, and its height is only 1 to 3 cm; the leaf shape is in linear, approximately 1 mm wide and 3 cm long. Extremely developed roots often form a dense interlayer with soil, and its thickness can be more than 10 cm (Li et al. 2010). Owing to the cold climate in the Qinghai-Tibet Plateau region, *K. pygmaea* starts to turn green in mid-early May and becomes yellow wilt in early October. The growth and expansion of leaves are slow, especially from May to early July, and the productivity of alpine meadows is maintained at a very low level. The dwarf shape of plants and linear leaves can effectively reduce the wind area; in addition, they also have the advantages of heat preservation, water retention and transpiration reduction, avoiding damage from physiological drought and day and night temperature differences (Jiang and Jia 1999). At the same time, the leaf stomata distributed on the abaxial surface with higher density and more sunken also reflected the adaptability to the low pressure and cold climate (Zhang et al. 2006; Zhao et al. 2007). The morphology and life characteristics of *K. pygmaea* exhibit adaptation to extreme adversity, and it is also the product shaped by alpine harsh habitats (Yamori et al. 2010). During the field experiments, we always found that the blade tip of *K. pygmaea* gradually emerges yellow and there are signs of drying up and dying; it has been considered mainly induced by extremely cold adversity such as frost at night (Li et al. 2003; Sun et al. 2007). Low temperature seems to constitute the main adversity restricting plant growth and development in the Qinghai-Tibet Plateau region, and frost frequently appears in the plant growing season and is the key factor accelerating leaf apoptosis.

The alpine *K. pygmaea* meadow is a unique vegetation type in Northwest China, and it has the widest distribution and largest area on the Qinghai-Tibet Plateau; its distribution is co-limited by low rainfall, a short growing season and livestock grazing (Li et al. 2010; Miede et al. 2019). Early studies have revealed that in the alpine region where the average annual temperature is -0.1 to -1.6 °C and the accumulated temperature greater than and/or equal to 5 °C is only 543.1–886.9 °C, *K. pygmaea* can still complete its growth and development well, demonstrating its strong ability of cold tolerance (Sun et al. 2007). We simulated 8°C/4°C (day/night) of the low-temperature treatment lasting for 5 days, compared with normal control growing condition of 23 °C/18 °C, and determined the influence of low-temperature adversity on the growth of leaves length. Similar to a typical finite growth process of plant organs,

plants, or even populations (Hunt 1982), the leaf length of *K. pygmaea* increased, and its average relative growth rate decreased gradually after mowing. The results indicated that the adversity of low temperature restrained leaf length growth and reduced the relative growth rate. However, this suppression of growth could partially or even fully recover after relief from low-temperature stress, meaning that the PSII photosynthetic function of *K. pygmaea* still maintained activity and that the potential growth trend was preserved. Therefore, the photosynthetic and physiological processes of this typical native species had strong adaptability and/or tolerance to low-temperature adversity.

The adversity of low temperature restricts the activity performance of the photosynthetic apparatus of *K. pygmaea* leaves

The PSII photochemical quantum efficiency of the photosynthetic apparatus is influenced by many biotic and abiotic factors and is very sensitive to variations in external environmental factors (Baker 2008). F_v'/F_m' is the PSII maximum efficiency, which represents the maximum quantum efficiency of the PSII opening trap in light-adapting leaves. A decrease in F_v'/F_m' means the deficiency of the energy capture traps. The PSII operating efficiency F_q'/F_m' is the actual primary light energy capture efficiency when the PSII reaction center is partially closed under actinic light, which is directly related to the linear electron transfer flux through the PSII reaction center; furthermore, F_q'/F_m' , also known as the PSII actual photochemical quantum efficiency (Φ_{PSII}), can reflect the actual operating efficiency of the PSII absorption light energy used in the reduction of the primary quinone electron acceptor Q_A . F_q'/F_m' is a fast reflection of the efficiency of PSII reaction centers under varied light intensities or other environmental conditions, and its reduction will not only limit the efficiency of light energy conversion in plants but also affect the demand for the accumulation of homogenization forces (ATP and NADPH) in the carbon assimilation process of the dark reaction (Xu 2002). In addition to driving the photochemical reactions of PSII reaction centers, excessive energy absorbed by the photosynthetic apparatus needs to be dissipated in safe ways. Non-radiative energy dissipation in the PSII reaction center is the main way to eliminate excessive excitation energy; it can effectively dissipate excited energy accumulation in the PSII core and reduce the probability of photoinhibition or even photodamage (Bilger and Björkman 1990; Govindjee 2002; Niyogi and Truong 2013). The non-photochemical quenching, NPQ, is thought to protect the photosynthetic apparatus under excessive light conditions via controlled dissipation of absorbed light energy as heat (Ruban 2016). Studies have shown that NPQ is susceptible to a variety of environmental factors; under the condition of adversity, the closure of leaf stomata can block the electron flow from CO_2 to O_2 , resulting in a decrease in the energy conversion

efficiency of the PSII reaction center and the accumulation of ATP and NADPH in the carbon assimilation process of the dark reaction (Sáez et al. 2013), which indicates that the change in energy status in chloroplasts is the key factor in determining the non-photochemical quenching process. In our studies, F_v'/F_m' , F_q'/F_m' and NPQ all decreased under low-temperature adversity; obviously, low temperature inhibited the PSII photochemical efficiency and the non-radiative heat dissipation capacity. It was clear that the light intensity played an important role in determining the photochemical efficiency of the PSII reaction center; however, the adversity of low temperature also led to a reduction in the PSII photochemical efficiency, and it could be improved to some extent after restoration to normal temperature growing conditions. As a result, the photosynthetic apparatus would accumulate a large amount of excessive light energy in the low-temperature state due to the reduction of F_v'/F_m' , F_q'/F_m' and NPQ, which easily induced photoinhibition and even photodamage in *K. pygmaea* when exposed to high light intensity. The relative electron transfer rate, rETR, was also sensitive to growing temperature. The light response curves showed that the photosynthetic electron transport capacity was limited by low temperature, while two days of recovery after removal of adversity could increase the rETR.

NPQ is linearly related to heat dissipation, and it is known as an important mechanism by which plants protect their photosynthetic apparatus in a rapidly varying light environment (Lima Neto et al. 2014). Recent studies suggest that most NPQ occurs in the light-harvesting antenna complex of PSII (LHCII), but the core antenna complexes CP43 and/or CP47 are also likely to be additional PsbS protein-dependent quenching sites in the PSII core (Nicol et al. 2019). The reduction in NPQ caused by cold stress is related to LHCII damage and the destruction of CP43 and/or CP47 proteins (Niyogi and Truong 2013; Tikkanen et al. 2013). When NPQ is increased under high light irradiation, most of the PSII reaction center on the thylakoid membrane of the matrix is in an inactive status; inactivated PSII can participate in the dissipation of excessive excitation energy together with light-harvesting antenna pigments, protecting the functional reaction center from injury (Murchie and Niyogi 2011). Usually, dark relaxation within a few minutes is considered to be a photoprotective process, which mainly depends on the presence of a low pH in the lumen of the thylakoid (ΔpH) and partly depends on the change in the light absorption cross-section and state transition of excited energy overflowing (Quick and Stitt 1989). For most cases, the relaxation over a time scale of a few minutes following the cessation of irradiation can be regarded as photoprotective processes (Maxwell and Johnson 2000); however, a long-term dark relaxation (lasting up to a few hours) is often seen as photoinhibition, which is associated with the presence of zeaxanthin and thought to occur in the LHCII complex (Lima

Neto et al. 2014). It has also been confirmed that photoinhibition itself is involved in both the protection and injury mechanisms of the PSII reaction center (Xu 2002). The NPQ was obviously affected by light intensity; however, the low-temperature growing conditions also influenced the value of NPQ. Six days of low-temperature adversity caused a relative decrease in NPQ, combined with a reduction in the PSII maximum photochemical quantum efficiency F_v/F_m and an enhancement in the relative photoinhibition of photosynthesis P_{inh} , which indicated an inhibition of the activity performance of the photosynthetic apparatus in the leaves of *K. pygmaea*. The values of NPQ and F_v/F_m showed some recovery or even more after 2 days of resuming in a normal temperature culture room, and P_{inh} also showed lower values than those after treatment in low-temperature adversity for 6 days. The injury to LHCII and destruction to CP43 and/or CP47 proteins caused by low-temperature adversity still needed a slow recovery process to reach the control level.

Furthermore, we divided the NPQ into two components, fast and slow relaxing quenching (NPQ_f and NPQ_s), by referring to Maxwell and Johnson (2000). The results were slightly different from those of alpine plants *Anisodus tanguticus* and *Rheum tanguticum* (Shi et al. 2007). Here, the NPQ_s occupied a main component, accounting for nearly 64% of NPQ on average, which probably resulted from the relatively lower growth light intensity in the culture room and artificial climate chamber. It has been proven that shaded plants of the same species exhibit reduced light use, and an increased likelihood of photoinhibition (Demmig-Adams et al. 1995). Our results demonstrated that the photosynthetic apparatus of *K. pygmaea* did not dissipate the accumulated excessive excitation energy quickly and effectively, and there was an increased chance of photoinhibition. During 15 min of dark relaxation, the NPQ_f of the control plants continued to decrease, while NPQ_s increased, and the variation tendency was relatively steady; it was slightly different from early soil drought trials conducted under the natural field sunlight conditions (Shi et al. 2015), and its recovery of photosynthetic function from the photoinhibition state was slower due to the unperfected development of the photosynthetic apparatus (Demmig-Adams et al. 1995). It should be kept in mind that the proportion of NPQ_s and NPQ_f only represented the value determined after 15 min of dark relaxation, providing a relative inter-comparison among the control group and the low-temperature group and its resumption treatment. It cannot be compared with the fully relaxed absolute state, which usually needs 30 min to one hour of dark relaxation (Maxwell and Johnson 2000). The NPQ and its two components, NPQ_f and NPQ_s, were relatively lower in the low-temperature group, but NPQ_f was slightly reduced; after the 2 days of resuming treatment, NPQ recovered slightly and mainly belonged to the increase in NPQ_f. The fast component NPQ_f in NPQ was

more sensitive to environmental variation (Shi et al. 2015); the decrease in photosynthetic function caused by low temperature was not permanent, but its recovery still required a longer time. Our results demonstrated that low-temperature adversity could aggravate the degree of photoinhibition or photodamage of photosynthesis, being a main environmental factor restricting plant growth and development; however, it did not constitute the completely irreversible damage to the photosynthesis apparatus of *K. pygmaea*, showing a strong tolerance to low-temperature stress.

Analysis of the interaction effect between low temperature and high light intensity

Two or more environmental factors often exhibit inter-effectiveness due to the synergistic effects of their ecological factors and/or the cross-tolerance or cross-adaptation of biological organisms to adversity during the period of acclimation (Shi et al. 2017). Does low-temperature stress exacerbate the effect of strong light intensity on PSII photochemical and non-photochemical quenching abilities in plants? Based on the univariate variance analysis, we found that although both low temperature and strong light intensity could cause a significant reduction in the PSII maximum efficiency F_v'/F_m' , there was no inter-effectiveness on F_v'/F_m' between light intensity and temperature; that is, the reduction in the maximum quantum efficiency of the PSII opening trap caused by low-temperature stress did not tend to be aggravated by the presence of strong light intensity. PSII operating efficiency F_q'/F_m' and efficiency factor F_q'/F_v' displayed inter-effectiveness, even though low temperature had no remarkable effect on F_q'/F_v' . The NPQ showed the interaction effect of two stress factors, but its contribution to the total variation (η^2) was small (Table 2). The inter-effectiveness was not remarkable when irradiated with light for only 5 and 10 min (the results were not listed), indicating that only long enough of high light illumination could aggravate the adverse impact caused by low-temperature adversity.

Table 2 shows that the contribution of high light intensity to total variation η^2 was much greater than that of low-temperature adversity, which further confirmed that light intensity was the main factor influencing the PSII photochemical efficiency and non-photochemical quenching ability in plant leaves. Except for the PSII efficiency factor F_q'/F_v' , the values of η^2 in other chlorophyll fluorescence parameters were as follows: high light intensity > low temperature > high light intensity × low temperature, which was completely consistent with early studies on the inter-effectiveness of strong light intensity and soil drought stress in *K. pygmaea* (Shi et al. 2017). Except for the PSII operating efficiency F_q'/F_m' , the η^2 was relatively small, which was also similar to early studies in soil drought trials. The results demonstrated that low-temperature adversity could aggravate the photosynthetic photoinhibition or that the photoinhibition caused by

Table 2 Analysis of the inter-effectiveness of light intensity and low temperature on chlorophyll fluorescence parameters

	Temperature			PPFD			Temperature × PPFD		
	<i>F</i>	Sig	η^2	<i>F</i>	Sig	η^2	<i>F</i>	Sig	η^2
NPQ	30.4	0	0.173	274.317	0	0.645	4.942	0.028	0.033
F_q'/F_m'	134.749	0	0.482	10,786.027	0	0.987	113.448	0	0.439
F_v'/F_m'	24.722	0	0.146	44.098	0	0.233	1.4	0.239	0.01
F_q'/F_v'	0.27	0.604	0.002	2661.95	0	0.948	8.102	0.005	0.053

F, Sig, and η^2 indicate the ratio of the average square, significance and contribution to the total variance, respectively

strong light intensity was more obvious in a low-temperature environment. Although the relative contributions of the interaction effect to total variation were all lower, the adverse impacts on physiological processes caused by low temperature and strong light intensity could be exacerbated. Combined with early studies on soil drought (Shi et al. 2015, 2017), we should think if other adverse factors, such as low air pressure, strong wind, hail attack coupling, or disastrous and extreme weather occurring frequently due to the global changes, can the activity performance of the photosynthetic apparatus of *K. pygmaea* still withstand harsh environmental adversity? remaining a matter of great concern.

In conclusion, the alpine plant *K. pygmaea* is a typical cold mesophyte, but low-temperature stress could still reduce the photosynthetic performance of leaves. Low temperature led to a decrease in PSII photosynthetic efficiency and caused an increase in the degree of photosynthetic photoinhibition or even photodamage, playing a main environmental factor restricting plant growth and development. The low temperature at 8 °C day/4 °C night did not constitute a complete irreversible damage to the photosynthetic apparatus, and the photosynthetic function could be partially recuperated after two days of resumption under normal temperature, reflecting the strong tolerance to low-temperature adversity. There was an interaction effect between low temperature and strong light intensity, and the adverse impact of low-temperature adversity could be aggravated by strong light intensity. Therefore, the frequent adversity of low temperature in the plant growing season on the Qinghai-Tibet Plateau is an important factor that affects the effective operation of the photosynthetic function of *K. pygmaea* and limits the productivity of alpine meadows.

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Author contributions SBS supervised the experiments and wrote, reviewed and edited the manuscript. RS and DWZ performed the statistical analysis of the data and prepared the draft of the manuscript. TCL and KJD performed the research design and participated in paper revision. XZG, JLM and FLW performed field data collection and laboratory experimental items. All authors contributed to data analysis, writing of the paper and discussion.

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Data Availability The data presented in this study are available in the article.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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