

## Article

# Responses of Rice Photosynthetic Carboxylation Capacity to Drought–Flood Abrupt Alternation: Implications for Yield and Water Use Efficiency

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## Abstract

Investigating how drought and flooding stresses interact during drought–flood abrupt alternation events and their impact on rice photosynthetic carboxylation capacity ( $V_{cmax}$ ) is critical for improving crop growth and yield models under environmental stress conditions. However, there is limited research on the specific role of these combined stresses on  $V_{cmax}$  in rice. This study aims to address this gap by examining the effects of drought and flooding on rice  $V_{cmax}$ . Using data from drought–flood experiments conducted in 2017 and 2018, we calculated  $V_{cmax}$  by combining observed gas exchange parameters with photosynthetic biochemical models. The results revealed that  $V_{cmax}$  damage caused by drought and flooding stresses was eventually repaired. Notably,  $V_{cmax}$  recovered more quickly when mild drought preceded flooding stress. In contrast, severe and moderate drought treatments showed synergistic effects, where the preceding drought and subsequent flooding exacerbated the damage to  $V_{cmax}$ . However, the pre-mild drought stress antagonistically mitigated the damage to  $V_{cmax}$  of rice induced by flooding stress, showing an antagonistic effect. Additionally, rice increased intrinsic water use efficiency ( $WUE_i$ ;  $A_n/g_s$ ) by increasing investment in  $V_{cmax}$  after drought and flooding stress, but rice yield was not improved. The preceding drought is probably beneficial for yield of rice experiencing subsequent flooding stress at relatively low  $V_{cmax}$ , while subsequent flooding stress exacerbated the reduction in yield of rice experiencing preceding drought stress. This research enhances our understanding of how the interaction between drought and flooding affects rice's photosynthetic capacity and emphasizes that appropriate drought and flooding management may have potential optimizing effects on rice yield and water use, and provides an important theoretical basis and practical guidance for paddy water management.

**Keywords:** drought; flooding; photosynthetic carboxylation rate; intrinsic water use efficiency; rice yield



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## 1. Introduction

As global climate change accelerates, drought and flooding stresses occur frequently, often occurring as abrupt alternations between drought and flooding (drought–flood abrupt alternation (DF)). Unlike conventional water stress, DF events refer to the phenomenon of

abrupt alternation between drought and flooding, which occurs frequently in monsoon climate zones [1]. Between 1981 and 2020, DF events occurred over 15 times in the Yangtze and Yellow River basins [2]. These extreme fluctuations pose significant challenges for plant adaptation and represent a serious threat to agricultural production [3]. Rice (*Oryza sativa* L.), a key global food source, is particularly vulnerable to extreme drought and flooding stress in southern China. Both drought and flooding have profound effects on various physiological processes, including plant water status [4], root growth [5], stomatal regulation [6], and photosynthesis [7]. Among them, photosynthesis plays a critical role in crop dry matter production and accumulation, directly influencing crop yields. Understanding how photosynthesis in plants responds to water stress is crucial for improving crop resilience and productivity.

In addition to the impact of stomatal conductance on CO<sub>2</sub> uptake, leaf photosynthesis is primarily determined by the photosynthetic capacity of the plant. Usually, photosynthetic capacity is characterized by using the maximum photosynthetic carboxylation rate ( $V_{cmax}$ ), which reflects the activity of Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase) in the chloroplasts. Rubisco activity directly governs the efficiency of carbon assimilation in response to intercellular CO<sub>2</sub> and plays a decisive role in dry matter production [8–10]. Changes in  $V_{cmax}$  under water stress conditions (i.e., drought and flooding) can sensitively reflect the response and adaptation of leaf photosynthesis to environmental stress [11,12]. Given its significance, understanding the response of rice  $V_{cmax}$  to drought–flood (DF) stress is essential for uncovering the physiological mechanisms that govern rice under extreme climatic conditions and optimizing rice management practices.

Both drought and flooding stresses in DF events impair rice photosynthesis. Drought stress typically causes stomatal closure, reducing CO<sub>2</sub> supply and inhibiting photosynthetic capacity [13–15]. Meanwhile, drought stress can damage leaf photosystem II and increase photorespiration, leading to greater carbon loss in rice [16,17]. In contrast, flooding stress can result in root hypoxia [5], impairing root function and inducing oxidative stress, which further reduces photosynthetic efficiency [18,19], further affecting the photosynthetic efficiency of plants [20]. Both stresses have a significant cumulative effect on rice photosynthetic capacity, ultimately resulting in reduced rice yields [10].

Under compound stress (i.e., DF stress) conditions, rice is exposed to a combination of complex and variable environmental factors [3,21,22], and the interaction effects between drought and flooding are even more complex. As such, studying the specific effects of such compound stresses on rice photosynthesis is crucial. Previous studies have revealed the damage and yield reduction effects of DF stress on rice growth, focusing on the responses of root growth [23], photosynthetic gas exchange processes [21], dry matter accumulation and partitioning [23], and yield component [24] under DF treatment. For instance, Zhu et al. [25] observed that mild drought exhibited antagonistic effects on gas exchange parameters in rice experiencing subsequent flooding stress, with recovery in both stomatal conductance and photosynthetic rate after DF stress. In contrast, Gao et al. [26] analyzed the yield components of rice experiencing DF stress and found that DF stress with severe drought resulted in a greater yield reduction (up to 34.50%) compared to mild or moderate drought conditions. The core issue with DF stress is its impairment of photosynthetic processes, including photosynthetic capacity, which directly affects yield. To address this, Liu et al. [3] explored the interaction between preceding drought and subsequent flooding in DF stress on the maximum photosynthetic carboxylation rate at 25 °C ( $V_{cmax25}$ ) for rice, based on a stomatal optimization model. Their findings revealed both antagonistic and synergistic effects between drought and flooding stresses on  $V_{cmax25}$ . However, this study simplified the analysis by only considering  $V_{cmax25}$  under different DF treatments, without addressing the non-stomatal limitations or the dynamic changes in photosynthetic capacity at different

stages of stress. This oversimplification may lead to a skewed understanding of how drought and flooding interact to affect photosynthetic capacity. Therefore, further research is needed to systematically explore the response of rice photosynthetic capacity to DF stress and the interactive effects on rice  $V_{cmax}$  between drought and flooding stresses.

To address this research gap, this study will delve into the following research points: (1) The extent of damage to rice  $V_{cmax}$  and its recovery dynamics by DF events; (2) the interaction between drought and flooding stress on  $V_{cmax}$  in DF treatment; and (3) the relationship between photosynthetic carboxylation capacity, water use efficiency, and their effect on rice yield. It is hoped that this study will provide valuable theoretical guidance and practical recommendations for the management and cultivation of rice in regions affected by drought–flood abrupt alternation events.

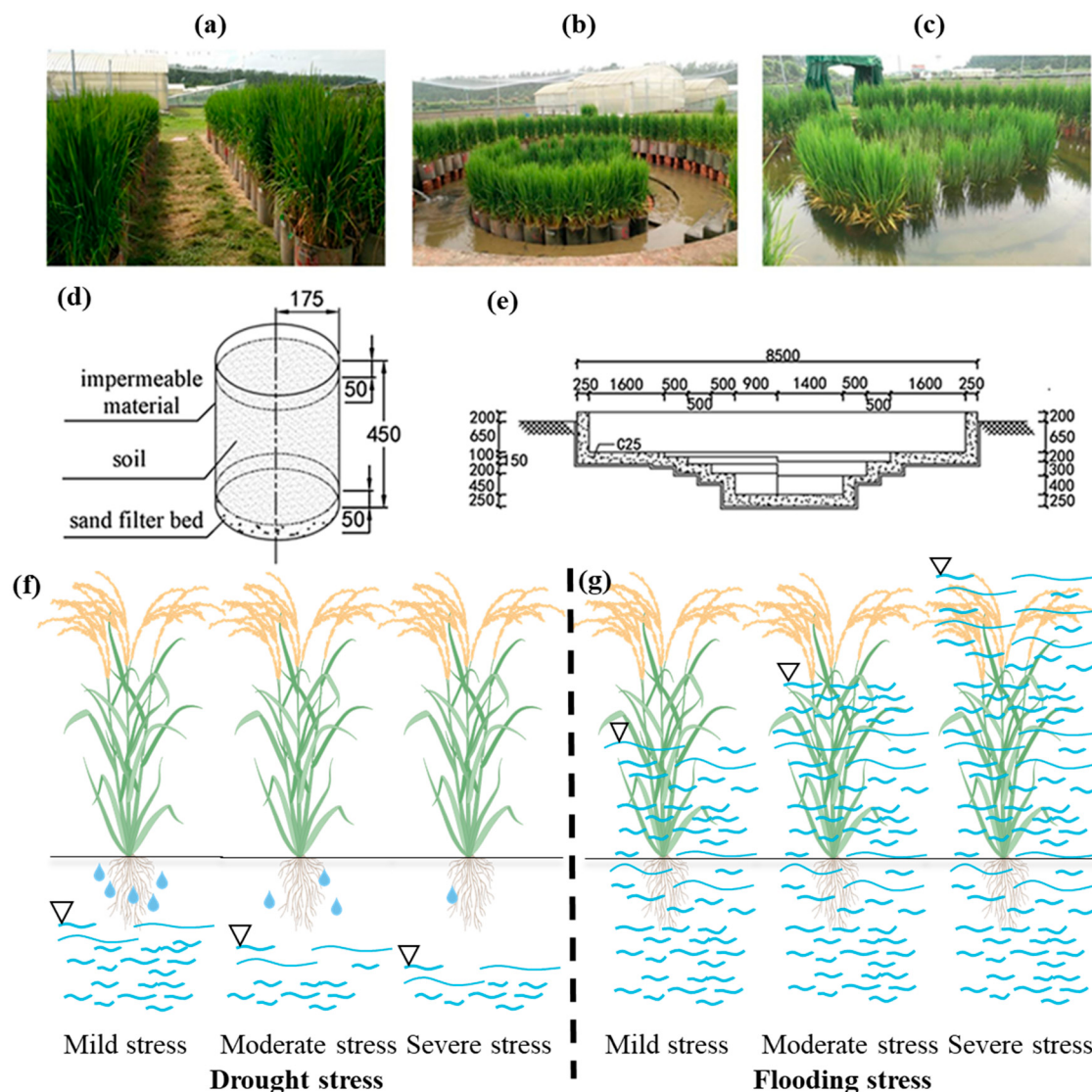
## 2. Materials and Methods

### 2.1. Experimental Site

The pot experiments were conducted during the rice-growing seasons of 2017 and 2018 at Bengbu, Anhui Province, China (117°21'34" E, 33°08'56" N), located in the Huaihe River Basin, where drought–flood abrupt alternation events frequently occur during summer and autumn. The average annual sunshine hours are 2167.5 h; the average temperature is 15.1 °C; the average annual precipitation is 905.4 mm; and the average frost-free period is 217 d. The region has a subtropical monsoon climate with mean annual precipitation of 905.4 mm, a mean temperature of 15.1 °C, and approximately 2167.5 h of sunshine per year, along with a frost-free period of about 217 days. The experimental site description follows Liu et al., where additional information about regional characteristics can be found [3].

### 2.2. Experimental Treatment

The experimental treatments included drought stress (DNF), flooding stress (NDF), drought–flood abrupt alternation (DF), and traditional flooding treatments (CK). Three levels of drought and flooding stress (mild, moderate, and severe) were designed in conjunction with short-, medium-, and long-duration stress periods, producing 28 total treatment combinations, with each treatment repeated 3 times (Figure 1 and Table S1). Based on historical data of the experimental site [27,28], the specific combination treatments are as follows: DF1, 50%FC + 5 d + 100% PH + 7 d; DF2, 50%FC + 10 d + 50% PH + 9 d; DF3, 50%FC + 15 d + 75%PH + 5 d; DF4, 60%FC + 5 d + 75%PH + 9 d; DF5, 60%FC + 10 d + 100%PH + 5 d; DF6, 60%FC + 15 d + 50% PH + 7 d; DF7, 70%FC + 5 d + 50% PH + 5 d; DF8, 70%FC + 10 d + 75% PH + 7 d; DF9, 70%FC + 15 d + 100%PH + 9 d. FC represents field capacity, and PH represents plant height. All treatments are shown in Table S1. Detailed descriptions about the experiment can be found in Liu et al. and Zhu et al. [3,25]. For the water management method, water status in each treatment was controlled using a gravimetric approach. All buckets were weighed twice daily at 7:00 and 18:00 with a portable digital balance, with the corresponding amount of water added or drained to maintain the prescribed soil moisture level. During flooding periods, the standing water depth of the soil surface was monitored using a fixed ruler and manually adjusted to match the target flooding degree. No additional irrigation was applied beyond the scheduled water control of each stress and CK treatment.



**Figure 1.** Experimental design for water stress testing. Figure (a–c) illustrate drought and flooding stress experiments. Figure (d) is the schematic diagrams of the soil column for the experiment. Figure (e) is pool for water control in experiment (unit: mm). Figure (f,g) shows the degree of drought and flooding stresses. The inverted triangle symbol indicates the water surface level associated with water stress treatment.

### 2.3. Measurement and Methods

#### 2.3.1. Experimental Observation Data

The leaf gas exchange data observed in experiments mainly included leaf stomatal conductance ( $g_s$ ), transpiration rate ( $E$ ), photosynthetic rate ( $A_{net}$ ), intercellular  $CO_2$  concentration ( $C_i$ ), atmospheric pressure ( $P_{atm}$ ), leaf temperature ( $T_{leaf}$ ), Photosynthetic Photon Flux Density (PPFD), and leaf air–water pressure deficit ( $VPD$ ). The experiment used a portable photosynthesis system (CI-340, CID, US) to measure photosynthetic metrics, mainly including  $g_s$ ,  $A_{net}$ , and  $E$  of the second-youngest fully expanded leaf in all treatments. The intrinsic water use efficiency ( $WUE_i$ ), calculated by  $A_{net}$  and  $g_s$ ,  $WUE_i = A_{net}/g_s$ . The observation periods were during the jointing and booting stage and the heading and flowering stage of rice after drought and flooding stress, and the specific observation periods for each treatment are shown in Table S1, and measurements were taken at 10:00 and 14:00 on the designated sampling days. Following harvest, the grains were dried in an oven at 40 °C for 12 h, threshed, and subsequently weighed to determine rice yield.

### 2.3.2. Photosynthetic Physiological Model

In this study, the maximum photosynthetic carboxylation rate ( $V_{cmax}$ ) was calculated using the photosynthetic biochemical model (Farquhar model) proposed by Farquhar et al. [29]. In the photosynthetic biochemical model, the net photosynthetic assimilation rate ( $A_{net}$ ) was calculated as follows, at steady state:

$$A_{net} = \frac{A_c + A_e - \left( (A_c + A_e)^2 - 4 \cdot c' \cdot A_c \cdot A_e \right)}{2 \cdot c'} \quad (1)$$

where  $A_c$  represents the Rubisco-limited photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), and  $A_e$  represents the Electron transport-limited photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), which were calculated as follows [30]:

$$A_c = \frac{V_{cmax}(C_i - \Gamma^*)}{C_i + K_c \cdot \left(1 + \frac{O_i}{K_o}\right)} \quad (2)$$

where  $C_i$  represents the intercellular  $\text{CO}_2$  concentration,  $O_i$  denotes the internal partial pressure of  $\text{O}_2$ , and  $\Gamma^*$  is the  $\text{CO}_2$  compensation point.  $K_c$  and  $K_o$  are the Michaelis–Menten coefficients of Rubisco activity with respect to  $\text{CO}_2$  and  $\text{O}_2$  ( $\Gamma^*$ ,  $K_c$ , and  $K_o$  values were adopted from Bernacchi et al. [31]).  $V_{cmax}$  is the maximum carboxylation rate and was estimated based on the photosynthetic carboxylation capacity at 25 °C ( $V_{cmax25}$ ) and leaf temperature ( $T_l$ ) as follows:

$$V_{cmax} = V_{cmax25} \cdot \frac{1 + \exp[(S_v \cdot T_0 - H_d)/(R \cdot T_0)] \cdot \exp[(H_a/(R \cdot T_0)) \cdot (1 - T_0/T_l)]}{1 + \exp[(S_v \cdot T_l - H_d)/(R \cdot T_l)]} \quad (3)$$

where  $H_a$ ,  $H_d$ , and  $S_v$  represent the energy of activation, deactivation, and an entropy term, respectively, and are in reference to Leuning [32], while  $T_0$  is the reference temperature and is equal to 298.2 K, and the  $T_l$  value is equal to the sum of 273.2 K and  $T_{leaf}$ .

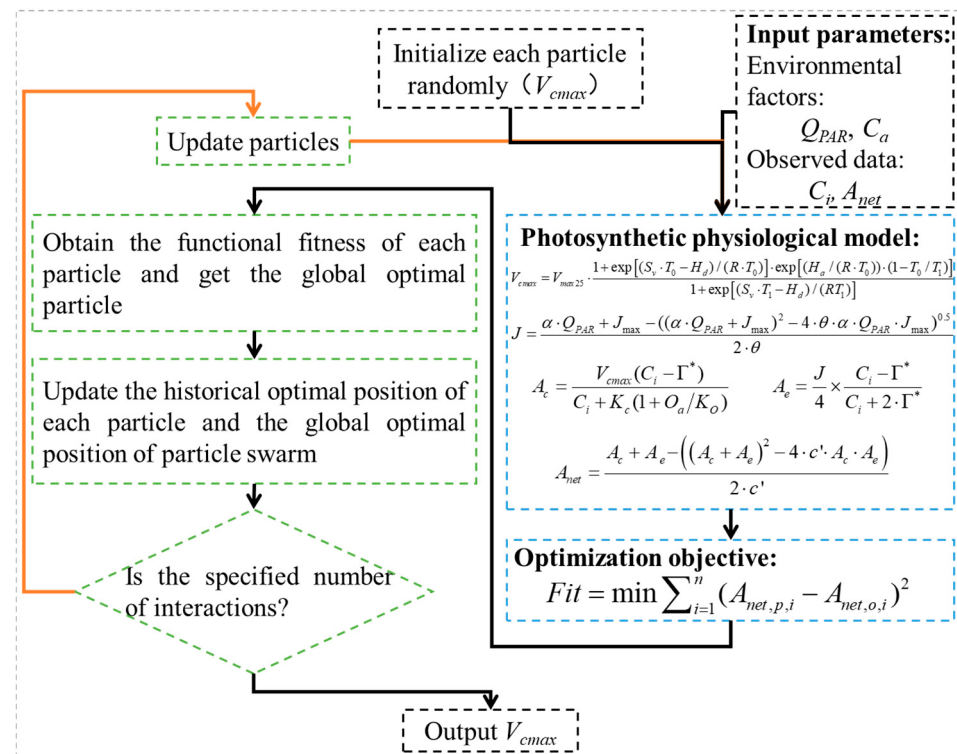
$$A_e = \frac{J}{4} \times \frac{C_i - \Gamma^*}{C_i + 2\Gamma^*} \quad (4)$$

$$J = \frac{\alpha \cdot Q_{PAR} + J_{max} - ((\alpha \cdot Q_{PAR} + J_{max})^2 - 4 \cdot \theta \cdot \alpha \cdot Q_{PAR} \cdot J_{max})^{0.5}}{2 \cdot \theta} \quad (5)$$

where  $J$  is the electron transport rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), the value of  $\alpha$  is 0.3 [30],  $Q_{PAR}$  = PPFD,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ),  $J_{max}$  is the maximum electron transport rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) calculated by  $J_{max25}$  and  $T_{leaf}$  [32], and the  $J_{max25}$  was used as a fixed ratio to  $V_{cmax25}$  ( $J_{max25} = 1.67V_{cmax25}$ ).  $\theta$  represents the curvature of the light response curve, and the value was taken to be 0.9 [30,33]. The computational process of  $V_{cmax}$  is shown in Figure 2.

### 2.4. Statistical Analysis

Microsoft Excel 2021 and OriginPro 2025 were used for data processing and graphical visualization. All statistical analyses were performed using IBM-SPSS Statistics (version 20). A one-way analysis of variance (ANOVA) was performed to examine differences among treatments, and Dunn's test was applied to evaluate significant differences at the 0.05 probability level.



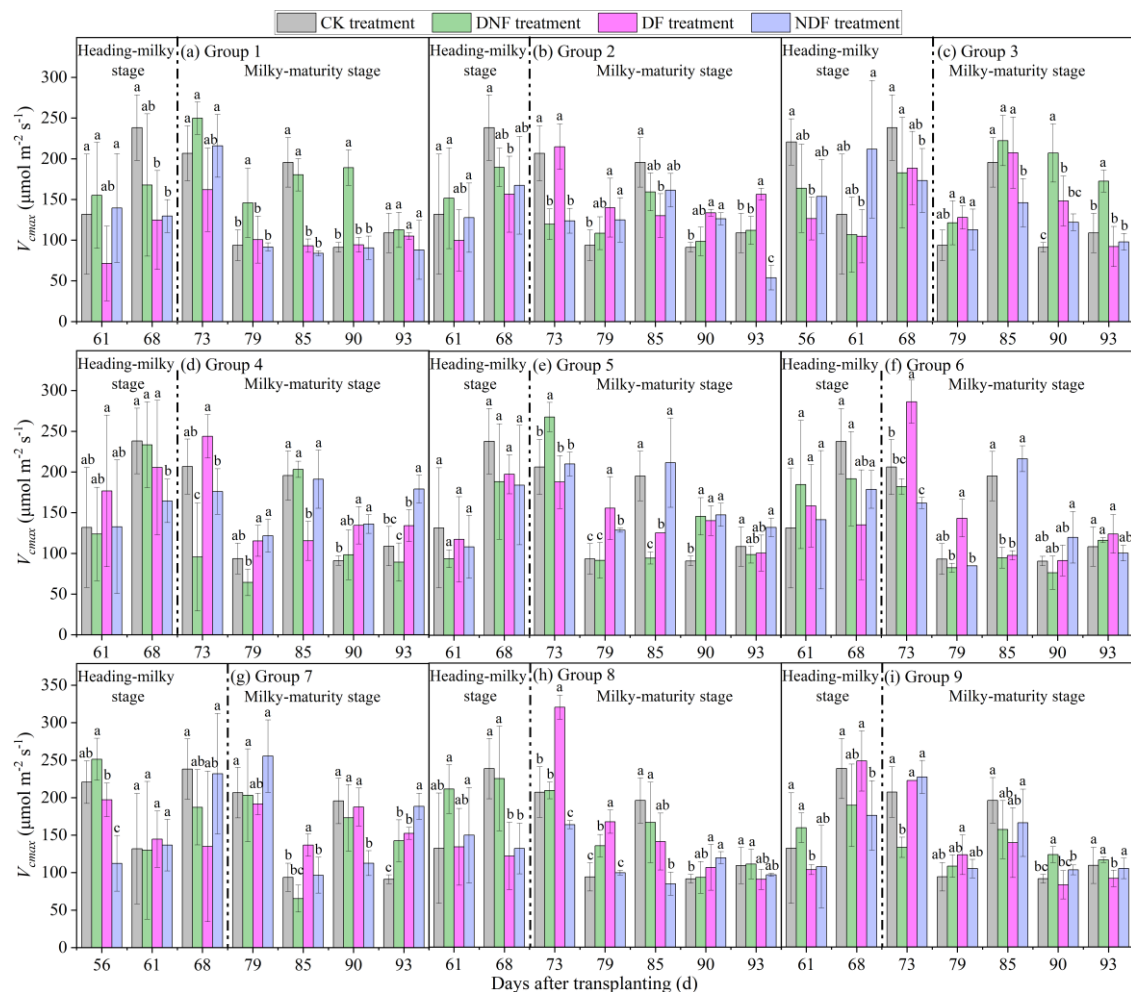
**Figure 2.** A schematic showing the calculation process for  $V_{cmax}$ . The particle swarm algorithm (PSO) was used to extract the maximum carboxylation rate ( $V_{cmax}$ ) using the observed intercellular  $CO_2$  concentration and photosynthetic rate ( $A_{net}$ ).

### 3. Results

#### 3.1. Effect of DF Events on $V_{cmax}$ of Rice

##### 3.1.1. Recovery Characteristics of Rice $V_{cmax}$ After Drought and Flood Stresses

Figure 3 illustrates the dynamic changes in maximum photosynthetic carboxylation rate ( $V_{cmax}$ ) under different drought and flooding stress during the two-year experiments. The dynamic changes in rice  $V_{cmax}$  during the various stress treatments closely resembled those in the Control (CK) treatment. In both cases,  $V_{cmax}$  increased at the heading–milky stage, then declined at the milky–maturity stage, with higher values observed at the heading–milky stage and lower values at the milky–maturity stage. Shortly after the drought–flood abrupt alternation (DF) events,  $V_{cmax}$  in most DF treatments was lower than that in the CK treatment, indicating an initial inhibitory effect of DF stress on rice  $V_{cmax}$ ; however,  $V_{cmax}$  gradually recovered, and by the milky–maturity stage, it was higher in rice exposed to DF stress compared to CK treatment, suggesting that the rice  $V_{cmax}$  was able to fully recover or even showed a compensatory effect. Notably, rice  $V_{cmax}$  recovered more rapidly following DF treatments when mild drought preceded flooding stress (Figure 3g–i). For example, in DF7, DF8, and DF9 treatments, rice  $V_{cmax}$  was higher than the values in CK treatment at 61 days, 61 days, and 68 days after transplanting, respectively. In contrast, the  $V_{cmax}$  of rice with preceding moderate or severe drought recovered more slowly after DF stress, with most DF treatments (e.g., DF1, DF2, DF3, DF5, DF6 treatments) showing higher  $V_{cmax}$  only after the milky–maturity stage. This result indicated that the severe drought stress negatively affected the recovery of rice  $V_{cmax}$  after experiencing DF stress.

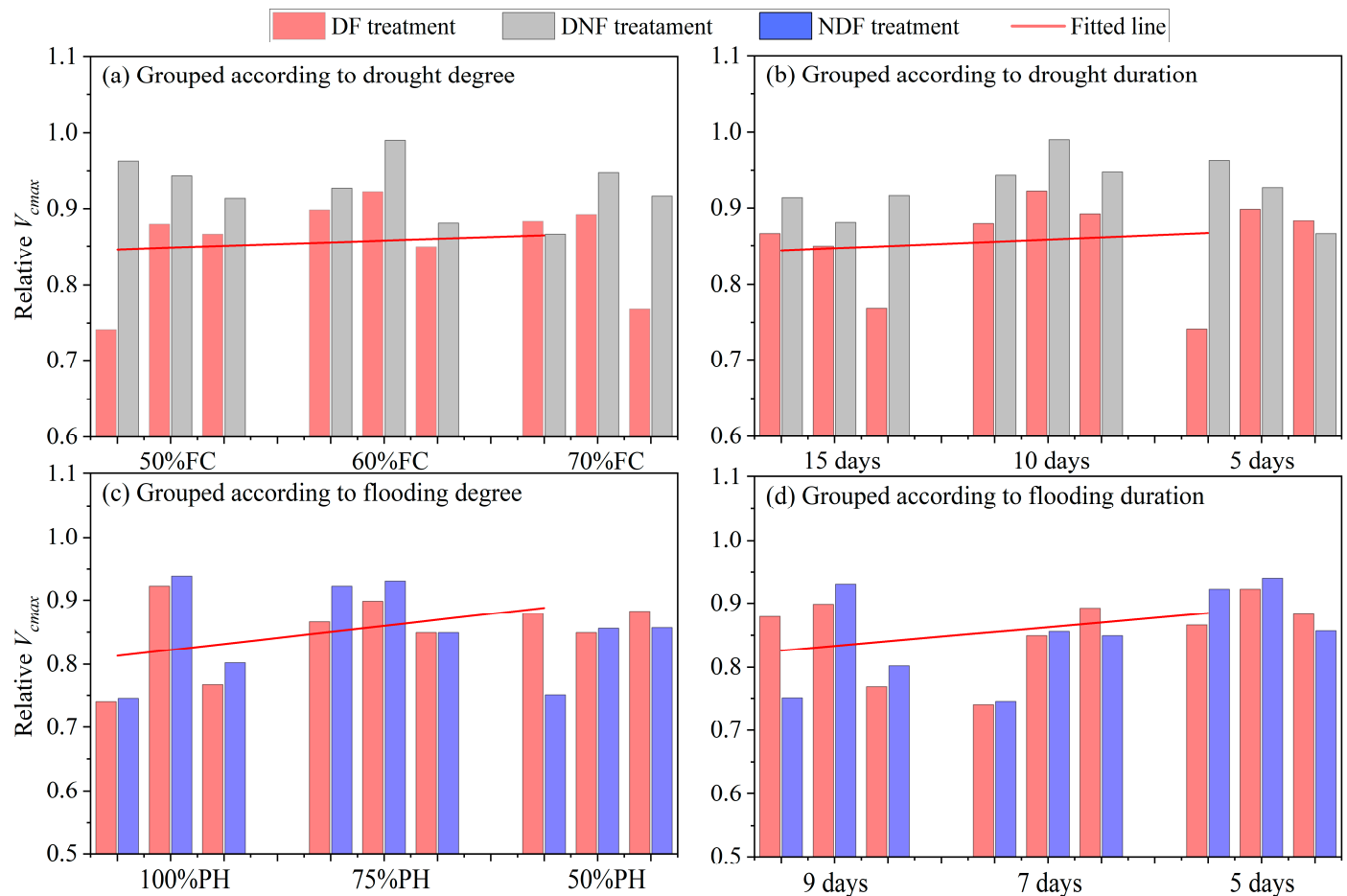


**Figure 3.** Dynamic changes in the maximum carboxylation activity ( $V_{cmax}$ ) of rice under different stress treatments in 2017 and 2018. Figure (a–i) correspond to Groups 1–9, respectively. Within each group, values are presented for the three treatments: DNF, DF, NDF, respectively. CK, control treatment; DF, drought–flood abrupt alternation; DNF, drought stress; and NDF, flooding stress. DF1, 50%FC + 5 d + 100% PH + 7 d; DF2, 50%FC + 10 d + 50% PH + 9 d; DF3, 50%FC + 15 d + 75%PH + 5 d; DF4, 60%FC + 5 d + 75% PH + 9 d; DF5, 60%FC + 10 d + 100%PH + 5 d; DF6, 60%FC + 15 d + 50% PH + 7 d; DF7, 70%FC + 5 d + 50% PH + 5 d; DF8, 70%FC + 10 d + 75% PH + 7 d; DF9, 70%FC + 15 d + 100%PH + 9 d. Each data point represents the mean of three replicates at each observation time, and the error bars represent the standard deviation (SD). Different lowercase letters (a, b, c) indicate significant differences among treatments ( $p < 0.05$ ). Shared letters (e.g., ab or bc) indicate no significant difference between treatments ( $p > 0.05$ ).

### 3.1.2. Role of Drought and Flooding Stress Degrees and Durations on $V_{cmax}$

To investigate the effects of different stress treatments on rice  $V_{cmax}$ , this study analyzed  $V_{cmax}$  in relation to the degree and duration of drought and flooding stress (Figure 4). It was found that the relative  $V_{cmax}$  of rice showed an elevated trend, with the slopes of different DF treatments above zero. This indicates that as the degree of preceding drought or subsequent flooding diminished and the stress duration shortened, the damaging effect of DF treatment decreased. In terms of drought stress, rice  $V_{cmax}$  in all DF treatments (across various drought degrees and durations) was lower than in the corresponding drought (DNF) treatments in both 2017 and 2018, demonstrating that subsequent flooding in DF stress generally exacerbated rice  $V_{cmax}$ . In contrast, rice  $V_{cmax}$  was generally lower in DF treatments with 100%PH, 75%PH, and flooding durations of 9 and 7 days compared to

flooding (NDF) treatments. Interestingly, in the cases with mild flooding stress, rice  $V_{cmax}$  in DF treatments was higher than in NDF treatments, indicating that the preceding drought was effective in mitigating rice  $V_{cmax}$  in rice that experienced subsequent mild flooding stress. The results suggest that flooding following drought exerts a stronger negative impact on leaf photosynthesis than the preceding drought, and that exposure to an appropriate level of drought can enhance rice resilience to flooding stress.

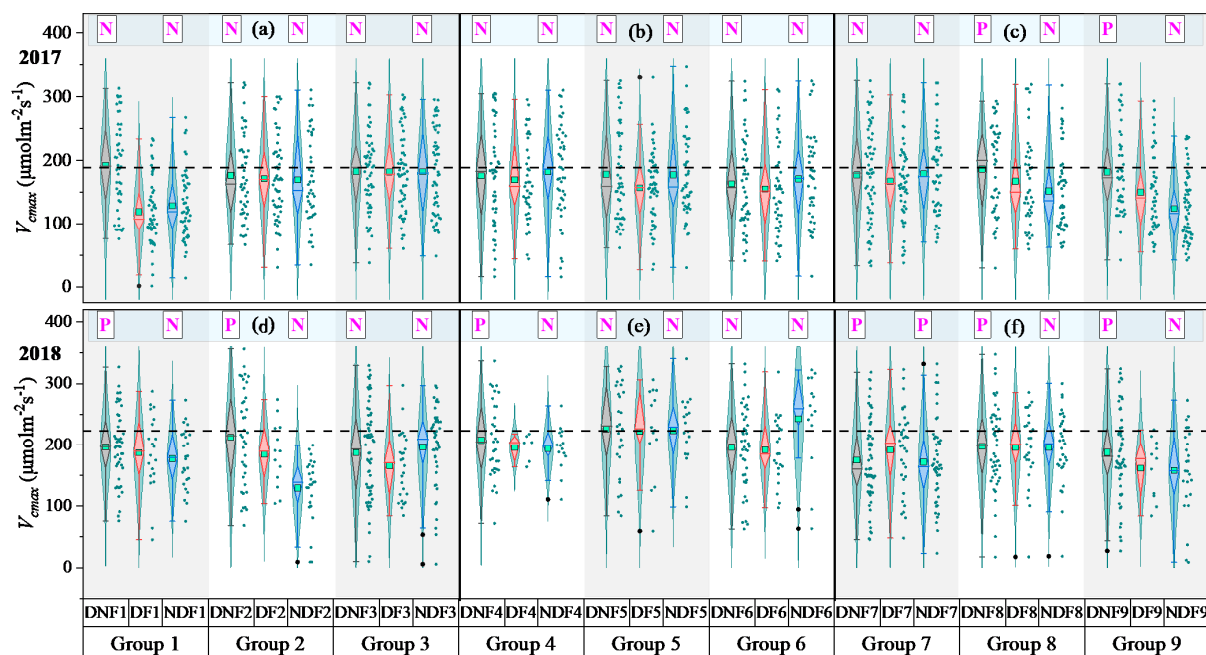


**Figure 4.** The relative maximum photosynthetic carboxylation rate ( $V_{cmax}$ ) under DF treatments at different drought degrees, flooding degrees, drought durations, and flooding durations in 2017 and 2018. Each data in the figure represents the average value of all measurements for two years of each treatment. FC and PH represent field capacity and plant height, respectively. The red line denotes the fitted regression trend between  $V_{cmax}$  under DF treatment and the corresponding stress factors.

### 3.2. Interactions Between Drought and Flooding on Rice $V_{cmax}$ in DF Events

The analysis in Figure 3 revealed that rice maximum photosynthetic carboxylation rate ( $V_{cmax}$ ) exhibited both inhibition and recovery phenomena following DF stress. The DF treatment, involving a sequence of preceding drought followed by subsequent flooding, produced a more complex interaction effect on rice  $V_{cmax}$ , as illustrated in Figure 5. The interaction between different degrees of drought and flooding had different impacts on rice  $V_{cmax}$ . In most DF treatments with preceding severe or moderate drought, rice  $V_{cmax}$  was consistently lower than in treatments with either drought or flooding treatments in both years, indicating that  $V_{cmax}$  was lower under DF treatments with preceding moderate and severe drought. These results suggest that preceding moderate or severe drought in DF treatments exacerbated the subsequent-flooding-induced  $V_{cmax}$  inhibition (e.g., Group 3 and Group 4 in Figure 5a,b), and thus limited photosynthetic capacity re-

covery after DF stress. Furthermore, subsequent flooding stress with moderate and severe degrees in DF treatments intensified the damage to  $V_{cmax}$  in rice experiencing preceding drought stress (e.g., Group 1 and Group 5 in Figure 5d,e).



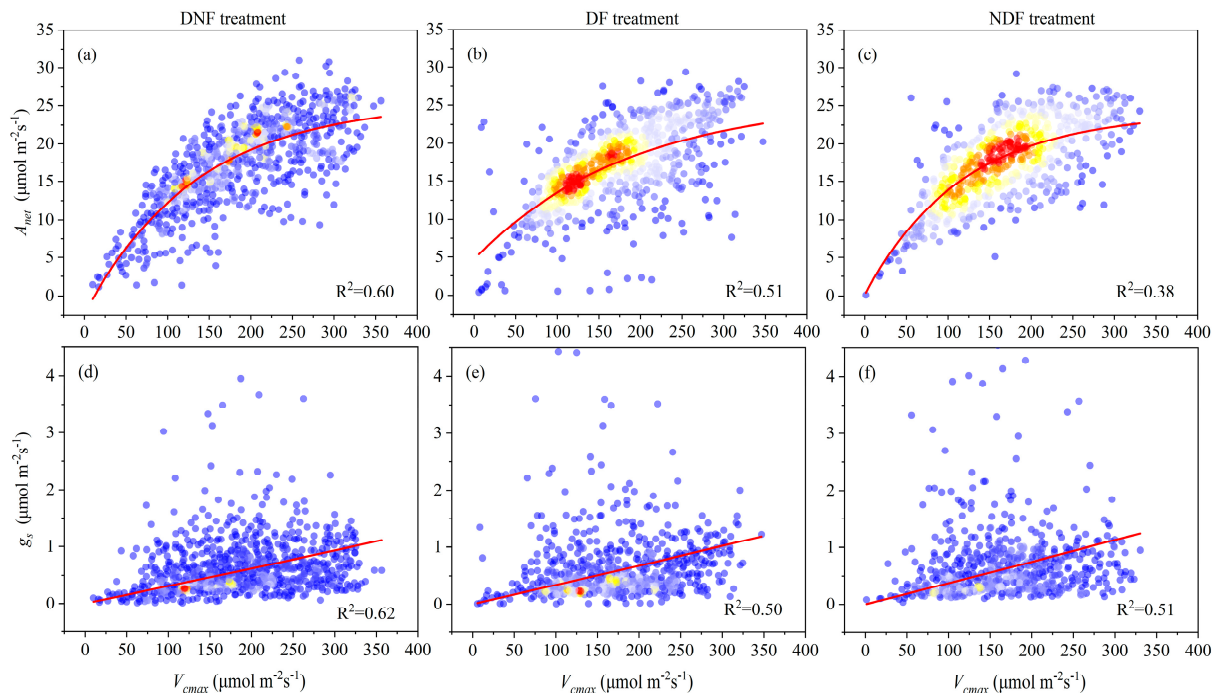
**Figure 5.** Effects of drought–flood abrupt alternation (DF) events on rice maximum carboxylation activity ( $V_{cmax}$ ) compared to single drought (DNF) or flooding (NDF) treatments in 2017 and 2018. Figure (a,d) show the treatment effects on  $V_{cmax}$  for Groups 1–3; Figure (b,e) correspond to Groups 4–6; and Figure (c,f) correspond to Groups 7–9. N and P indicate the effects of drought (flooding) on flooding (drought) in DF events. Specifically, N and P represent negative and positive effects, respectively. Groups 1–3: DF treatments with severe drought in the early stage, Groups 4–6: moderate drought in the early stage, and Groups 7–9: mild drought in the early stage. Similarly, Group 1, Group 5, and Group 9 are DF treatments with severe flooding in the later stages; Group 3, Group 4, and Group 8 are DF treatments with moderate flooding in the later stages; and Group 2, Group 6, and Group 7 are DF treatments with mild flooding in the later stages.

In DF treatments, mild preceding drought resulted in a more intricate interaction between drought and flooding on  $V_{cmax}$  compared to DF events with moderate or severe drought (Figure 5c,f), showing a coexistence of synergistic and antagonistic effects. In these cases, rice  $V_{cmax}$  treated with DF stress was intermediate between the treatments with mild drought and flooding stress (e.g., Group 8 and Group 9 in both years). The inhibitory effect of pre-mild drought in DF stress on  $V_{cmax}$  in rice experiencing subsequent flooding stress was reduced, promoting  $V_{cmax}$  recovery. This result indicates an antagonistic effect of mild drought, suggesting that mild drought before flooding can mitigate the negative impact of flooding stress. However, the subsequent flooding stress exacerbated the inhibitory effect of  $V_{cmax}$  in rice experiencing mild drought, demonstrating a synergistic effect between subsequent flooding and preceding drought on rice  $V_{cmax}$ .

### 3.3. The Interrelation Between $V_{cmax}$ and Intrinsic Water Use Efficiency in Different Treatments

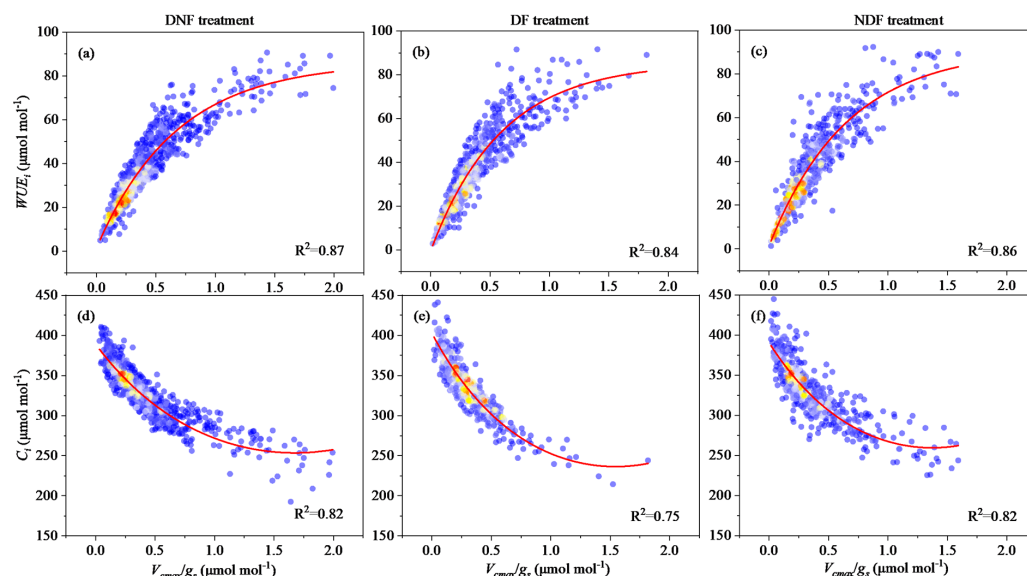
At the leaf level, intrinsic water use efficiency ( $WUE_i$ ), calculated by the ratio between photosynthesis and transpiration (stomatal conductance), is a key measure of the water use efficiency of rice. Additionally, intercellular  $CO_2$  concentration ( $C_i$ ) is a key parameter reflecting the trade-off between photosynthesis and water use of plants under varying environmental conditions. After exposure to drought and flooding stress, interactions were

observed between different rice gas exchange parameters and photosynthetic carboxylation rate (Figure 6). A significant curvilinear relationship between rice maximum photosynthetic carboxylation rate ( $V_{cmax}$ ) and  $A_{net}$  ( $R^2$  of 0.60, 0.51, and 0.38 for flooding (DNF), drought–flood abrupt alternation (DF), and drought (NDF) events, respectively). As  $V_{cmax}$  increased, rice  $A_{net}$  also increased under different water stress treatments, eventually reaching a gradual tendency to photosynthesis saturation. In contrast,  $g_s$  showed a correlation with  $V_{cmax}$ , with  $R^2$  of 0.62, 0.50, and 0.51 for DNF, DF, and NDF treatments, respectively.

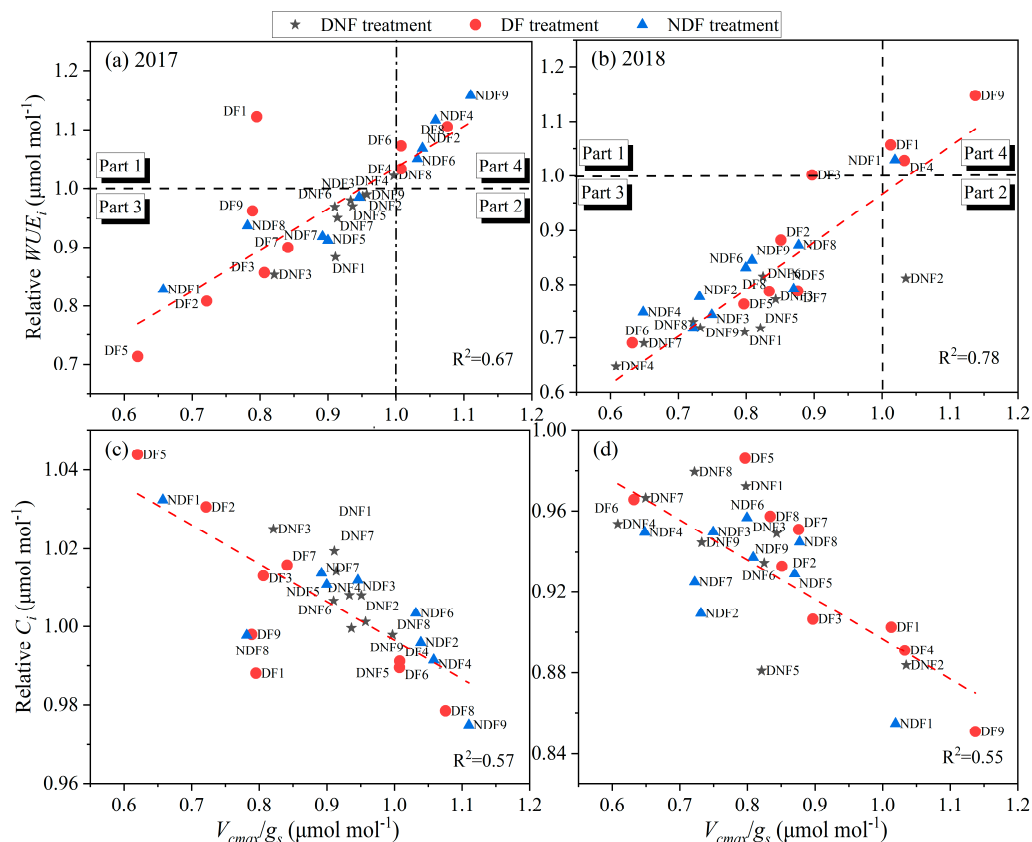


**Figure 6.** Observed relationship between gas exchange parameters ( $A_{net}$  and  $g_s$ ) and the maximum carboxylation activity ( $V_{cmax}$ ) in different treatments according to the datasets measured in 2017 and 2018. Figure (a–c) show the correlation between  $A_{net}$  and  $V_{cmax}$  under the DNF, DF, and NDF treatments, respectively. Figure (d–f) show the corresponding correlation between  $g_s$  and  $V_{cmax}$ . In this figure, DNF represents all the groups that experienced drought stress, including DNF 1–9; NDF represents all groups that experienced flooding stress, including NDF 1–9; and DF represents all groups experienced drought–flood abrupt alternation, including DF 1–9.

Photosynthesis is primarily determined by photosynthetic capacity (e.g., photosynthetic carboxylation capacity,  $V_{cmax}$ ), which indirectly regulates rice water use efficiency. For this purpose, this study further examined the response relationship between intrinsic water use efficiency ( $WUE_i$ ),  $C_i$ , and  $V_{cmax}/g_s$  in rice undergoing DF stress treatments (Figure 7). The analysis revealed that rice showed the strongly curvilinear relationship between  $WUE_i$  and  $V_{cmax}/g_s$  after experiencing drought, flooding, and DF treatments (Figure 7a–c,  $R^2$  of 0.87, 0.84, and 0.86 for DNF, DF, and NDF treatments, respectively). As  $V_{cmax}/g_s$  increased, rice photosynthetic rate improved, leading to higher  $WUE_i$  and lower  $C_i$ . Moreover,  $WUE_i$ ,  $C_i$ , and  $V_{cmax}/g_s$  showed strong correlations across the different drought and flooding treatments, with higher  $WUE_i$  observed in treatments with higher  $V_{cmax}/g_s$  (Figure 8). DF4, DF6, and DF8 treatments in 2017 and DF1, DF4, and DF9 treatments in 2018 showed higher  $WUE_i$  and  $V_{cmax}/g_s$  values compared to the CK treatment (Part 4 in Figure 8), while the  $C_i$  was lower in these treatments.



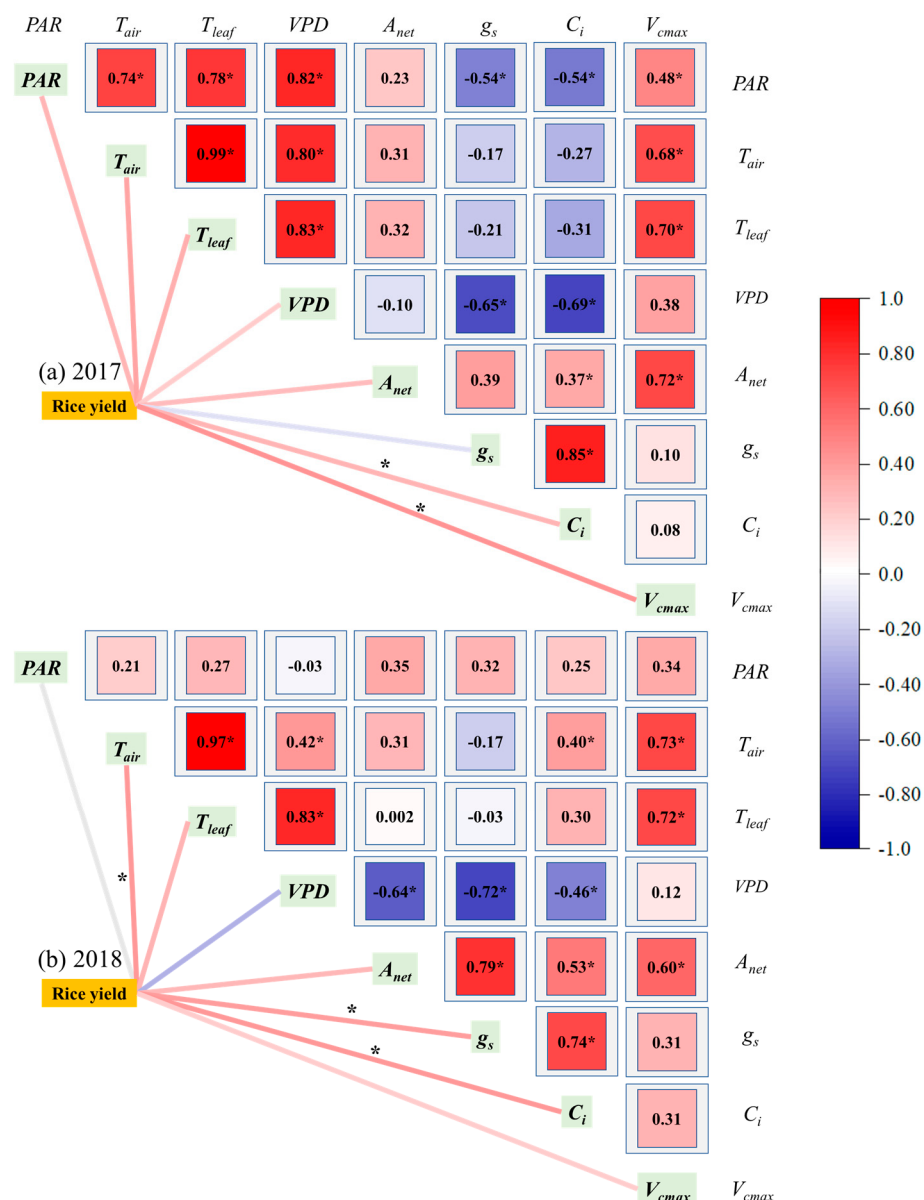
**Figure 7.** Observed relationship between the intercellular  $CO_2$  concentration ( $C_i$ ) and intrinsic water use efficiency ( $WUE_i$ ) and the ratio of the maximum carboxylation Rubisco activity ( $V_{cmax}$ ) to stomatal conductance ( $g_s$ ). Figure (a–c) show the correlation between  $WUE_i$  and  $V_{cmax}/g_s$  under the DNF, DF, and NDF treatments, respectively. Figure (d–f) show the corresponding correlation between  $C_i$  and  $V_{cmax}/g_s$ . In this figure, DNF represents all the groups that experienced drought stress, including DNF 1–9; NDF represents all groups that experienced flooding stress, including NDF 1–9; and DF represents all groups that experienced drought–flood abrupt alternation, including DF 1–9.



**Figure 8.** Relationship between intrinsic water use efficiency ( $WUE_i$ ) and the ratio of photosynthetic carboxylation capacity ( $V_{cmax}$ ) to stomatal conductance ( $g_s$ ) in rice experiencing different drought and flooding stresses. Each data in the figure represents the average value of all measurements for each treatment. Figure (a,c) is the observed data from 2017. Similarly, Figure (b,d) is the observed data from 2018.

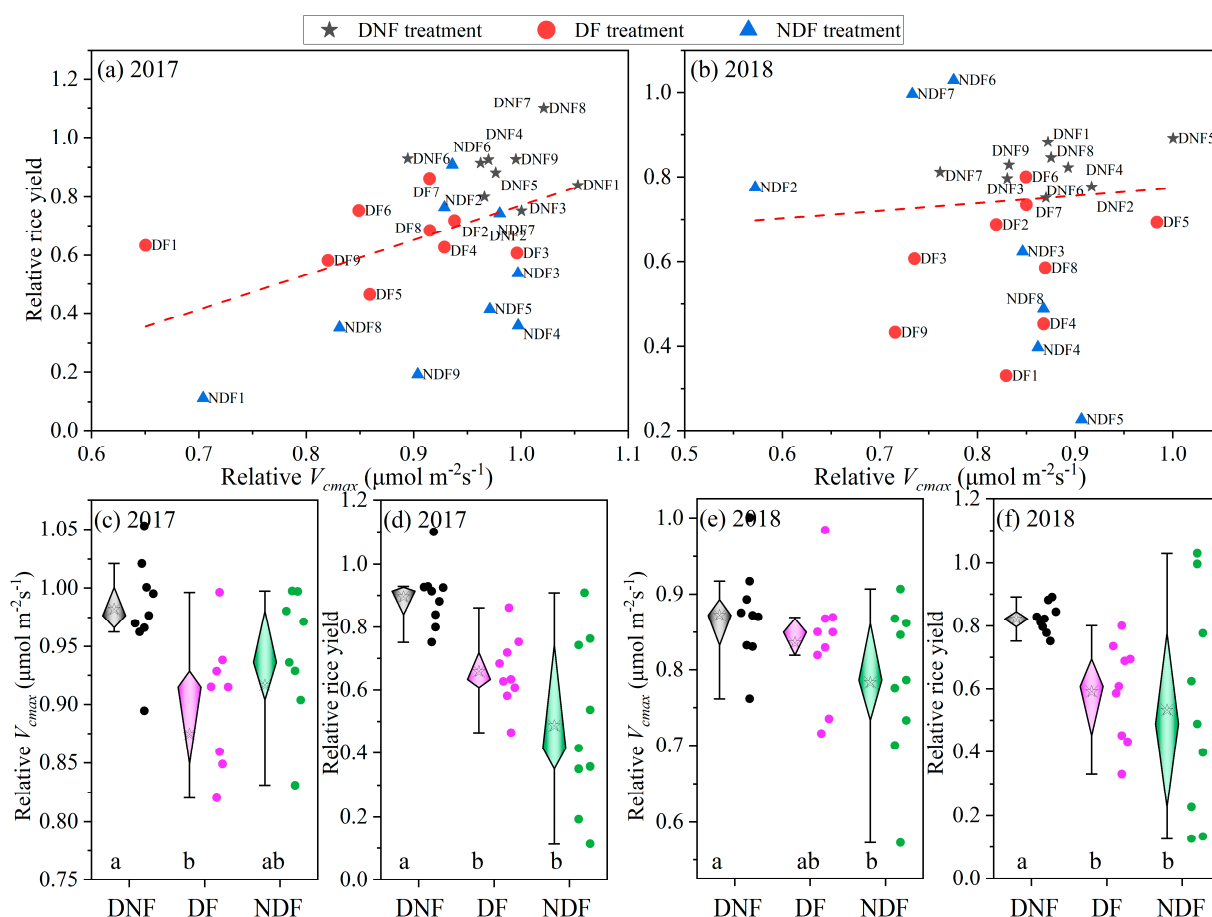
### 3.4. Correlation of Rice Yield with Key Biotic and Abiotic Factors

Pearson correlation analysis was used to explore the relationships between rice yield and key biotic and abiotic factors. As shown in Figure 9, rice maximum photosynthetic carboxylation rate ( $V_{cmax}$ ) was positively correlated with photosynthetic active radiation (PAR), atmospheric temperature ( $T_{air}$ ), leaf temperature ( $T_{leaf}$ ), and photosynthetic rate ( $A_n$ ) ( $p < 0.05$ ). Rice yield was influenced by a combination of key abiotic and biotic factors (Figure 9). In the 2017 experiment, rice yield showed a significant positive correlation with  $C_i$  and  $V_{cmax}$ , and a negative correlation with stomatal conductance ( $g_s$ ). In contrast, the 2018 experiment showed a significant positive correlation with  $g_s$  and  $C_i$  ( $p < 0.05$ ), and an insignificant and positive correlation with  $V_{cmax}$  ( $P > 0.05$ ). These results suggest that while increased  $V_{cmax}$  investment may positively influence rice yield, its effects can be modulated by environmental and growth conditions.



**Figure 9.** Correlation analysis of rice yield with key biotic and abiotic factors. Figure (a,b) represent the results for 2017 and 2018, respectively. “\*” indicates a significant correlation between both parameters.

Further analysis of yield differences under various drought and flooding stress treatments revealed that high  $V_{cmax}$  investments in DF4, DF6, and DF8 treatments in 2017, and DF1, DF4, and DF9 treatments in 2018 did not lead to improved rice yield. These treatments resulted in relatively low yields (0.69 and 0.40 of the two-year CK treatments). Additionally, rice under drought, flooding, or DF treatments showed no significant difference in  $V_{cmax}$  (Figure 10c,e). This result suggests that although increased  $V_{cmax}$  investment in response to DF and flooding stress improved water use efficiency ( $WUE_i$ ) at the leaf level, it did not necessarily translate into improved water use efficiency at the whole-plant level (Figure 10). Differently, the relative rice yield in drought treatments were higher than those of flooding and DF stresses across both years, with the rice yield in most DF treatments between the DNF and NDF treatments in 2017 (Figure 10a). This result suggests that the preceding drought can largely mitigate the loss of rice yield caused by subsequent flooding stress, confirming the antagonistic effect of the preceding drought on rice yield under DF treatments. Meanwhile, subsequent flooding treatments showed lower relative rice yield than drought stress, indicating that subsequent flooding in DF treatments enhanced the detrimental effect of the preceding drought on yield. Therefore, the preceding drought is probably beneficial for yield of rice experiencing subsequent flooding stress at relatively low  $V_{cmax}$ , while subsequent flooding stress exacerbated the reduction in yield of rice experiencing preceding drought stress.



**Figure 10.** Relationship between rice yield and the photosynthetic carboxylation capacity ( $V_{cmax}$ ) in rice experiencing different drought and flooding stresses. Each data in Figure (a,b) represents the average value of all measurements for each treatment. Each data point in Figure (c–f) represents the mean value calculated from all corresponding treatments. Different lowercase letters (e.g., a, b) indicate significant differences among treatments ( $p < 0.05$ ). Shared letters (e.g., ab) indicate no significant difference between treatments ( $p > 0.05$ ).

## 4. Discussion

### 4.1. Effects of DF Stress on Photosynthetic Carboxylation Capacity ( $V_{cmax}$ )

During the reproductive stage, rice photosynthetic capacity adapts continuously in response to environmental changes [8]. In this study, most drought–flood abrupt alternation (DF) treatments showed an initial reduction in photosynthetic carboxylation capacity following drought and flooding stresses, followed by a recovery in photosynthetic capacity (Figure 3). DF treatments with rice experiencing pre-mild drought showed compensatory effects, with  $V_{cmax}$  surpassing that of the control (CK) treatment at the milky stage. Similar compensatory responses have been reported previously, where rice photosynthesis recovered or even exceeded control levels after drought and flooding stress [25,34]. In contrast, this study also shows that when severe drought occurred before flooding,  $V_{cmax}$  exhibited limited recovery. These findings align with a study by Lu et al. [35], which found that severe drought in the preceding stage of DF stress significantly inhibited the leaf growth of rice (e.g., the maximum decrease in leaf area was 64.45%), which negatively affected the restoration efficiency of photosynthetic capacity. Moreover, chlorophyll content in leaves decreased remarkably, impairing leaf photosynthesis [34], which further restricted light energy capture during photosynthesis, Yu et al. [36], with reductions in light energy conversion efficiency ( $F_v/F_m$ ) and non-photochemical quenching coefficient ( $q_p$ ) by 2.59% and 6.92%, respectively. These reported mechanisms support the observation in this study that DF treatments with pre-severe drought exhibited prolonged photosynthetic limitations.

DF treatments preceded by mild drought showed greater recovery in stomatal opening and photosynthetic rate than those preceded by moderate or severe drought [25]. This can be attributed to the fact that appropriate water stress can enhance rice root development. A more active root system ensures a better supply of water and nutrients, supporting stomatal opening and photosynthesis [37]. Consequently, rice experiencing DF stress with pre-mild drought showed quicker compensation of photosynthetic physiological processes, facilitating the recovery of photosynthetic carboxylation capacity (Figure 3g–i). Furthermore, an active root system helps maintain the water balance within the plant, supporting stomatal opening and enhancing photosynthetic efficiency [38]. Hence, this study highlights that rice exposed to DF stress with pre-mild drought is able to recover photosynthetic capacity more effectively, showing compensatory effects after the stress period.

### 4.2. Interactions Between Drought and Flooding on $V_{cmax}$

The interaction effects of preceding drought and subsequent flooding on rice  $V_{cmax}$  differed across different DF treatments in 2017 and 2018 experiments (Figure 5). Overall, the damage to rice  $V_{cmax}$  in DF treatments was less pronounced as the intensity and duration of both drought and flooding stresses decreased (Figure 4). In particular, subsequent flooding in DF treatments commonly exacerbated the damage to  $V_{cmax}$  in rice subjected to drought stress. All degrees of flooding (i.e., mild, moderate, and severe) stresses demonstrated a synergistic impact on  $V_{cmax}$  induced by drought stress. These findings suggest that rice, when subjected to drought stress followed by flooding, experience greater photosynthetic inhibition, with the preceding drought intensifying the negative effects of flooding on  $V_{cmax}$ . This result is consistent with previous studies on rice, which also found reduced photosynthetic capacity when rice underwent flooding stress [3]. Liu et al. [3] reported that rice subjected to both drought and DF treatments exhibited lower photosynthetic carboxylation capacity than those experiencing only flooding or drought stress. The synergistic damage caused by flooding stress is primarily attributed to the hypoxic or anaerobic conditions that alter the intracellular biochemical reactions in leaf cells under flooding conditions [34,39]. Under low oxygen conditions, pyruvate can be converted to lactate or ethanol in the cytosol,

constraining the Calvin–Benson cycle and inhibiting photosynthesis [21]. Furthermore, Guo et al. [40] found that severe flooding in DF treatments significantly increased leaf malondialdehyde content by more than 35%, accelerating lipid peroxidation in the leaf membrane, leading to leaf senescence and a significant reduction in photosynthetic rates. In addition to limiting the oxygen of rice, excess water under flooding stress also disrupts the osmotic function of leaf cells, damages chlorophyll structure, reduces photosynthetic pigment concentration, and disrupts the photosynthetic electron transport system [41,42], further hindering photosynthetic carboxylation reaction process.

In contrast, the effect of preceding drought in DF treatments on  $V_{cmax}$  in rice subjected to subsequent flooding depended on the degree of drought stress. Moderate and severe drought stress showed a synergistic damaging effect on  $V_{cmax}$  in rice experiencing subsequent flooding stress (Groups 1 to 6 in Figure 5a,b,d,e). This exacerbation was confirmed by the analysis of photosynthetic rate, which showed a significant correlation with  $V_{cmax}$  ( $R > 0.51$ ). Similarly, Zhu et al. [25], found that preceding drought stress worsened the impact of flooding stress on rice photosynthetic rate. The synergistic effect was linked to changes in root vigor and plant water status induced by severe drought stress. Huang et al. [23] reported that the pre-severe drought exacerbated the reduction in root vigor before flooding stress, and the rice growth (e.g., root length and volume) was restricted, which was not conducive to water uptake by rice root. The low water supply of rice root resulted in maintaining leaf water status at low level, contributing to the reduction/closure of leaf stomatal openings [43]. More importantly, drought suppresses photosynthesis through both stomatal and non-stomatal limitations. Reduced stomatal conductance restricts  $CO_2$  availability, diminishing Rubisco carboxylation efficiency. Prolonged stress impairs the photosynthetic electron transport chain, such as causing the decoupling of D1 protein and negatively influencing photolysis and downstream biochemical reactions [42,44,45]. Although water is a substrate for light reactions, drought rarely inhibits photosynthesis primarily by limiting water-splitting at PSII. Instead, the dominant impacts are restricted  $CO_2$  diffusion and impaired biochemical processes downstream of the electron transport chain (Equations (2) and (4)), ultimately resulting in a compromised photosynthetic capacity [46].

Unlike severe and moderate drought, the pre-mild drought alleviated the damage effect on  $V_{cmax}$  induced by subsequent flooding stress, which showed antagonistic effects (Group 8 and 9 in Figure 5). This antagonistic effect was mainly attributed to the limitation effect of pre-mild drought stress on rice was lower than those of other stress treatments. A previous study has shown that the pre-mild drought in DF treatment was antagonistic to rice hydraulic efficiency induced by subsequent flooding stress [3], which alleviated the damage of flooding stress on rice hydraulic transport capacity. Maintaining relatively high hydraulic efficiency in rice experiencing DF stress facilitates the maintenance of leaf water status [47]. Lu et al. [35] found that there was no significant difference between the leaf water content of rice in the DF treatment with pre-mild drought, and the CK treatment, i.e., a normal hydraulic transport system was maintained. The normal level of leaf water content promoted leaf stomatal water–carbon exchange and photosynthesis in rice experiencing DF stress [47], which allowed rice to achieve photosynthetic carbon assimilation at a relatively high  $V_{cmax}$ . The antagonistic effects on  $V_{cmax}$  found in this study, contrary to those reported by Xiong et al. [24] for photosynthetic enzymes, verify the antagonistic effects found in Liu et al. [3] and Zhu et al. [25] for rice hydraulic efficiency, photosynthetic capacity, and gas exchange parameters. Also, the DF events with mild drought promoted root and increased the ratio of root to crown [23], which are characteristics of flood-tolerant rice [48]. Thus, mild drought stress increased the flooding tolerance of rice and reduced the impairment of physiological states in rice experiencing DF events.

#### 4.3. Rice Improving Water Use by Investing in $V_{cmax}$

At the leaf level, intrinsic water use efficiency ( $WUE_i$ ) and intercellular  $CO_2$  concentration ( $C_i$ ) are key measures of the efficiency of rice in utilizing water resources and photosynthesis [49–51]. Maintaining high photosynthesis under water stress while improving  $WUE_i$  requires improving the biochemical capacity for  $CO_2$  assimilation, in which Rubisco characteristics ( $V_{cmax}$ ) play a crucial role [52]. The analysis in this study revealed that  $WUE_i$  increased with rising  $V_{cmax}/g_s$  in drought, flooding and DF treatments; however, the growth rates showed a decreasing trend, and  $C_i$  also showed a decreasing trend (Figure 7b,e). This finding suggests that the relative cost of rice inputs for  $V_{cmax}$  gradually increased with increasing  $WUE_i$ , but may lead to a decrease in photosynthetic potential. Similarly, various drought and flooding stresses demonstrated the same pattern, with increasing  $WUE_i$  and decreasing  $C_i$ , where some DF treatments (e.g., DF4, DF6, and DF8 treatment in 2017) exhibited higher  $WUE_i$  and lower  $C_i$  than the CK treatment (Figure 8). These results confirmed an important argument: concerning a reduction in water availability: rice experiences an apparent “over-investment” in  $V_{cmax}$ , which marginally enhances its  $WUE_i$ , but may constitute a decrease in the marginal gain of increasing  $V_{cmax}$  on  $WUE_i$ , thereby further affecting the balance between photosynthetic efficiency and water use efficiency. These findings are consistent with the conclusion of [52], who found that over-investment in photosynthetic capacity progressively diminishes the corresponding increases in  $A_n/g_s$  ( $WUE_i$ ), thereby causing greater imbalances between photosynthetic capacity and stomatal conductance.

Furthermore, rice subjected to drought and flooding stresses altered  $WUE_i$  by regulating  $V_{cmax}$ ; however, an increase in intrinsic water use efficiency at the leaf level may not necessarily result in an improvement in water use efficiency at the whole-plant level. In this study, it was found that the increase in  $V_{cmax}$  favored rice yield, but all of these DF treatments (e.g., DF4, DF6, and DF8 treatments in 2017) with higher  $WUE_i$  than CK treatment had relatively low rice yields (Figure 10b). This phenomenon occurs because, under different growth environments, the positive effects of increasing  $WUE_i$  may be counteracted by environmental and growth factors (Figure 9) that are not entirely independent of the manipulation of  $WUE_i$  [52]. Among these factors, enhanced leaf growth leading to greater self-shading within the canopy and increased rice respiration played dominant roles in limiting improvements in whole-plant water use efficiency through improved  $WUE_i$  [53,54]. Moreover, excessive increase in rice investment in  $V_{cmax}$  reduces the relative cost of  $g_s$ , decreases  $C_i$  (Figure 8), and negatively counteracts rice yield (Figure 9). Therefore, although rice increases its investment in  $V_{cmax}$  to enhance intrinsic water use efficiency ( $WUE_i$ ) under drought and flooding stress, the resulting physiological responses may, to some extent, constrain the further enhancement of rice’s productivity and water use efficiency under various climatic conditions.

## 5. Conclusions

In summary, the damage to rice induced by drought–flood abrupt alternation (DF) stresses gradually recovered by the end of the stress stage, although DF treatments involving severe drought stress hindered the recovery of photosynthetic carboxylation capacity ( $V_{cmax}$ ). The subsequent flooding stress of varying intensities in the DF treatments synergistically exacerbated the damage to rice  $V_{cmax}$  induced by the preceding drought stress. DF stresses altered rice  $V_{cmax}$  in distinct ways depending on drought severity. The pre-mild drought in the DF treatments had an antagonistic effect on  $V_{cmax}$  in rice experiencing subsequent flooding stress, while severe and moderate drought exacerbated the subsequent flooding-induced  $V_{cmax}$  inhibition. Although drought and flooding stresses temporarily improved  $WUE_i$  at the leaf level through increased  $V_{cmax}$  investment, this enhancement

did not translate into yield benefits. The preceding drought is probably beneficial for yield of rice experiencing subsequent flooding stress, particularly at lower  $V_{cmax}$  values, whereas subsequent flooding stress exacerbated the yield reduction in rice experiencing preceding drought stress. This study provides valuable insights into the physiological regulatory mechanisms of rice experiencing compound drought and flooding stress, and emphasizes that appropriate drought and flooding management may have potential optimizing effects on rice yield and water use, providing an important theoretical basis and practical guidance for paddy water management.

**Supplementary Materials:** The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agronomy15112573/s1>; Table S1 Experimental treatment in 2017 and 2018.

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