



Research paper

Leaf area modulates the chlorophyll fluorescence of *Leymus chinensis* in response to different drought scenarios

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ABSTRACT

The photosynthetic response of plants to drought has been widely explored, primarily through indoor cultivation or short-term physiological monitoring. However, studies linking the photosynthesis of forage with plant traits and production under various drought conditions, especially in the context of global precipitation changes, are limited. We conducted a four-year field experiment involving different precipitation treatments: ambient precipitation, intense drought (ID, precipitation exclusion during June), chronic drought (CD, reducing half precipitation amount from June to August), and reducing half precipitation frequency from June to August (RF, precipitation redistribution without changing precipitation amount). Our results showed that ID and CD significantly decreased the actual maximum photochemical quantum yield of PSII (Φ_{PSII}) and maximum photochemical quantum yield (F_v/F_m), indicating a decline in photosynthetic capacity in *Leymus chinensis*. Meanwhile, the increase in regulatory energy dissipation quantum yield ($\Phi(NPQ)$) highlighted enhanced photoprotection. Additionally, the CD increased the non-regulatory energy dissipation quantum yield ($\Phi(NO)$), indicating that the photoprotection mechanism was insufficient to dissipate excess excitation energy, leading to photodamage at the reaction center. In contrast, under the RF scenario, plants effectively managed excess excitation energy by increasing $\Phi(NPQ)$, which prevented damage and maintained stable Φ_{PSII} and F_v/F_m levels. Through regulating leaf area, drought increased $\Phi(NO)$ and decreased F_v/F_m . Although this strategy mitigated further photosynthetic damage, it also reduced photosynthetic efficiency and productivity of *L. chinensis*. This study represents the first exploration of patterns and mechanisms of plant photosynthetic processes in response to diverse drought scenarios. It underscores the crucial role of key plant traits, i.e. leaf area, in regulating photosynthetic responses amid changing precipitation patterns, and provides valuable information for grassland management and continuous forage supply.

1. Introduction

Global climate models project that drought will increase in duration and frequency in many regions of the world, characterized by a decline

in total precipitation, longer drought durations, and more frequent extreme precipitation events (Masson-Delmotte et al., 2021). It is estimated that drought may cause a 52 % decrease in forage yield in Inner Mongolia grasslands in China (Li et al., 2020). Drought stress disrupts

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key physiological and biochemical processes in plant tissues, primarily by inducing stomatal closure, reducing water flux, and limiting carbon dioxide fixation (Mahajan and Tuteja, 2005; Wang et al., 2024). These processes hinder photosynthesis, subsequently affecting plant growth and survival. Additionally, drought would decrease plant photosynthesis by causing the breakdown of photosynthetic pigments, antioxidant activity, rubisco degradation, and reduced enzyme activity in the Calvin cycle (Lawlor and Cornic, 2002; Lü et al., 2019), ultimately reducing yield. The physiological and biochemical responses of plants not only reflect their sensitivity to drought but also their adaptation mechanisms under drought. Different drought intensities and frequency may elicit distinct physiological responses in plants. Intense droughts often induce acute stress that rapidly suppresses stomatal conductance and photosynthesis (Yang et al., 2023), while reduced rainfall frequency causes more gradual water stress and slower recovery (Heisler-White et al., 2009; Zhang et al., 2025). Reductions in precipitation amount, rather than changes in rainfall frequency, leading to changes in leaf traits to minimize water loss, might have stronger negative effects on plant growth (Li et al., 2009; Luo et al., 2024), but both can alter the coordination between plant physiology and performance.

Moreover, plant morphological traits can provide important insights into strategies for plants adapting to drought stress (Rowland et al., 2023). Plants adjust their photosynthetic physiology by modifying leaf morphology and structural traits to maximize their photosynthetic efficiency, reflecting an adaptive strategy that balances plant survival with environmental challenges (Xia et al., 2017). Studies have shown that leaf area (LA) and leaf mass per area (LMA) are crucial traits linked to

plant photosynthesis (Lambers and Poorter, 1992), and leaf dry matter content (LDMC) is closely associated with a plant's ability to tolerate environmental stress (Fenollosa et al., 2024). Empirically, under drought scenarios, LA tends to decrease to prevent excessive water loss through evapotranspiration and enhance drought tolerance, although this may also decrease light capture and reduce photosynthetic capacity (Li et al., 2009). This trade-off indicates the importance of morphological plasticity in balancing water conservation and energy acquisition under varying environmental conditions (Kaproth et al., 2023). However, LMA and LDMC typically increase (Fig. 1, step A) due to constraints on cell growth and division under drought conditions, leading to smaller and more tightly arranged cells and thicker leaf cell walls (Sack and Scoffoni, 2013; Wright et al., 2004). The changes in these leaf traits represent important photoprotective strategies of plants under drought stress, reducing the risk of photoinhibition or photooxidative stress by limiting light energy input and water loss through evaporation (Bhusal et al., 2021). The adaptation enhances resource conservation and environmental tolerance (Bushey et al., 2023). Additionally, plants growing in drought conditions are generally low in height, which shortens the distance from leaves to rhizosphere moisture, facilitating effective moisture transport (Awad et al., 2010).

Photosynthesis forms the base of plant growth, and plants respond to adversity through continually altering photosynthesis-related physiological and biochemical characteristics (Zhou et al., 2023). Assessing photosynthesis is crucial for a mechanistic understanding of how plants respond to various stress conditions (Khatri and Rathore, 2019). Extensive studies have established the drought sensitivity of

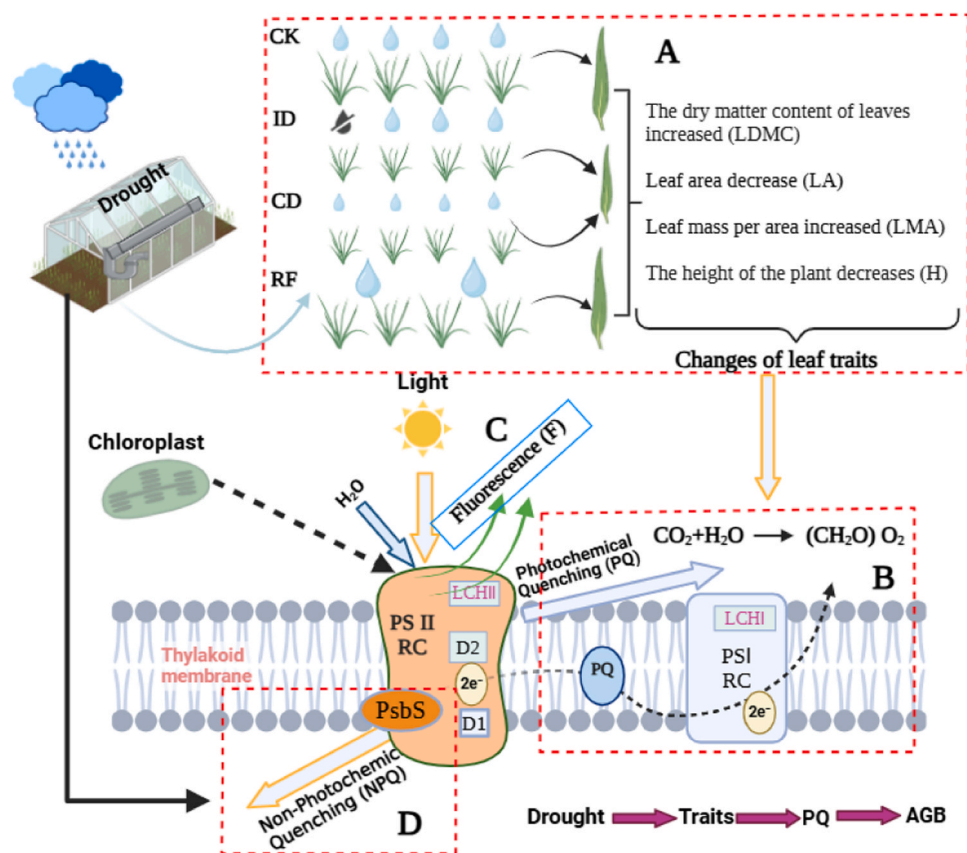


Fig. 1. Schematic representation of the relationship between leaf traits, chlorophyll fluorescence yield, and photosynthetic electron transport chain under different drought scenarios. A: Changes in leaf traits of *L. chinensis*; The processes B, C, and D in the figure represent the possible fate of an absorbed photon as photochemical quenching (PQ), non-photochemical quenching (NPQ), or fluorescence release (F). Here is also described in the electron transfer process of photosynthesis, where PSII RC and PSI RC represent the photosystem II reaction center and photosystem I reaction center. The LCH II and LCH I points represent the light-harvesting complex II and light-harvesting complex I and the blue oval represents the plastoquinone (PQ). D1 and D2 are important proteins involved in light capture and energy transmission in photosystem II, and PsbS is the key protein affecting NPQ. CK, ambient precipitation; ID, intense drought; CD, chronic drought; RF, reducing half precipitation frequency.

photosystem II (PSII) in higher plants (Falk et al., 1996; Hu et al., 2023). During photosynthesis, chlorophyll absorbs light and generates protons, while photons entering photochemistry conduct acyclic electron transfer through PSII, ultimately providing electrons for chloroplast metabolism and the Calvin cycle (Fig. 1, step B) (Helm et al., 2020). Additionally, light absorbed by chlorophyll can be dissipated as fluorescence (Fig. 1, step C) or heat (Fig. 1, step D) (Demmig-Adams and Adams III, 2006). In higher plants, chlorophyll fluorescence has been used as a non-destructive technique to elucidate various aspects of the physiological condition of photosynthetic organs (Krause and Weis, 1991). Chlorophyll fluorescence kinetics plays an irreplaceable role in light energy absorption, transmission, dissipation, and distribution (Li et al., 2019a). Research on chlorophyll fluorescence can enhance our understanding of plant stress response mechanisms and offer practical agricultural benefits, such as identifying stress-tolerant crops and improving growth conditions to boost productivity amid environmental challenges (Masson-Delmotte et al., 2021; Moustakas et al., 2019; Pleban et al., 2020). While extensive research has characterized individual relationships between chlorophyll fluorescence parameters, plant traits, forage yield, and soil properties, a critical knowledge gap persists regarding their synergistic responses to various drought scenarios in natural grassland ecosystems, as no study to date has concurrently examined these interactions within an integrated experimental design.

In contrast to the gas exchange index that primarily reflects the "apparent" characteristics, chlorophyll fluorescence parameters better capture the "internal" characteristics of plant photosynthesis (Schreiber et al., 1995). The maximum quantum yield of PSII (F_v/F_m), actual quantum yield of PSII (Φ_{PSII}), and photochemical quenching coefficient (q_p , q_L) reflect the strength of a plant's photosynthetic capacity, while non-photochemical quenching (NPQ) represents the plant's ability for light energy dissipation (Li et al., 2019a). These indicators serve as important indicators of the regulatory mechanism of plant photosynthesis in adverse circumstances (Schreiber et al., 1995). Empirical research has demonstrated that drought stress adversely affects F_v/F_m and Φ_{PSII} (Gallé et al., 2007; Larouk et al., 2021) while also reducing q_p and q_L , indicating decreased openness of PSII reaction centers, thereby weakening the plant's photosynthetic efficiency (Terzi et al., 2010; Yamada et al., 1996). In drought conditions, when plants are unable to fully utilize the absorbed light energy for photosynthesis, the excess energy leads to the production of reactive oxygen species (ROS), which will damage the photosynthetic system and reduce F_v/F_m (Krause and Weis, 1991). To safeguard the photosynthetic system from this damage, plants dissipate excess light energy by increasing NPQ, with the PsbS protein acting as a necessary sensor to trigger NPQ (Fig. 1, step D) (Bassi and Dall'Osto, 2021).

In addition to chlorophyll fluorescence parameters, enzyme activity is also an important indicator of changes in plant physiological activity (Shirvani et al., 2024). Under drought stress, plants have been observed to increase the activities of antioxidant enzymes such as superoxide dismutase (SOD) and peroxidase (POD) (Lü et al., 2019; Pandey et al., 2023). The antioxidative capacities of SOD and POD can effectively balance ROS levels within cells (Saxena et al., 2016; Thakur and Anand, 2021). Although previous research has explored the chlorophyll fluorescence response of plants to drought stress (Larouk et al., 2021; Li et al., 2019a), long-term field observations focusing on forage grasses remain remarkably scarce.

The Inner Mongolian grassland is a crucial part of the Eurasian grassland region, contributing significantly to economic output and ecological importance (Xu et al., 2024). This ecosystem is highly sensitive to environmental changes and will face increasing frequency and intensity of drought (Guo et al., 2023). As a dominant perennial rhizomatous grass in this region, *L. chinensis* contributes over 60 % of the total grassland biomass (Yao et al., 2021). It is highly valued for its nutrient content and livestock palatability and is also highly responsive to climate change (Ren et al., 2017). Although *L. chinensis* plays a crucial role in both forage production and ecosystem services, our

understanding of its drought response mechanisms, especially at the photosynthetic and physiological levels, remains limited.

This study investigated continuous changes in leaf fluorescence indices and functional traits of *L. chinensis* under different drought scenarios across three growing seasons in a temperate semiarid steppe. Our objective is to explore two key questions: a) How do different drought scenarios affect the chlorophyll fluorescence characteristics of *L. chinensis*? b) What are the biotic and abiotic mechanisms by which different drought scenarios regulate chlorophyll fluorescence parameters to drive changes in the aboveground biomass of *L. chinensis*? We hypothesize that: a) drought will reduce the chlorophyll fluorescence parameters of *L. chinensis*, including q_p , q_L , Φ_{PSII} , and F_v/F_m , while increasing NPQ. b) Drought will reduce plant height and leaf area of *L. chinensis*, thereby limiting photosynthesis and ultimately leading to a decline in aboveground biomass.

2. Materials and methods

2.1. Study site

The study was conducted at the Observation and Research Station for the Typical Steppe Ecosystem of the Ministry of Education (44°10'02"N, 116°28'56"E, elevation 1324 m a.s.l.), located in Xilinhot, Inner Mongolia, China. The average frost-free period is 150 days, with an average annual temperature of 2.8 °C. The average temperature during the growing season (May to September) is 17.2 °C, and the annual precipitation is 299.4 mm (from 1973 to 2017), with about 87.1 % occurring during the growing season (Du et al., 2024). This region is an important base for forage and agricultural livestock production, as well as a core area for pastoral development, playing a crucial role in the regional economy and ecological balance. The soil is chestnut, which corresponds to Calcic-orthic Aridisols in the US soil taxonomy classification, and the plant community is dominated by *L. chinensis* and *Cleistogenes squarrosa*, and the associated species were *Corispermum mongolicum*, *Thermopsis lanceolata*, *Artemisia frigida*, and *Astragalus galacties*.

2.2. Experimental design

The drought treatment design was based on an analysis of nearly 40 years of historical precipitation data in the study region. June had the highest likelihood of extended dry spells (e.g., no rainfall for 28 consecutive days in June 2009). Furthermore, since 2000, there has been a significant decline in rainfall during the period from June to August, with a 50 % reduction in precipitation in some years, accompanied by a concurrent decrease in rainfall frequency (Luo et al., 2024). This field experiment was initiated in early May 2018. Five 23 m × 5 m blocks with naturally assembled communities were established. Each block contained four 5 m × 5 m plots, with a 2-meter buffer zone between adjacent plots to minimize water movement and edge effects. The five blocks were spaced 2 m apart. To prevent surface runoff and subsurface water movement between adjacent plots, we installed iron sheets around each plot, extending 100 cm belowground and 20 cm aboveground. Each plot was randomly assigned to one of four drought treatments: control (ambient precipitation), intense drought (ID, precipitation exclusion during June), chronic drought (CD, reducing half precipitation amount of each rainfall event from June to August), and reducing precipitation frequency (RF, halving the number of rainfall events by collecting every two adjacent rainfalls and reapplying them together onto the original plot from June to August). A 5 m × 5 m rain shelter was erected in each drought plot, with the roof covered by arched transparent polyethylene (light transmittance is about 90 %) to intercept natural rainfall. The detailed information about the rain shelter design and experimental treatments can be found in our recent study (Luo et al., 2024). The rain shelter roofs were promptly removed after the drought treatment period.

Each 5×5 m plot was divided into two subplots (2.25×5 m each), separated by a 0.5 m buffer zone, with ambient nitrogen and nitrogen addition treatment randomly assigned to one of the two subplots. As part of the experiment, this study focused solely on the effects of drought. We established experimental plots in areas with uniformly distributed vegetation to ensure no inherent differences existed among plots prior to the drought treatments. We analyzed pre-treatment soil moisture, pH, electrical conductivity, organic carbon, and organic matter (measured in May in 2018), and found no significant differences in soil characteristics among treatments (all $P > 0.05$), indicating no baseline variations between subplots before treatment initiation. The drought treatments were implemented from 2018 to 2021.

2.3. Chlorophyll fluorescence measurements

From June to September each year from 2019 to 2021, chlorophyll fluorescence parameters of *L. chinensis* were determined every five days using the chlorophyll fluorescence instrument Mini-PAM-II (Walz, effeltrich, Germany). The fully expanded leaves with the same leaf position and similar growth status from three healthy *L. chinensis* individuals in each subplot were selected for measurement (Only one leaf is measured for each plant). To ensure consistency, measurements were taken between 8 a.m. and 11:00 a.m. on the fixed individuals which were marked with tags. Walz manufacturer's protocol explicitly recommends dark adaptation of 20–30 min for herbaceous species. Based on pre-tests showing F_0 stabilization at approximately 20 minutes in *L. chinensis*, we extended the dark adaptation to 25 minutes according to standard chlorophyll fluorescence protocols (Maxwell and Johnson, 2000) to guarantee full dissipation of energy-dependent quenching mechanisms. Before measurement, leaves were dark-adapted for 25 minutes using a blade clamp (DLC-8). Then, initial fluorescence (F_0) was recorded by turning on the weak measuring light ($< 0.1 \mu\text{mol photon m}^{-2}\text{s}^{-1}$). A saturation pulse ($6000 \mu\text{mol photon m}^{-2}\text{s}^{-1}$) was applied to obtain maximum fluorescence yield (F_m). Subsequently, leaves were continuously irradiated with white actinic light with an intensity of $1300 \mu\text{mol photon m}^{-2}\text{s}^{-1}$, and steady-state fluorescence values (F_s) were recorded. Following this, a second saturation pulse of $6000 \mu\text{mol photon m}^{-2}\text{s}^{-1}$ was used to measure maximum fluorescence yield under actinic light (F_m'). The minimum fluorescence level (F_0') was measured under far-red light within 5 seconds after removing the photochemical light. The calculation method and meaning of the parameters are shown in Table 1.

2.4. Measurements of leaf traits, chemistry, and above-ground biomass

In late July of each year from 2019 to 2021, we conducted destructive sampling of *L. chinensis* within a randomly placed $0.5 \text{ m} \times 0.5 \text{ m}$ square iron frame in each subplot to measure leaf mass per area (LMA), leaf area (LA), specific leaf area (SLA), leaf dry matter content (LDMC), leaf nitrogen concentration (LNC), and stem-leaf ratio (SLR). Specifically, the entire aboveground part of each plant was harvested and transported to the laboratory. In the lab, stems and leaves were separated. Fresh weights of leaves and stems were recorded, and leaf area was measured using a scanner (SigmaScan 4.1). Samples were then oven-dried at 65°C for 48 h to determine dry weights. These measurements were used to calculate SLA, LMA, LDMC, and SLR. In late July 2021, five uniformly growing individuals of *L. chinensis* were randomly selected from each subplot, and three to five leaves were collected from each individual for biochemical analyses. The collected leaves did not include individuals of *L. chinensis* used for chlorophyll fluorescence measurements, as those plants were tagged and needed to remain intact for subsequent assessments to avoid any destructive interference. Fresh leaves were stored in liquid nitrogen and transported to the laboratory for subsequent measurements of the activities of superoxide dismutase (SOD), peroxidase (POD), and the concentrations of malondialdehyde (MDA) and chlorophyll (*Chl*). The SOD was determined via nitro blue

Table 1

Basic physiological interpretation of fluorescence parameters.

Fluorescence parameters	Basic definition	Interpretation of parameters
$F_v/F_m = (F_m - F_0)/F_m$	Maximum quantum yield of PSII photochemistry.	It reflects the potential photosynthetic capacity of plants.
$\Phi_{PSII} = (F_m' - F_s)/F_m'$	Actual photochemical quantum yield of PSII photochemistry.	The part involved in the photochemical reaction.
$q_p = (F_m' - F_s)/(F_m' - F_0)$	Photochemical quenching based on the puddle model.	q_p is a parameter of the degree of openness of the photosystem II.
$q_L = q_p \times F_0'/F_s$	Photochemical quenching based on lake models. Both q_p and q_L are photochemical quenching coefficients, and the difference is that the two parameters are calculated based on different model algorithms.	q_L is a parameter of the degree of openness of the photosystem II.
$NPQ = F_m/F_m' - 1$	Non-photochemical quenching.	It indicates how well plants can convert surplus light energy into heat, preventing damage from overexposure.
$\Phi(NO) = F_s/F_m$	$\Phi(NO)$ reflects the quantum yield of constitutive non-regulatory (basal or dark) nonphotochemical dissipation processes.	The excess excitation energy is dissipated by destroying the photosynthetic system after reaching the light reaction center.
$\Phi(NPQ) = F_s/(F_m' - F_s)/F_m$	Regulatory energy dissipation quantum yield ($\Phi(NPQ)$), which reflects basal quenching processes.	The excess excitation energy is reasonably dissipated before reaching photosystem II.

tetrazolium photoreduction, and the POD activity was determined using guaiacol colorimetry. The MDA concentration was determined via thiobarbituric acid colorimetry, and chlorophyll concentration was determined by ethanol colorimetry. The concentrations of magnesium (Mg) and phosphorus (P) in the leaves of *L. chinensis* were determined using inductively coupled plasma. These elements were selected for analysis due to their essential roles in plant growth, particularly their close relationship to photosynthesis (Messant et al., 2023; Sardans et al., 2008). Before elemental analysis, all dried plant samples were ground to a fine powder. For the determination of Mg and P concentrations, approximately 0.2 g of ground leaf material was digested with concentrated nitric acid (HNO_3) using a microwave digestion system before measurement. Leaf nitrogen concentration (LNC) was determined by an elemental analyzer (Vario MACRO cube, Elementar, Germany) on finely ground leaf samples that had passed through a 0.25 mm sieve. In late August of each year, all aboveground living tissues of *L. chinensis* within a randomly selected $0.5 \text{ m} \times 0.5 \text{ m}$ quadrat were harvested in all treatments to determine their aboveground biomass (AGB).

2.5. Measurements of meteorological data and soil characteristics

The continuous data of natural precipitation amount, air temperature as well as soil moisture and temperature at a depth of 10 cm were collected from 2019 to 2021 using a meteorological station (CR1000X, CAMPBELL SCIENTIFIC, USA) installed at our experimental site. Annual precipitation from 2019 to 2021 was recorded as 293.5 mm, 367.1 mm, and 408.1 mm, respectively (Fig. S1)

2.6. Data analyses

We employed linear regression analyses to investigate relationships between soil moisture, leaf physiological and biochemical traits, AGB of *L. chinensis*, and chlorophyll fluorescence parameters. Linear mixed-effects models were used to assess the effects of drought scenarios on fluorescence parameters and leaf related index with drought scenarios as fixed factors and blocks as random factors in the models. Duncan's multiple range comparison tests were utilized to examine the differences

among different drought scenarios at 0.05 level.

We examined the pairwise relationships among continuous variables, including soil characteristics, leaf traits, chlorophyll fluorescence indicators, and AGB, under each drought scenario. Subsequently, we constructed a-prior structural equation model (SEM) based on the binary relationships to investigate the direct and indirect pathways by which drought influences AGB (Fig. S2; Table S1). All the data were standardized prior to SEM analysis. Model fit was evaluated using Shipley's test of d-separation, Fisher's C statistic, and Akaike Information Criteria (AIC). All models used in this study satisfied the established assessment standards. We constructed an SEM, with each model including data from both the specific drought treatment and the control to evaluate drought effects and associated pathways. The SEM analyses were carried out utilizing the *piecewiseSEM* package in R (version 4.3.1), while other statistical analyses were conducted with SPSS 24.0 (SPSS, Inc., Chicago, USA). Standardized total effects, including both direct and indirect effects, were calculated using the *coefs()* function from the *piecewiseSEM* package in R (version 4.3.1). To construct and visualize the network of hypothesized and retained pathways, we used the *igraph*, *tidygraph*, and *ggraph* packages in R (version 4.3.1).

3. Results

3.1. Effects of drought on F_0 , F_m , and F_v/F_m

Intense drought increased the initial fluorescence (F_0) of *L. chinensis* in 2019 and 2020 ($P < 0.05$; Fig. 2a-c), with no observed effect in 2021

(Fig. 2c). Chronic drought increased F_0 by 14.1 %, 16.3 %, and 11.5 % in 2019, 2020, and 2021, respectively (all $P < 0.05$), while reducing half summer precipitation frequency showed no effect on F_0 in each sampling year (Fig. 2a-c, Table 2, Table S2). F_m showed no response to drought treatments, except it increased with reducing half precipitation frequency in 2021 (Fig. 2d-f, Table 2, Table S2). Intense drought significantly decreased F_v/F_m in 2019 but showed no effects in the subsequent two years (Fig. 2g-i). Chronic drought significantly decreased F_v/F_m in the normal precipitation year in 2019 and 2020 (Fig. 2g), but had no effect in the wetter year of 2021 (Fig. 2h-i). Overall, intense and chronic drought had the greatest effects on F_0 , F_m , and F_v/F_m , while reducing precipitation frequency had little effect. That is, more severe or persistent drought impairs photochemical performance, whereas moderate changes in precipitation frequency have a limited impact.

3.2. Effects of drought on q_p , q_L , and $\Phi PSII$

Intense drought significantly reduced the photochemical fluorescence quenching coefficient (q_p) throughout the growing season in 2019. Following intense drought, q_p gradually increased, with similar dynamics observed for q_L and q_p , both initially decreasing and then increasing (Fig. 3a-f). Reducing precipitation frequency significantly decreased q_p in 2019 ($P < 0.05$, Fig. 3a), but did not affect q_L (Fig. 3d). From 2019–2021, both q_p and q_L significantly decreased under chronic drought ($P < 0.05$). Intense drought also significantly reduced $\Phi PSII$ ($P < 0.05$, Fig. 3g-i, Table 2, Table S2), with $\Phi PSII$ remaining low even after natural precipitation resumed. Reducing half precipitation

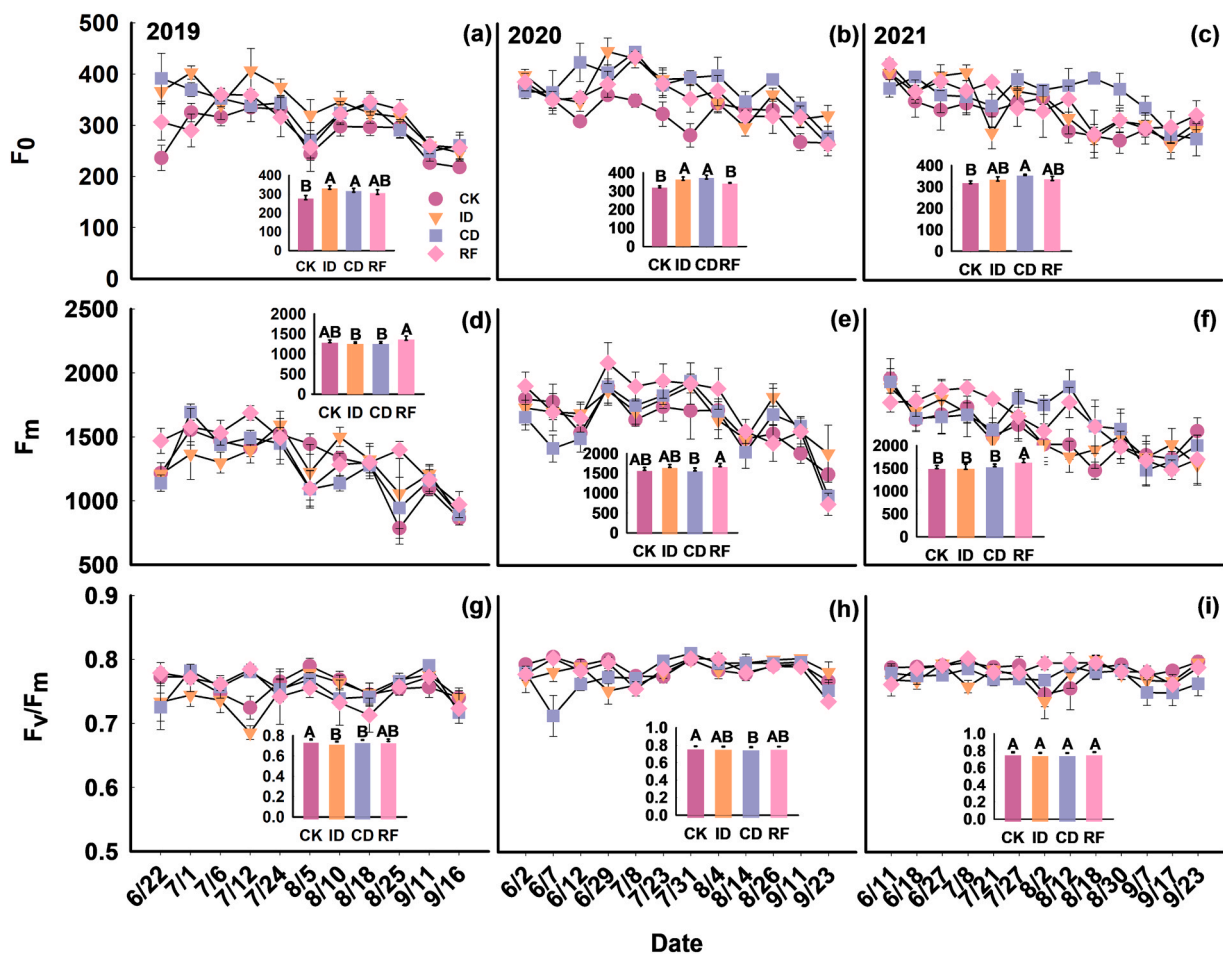


Fig. 2. The response of F_0 , F_m , and F_v/F_m of *L. chinensis* to different drought scenarios over three growing seasons (2019–2021). CK, ambient precipitation; ID, intense drought; CD, chronic drought; RF, reducing half precipitation frequency. Bars indicate means \pm SE ($n = 5$ per treatment). Significant differences ($P < 0.05$) between drought treatments are denoted by different superscript letters ($P < 0.05$).

Table 2
Results (*F*-values) of linear mixed-effects models on the effects of intense drought (ID), chronic drought (CD), and reducing half summer precipitation frequency (RF) on fluorescence parameters.

Year	2019			2020			2021		
Treatment	ID	CD	RF	ID	CD	RF	ID	CD	RF
F_0	70.44 **	10.19*	3.02	19.47*	20.08*	14.57*	2.47	24.09 **	8.09*
F_m	1.28	11.16*	2.38	2.07	0.075	5.11*	0.011	1.45	6.38*
F_v/F_m	7.22*	47.22 **	1.52	0.95	5.19*	2.7	0.74	4.17	0.12
Φ_{PSII}	4.49	17.17*	10.44*	13.80*	15.09*	21.01*	1.39	5.89*	0.58
q_P	17.28*	15.33*	8.63*	17.93*	8.77*	0.24	10.07*	13.57*	0.67
q_L	4.37	6.92*	2.75	11.29*	19.37*	2.29	2.2	6.21*	0.022
NPQ	9.64*	13.82*	7.92*	46.28 **	10.65*	21.47 **	2.91	4.29	3.08
$\Phi(NO)$	5.22*	9.97*	5.97*	0.082	9.39*	1.21	4.71	21.44 **	0.001
$\Phi(NPQ)$	5.74*	17.07*	10.77*	19.39*	6.11*	32.38 **	0.77	3.62	2.75

* and ** denote significant differences at $P < 0.05$ and $P < 0.01$, respectively.

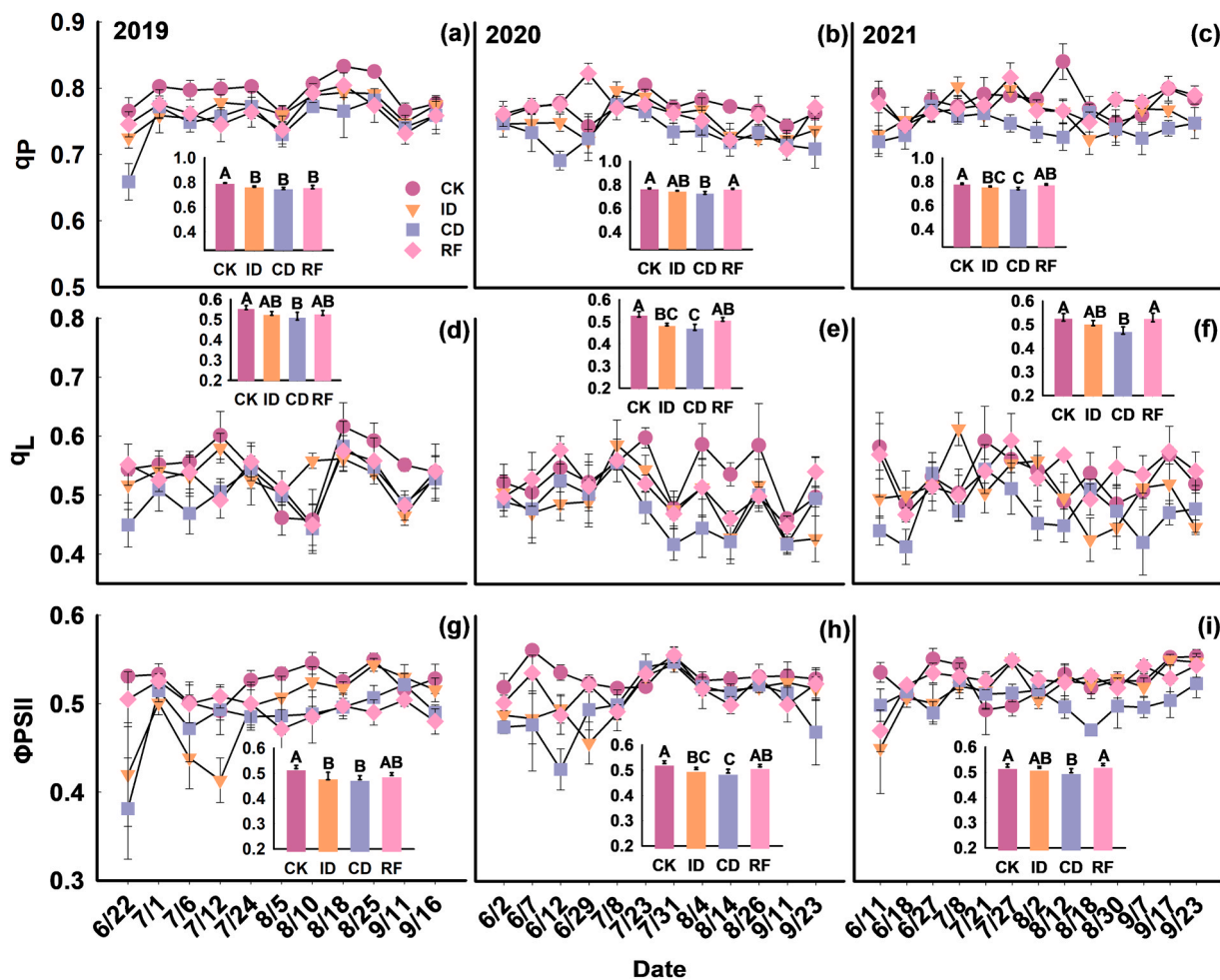


Fig. 3. The response of q_P , q_L , and Φ_{PSII} of *L. chinensis* to different drought scenarios over three growing seasons (2019–2021). CK, ambient precipitation; ID, intense drought; CD, chronic drought; RF, reducing half summer precipitation frequency. Bars indicate means \pm SE ($n = 5$ per treatment). Significant differences ($P < 0.05$) between drought treatments are denoted by different superscript letters ($P < 0.05$).

frequency had no effect on Φ_{PSII} , while chronic drought decreased Φ_{PSII} by 7.3 %, 6.6 %, and 4.0 %, respectively, from 2019 to 2021 (Fig. 3g-i, Table 2, Table S2). In general, intense drought had the strongest negative impact on photochemical parameters (q_P , q_L , and Φ_{PSII}), followed by chronic drought, while reduced precipitation frequency showed the weakest or no significant effects.

3.3. Effects of drought stress on NPQ, $\Phi(NPQ)$, and $\Phi(NO)$

Intense drought, chronic drought, and reducing precipitation

frequency consistently increased NPQ from 2019 to 2021 (all $P < 0.05$, Fig. 4a-c, Table 2, Table S2). Intense drought significantly increased $\Phi(NPQ)$ during treatment, although the extent of this increase gradually diminished after the drought period (Fig. 4d-f). Intense drought also increased $\Phi(NO)$ in 2019, but not in 2020 and 2021 (Fig. 4g-i). Under the reducing precipitation frequency treatment, $\Phi(NPQ)$ increased significantly, but $\Phi(NO)$ remained unaffected (Fig. 4d-i). Chronic drought increased $\Phi(NPQ)$ and $\Phi(NO)$ in each sampling year (all $P < 0.05$, Fig. 4d-i). Collectively, chronic drought exerted the most consistent and sustained effects on non-photochemical energy

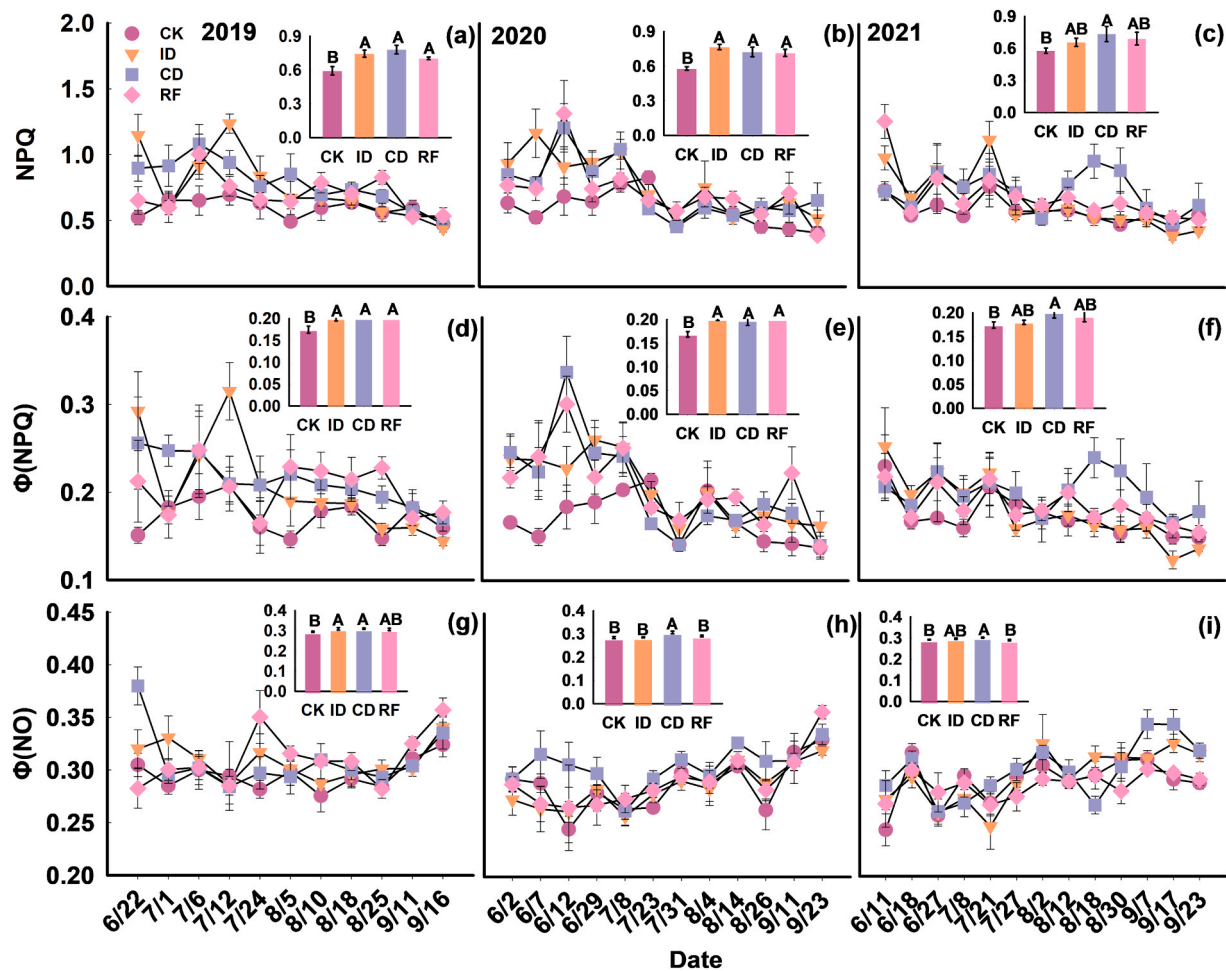


Fig. 4. The response of NPQ, $\Phi(\text{NPQ})$, and $\Phi(\text{NO})$ of *L. chinensis* to different drought scenarios over three growing seasons (2019–2021). CK, ambient precipitation; ID, intense drought; CD, chronic drought; RF, reducing half precipitation frequency. Bars indicate means \pm SE ($n = 5$ per treatment). Significant differences ($P < 0.05$) between drought treatments are denoted by different superscript letters ($P < 0.05$).

dissipation parameters (NPQ, $\Phi(\text{NPQ})$, and $\Phi(\text{NO})$), followed by intense drought, whereas reduced precipitation frequency had relatively minor effects.

3.4. Responses of chlorophyll concentration, SOD, and MDA

Intense drought and chronic drought, but not reducing half precipitation frequency, significantly (both $P < 0.05$) reduced the concentrations of chlorophyll, chlorophyll *a*, and chlorophyll *b* in *L. chinensis* leaves (Fig. S3a–c). Different drought treatments had no significant effect on the *Chl a/b* ratio (Fig. S3d). The SOD enzyme activity significantly increased under both intense and chronic drought but did not respond to reducing precipitation frequency (Fig. S3e). The MDA concentration significantly increased under chronic drought (Fig. S3f).

3.5. Relationships between chlorophyll fluorescence parameters and soil and leaf traits, and annual precipitation under different drought scenarios

AGB of *L. chinensis* was positively related to ΦPSII , F_v/F_m and soil moisture, and negatively correlated with NPQ (Fig. S4a–d). Both F_v/F_m and ΦPSII were significantly positively related to LA and negatively correlated with LMA (Fig. S4e–h). F_v/F_m was positively related to ΦPSII (Fig. S4i). There were significant negative relationships of chlorophyll concentration with NPQ and $\Phi(\text{NPQ})$, and positive relationships between MDA and SOD (Fig. S4j–l). Additionally, we found significant positive relationships between annual precipitation and F_m , F_v/F_m , q_p ,

and ΦPSII , and significant negative relationships with NPQ, $\Phi(\text{NPQ})$, and $\Phi(\text{NO})$ (Fig. S4b–d, f, g–i).

The final SEM showed that intense drought stimulated $\Phi(\text{NO})$ both directly and indirectly by increasing soil temperature, but decreased LA by directly and indirectly reducing soil moisture, which together with the increased soil temperature reduced F_v/F_m , leading to a decrease in AGB of *L. chinensis*, and the decreased LA also directly reduced AGB of *L. chinensis* (Fig. 5A, D). Chronic drought increased $\Phi(\text{NO})$ and decreased LA and F_v/F_m through very similar pathways as those observed under intense drought, except for the missing effect of soil temperature on $\Phi(\text{NO})$ and the insignificant effect of $\Phi(\text{NO})$ on F_v/F_m , causing decrease in AGB of *L. chinensis* (Fig. 5B, E). In contrast, reducing precipitation frequency increased soil moisture, leading to increased LA and decreased soil temperature, which stimulated F_v/F_m and then AGB of *L. chinensis*, increased LA also directly increased AGB. However, these positive effects of reducing precipitation frequency on AGB of *L. chinensis* were largely counteracted by the negative impact through increasing $\Phi(\text{NO})$, resulting in no change in AGB of *L. chinensis* (Fig. 5C, F).

4. Discussion

This study investigated the physiological responses of *L. chinensis* to three drought scenarios, based on chlorophyll fluorescence parameters and leaf traits. Our findings demonstrate that intense and chronic drought exerted more pronounced and persistent negative effects on PSII

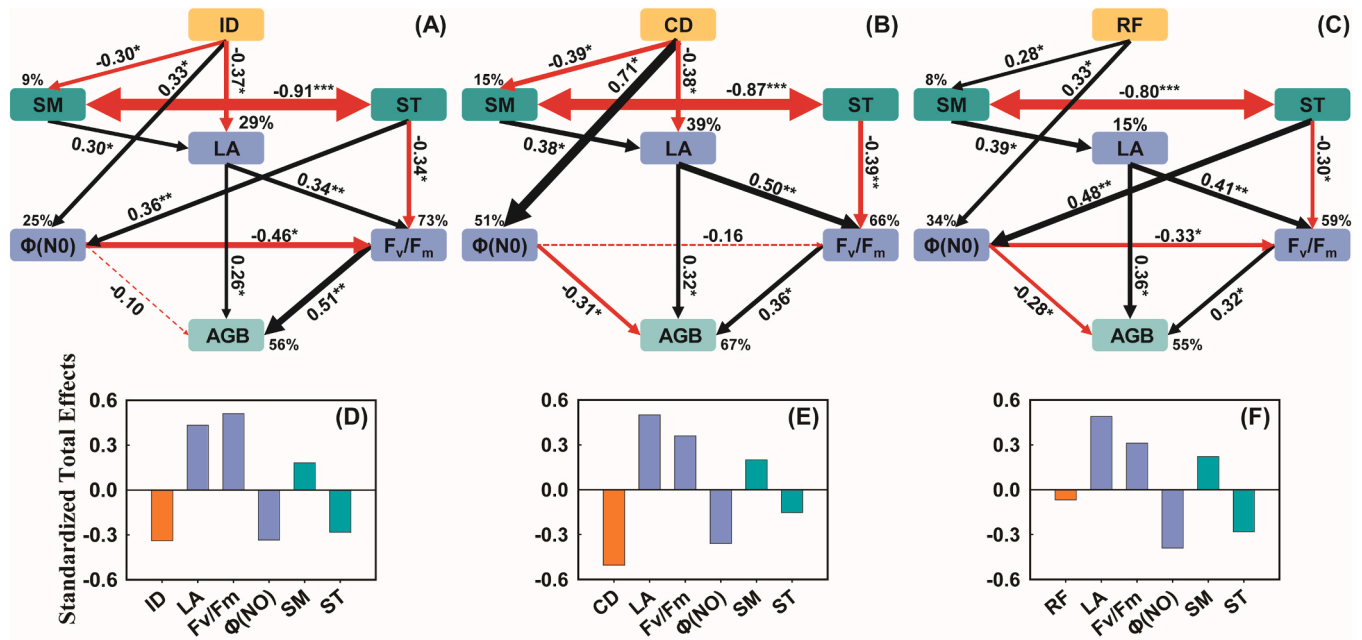


Fig. 5. The final SEM relating intense drought (ID), chronic drought (CD), reducing half precipitation frequency (RF), soil moisture (SM), soil temperature (ST), leaf area (LA), the quantum yield of non-regulated energy ($\Phi(\text{NO})$), and F_v/F_m to AGB of *L. chinensis*. The final SEM fully fitted the data (A: intense drought, Fisher's $C = 11.19$, $P = 0.798$, $df = 16$, $AIC = 380.89$; B: chronic drought, Fisher's $C = 24.60$, $P = 0.136$, $df = 18$; $AIC = 254.08$; C: reducing half frequency of precipitation, Fisher's $C = 16.90$, $P = 0.530$, $df = 18$, $AIC = 294.58$). The number adjacent to the arrow represents the path coefficient, indicating the magnitude of the relationship effect. The explained variance ratio (R^2) is shown together with the response variables in the model. The arrow indicates significant (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$), positive (black), negative (red) pathways and dashed lines indicate irrelevance. The width of the arrow corresponds to the strength of the relationship. The standardized total effects of predictors on AGB (D–F) in intense drought, chronic drought, and reducing half summer precipitation frequency, respectively.

activity, energy dissipation processes, and biomass production than reduced precipitation frequency. These results indicate the heightened vulnerability of photosynthetic systems under severe or prolonged water stress, while also highlighting the capacity of plants to adapt through energy dissipation and trait plasticity. Our study highlights the photosynthetic response strategies of the dominant grass under different drought scenarios, providing important insights for predicting grassland dynamics and offering a practical basis for adaptive management under future climate change.

4.1. Effects of drought on F_0 and F_m

In our study, both intense and chronic drought significantly increased F_0 and reduced F_m , while reducing half precipitation frequency had no significant effect on them. This result aligns with previous research (Li et al., 2019a). It has been found that drought stress will increase F_0 (Li et al., 2019a), which is a characteristic of photosynthetic system inactivation (Baker, 1988). Under drought stress, the dissociation of the light-harvesting antenna from the PSII core complex may lead to the irreversible inactivation of PSII, causing an increase in F_0 (Baker, 1988). Moreover, both intense and chronic drought treatments showed a decreasing trend in F_m , with reductions of 3.8 % and 5.9 %, respectively, corroborating empirical studies (Arab et al., 2023; Li et al., 2019a; Mathobo et al., 2017). Drought stress alters the structure of the catch light composite in PSII, reduces the efficiency of capturing light energy, and blocks electron transfer, which in turn decreases the F_m (Mathobo et al., 2017). However, reducing half precipitation frequency did not reduce F_m . Although reducing precipitation frequency lengthens rainfall intervals, the increased rainfall per event enhances soil water availability, offsetting potential adverse effects of prolonged dry periods, resulting in unchanged F_m (Heisler-White et al., 2009). The decrease in F_0 and F_m may be due to damage to the plant's photosynthetic system caused by drought. This finding provides a valuable reference for assessing plant stress responses and their adaptability to water-limited

environments. However, this response varied among years. In wetter years with higher precipitation, drought treatments had less impact on F_m and F_0 ; F_m increased significantly with annual precipitation (Fig. S6b), indicating that higher rainfall may alleviate drought-induced damage to the photosynthetic system.

4.2. Effects of different drought scenarios on NPQ

We identified a significant increase in NPQ under intense drought, chronic drought, and reduced precipitation frequency, with the strongest responses observed in the former two treatments, suggesting a robust photoprotective adjustment to prolonged water stress. Plants can dissipate excess energy as heat through a photoprotection mechanism, which is achieved by an increase in NPQ (Kromdijk et al., 2016; Muhammad et al., 2021). In this study, NPQ increased under scenarios of intense drought and chronic drought, aligning with the empirical study in a farmland ecosystem (Yu et al., 2021), highlighting the occurrence of photoprotection mechanisms through the NPQ pathway. However, the enhanced NPQ will weaken the carbon fixation capacity and reduce plant yield (Mathobo et al., 2017). Consistently, we observed that intense drought and chronic drought both decreased the AGB of *L. chinensis* by 20 % and 35 %, respectively (Fig. S5). Interestingly, our results demonstrate that NPQ significantly increased in the early stage of intense and chronic drought treatments but gradually decreased after July each year; in years with higher rainfall (2021), NPQ also decreased. With the increase in rainfall, the dissipation of thermal energy decreases (Fig. S6g), the photosynthetic efficiency of plants increases, and the energy conversion for carbon dioxide fixation improves (Kromdijk et al., 2016).

4.3. Differences in heat dissipation processes under different drought scenarios

Our study revealed distinct patterns in the distribution of light

energy under different drought scenarios, with $\Phi(\text{NPQ})$ and $\Phi(\text{NO})$ showing significant and stress-dependent responses that reflect the balance between protective energy dissipation and photodamage risk. The summed quantum yield of PSII, including $\Phi(\text{NPQ})$, $\Phi(\text{NO})$, and ΦPSII , equals 1, and its balance reflects the distribution of light energy (Kramer et al., 2004). $\Phi(\text{NPQ})$ reflects the potential of non-destructive heat dissipation of leaves under stress and will enhance with the increase in intensity and duration of drought stress (Stefanov et al., 2023; Yu et al., 2021). A greater $\Phi(\text{NPQ})$ signifies that photosynthetic organisms absorbed excess light intensity, but can still eliminate the potential damage from excess excitation energy by regulating the heat dissipation mechanism (Pandey et al., 2023). Thus, $\Phi(\text{NPQ})$ can be used as an important index of energy dissipation of light protection mechanisms. The increase of $\Phi(\text{NO})$ signifies that the photochemical energy conversion and protective regulation are insufficient to dissipate the absorbed light energy. In that case, excess light energy reaches the light reaction center, which will cause permanent and irreversible damage to the plant's photosynthetic system (Kramer et al., 2004; Takahashi et al., 2010). Our study found that $\Phi(\text{NPQ})$ increased under intense drought in all years, while $\Phi(\text{NO})$ increased significantly with intense drought only in the dry year of 2019. Both $\Phi(\text{NPQ})$ and $\Phi(\text{NO})$ showed significant negative relationships with annual precipitation (Fig. S6h, i), suggesting that interannual variation in rainfall may underlie the differing responses of these parameters to drought across years. Both $\Phi(\text{NO})$ and $\Phi(\text{NPQ})$ increased significantly with chronic drought, indicating that within their tolerance range, plants can eliminate the potential harm of excessive excitation energy by activating the heat dissipation mechanism. However, in dry years, the protective regulation mechanism is insufficient to dissipate the excess light energy absorption, leading to damage to the photosynthetic system of plants and a subsequent decline in productivity (Sperdoui et al., 2021; Stefanov et al., 2023). Reducing precipitation frequency increased $\Phi(\text{NPQ})$ but showed no effect on $\Phi(\text{NO})$, indicating that plants can eliminate the potential harm from excess excitation energy by increasing the adjustable non-photochemical energy loss under this drought scenario (Moinuddin et al., 2017). The changes in $\Phi(\text{NPQ})$ and $\Phi(\text{NO})$ can serve as key indicators for evaluating the mechanisms of plant photoprotection and stress response, providing important references for improving the stress resistance and management strategies of forage under different drought scenarios.

4.4. Other chlorophyll fluorescence parameters (q_p , q_L , ΦPSII , and F_v/F_m)

Our study revealed that, except for reducing precipitation frequency, q_p , q_L , and ΦPSII significantly decreased with intense drought and chronic drought, which indicates a decrease in the energy absorbed by chlorophyll for photochemical reactions and a diminished capacity of PSII to convert light energy into potential energy (Yamada et al., 1996). The similar variation tendencies observed between q_p and ΦPSII in our findings align with previous research (Li et al., 2019a). This means that, under drought conditions, ΦPSII depends mainly on the proportion of photochemically "open" reaction centers (denoted by q_p and q_L) (Pandey et al., 2023; Zlatev and Lidon, 2012). The results emphasize that intense drought and chronic drought lead to decreased PSII reaction center availability and decreased photosynthetic efficiency in plants.

The variation in F_v/F_m is used to evaluate species tolerance caused by drought or environmental stress factors, and the decrease in F_v/F_m is a mechanism by which plants respond to drought (Falqueto et al., 2017). Our research indicated that both intense drought and chronic drought significantly decreased F_v/F_m in 2019, whereas in 2020, only chronic drought caused a significant decrease in F_v/F_m . However, reducing precipitation frequency had no effect on F_v/F_m . We found a significant positive correlation between F_v/F_m and annual precipitation (Fig. S6c), which may be the main reason for the observed interannual variation. Under drought stress, the PSII oxygen evolution complex and PSII

activity may be damaged or inhibited, resulting in the degradation of the D1 protein (Mathobo et al., 2017). The inability of F_v/F_m to recover to control levels following drought stress indicates irreversible damage to PSII. This is similar to previous research results, where the F_v/F_m of plants can only partially recover after experiencing drought (Xu et al., 2009). This decline may also result from a disorder in the Calvin cycle, subsequently reducing the efficiency of the photochemical transformation process (Silvestre-Carbonell et al., 2023). Therefore, drought stress during the early stages of plant growth or chronic drought may become a key determinant of photosynthesis and other key metabolic activities, and a key determinant of growth, development, and even productivity (Li et al., 2019a).

4.5. Relationships between chlorophyll fluorescence parameters and leaf traits

Our results demonstrate that *L. chinensis* adapts to drought stress through a coordinated strategy involving *Chl* degradation and enhanced antioxidant defense. The MDA concentration in plant tissues serves as an indicator of membrane damage, which can lead to protein inactivation and *Chl* degradation (Bagheri et al., 2019). Our findings indicate that MDA concentration in *L. chinensis* leaves increased with intense drought and chronic drought, suggesting *Chl* degradation. Empirical studies suggested that *Chl* affects NPQ and $\Phi(\text{NPQ})$ (Li et al., 2019a; Ruban, 2016). In our study, *Chl* concentration showed a negative relationship with NPQ and $\Phi(\text{NPQ})$, suggesting a potential drought response in *L. chinensis* via reduced chlorophyll content and enhanced energy dissipation. Additionally, SOD activity increased significantly under both intense and chronic drought, which is consistent with previous findings highlighting its role in ROS scavenging and drought tolerance (Saxena et al., 2016; Song et al., 2011; Zhou et al., 2023). These findings offer valuable insight, though their interpretation is limited by the single sampling year and time point.

The SEM analyses showed that drought affected the photosynthetic process, ultimately influencing the AGB of *L. chinensis* by changing the key plant trait, i.e. LA. The decrease in LA results in reduced interception of light energy, which leads to diminished photosynthetic efficiency (F_v/F_m), but can mitigate photodamage from excessive light energy under drought conditions (Ackerly et al., 2002). In addition, a decrease in LA will increase the contact between mesophyll tissue and leaf veins, potentially increasing the water supply capacity of leaves (Li et al., 2009; Sack and Scoffoni, 2013). LA is a vital index for assessing photosynthetic capacity (Abdelgawad et al., 2020), leaves become smaller and narrower under drought stress, which helps to provide a high surface area for CO_2 uptake through diffusion, ensuring the demand for CO_2 in the most basic photosynthesis of plants, and effectively reduce plant transpiration (Coe et al., 2019). Drought stress often induces structural modifications in plant leaf vasculature, such as narrower xylem vessels and increased vein density (Baird et al., 2025). These structural changes enhance leaf hydraulic safety by minimizing the risk of embolism, maintaining residual water transport under severe drought, and sustaining cell turgor to support physiological functions (Awad et al., 2010). Through the plasticity of leaf morphology and structure, plants change the photosynthetic physiological process of leaves to reduce photosynthetic damage under drought stress, which reflects the survival strategy of coordinated adaptation between plants and the environment (Ackerly et al., 2002; Xia et al., 2017). Our findings show that both intense drought and chronic drought reduce LA, whereas reducing precipitation frequency increases it. This suggests that the amount of precipitation is more important than the precipitation frequency in maintaining photosynthetic efficiency without photodamage. This observation aligns with empirical studies indicating that reduced precipitation significantly decreases the productivity of grassland ecosystems (Luo et al., 2021). Additionally, $\Phi(\text{NO})$ and F_v/F_m serve as indicators for predicting the health and yield of *L. chinensis* under drought stress, supporting their utility as important predictors of plant

adaptability (Li et al., 2019b).

5. Conclusion

This study presents the first evidence for the patterns and mechanisms of plant photosynthetic processes in response to different drought scenarios in grassland ecosystems. Our three-year field manipulative experiment clearly demonstrated that both intense drought and chronic drought decrease leaf area and chlorophyll concentration, inhibiting key photosynthetic processes (Φ PSII, F_v/F_m , q_p , and q_L). Simultaneously, both intense drought and chronic drought stimulate energy dissipation processes (Φ (NPQ) and Φ (NO)) in *L. chinensis*, which decrease light absorption and reduce photosynthetic damage, serving as adaptive mechanisms to cope with drought stress, although decrease the production of *L. chinensis*. In contrast, reducing precipitation frequency does not affect these chlorophyll fluorescence parameters or plant production, as the potential damage caused by excess excitation energy could be mitigated by increasing Φ (NPQ). Our results strongly indicate that the amount of growing season precipitation is more crucial than its frequency in determining photosynthetic processes and plant production in temperate steppe. This study also underscores the significance of key plant traits in modulating photosynthetic responses of plants to changing precipitation patterns, providing valuable insights for managing grassland ecosystems and predicting forage grass adaptability in the face of climate variability.

CRediT authorship contribution statement

Mai-He Li: Writing – review & editing, Visualization, Data curation. **Jiatao Zhang:** Writing – original draft, Validation, Software. **Jordi Sardans:** Validation, Software, Funding acquisition. **Josep Penuelas:** Writing – review & editing, Validation, Funding acquisition, Conceptualization. **Na Li:** Validation, Software. **Ru Tian:** Software, Data curation. **Xingguo Han:** Validation, Methodology. **Jinbao Zhang:** Software, Formal analysis. **Zuoqiang Yuan:** Software, Data curation. **Lan Du:** Visualization, Software. **Yan Shen:** Software, Data curation. **Yonghong Luo:** Investigation, Data curation. **Haiyan Ren:** Validation, Formal analysis. **Mohsin Mahmood:** Validation, Investigation. **Zhuwen Xu:** Writing – review & editing, Validation, Supervision, Funding acquisition.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.envexpbot.2025.106175](https://doi.org/10.1016/j.envexpbot.2025.106175).

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