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Effects of water stress and fertilization on leaf gas exchange and photosynthetic light-response curves of *Bothriochloa ischaemum* L.

W.Z. XU[*], X.P. DENG[*,**], and B.C. XU[*,**,+]

State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling, Shaanxi 712100, China[*]
Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, Yangling, Shaanxi 712100, China[**]

Abstract

Bothriochloa ischaemum L. is an important species in many temperate regions, but information about the interactive effects of water stress and fertilization on its photosynthetic characteristics was inadequate. A pot experiment was conducted to investigate the effects of three water [80% (HW), 40% (MW), and 20% (LW) of field capacity (FC)] and four fertilization regimes [nitrogen (N), phosphorus (P), nitrogen with phosphorus (NP), and no fertilization] on leaf photosynthesis. Leaf gas exchange and photosynthetic light-response curves were measured at the flowering phase of *B. ischaemum*. Water stress decreased not only the leaf gas-exchange parameters, such as net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and water-use efficiency (WUE) of *B. ischaemum*, but also downregulated P_N-photosynthetically active radiation (PAR) curve parameters, such as light-saturated net photosynthetic rate (P_{Nmax}), apparent quantum efficiency (AQE), and light compensation point (LCP). Fertilization (N, P, and NP) enhanced the daily mean P_N values and P_{Nmax} under the HW regime. Addition of N (either alone or with P) improved the photosynthetic capacity of *B. ischaemum* under the MW and LW regimes by increasing P_N, P_{Nmax}, and AQE and reducing dark respiration rate and LCP, but the addition of P alone did not significantly improve the photosynthetic performance. Decline in P_N under each fertilization regime occurred during the day and it was caused mainly by nonstomatal limitation. Our results indicated that water was the primary limiting factor for photosynthesis in *B. ischaemum*, and that appropriate levels of N fertilization improved its potential photosynthetic capacity under water-deficit conditions.

Additional key words: diurnal variation; nitrogen; phosphorus; photosynthetic capacity; soil water deficit.

Introduction

Plants growing in natural conditions are often limited by multiple environmental factors, requiring a balance of resources (*i.e.*, light, water, and nutrition) in order to maintain their optimal growth (Chapin 1987, Poorter and Nagel 2000, Guo *et al*. 2007). Water deficits and soil infertility are two main factors that restrict plant growth and production in semiarid regions (Walker and Langridge 1996, Shan and Xu 2009). An efficient use of limited water and nutrient resources and better growth under these limiting conditions would be desirable traits for plants in drought-stricken environments (Shangguan *et al*. 2000, Davis *et al*. 2011). Photosynthesis is the main driving force influencing dry matter partitioning and organ formation (Iqbal *et al*. 2011), and it is the basis of plant production (Zlatev and Fernando 2012). However, factors, such as water and fertilization, which regulate the capacity of plants to utilize photosynthates, are the major determinants of potential productivity (Zhai and Li 2006,

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[+]Corresponding author; tel: +86-29-87016571, fax: +86 -29-87012210, e-mail: Bcxu@ms.iswc.ac.cn
Abbreviations: AQE – apparent quantum efficiency; C_a – ambient CO_2 concentration; C_i – intercellular CO_2 concentration; CK – no fertilization (control); E – transpiration rate; FC – field capacity; g_s – stomatal conductance; HW – 80% of field capacity; LCP – light compensation point; L_s – stomatal limitation value; LSP – light saturation point; LW – 40% of field capacity; MW – 60% of field capacity; N – nitrogen; NP – nitrogen with phosphorus; P – phosphorus; PAR – photosynthetically active radiation; P_N – net photosynthetic rate; P_{Nmax} – light-saturated net photosynthetic rate; R_D – dark respiration rate; RH – relative humidity; SD – standard deviation; T_{AIR} – air temperature; T_{LEAF} – leaf temperature; VPD – vapour pressure deficit; WUE – water-use efficiency.
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Shen and Li 2011). Water stress is often accompanied by other limiting factors (*i.e.*, high temperature and strong irradiance, vapour pressure deficits). Plants with the high capacity for photosynthesis are able to survive better under the combination of these limiting factors (Valladares and Pearcy 1997, Flexas *et al*. 1999). Research concerning the influence of water and nutrient on plant ecophysiological characteristics is scarce (Tezara *et al*. 1999, Makoto and Koike 2007). Therefore, it is necessary to investigate the influence of an interaction between water and fertilization on the photosynthetic response of plants, especially under water-stress conditions (Wu *et al*. 2008).

Bothriochloa ischaemum (L.) Keng. is a dominant, drought-tolerant, and warm-season, C_4 perennial grass species, which is mainly found in temperate zones around the world. In the semiarid, loess, hilly-gully region on the Loess Plateau of China, it is an excellent native pasture and forage species due to its ability to adapt to the local conditions and due to the palatability and forage quality of its leaves (Xu *et al*. 2006, 2011). Its elaborate root system and high degree of cover make it an important species in reducing soil and water losses, and in maintaining the distinctive natural scenery of the area (Xu *et al*. 1997, Jiao *et al*. 2009). However, information about the interaction between water, fertilization, and the photosynthetic characteristics of this species is currently inadequate, although it is necessary in order to evaluate its adaptation to different environments. Therefore, this study was conducted to obtain such information by investigating the leaf gas exchange and photosynthetic light-response curve of *B. ischaemum* subjected to different water regimes and fertilization in a controlled, pot experiment. The objectives were: (*1*) to compare the diurnal patterns of leaf gas-exchange parameters, stomatal limitation value (L_s) and WUE under different water and fertilization regimes, (*2*) to clarify its P_N-PAR response curves under different water and fertilization regimes, and (*3*) to determine whether fertilization improves the photosynthetic capacity under water deficit.

Materials and methods

Plant material and growth conditions: The pot experiment was conducted in Yangling, Shaanxi Province, China (34°12′N, 108°7′E, 530 m a.s.l.), which has a mean annual temperature of 12.9°C, with a maximum monthly temperature of 26.7°C in July, a minimum temperature of −1 to −2°C in January, and mean annual rainfall of 637.6 mm.

Seeds of *B. ischaemum* were collected in the autumn of 2009 from the experimental fields at the Ansai Research Station (ARS) of the Chinese Academy of Sciences (CAS) (36°51′30″N, 109°19′23″E, 1,068–1,309 m a.s.l.) located at the center of the semiarid, hilly-gully region on the Loess Plateau. After drying for one week outside in direct sunlight, the seeds were placed in a sealed container and stored in a laboratory.

The loessial soil used in the experiment was collected from the upper 20 cm of a cultivated field at ARS, and it was air-dried and passed through a 2-mm mesh. The soil gravimetric moisture content at a field capacity (FC) and wilting point were 20.0% and 4.0%, respectively. The soil pH was 8.2, and the soil organic matter content was 0.27%. The soil total N, total P, and total K contents were 0.017%, 0.063%, and 1.97%, and the soil available N, P, and K contents were 11.22, 6.55, and 94.85 mg kg^{-1}, respectively.

The experiment began on 15 March 2010. The air-dried soil of 9.0 kg was packed into each pot (20 cm in a diameter and 30 cm in a depth). A vertical plastic pipe was placed adjacent to the inner wall of each pot; it was used to supply water to the base of the pot. Seeds were sown in 12 equally spaced dibbles in each pot on 20 March, 2010. Pots were initially well-watered in order to ensure seedling establishment. Shortly after the emergence, seedlings were thinned to one plant per dibble, *i.e.*, to 12 plants per pot.

Experimental design: Four fertilization regimes [nitrogen (N, 0.481 g(CON_2H_4) pot^{-1}), phosphorus (P, 3.949 g(KH_2PO_4) pot^{-1}], nitrogen + phosphorus (NP, a mixture of 0.481 g CON_2H_4 and 3.949 g KH_2PO_4 per pot), and no fertilization (CK, control)] were applied. When seedlings of *B. ischaemum* had 5 leaves and they were about 0.10 m high, 3 water regimes [80 ± 5% FC (HW), 60 ± 5% FC (MW), and 40 ± 5% FC (LW)] were initiated on 30 May 2010.

Before watering, a layer of perlite was spread on the soil surface of each pot (20 g, about 2.0 cm deep) in order to reduce evaporation from the soil surface. Daily evapotranspiration was assessed at 18:00 h by weighing the pots, and the water losses were substituted *via* the plastic pipes in order to maintain the desired water regime. The pots were distributed in a completely randomized design with five replicates for each of four fertilizations and three water regimes, and all were covered by a rainout shelter on rainy days.

Gas-exchange parameters were measured during three consecutive, completely sunny days (August 13–15, 2010), when the plants were in the flowering phase. Three newly expanded, healthy leaves, at similar positions in each pot, were chosen and repetitively measured using a portable photosynthesis system (*LI-6400, LI-COR Inc*., Lincoln, NE, USA) under natural sunlight. The middle parts of the selected leaves were measured at 2-h intervals from 07:30 to 19:30 h. The parameters obtained included: P_N, E, g_s, intercellular CO_2

concentration (C_i), air temperature (T_{AIR}), vapour pressure deficient (VPD), PAR, leaf temperature (T_{LEAF}), relative humidity (RH), and ambient CO_2 concentration (C_a). WUE was calculated as P_N/E (Nijs *et al* 1997), and L_s using the following formula: $L_s = 1 - C_i/C_a$ according to Berry and Downton (1982). Diurnal mean values of P_N, E, g_s, C_i, L_s, and WUE were calculated from the means of the measured values made between 07:30 and 19:30 h during all three days.

***P*$_N$-PAR response curves** of the leaves were determined on the same three sunny days and at the same times as the previous measurements, using the *LI-6400* portable photosynthesis system with a red-blue LED light source (*6400-02B*). P_N-PAR responses were determined for PAR values of 2,000; 1,600; 1,200; 1,000; 800, 600, 400, 300, 200, 160, 120, 80, 40, and 0 μmol(photon) m^{-2} s^{-1} under the same conditions [C_a of 330–370 μmol(CO_2) mol^{-1}, T_{LEAF} of 25 ± 3°C, and RH of 50–55%] inside the leaf chamber between 09:00 and 11:30 h. The resulting P_N-PAR curves were fitted by a modified rectangular hyperbolic model (Ye 2007, Ye and Yu 2008). Parameter estimation was accomplished by using the nonlinear regression module of the *SPSS* statistical package (Version *16 for Windows*, *SPSS*, Chicago, IL, USA). The regression equation is expressed as:

$$P_N = \alpha \frac{1 - \beta\, PAR}{1 + \gamma\, PAR}(PAR - LCP) \quad (1)$$

where LCP is the light compensation point; α, β , and γ are coefficients independent of PAR. Dark respiration rate (R_D) was obtained by the above equation, when PAR = 0. Light saturation point (LSP) and P_{Nmax} were given by following formulae:

$$LSP = \frac{\sqrt{(\beta + \gamma)(1 + \gamma\, LCP)/\beta} - 1}{\gamma} \quad (2)$$

$$P_{N\,max} = \alpha \frac{1 - \beta\, LSP}{1 + \gamma\, LSP}(LSP - LCP) \quad (3)$$

AQE was estimated from the linear part of the P_N-PAR curves, which occurred in the PAR range of 0–200 μmol(photon) m^{-2} s^{-1} (Singsaas *et al*. 2001).

Statistical analysis was performed using the *SPSS* statistical package. Effects of water, fertilization, and their interactions on photosynthetic parameters were analyzed using two-way *ANOVA* ($P<0.05$). Differences between the treatment means among 3 water regimes or 4 fertilizations were compared using least significant difference (LSD) multiple range tests at the 0.05 probability levels.

Results

Diurnal pattern of environmental factors: PAR ranged between 132 and 1,789 μmol(photon) m^{-2} s^{-1} during the daytime; the highest daily values were observed at 11:30 h (Fig. 1*A*). C_a was the highest [453.1 μmol(CO_2) mol^{-1}] at 07:30 h, and decreased to the daily minimum value at 17:30 h [369.2 μmol(CO_2) mol^{-1}], then again increased (Fig. 1*A*). The diurnal patterns of T_{AIR} and T_{LEAF} were similar, increasing consistently to a maximum temperature of around 39.5°C at about 13:30 h before decreasing (Fig. 1*B*). RH was the highest (62.5%) in the morning at 07:30 h and decreased to the daily minimum value (25.1%), which occurred at 13:30 h, then increased gradually. The diurnal pattern of VPD was the opposite to that of RH (Fig. 1*C*).

Diurnal changes of photosynthetic parameters: Diurnal variations of P_N, E, and g_s exhibited single-peak curves under all regimes (Fig. 2). The phenomenon of midday photosynthetic depression did not occur in the *B. ischaemum* plants (Fig. 2*A*,*B*,*C*).

***P*$_N$**: Among four fertilizations (N, P, NP, and CK), the highest values [16.3, 15.9, 17.2, and 15.7 μmol(CO_2) m^{-2} s^{-1}, respectively] were reached in plants at 13:30 h under HW. In contrast, the respective peak values occurred earlier at 11:30 h under both MW [16.63, 12.63, 15.30, and 13.30 μmol(CO_2) m^{-2} s^{-1}, respectively] and LW [12.8, 9.6, 13.8, and 9.2 μmol(CO_2) m^{-2} s^{-1}, respectively] (Fig. 2*A*,*B*,*C*). Water, fertilization, and their interactions significantly affected P_N (Table 1). Under HW, daily mean P_N values of the fertilized plants (N, P, and NP plants) were all significantly higher than those of CK plants, and those of the NP plants were higher than those of N or P plants (Table 2). Under water stress conditions (MW and LW), the daily mean P_N values of the N or NP plants were significantly higher than those of P and CK plants, while there were no significant differences between P and CK plants (Table 2).

E: The highest values were found at 13:30 h under both the HW [5.62, 5.64, 5.40, and 4.73 mmol(H_2O) m^{-2} s^{-1}, respectively] and the MW [5.30, 4.51, 5.44, and 3.41 mmol(H_2O) m^{-2} s^{-1}] respectively in the N, P, NP, and CK plants (Fig. 2*D*,*E*). In contrast, under LW, the highest corresponding E values were 3.77, 3.94, 3.92, and 2.62 mmol(H_2O) m^{-2} s^{-1}, and they occurred earlier, at 11:30 h (Fig. 2*F*). Water, fertilization, and their interactions significantly affected E (Table 1). Under all three water regimes, the daily mean E significantly differed among the fertilization, with the highest values occurring in the fertilized plants (N, P, and NP plants), and the lowest in the CK plants (Table 2). The daily mean E values in the P plants were significantly higher than those of the N and NP plants under HW, but under MW, they were significantly lower than those of the N and NP plants (Table 2). Under LW, the daily mean E values of the

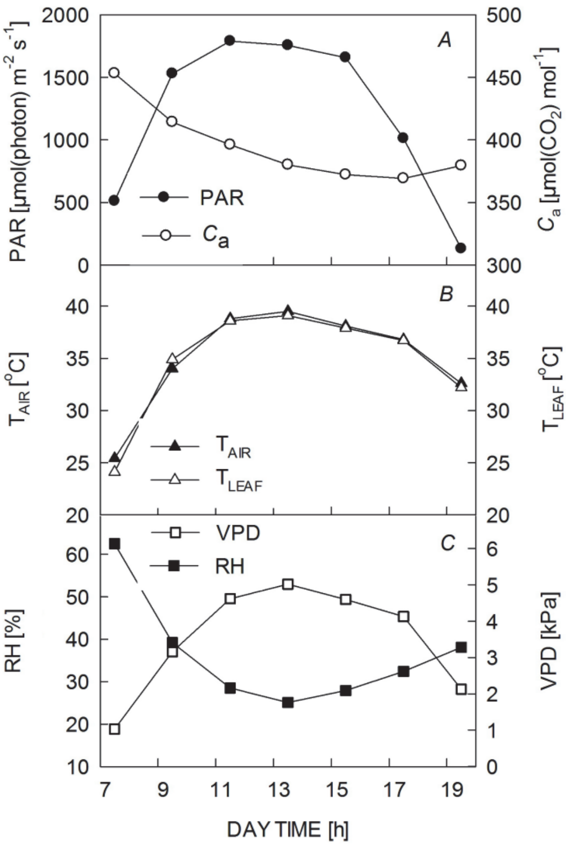

Fig. 1. Diurnal changes of photosynthetically active radiation (PAR) (*A*;●), ambient CO_2 concentration (C_a) (*A*;○), air temperature (T_{AIR}) (*B*;▲), leaf temperature (T_{LEAF}) (*B*;△), relative humidity (RH) (*C*;■), and vapour pressure deficient (VPD) (*C*;□). Values are means ± SD; n = 5.

NP plants were significantly higher than those of the N or P plants (Table 2).

g_s: The highest values occurred earlier during the day under water stress (Fig. 2*G*,*H*,*I*). Thus, the highest g_s of the N, P, NP, and CK plants [0.114, 0.101, 0.112, and 0.113 mol(H_2O) m^{-2} s^{-1}, respectively] occurred at 13:30 h under HW (Fig. 2*G*). Under MW, the highest g_s of the N, P, NP, and CK plants [0.109, 0.097, 0.112, and 0.106 mol(H_2O) m^{-2} s^{-1}, respectively] occurred earlier, at 11:30 h (Fig. 2*H*). Under LW, the highest g_s of the N, P, NP, and CK plants [0.095, 0.091, 0.104, and 0.085 mol(H_2O) m^{-2} s^{-1}, respectively] were observed at 09:30 h (Fig. 2*I*). Water or fertilization significantly affected g_s, except for the interaction of water *vs*. fertilizer (Table 1). The daily mean g_s values of all fertilized plants grown under HW showed no significant differences, but they were significantly higher than those of the CK plants (Table 2). There were significant differences in daily mean g_s values between the P and NP plants under MW, and both were significantly higher than the CK plants, while there were no significant differences between the N and CK plants (Table 2). Under LW, the daily mean g_s values of all fertilized plants were significantly higher than those of the CK plants, and those values of the NP plants were significantly higher than the P plants, but they were similar to those of the N plants (Table 2).

C_i: The highest values appeared at 07:30 h, then decreased to the daily minimum values at about 11:30 h and they maintained at low levels for about 4 h until 15:30 h (Fig. 2*J*,*K*,*L*). The diurnal variations in L_s followed opposite trend to those of C_i (Fig. 2*M*,*N*,*O*). Water and fertilization significantly affected both C_i and L_s, while the effects of their interaction were only significant for L_s (Table 1). Under HW, the daily mean C_i values of the N, P, and CK plants were significantly higher than those of the NP plants, while their daily mean L_s values were significantly lower (Table 2). There were no significant differences in the daily mean C_i or L_s among different fertilizations under MW (Table 2). Under LW, the daily mean C_i values of the P or CK plants were significantly higher than those of the N and NP plants, while the daily mean L_s values of the P or CK plants were significantly lower than those of the N and NP plants (Table 2).

WUE: Diurnal variations followed "L" pattern curves for all water and fertilization regimes. WUE was the greatest at 07:30 h, declined sharply within 2 h to the daily minimum value at about 09:30 h, and after remaining at low values for about 8 h, it increased slightly at about 17:30 h (Fig. 2*P*,*Q*,*R*). Water, fertilization, and their interactions significantly affected WUE (Table 1). Under the HW or MW, the daily mean WUE values in the CK plants were significantly higher than that in all fertilized plants (N, P, and NP). There were also significant differences among all fertilized plants under HW, with the highest values in NP plants, and the lowest in the P plants, but the differences among fertilized plants under MW were not significant (Table 2). Under LW, the daily mean WUE values in the NP or CK plants were significantly higher than those of the N and P plants (Table 2).

P_N-PAR curves: The P_N-PAR response curves were well fitted by the modified rectangular hyperbolic model as indicated by R^2 values, which were greater than 0.996 (Table 3), and they indicated obvious light saturation phenomenon (Fig. 3*A*,*B*,*C*). Water, fertilization, and their interactions significantly affected P_{Nmax}, AQE, R_D, LCP, and LSP (Table 4).

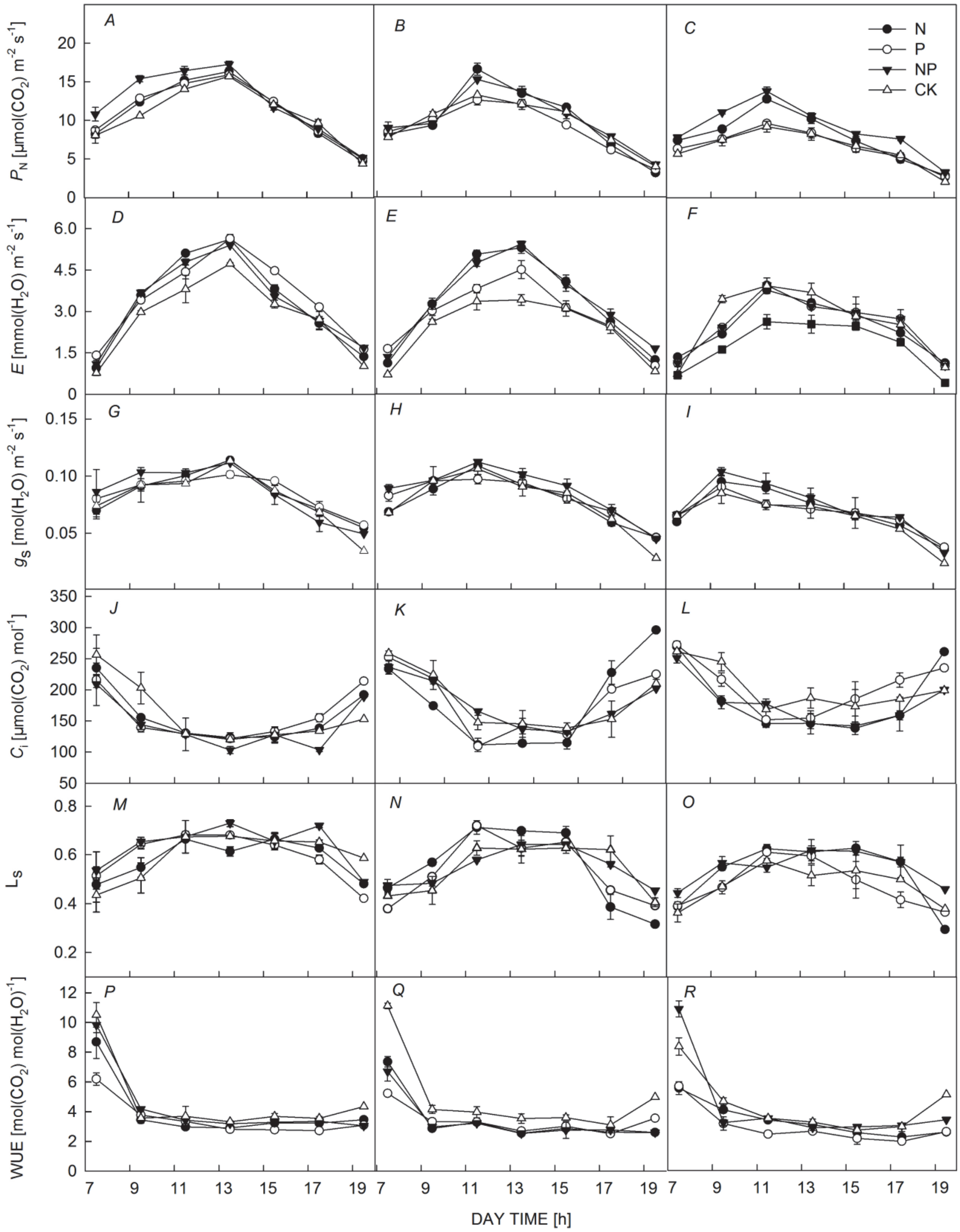

Fig. 2. Diurnal changes of net photosynthetic rate (P_N) (*A*,*B*,*C*), transpiration rate (*E*) (*D*,*E*,*F*), stomatal conductance (g_s) (*G*,*H*,*I*), intercellular CO_2 concentration (C_i) (*J*,*K*,*L*), stomatal limitation value (L_s) (*M*,*N*,*O*), and water-use efficiency (WUE) (*P*,*Q*,*R*) in *Bothriochloa ischaemum* grown under different water regimes and fertilizations. Left side (*A*,*D*,*G*,*J*,*M*,*P*), middle (*B*,*E*,*H*,*K*,*N*,*Q*) and right side (*C*,*F*,*I*,*L*,*O*,*R*) represented HW, MW, and LW regime, respectively. HW – 80% of field capacity; MW – 60% of field capacity; LW – 40% of field capacity; N – nitrogen (●); P – phosphorus (○); NP – nitrogen with phosphorus (▼) ; CK – no fertilization (control; △). The bars indicate the standard deviation of the mean (SD, n = 5).

Table 1. The analysis of variance for the effects of water, fertilization, and their interactions on net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), stomatal limitation value (L_s), and water-use efficiency (WUE) in *Bothriochloa ischaemum*.* – significant at $P<0.05$; ** – significant at $P<0.01$; NS – not significant. df – degree of freedom.

Effect	df	P_N	E	g_s	C_i	L_s	WUE
Water	2	**	**	**	**	**	**
Fertilization	3	**	**	**	**	**	**
Water × fertilization	6	**	**	NS	NS	*	**

Table 2. Daily mean net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), stomatal limitation value (Ls), and water use efficiency (WUE) in *Bothriochloa ischaemum* under different water regimes and fertilizations. Values are means ± standard deviation (SD) (n = 5). HW – 80% of field capacity; MW – 60% of field capacity; LW – 40% of field capacity; N – nitrogen; P – phosphorus; NP – nitrogen with phosphorus; CK – no fertilization (control). *Different superscript letters in parentheses* correspond to significant water regime differences for each parameter within a given fertilization ($P<0.05$). *Different superscript letters without parentheses* correspond to significant differences between fertilizations under each water regime ($P<0.05$).

Parameter	Treatment	N	P	NP	CK
P_N [µmol(CO_2) m^{-2} s^{-1}]	HW	$11.07 \pm 0.09^{b(a)}$	$11.20 \pm 0.12^{b(a)}$	$12.23 \pm 0.02^{a(a)}$	$10.64 \pm 0.10^{c(a)}$
	MW	$9.93 \pm 0.09^{a(b)}$	$9.01 \pm 0.17^{b(b)}$	$10.12 \pm 0.03^{a(b)}$	$9.53 \pm 0.15^{b(b)}$
	LW	$7.78 \pm 0.07^{b(c)}$	$6.61 \pm 0.13^{c(c)}$	$8.90 \pm 0.09^{a(c)}$	$6.40 \pm 0.07^{c(c)}$
E [mmol(H_2O) m^{-2} s^{-1}]	HW	$3.28 \pm 0.03^{b(a)}$	$3.45 \pm 0.03^{a(a)}$	$3.27 \pm 0.03^{b(a)}$	$2.75 \pm 0.05^{c(a)}$
	MW	$3.24 \pm 0.05^{a(a)}$	$2.80 \pm 0.03^{b(b)}$	$3.32 \pm 0.04^{a(a)}$	$2.34 \pm 0.05^{c(b)}$
	LW	$2.40 \pm 0.03^{b(b)}$	$2.48 \pm 0.05^{b(c)}$	$2.58 \pm 0.06^{a(b)}$	$1.74 \pm 0.01^{c(c)}$
g_s [mol(H_2O) m^{-2} s^{-1}]	HW	$0.084 \pm 0.001^{a(a)}$	$0.085 \pm 0.001^{a(a)}$	$0.085 \pm 0.001^{a(a)}$	$0.080 \pm 0.002^{b(a)}$
	MW	$0.078 \pm 0.001^{bc(b)}$	$0.081 \pm 0.001^{b(b)}$	$0.087 \pm 0.001^{a(a)}$	$0.077 \pm 0.002^{c(a)}$
	LW	$0.069 \pm 0.001^{ab(c)}$	$0.067 \pm 0.001^{b(c)}$	$0.072 \pm 0.002^{a(b)}$	$0.063 \pm 0.001^{c(b)}$
C_i [µmol(CO_2) mol^{-1}]	HW	$157.0 \pm 3.0^{a(b)}$	$158.3 \pm 0.4^{a(c)}$	$143.6 \pm 3.8^{b(b)}$	$160.5 \pm 3.6^{a(c)}$
	MW	$181.7 \pm 4.0^{a(a)}$	$182.3 \pm 0.5^{a(b)}$	$178.5 \pm 2.8^{a(a)}$	$182.4 \pm 6.8^{a(b)}$
	LW	$186.1 \pm 4.3^{b(a)}$	$204.5 \pm 6.2^{a(a)}$	$179.4 \pm 1.5^{b(a)}$	$202.7 \pm 3.7^{a(a)}$
L_s	HW	$0.582 \pm 0.007^{b(a)}$	$0.594 \pm 0.001^{b(a)}$	$0.636 \pm 0.009^{a(a)}$	$0.598 \pm 0.009^{b(a)}$
	MW	$0.548 \pm 0.011^{a(b)}$	$0.533 \pm 0.001^{a(b)}$	$0.548 \pm 0.007^{a(b)}$	$0.541 \pm 0.014^{a(b)}$
	LW	$0.524 \pm 0.011^{a(b)}$	$0.477 \pm 0.016^{b(c)}$	$0.546 \pm 0.004^{a(b)}$	$0.476 \pm 0.009^{b(c)}$
WUE [mol(CO_2) mol(H_2O)$^{-1}$]	HW	$4.00 \pm 0.07^{c(a)}$	$3.53 \pm 0.05^{d(a)}$	$4.34 \pm 0.34^{b(a)}$	$4.67 \pm 0.16^{a(b)}$
	MW	$3.45 \pm 0.07^{b(b)}$	$3.38 \pm 0.02^{b(b)}$	$3.36 \pm 0.05^{b(b)}$	$4.92 \pm 0.11^{a(a)}$
	LW	$3.35 \pm 0.06^{b(b)}$	$2.94 \pm 0.09^{c(c)}$	$4.26 \pm 0.03^{a(a)}$	$4.36 \pm 0.04^{a(c)}$

P_{Nmax}: Under all water regimes, the values were significantly higher in the N or NP plants than in the P and CK plants (Table 3). Within a given fertilization, there were significant differences in P_N among all water regimes, the highest and the lowest P_{Nmax} occurred under HW and LW, respectively (Table 3).

AQE: Under HW, the values in N, P, and NP plants were significantly higher than that of the CK plants (Table 3). AQE of the N, NP, and CK plants were significantly higher than those of the P plants under MW and LW (Table 3). There were no significant differences in AQE among the N, NP, and CK plants under MW, but AQE in the N or NP plants were significantly higher than those of the CK plants under LW (Table 3). For any given fertilization, the highest and the lowest AQE values occurred under HW and MW, respectively (Table 3). However, in plants grown under MW and LW, the AQE values were not significantly different in N or CK plants, but they were significantly different, when the plants were fertilized with P (P and NP plants) (Table 3).

R_D of the N, P, and NP plants were significantly higher than those of the CK plants under HW. In contrast, under MW and LW, R_D of the N, P, and NP plants were significantly lower than that of the CK plants (Table 3). In all fertilized plants, the highest and the lowest R_D occurred under HW and LW, respectively, but the opposite trend was observed in the CK plants as water stress increased (Table 3).

Table 3. Light saturated net photosynthetic rate (P_{Nmax}), apparent quantum efficiency (AQE), dark respiration rate (R_D), light compensation point (LCP), and light saturation point (LSP) in *Bothriochloa ischaemum* under different water regimes and fertilizations. Values are means ± standard deviation (SD) (n = 5). HW – 80% of field capacity; MW – 60% of field capacity; LW – 40% of field capacity; N – nitrogen; P – phosphorus; NP – nitrogen with phosphorus; CK – no fertilization (control); R^2 – coefficient of determination. *Different superscript letters in parentheses* correspond to significant water regimes differences for each parameter within a given fertilization (P<0.05). *Different superscript letters without parentheses* correspond to significant differences between fertilizations for each water regime (P<0.05).

Parameter	Treatment	N	P	NP	CK
P_{Nmax} [µmol(CO_2) m^{-2} s^{-1}]	HW	$20.6 \pm 0.23^{b(a)}$	$17.2 \pm 0.03^{c(a)}$	$21.5 \pm 0.23^{a(a)}$	$16.1 \pm 0.12^{d(a)}$
	MW	$16.3 \pm 0.15^{a(b)}$	$14.2 \pm 0.15^{b(b)}$	$16.7 \pm 0.57^{a(b)}$	$14.1 \pm 0.57^{b(b)}$
	LW	$13.9 \pm 0.26^{a(c)}$	$12.2 \pm 0.17^{b(c)}$	$13.9 \pm 0.17^{a(c)}$	$12.1 \pm 0.05^{b(c)}$
AQE [µmol(CO_2) µmol(photon)$^{-1}$]	HW	$0.052 \pm 0.001^{a(a)}$	$0.051 \pm 0.002^{a(a)}$	$0.051 \pm 0.001^{a(a)}$	$0.045 \pm 0.001^{b(a)}$
	MW	$0.043 \pm 0.001^{a(b)}$	$0.031 \pm 0.001^{b(c)}$	$0.041 \pm 0.001^{a(c)}$	$0.040 \pm 0.001^{a(b)}$
	LW	$0.043 \pm 0.001^{a(b)}$	$0.037 \pm 0.001^{c(b)}$	$0.046 \pm 0.001^{a(b)}$	$0.042 \pm 0.001^{b(ab)}$
R_D [µmol(CO_2) m^{-2} s^{-1}]	HW	$2.03 \pm 0.14^{a(a)}$	$1.73 \pm 0.21^{b(a)}$	$2.11 \pm 0.08^{a(a)}$	$1.05 \pm 0.05^{c(c)}$
	MW	$1.21 \pm 0.06^{b(b)}$	$1.44 \pm 0.05^{b(ab)}$	$1.35 \pm 0.04^{b(b)}$	$1.75 \pm 0.05^{a(b)}$
	LW	$1.04 \pm 0.16^{bc(b)}$	$1.32 \pm 0.07^{b(b)}$	$0.86 \pm 0.04^{c(c)}$	$2.60 \pm 0.09^{a(a)}$
LCP [µmol(photon) m^{-2} s^{-1}]	HW	$26.2 \pm 2.2^{a(a)}$	$19.7 \pm 2.1^{b(b)}$	$27.0 \pm 2.1^{a(a)}$	$16.1 \pm 1.0^{c(c)}$
	MW	$18.6 \pm 1.1^{b(b)}$	$34.5 \pm 1.4^{a(a)}$	$21.3 \pm 0.6^{b(b)}$	$32.6 \pm 1.3^{a(b)}$
	LW	$14.1 \pm 1.8^{c(c)}$	$23.7 \pm 2.3^{b(b)}$	$10.0 \pm 0.1^{c(c)}$	$41.1 \pm 0.4^{a(a)}$
LSP [µmol(photon) m^{-2} s^{-1}]	HW	$1,348.7 \pm 5.0^{c(c)}$	$1,406.5 \pm 8.5^{b(b)}$	$1,344.7 \pm 7.7^{c(c)}$	$1,476.8 \pm 3.2^{a(a)}$
	MW	$1,446.5 \pm 6.4^{b(a)}$	$1,496.3 \pm 6.6^{a(a)}$	$1,516.0 \pm 11.0^{a(a)}$	$1,462.0 \pm 13.5^{b(a)}$
	LW	$1,403.3 \pm 7.7^{b(b)}$	$1,387.7 \pm 10.1^{b(b)}$	$1,451.3 \pm 2.7^{a(b)}$	$1,234.9 \pm 0.8^{c(b)}$
R^2	HW	0.998	0.997	0.997	0.998
	MW	0.999	0.999	0.998	0.999
	LW	0.997	0.999	0.996	0.997

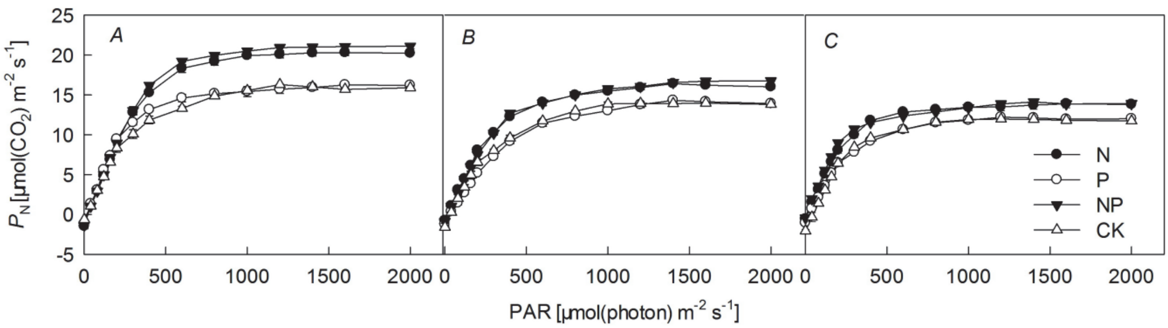

Fig. 3. Net photosynthetic rate (P_N)-photosynthetically active radiation (PAR) response curves for *Bothriochloa ischaemum* grown under different water regimes and fertilizations. HW – 80% of field capacity (*A*); MW – 60% of field capacity (*B*); LW – 40% of field capacity (*C*); N – nitrogen (●); P – phosphorus (○); NP – nitrogen with phosphorus (▼); CK – no fertilization (control; △). The bars indicate the standard deviation of the mean (SD, n = 5).

Table 4. The analysis of variance for the effects of water, fertilization, and their interactions on light saturated net photosynthetic rate (P_{Nmax}), apparent quantum efficiency (AQE), dark respiration rate (R_D), light compensation point (LCP), and light saturation point (LSP) in *Bothriochloa ischaemum*.** – significant at P<0.01. df – degree of freedom.

Effect	df	P_{Nmax}	AQE	R_D	LCP	LSP
Water	2	**	**	**	**	**
Fertilization	3	**	**	**	**	**
Water × fertilization	6	**	**	**	**	**

LCP: Under HW, the values in the fertilized plants were significantly higher than those of the CK plants. In contrast, LCP of the fertilized plants were significantly lower than that of the CK plants under LW, LCP of the P or CK plants were significantly higher than other plants under MW (Table 3). The highest and lowest LCP occurred under HW and LW in the plants with N addition (either alone or with P), respectively, while in the CK plants, they were found under LW and HW, respectively (Table 3).

LSP: Under the HW regime, the values in the fertilized plants were significantly lower than those of the CK plants (Table 3). In contrast, under LW, LSP of the fertilized plants were significantly higher than those of the CK plants (Table 3). Under MW, only the plants fertilized with P (either alone or with N) had significantly higher values than the CK plants (Table 3). The highest and lowest LSP in the fertilized plants were observed under MW and HW, respectively (Table 3). In contrast, the highest and the lowest LSP of the CK plants was found under HW and LW, respectively, although there were no significant differences between HW and MW (Table 3).

Discussion

Water stress significantly decreased the leaf gas-exchange parameters of *B. ischaemum* (*i.e.,* P_N, E, and g_s), and the fertilization played a positive role in improving photosynthesis (Fig. 3, Table 1). In this study, compared with sufficient water regime (HW), the daily P_N peak values occurred earlier in the daytime under water stress conditions (MW and LW) (Fig. 2), indicating that these conditions might intensify the decline in photosynthetic capacity and photoinhibition during the day (Zlatko and Fernando 2012). The significantly higher P_N maximum values of the plants fertilized with N (either alone or with P), implied that appropriate N addition could alleviate the detrimental effects of water stress and improve photosynthetic performance under water stress (Wu *et al*. 2008).

According to Mudrik *et al*. (2003), soil water stress can decrease the photosynthetic activity by inducing stomata closure, decreasing carboxylation efficiency, and inhibiting the light reaction mechanism. The control of stomata opening and transpiration is an effective adaptive strategy of plants responding to water stress in semiarid regions (Xoconostle-Cazares *et al*. 2010, Shan 2009). Stomata closure was the earliest response of plants to water stress under mild to moderate drought conditions, whereas nonstomatal limitation factors became dominant under severe drought conditions (Flexas and Medrano 2002, Jia *et al*. 2012). Stomatal factor was dominant and limiting only if P_N decreased and C_i and L_s increased at the same time, otherwise it was considered as nonstomatal limitation (Farquhar and Sharkey 1982). In this study, the reductions in P_N observed in all plants under all water regimes between 11:30 and 15:30 h were caused by nonstomatal limitation, except for those observed in the NP plants grown under MW and LW and the N plants grown under LW (Fig. 2). The occurrence of the nonstomatal limitation might be attributed to the increased stress caused by the severity, especially due to of high T_{AIR} and high PAR (Fig. 1), as well as to the low soil moisture content. This suggested that nonstomatal controls plays an important role in the daily photosynthetic processes of *B. ischaemum* (Cheng *et al*. 2004). The results further indicated that appropriate levels of N fertilization could alleviate the responses to water stress (Wu *et al*. 2008).

In agreement with the results of Yin *et al.* (2006), water stress significantly decreased P_{Nmax} in our study. Daily mean P_N values and P_{Nmax} of all the fertilized plants were significantly higher than those of the unfertilized plants under HW regime, suggesting that fertilization could improve the potential photosynthetic capacity of *B. ischaemum* under water-sufficient condition. However, under water stress, P fertilization alone did not significantly improve the daily mean P_N and P_{Nmax} values, indicating that addition of N and NP could enhance the photosynthetic capacity under water stress.

WUE indicates the performance of plant growth in the presence of any environmental constraint (Guo *et al*. 2011). In this study, the daily mean values of WUE decreased with declining soil water content, and under all three water regimes, all the fertilized plants had significantly lower WUE than the corresponding unfertilized plants, except for the NP plants grown under LW. This was due to the relative reduction in P_N being greater than that of E under water stress, and to the application of fertilizer further increasing E (Anyia and Herzog 2004, Table 1), which indicated that the reduced WUE might affect undesirably survival of the plant, while fertilization is recommended to improve photosynthetic capacity.

The P_N-PAR curves of *B. ischaemum* were fitted accurately by the modified rectangular hyperbolic model, as indicated by the coefficient of determination (R^2) values (Table 3). AQE is an estimate of the maximum efficiency of light harvesting during the assimilation of CO_2 (Bernacchi *et al.* 2003). Thus, in the absence of water stress (HW), the light-harvesting efficiency of fertilized plants was significantly higher than that of the unfertilized plants. Water stress significantly decreased the light-harvesting efficiency at each fertilization. However, the addition of N, either alone or with P, resulted in significantly higher AQE under LW regime, suggesting that addition of N could enhance the utilization of sunlight by *B. ischaemum*, even under water stress (Table 3).

R_D plays an important role in carbon sequestration for individual plants, plant communities, and even ecosystems, and it is sensitive to water stress, depending on the severity of the stress (Qiao *et al*. 2007). In this study, increasing water stress significantly increased R_D of the unfertilized plants, while in the fertilized plants, it tended to decrease R_D (Table 3). Under LW, the addition of N and NP decreased both R_D and LCP and increased LSP. This response could enhance the assimilation during daylight and reduce the dissimilation during night. Therefore, N and NP fertilization could stimulate effective photosynthesis in the daytime, while it reduces plant consumption at night, which benefits plant growth and productivity (Wu *et al*. 2008, Yin *et al*. 2006).

Water deficit and fertilization significantly affected leaf gas exchange and photosynthetic light-response curves of *B. ischaemum*. Water stress limited photosynthesis not only by decreasing the daily peak P_N, daily mean P_N, E, g_s, WUE, P_{Nmax}, AQE, and LSP, but also by increasing R_D and LCP. Fertilization improved daily mean P_N, P_{Nmax}, and AQE in the presence of sufficient amount of water. The addition of N and NP also improved the daily peak P_N, daily mean P_N, P_{Nmax}, AQE, and LSP, and decreased R_D and LCP under water stress, but the addition of P alone did not significantly improve the photosynthetic performance. These results suggested that appropriate levels of N fertilization could improve the potential photosynthetic capacity of *B. ischaemum* under water stress.

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