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CO_2 ENRICHMENT IMPROVES RECOVERY OF GROWTH AND PHOTOSYNTHESIS FROM DROUGHT AND NITROGEN STRESS IN MAIZE

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Abstract

In the context of the increasing risk of extreme drought as a result of climate change and increasing CO_2 in the future of northwest China, evaluation of crops' ability to recover and survive droughts requires further attention. To test the effects of re-watering on plants suffering water and nitrogen limitations in the presence of elevated CO_2, maize (*Zea mays*) was planted to experience combined elevated CO_2 (380 or 750 μmolmol^{-1}, climate chamber), water stress (15% PEG-6000) and nitrogen limitation (5 or 15mM N in Hoagland solutions) and then re-watered at three levels (300mL, 600mL, 900mL per pot of distilled water). When plants were re-watered, drought stressed and N limited plants with ambient CO_2 increased their water content more than that of elevated CO_2, while the enhancement of growth rate were negatively related to the increasing plant water content. Elevated CO_2 could help re-watered seedlings to have higher photosynthetic capacity (F_v/F_m, Φ_{PSII}, P_n,P_n/T_r and P_n/G_s) and new leaf growth under low water content, apart from nitrogen deficiency. The results demonstrated that elevated CO_2 could help drought stressed seedlings to maintain higher carbon assimilation rates under low water content, as a result to improve leaf water use efficiency.

Introduction

Projections of population growth and energy-use scenarios show that adverse global climatic changes exerts remarkable influence on CO_2 concentration (Solomon, 2007). Rises in ambient CO_2 and other greenhouse-effect gases are expected to cause global climate changes, including increases in air temperature and shifts of regional scale rainfall patterns, which lead to decreased soil water availability in some areas of the world. Elevated CO_2 probably lead to the suppression of plant N availability that limits the effect of CO_2 enrichment (Luo *et al*., 2004; Reich *et al*., 2006). How the interaction of drought, N limitation and increasing CO_2 affect plant production, community composition, and plant function remains unclear.

In an arid ecosystem, drought and re-watering can trigger plant productivity fluctuation (Reynolds *et al*., 2004; Yahdjian & Sala, 2006; Xu *et al*., 2009; Bakhsh *et al*., 2012). The influence of drought and re-watering on plant growth must be further investigated. Water stress induces soluble sugar accumulation in meristem (Dosio *et al*., 2011; Shinwari *et al*., 1998), reduces tissue expansion, induces substantial stomatal closure and decreases WUE (Xie *et al*., 2010; Khakwani *et al*., 2012; Hamayun *et al*., 2010). The role of metabolic restoration (such as Φ_{PSII} and F_v/F_m) and stomatal acclimation in photosynthetic recovery during re-watering is under debate. Many studies suggest that metabolic activity restoration contribute greatly to photosynthesis recovery after drought acclimation (Hu *et al*., 2010). But the key influence in photosynthesis recovery is stomatal acclimation (Galle *et al*., 2009; Hu *et al*., 2010). Hu *et al*., (2010) reported that ability of stomatal restoration from medium drought stress may be critical for C_3 perennial grass to resume photosynthetic capacity. Other reports showed that the role of G_s depends on species and severity of stress. Posch & Posch (2009) reported that photosynthesis potential of moderate stressed seedlings recovered quicker than severely stressed seedlings. Varone (2012) compared recovering capacities of seedlings and saplings of Mediterranean species, demonstrating that seedling P_n decreased slowly due to stomatal limitation and sapling P_n decreased fast, which is mainly associated with non-stomatal limitation. After seedlings and saplings were rewatered, P_n recovered and peaked within a few days, P_n of saplings recovered slowly and incompletely. Thus, the issue remains debatable and needs to be elucidated(Xu *et al.*, 2009).

Furthermore, recent climatic factors, such as increasing atmospheric CO_2, inevitably influence the cycles of elements, such as N, P and K (Hamayun *et al.*, 2011). Nitrogen is the main nutrient for crop production that frequently limits plant growth (Reich *et al.*, 2006). N deficiency reduces leaf N content, N use efficiency, and N reserves in response to elevated CO_2 (Dyckmans & Flessa, 2001; Watanabe *et al.*, 2011). Elevated CO_2 can increase N availability and N-remobilizing capacities of crops (Franzaring *et al.*, 2011; Kim *et al.*, 2011). In desert plants, elevated CO_2 decrease leaf nitrogen and leaf soluble protein concentrations under soil drought (Xu *et al.*, 2007). Changes in precipitation pattern and N cycling across the globe are likely to affect primary productivities and CO_2 exchanges of ecosystems, especially in arid and semi-arid areas. The relations between crop productivity and N availability have been extensively studied, although restoring capacities of N limited plant in drought and re-watering environments are not fully understood.

Predictions of future ecosystem functions and food supply by staple C_4 crops, such as maize, depend on the elucidation of the mechanisms by which environmental change and growing conditions interact to determine plant performance in the future. The main objective of this study was to investigate N and water limitation effects on plant recovery, water use efficiency, photosynthesis, and growth in CO_2 enriched environment.

Materials and Methods

Plant material and treatments: Maize (*Zea mays* L. cvs Zhengdan 958) seeds were obtained from the Northwest Agriculture and Forestry University (Yangling, Shaanxi, China), disinfected with 20% (w/v) sodium hypochlorite for 30 min to prevent fungal infection, rinsed with distilled water, and placed on moist filter paper at 30°C in a dark chamber for about 2 days. Seedlings were hydroponically cultured in the sand in two closed climate chambers (AGC-D001P, Qiushi Corp., China), which were programmed to have11 dark hours (18℃, RH50%) and13 light hours (25°C, RH40%, 300 μmol photons m^{-2} s^{-1} from 7:30 AM to 20:30 PM), by 1/2 modified Hoagland nutrient solution injected into the sand. When more than 80% of the seedlings fully expanded their first leaf, the two closed climate chambers were programmed have the CO_2 concentration of 380 or 750 μmolmol^{-1} CO_2 until the end of the study. When more than 80% of the seedlings expanded their third leaves, the seedlings were irrigated with 15% PEG-6000 (-0.4 M Pa) in the Hoagland solutions as well as different N solutions (5 mM N as the nitrogen deficiency treatment and 15 mM N as the control), and then the seedlings were allowed to grow for 25 d. The pH of Hoagland solutions was adjusted to be 5.8-6.2 with KOH and HCL. Every three days the Hoagland solutions were renewed with the used solutions washed away with 1000 mL distilled water, and new Hoagland solutions injected into the sand. On the 12th day under drought stress, the seedlings were rewatered at different levels. All relevant gas exchange and chlorophyll a fluorescence parameters of the seedlings were measured on the fifth day during their re-watering, and they were harvested on the sixth day during re-watering.

The experimental design was a completely randomized block design with four replications. The treatments are presented in Table 1.

Table 1. Treatments and abbreviations used in this study.

CO_2 concentration (μmolmol^{-1})	Treatments	Nitrogen status (mM)	Drought status	Re-watering levels (mL)	Abbreviation
380	Control	15	well watered	-	Control
	D (Drought)	15	15% PEG	-	D_0
				300	D_{300}
				600	D_{600}
				900	D_{900}
	N (N limitation)	5	well watered	-	N
	D-N (Drought + N limitation)	5	15% PEG	-	D_0-N
				300	D_{300}-N
				600	D_{600}-N
				900	D_{900}-N
750	Elevated CO_2	15	well watered	-	C
	C+D (Elevated CO_2 + Drought)	15	15% PEG	-	C+D_0
				300	C+D_{300}
				600	C+D_{600}
				900	C+D_{900}
	Elevated CO_2 + N limitation	5	well watered	-	C-N
	C+D-N (Elevated CO_2 + Drought + N limitation)	5	15% PEG	-	C+D_0-N
				300	C+D_{300}-N
				600	C+D_{600}-N
				900	C+D_{900}-N

Biomass measurements: At the harvesting time of the study, the plants of four pots were taken from each treatment, oven dried at 80°C to a constant weight and weighed.

Relative growth rate (RGR) was calculated by the formula of (Lindroth *et al.*, 2001):

$$\mathrm{RGR}(\mathrm{mg\,g^{-1}day^{-1}}) = \frac{\mathrm{Ln}_{\text{dry mass of } t_2} - \mathrm{Ln}_{\text{dry mass of } t_1}}{t_2 - t_1} \times 1000 \qquad (1)$$

In order to assess growth limitation during drought, the percentage of drought limitation (PDL) was estimated by the following formula (Xu *et al.*, 2009):

$$\mathrm{PDL}_{stress}(\%) = \frac{\mathrm{values}_c - \mathrm{values}_s}{\mathrm{values}_c} \times 100 \qquad (2)$$

In which, *value*$_c$ is growth traits (biomass or relative growth rate) of control plant (without experienced drought during the entire experimental period), and *value*$_s$ is growth traits (biomass or relative growth rate in plant experienced drought stress.

In order to assess re-watering stimulation of plant growth, the percentage of enhancement (PE) of growth traits (relative growth rate and water content) was estimated by the following formula：

$$\mathrm{PDL}_{re-watering}(\%) = \frac{\mathrm{values}_s - \mathrm{values}_c}{\mathrm{values}_c} \times 100 \qquad (3)$$

In which, *value*$_c$ is growth traits (relative growth rate or water content) of control plant (without experiencing drought during the entire experimental period), and *value*$_s$ is growth trait (relative growth rate or water content) of plants following re-watering.

Leaf gas exchange and chlorophyll a fluorescence measurements: The gas exchange parameters of the fully expanded leaves were measured with a portable open gas exchange system (*LI-6400*, *Li-Cor*, USA). The irradiation was provided with LED model *6400-02B* (*Li-Cor*). The gas entry was connected to a gas pole 3 m above ground. The open pathway was turned on to adjust the airflow rate to 0.5 cm^3 min^{-1}, and the cuvette temperature was approximately to air temperature.

The photochemical efficiency (F_v/F_m) and actual photochemical efficiency of photosystem II (Φ_{PSII}) of the fully expanded leaves were measured with an *FMS 2.02* pulse modulation fluorescence meter (*Hansatech*, King's Lynn, UK). The initial fluorescence (F_0) of the leaves was measured after 30 min dark-adaptation, and their maximal fluorescence (F_m) was measured after they received a strong flash (6000 μmol m^{-2} s^{-1}, 0.7 s pulse duration). After the steady-state fluorescence of the leaves were measured under natural irradiance (F_s), and their maximum fluorescence at adaptation to irradiance (F_m') was measured after their exposure to a strong flash. The photochemistry capacity of PSII were finally calculated by the formulae of F_v/F_m= (F_m -F_0)/F_m; Φ_{PSII}= (F_m' - F_s)/F_m' (Schreiber *et al.* , 1986). All the measurements were taken between 09:00 and 11:30.

Statistical analysis: Data from the independent experiments presented in figures are in the form of means ± standard deviation. Significance of differences were (at $p<0.05$) tested using SAS for Windows V8 (SAS Institute, Cary, NC, USA), and the differences among the treatments were tested by Duncan's multiple range test.

Results

Variations of plant growth rate: Figure 1A presents the PDL of the relative growth rates in the drought treatments. The PDL of RGR in leaf sheath significantly increased in C+D-N treatment. PDL of RGR significantly increased in leaves of D and D-N. Figure 1B presents D significantly increased the PDL of sheathes and leaves. C+D, C+D-N significantly increased the PDL of the roots.

The PE of different organs was measured on the 6th d during their re-watering (Fig. 2). The PE of RGR was significantly increased by re-watering except in D treatments. The PE of biomass significantly increased in leaves but decreased in roots and sheathes in D_{300}-N and D_{600}-N. The PE of biomass in C+D_{300}-N and C+D_{600}-N increased in leaves and sheathes.

Variations of plant water status: Figure 3A presents the water contents in the different treatments with different re-watering levels. In the treatments with the different re-watering levels, the water contents were higher at CO_2=380 μmolmol^{-1} than at CO_2=750 μmolmol^{-1}, respectively. The relationship between PE of RGR and PE of plant water content was fitted to a quadratic function [PE_{RGR}= 0.25+123.41PE_{WC}-4579.75$PE_{WC}{}^2$; R^2=0.7811, $p<0.01$, Fig 3B]. The PE of RGR gradually decreased as the PE of plant water content increased.

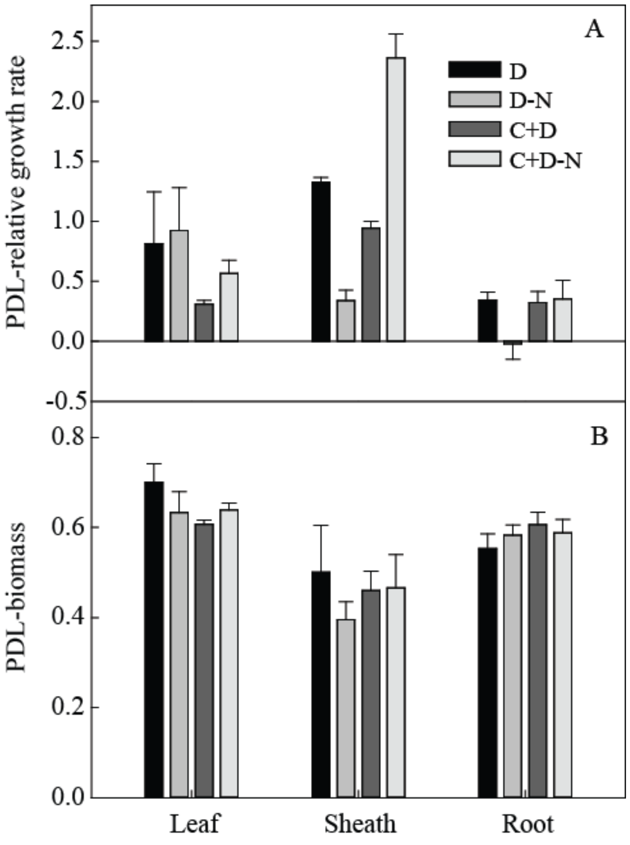

Fig. 1. (A)PDL of the relative growth rates (RGR) and (B) PDL of the plant biomasses on the twelfth day during water stress. In all the treatments, the values are means ± SE (n=4).

Photosynthetic potential to water content: As shown in Fig. 4, the F_v/F_m responses to the water contents were fitted to a trinary polynomial function in the form constructed by non-linear regression [F_v/F_m = -10.52+34.95WC +38.68 WC^2+14.27WC^3; R^2=0.7111, $p<0.01$, Fig 4A]. As the water content increased, the F_v/F_m initially increased and then peaked and that stabilized at 0.8263 when the water content was 89.64%. In addition , The ΦPSII and P_n were closely related to water content, and the relations were fitted to a trinary polynomial function [Φ_{PSII} = -27.77+ 92.57WC-102.86 WC^2+38.09WC^3; R^2=0.7570, $p<0.01$, Fig. 4B for Φ_{PSII}, and P_n= -10.77+ 35.81WC-39.7WC^2 +14.67WC^3; R^2=0.7366, $p<0.01$, Fig. 4D for P_n].The inflection points of the curves were at the middle plant water content (the maximum Φ_{PSII} of 0.6933 and the maximum P_n of 18.05μmol (CO_2) m^{-2} s^{-1} appearing at the WC of 88.6%). The P_n/G_s also were closely related to water content, and the relations were fitted to a binomial function [P_n/Gs = -526.6+11.7WC -0.065WC^2; R^2= 0.4772, p=0.015, Fig. 4F], with their maximum of 522.69 at water content of 89.87%. The T_r was closely related to the water content. NPQ was negatively related to water content [NPQ = 30.22-99.53WC +109.24WC^2 +39.94WC^3; R^2=0.6227; $p<0.01$, Fig. 4C].

Photosynthetic rate were determined under both drought and re-watering conditions. As shown in Table 2, significant changes in gas exchange parameters resulted from re-wetted sand. Plants in elevated CO_2 had higher P_n, P_n /T_r and P_n /G_s than in ambient CO_2. During re-watering, P_n and P_n /G_s reached the max level in C+D_{600}-N treatment and P_n /T_r reached the max level inC+D_{300}treatment.

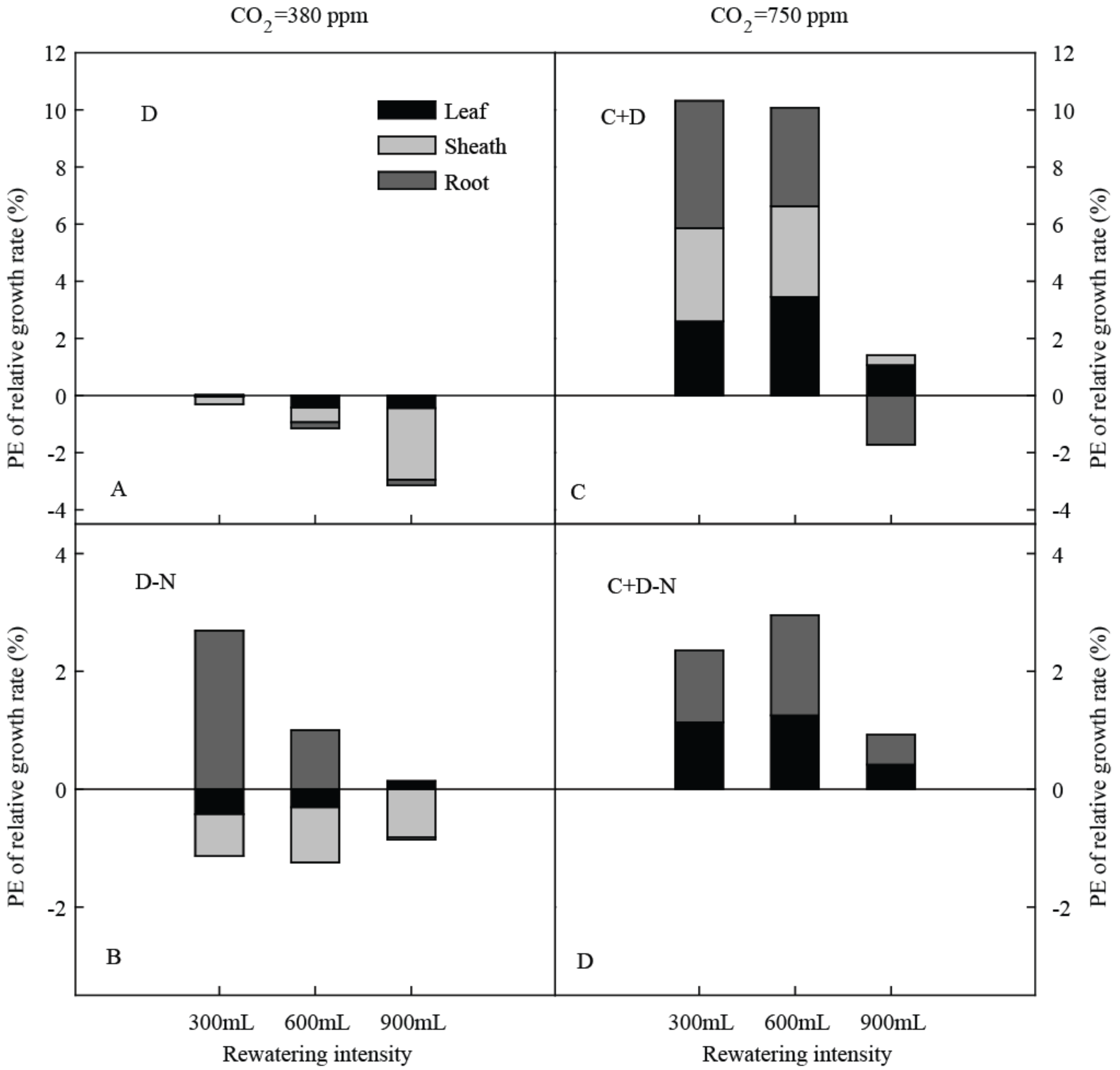

Fig. 2. Percent enhancements (PE) of the relative growth rates during re-watering (the 6 d period during re-watering). In all the treatments, the data were based on four replicates.

New leaf Recovery following re-watering: Figure 5A demonstrates that C+D_{300} and C+D_{600} significantly increased the PE of RGR in new leaves. D_{300}-N, D_{600}-N, C+D_{300}-N and C+D_{600}-N obviously stimulated the PE, but D_{900}-N and C+D_{900}-N, especially in D_{900}-N, restrained them. Re-watering exerted no effects on the new leaf growth PE under D. As shown in Fig. 5B, the relations between new leaf RGR and plant RGR were better fitted with a linear function of $RGR_{new\ leaf} = 38.38+0.54RGR_{plant}$; $R^2=0.4832$, $p<0.01$. The plant RGR increased gradually as the new leaf RGR improved.

The PSII function performances of the plants were determined on the 5th d during they were re-watered. As shown in Fig. 6, under D, the F_m gradually reduced as the re-watering levels increased. Under D-N and C+D, the F_m increased as the re-watering levels increased from 0 to 600 mL but decreased at the re-watering intensity of 900 mL. The F_m was higher under C+D_{300}-N, C+D_{600}-N and C+D_{900}-N than under C+D_0-N. Re-watering also significantly affected the F_v/F_m under D exerted no influence on those under the other treatments.

Discussion

In this study, the effects of re-watering at the two CO_2 concentrations could lead to increased plant water content. Photosynthetic capacity and biomass re-growth of the plants could be improved by maintaining proper water content but restrained due to unnecessarily abundant water contents (more than 90.5%). Seedlings with elevated CO_2 kept having an obvious potentials by their re-watering, and that biomass re-growth escalate by accelerating their new leaf growth and enhancing photosynthetic activity following re-watering.

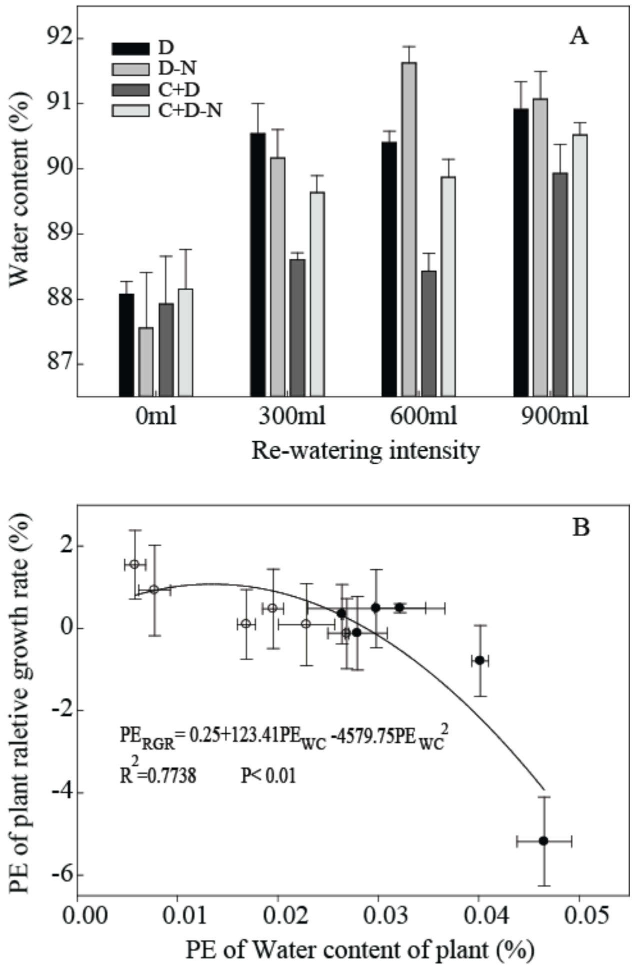

Fig. 3. (A), Plant Water contents (WC) during re-watering. (B), relation between the PE of the plant relative growth rates and the PE of the plant water content. The Open circles stands for CO_2=750 ppm; the solid circles stand for CO_2=380 ppm. In all the treatments, the values are means ± SE (n=4).

Re-watering water-stressed plants under ambient CO_2 increased water contents to 90.5-91.0%. Under D, especially D_{900}, re-watering the plants caused their total biomass accumulations to decline because they significantly reduced sheath growth (Fig. 2A, C), indicating that they had weak acclimation capacity while they were provided with abundant water at drought. Results from barley plants indicated elevated CO_2 could mitigate the effects of drought stress, as well as make the plants to more rapidly recover during re-watering (Robredo *et al.* , 2011). In current study, re-watering water-stressed plants with elevated CO_2 maintained their water contents between 88.4% and 89.9%. Biomass accumulation of C+D_{300} and C+D_{600} increased but that of C+D_{900} decreased with redundant water (89.9% of WC) because it led to root production reduction. A meta-analytical synthesis from Wang & Taub (2010) indicated that lower soil water increased root biomass fraction in whole plant, but to a greater extent at elevated than at ambient CO_2. Our results further demonstrated that root of water-stressed maize recovered better in elevated CO_2. The increased root production may contribute to whole plant recovery (Iversen *et al.*, 2008; Pritchard *et al.*, 2008; Yang *et al.*, 2008; Franklin *et al.*, 2009).

Descriptive information around amelioration of water-stressed plant with elevated CO_2 have been widely proved(Reich *et al.*, 2006; Albert *et al.*, 2011), even in water and N co-limited conditions (Markelz *et al.*, 2011). In this study, re-watering increased root production of the seedlings subjected to N limitation and water-stress at the both CO_2 concentrations. Under D-N, the plants did not restore their sheath production and leaf expansion while they were rewatered, although they obviously increased their root production. The plants restored their leaf expansion better under C+D-N than under D-N and the reason for this was probably partially that they enhanced their 'intrinsic water use efficiency' for CO_2 uptake while they were rewatered (Fig. 4F).

Table 2. Gas exchange parameters under drought limitation and re-watering.

CO_2 concentration (μmolmol^{-1})	**Treatment**	**P_n (μ mol (CO_2) m^{-2} s^{-1})**	**P_n /T_r (μ mol m^{-2} s^{-1})**	**P_n /G_s (μ mol m^{-2} s^{-1})**
			Drought limitation	
380	D_0	0.01 ± 0.12 c	0.02 ± 4.62 d	0.26 ± 0.02 c
	D_0-N	2.85 ± 1.26 b	14.74 ± 6.22 c	130.53 ± 15.07 b
750	C+D_0	10.75 ± 2.04 a	202.14 ± 26.14 a	184.30 ± 21.28 a
	C+D_0-N	9.44 ± 2.63 a	29.87 ± 7.04 b	212.86 ± 24.58 a
			Re-watering	
380	D_{300}	9.70 ± 0.64 d	13.01 ± 5.96 e	507.32 ± 58.59 a
	D_{600}	8.04 ± 2.20 de	15.73 ± 6.99 de	420.39 ± 48.55 c
	D_{900}	7.56 ± 1.82 e	15.47 ± 6.95 de	287.23 ± 33.17 e
	D_{300}-N	9.40 ± 2.63 d	16.25 ± 6.37 d	423.50 ± 48.91 bc
	D_{600}-N	7.37 ± 3.22 e	16.33 ± 7.15 d	337.30 ± 38.95 d
	D_{900}-N	8.40 ± 3.37 d	19.21 ± 8.05 d	194.25 ± 22.43 f
750	C+D_{300}	18.05 ± 1.44 a	105.56 ± 18.67 a	475.31 ± 54.89 ab
	C+D_{600}	16.27 ± 2.09 ab	34.97 ± 9.13 b	461.47 ± 53.29 b
	C+D_{900}	18.27 ± 2.43 a	30.81 ± 9.05 b	422.59 ± 48.80 bc
	C+D_{300}-N	12.67 ± 0.88 c	34.64 ± 7.63 b	330.03 ± 38.11 d
	C+D_{600}-N	18.33 ± 1.97 a	26.51 ± 6.45 c	522.69 ± 60.36 a
	C+D_{900}-N	13.33 ± 0.95 c	26.03 ± 7.80 c	313.54 ± 36.21 d

P_n is the net CO_2 accumulation rate (μ mol (CO_2) m^{-2} s^{-1}); P_n /T_r is water use efficiency of leaf (μ mol m^{-2} s^{-1}), P_n /G_s is CO_2 accumulation rate per stomatal conductance. Asterisks indicate significance at the 0.05 level, compared with those of the control value

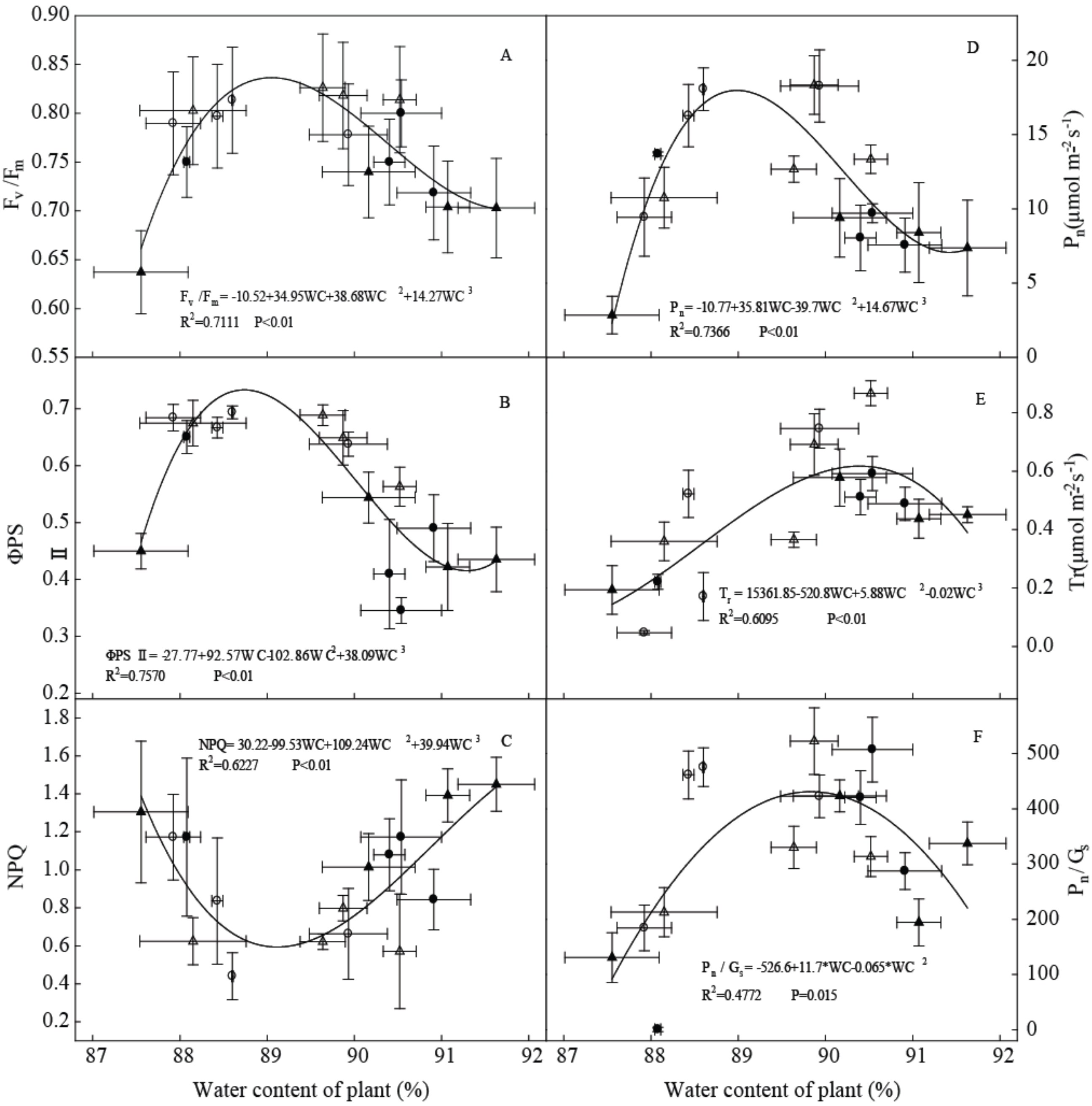

Fig. 4. Responses of photosynthetic potential to water content (WC). Open circles, C+D; Open triangle, C+D-N; Filled circles, D; Filled circles, D-N. (A) the maximal efficiency of PSII photochemistry (F_v/F_m); (B) the actual PSII efficiency (Φ_{PSII}); (C) non-photochemical quenching (NPQ); (D) net photosynthesis rate (P_n); (E) stomatal Conductance (G_s); (F) intrinsic water use efficiency (P_n/G_s). In all the treatments, the data were based on five replicates.

Previous studies demonstrated that elevated CO_2 can increase N availability and remobilizing capacities of crops (Franzaring *et al.*, 2011; Kim *et al.*, 2011). Results from barley suggested that elevated CO_2 could help plants having a rapid recovery following water stress by improving nitrogen metabolism (Robredo *et al.* , 2011). In the study, elevated CO_2 could make the N limited seedlings restore their leaf growth more than ambient $CO_2$when they were rewatered. The better recovery on leaf growth might result from the improved nitrogen availability in plant.

Currently, physiological processes of plants that modulate photosynthetic acclimation to variable environments are subjects of intense discussion (Posch & Bennett, 2009; Galle *et al.*, 2011; Markelz *et al.*, 2011; Kidokoro *et al.*, 2009). The impacts of metabolic restoration (such as Φ_{PSII} and F_v/F_m) and stomatal acclimation in photosynthetic recovery are varied depend on species and water status (Galle & Feller, 2007; Hu *et al.*, 2010). In the study, the response of F_v/F_m to seedlings suffered drought and re-watering is similar to that of P_n (Fig. 4). The result agrees with previous reports that the recuperative ability of photosynthesis is largely correlated to the restoration ability of metabolic activity (Hu *et al.* , 2010). In addition, re-watering the seedlings with elevated CO_2 increased F_v/F_m and Φ_{PSII} but decreased NPQ as compared to ambient CO_2, indicating that PSII acclimation is better with elevated CO_2.

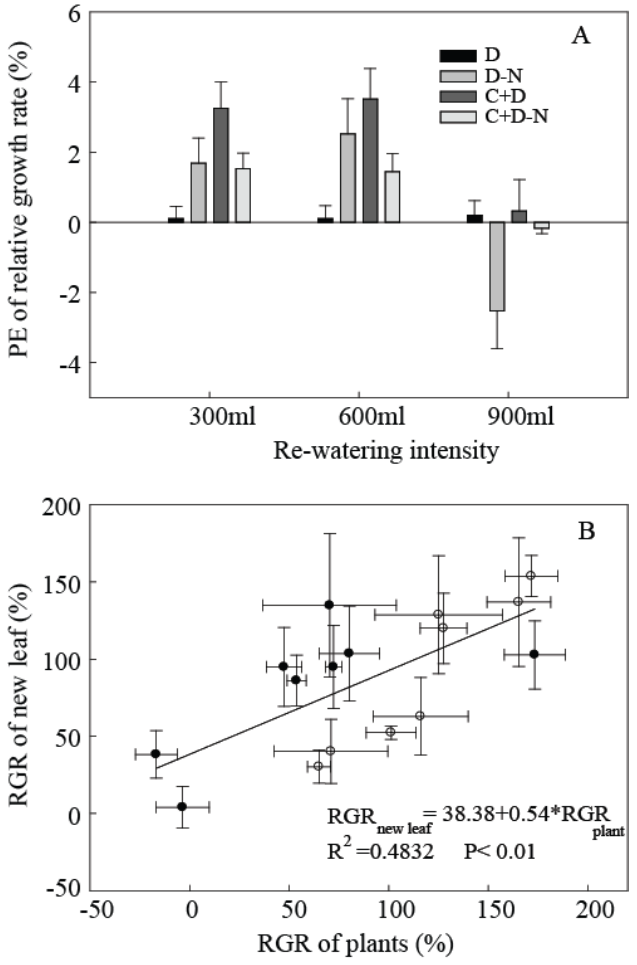

Fig. 5. (A) PE of the new leaf growth rates and (B) the relations between the RGR of the new leaf growth and the RGR of the plant growth during re-watering. The Open circles stand for CO$_2$=750 ppm; the solid circles stand for CO$_2$=380 ppm. In all the treatments, the values are means ± SE (n=4).

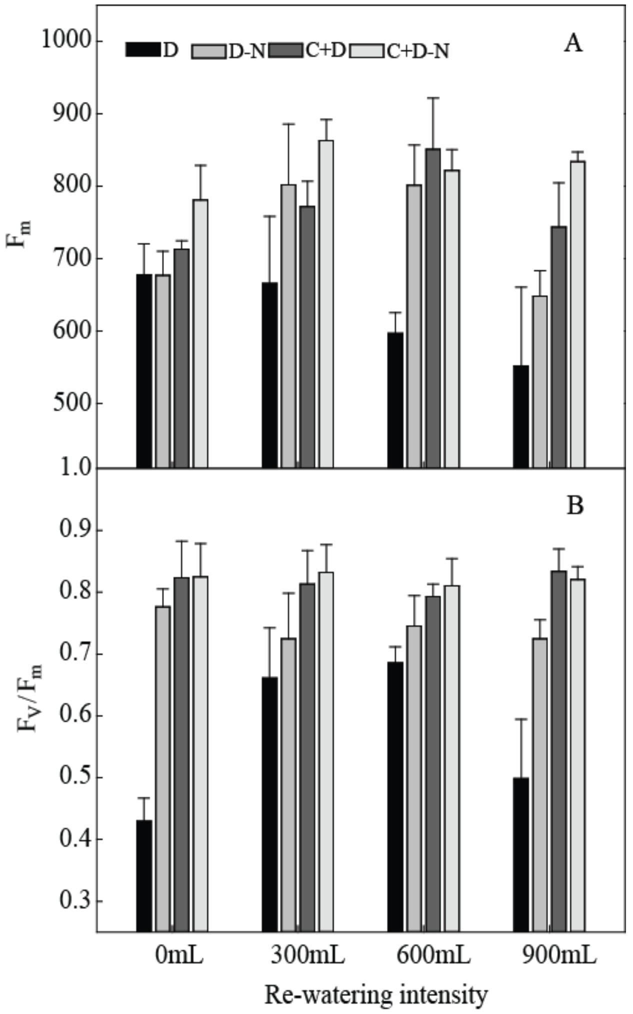

Fig. 6. Photosynthetic potentials of the new leaves. (A) the maximum Chl a fluorescence (F_m); (B) the maximal efficiency of PSII photochemistry (F_v/F_m). All the values are means ± SE (n=4).

Furthermore, our results indicated that there was a strong relationship between the plant photosynthetic capacities and plant water content. As water contents increased, P_n than T_r and G_s in seedlings with ambient CO$_2$ more significantly decreased so that they had lower P_n/T_r and P_n/G_s ratios (Fig. 4, Table 2). Thus, with ambient CO$_2$, water-stressed maize plants had higher water contents and weak photosynthesis restoring capacities when they were rewatered. As water contents increased, maize seedlings in elevated CO$_2$ (eg. C+D$_{600}$, C+D$_{900}$, C+D$_{600}$-N and C+D$_{900}$-N) had lower water contents than those in ambient CO$_2$, better restored P_n than T_r and G_s so that they had higher P_n/T_r and P_n/G_s ratios. Hence, elevated CO$_2$ could help drought stressed seedlings to maintain higher carbon assimilation rates under low water uptakes, as a result to improve water use efficiency. This is similar to results on cotton plants (Ephrath *et al*., 2011).

The N supply limitation could exacerbate stomatal limitations under drought, but elevated CO$_2$ delayed and relieved the limitations to P_n (Markelz *et al.*, 2011). Seedlings suffered N and water deficits in the study significantly had higher T_r and G_s with elevated CO$_2$ than with ambient CO$_2$, indicating low stomatal limitation in elevated CO$_2$. The P_n increased as T_r and G_s increased. Interestingly, as water content increased, the P_n restoration of C+D$_{600}$-N and C+D$_{900}$-N were faster than T_r and G_s, increasing 'intrinsic water use efficiency' for CO$_2$ uptake. Thus, promoting stomatal recovery from drought stress may be important for plants to resume photosynthetic capacity (Hu *et al.*, 2010). But elevated CO$_2$ reduced the limitation of stomatal restoration ability in photosynthesis recovery and then improved leaf water use efficiency.

Plant productivity promotion by re-watering has also been investigated by other researchers (Pinheiro *et al.*, 2004; Xu *et al.*, 2009; Nure *et al.*, 2010). Immediately when they were rewatered, plants will recover their growth and photosynthesis by growing new plant parts, re-opening their stomata, and decreasing their per-oxidation; how much recovery after being rewatered strongly depend on drought intensity and duration, and species(Xu *et al.* , 2010). When they were rewatered, the rapid new organ growth (e.g. new leaf) probably contributes to their high RGR (Fig. 5B). How much the new growth of the plants offset drought restraint that they suffered was determined by their water content after their being rewatered. When rewatered, the plants grew new leaves more rapidly under C+D than under D, thus rapidly recovering their F_m and F_v/F_m.

While, N deficiency seedlings had more new leaves at both of the CO_2 concentrations under the re-watering levels of 300mL and 600mL, but had less new leaves at the re-watering intensity of 900mL. Furthermore, the decreased new leaf growth was lower under C+D_{900}-N than under D_{900}-N (Fig. 5A), demonstrating a better leaf production restoration in elevated CO_2 (Fig. 2B and D). Because of organs development dependent on N recycles and new proteins synthetize in plant(Lattanzi, 2005), the increased N availability and remobilizing capacities of plant with elevated CO_2 (Franzaring *et al.*, 2011; Kim *et al.*, 2011) are supposed helpful to induce a better restoration of new organ from re-watering, even in N deficient conditions. Our results support this conclusion.

Re-watering exerted no obvious enhancement in the F_v/F_m at the elevated CO_2 concentration, but significantly increased the F_m (Fig. 6), indicating a short recovery on light absorption capacity instead of maximum light energy conversion efficiency. Such F_m improvements were also true under D_{300}-N and D_{600}-N, but not true under D_{300}, D_{600} and D_{900}. Maize seedlings suffering combined N limitation and drought had a better recovery of new leaf photosynthetic potential than those suffering only drought with ambient CO_2.

Conclusions

In the study, the RGR recoveries during re-watering were negatively proportional to plant water content. The plants were able to maintain favorable water content as well as enhance their biomass accumulation, photochemistry activity, leaf water use efficiency and new leaf growth recoveries with elevated CO_2. Therefore, it is clear that plants presented a unique acclimation feature with elevated CO_2 when they were rewatered even under N limited condition, which allows the plants to have a better performance under drought following re-watering.

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