Detection of Photosynthetic Performance of *Stipa bungeana* Seedlings under Climatic Change using Chlorophyll Fluorescence Imaging

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In this study, the impact of future climate change on photosynthetic efficiency as well as energy partitioning in the *Stipa bungeana* was investigated by using chlorophyll fluorescence imaging (CFI) technique. Two thermal regimes (room temperature, T₀: 23.0/17.0°C; High temperature, T₆: 29.0/23.0°C) and three water conditions (Control, W₀; Water deficit, W₋30; excess precipitation, W₊30) were set up in artificial control chambers. The results showed that excess precipitation had no significant effect on chlorophyll fluorescence parameters, while water deficit decreased the maximal quantum yield of photosystem II (PSII) photochemistry for the dark-adapted state ($F_v/F_m$) by 16.7%, with no large change in maximal quantum yield of PSII photochemistry for the light-adapted state ($F_v'/F_m'$) and coefficient of the photochemical quenching ($q_P$) at T₀ condition. Under T₆ condition, high temperature offset the negative effect of water deficit on $F_v/F_m$ and enhanced the positive effect of excess precipitation on $F_v/F_m$, $F_v'/F_m'$, and $q_P$, the values of which all increased. This indicates that the temperature higher by 6°C will be beneficial to the photosynthetic performance of *S. bungeana*. Spatial changes of photosynthetic performance were monitored in three areas of interest (AOIs) located on the bottom, middle and upper position of leaf. Chlorophyll fluorescence images ($F_v/F_m$, actual quantum yield of PSII photochemistry for the light-adapted state ($Φ_{PSII}$), quantum yield of non-regulated energy dissipation for the light-adapted state ($Φ_{NO}$) at T₀ condition, and $Φ_{PSII}$ at T₆ condition) showed a large spatial variation, with greater value of $Φ_{NO}$ and lower values of $F_v/F_m$ and $Φ_{PSII}$ in the upper position of leaves. Moreover, there was a closer relationship between $Φ_{PSII}$ and $Φ_{NO}$, suggesting that the energy dissipation by non-regulated quenching mechanisms played a dominant role in the yield of PSII photochemistry. It was also found that, among all measured fluorescence parameters, the $F_v/F_m$ ratio was most sensitive to precipitation change at T₀, while $Φ_{PSII}$ was the most sensitive indicator at T₆.

Keywords: *Stipa bungeana*, chlorophyll fluorescence imaging, photosynthetic efficiency, energy partitioning, high temperature, precipitation change
INTRODUCTION

High temperature and water stress as abiotic stress factors will limit plant growth and reduce crop productivity (Boyer, 1982; Wahid et al., 2007), and they always occur simultaneously in that high temperature increases both evaporation and potential evapotranspiration and exacerbates the negative influence of water deficit (Machado and Paulsen, 2001; Osório et al., 2011). Models of global climate change have predicted that the globally averaged surface temperature will be 1.5–4.0°C higher till 2100 and the extreme precipitation events will occur more frequently than before (IPCC, 2013). According to the study by Xu et al. (2009), temperature and precipitation change determine the physiological response of perennial grass to new environmental conditions to a large extent. Among all plant physiological functions, photosynthesis plays a pivotal role in plant carbon uptake, plant growth and biomass accumulation. It is commonly considered that stomatal limitation which influences the substomatal CO$_2$ concentration is the main reason for the reduction of photosynthesis under moderate water deficit (Cornic, 2000). The limitation on CO$_2$ assimilation may damage the balance between photochemical activity in photosystem II (PSII) and electron requirement for photosynthesis, resulting in the photodamage of PSII centers. Although plant photosynthetic apparatus appears to be highly resistant to water deficit (Giardi et al., 1996; Petsas and Grammatikopoulos, 2009; Zivcak et al., 2014), temperature rising can change the response of photosynthesis to water stress (Chaves et al., 2002). Among all plant physiological activities, photosynthesis has been proved to be most sensitive to high temperature and can be inhibited entirely by heat stress before other plant physiological symptoms occur (Berry and Bjorkman, 1980). High temperature damages several photosynthetic functions, such as Calvin cycle, photosystem I (PSI) and PSII. Many studies have reported that the cooperative effect of water stress and high temperature is more drastic than their single effect (Albert et al., 2011; Thomey et al., 2011; Bauweraerts et al., 2013). When water and heat stress occur simultaneously, water stress may impose a certain effect on the photosynthesis together with temperature through oxidative damage (Chaves et al., 2002). On this basis, the inhibitory effect and damage on photosynthesis can be studied when the two stresses coexist, even at a low light intensity.

For the quantitative detection of the changes in the photosynthetic apparatus and photosynthetic activity under various environmental stresses, chlorophyll fluorescence measurement has been demonstrated to be a fast, non-destructive, sensitive and reliable method (Berry and Bjorkman, 1980; Havaux, 1992; Martinez-Carrasco et al., 2002; Mielenz et al., 2003; Xu et al., 2004; Xu and Zhou, 2006; Swoczyna et al., 2010; Tuba et al., 2010; Ogaya et al., 2011; Brestic et al., 2014; Kalaji et al., 2014; Lazár, 2015). However, the conventional chlorophyll fluorescence measurement approach is based on point measurements and cannot exhibit the physiological status of a whole plant (Lichtenhaler and Miché, 1997; Ehler et and Hincha, 2008). Furthermore, habitual heterogeneity of photosynthetic activity over the leaf surface makes this approach highly error-prone (Ehler and Hincha, 2008). To overcome these problems, a more advanced technique, chlorophyll fluorescence imaging (CFI), was developed to take a powerful role in identifying spatial heterogeneity of leaf photosynthetic performance (Omasa et al., 1987; Baker and Rosenqvist, 2004; Ivanov and Bernards, 2015). This provides new possibilities to understand the regulation mechanism of photosynthesis, and to assess the properties of the photosynthetic apparatus and the extent to which the plants are affected by different stresses (Gorbe and Calatayud, 2012; Shaw et al., 2014; Humplík et al., 2015; Ivanov and Bernards, 2015). One of the first works on experiments with CFI were carried out by Omasa et al. (1987). In their work, the analysis of CFI was proved to be a useful method in early warning diagnosis, functional analysis of disorders during environment stress and plant’s ability to recover. CFI can also be used to study plant response to dynamic climate control as image information is the most intuitive, easily comprehensible, and provides useful information on plant status (Omasa, 1990; Calatayud et al., 2006; Gorbe and Calatayud, 2012). Because CFI detects fluorescence signal pixel-by-pixel, it also provides huge amount of data which can be used for a sophisticated statistical treatments which can lead to an early detection of plant stress (Lazár et al., 2006).

As a key vegetation type dominating the typical steppe in Loess Plateau, *Stipa bungeana* is a useful plant species which can control water loss and soil erosion and improve the ecological environment effectively for its developed root system. It is also a type of appetizing forage with high nutritive value for livestocks. Hence, the research concerning the photosynthetic physiological responses of *S. bungeana* to the major stresses becomes increasingly important in the context of the predicted future climatic changes. In the present study, it was confirmed that CFI is a useful and convenient method for detecting the physiological mechanism of response to higher temperature and precipitation change. Moreover, the spatial variations of chlorophyll a (Chl a) fluorescence parameters of *S. bungeana* under different environmental stresses were analyzed. This work aims to evaluate the impact of high temperature and precipitation change on the photosynthetic performance and the utilization of excess excitation energy in photosynthetic apparatus of *S. bungeana*. Specifically, the following questions were addressed: (1) Are there any negative or positive impact of high temperature and precipitation change on the photosynthetic apparatus of *S. bungeana*? (2) What are the mechanisms of PSII photoprotection for *S. bungeana*? (3) Which is the most sensitive fluorescence parameter in predicting the impact of future climate change on *S. bungeana*?

**Abbreviations:** AOD, area of interest; Chl, chlorophyll; CFI, chlorophyll fluorescence imaging; PSII, photosystem II; $F_{v}/F_{m}$, maximal quantum yield of PSII photochemistry for the dark-adapted state; $q_{P}$, coefficient of the photochemical quenching; $\Phi_{PSII}$, actual quantum yield of PSII photochemistry for the light-adapted state; $\Phi_{NPQ}$, quantum yield of regulated energy dissipation of PSII for the light-adapted state; $\Phi_{SC}$, quantum yield of non-regulated energy dissipation of PSII for the light-adapted state; $F_{v}/F_{m}'$, maximal quantum yield of PSII photochemistry for the light-adapted state.
**MATERIALS**

**Plant Material and Growth Conditions**
To understand the effects of high temperature and precipitation change on *S. bungeana*’s photosynthesis characteristics, the changes of water and heat conditions were controlled for the seedlings germinated from seeds. The experiment was carried out at the Institute of Botany, Chinese Academy of Sciences. The seeds of *S. bungeana* were obtained from the grassland in Dongsheng (39°82’N, 110°00’E), Inner Mongolia. They were sterilized by soaking in 0.7% potassium permanganate solution for 8 min and rinsed. Then, these seeds were sown in plastic pots wrapped with plastic film. Each plastic pot was filled with 4.08 kg of dry soil and planted with four plants. In the chestnut pots, the organic carbon content was 12.3 g·kg⁻¹ and the total nitrogen content was 1.45 g·kg⁻¹. Polyethylene nets were used as the experimental containers, which were lined with plastic bags to prevent water leakage.

Different temperature and precipitation treatments were set according to the monthly average temperature and precipitation during *S. bungeana*’s blooming stage in the past 30 years (1978-2007). Considering the diurnal temperature variations, two temperature treatments 23.0/17.0°C (T₀) and 29.0/23.0°C (T₆) were selected for experiment. Three precipitation regimes were set: average monthly precipitation over 30 years (W₀: 82.3 mm); the average increased by 30% (W₀+30); the average decreased by 30% (W₀-30). All the plants were grown in a naturally illuminated glasshouse (the CO₂ concentration was maintained at 390 ppm with a photosynthetic photon flux density of 1000 µmol photons m⁻²·s⁻¹) and the timing used for day/night regime was 16 h light/8 h dark.

**Imaging of Chlorophyll Fluorescence Measurement**
In order to investigate the spatial heterogeneity of Chl fluorescence parameters, Chl fluorescence imaging of leaves was performed by using an imaging-PAM fluorometer (Walz, Effeltrich, Germany). Chl a fluorescence parameter was measured in the healthy and fully expanded leaves of three plants from each treatment. To evaluate spatial heterogeneity, three areas of interest (AOI, AOI type: Rectangle) in the same leaf were used for AOI determination. All plants were placed in dark for 10 min before measurement. Images of maximum fluorescence in the dark-adapted state, *Fₚ*, was determined by applying a blue saturation pulse. The saturation pulse intensity was 8000 µmol photons m⁻²·s⁻¹ for 0.8 s. Minimum Chl fluorescence yield *F₀* was determined using low frequency light pulses (0.5 µmol photons m⁻²·s⁻¹). Then the images of maximal quantum yield of PSII photochemistry in the dark-adapted state *Fₚ/Fₚ* were captured, and *F₀/Fₚ* ratio were obtained as 

\[ \frac{Fₚ - F₀}{Fₚ} \]

To determine the maximum fluorescence yield in the light-adapted state (*Fₚ*) and Chl fluorescence during actinic illumination (*Fₐ*), actinic illumination (336 µmol photons m⁻²·s⁻¹) was switched on and saturating pulses were applied at 20 s intervals for 5 min. All the fluorescence levels for the light-adapted state of the sample were determined at the end of 5 min. The maximal quantum yield of PSII photochemistry for the light-adapted state was estimated by the *Fₐ/Fₚ* and was calculated by measuring the above same parameters (*F₀* and *Fₚ*) on light-adapted leaves. In light-adapted state, the *F₀* level was estimated using the approximation of Oxborough and Baker (1997): 

\[ F₀ = \frac{Fₚ}{(Fₚ/Fₚ) + (F₀/Fₚ)} \]

The actual quantum yield of PSII photochemistry for the light-adapted state (Φₚₛₛ) could be calculated by the formula: 

\[ Φₚₛₛ = \frac{Fₚ - F₀}{Fₚ} = \frac{Fₚ}{Fₚ} \]

The coefficient of the photochemical quenching (qP), which was used for the estimation of the fraction of open PSII centers, was calculated as: 

\[ qp = 1 - \frac{Fₚ - F₀}{Fₚ} \]

The quantum yields of PSII photochemical energy dissipation (Φₚₛₛ), non-regulated (Φₙₒₕ), and regulated (Φₙₚₕ) thermal energy dissipation for the light-adapted state could be used to reflect the utilization of photons which are absorbed by the PSII antenna (Lazár, 2015). It has been proved that 

\[ Φₚₛₛ + Φₙₒₕ + Φₙₚₕ = 1 \]

Φₙₒₕ in PSII was calculated by the equation 

\[ Φₙₒₕ = \frac{1}{1 - \frac{(NPQ + 1)}{(NPQ + 1)}} \]

Φₙₚₕ was calculated by 

\[ Φₙₚₕ = 1 - Φₚₛₛ \]

Statistical Analysis
All statistical analysis was performed using SPSS 18.0 (SPSS, Chicago, Illinois, USA). The mean with standard deviation (±SD) of each treatment was shown. The parameters were analyzed by one-/two-way analysis of variance (ANOVA) followed by Duncan’s multiple range test (Duncan, 1955). The graphing were performed using Origin 9.0 software (Origin Lab, USA).

**RESULTS AND DISCUSSION**
In this study, fluorescence imaging technique was used to provide real-time information of photosynthetic performance of *S. bungeana* under different heat and water conditions. The change of images revealed the spatial variation of photosynthetic efficiency in the leaves of *S. bungeana* under different climate environments.

**Chlorophyll Fluorescence Parameters in Temperature Warming and Precipitation Change Leaves**
Different chlorophyll fluorescence parameters were measured for the leaves of *S. bungeana* to determine the impact of high temperature and precipitation change on the photosynthesis. Maximal quantum yield of PSII photochemistry for the dark-adapted state (*Fₐ/Fₚ*) has been widely used as an indicator.
of environmental stress. It can reveal the potential electron transport of maximal PSII quantum yield in the dark-adapted state. The imaging of $q_P$ and $F_v/F_m'$ facilitates the evaluation of their variations (Oxborough and Baker, 1997). The fraction of the open PSII can be quantified by the parameter $q_P$ (Lazár, 2015). The light-induced non-photochemical quenching is a process that regulates energy conversion in PSII to protect plants from photoinhibition. It represents the plant’s ability to dissipate excess light energy that cannot be utilized in CO$_2$ assimilation (Müller et al., 2001). Our results showed that under T$_6$ condition, $F_v/F_m$ in water-deficient (W$_{-30}$) plants significantly decreased by 16.7% compared with the normally-watered (W$_0$) plants. There is no significant difference between the over-watered (W$_{+30}$) plants and W$_0$ plants. Moreover, there is a great change in $F_m$ but not $F_0$ under W$_{-30}$ condition, suggesting that the decrease in $F_v/F_m$ was due to the decrease in $F_m$. Except for $F_v/F_m$, there were no significant changes in the other chlorophyll fluorescence parameters such as $F_v'/F_m'$ and $q_P$ in both W$_{-30}$ and W$_{+30}$ plants. The results indicate that (1) excess precipitation had no effect on S. bungeana at room temperature; (2) S. bungeana suffered from water deficit (decrease in $F_v/F_m$), and water stress inhibited plant’s ability in thermal energy dissipation (Zivcak et al., 2014). This can be explained by the fact that an extreme decrease in trans-thylakoid pH gradient was not generated owing to cooperative consumption of light energy by CO$_2$ fixation and photorespiration (Müller et al., 2001).

At the T$_6$ condition, high temperature offset the negative effect of water deficit on $F_v/F_m$, and enhanced the positive effect of excess precipitation on $F_v/F_m$, $F_v'/F_m'$ and $q_P$, leading to the increase in value. This indicates that the temperature higher by 6°C will be beneficial to the photosynthetic performance of S. bungeana. However, in the study by Xu and Zhou (2006), the combination of severe water stress and high temperature exhibited adverse effects on the PSII function of Leymus chinensis, which is similar to Petsas and Grammatikopoulos (2009)’s conclusion that PSII function of Phlomis fruticosa was progressively suppressed under long-term water deficit. This obvious difference may be explained by that S. bungeana can well adapt to stress environment for its well-developed root system (Cheng et al., 2012).

Furthermore, in the leaves of plants under optimum temperature and water condition, the mean value of $F_v/F_m$ was 0.678 (Table 1), which was lower than the typical value of 0.83 for non-photoinhibited leaves (Björkman and Demmig, 1987). There are two possible reasons to explain the difference. One is the usage of a different intensity of illumination during plants growing (Brestit et al., 2014) and the timing used for day/night regime was also different when compared to natural conditions. The other one is S. bungeana grown in the plastic pots with a small size under weak illumination may limit S. bungeana’s normal growth (Xu and Zhou, 2006). Therefore, the use of pots inside the greenhouse still requires further investigation.

**Utilization of Excess Excitation Energy under High Temperature and Abnormal Water Conditions**

$F_v/F_m$ is known to be a sensitive indicator of plant photosynthetic performance (Björkman and Demmig, 1987). It reflects the maximum efficiency of photosynthetic apparatus converting the absorbed light energy into chemical energy, and has been widely used for the detection of photoinhibition (Dickmann et al., 1992; Herppich and Peckmann, 2000). The plants under stress have a lower value of $F_v/F_m$ than those under normal environment (Papageorgiou and Govindjee, 2004; Tuba et al., 2010; Shaw et al., 2014). Calatayud et al. (2013) proposed several reasons for why the $F_v/F_m$ ratio is preferable for the research of environmental stress. Firstly, it can be measured rapidly for the dark-adapted samples. Secondly, it is very useful for quick screening of stress-suffered plants in large quantities. Lastly, unlike the other parameters such as $\Phi_{PSII}$ or $q_P$, it does not need an extended period of illumination as a single saturation pulse is enough. However, the decrease of $F_v/F_m$ can only reflect the degree of environmental stress, while the utilization of excess excitation energy is still unknown. To solve the problem, three fluorescence parameters which divides the allocation of absorbed light energy into three fractions: (1) utilized by PSII photochemistry ($\Phi_{PSII}$); (2) thermally dissipated via $\Delta$H and xanthophyll-dependent energy quenching ($\Phi_{NQ}$); (3) non-regulated energy dissipation ($\Phi_{NO}$) (Demmig-Adams et al., 1996; Lazár, 2015). Among the

### TABLE 1 | Effects of precipitation treatments on maximum and minimum fluorescence yield in dark ($F_m$ and $F_0$, respectively), maximal quantum yield of PSII photochemistry for the dark-adapted state ($F_v/F_m$), coefficient of the photochemical quenching ($q_P$) and maximal quantum yield of PSII photochemistry for the light-adapted state ($F_v'/F_m'$) of Stipa bungeana leaf under ambient temperature ($T_0$) and high temperature ($T_6$) conditions.

| Temperature treatments | Water treatments | $F_m$ | $F_0$ | $F_v/F_m$ | $F_v'/F_m'$ | $q_P$ |
|------------------------|------------------|-------|-------|-----------|-------------|-------|
| $T_0$                  | $W_{+30}$        | 0.557 ± 0.130 a | 0.170 ± 0.048 a | 0.691 ± 0.076 a | 0.368 ± 0.109 a | 0.627 ± 0.158 a |
|                        | $W_0$            | 0.577 ± 0.121 a | 0.181 ± 0.031 a | 0.678 ± 0.078 a | 0.426 ± 0.129 a | 0.690 ± 0.150 a |
|                        | $W_{-30}$        | 0.417 ± 0.181 b | 0.172 ± 0.054 a | 0.565 ± 0.096 b | 0.448 ± 0.066 a | 0.560 ± 0.195 a |
| $T_6$                  | $W_{+30}$        | 0.555 ± 0.090 a | 0.164 ± 0.036 a | 0.702 ± 0.058 a | 0.524 ± 0.100 a | 0.600 ± 0.088 a |
|                        | $W_0$            | 0.548 ± 0.122 a | 0.193 ± 0.069 a | 0.662 ± 0.081 b | 0.461 ± 0.069 ab | 0.461 ± 0.113 b |
|                        | $W_{-30}$        | 0.522 ± 0.136 a | 0.233 ± 0.112 a | 0.569 ± 0.111 b | 0.409 ± 0.129 b | 0.400 ± 0.121 b |

Different letters indicate significant differences ($p < 0.05$) between water treatments according Duncan test. Values shown are means ± standard deviation (SD) of nine to twelve replicates.
latter three fluorescence parameters, $\Phi_{\text{PSII}}$ reflects light-induced protective mechanism while $\Phi_{\text{NO}}$ reflects a basal quenching which is not regulated by light. They shed a light on the study of plant's capacity to cope with excess excitation energy, and have been widely used to determine QA redox state and excitation energy fluxes in order to gain a better understanding of the stress response mechanisms (Calatayud et al., 2006; Osório et al., 2011).

As seen in Figure 1, the precipitation change had no significant effect ($p > 0.05$) on $\Phi_{\text{PSII}}$, $\Phi_{\text{NPQ}}$, and $\Phi_{\text{NO}}$ at T\(_0\) condition, though $\Phi_{\text{PSII}}$ decreased by 20.8 and 16.7% under W\(_{+30}\) and W\(_{-30}\) conditions and $\Phi_{\text{NO}}$ increased by 21.0 and 23.9%, respectively. At the T\(_6\) condition, high temperature slightly decreased the value of $\Phi_{\text{PSII}}$ at W\(_0\) and W\(_{-30}\) conditions. This suggests that heat dissipation of the excess light energy was activated to protect the photosynthetic apparatus from photoinhibitory damage (Ort and Baker, 2002; Omasa and Takayama, 2003). Whereas excess precipitation under T\(_6\) condition significantly increased $\Phi_{\text{PSII}}$, indicating that the increased precipitation can enhance the protective mechanism of PSII. In addition, $\Phi_{\text{NPQ}}$ showed no obvious change under all environment conditions and the change of $\Phi_{\text{NO}}$ was opposite to $\Phi_{\text{PSII}}$. This means that there was much overlap between $\Phi_{\text{PSII}}$ and $\Phi_{\text{NO}}$, indicating that energy dissipation by non-regulated quenching mechanisms tends to dominate the yield of PSII photochemistry under drought and heat stress, with the xanthophyll cycle-mediated thermal dissipation playing possibly a less important role. Similar results were also reported by Osório et al. (2011).

### Spatial Heterogeneity of Chlorophyll Fluorescence Parameters under Various Temperature and Water Conditions

In Table 2, the value of Chl a fluorescence was obtained from three leaves in each treatment. CFI reveals spatial changes in three areas of interest (AOIs) of the same leaves of *S. bungeana*, i.e., the bottom position of leaf, middle position of leaf and upper position of leaf as shown in Figure 2. For each AOI, the values of fluorescence parameter of all pixels within this area were averaged. In Figure 2, the images of a single leaf are used to show the heterogeneous distribution of light utilization (changes in $F_v/F_m$) and photosynthetic activity (change in $\Phi_{\text{PSII}}$, $\Phi_{\text{NPQ}}$, and $\Phi_{\text{NO}}$) over the surface of the whole leaf. The observation of changed image color is an intuitive process. Pixel-value images of $F_v/F_m$, $\Phi_{\text{PSII}}$, $\Phi_{\text{NPQ}}$, and $\Phi_{\text{NO}}$ were displayed with the help of a false color code, ranging from black (0.000) to pink (ending 1.000).

According to Table 2, it was found that CFI exhibited the spatial changes in different AOIs of the leaf of *S. bungeana*. Three different AOI were considered for each leaf. Each datum in the table is the mean value of the corresponding AOI from all leaves. At T\(_0\) condition, excess precipitation did not alter the

![Figure 1](image.png)
TABLE 2 | Effect of high temperature and precipitation change on maximal quantum yield of PSII photochemistry for the dark-adapted state ($F_v/F_m$), actual quantum yield of PSII photochemistry for the light-adapted state ($\Phi_{PSII}$), quantum yield of regulated energy dissipation of PSII for the light-adapted state ($\Phi_{NPQ}$), and quantum yield of non-regulated energy dissipation of PSII for the light-adapted state ($\Phi_{NO}$) of S. bungeana in different AOIs.

| Precipitation change | $T_0$ | $T_6$ |
|----------------------|-------|-------|
|                      | $F_v/F_m$ | $\Phi_{PSII}$ | $\Phi_{NPQ}$ | $\Phi_{NO}$ | $F_v/F_m$ | $\Phi_{PSII}$ | $\Phi_{NPQ}$ | $\Phi_{NO}$ |
| AOI1                 |        |       |       |       |        |       |       |       |
| W_{40}               | 0.73 ± 0.02 a | 0.31 ± 0.05 a | 0.44 ± 0.04 a | 0.25 ± 0.01 a | 0.72 ± 0.05 a | 0.37 ± 0.12 a | 0.32 ± 0.07 a | 0.31 ± 0.06 ab |
| W_{0}                | 0.76 ± 0.00 a | 0.35 ± 0.05 a | 0.42 ± 0.14 a | 0.23 ± 0.08 a | 0.71 ± 0.02 a | 0.28 ± 0.06 a | 0.44 ± 0.10 a | 0.28 ± 0.03 b |
| W_{−30}              | 0.66 ± 0.02 b | 0.30 ± 0.02 a | 0.46 ± 0.06 a | 0.24 ± 0.06 a | 0.64 ± 0.10 a | 0.23 ± 0.10 a | 0.39 ± 0.09 a | 0.38 ± 0.05 a |
| AOI2                 |        |       |       |       |        |       |       |       |
| W_{40}               | 0.54 ± 0.10 b | 0.24 ± 0.05 ab | 0.45 ± 0.08 a | 0.31 ± 0.10 a | 0.70 ± 0.04 a | 0.31 ± 0.03 a | 0.36 ± 0.06 a | 0.33 ± 0.04 a |
| W_{0}                | 0.68 ± 0.02 a | 0.31 ± 0.04 a | 0.42 ± 0.11 a | 0.27 ± 0.08 a | 0.63 ± 0.11 a | 0.19 ± 0.05 b | 0.45 ± 0.02 a | 0.36 ± 0.05 a |
| W_{−30}              | 0.73 ± 0.02 a | 0.22 ± 0.03 b | 0.47 ± 0.08 a | 0.31 ± 0.05 a | 0.53 ± 0.11 a | 0.13 ± 0.03 b | 0.43 ± 0.11 a | 0.44 ± 0.12 a |
| AOI3                 |        |       |       |       |        |       |       |       |
| W_{40}               | 0.49 ± 0.04 a | 0.14 ± 0.04 a | 0.51 ± 0.08 a | 0.35 ± 0.09 a | 0.68 ± 0.09 a | 0.27 ± 0.11 a | 0.42 ± 0.13 a | 0.31 ± 0.04 a |
| W_{0}                | 0.59 ± 0.03 a | 0.21 ± 0.11 a | 0.54 ± 0.15 a | 0.26 ± 0.08 a | 0.62 ± 0.09 a | 0.17 ± 0.05 a | 0.37 ± 0.16 a | 0.46 ± 0.15 a |
| W_{−30}              | 0.61 ± 0.09 a | 0.20 ± 0.06 a | 0.41 ± 0.09 a | 0.39 ± 0.04 a | 0.53 ± 0.12 a | 0.13 ± 0.03 a | 0.40 ± 0.10 a | 0.48 ± 0.12 a |

Different letters indicate significant differences between water treatments at the same part of leaf ($p < 0.05$) according Duncan test. Values shown are means ± standard deviation (SD) of three replicates.

$F_v/F_m$ in AOI1, but reduced the $F_v/F_m$ by 13.0 and 9.2% in AOI2 and AOI3, respectively. Water deficit significantly decreased the $F_v/F_m$ by 15.0% in AOI1, but there was no significant change in AOI2 and AOI3. At $T_6$ condition, excess precipitation increased the $F_v/F_m$ in AOI1, AOI2, and AOI3 by 2.8, 12.6, and 9.0%, respectively. This means that a 6°C higher temperature is beneficial for $F_v/F_m$ under abundant water condition. In contrast, water deficit decreased the $F_v/F_m$ in AOI1, AOI2, and AOI3 by 9.2, 14.9, and 16.8%, respectively. This suggests a reduction in light energy utilization by chloroplasts in the photosynthesis. According to the changes of $F_v/F_m$ and the results of One-way ANOVA in Table 2, it can be concluded as follows. The middle position of leaf (AOI2) is most sensitive to excess precipitation under both $T_0$ and $T_6$ condition, while the bottom position (AOI1) and upper position (AOI3) are most sensitive to water deficit under both temperature conditions.

The contribution of different pathways to energy partitioning in PSII complexes is shown in Table 2. In AOI1 and AOI3, the actual quantum yield of PSII photochemistry for the light-adapted state ($\Phi_{PSII}$) which can indirectly reflect linear electron transport was not affected by precipitation change at both $T_0$ and $T_6$ treatment. This confirms that photoinhibition is not induced under these conditions. In AOI2, high temperature ($T_6$) improved the effect of $W_{−30}$ on $F_v/F_m$ by 63.6%. This change of $\Phi_{PSII}$ resulted from changes in the total non-photochemical quenching capacity ($\Phi_{NPQ} + \Phi_{NO}$). The quantum yield of regulated energy dissipation ($\Phi_{NPQ}$) was quite similar in all AOIs under different environment conditions, indicating that no excess light energy was produced by precipitation change and high temperature. The values of quantum yield of non-regulated energy dissipation ($\Phi_{NO}$) were low and similar in all AOIs at $T_0$. This means that there were sufficient photochemical conversion and protective regulatory mechanisms in the whole leaf. At $T_6$ condition, water deficit increased $\Phi_{NO}$ in whole leaf, indicating that high temperature exacerbated the negative effect of water deficit on energy dissipation. Both photochemical energy conversion and protective regulatory mechanism were not enough.

The Two-way ANOVA (Table 3) indicated that, $F_v/F_m$ was significantly influenced by precipitation change at $T_6$ condition and varied greatly at different AOIs ($p < 0.01$), exhibiting significant interaction of AOI and precipitation change ($p < 0.01$). The other chlorophyll fluorescence parameters such as $\Phi_{PSII}$ and $\Phi_{NO}$ exhibited significant difference across AOIs, but were not affected by precipitation change. Under condition, only $\Phi_{PSII}$ was significantly affected by both AOI and precipitation change, but the interaction was not significant ($p > 0.05$). To conclude, $F_v/F_m$ is most sensitive to precipitation change at $T_0$ condition, while $\Phi_{PSII}$ is the most sensitive indicator at $T_6$ condition. However, in the study by Lazár et al. (2006), even if there are no changes in the mean value of a fluorescence parameter, there can be the changes in shapes of statistical distributions of fluorescence parameter which is an early indication of a plant stress. Base on this, we should not only find the most sensitive parameter (Kalaji et al., 2014) and the most sensitive species (Swoczyna et al., 2010) but also find the best (statistical) method for detection of the stresses is more important. The use of fluorescence imaging and the detection of photosynthetic performance of S. bungeana response to climatic change still requires further investigation.

CONCLUSION

Chlorophyll fluorescence imaging provided detailed intuitive information on the spatial heterogeneity of chlorophyll fluorescence parameters of S. bungeana and facilitated the investigation of plant photosynthetic performance under various temperature and water conditions. Our results showed that S. bungeana has strong ability in protecting photosynthetic apparatus against the photoinhibitory damage from drought, and a 6°C higher temperature could offset the negative effect of water deficit to a certain extent. On the other hand, excess precipitation...
FIGURE 2 | Use of chlorophyll fluorescence imaging of whole plant *Stipa bungeana* under different temperature and precipitation conditions. This figure illustrates several images of the same leaf of *Stipa bungeana* showing the spatial variation in the parameters \( \Phi_{\text{PSII}} \), \( \Phi_{\text{NPQ}} \), and \( \Phi_{\text{NO}} \) at steady state with actinic illumination of 336 \( \mu \text{mol photons m}^{-2} \cdot \text{s}^{-1} \), and in the parameter \( F_{\text{v}}/F_{\text{m}} \) after dark adaptation. The color scale showed at the bottom of the figure stands for values from 0 (black) to 1 (pink) based on Imaging Win v2.32 software. The three little red boxes in each image display the mean values of the selected fluorescence parameters within the AOI of one leaf.

| T_0 | AOI   | df | F    | P    | AOI × Precipitation | df | F    | P    |
|-----|-------|----|------|------|---------------------|----|------|------|
| T_0 | Water | 2  | 20.918 | 0.000 |                     | 2  | 14.455 | 0.000 |
| T_0 | Precipitation | 2 | 8.041 | 0.003 |                     | 2  | 3.051 | 0.072 |
|      | AOI × Precipitation | 4 | 5.160 | 0.006 |                     | 4  | 0.692 | 0.607 |

| T_6 | AOI   | df | F    | P    | AOI × Precipitation | df | F    | P    |
|-----|-------|----|------|------|---------------------|----|------|------|
| T_6 | Water | 2  | 2.166 | 0.144 |                     | 2  | 4.713 | 0.023 |
| T_6 | Precipitation | 2 | 5.272 | 0.016 |                     | 2  | 10.366 | 0.001 |
|      | AOI × Precipitation | 4 | 0.198 | 0.936 |                     | 4  | 0.075 | 0.989 |

**TABLE 3 | Multiple range test among effects of areas of interest (AOI) and watering treatments on *Stipa bungeana* leaf Chl fluorescence parameters under ambient temperature (T_0) and high temperature (T_6) conditions based on the Two-way ANOVA.**

\[ df, \text{Degree of freedom}; \ F, \text{F-value, used for Homogeneity of variance test}; \ P, \text{Significant level.} \]

had no significant effect on PSII at room temperature. But high temperature had a positive effect on PSII and significantly enhanced the photosynthesis of *S. bungeana*. We also found that it is energy dissipation by non-regulated quenching mechanisms rather than the xanthophyll cycle-mediated thermal dissipation that plays an important role in dominating the yield of PSII photochemistry under climate change. This study also found that \( F_{\text{v}}/F_{\text{m}} \) measured in A0Is was the most sensitive indicator for the response of PSII to climate change.
to precipitation change under room temperature, while $\Phi_{\text{PSII}}$ is more sensitive to precipitation change at higher temperature.

**AUTHOR CONTRIBUTIONS**

GZ and YW conceived the experiment, YW, XL, and ZX conducted the experiment, XS analyzed the results and wrote the manuscript. All authors reviewed the manuscript. No conflict of interest exits in the submission of this manuscript, and the manuscript is approved by all authors for publication. We would like to declare that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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