Effects of Elevated CO₂ Concentration on Water Relations and Photosynthetic Responses to Drought Stress and Recovery during Rewatering in Tall Fescue

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ADDITIONAL INDEX WORDS. *Festuca arundinacea*, osmotic potential, Rubisco activity, sugar

ABSTRACT. Water availability for plant growth is becoming increasingly limited, whereas rising atmospheric carbon dioxide concentration may have interactive effects with drought stress. The objectives of this study were to determine whether elevated CO₂ would mitigate drought-induced water deficit and photosynthesis inhibition and enhance recovery from drought damages on rewatering and to determine whether the mitigating effects during drought stress and the recovery in photosynthesis during rewatering by elevated CO₂ were the result of the regulation of stomatal movement or carboxylation activities in tall fescue (*Festuca arundinacea* Schreb. cv. Rembrandt). Plants were grown in controlled-environment chambers with ambient CO₂ concentration (400 $\mu\text{mol}\cdot\text{mol}^{-1}$) or elevated CO₂ concentration (800 $\mu\text{mol}\cdot\text{mol}^{-1}$) and maintained well watered (control) or subjected to drought stress and subsequently rewatered. Elevated CO₂ reduced stomatal conductance (g_s) and transpiration rate of leaves during both drought stress and rewatering. Osmotic adjustment and soluble sugar content were enhanced by elevated CO₂. Elevated CO₂ enhanced net photosynthetic rate with lower g_s but higher Rubisco and Rubisco activase activities during both drought and rewatering. The results demonstrated that elevated CO₂ could improve leaf hydration status and photosynthesis during both drought stress and rewatering, and the recovery in photosynthesis from drought damages on rewatering was mainly the result of the elimination of metabolic limitation from drought damages associated with carboxylation enzyme activities.

Drought stress is one of the most detrimental abiotic stresses for plant growth. Water deficit in plants leads to stomatal closure and reduces photosynthesis resulting from restricted CO₂ diffusion through leaf stomata (stomatal limitation) and inhibition in carboxylation activity (metabolic limitation) (Flexas et al., 2004). Therefore, minimizing cellular dehydration and maintaining active photosynthesis are key strategies for plant survival or persistence through dry-down periods (Nilsen and Orcutt, 1996). In addition, rapid recovery of damaged plant tissues or rehydration and resumption of photosynthesis after drought stress when water becomes available is particularly important for perennial plant species to ensure rapid regrowth and stand re-establishment in areas with alternating drought and rewatering events. Post-drought recovery is largely dependent on the existing leaves to be able to resume rapidly physiological activities such as photosynthesis; however, regulating factors for cellular rehydration and photosynthesis resumption from drought damage on rewatering have received limited attention.

The atmospheric CO₂ concentration has been increasing, which has been shown to promote drought tolerance or mitigate drought damages in various plant species, particularly effective for C₃ species (Kirkham, 2011), including perennial grass species such as tall fescue (Yu et al., 2012a, 2012b, 2014) and kentucky bluegrass (*Poa pratensis* L.) (Song et al., 2014). The positive effects of elevated CO₂ on drought tolerance of plants are mainly the result of the reduction in water use as the result of CO₂ induction of stomatal closure and promotion of photosynthesis (Kirkham, 2011; Wullschlegel et al., 2002). The promotive effects of elevated CO₂ on photosynthesis have been mainly associated with overcoming metabolic limitations from drought stress by reducing photorespiration and increasing ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) carboxylation (Leakey et al., 2006; Reddy et al., 2010). Many previous studies have examined effects of elevated CO₂ on various physiological changes during drought stress; however, limited information is available for effects of elevated CO₂ on physiological recovery of plants from drought stress on rewatering (Widodo et al., 2003). It is not clear whether effects of elevated CO₂ would promote rehydration and resumption of photosynthesis when plants are rewatered and whether the recovery in photosynthesis is related to the elimination of stomatal limitation (i.e., stomatal reopening) and/or metabolic limitations (i.e., Rubisco activation) from drought damages.

Received for publication 27 Aug. 2014. Accepted for publication 7 Oct. 2014. We thank Dr. Marshall Bergen, Dr. James White, and Dr. Chenping Xu for their technical support and Patrick Burgess and David Jespersen for critical reviewing the manuscript. Thanks also go to Rutgers Centre of Turfgrass Science and China National Science Foundation (30871735 and 31272191) for support.

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Tall fescue is a widely used C_3 perennial turfgrass species. It has superior drought avoidance compared with many other commonly used cool-season turfgrass species as a result of its deep extensive root system, but has limited capacity of dehydration tolerance and recuperative ability from drought damages (Fry and Huang, 2004; Qian et al., 1997). The physiological effects of CO_2 and rewatering of drought responding on tall fescue provide further insights into mechanisms imparting of perennial grass species adapted to drought and rewatering in the environmental atmospheric CO_2 concentrations and would also be important for developing practices of management to promote drought tolerance and recovery for widely used turfgrass species. Therefore, the objectives of the research were to study whether the elevated CO_2 would mitigate drought-induced water deficit and photosynthesis inhibition and enhance recovery from drought damages on rewatering and to determine whether the mitigating effects during drought stress and the recovery in photosynthesis during rewatering by elevated CO_2 were the result of the regulation of stomatal movement or carboxylation activities in tall fescue.

Materials and Methods

PLANT MATERIAL AND GROWTH CONDITIONS. Tall fescue ('Rembrandt') sod (10 cm diameter) was obtained on 10 June 2011 from the turf research field at Rutgers University in North Brunswick, NJ, and were established in plastic pots (10 cm diameter and 60 cm deep) filled with a mixture of sand and topsoil (fine, montmorillonitic, mesic, aquic arqui-dolls) (1:3, v/v). Plants were maintained in a greenhouse for 2 months for establishing the canopy and roots with an average temperature at 23/16 °C (day/night) and $760 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetically active radiation (*PAR*). Plants were moved to a growth chamber (GC15; Environmental Growth Chambers, Chagrin Falls, OH) for the acclimation to controlled-environment conditions for 14 d before imposing CO_2 and watering treatments. The growth chambers were set at 11-h photoperiod with $680 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ *PAR* and day/night temperatures at 23/16 °C. During plant establishment in the greenhouse or growth chamber, plants were trimmed weekly to ≈ 10 cm, watered every 2 d, and fertilized weekly with half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950).

TREATMENTS AND EXPERIMENTAL DESIGN. The experiment consisted of two CO_2 concentrations and two watering treatments, which were arranged in a randomized complete block split-plot design with CO_2 concentrations as main plots and watering treatments as subplots. Each CO_2 treatment was replicated in four growth chambers. Each watering treatment was replicated in four pots placed inside each growth chamber under each CO_2 treatment.

The CO_2 treatments included ambient CO_2 concentration ($400 \pm 10 \mu\text{mol}\cdot\text{mol}^{-1}$) and elevated CO_2 concentration ($800 \pm 10 \mu\text{mol}\cdot\text{mol}^{-1}$). Plants were grown at the two CO_2 levels in growth chambers for 14 d before and then during drought (15 d) and rewatering (6 d) treatments for a total of 35 d. The CO_2 controlling setup of growth chambers followed the design as described in Yu et al. (2012a, 2012b). The concentration of CO_2 inside each growth chamber was maintained through an automated, open-chamber CO_2 control system connected to a gas tank containing 100% CO_2 (Airgas, Radnor, PA). The CO_2 levels were continuously monitored through an infrared gas analyzer (LI-820; LI-COR, Lincoln, NE) and controlled using an automatic system consisting of a programmable logic

controller unit, solenoid valves, and a laptop computer with monitoring software accurate to within $10 \mu\text{mol}\cdot\text{mol}^{-1}$.

After 14 d of pre-exposure to CO_2 treatments, plants were then exposed to two levels of water treatments under either ambient or elevated CO_2 concentration: 1) well-watered control by watering plants every 2 d until water drainage from the bottom of the pot to maintain soil volumetric water content (SWC) at the pot capacity ($\approx 25\%$); 2) drought stress by withholding irrigation for 15 d when SWC decreased to 4.98%; and 3) rewatering for 6 d with irrigating previously drought-stressed plants and SWC resumed to the pot capacity.

PHYSIOLOGICAL MEASUREMENTS. Leaf relative water content (RWC) of fully expanded leaves was determined at 0, 5, 10, and 15 d of drought stress and at 2 and 6 d of rewatering based on fresh weight (FW), turgid weight (TW), and dry weight (DW) using the following formula: $\text{RWC} (\%) = [(FW - DW)/(TW - DW)] \times 100$. Leaf fresh weight was immediately weighed after excised from the plants and then was soaked in deionized water for 6 h at 23 °C. Leaf samples were then blotted dry and immediately weighed for determination of TW. Samples were then dried in an oven at 80 °C for 72 h and weighed again for DW (Bagatta et al., 2008).

Leaf osmotic potential (ψ_s) was measured at 5, 10, and 15 d of drought stress following the method described by Fu et al. (2010). Briefly, leaf samples were soaked in deionized water for 12 h at 4 °C and blotted dry. Leaves were submerged in liquid nitrogen for rapid freezing and then stored at -20 °C until analyzed. Frozen samples were thawed for 30 min and cell sap was pressed using a laboratory press (Fred S. Carver, Wabash, IN). A 10- μL aliquot of the expressed sap was pipetted onto a filter paper disc that was placed in the sampling chamber of an osmometer (Wescor, Logan, UT) for analyzing solute concentration [C (millimoles per kilogram)]. Osmolarity of leaf sap was converted from millimoles per kilogram to megapascals using the formula: $\text{MPa} = -C \times 2.58 \times 10^{-3}$ (Bajji et al., 2001)

Soluble sugar content of leaves was measured at 5, 10, and 15 d of drought stress using the spectrophotometric method described by Pei et al. (2010) with modifications. Briefly, 0.2 g fresh leaves were boiled in 10 mL distilled water for 30 min. The extract was filtered through two layers of cheesecloth. The filtrate (0.5 mL) was mixed with 1.5 mL distilled water and 1 mL of 9% phenol and 5 mL H_2SO_4 . Tubes with this mixture were left at 23 °C for 30 min. The absorbance of the solution was measured with a spectrophotometer (Spectronic Genesys 2; Thermo Electron Corp., Madison, WI) at 485 nm. The soluble sugar concentration was determined using a standard curve.

Leaf net photosynthesis (P_n), transpiration rate (T_r), and g_s were determined on the second fully expanded leaves at 0, 5, 10, and 15 d of drought and 2 and 6 d of rewatering using an infrared gas analyzer (LI-6400; LI-COR). Ten individual leaves attached to the plants were taken from each pot and were placed in the leaf chamber with a built-in red and blue light source of the LI-6400, and all measurements were taken on at the level of $800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetic photon flux. The CO_2 concentration settings in leaf chamber were 400 ($\mu\text{mol}\cdot\text{mol}^{-1}$) for ambient CO_2 treatments and 800 ($\mu\text{mol}\cdot\text{mol}^{-1}$) for elevated CO_2 treatments, respectively. Leaf instantaneous water use efficiency (WUE) was calculated as the ratio of P_n to T_r . Stomatal opening and closure in response to elevated CO_2 concentration, drought stress, and rewatering was examined on the second fully expanded from the top of plants from each treatment and

photographs were taken using a camera fitted to a microscope (H600L; Nikon Instruments, Tokyo, Japan).

Rubisco extraction and activity assays were conducted using methods described by Hu et al. (2010) with modifications. Samples of fresh leaves (0.2 g) collected at 10 and 15 d of drought and 2 d of rewatering were immediately frozen in liquid nitrogen and kept at -80°C before extraction. For extraction, the leaf tissue was ground in liquid nitrogen with 3 mL extraction buffer containing 50 mM Hepes-KOH (pH = 7.5 at 25°C), 10 mM MgCl_2 , 2 mM ethylene diamine tetraacetic acid, 10 mM dithiothreitol, 10% glycerol (v/v), 1% bovine serum albumin (w/v), and 1% Triton X-100 (v/v) (Sigma, St. Louis, MO). The supernatant was isolated by centrifugation at $14,000 g_n$ for 10 min at 4°C and used immediately for Rubisco activity assays. Rubisco activity was measured by adding RuBP to the assay solution [100 mM Bicine (pH = 8.0), 25 mM KHCO_3 , 20 mM MgCl_2 , 3.5 mM adenosine-5'-triphosphate, 5 mM phosphocreatine, 5 units glyceraldehyde-3-phosphate dehydrogenase, 5 units 3-phosphoglyceric phosphokinase, 17.5 units creatine phosphokinase, and 0.25 mM NADH] and absorbance measured at 340 nm with a spectrophotometer (Helios Alpha; Thermo-spectronic, Rochester, NY). Initial activity was measured immediately after adding RuBP and total activity was measured after incubating samples at 25°C for 5 min. Rubisco activation state was determined by the ratio of initial to total activity. Fifty microliters of leaf extract was used to determine soluble protein concentration (Bradford, 1976). Total soluble protein was estimated from standard curves prepared with bovine serum albumin and used to calculate Rubisco activity expressed as micromoles CO_2 per second per milligram protein.

STATISTICAL ANALYSIS. All data were analyzed using SAS statistics software (Version 9.0; SAS Institute, Cary, NC). The analysis of variance with a fixed model was used to determine differences among treatments in RWC, P_n , g_s , T_r , WUE, ψ_s , soluble sugar content, Rubisco activity, and Rubisco activation state. Means of different treatments were tested with least significant difference at a probability level of 0.05.

Results and Discussion

EFFECTS OF ELEVATED CO_2 CONCENTRATION ON LEAF INTERNAL WATER STATUS AND WATER USE EFFICIENCY UNDER DROUGHT STRESS AND REWATERING. Leaf water retention capacity, water use rate, and WUE are important factors controlling plant water status during drought stress and ultimately whole-plant drought resistance. RWC is a good indicator of leaf hydration status and the level of drought tolerance (Flexas and Medrano, 2002). In this study, RWC of tall fescue was maintained at an average of 89.87% in well-watered plants during the experimental period, regardless of the CO_2 treatments, but it declined below the well-watered control level after 10 d of drought stress in both ambient and elevated CO_2 treatments (Fig. 1). The decline in RWC was less severe in the elevated CO_2 with drought treatment than in the ambient CO_2 with drought treatment. A significantly higher leaf RWC in tall fescue was observed in elevated CO_2 with drought-stressed plants than those in ambient CO_2 with drought treated at 10 and 15 d of drought stress. After 2 d of rewatering, plants treated with elevated CO_2 under drought recovered more rapidly than those treated with ambient CO_2 under drought. These results suggested that leaves treated with elevated CO_2 had a greater capacity of retaining water or resistance to dehydration during

Fig. 1. Changes in leaf relative water content (RWC) of tall fescue in response to 15 d of drought stress and 6 d of rewatering under ambient CO_2 concentration ($400 \mu\text{mol}\cdot\text{mol}^{-1}$) or elevated CO_2 concentration ($800 \mu\text{mol}\cdot\text{mol}^{-1}$). 800-D = drought-stressed plants under elevated CO_2 concentration; 800-W = well-watered plants under elevated CO_2 concentration; 400-D = drought-stressed plants under ambient CO_2 concentration; 400-W = well-watered plants under ambient CO_2 concentration. Vertical bars indicate least significant different values ($P \leq 0.05$) for the comparison among treatments at a given day of drought or rewatering treatment.

drought stress as well as rapid rehydration or recovery potential on rewatering.

Cellular hydration status of leaves during drought stress depends on the balance between water retention and water loss, which is governed by stomatal behaviors and osmotic adjustment among various other physiological factors (Farooq et al., 2009; Kramer and Boyer, 1995). Osmotic adjustment helps to maintain the cell water balance with active accumulation of solutes in the cytoplasm, thereby minimizing the harmful effects of drought (Clifford et al., 1998). Elevated CO_2 could alleviate leaf dehydration through osmotic adjustment under drought stress. In this study, elevated CO_2 treatment promoted osmotic adjustment, as shown by the decreases in the level of ψ_s under drought stress conditions (Fig. 2). Osmotic adjustment is associated with the accumulation of some osmoregulation substances such as water-soluble carbohydrates (Geissler et al., 2009; Pérez-López et al., 2010; Yoshida et al., 1997). Soluble sugars are the predominant forms of osmoregulators in C_3 perennial grasses during the early phase or a moderate level of drought stress (DaCosta and Huang, 2006) and decreases in ψ_s during drought stress are accompanied by increases in sucrose content in tall fescue (Fu et al., 2010). In the present study, soluble sugar content increased along with lowering ψ_s under elevated CO_2 concentration, particularly under drought conditions (Fig. 3). These results suggested that elevated CO_2 could facilitate osmotic adjustment in association with soluble sugar accumulation and consequently retained more water in leaves of tall fescue. Other solutes such as proline and glycine betaine also play roles in osmotic adjustment. How elevated CO_2 may affect osmotic adjustment in relation to the accumulation of those solutes is yet to be determined. Several previous studies also reported enhanced osmotic adjustment by elevated CO_2 in different plant species (Ferris and Taylor, 1994; Pérez-López et al., 2010). Some other studies have demonstrated that the elevated CO_2 concentration had minimal effect on ψ_s

Fig. 2. Osmotic potential of leaves in tall fescue at 15 d of drought stress under ambient CO_2 concentration ($400 \mu\text{mol}\cdot\text{mol}^{-1}$) or elevated CO_2 concentration ($800 \mu\text{mol}\cdot\text{mol}^{-1}$). 800-D = drought-stressed plants under elevated CO_2 concentration; 800-W = well-watered plants under elevated CO_2 concentration; 400-D = drought-stressed plants under ambient CO_2 concentration; 400-W = well-watered plants under ambient CO_2 concentration. Columns marked with different letters were significantly different among treatments at a given day of treatment based on a least significant difference test at $P \leq 0.05$.

Fig. 3. Total soluble sugar content of leaves in tall fescue at 15 d of drought stress under ambient CO_2 concentration ($400 \mu\text{mol}\cdot\text{mol}^{-1}$) or elevated CO_2 concentration ($800 \mu\text{mol}\cdot\text{mol}^{-1}$). 800-D = drought-stressed plants under elevated CO_2 concentration; 800-W = well-watered plants under elevated CO_2 concentration; 400-D = drought-stressed plants under ambient CO_2 concentration; 400-W = well-watered plants under ambient CO_2 concentration. Columns marked with different letters were significantly different among treatments at a given day of treatment based on a least significant difference test at $P \leq 0.05$.

during drought stress (Aranda et al., 2008; Polley et al., 1999; Robredo et al., 2007), and a few studies even showed decreased osmotic adjustment in response to drought under elevated CO_2 concentration (Tschaplinski, et al., 1993, 1995). The variable results of elevated CO_2 effects on osmotic adjustment under drought stress from different studies may be the result of different plant species, growth stages, drought severity levels or duration as well as CO_2 concentrations examined in different experiments. Nevertheless, our results suggested that the

Fig. 4. Changes in transpiration rate [T_r (A)], stomatal conductance [g_s (B)], and water use efficiency [WUE (C)] of tall fescue in response to 15 d of drought stress and 6 d of rewatering under ambient CO_2 concentration ($400 \mu\text{mol}\cdot\text{mol}^{-1}$) or elevated CO_2 concentration ($800 \mu\text{mol}\cdot\text{mol}^{-1}$). 800-D = drought-stressed plants under elevated CO_2 concentration; 800-W = well-watered plants under elevated CO_2 concentration; 400-D = drought-stressed plants under ambient CO_2 concentration; 400-W = well-watered plants under ambient CO_2 concentration. Vertical bars indicate least significant different values ($P \leq 0.05$) for the comparison among treatments at a given day of drought or rewatering treatment.

enhanced osmotic adjustment in association with sugar accumulation by elevated CO_2 concentration could play roles in maintaining greater RWC in tall fescue leaves under drought stress in this study.

Elevated CO₂ concentration could also alleviate leaf dehydration through lowering the rate of water loss. In this study, T_r (Fig. 4A) was suppressed by elevated CO₂ under both well-watered conditions and drought stress. Drought stress led to a rapid decline in T_r under both CO₂ regimes. Plants under the elevated CO₂ treatment had significantly lower T_r than those under the ambient CO₂ treatment during the first 10 d of drought stress and by 15 d of drought stress, T_r declined to the lowest level and there were no significant differences between the two CO₂ treatments. The reduction in T_r by elevated CO₂ concentration also has been reported by others (Assmann, 1993; Morison and Gifford, 1983; Tyree and Alexander, 1993). Along with T_r decline, g_s decreased in plants exposed to both drought and elevated CO₂ but increased during rewatering (Fig. 4B).

The partial closure of stomata induced by elevated CO₂ may contribute to the increases in leaf instantaneous WUE in tall fescue. In this study, WUE of tall fescue increased with drought stress under both CO₂ treatments, and it was significantly enhanced by elevated CO₂ under drought stress (Fig. 4C). The WUE in plants treated with the elevated CO₂ concentration was 1.54-, 2.09-, and 1.85-fold greater than those plants under the ambient CO₂ treatment at 5, 10, and 15 d of drought stress, respectively. Under rewatering conditions, no differences in WUE were detected in the two CO₂ treatments. Improved WUE by elevated CO₂ concentration has also been reported in various plant species exposed to drought stress (Centritto et al., 2002; Chun et al., 2011; Ghannoum et al., 2002; Laila and Adel, 2002; Retuerto and Woodward, 1993; Tschaplinski et al., 1995). WUE has been suggested as an effective criterion to select for superior drought-performing turfgrasses (Ebdon and Kopp, 2004). The improvement in WUE with elevated CO₂ has a great potential for water conservation and reducing irrigation in turfgrass management.

Stomata remained open in well watered and excessive CO₂ conditions (Fig. 5A) but closed under drought stress with ambient CO₂ (Fig. 5B) or under elevated CO₂ with adequate water (Fig. 5C) or under drought stress with elevated CO₂ (Fig. 5D). On rewatering, stomata of drought-stressed leaves reopened under ambient CO₂ (Fig. 5E) but remained at least partially closed under elevated CO₂ (Fig. 5F). These results suggested that elevated CO₂ concentration could induce stomatal closure at least partially, leading to the reduction in the rate of water loss through transpiration either under well-watered conditions or drought stress. However, CO₂-induced stomatal closure may inhibit photosynthetic capacity under drought stress and photosynthetic recovery after rewatering, as discussed subsequently.

EFFECTS OF ELEVATED CO₂ CONCENTRATION ON PHOTOSYNTHETIC RATE AND CARBOXYLATION ENZYME ACTIVITY UNDER DROUGHT STRESS AND REWATERING. Leaf P_n of tall fescue was enhanced by elevated CO₂ under both well-watered conditions and drought stress (Fig. 6A). Leaf P_n under both CO₂ regimes declined during 15-d drought stress, but the decline was less pronounced (36% reduction from 8.21 to 5.23 μmol·m⁻²·s⁻¹) under the elevated CO₂ treatment than that under the ambient CO₂ treatment (58% reduction from 7.42 to 3.12 μmol·m⁻²·s⁻¹). Elevated CO₂-enhanced P_n has been reported under non-stress and stress conditions in other plant species (Albert et al., 2011; Campbell et al., 1988; Sage et al., 1989; Vu et al., 1997), but few reported effects of elevated CO₂ on recovery in photosynthesis in response to rewatering (Widodo et al., 2003). In this study, on rewatering, leaf P_n with the elevated CO₂ treatment recovered more rapidly than that did with the ambient CO₂ treatment, and at 2 d of rewatering, P_n of elevated CO₂-treated plants was ≈37% greater than that under ambient CO₂ treatment. However, leaves under elevated CO₂ still maintained lower g_s compared with those exposed to ambient CO₂ after rewatering, which could

Fig. 5. Microscopic images of leaf epidermis showing stomatal aperture of tall fescue leaves in well-watered plants with ambient CO₂ concentration (400 μmol·mol⁻¹) (A), drought-stressed plants (10 d) with ambient CO₂ concentration (B), well-watered plants with elevated CO₂ concentration (800 μmol·mol⁻¹) (C), drought-stressed plants (10 d) with elevated CO₂ concentration (D), rewatered plants (6 d) with ambient CO₂ concentration (E), and rewatered plants (6 d) with elevated CO₂ concentration (F).

Fig. 6. Changes in net photosynthetic rate (P_n) of tall fescue leaves in response to 15 d of drought stress and 6 d of rewatering under ambient CO_2 concentration ($400\ \mu\text{mol}\cdot\text{mol}^{-1}$) or elevated CO_2 concentration ($800\ \mu\text{mol}\cdot\text{mol}^{-1}$) (A), and effects of elevated CO_2 concentration on Rubisco activity (B) and Rubisco activation state (C) at 10 and 15 d of drought and 2 d of rewatering. 800-D = drought-stressed plants under elevated CO_2 concentration; 800-W = well-watered plants under elevated CO_2 concentration; 400-D = drought-stressed plants under ambient CO_2 concentration; 400-W = well-watered plants under ambient CO_2 concentration. Vertical bars in (A) indicate least significant different (LSD) values ($P \leq 0.05$) for the comparison among treatments at a given day of drought or rewatering treatment. Columns in (B) and (C) marked with different letters were significantly different among treatments at a given day of treatment based on a LSD test ($P \leq 0.05$).

have limited the recovery potential in P_n (Fig. 6A) even in fully rehydrated leaves (Fig. 1) during rewatering. Factors regulating the recovery in P_n under elevated CO_2 concentration on rewatering after prolonged periods of drought stress were not well understood. The results in this study indicated that greater P_n during rewatering in CO_2 -enriched plants relative to that under ambient CO_2 was not the result of the elimination in stomatal limitation, but could be the result of other factors such as metabolic activity of carboxylation.

Rubisco is the key enzyme for carbon fixation of photosynthesis, and its activity is controlled by another enzyme, Rubisco activase. Rubisco activity showed a significant decline compared with the well-watered control at 10 and 15 d of drought stress under both CO_2 treatments (Fig. 6B). No significant differences were observed in Rubisco activity between ambient CO_2 and elevated CO_2 treatments under well-watered conditions, but Rubisco activity was significantly higher in CO_2 -enriched plants than those with ambient CO_2 at 10 and 15 d of drought stress (Fig. 6B). Rubisco activation state decreased to a significantly lower level at 10 and 15 d of drought stress than that of the well-watered control plants under both ambient and elevated CO_2 treatments (Fig. 6C). The drought-induced decline in Rubisco activation state was to a lesser extent under the elevated CO_2 (reduction by 19%) than that under ambient CO_2 treatment (reduction by 35%). Drought-induced decline in Rubisco activity and activation state has been reported in other plant species (Erice et al., 2006; Vu et al., 1998). At 10 and 15 d of drought stress, Rubisco activation state was significantly greater under the elevated CO_2 treatment than that under the ambient CO_2 treatment, suggesting that increasing CO_2 concentration could enhance the photosynthetic capacity of tall fescue by stimulating Rubisco carboxylation activities under drought stress. Previous studies reported enhancement in leaf photosynthesis by elevated CO_2 under heat stress was associated with the up-regulation of Rubisco (Prasad et al., 2009). After 2 d of rewatering, both Rubisco activity and activation state increased to a significantly higher level than that at 15 d of drought but did not fully recover to the well-watered control level under either ambient or elevated CO_2 treatment. Both Rubisco activity and activation state were significantly greater in plants exposed to elevated CO_2 than those under ambient CO_2 concentration at 2 d of rewatering. These results indicated that the resumption of Rubisco activity and activation state could contribute to the greater recovery of P_n on rewatering after drought stress, particularly under elevated CO_2 .

In summary, elevated CO_2 improved water status in tall fescue during drought stress, which could be the result of CO_2 -induced stomatal closure with limited transpirational water loss and enhanced osmotic adjustment in association with soluble sugar accumulation. Elevated CO_2 enhanced net photosynthetic rate in tall fescue leaves even with lower g_s but enhanced Rubisco and Rubisco activase activity during both drought and rewatering. The mitigating effects of elevated CO_2 on drought inhibition of photosynthesis and the enhanced recovery in photosynthesis on rewatering were mainly the result of the elimination of metabolic limitation from drought damages associated with increased enzyme activities for carboxylation.

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