

## Article

# Photosynthetic and Antioxidant Responses of *Gymnocarpos przewalskii* to Simulated Rainfall Changes

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**Abstract:** *Gymnocarpos przewalskii* is a rare Tertiary relict species, mainly distributed in desert areas of northwestern China. Changes in rainfall have a significant impact on the physiological characteristics of desert plants. In the present study, the effects of five simulated rainfall levels on the gas exchange parameters, chlorophyll fluorescence characteristics, and antioxidant system of *G. przewalskii* were studied. The results show that with increased rainfall the net photosynthetic rate ( $P_n$ ) and transpiration rate increase significantly. The reduction in  $P_n$  is caused by stomatal and non-stomatal limitations under decreased rainfall. Decreased rainfall markedly improves the instantaneous water-use efficiency of leaves. With increased rainfall, the maximum photosynthetic rate, apparent quantum efficiency, and light utilization range significantly increase. Under reduced rainfall, the chlorophyll content, maximum photochemical efficiency of PSII, and steady-state optical quantum efficiency decrease and photoinhibition is caused in the PSII system. A rainfall reduction of 30% leads to massive production of superoxide anions and hydrogen peroxide, causing obvious peroxidation damage. Meanwhile, superoxide dismutase, peroxidase, and catalase in the leaves are significantly enhanced to remove excess reactive oxygen species and alleviate the injury to photosynthetic apparatus. Our study reveals the effect of rainfall changes on the photosynthetic characteristics and antioxidant system of *G. przewalskii*, and can improve understanding of the adaptive strategies of desert plants under future precipitation changes in northwestern China.

**Keywords:** simulated rainfall; *Gymnocarpos przewalskii*; photosynthesis; chlorophyll fluorescence; reactive oxygen species; antioxidant enzymes



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## 1. Introduction

Climate change has a significant effect on the water cycle process at regional and global scales, causing increases or decreases in precipitation [1]. Northwestern China is one of the most arid areas of East Asia. Because of its complex terrain, precipitation shows a distinctly different changing trend. The western region, i.e., Xinjiang, Qilian mountainous areas, and parts of Qinghai, experienced a change from dry to wet during the second half of the 20th century [2–4]. In the eastern part of northwest China, including east Qinghai, Ningxia, and Shaanxi, annual precipitation has shown a downward trend [3]. Ecosystems located in areas with low annual precipitation are predicted to be the most susceptible to precipitation changes [5]. Compared to other ecosystems, desert ecosystems are more sensitive to precipitation changes [6], and plants inhabiting desert ecosystems have evolved specific physiological mechanisms to adapt to rainfall changes [7]. In recent years, the effects of precipitation patterns on plant physiological characteristics have become an important research topic [8–10]. The ability of leaves to obtain carbon by photosynthesis and regulate water loss by transpiration is very sensitive to precipitation [11]. In the southwestern United States, both an increase in precipitation by 30% and a decrease in precipitation by 45% had significant effects on the gas conductance ( $G_s$ ) and net photosynthetic rate ( $P_n$ ) of *Pinus edulis* and *Juniperus monosperma*; the  $P_n$  and  $G_s$  of *P. edulis* were more responsive to increased precipitation, whereas the  $P_n$  and  $G_s$  of *J. monosperma* were

more sensitive to sheltering precipitation [12]. By fitting photosynthesis–light response curves, it is possible to obtain important photosynthetic parameters [13] such as apparent quantum efficiency ( $A_{QY}$ ), dark respiratory rate ( $R_d$ ), light saturation point ( $L_{sp}$ ), light compensation point ( $L_{cp}$ ), and maximum net photosynthetic rate ( $P_{nmax}$ ). These parameters can be used to determine whether the photosynthesis apparatus operates normally and to determine a plant's photosynthetic adaptability [14,15]. Compared to gas exchange parameters, chlorophyll fluorescence parameters can better reflect the internal mechanism of photosynthesis [16,17]; therefore, the comprehensive analysis of gas exchange, light–response curves, and chlorophyll fluorescence parameters are a better choice for verifying the photosynthetic characteristics of plants under changing precipitation conditions.

Water stress often causes partial stomatal closure to conserve water [10] while simultaneously limiting  $CO_2$  entry, reducing carboxylation efficiency of RuBisCO, and changing how the electrons in the electron transport chain are accepted by  $O_2$ , accordingly enhancing oxygenation and photorespiration and increasing ROS production [18]. In addition, excess light energy in the PSII system causes changes in the photochemistry of leaves subjected to drought stress, leading to excess accumulation of ROS [19]. Enhancement of antioxidant enzyme activity plays a vital role in scavenging excessive ROS, inhibiting membrane lipid peroxidation, and maintaining normal operation of the photosynthetic system [20]. It has been shown that peroxidase (POD) and catalase (CAT) activities in the leaves of *Caragana microphylla* and *Salsola collina* improve diurnal  $P_n$  and photosynthesis recovery [21].

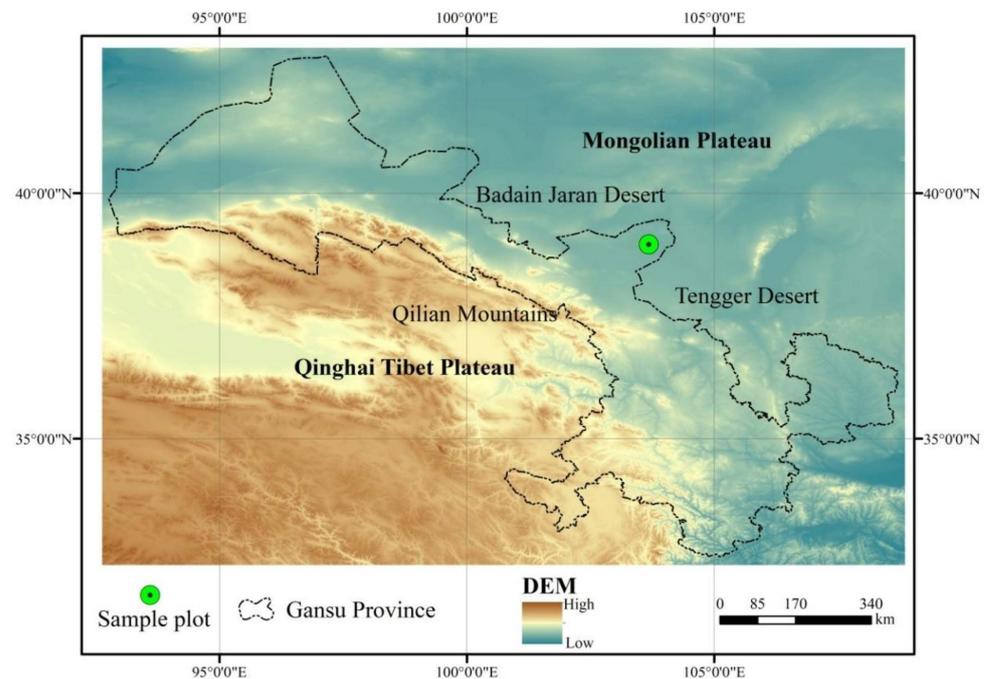
*Gymnocarpos przewalskii* originates from the Mediterranean region, and is a rare plant in the desert areas of Central Asia. Its distribution is mainly restricted to the desert areas of northwestern China, including the Xinjiang Uygur Autonomous Region, Inner Mongolia Autonomous Region, Gansu Province, and Qinghai Province, which are characterized by low precipitation and high evaporation [22]. It has high tolerance to drought, saline-alkaline conditions, wind erosion, and sand burial, and plays an important ecological role in combating desertification and maintaining ecological balance in desert areas [23,24]. Its survival and evolution can provide good evidence for researching the evolution of plants in the arid regions of northwestern China. However, over the past few decades, increasing human activity in its natural habitat has resulted in a serious loss of individuals and habitat fragmentation [25]. At present, research related to the water adaptability of *G. przewalskii* mainly involves population structure [23], potential geographical distribution [26], phylogeography [22], osmoregulation, antioxidant protection, and biomass accumulation [24,27]. Research has shown that precipitation has the greatest effect on the distribution of *G. przewalskii*, with mean annual precipitation being the most important factor [26]. However, the response of its physiological characteristics to changing precipitation in arid desert area has not been elucidated. The main objective of this study is to explore the effect of increased and decreased rainfall on the photosynthetic characteristics and antioxidant system of *G. przewalskii* and to provide a scientific basis for evaluating the adaptability of desert plants to future precipitation patterns in northwestern China. The hypotheses of our study are as follows: (1) photosynthetic capacity is inhibited by decreased rainfall and improved by increased rainfall; (2) decreased rainfall enhances water-use efficiency; (3) photoinhibition in the PSII system occurs under rainfall reduction; and (4) regulation of antioxidant enzyme activity plays an important role in stabilizing the photosynthetic apparatus.

## 2. Materials and Methods

### 2.1. Study Area

The experimental site was located in Minqin County (38°57'25" N, 103°40'48" E) in the lower reaches of the Shiyang River, Gansu Province, northwest China, which is surrounded by the Badain Jaran Desert in the west and north and the Tengger Desert in the east (Figure 1). The average altitude of the area is 1320 m. The area has a typical arid continental climate with an average temperature of 7.8 °C. The mean annual precipitation is 113.2 mm, and the potential annual evaporation is 2624 mm. The annual sunshine hours are 3028 h, with a frost-free period lasting approximately 163 d. The soil type is sandy soil,

average soil bulk density is  $1.3 \text{ g cm}^{-3}$ , average field capacity is 25.0%, and average soil organic matter content is  $0.80 \text{ g kg}^{-1}$ .



**Figure 1.** Location of the study area.

## 2.2. Plant Material and Test Design

The test materials consisted of two-year-old *G. przewalskii* seedlings. The test area was  $15 \text{ m}^2$ , including 90 seedlings, and the plants were fully irrigated at the beginning of April. According to rainfall records over the last 60 years in Minqin County, the mean annual rainfall was 116.4 mm and rainfall from May to August accounts for approximately 84% of the total. The maximum and minimum rainfall over the same years were 154.2 mm and 81.5 mm, respectively, and the change amplitude was approximately 30%. In this study, five rainfall gradients were simulated as follows: mean monthly rainfall from May to August over the past 60 years ( $R_0$ ),  $R_0$  increased by 30% ( $R_{+30}$ ),  $R_0$  increased by 15% ( $R_{+15}$ ),  $R_0$  decreased by 30% ( $R_{-30}$ ), and  $R_0$  decreased by 15% ( $R_{-15}$ ). Each treatment included three test plots with an area of  $1 \text{ m} \times 1 \text{ m}$ , six seedlings per plot were included, and a 40 cm deep trench was dug between the two adjacent plots to prevent lateral water infiltration. The awning was opened on cloudy and rainy days to prevent the impact of natural rainfall. Irrigation was conducted every 5 d. Irrigation volume was determined by multiplying monthly rainfall by the plot area. The average monthly rainfall and irrigation volume for each treatment are presented in Table 1.

**Table 1.** Average monthly rainfall from May to August during the period of 1961 to 2018 and irrigation volume in the present study.

Month	Rainfall (mm)	Irrigation Volume (mL)				
		$R_{-30}$	$R_{-15}$	$R_0$	$R_{+15}$	$R_{+30}$
May	11.7	1366.4	1659.2	1952.0	2244.7	2537.5
June	14.5	1686.4	2047.7	2409.1	2770.4	3131.8
July	23.8	2776.1	3370.9	3965.8	4560.7	5155.5
August	28.7	3347.5	4064.8	4782.1	5499.4	6216.7

$R_0$  = monthly rainfall from May to August during the period from 1961 to 2018;  $R_{+30}$ ,  $R_{+15}$  =  $R_0$  increased by 30% and 15%, respectively;  $R_{-30}$ ,  $R_{-15}$  =  $R_0$  decreased by 30% and 15%, respectively.

### 2.3. Measurement of Gas Exchange Parameters

A portable photosynthesis system (LCpro-SD, ADC BioScientific Ltd., Hoddesdon, UK) was used to measure light response and gas exchange parameters. Eleven photosynthetic active radiation (PAR) gradients (2000, 1800, 1500, 1200, 1000, 800, 500, 300, 100, 50, and 0  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) were set, and were supplied by red and blue light sources. During the measurements, the air temperature was  $28 \pm 1.5$  °C, the concentration of  $\text{CO}_2$  in the leaf chamber was  $380 \pm 5$   $\mu\text{mol mol}^{-1}$ , and the airflow velocity in the leaf chamber was  $280 \pm 5$   $\mu\text{mol mol}^{-1}$ . Each PAR setting was measured for 120 s and repeated three times. The portable photosynthesis system automatically recorded the PAR,  $P_n$ , transpiration rate ( $T_r$ ),  $G_s$ , intercellular  $\text{CO}_2$  concentration ( $C_i$ ), and air  $\text{CO}_2$  concentration ( $C_a$ ). Instantaneous water-use efficiency (WUE) and stomatal limitation value ( $L_s$ ) were calculated according to the equations  $\text{WUE} = P_n/T_r$  [28] and  $L_s = 1 - C_i/C_a$  [29,30], respectively. Light-response parameters, including  $A_{QY}$ ,  $R_d$ ,  $P_{n\text{max}}$ ,  $L_{sp}$ , and  $L_{cp}$ , were estimated according to the mechanism model proposed by Ye et al. [13].

### 2.4. Measurement of Chlorophyll Fluorescence Parameters and Chlorophyll Content

Chlorophyll fluorescence parameters were measured using the FluorCam fluorescence imaging system (Beijing Yiketai EcoTech Co., Ltd., Beijing, China), which recorded initial fluorescence ( $F_o$ ), maximum fluorescence ( $F_m$ ), steady-state photochemical quenching coefficient ( $qL\_Lss$ ), steady-state non-photochemical quenching coefficient (NPQ\_Lss), steady-state optical quantum efficiency (QY\_Lss), and steady-state fluorescence decay rate (Rfd\_Lss). The maximal photochemical quantum efficiency ( $F_v/F_m$ ) and potential activity of PSII ( $F_v/F_o$ ) were calculated according to the following equations:  $F_v/F_m = (F_m - F_o)/F_m$  and  $F_v/F_o = (F_m - F_o)/F_o$ . The chlorophyll fluorescence parameters were calculated according to the methods reported by Baker [31] and Roháček [32]. All measurements were taken on the leaves at the same position of the test plants, and the plants were dark-adapted for 20 min using clips before measurements. Chlorophyll content was determined according to the method described by Aron [33].

### 2.5. Measurement of $\text{O}_2^-$ , $\text{H}_2\text{O}_2$ MDA Content, and Antioxidant Enzyme Activities

The content of  $\text{O}_2^-$  and  $\text{H}_2\text{O}_2$  was determined according to Jiao et al. [34], and MDA content was determined according to Farooq et al. [35]. For the enzyme assays, the crude extract was prepared as follows: frozen leaves (0.3 g) were crushed into a fine powder under liquid nitrogen using a mortar and pestle, then the leaf powder was homogenized in a 50 mM potassium phosphate ( $\text{K}_2\text{HPO}_4$ ) buffer (pH 7.0) containing 1 mM EDTA- $\text{Na}_2$  and 1% polyvinylpyrrolidone (PVP). The homogenate was centrifuged at  $12,000 \times g$  for 20 min at 4 °C and the supernatant was used for subsequent enzyme assays. SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) according to the method reported by Hwang et al. [36]. One unit of SOD activity was defined as the amount of enzyme that caused 50% inhibition of NBT reduction at 560 nm [37]. CAT activity was determined as the decline in absorbance for 120 s at 240 nm [38]. POD activity was determined according to the method described by Ekmekca and Terzioglu [39], and the change in absorbance was recorded at 470 nm for 120 s.

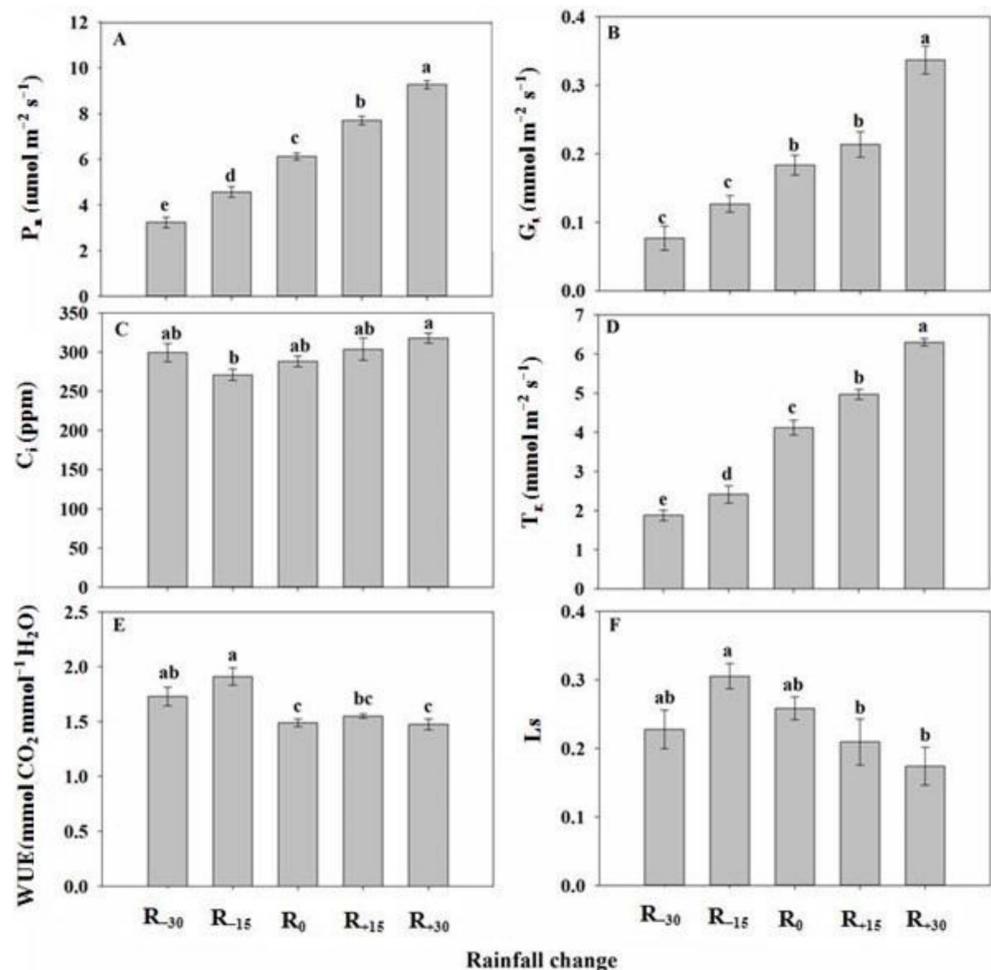
### 2.6. Statistical Analysis

SPSS 24.0 software (Microsoft Inc., Redmond, WA, USA) was used for one-way analysis of variance (ANOVA), and significant differences between the treatments were determined by Duncan's test at a significance level of 0.05. Pearson correlation analysis was used to analyze the correlation between photosynthetic parameters. All graphs were drawn using SigmaPlot 12.5 (Systat Software Inc., Chicago, IL, USA).

### 3. Results

#### 3.1. Effect of Rainfall Change on Photosynthetic Parameters

$P_n$  and  $T_r$  increased with the increase in rainfall, and the differences among the treatments were significant ( $p < 0.05$ ; Figure 2A,D). Under rainfall reduction ( $R_{-15}$  and  $R_{-30}$ ), the decrease in  $T_r$  was more obvious than that in  $P_n$ , resulting in an increase in WUE by 16.0% and 28.3%, respectively. When the rainfall was increased, the change in WUE was not significant compared to that under  $R_0$  ( $p > 0.05$ ; Figure 2E). The change in  $G_s$  (Figure 2B) was consistent with the changes in  $P_n$  and  $T_r$ ; when the rainfall was reduced ( $R_{-15}$  and  $R_{-30}$ ),  $G_s$  decreased significantly by 30.9% and 58.1%, respectively, whereas it increased significantly by 83.6% under  $R_{+30}$  ( $p < 0.05$ ). Compared with  $R_0$ ,  $C_i$  and  $L_s$  (Figure 2C,F) did not change significantly ( $p > 0.05$ ) when the rainfall increased or decreased.

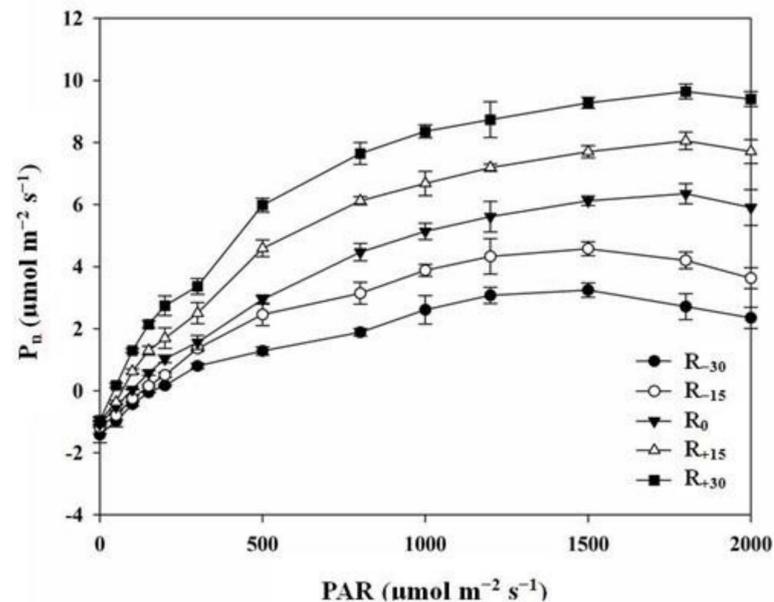


**Figure 2.** Changes in the photosynthetic characteristic parameters of *Gymnocarpos przewalskii* leaves under rainfall change. (A):  $P_n$ ; (B):  $G_s$ ; (C):  $C_i$ ; (D):  $T_r$ ; (E): WUE; (F):  $L_s$ ;  $P_n$ , net photosynthetic rate;  $G_s$ , stomatal conductance;  $T_r$ , transpiration rate;  $C_i$ , intercellular  $\text{CO}_2$  concentration; WUE, water-use efficiency;  $L_s$ , stomatal limitation;  $R_0$ , mean monthly rainfall from May to August during the period from 1961 to 2018;  $R_{-30}$ ,  $R_0$  decreased by 30%;  $R_{-15}$ ,  $R_0$  decreased by 15%;  $R_{+15}$ ,  $R_0$  increased by 15%;  $R_{+30}$ ,  $R_0$  increased by 30%. The vertical bars represent the means  $\pm$  SE ( $n = 3$ ), and different lowercase letters above the bars indicate significant differences among the treatments ( $p < 0.05$ ).

#### 3.2. Effect of Rainfall Change on Light Response Parameters

The light-response curves exhibited a consistent trend under the tested rainfall conditions (Figure 3). With increasing PAR,  $P_n$  increased at first, was saturated for a certain period, and finally decreased slightly. When PAR was above  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ , the dif-

ference in  $P_n$  among the treatments increased. The  $P_n$  was nearly saturated at the PAR of approximately  $1400 \mu\text{mol m}^{-2} \text{s}^{-1}$  under  $R_{-30}$  and  $R_{-15}$ , and decreased when PAR was higher than  $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The  $P_n$  of the other three treatments decreased when PAR was higher than  $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ .



**Figure 3.**  $P_n$ -PAR curves under rainfall change.  $P_n$ , net photosynthetic rate; PAR, photosynthetic active radiation;  $R_0$ , mean monthly rainfall from May to August during the period from 1961 to 2018;  $R_{-30}$ ,  $R_0$  decreased by 30%;  $R_{-15}$ ,  $R_0$  decreased by 15%;  $R_{+15}$ ,  $R_0$  increased by 15%;  $R_{+30}$ ,  $R_0$  increased by 30%. Values are shown as means  $\pm$  SE ( $n = 3$ ).

The  $A_{QY}$ ,  $P_{nmax}$ , and  $L_{sp}$  increased with the increase in rainfall, whereas  $R_d$  and  $L_{cp}$  showed the opposite trend. The  $A_{QY}$  and  $P_{nmax}$  under  $R_{-30}$  and  $R_{+30}$  were significantly different from those under  $R_0$  ( $p < 0.05$ , Table 2). Compared to  $R_0$ , the  $L_{sp}$  under  $R_{-15}$  and  $R_{-30}$  decreased significantly ( $p < 0.05$ ). The difference in  $R_d$  among the treatments was not significant ( $p > 0.05$ ).  $L_{cp}$  was sensitive to rainfall changes; compared with  $R_0$ , it decreased by 96.0% under  $R_{-30}$  and increased by 59.7% under  $R_{+30}$ .

**Table 2.** Light response parameters of *Gymnocarpus przewalskii* leaves under different rainfall treatments.

Treatment	$A_{QY}$	$R_d/$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$P_{nmax}/$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$L_{sp}/$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$L_{cp}/$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )
$R_{-30}$	$0.006 \pm 0.001^c$	$1.16 \pm 0.24^a$	$3.01 \pm 0.47^d$	$1408.96 \pm 45.11^b$	$195.88 \pm 9.86^a$
$R_{-15}$	$0.009 \pm 0.002^{bc}$	$1.12 \pm 0.11^a$	$4.39 \pm 0.38^{cd}$	$1423.50 \pm 27.93^b$	$134.28 \pm 2.31^b$
$R_0$	$0.010 \pm 0.003^{bc}$	$0.96 \pm 0.25^a$	$6.17 \pm 0.45^{bc}$	$1719.98 \pm 46.44^a$	$99.94 \pm 4.64^c$
$R_{+15}$	$0.016 \pm 0.004^{ab}$	$0.99 \pm 0.23^a$	$7.86 \pm 0.53^{ab}$	$1860.54 \pm 30.13^a$	$65.03 \pm 3.00^d$
$R_{+30}$	$0.022 \pm 0.003^a$	$0.84 \pm 0.36^a$	$9.50 \pm 0.46^a$	$1938.27 \pm 56.28^a$	$40.25 \pm 5.11^e$

$A_{QY}$ , apparent quantum efficiency;  $R_d$ , dark respiratory rate;  $P_{nmax}$ , maximum net photosynthetic rate;  $L_{sp}$ , light saturation point;  $L_{cp}$ , light compensation point;  $R_0$ , mean monthly rainfall from May to August during the period from 1961 to 2018;  $R_{-30}$ ,  $R_0$  decreased by 30%;  $R_{-15}$ ,  $R_0$  decreased by 15%;  $R_{+15}$ ,  $R_0$  increased by 15%;  $R_{+30}$ ,  $R_0$  increased by 30%. Values are shown as mean  $\pm$  SE ( $n = 3$ ). Different lower-case letters in the same column indicate significant differences among the treatments at  $p < 0.05$ .

### 3.3. Effect of Rainfall Change on Chlorophyll Content

With the increase in rainfall, the content of Chla, Chlb, total Chl, and Chl a/b showed an increasing trend (Table 3). Compared with  $R_0$ , the content of Chla and total Chl decreased significantly under  $R_{-15}$  and  $R_{-30}$ , and increased significantly under  $R_{+15}$  and  $R_{+30}$  ( $p < 0.05$ ). The Chlb content under decreased rainfall was significantly lower than that

under increased rainfall ( $p < 0.05$ ). Furthermore, under reduced rainfall ( $R_{-30}$  and  $R_{-15}$ ), the Chl a/b was decreased significantly, to 76.4% and 66.7% of that under  $R_0$ , whereas it rose remarkably under increased rainfall, to 1.2 times that under  $R_0$  ( $p < 0.05$ ).

**Table 3.** Changes in the chlorophyll content of *Gymnocarpos przewalskii* leaves under different rainfall treatments.

Treatment	Chla (mg g <sup>-1</sup> FW)	Chlb (mg g <sup>-1</sup> FW)	Chl (mg g <sup>-1</sup> FW)	Chla/b
$R_{-30}$	0.74 ± 0.12 <sup>e</sup>	1.55 ± 0.22 <sup>d</sup>	2.29 ± 0.03 <sup>e</sup>	0.48 ± 0.01 <sup>c</sup>
$R_{-15}$	0.91 ± 0.08 <sup>d</sup>	1.69 ± 0.15 <sup>c</sup>	2.60 ± 0.98 <sup>d</sup>	0.55 ± 0.92 <sup>c</sup>
$R_0$	1.25 ± 0.06 <sup>c</sup>	1.76 ± 0.07 <sup>bc</sup>	3.01 ± 0.37 <sup>c</sup>	0.71 ± 0.06 <sup>b</sup>
$R_{+15}$	1.55 ± 0.98 <sup>b</sup>	1.86 ± 0.87 <sup>b</sup>	3.41 ± 0.87 <sup>b</sup>	0.83 ± 0.07 <sup>a</sup>
$R_{+30}$	1.67 ± 0.04 <sup>a</sup>	2.00 ± 0.17 <sup>a</sup>	3.67 ± 0.67 <sup>a</sup>	0.84 ± 0.05 <sup>a</sup>

Chla, chlorophyll a; Chlb, chlorophyll b; Chl, total chlorophyll; Chla/b, ratio of chlorophyll a to chlorophyll b;  $R_0$ , mean monthly rainfall from May to August during the period from 1961 to 2018;  $R_{-30}$ ,  $R_0$  decreased by 30%;  $R_{-15}$ ,  $R_0$  decreased by 15%;  $R_{+15}$ ,  $R_0$  increased by 15%;  $R_{+30}$ ,  $R_0$  increased by 30%. Values are shown as mean ± SE (n = 3). Different lowercase letters in the same column indicate significant differences among the treatments at  $p < 0.05$ .

### 3.4. Effect of Rainfall Change on Chlorophyll Fluorescence Parameters

In general, changes in the chlorophyll fluorescence parameters were more obvious under decreased rainfall than under increased rainfall (Figure 4). With increased rainfall, the  $F_0$  and NPQ\_Lss decreased, whereas the  $F_v/F_0$ ,  $F_v/F_m$ , QY\_Lss, Rfd\_Lss, and qL\_Lss increased. Compared to  $R_0$ , the  $F_m$  decreased under reduced rainfall and changed slightly under increased rainfall (Figure 4B). The difference in  $F_0$  under rainfall change was not significant compared to that under  $R_0$  ( $p > 0.05$ ; Figure 4A). The  $F_m$ ,  $F_v/F_0$ , Rfd\_Lss, and qL\_Lss under decreased rainfall were significantly lower than those under  $R_0$  (Figure 4B,C,F,H). The  $F_v/F_m$ , and QY\_Lss decreased significantly under  $R_{-30}$ , equaling only 77.8% and 51.1% of those under  $R_0$ , respectively (Figure 4D,E). Compared to  $R_0$ , the NPQ\_Lss did not change significantly with rainfall change, except for that under  $R_{+30}$  ( $p > 0.05$ ; Figure 4G).

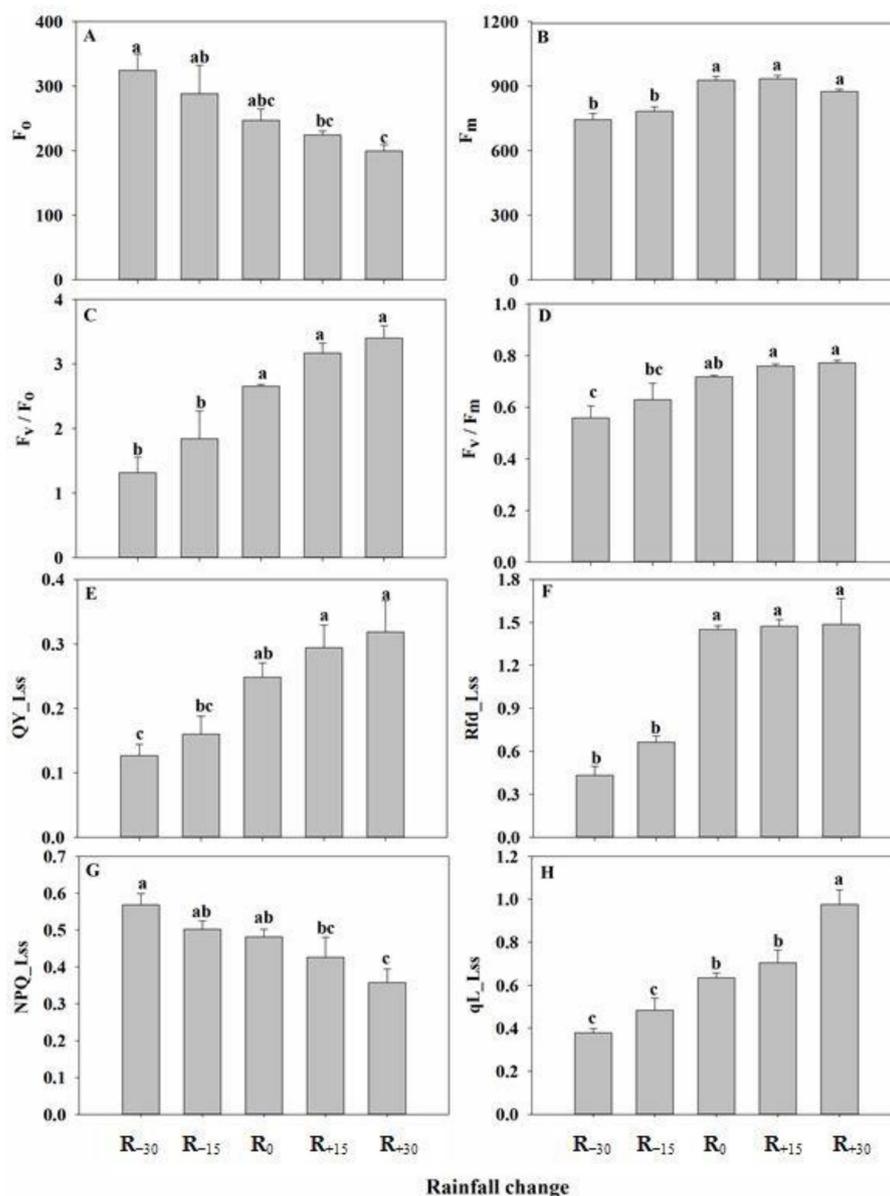
### 3.5. Correlation between Fluorescence and Light Response Parameters

As shown in Table 4,  $F_v/F_0$  and  $F_v/F_m$  were significantly positively correlated with  $A_{QY}$  ( $p < 0.05$ ),  $P_{nmax}$ , and  $L_{sp}$  ( $p < 0.01$ ), and significantly negatively correlated with  $L_{cp}$  ( $p < 0.01$ ). The QY\_Lss and qL\_Lss showed significant positive correlations with  $A_{QY}$ ,  $P_{nmax}$ , and  $L_{sp}$  ( $p < 0.01$ ) and a significant negative correlation with  $L_{cp}$  ( $p < 0.01$ ). Moreover, significant negative correlations of the NPQ\_Lss with  $A_{QY}$  ( $p < 0.05$ ),  $P_{nmax}$ , and  $L_{sp}$  were observed ( $p < 0.01$ ), as well as a significant positive correlation between the NPQ\_Lss and  $L_{cp}$  ( $p < 0.01$ ).

**Table 4.** Correlation between the characteristic parameters of the light response curves and fluorescence parameters.

Parameters	$F_v/F_0$	$F_v/F_m$	QY_Lss	NPQ_Lss	qL_Lss	$A_{QY}$	$R_d$	$P_{nmax}$	$L_{sp}$	$L_{cp}$
$F_v/F_0$	1									
$F_v/F_m$	0.973 **	1								
QY_Lss	0.826 **	0.763 **	1							
NPQ_Lss	-0.752 **	-0.662 **	-0.698 **	1						
qL_Lss	0.835 **	0.766 **	0.891 **	-0.770 **	1					
$A_{QY}$	0.635 *	0.548 *	0.676 **	-0.558 *	-0.792 **	1				
$R_d$	-0.234	-0.128	-0.133	0.062	-0.177	-0.358	1			
$P_{nmax}$	0.815 **	0.716 **	0.828 **	-0.797 **	0.892 **	0.812 **	-0.229	1		
$L_{sp}$	0.932 **	0.878 **	0.905 **	-0.684 **	0.863 **	0.684 **	-0.140	0.817 **	1	
$L_{cp}$	-0.909 **	-0.848 **	-0.836 **	0.813 **	-0.872 **	-0.761 **	0.319	-0.888 **	-0.854 **	1

$F_v/F_0$ , potential activity of PSII;  $F_v/F_m$ , maximum quantum yield of PSII; QY\_Lss, steady-state optical quantum efficiency; NPQ\_Lss, steady-state non-photochemical quenching coefficient; qL\_Lss, steady-state photochemical quenching coefficient;  $A_{QY}$ , apparent quantum efficiency;  $R_d$ , dark respiratory rate;  $P_{nmax}$ , maximum net photosynthetic rate;  $L_{sp}$ , light saturation point;  $L_{cp}$ , light compensation point; \*  $p < 0.05$ ; \*\*  $p < 0.01$ .

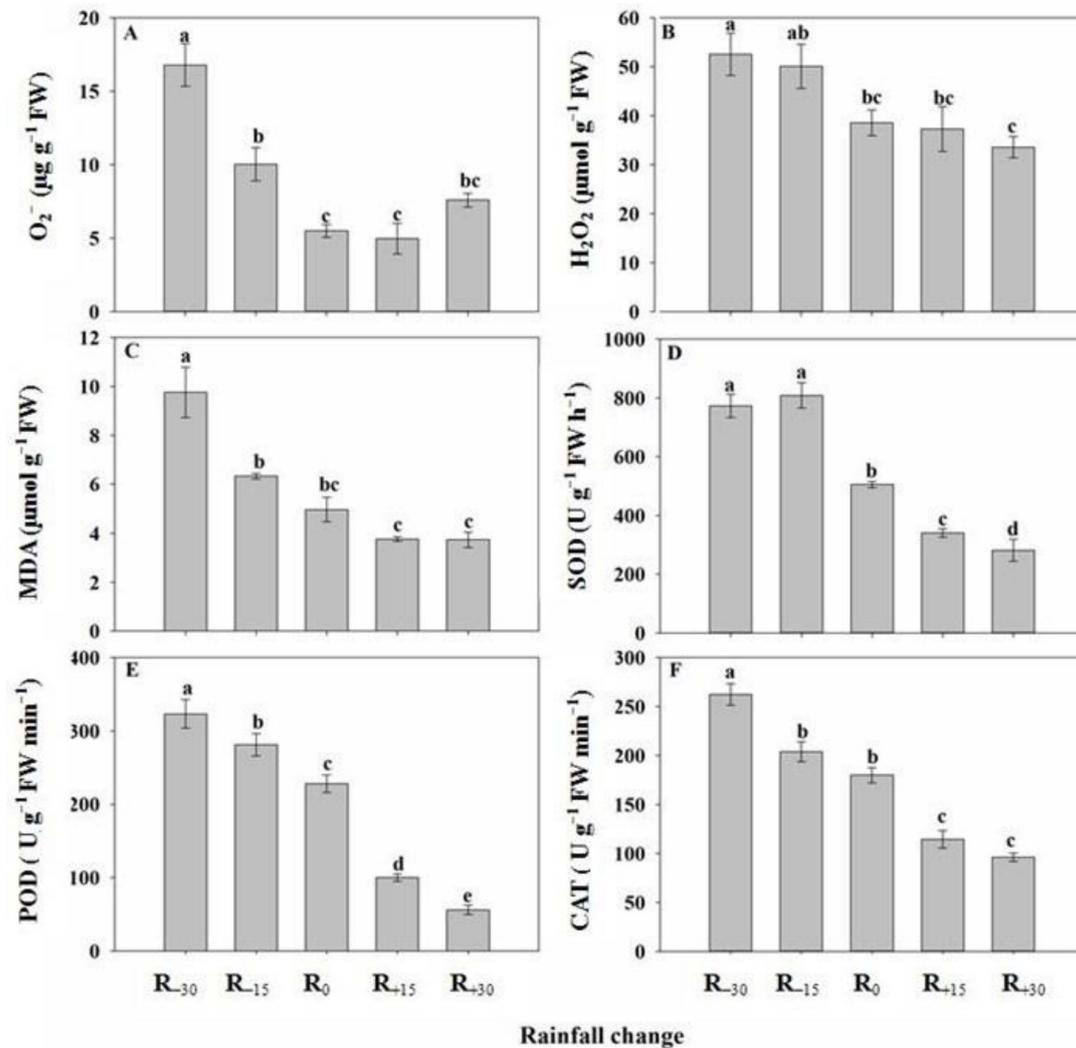


**Figure 4.** Changes in chlorophyll fluorescence parameters in *Gymnocarpos przewalskii* leaves under rainfall change. (A): F<sub>0</sub>; (B): F<sub>m</sub>; (C): F<sub>v</sub>/F<sub>0</sub>; (D): F<sub>v</sub>/F<sub>m</sub>; (E): QY<sub>LSS</sub>; (F): Rfd<sub>LSS</sub>; (G): NPQ<sub>LSS</sub>; (H): qL<sub>LSS</sub>; F<sub>0</sub>, initial fluorescence; F<sub>m</sub>, maximum fluorescence; F<sub>v</sub>/F<sub>0</sub>, potential activity of PSII; F<sub>v</sub>/F<sub>m</sub>, maximum quantum yield of PSII; QY<sub>LSS</sub>, steady state optical quantum efficiency; Rfd<sub>LSS</sub>, steady state fluorescence decay rate; NPQ<sub>LSS</sub>, steady state non-photochemical quenching coefficient; qL<sub>LSS</sub>, steady state photochemical quenching coefficient; R<sub>0</sub>, mean monthly rainfall from May to August during the period from 1961 to 2018; R<sub>-30</sub>, R<sub>0</sub> decreased by 30%; R<sub>-15</sub>, R<sub>0</sub> decreased by 15%; R<sub>+15</sub>, R<sub>0</sub> increased by 15%; R<sub>+30</sub>, R<sub>0</sub> increased by 30%. Vertical bars represent the means ± SE (n = 3), and different lowercase letters above the bars indicate significant differences among the treatments (p < 0.05).

### 3.6. Effect of Rainfall Change on O<sub>2</sub><sup>-</sup>, H<sub>2</sub>O<sub>2</sub>, and MDA Content

With increased rainfall, the content of O<sub>2</sub><sup>-</sup>, H<sub>2</sub>O<sub>2</sub>, and MDA generally decreased (Figure 5A–C). O<sub>2</sub><sup>-</sup> significantly accumulated under R<sub>-15</sub> and R<sub>-30</sub> (p < 0.05), being 1.8 and 3.1 times that under R<sub>0</sub> (Figure 5A). Compared to R<sub>0</sub>, R<sub>-30</sub> significantly increased H<sub>2</sub>O<sub>2</sub> and MDA levels, by 36.2% and 96.4%, respectively (p < 0.05). When rainfall increased (R<sub>+15</sub>, R<sub>+30</sub>), the MDA content decreased markedly compared with that under decreased

rainfall treatments ( $R_{-15}$  and  $R_{-30}$ ,  $p < 0.05$ ). Compared with  $R_0$ , increased rainfall had no significant effect on the accumulation of  $O_2^-$ ,  $H_2O_2$ , or MDA ( $p > 0.05$ ).



**Figure 5.** Changes in the antioxidant enzyme activities in *Gymnocarpus przewalskii* leaves under rainfall change. (A):  $O_2^-$ ; (B):  $H_2O_2$ ; (C): MDA; (D): SOD; (E): POD; (F): CAT;  $O_2^-$ , superoxide anion;  $H_2O_2$ , hydrogen peroxide; MDA, malondialdehyde; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase;  $R_0$ , mean monthly rainfall from May to August during the period from 1961 to 2018;  $R_{-30}$ ,  $R_0$  decreased by 30%;  $R_{-15}$ ,  $R_0$  decreased by 15%;  $R_{+15}$ ,  $R_0$  increased by 15%;  $R_{+30}$ ,  $R_0$  increased by 30%. Vertical bars represent the means  $\pm$  SE ( $n = 3$ ), and different lowercase letters above the bars indicate significant differences among the treatments ( $p < 0.05$ ).

### 3.7. Effect of Rainfall Change on Antioxidant Enzyme Activity

With increased rainfall, the SOD, POD, and CAT activities all decreased (Figure 5D–F). Compared with  $R_0$ , both decreased and increased rainfall caused significant changes in SOD and POD activities ( $p < 0.05$ ). The SOD activity was the highest under  $R_{-15}$ , being 1.6, 2.4, and 2.9 times that under  $R_0$ ,  $R_{+15}$ , and  $R_{+30}$ , respectively. The SOD activity under increased rainfall ( $R_{+15}$  and  $R_{+30}$ ) markedly decreased by 48.4% and 79.5%, respectively, compared with that under  $R_0$  ( $p < 0.05$ ). The POD activity was sensitive to rainfall changes, and the differences in POD activity among all treatments were significant (Figure 5E,  $p < 0.05$ ). The CAT activity showed a changing trend similar to that of POD activity (Figure 5F), reaching its maximum under  $R_{-30}$ , where it was significantly higher (46.0%) than under

$R_0$  ( $p < 0.05$ ). Compared with  $R_0$ , CAT activity under increased rainfall ( $R_{+15}$  and  $R_{+30}$ ) decreased significantly, by 36.3% and 46.5%, respectively ( $p < 0.05$ ).

#### 4. Discussion

Precipitation is the main source of soil moisture in arid desert regions. With changes in global climate, precipitation varies greatly, precipitation interval lengthens, and extreme weather occurs more often [10]. This leads to prolonged dry periods, affects water utilization by plants, and further induces plant physiological change [40]. Studies have indicated that additional precipitation can improve plant photosynthesis, whereas water deficit has the opposite effect [41,42]. With increasing precipitation, the carbon assimilation rate of *Artemisia tridentata* increased significantly more than that of *Purshia tridentata* [43]. The daily mean  $P_n$  of *Nitraria tangutorum* markedly increased when increasing rainfall by 75% and 100% [9]. The  $P_n$  of *Reaumuria soongorica* increased significantly under 15% and 30% increased precipitation, and was reduced under 15% and 30% decreased rainfall [42]. In the present study, the  $P_n$  response of *G. przewalskii* seedlings to rainfall variation was similar to that of *R. soongorica*; however, it showed higher responsiveness. The reduction in  $P_n$  was possibly because the seedlings suffered from drought stress under decreased rainfall, inhibiting the carbon assimilation process [43].

Factors of water stress that impact plant photosynthesis are divided into stomatal and non-stomatal factors [29,44]. The change trends of  $P_n$ ,  $C_i$ , and  $L_s$  can be used to judge whether the decrease in  $P_n$  is attributed to stomatal or non-stomatal factors [15]. In this study, we observed that the decrease in  $P_n$  under  $R_{-15}$  was accompanied by a decrease in  $G_s$  and  $C_i$  and an increase in  $L_s$ , suggesting that stomatal limitation was responsible for the  $P_n$  decrease in *G. przewalskii*. However, the decrease in  $P_n$  under  $R_{-30}$  was accompanied by a decrease in  $L_s$  and an increase in  $C_i$ , indicating that non-stomatal limitation was the dominant factor. The turning point from stomatal to non-stomatal limitation in photosynthesis varies depending on the plant species, drought stress period, and stress intensity [45–47]. For photosynthesis of *G. przewalskii*, the turning point was within the range of 15% and 30% rainfall reduction; when the water conditions are below the critical point over the long term, biochemical and photochemical metabolic pathways may be significantly damaged [47,48]. Plant WUE is the result of a trade-off between carbon assimilation and transpiration loss, and the literature has reported that WUE is improved by water stress [45,49,50]. In our study, decreased rainfall enhanced the WUE of *G. przewalskii* by decreasing transpiration more than photosynthesis, indicating that for *G. przewalskii* improving WUE is a way of adapting to drought. WUE was not improved continuously under water stress, showing a threshold effect [51]. The maximum WUE of *G. przewalskii* may occur between rainfall decreased by 15% and 30%, which needs to be studied further.

The photosynthetic physiological processes of plants are highly sensitive to water variation [52]. Light response parameters can reflect plant photosynthetic potential [53,54], and  $P_{nmax}$  represents the maximal photosynthetic capacity of plants [55]. In the present study, the  $P_{nmax}$  of *G. przewalskii* increased with increased rainfall, indicating that the increase in rainfall improved its photosynthetic ability. On the other hand, it declined significantly under  $R_{-30}$ , suggesting that the seedlings were subjected to severe drought and their photosynthetic potential was limited. Decreased rainfall caused a decrease in  $L_{cp}$  and  $A_{QY}$ , indicating that water deficit reduced light-use efficiency to weak light. In this study, the  $A_{QY}$  was between 0.006 and 0.022, which is far below that of certain plants grown under drought conditions [15,56], indicating that the capability of *G. przewalskii* to use low light was weak. With increased rainfall, the  $A_{QY}$  and  $L_{sp}$  increased and the utilization range of light intensity was enlarged. Under  $R_{+30}$ , the ability to use weak light was significantly improved, possibly because the pigment–protein complex increased, further enhancing the light conversion efficiency [49]. In our study, the  $R_d$  of *G. przewalskii* gradually increased with decreased rainfall, though the effect of rainfall change was not significant. The  $R_d$  of *Phragmites australis* was found to increase under water stress [47]. In this study, the  $R_d$  of *G. przewalskii* varied in the range of 0.83–1.16  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , whereas that of *Periploca*

*sepium* was previously found to be in the range of  $1.37\text{--}2.08 \mu\text{mol m}^{-2} \text{s}^{-1}$  [56], showing great differences in utilization of weak light. This may be because *G. przewalskii* grows in desert areas characterized by high light intensity and drought, and adapts to this habitat by maintaining a lower consumption of photosynthetic products.

Chlorophyll content is an important indicator reflecting plant drought resistance [57]. Water stress usually causes a reduction in chlorophyll content [10,16], which is probably related to the inhibition of chlorophyll biosynthesis [58] or oxidative damage of ROS to chlorophyllase [59]. Our findings show that the contents of Chla, Chlb, and total Chl decreased with declining rainfall and that total Chl content was significantly negatively correlated with  $\text{O}_2^-$ ,  $\text{H}_2\text{O}_2$ , and MDA content ( $r = -0.698$ ,  $r = -0.655$ , and  $r = -0.853$ , respectively;  $p < 0.01$ ), indicating that the decline in chlorophyll content was related to the damage of ROS.

Gas exchange parameters cannot completely represent the effects of drought stress on the photochemical processes [60]. Variations in chlorophyll fluorescence can be used to explore the effect of water stress on PSII structure and function [61], and chlorophyll fluorescence parameters are intrinsically compared with the gas exchange parameters [31].  $F_o$  and  $F_m$  can be used to detect damage to PSII [47]. An increase in  $F_o$  coupled with a decrease in  $F_m$  is a possible sign of PSII center inactivation [16]. Our study found a similar law under decreased rainfall treatments, indicating that initial photochemical reaction process was inhibited, electron transport efficiency was reduced, and inactivation in PSII reaction center occurred.  $F_v/F_m$  reflects the maximum photochemical efficiency [62]; it is relatively constant under normal conditions and usually decreases under drought stress [16], and as such can be used to detect possible photoinhibition. In the present study, decreased rainfall caused a decline in  $F_v/F_m$  and the decrease was significant under  $R_{-30}$ , indicating that the original light energy conversion efficiency was significantly reduced and the potential active center was damaged [63].  $QY\_Lss$  is positively correlated with PSII activity, and can be used to evaluate the electron transfer efficiency of PSII [64,65]. Our results show that  $QY\_Lss$  decreased significantly under  $R_{-30}$  compared with  $R_0$ , indicating that this rainfall condition caused evident photoinhibition.  $qL\_Lss$  represents the openness of the PSII reaction center [66], and is positively related to electron transfer activity. Our results show that rainfall reduction caused a significant decline in  $qL\_Lss$ , implying that electron transfer activity was inhibited in the PSII reaction center [67]. On the other hand, increased rainfall improved the  $qL\_Lss$  of *G. przewalskii*, suggesting that these rainfall conditions increased the openness of the PSII centers, providing sufficient energy and reduction capacity for photosynthetic carbon assimilation. Under drought stress, energy dissipation mechanisms such as heat dissipation or photorespiration are enhanced in order to prevent or reduce photoinhibition [68].  $NPQ\_Lss$  represents the capacity for heat dissipation [69]. The reduction in rainfall caused an increase in  $NPQ\_Lss$ , indicating that thermal dissipation was induced in *G. przewalskii* seedlings, which played a role in alleviating damage caused by excess excitation energy in the photosynthetic apparatus [65].

Under water stress, availability for photosynthesis is limited and excess light energy in PSII results in production of highly reactive ROS [70], including  $\text{O}_2^-$ ,  $\text{H}_2\text{O}_2$ ,  $\text{OH}^-$ ,  $^1\text{O}_2$ , etc. This can cause peroxidation damage to the photosynthetic apparatus [71,72]; the degree of injury can be measured by the change in MDA content [10]. Drought stress has been found to increase  $\text{O}_2^-$  and  $\text{H}_2\text{O}_2$  production in leaves of Kentucky bluegrass [73] and the roots of *G. przewalskii* [24]. In the present study, the  $\text{O}_2^-$ ,  $\text{H}_2\text{O}_2$ , and MDA contents were significantly increased under  $R_{-30}$  compared with  $R_0$ , indicating that changes in the rainfall level caused obvious oxidative damage to the cell membrane of *G. przewalskii*. In addition, the contents of  $\text{O}_2^-$  and  $\text{H}_2\text{O}_2$  were negatively correlated with  $P_n$  ( $r = -0.648$ ,  $r = -0.703$ ,  $p < 0.01$ ) and  $F_v/F_m$  ( $r = -0.662$ ,  $R = -0.840$ ,  $p < 0.01$ ), indicating that ROS action was closely related to a decrease in photosynthesis. To scavenge excessive ROS in adverse environments, plants have evolved a highly efficient antioxidant defense system that includes both enzymatic and non-enzymatic constituents, such as SOD, POD, CAT, ascorbate peroxidase (APX), ascorbate, reduced glutathione, and carotenoids [74,75]. SOD constitutes

the first line of defense against ROS, as it catalyzes  $O_2^-$  into  $H_2O_2$  [76], while CAT and POD are mainly responsible for eliminating  $H_2O_2$  [13,77]. The SOD and CAT activities of *Manihot esculenta* became enhanced to maintain the balance of ROS when drought intensity was increased [78]. For *Iris lactea* var. *chinensis*, POD activity was significantly reduced under both moderate and severe drought stress, whereas SOD activity was reduced under moderate stress and increased under severe stress [79]. In *G. przewalskii*, SOD activity significantly increased under  $R_{-15}$  and decreased under  $R_{-30}$  compared with  $R_0$ , indicating that rainfall reduction induced SOD to remove excess  $O_2^-$ ; however, its scavenging capacity was limited under severe drought, showing a similar response pattern to that of *Potentilla fruticosa* [80]. Our results show that the POD activity continuously increased with decreasing rainfall, showed significant differences among rainfall levels ( $p < 0.05$ ), and had an extremely significant positive correlation with  $H_2O_2$  content ( $r = 0.625$ ,  $p < 0.01$ ). This indicates that POD is the key enzyme in removing  $H_2O_2$  under drought conditions. The changes in CAT activity were generally consistent with POD and were significantly positively related to  $H_2O_2$  content ( $r = 0.650$ ,  $p < 0.01$ ), suggesting that CAT plays an important role in reducing  $H_2O_2$ . In accordance with our findings, the POD and CAT activities of *Cleome spinosa* were found to be significantly enhanced under drought stress [81]. Under increased rainfall, the SOD, POD, and CAT activities were markedly lower than under  $R_0$  ( $p < 0.05$ ), and the MDA and  $H_2O_2$  contents were lower than under  $R_0$ , indicating that water status was improved and ROS metabolism tended to be balanced [82]. This indicates that antioxidant protection is one of the main mechanisms by which *G. przewalskii* responds to water changes.

Seedlings face greater water stress versus adult individuals, possibly due to their smaller root system [83] and reduced photosynthetic physiological activity relative to older and larger individuals [84]. With the increase in drought conditions, SOD and APX activity increased first and then decreased in adult individuals of *G. przewalskii* at different ages (about 15 a and 53 a); under severe drought, CAT and POD activity significantly increased, and older individuals had stronger drought resistance [27]. These results indicate that there are both connections and differences between seedlings and adult individuals of *G. przewalskii* under different water conditions. Thus, it is necessary to explore the response of both *G. przewalskii* seedlings and adult individuals to rainfall changes. This would be helpful for a full understanding of the water adaptation mechanism.

Extreme precipitation has been determined as a potential threat to the global ecosystem [85], with wet areas expected to be wetter and dry areas predicted to become drier [86]. In more extreme climatic conditions, what will happen to the physiological characteristics of *G. przewalskii*? Based on the changes in indicators more associated with biomass production and resource utilization under simulated rainfall, it can be inferred that photosynthesis ( $P_n$ ,  $P_{nmax}$ ) will be more sensitive to extremely increased rainfall than that to extremely decreased rainfall, being significantly enhanced with increased rainfall, while the photochemical efficiency of PSII ( $F_v/F_m$ ,  $QY_{Lss}$ ) will be more responsive to extremely decreased rainfall and significantly reduced with declining rainfall. Improvements in WUE will be reduced under extremely decreased rainfall, and the range of light energy utilization ( $L_{sp}$ ,  $L_{cp}$ ) will be narrowed significantly. The atmospheric  $CO_2$  concentration and soil moisture always interact with each other, affecting the growth, development, productivity of plants and ultimately affecting the function of terrestrial ecosystems [42,87]. To better understand the adaptation of desert plants to future climate changes, it is necessary to further research the interactive effects of elevated  $CO_2$  and precipitation changes on the biomass and physiological characteristics of *G. przewalskii* by designing extreme precipitation gradients.

## 5. Conclusions

In the present study, we conducted a controlled experiment to explore the physiological responses of *G. przewalskii* to changes in rainfall. Our conclusions are as follows. (1) With increasing rainfall, the  $P_n$  and  $T_r$  of *G. przewalskii* seedlings were significantly increased. The decline in  $P_n$  was caused by stomatal and non-stomatal limitations when rainfall

decreased by 15% and 30%, respectively. Rainfall reduction significantly improved the WUE. A decrease in rainfall by 30% significantly reduced the maximum photosynthetic capacity of *G. przewalskii* as well as the ability to use low light. (2) Decreased rainfall caused an obvious decline in Chla and total Chl content, while a 30% decrease in rainfall caused significant photoinhibition and reduced the photochemical activity. (3) A reduction in rainfall of 30% caused significant peroxide damage to the photosynthetic apparatus, while SOD, POD, and CAT synergistically mitigated peroxidation.

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