

RESEARCH PAPER

Are plant growth and photosynthesis limited by pre-drought following rewatering in grass?

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Abstract

Although the relationship between grassland productivity and soil water status has been extensively researched, the responses of plant growth and photosynthetic physiological processes to long-term drought and rewatering are not fully understood. Here, the perennial grass (*Leymus chinensis*), predominantly distributed in the Euro-Asia steppe, was used as an experimental plant for an irrigation manipulation experiment involving five soil moisture levels [75–80, 60–75, 50–60, 35–50, and 25–35% of soil relative water content (SRWC), i.e. the ratio between present soil moisture and field capacity] to examine the effects of soil drought and rewatering on plant biomass, relative growth rate (RGR), and photosynthetic potential. The recovery of plant biomass following rewatering was lower for the plants that had experienced previous drought compared with the controls; the extent of recovery was proportional to the intensity of soil drought. However, the plant RGR, leaf photosynthesis, and light use potential were markedly stimulated by the previous drought, depending on drought intensity, whereas stomatal conductance (g_s) achieved only partial recovery. The results indicated that g_s may be responsible for regulating actual photosynthetic efficiency. It is assumed that the new plant growth and photosynthetic potential enhanced by pre-drought following rewatering may try to overcompensate the great loss of the plant's net primary production due to the pre-drought effect. The present results highlight the episodic effects of drought on grass growth and photosynthesis. This study will assist in understanding how degraded ecosystems can potentially cope with climate change.

Key words: Chlorophyll fluorescence, gas exchange, grassland ecosystem, relative plant growth rate (RGR), rewatering, water stress.

Introduction

Water shortage has a major limiting effect on plant productivity in many terrestrial ecosystems, especially in arid and semi-arid areas, and the occurrence of drought usually fluctuates at different temporal and spatial levels (Knapp *et al.*, 2001; Bai *et al.*, 2004; Potts *et al.*, 2006a, b; Sponseller, 2007; Swemmer *et al.*, 2007). Global climatic change may result in not only a change in total precipitation but also the occurrence of episodic drought (IPCC, 2007; Wang *et al.*, 2007). Clearly, a more robust assessment of the degree to which changes in water availability affect the

ecological processes under present climatic conditions is urgently needed before predictions can be made for future responses to climatic scenarios (Sponseller, 2007). Moreover, how both continuous drought and rewatering induced by adverse climatic change events affect plant production, community composition, and plant function remains relatively unknown, especially for grassland plants that are more sensitive to drought.

Fernández (2007) indicated that information about the response of plants to rainfall events in some arid areas is

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often lacking. However, even a small rainfall pulse can trigger an increase in plant productivity in a desert ecosystem (Reynolds *et al.*, 2004). For example, Siopongco *et al.* (2006) recently reported that rice plant growth may be remarkably stimulated by rewatering following drought. An investigation into the stimulated effect of pre-drought treatment following rewatering in wheat by Liu *et al.* (2001) indicated that severe water stress increased the leaf area more than moderate water stress after rewatering. Recently, however, Yahdjian and Sala (2006) reported that pre-drought can constrain the response of aboveground net primary production (NPP) to current precipitation in the Patagonian steppe. Thus, the effect of pre-drought and rewatering on plant growth and productivity must be further clarified.

The leaf net photosynthetic rate (A) of plants subjected to moderate drought (MD) may be largely attributed to stomatal limitation, rather than biochemical factors such as Rubisco activity, which is under debate (Lawlor and Cornic, 2002; Bota *et al.*, 2004; Flexas *et al.*, 2006; Galmés *et al.*, 2007a). An obvious decrease in mesophyll conductance (g_s) under severe drought (SD), however, can be partly responsible for an increase in non-stomatal limitations (Flexas *et al.*, 2002; Grassi and Magnani, 2005; Galmés *et al.*, 2007a). More SD may result in cell membrane damage (Benhassaine-Kesri *et al.*, 2002) and decreased photosynthetic capacity, including photosystem II (PSII) activity (Ghannoum *et al.*, 2003; Xu and Zhou, 2006b; Gallé and Feller, 2007). Recent reports indicated that discrete precipitation events can trigger brief but important episodes of biological activity, including both photosynthesis and respiration in water-limited ecosystems (Potts *et al.*, 2006a; Loik, 2007). Leaf A of beech saplings can be completely restored after rewatering (Gallé and Feller, 2007), while stomatal conductance (g_s) remains permanently lower in stressed plants than in control plants, resulting in increased intrinsic water use efficiency (WUE; Gallé and Feller, 2007; Pou *et al.*, 2008) and thus demonstrating a high tolerance to episodic drought. However, Gomes *et al.* (2008) indicated that rewatering also leads to the incomplete recovery of leaf A rate, mainly due to photochemical impairment under SD. Thus, the issue remains debatable and needs to be elucidated.

Precipitation pulse obviously affects ecophysiological function processes, particularly in an arid ecosystem, from biochemical to ecosystem levels, and is of particular interest to many researchers (Reynolds *et al.*, 2004; Yahdjian and Sala, 2006; Fernández, 2007; Yang *et al.*, 2008). Nevertheless, the relationship between plant growth and ecophysiological performances in response to drought and rewatering is not yet fully understood (Wiegand *et al.*, 2004; Yahdjian and Sala, 2006; Fernández, 2007; Xu and Zhou, 2008). Moreover, drought limitation and recovery following rewatering may have different adaptive mechanisms, as plants were subjected to erratic drought or rainfall in semi-arid regions (Gazanchian *et al.*, 2007; Izanloo *et al.*, 2008). Therefore, the main objective of the present experiment was to examine the responses of plant growth and photosynthe-

sis to soil drought, particularly their recovery after rewatering, as plants were subjected to different intensities of long-term previous drought. It was hypothesized that although rewatering after long-term drought can overcompensate plant biomass limitation due to previous drought by increasing relative growth rate (RGR) and photosynthetic activity, the final biomass of plants that experienced previous SD may be less than the biomass of plants that were always under well-watered conditions.

Materials and methods

Plant culture

Grassland dominated by *Leymus chinensis* (Trin.) Tzvel., a native perennial grass with good palatability and high forage value, is widespread in the Euro-Asia steppe. However, an increase in grazing pressure and adverse climatic change have led to a substantial reduction in productivity throughout most of the grassland. When this condition is combined with drought and summer high temperature, soil moisture becomes more critical as daily temperatures increase during the summer. Thus, drought has become a major limiting factor for *L. chinensis*, especially in recent decades (Wang and Gao, 2003; Bai *et al.*, 2004, 2008; Wang *et al.*, 2007). During the last year of the experiment, seeds were obtained from a grassland in Xilinhot, Inner Mongolia, China (44°08'N, 117°05'E), at 1100 m above sea level. The region is continental temperate semi-dry grassland with mild temperatures during spring and autumn, cool and dry winters, and wet but hot summers. The annual mean temperature was 2 °C, and the annual precipitation was 350 mm over the previous 50 years.

Seeds of *L. chinensis* were sterilized by a 0.7% potassium permanganate solution for 8 min, rinsed, and then immersed in water for 7 d before being placed into a refrigerator below 0 °C. They were sown in plastic pots (5.1 l, 18 cm in diameter, 20 cm in height) wrapped with plastic film. Each plastic pot was filled with 4.08 kg of dry soil obtained from a natural field in the Xilinguole grassland (Inner Mongolia of China) and planted with a density of eight plants per pot. In the chestnut-coloured soil, the organic carbon concentration was 19.60 ± 0.18 g kg⁻¹ and the total nitrogen was 4.18 ± 0.11 g kg⁻¹. All experimental pots were placed in a naturally illuminated greenhouse at the daily maximum photosynthetic photon flux density (PPFD) of 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ above the plant canopy on a clear day, provided by a combination of cool-white fluorescent and incandescent lamps in the greenhouse, with a day/night temperature of 28–32/20–24 °C.

Soil water-withholding treatments were performed until a third leaf appeared (44 d after sowing). The soil relative water contents (SRWCs) (ratio between the present soil moisture and field capacity) were sorted into five levels: control (75–80%), light drought (LD) (60–75%), MD (50–60%), SD (35–50%), and extreme drought (ED) (25–35%).

Water was added to return the soil moisture to the upper limit by irrigation at 17:00 h every 2–3 d. All additions of water were recorded for all treatments to obtain the amount of evapotranspiration. The SRWC is expressed as:

$$\text{SRWC} = (W_{\text{soil}} + W_{\text{pot}} - DW_{\text{soil}}) / (W_{\text{FC}} - W_{\text{pot}}),$$

where W_{soil} is the current soil weight, W_{pot} is the weight of the empty pot, DW_{soil} is the dry soil weight, and W_{FC} is the soil weight at field capacity.

At 51 d after the start of water withholding, the treated pots were divided into two groups: one was rewatered, while the other continued water-withholding treatments. The experimental procedure, including the time-sampled biomass, is presented in Table 1.

Biomass and leaf area measurements

Plant biomass was sampled in three or four pots per treatment at each harvesting time, and dried at 80 °C to a constant weight, then weighed. RGR ($\text{mg g}^{-1} \text{day}^{-1}$) was expressed as $[\ln(\text{harvest dry mass of } t_2) - \ln(\text{initial dry mass of } t_1)] / (t_2 - t_1) \times 1000$ (Lindroth *et al.*, 2001).

In order to assess the limitation of pre-drought after rewatering, the percentage of pre-drought limitation (PDL) was estimated as:

$$\text{PDL after rewatering (\%)} = [(\text{growth or photosynthetic traits under control} - \text{growth or photosynthetic traits undergone drought}) / \text{growth or photosynthetic traits under control}] \times 100,$$

where growth or photosynthetic traits under control denoted the plants that had not experienced drought during the entire experimental period, and growth or photosynthetic traits undergone drought denoted those plants that had experienced pre-drought and were measured following recovery.

The plant leaf area was measured using a Li-3000 leaf area meter (Li-Cor, Inc., Lincoln, NE, USA); the specific leaf area (SLA) was calculated from the measurements of leaf area and dry matter.

Leaf gas exchange and chlorophyll fluorescence measurements

Leaf gas exchange measurements were made using an open gas exchange system (LI-6400, LI-COR Bioscience, Lincoln, NE, USA) concurrently with a leaf chamber fluorometer (LI-6400-40). The data were initially analysed with data acquisition

software (OPEN Software version 5.1, LI-COR Bioscience). Illumination was supplied to the leaf from a red-blue light-emitting diode (LED) light source. Prior to measurement, leaves were acclimated in the chamber for >10 min at a relative consistent temperature (25–27 °C), an ambient CO_2 concentration ($380 \mu\text{mol mol}^{-1}$), and a PPFD of $900 \mu\text{mol m}^{-2} \text{s}^{-1}$ (a value at which photosynthesis is nearly saturated in the present experimental conditions). The gas exchange parameters were determined in the 3–4 youngest and fully expanded leaves of different individuals (one plant per pot, total of three pots) for all replicates, generally between 08:30 h and 14:30 h daily. For the actual measurement, the vapour pressure deficit (VPD) in the cuvette was maintained below 2.0 kPa to minimize its effect, showing that no significant changes in the slope of the initial part of the A/C_i curve occurred below the VPD threshold.

The leaves used for measurement of fluorescence parameters were the same leaves as used for determination of gas exchange; after 30 min of dark adaptation at the relative consistent temperature, the minimal fluorescence yield (F_0) was measured by using modulated light that was sufficiently low ($<0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$), and the maximal fluorescence yield (F_m) was determined by a 0.8 s saturating pulse at $8000 \mu\text{mol m}^{-2} \text{s}^{-1}$ in dark-adapted leaves. The leaves were then continuously illuminated with white actinic light at an intensity of $900 \mu\text{mol m}^{-2} \text{s}^{-1}$ for 30 min. The steady-state value of fluorescence (F_s) was thereafter recorded, and the second saturating pulse at $8000 \mu\text{mol m}^{-2} \text{s}^{-1}$ was imposed to determine the maximal light-adapted fluorescence level (F'_m). The fluorescence parameters were obtained from formulae (Genty *et al.*, 1989; van Kooten and Snel, 1990): the maximal efficiency of PSII photochemistry [$F_v/F_m = (F_m - F_0)/F_m$], actual PSII efficiency [$\Phi_{\text{PSII}} = (F'_m - F_s)/F'_m$], and non-photochemical quenching [$\text{NPQ} = (F_m - F'_m)/F'_m$].

Estimation of light response parameters

After acclimation, the PPFD was sequentially lowered to 1200, 900, 800, 600, 400, 200, 100, 50, and $20 \mu\text{mol m}^{-2} \text{s}^{-1}$. The responses of photosynthesis parameters to light were estimated according to a quadratic equation (Prioul and Chartier, 1977; Long *et al.*, 1993):

$$A = \left\{ \alpha \times \text{PPFD} + [(\alpha \times \text{PPFD} + A_{\text{sat}})^2 - 4\alpha \times \text{PPFD} \times \theta \times A_{\text{sat}}]^{0.5} \right\} / (2\theta) - R_d,$$

where A is the net photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$), A_{sat} is the maximum CO_2 accumulation rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$),

Table 1. Experimental procedure

Date	10 June	24 July	13 August	13 September	13 October	2 November	22 November
Period (d)	0	44	20	31	30	20	20
Activities	Sowing	Start of water withholding	Well treated	First harvesting ^a	Second harvesting	Third harvesting	Fourth harvesting

^a On 13 September 2005, the treated pots were divided into two groups. One was rewatered, while the other continued water deficit treatments.

PPFD is the photosynthetic photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$), α is the leaf maximum apparent quantum yield of CO_2 uptake, and θ is the convexity of the transit from light-limited to light-saturated photosynthesis. Instantaneous determinations of g_s were obtained after illumination at saturating light ($900 \mu\text{mol photon m}^{-2} \text{s}^{-1}$) for at least 10 min at 25–27 °C.

Estimation of a response to C_i

As detailed above, light acclimation was conducted before measurements of the A/C_i response. The CO_2 concentration gradients to produce A/C_i curves were 380, 300, 200, 100, 50, 20, 380, 380, 600, 800, and 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ step by step. The two 380s in a row after the low value was not an error, but a trick to give the leaf some recovery time after the low CO_2 measurement. Later, the first of those readings could be eliminated if it could not be fitted in. Curve-fitting software was used to analyse the A/C_i responses using the function of the form from the photosynthesis model of Farquhar *et al.* (1980) and the modification with g_i by Ethier and Livingston (2004). The CO_2 g_i was measured by the methods of Loreto *et al.* (1992).

$$A_c = (C_i - \Gamma^*) V_{c,\max} / [C_i + K_c(1 + O/K_o) + 2\Gamma + 0.5(V_{c,\max} - R_d)/g_i],$$

where A_c is the RuBP-saturated CO_2 assimilation rate, C_i is the intercellular CO_2 concentration, $V_{c,\max}$ is the maximum carboxylation velocity, and R_d is mitochondrial respiration in the light. The other parameters are indicated in Table 2.

The electron transport rate (J) was expressed as $J = \Phi_{\text{PSII}} \times f I \alpha_{\text{leaf}}$, where f is the fraction of absorbed quanta that is used by PSII and is typically assumed to be 0.5 for C_3 plants (Ögren and Evan, 1993), I is actinic PPFD, and α_{leaf} is the effective leaf absorptance, ranging from 0.88 to 0.95 (here assumed to be 0.95) (Flexas *et al.*, 2007). This g_i can be calculated by the variable J method (Harley *et al.*, 1992):

$$g_i = A / \{C_i - \Gamma^* [J + 8(A + R_d)] / [J - 4(A + R_d)]\}.$$

Statistical analysis

All data analyses were conducted with SPSS 10.0 statistical software (SPSS, Chicago, IL, USA). The experiments were repeated at least three times and means \pm SE of values are

Table 2. Kinetic constants of Rubisco in vivo used to parameterize in the photosynthesis model (from Bernacchi *et al.*, 2001, 2002)

Γ^*	33.86 $\mu\text{mol mol}^{-1}$	Chloroplasmic CO_2 photocompensation point
Γ	42.89 $\mu\text{mol mol}^{-1}$	CO_2 photocompensation point
O	210 mmol mol^{-1}	O_2 concentration
K_c	406.07 $\mu\text{mol mol}^{-1}$	Michaelis–Menten constant for RuBP carboxylation
K_o	276.9 mmol mol^{-1}	Michaelis–Menten constant for oxygenation

given. The parameters were analysed by one-/two-way analysis of variance (ANOVA) followed by Duncan's multiple range test (Duncan, 1955). In order to test the effect of g_s on A_{sat} , a three-component exponential function of the form by a non-linear regression estimate was used:

$$A_{\text{sat}} = a \times (1 - e^{-b \times g_s}) + c.$$

Using this function, $A_{\text{max, sat}}$ in response to g_s was expressed as $a+c$, and the slope ϕ was calculated as $b(a+c)$. Thus, the value of the threshold of g_s (the g_s onset as A_{sat} levels off at $A_{\text{max, sat}}$) estimate can be calculated by the linear function described by ϕ and c to its intersection with $A_{\text{max, sat}}$ (i.e. a/ϕ). Otherwise, linear regression analysis was also used in this study.

Results

Responses of leaf water status to drought and rewetting

The effect of drought was determined by the leaf's RWC ($F=28.065$, $P<0.001$), and SD and ED led to significant RWC decreases, according to Duncan's multiple range test ($P<0.05$) (Fig. 1). As the plants under MD, SD, and ED were rewatered, leaf RWC exhibited no significant differences from well-watered plants (control treatment) ($P>0.05$) and within the three rewatered treatments ($F=0.101$, $P=0.905$), indicating that leaf RWC completely recovered following rewetting.

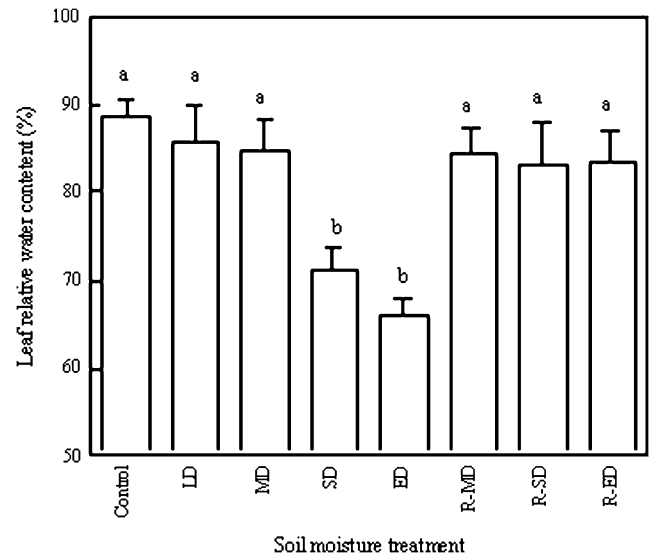


Fig. 1. Leaf relative water content (RWC) under soil drought and rewetting (measured on 19 October 2005). Five SRWCs are indicated by control (75–80%), LD (6–75%), MD (50–60%), SD (35–50%), and ED (25–35%); R-MD, R-SD, and R-ED represent rewetting after the corresponding drought. All values are means \pm SE for $n=3-4$. Bars with different lowercase letters are significantly different ($P<0.05$).

Recovery of new leaf following rewatering

As shown in Fig. 2A, new leaf area per plant following rewatering in pots that had experienced previous SD and ED treatments was significantly affected by previous drought treatment, according to the F test ($F=3.896$, $P=0.037$), and was significantly higher ($P < 0.05$) than that of the control; however, the pre-drought treatment did not result in a significant change in new leaf dry matter ($F=1.012$, $P=0.446$). For the SLA of the new leaves, the effect of drought was significant, and the effect of SD was significantly higher ($P < 0.05$) than that of the control.

Responses of plant growth to drought and rewatering

Figure 2B illustrates the changes in tiller number per pot from initial drought to continuous drought, after rewater-

ing. A two-way ANOVA indicated significant effects on main factors ($F=66.67$, $P < 0.001$ for time; $F=10.03$, $P < 0.001$ for soil moisture) and their interaction ($F=2.45$, $P=0.013$). Under the three continuous drought conditions, withholding soil water led to significant variations ($P < 0.05$); however, following rewatering, the obvious changes were negated due to previous soil drought ($F=1.13$, $P=0.396$). Compared with the effects of control treatment, the effects of SD and ED significantly declined at all water-withholding stages ($P < 0.05$), but tillers of the plants subjected to previous SD and ED had already exceeded or approached the level of the control treatment following rewatering.

Figure 2C demonstrates the responses of biomass in various organs and whole plants to drought and rewatering.

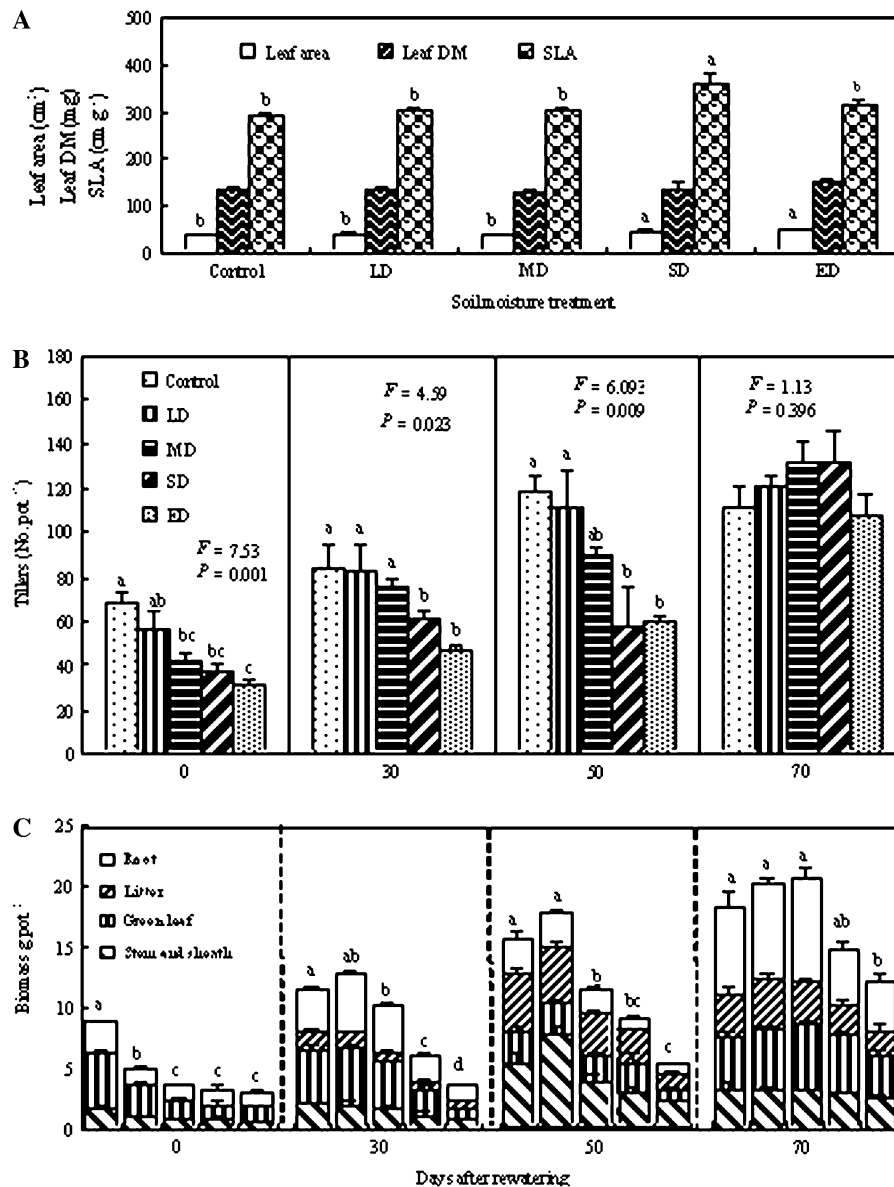


Fig. 2. (A) Leaf area, leaf dry matter, and specific leaf area (SLA) of the new leaves following rewatering after plants experienced previous different drought severities; (B) plant tillers, and (C) plant biomass at soil drought, continual drought, and following rewatering (right panel) after plants experienced different previous drought severities. Five SRWCs are indicated by control (75–80%), LD (60–75%), MD (50–60%), SD (35–50%), and ED (25–35%). All values are means \pm SE for $n=3-6$. Bars with different lowercase letters are significantly different ($P < 0.05$).

Before rewatering, during the initial water-withholding treatment, drought of more than LD (SRWC <75%) significantly decreased the biomass of various organs and the whole plant ($P < 0.05$). However, with continual drought, LD no longer reduced the biomass, and even slightly increased root and whole plant biomass ($P > 0.05$), although more than MD still decreased their biomass ($P < 0.05$). On the 70th day following rewatering, the treatments still produced significant differences for the organs ($F=7.304$, $P=0.005$ for green leaf; $F=3.823$, $P=0.039$ for litter; $F=4.667$, $P=0.022$ for root; and $F=3.649$, $P=0.044$ for whole plant), except for the stem and sheath ($F=1.029$, $P=0.438$). Following rewatering, comparison between the treatments indicated that only ED significantly decreased the biomass of the green leaf, litter, root, and whole plant ($P < 0.05$), but not that of the stem and sheath. However, previous drought of less than ED intensity did not reduce their biomass, indicating that MD did not limit growth following rewatering.

Responses of RGR to pre-drought and rewatering

The RGR changes in different organs of the plants were measured after rewatering after the continuous soil drought treatment (101 d, Fig. 3A) and after rewatering from the starting soil drought treatment (81 d, Fig. 3B). It was found that the RGR was significantly stimulated by more than SD treatments when the two durations of drought treatments were completed, except for stem and litter organs with the previous long-term drought treatment. For the long-term drought treatment (Fig. 3A), the greatest RGR occurred in root, followed by green leaf; in contrast, for short-term drought (Fig. 3B), the maximum values were in litter, followed by stem and green leaf, possibly due to the lower amount of litter with the short-term drought treatment.

Nevertheless, following rewatering, previous SD still caused a significant RGR increase of the whole plant for both durations of soil drought, indicating that plant RGR was remarkably stimulated by the previous SD.

PDL of both biomass and RGR due to previous soil drought

Figure 4A demonstrates the relative PDL biomass from the previous soil drought treatment. SD, especially ED, significantly increased the PDL in stem, green leaf, litter, root, and total biomass ($P < 0.05$). However, LD and MD did not produce positive PDL values; in contrast, negative values resulted, indicating that LD and MD significantly stimulated plant productivity rather than limitation.

However, when the RGR's PDL was determined, an obvious difference from the PDL of biomass was found (Fig. 4B). Previous drought led to significant changes in the RGR's PDL in stem ($F=5.01$, $P=0.018$), green leaf ($F=33.00$, $P < 0.001$), litter ($F=3.88$, $P=0.037$), root ($F=10.02$, $P=0.002$), and whole plant ($F=28.35$, $P < 0.001$). Values for all organs and whole plants were negative, except for green leaf at LD. SD and ED produced more negative values than LD and MD. For example, values >1000-fold for stimulating green leaf RGR and >300-fold for stimulating whole-plant RGR were observed under SD and ED conditions ($P < 0.01$), clearly indicating that previous soil drought of <50% SRWC dramatically accelerated plant growth after rewatering.

Responses of photosynthesis and PSII function to drought and rewatering

Photosynthetic and PSII function performances were further determined under both soil drought and rewatering conditions. As shown in Table 3, significant changes in g_s ,

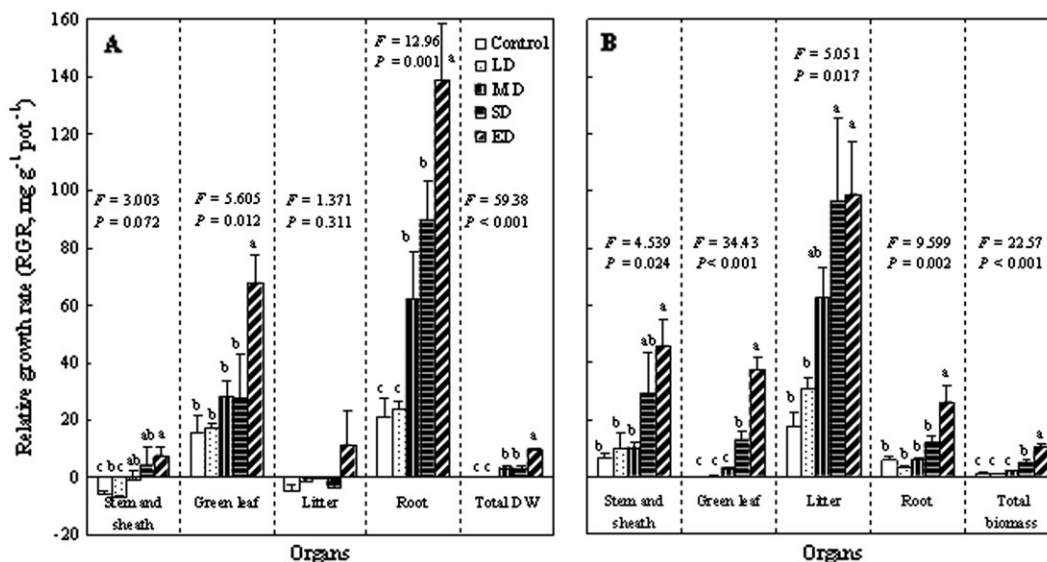


Fig. 3. (A) Relative growth rate (RGR) after rewatering from the continuous soil drought treatment (101 d), and (B) after rewatering from the start of soil drought treatment (81 d). Five SRWCs are indicated by control (75–80%), LD (60–75%), MD (5–60%), SD (35–50%), and ED (25–35%). All values are means \pm SE for $n=5$. Bars with different lowercase letters are significantly different in the same organs ($P < 0.05$).

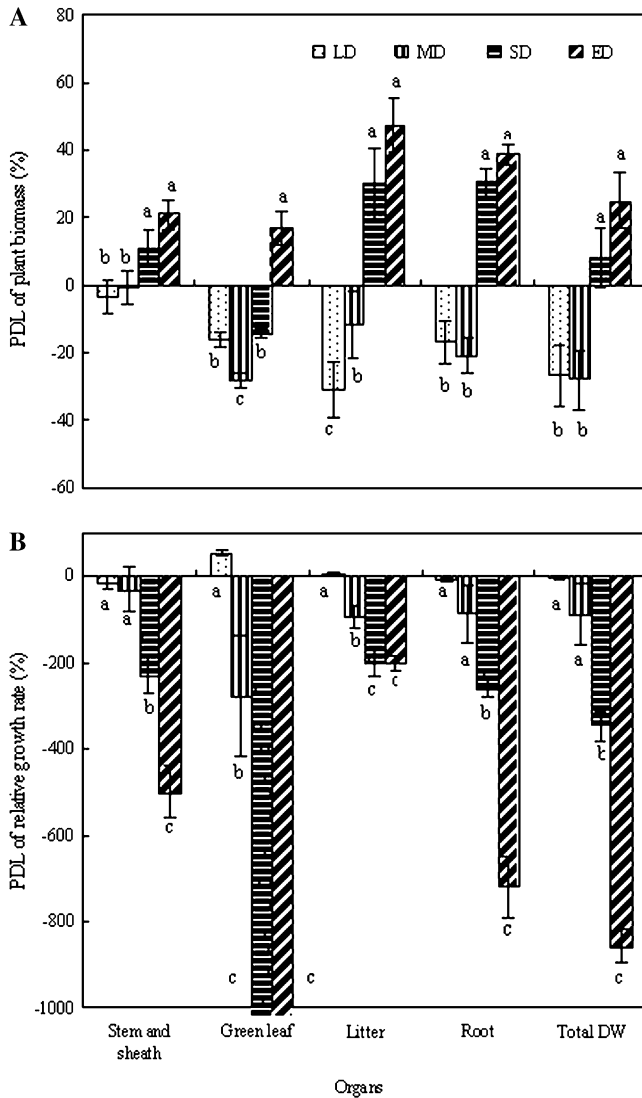


Fig. 4. (A) Pre-drought limitation (PDL) of plant biomass and (B) relative growth rate (RGR) (40 d period after rewatering). Five SRWCs are indicated by control (75–80%), LD (60–75%), MD (50–60%), SD (35–50%), and ED (25–35%). All values are means \pm SE for $n=3$. Bars with different lowercase letters are significantly different in the same organs ($P < 0.05$)

and g_i resulted from soil moisture treatments ($P < 0.01$), and significant reductions were induced by a drought of more than MD compared with the control treatment. Soil drought also significantly affected leaf photosynthetic potential indicated by A_{sat} ($F=83.63$, $P < 0.01$), and light use efficiency indicated by α ($F=45.42$, $P < 0.01$). Compared with the control, SD decreased A_{sat} by 22.3% and ED significantly decreased A_{sat} by 74.9%; in contrast, LD and MD did not significantly affect A_{sat} , although α significantly decreased due to the drought treatments of more than MD. Soil water-withholding treatments also resulted in significant changes in $V_{\text{c,max}}$ ($F=4.90$, $P=0.019$), which significantly decreased at SD and ED, indicating that Rubisco activity *in vivo* was severely affected by severe water deficit stress. However, following watering, substantial increases were observed in g_s , g_i , A_{sat} , $V_{\text{c,max}}$, and α compared with

pre-irrigation values, leading to a lower variation from previous drought treatments ($P > 0.05$), although g_s and g_i did not reach the control levels. These results indicated that the photosynthetic potential, the potential of light use efficiency, and Rubisco activity *in vivo* could be recovered following rewatering.

The PSII function and its photochemistry status were also determined. As many reports have pointed out (e.g. Baker and Rosenqvist, 2004), the maximal efficiency of PSII photochemistry (F_v/F_m) can represent the greatest photochemical efficiency or the primary efficiency of light energy transitions; the actual quantum yield (Φ_{PSII}) indicates the efficiency of transfer of absorbed photons to the reaction centre of PSII; and NPQ is the portion of light energy absorbed by antenna pigment but not used in electron transport and dissipated as thermal energy. As shown in Table 3, significant changes in F_v/F_m , Φ_{PSII} , and NPQ were observed when the plants were subjected to soil moisture treatments ($P < 0.01$). A significant decline in F_v/F_m was observed under more than SD, and a decline of Φ_{PSII} was observed under more than MD ($P < 0.05$); however, a significant increase in NPQ ($P < 0.05$) occurred at SD and ED. Following rewatering, complete recovery of F_v/F_m and Φ_{PSII} occurred in the leaves of plants subjected to pre-drought ($P > 0.05$), but NPQ did not significantly change after rewatering.

The PDL of the photosynthetic and photochemical parameters was also calculated (bottom section in Table 3). The previous different drought levels did not lead to significant changes of the parameters except for α ($F=5.4$, $P < 0.05$), but greater positive values of RDL of g_s , g_i , α , and Φ_{PSII} indicated that inhibition of four parameters following rewatering existed from pre-drought. However, the PDLs of the NPQ were significant negative values, indicating that the dissipating heat mechanism of PSII induced by pre-drought was maintained following rewatering.

Relationships between photosynthetic parameters

As shown in Fig. 5, the response of A_{sat} to g_s was better fitted with a three-component exponential function of the form estimated by non-linear regression [$A_{\text{sat}}=54.58 \times (1 - e^{-11.21 \times g_s}) - 35.15$; $R^2=0.74$, $P < 0.01$; Fig. 5A], rather than a linear function. Initially, A_{sat} rapidly increased with increasing g_s , then levelled off at a maximum of $19.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ at a g_s of $0.25 \text{ mol m}^{-2} \text{s}^{-1}$. However, the correlation of $V_{\text{c,max}}$ with g_s was obviously scattered ($R^2=0.17$), indicating that no close association existed.

The α and Φ_{PSII} also had close relationships with g_s , which are well expressed by the three-component exponential functions [$\alpha=0.148 \times (1 - e^{-5.92 \times g_s}) - 0.057$; $R^2=0.64$, $P < 0.01$ for α ; and $\Phi_{\text{PSII}}=0.467 \times (1 - e^{-4.04 \times g_s}) - 0.0006$; $R^2=0.71$, $P < 0.01$ for Φ_{PSII} ; Fig. 5B], with inflection points at MD soil moisture (g_s was 0.275 with the maximum α of 0.091 and 0.248 with the maximum Φ_{PSII} of 0.47), again indicating that the limitation to light use efficiency only occurred below the threshold. NPQ was significantly and

Table 3. Photosynthetic and PSII function performances under soil drought and rewatering (measured on 15–18 October 2005), with soil relative water contents (SRWCs) of control (75–80%), LD (60–75%), MD (50–60%), SD (35–50%), and ED (25–35%)

R-MD, R-SD, and R-ED represent rewatering after the plants have undergone the corresponding pre-drought treatments.

	g_s	g_i	A_{sat}	α	$V_{c,max}$	F_v/F_m	Φ_{PSII}	NPQ
Control	0.415±0.076 a	0.225±0.027 a	17.01±0.99 a	0.078±0.004 a	103.34±5.68 a	0.814±0.010 a	0.393±0.002 a	0.523±0.080 c
LD	0.412±0.048 a	0.217±0.029 a,b	18.00±1.36 a	0.080±0.003 a	100.12±1.82 a,b	0.816±0.003 a	0.369±0.024 a	1.064±0.081 b
MD	0.269±0.017 b	0.157±0.007 b,c	17.82±0.57 a	0.053±0.002 b	88.63±3.25 a,b	0.810±0.004 a	0.309±0.011 b	1.358±0.126 b
SD	0.218±0.012 b,c	0.120±0.017 c	13.21±0.58 b	0.030±0.001 c	90.62±1.37 b c	0.791±0.002 b	0.246±0.037 b	2.090±0.070 a
ED	0.146±0.019 c	0.057±0.009 d	4.27±0.46 c	0.028±0.002 c	84.89±3.43 c	0.749±0.005 c	0.203±0.007 b	2.483±0.248 a
F	7.91	12.58	45.42	83.63	4.79	24.12	16.42	32.90
P	0.004	<0.01	<0.01	<0.01	0.020	<0.01	<0.01	<0.01
Performances after rewatering								
R-MD	0.272±0.030 a*	0.180±0.013 a	18.18±0.95 a	0.077±0.001 a	107.71±20.68 a	0.820±0.003 a	0.312±0.012 a*	1.625±0.147 a*
R-SD	0.254±0.019 a*	0.148±0.012 a,b*	17.89±0.56 a	0.070±0.004 a,b	113.09±3.47 a	0.819±0.003 a	0.295±0.002 a*	1.824±0.042 a*
R-ED	0.249±0.009 a*	0.126±0.005 b*	18.95±1.49 a	0.067±0.003 b*	122.18±6.77 a*	0.767±0.054 a	0.268±0.021 a*	1.935±0.153 a*
F	0.94	5.88	0.26	2.64	0.32	0.96	2.52	1.58
P	0.441	0.069	0.778	0.150	0.736	0.44	0.160	0.28
PDL (%)								
R-MD	34.41±8.02	19.82±5.87 a	-6.88±5.64	1.28±4.95 b	-4.23±20.01	-0.74±0.82	20.61±2.60	-210.71±22.92
R-SD	38.75±8.20	34.26±5.87 a,b	-5.17±7.46	10.26±1.04 a,b	-9.44±3.36	-0.61±0.98	24.94±0.22	-248.76±41.52
R-ED	39.95±9.5	43.82±2.37 b	-11.41±10.43	14.10±1.5 2a	-18.24±7.28	5.77±5.94	31.81±4.97	-269.98±34.91

A_{sat} is the light-saturated CO_2 accumulation rate ($\mu mol mol^{-1}$); F_v/F_m is the maximal efficiency of PSII photochemistry (dimensionless); g_s is stomatal conductance ($mmol mol^{-1}$); g_i is mesophyll conductance ($mmol mol^{-1}$); NPQ is non-photochemical quenching (dimensionless); PDL is pre-drought limitation; $V_{c,max}$ is maximum carboxylation velocity ($\mu mol mol^{-1}$); α is the maximum photosynthetic quantum yield of CO_2 uptake (dimensionless); and Φ_{PSII} is the actual PSII efficiency (dimensionless). Asterisks indicate significance at the 0.05 level, compared with those of the control value.

negatively linearly related to g_s along the soil moisture gradient ($R^2=0.56$, $P<0.001$), but no strong relationship was observed between F_v/F_m and g_s ($R^2=0.19$, $P=0.015$) (Fig. 5C).

Discussion

The sensitivity of ecosystems to precipitation change plays a considerably important role in assessing and predicting ecological responses to climate change (Knapp *et al.*, 2001; O'Connor *et al.*, 2001; Yahdjian and Sala, 2006; Swemmer *et al.*, 2007). The limitation to plant growth and productivity under current well-watered environmental conditions may be due to the drought effects of previous years (Wiegand *et al.*, 2004; Yahdjian and Sala, 2006). However, in central North American grasslands, plant richness and growth increased most in wet years that followed dry years (Adler and Levine, 2007). The present results indicated that long-term SD remarkably leads to a dramatic decline in plant production, and that biomass resumption from the great loss caused by previous SD can be escalated by accelerating the growth of new parts of the plant and enhancing photosynthetic activity following rewatering.

The current experiment indicated that plant biomass was remarkably limited after rewatering (Fig. 2C), whereas RGR was stimulated drastically by previous SD (Figs 3, 4B). The limitation of the former has been emphasized by Yahdjian and Sala (2006). However, the stimulation of plant growth by rewatering has also been highlighted by other investigators (Liu *et al.*, 2001; Reynolds *et al.*, 2004;

Siopongco *et al.*, 2006). The rapid growth of new organs (e.g. new leaf) may contribute to the high RGR, which is favoured by easily available useful nutritional factors (e.g. nitrogen) in rewetted soil (Heckathorn *et al.*, 1994; Xu and Zhou, 2006b). Moreover, plant growth is relatively sensitive to environmental water conditions (Hsiao, 1973). For example, upon rewatering, *Lupinus albus* plants could rapidly produce new leaves with quickly re-restored plant water status, although the levels of other metabolites (e.g. the sugar level) recovered more slowly (Pinheiro *et al.*, 2004). The leaf length of maize plants that have experienced one or more days of drought stress could reach the levels of the control leaves after rewatering, but their growth rate could not exceed that of the latter, indicating that the growth may be only a resumption of the postponed event, not overcompensation (Acevedo *et al.*, 1971). The extent and speed of resumption may depend on drought stress intensity and duration (Hsiao, 1973). Thus, the extent of compensation for the limitation of pre-drought by stimulating new growth following rewatering would determine the final plant biomass and RGR, which may be closely associated with the severity and duration of soil drought.

Benson *et al.* (2004) indicated that in a tallgrass prairie plant population, drought may limit the growth of the plant organs (e.g. roots and branches), thereby reducing meristems and finally decreasing the capacity of vegetation to respond to high resource availability. As a result of meristem limitation, it is suggested that grassland production may be lower in wet years preceded by dry years than in wet years preceded by wet or normal years (Benson

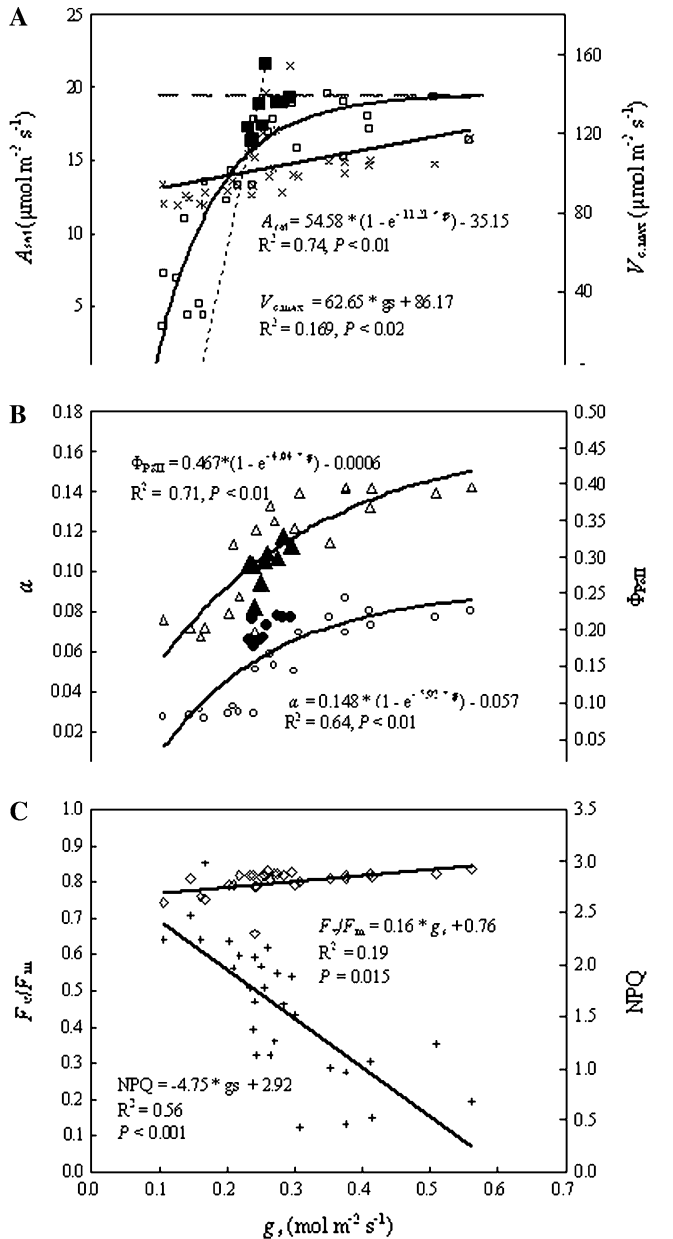


Fig. 5. Responses of photosynthetic potential and photochemical activities to stomatal conductance (g_s). (A) Open squares, A_{sat} light-saturated CO_2 accumulation rate; filled squares, A_{sat} after rewatering; crosses, $V_{c,\text{max}}$ maximum carboxylation velocity. The dotted line denotes the initial A_{sat} response to g_s ; the dashed line denotes $A_{\text{sat}} = \text{constant}$ value of $A_{\text{sat,max}}$ estimated; and the intersection of the two lines represents a point at which A_{sat} initially levelled off. (B) Open circles, α maximum photosynthetic quantum yield of CO_2 uptake; filled circles, α after rewatering; open triangles, Φ_{PSII} the actual PSII efficiency; filled triangles, Φ_{PSII} after rewatering. (C) Open squares, the maximal efficiency of PSII photochemistry (F_v/F_m); open diamonds, non-photochemical quenching (NPQ).

et al., 2004; Yahdjian and Sala, 2006). Recently, a central North American grassland study by Adler and Levine (2007) suggested that ‘how quickly this long-term response develops may depend on the colonization rates of species

better adapted to the altered rainfall regime’. In the present study, although SD led to declines in tiller and plant production (Fig. 2B, C), full recovery occurred as the plants were subjected to previous MD. Thus, the present results suggested that the limitation to meristems due to pre-drought following rewatering may also depend on pre-drought severity and duration.

SLA is a marker for the regulation of plant leaf following abiotic stress factors including drought (Monclus *et al.*, 2006), implying that its high negative relationship with the leaf elastic modulus (ϵ) may be associated with leaf elasticity to water stress, depending on the species (Galmés *et al.*, 2007a). Moreover, plants with a higher SLA can allocate a larger proportion of their leaf nitrogen to Rubisco instead of chlorophyll, enhancing their photosynthetic capacity and photosynthetic nitrogen use efficiency (PNUE), rather than their light capture (Poorter and Bongers, 2006). In previous reports, an SLA decrease under drought would be consistent with this suggestion (Xu and Zhou, 2006a); in the current experiment, an SLA increase in the new leaves of severely stressed plants after rewatering suggests that rewatering might cancel the elastic regulation mechanism of environmental stress to save energy for new growth (Fig. 2A).

The quantified changes in chlorophyll fluorescence parameters can indicate the PSII function in response to different environmental variables (Baker and Rosenqvist, 2004). For example, SD leads to a decline in PSII function, which may depend on species, growth stage, and stress intensity (Xu and Zhou, 2006a; Gallé *et al.*, 2007). Consistent resilience of the maximal efficiency of F_v/F_m that declined only at SD below an SRWC of 50% (Marques da Silva and Arrabaca, 2004; Table 3) has been observed, but Φ_{PSII} decreased concomitantly with declines in photosynthetic capacity from MD to ED intensification, depending on the species (Lu and Zhang, 1999; Tezara *et al.*, 1999; Ghannoum *et al.*, 2003; Liorens *et al.*, 2003). The results of Gallé *et al.* (2007) indicated partial restoration of F_v/F_m and Φ_{PSII} after only 1 d of rewatering, and complete restoration of all fluorescence parameters after 4 weeks. SD induced an increase in NPQ, and a high level remained after rewatering, implying that heat dissipation may involve photosynthetic acclimation to a change in water status (Table 3; Gomes *et al.*, 2008). In the present experiment, a new insight is provided into the effects of different degrees of drought on recovery from rewatering, showing that only SD affected the PSII function, and overcompensation recovery was observed after rewatering (Table 3). It is suggested that the activity of the photosynthetic electron chain may cope with CO_2 fixing under water stress and rewatering.

The g_s response to water stress and rewatering largely depends on the species’ growth forms and leaf habits (Potts *et al.*, 2006a; Galmés *et al.*, 2007a; Brodrribb and Cochard, 2009). Gallé and Feller (2007) reported a completely restored photosynthetic rate (A) 4 weeks after rewatering, but g_s remained at a lower level, consequently resulting in an increase in WUE in *Fagus sylvatica* saplings. The asynchrony between A and g_s appeared in *Quercus pubescens* in

the first 2 weeks after rewatering, although both g_s and photosynthesis could completely recover from drought-induced suppression 4 weeks after rewatering (Gallé *et al.*, 2007). In the drought-adapted *Vitis* hybrid Richter-110, Pou *et al.* (2008) reported that g_s maintained a lower level than in control plants, despite an increase in WUE. The lasting decrease in g_s after rewatering was related to drastically decreased hydraulic conductivity, which differed from that in plants that were still drought stressed (Pou *et al.*, 2008). A severely impaired vascular capacity for water transport due to previous drought may be responsible for the incomplete recovery of photosynthesis and g_s in SD-stressed plants after rewatering (Resco, 2008; Brodribb and Cochard, 2009). Gallé and Feller (2007) indicated that non-stomatal limitations are mainly responsible for drought-induced photosynthetic inhibition seen after recent rewatering, but Marques da Silva and Arrabaca (2004) addressed the major role of stomatal limitation. In the present experiment, the initial rapid reduction in A_{sat} , α , and Φ_{PSII} occurred in parallel with a decrease in g_s , but they levelled off as g_s reached a threshold of $0.25\text{--}0.28\text{ mol m}^{-2}\text{ s}^{-1}$ (Table 3, Fig. 5), consistent with reports of Ghannoum *et al.* (2003) and Flexas *et al.* (2006). This result suggests that stomatal limitation may lead to an inhibition of photosynthesis only below the g_s threshold with a change in water status. Galmés *et al.* (2007b) reported that although stomatal limitation to photosynthesis under water stress is still a major factor, photosynthetic recovery of severely stressed plants after rewatering generally showed a major biochemical limitation rather than stomatal closure, and SD can lead to a decline in photosynthetic-biochemical capacity such as Rubisco activity (Xu and Baldocchi, 2003; Flexas *et al.*, 2006). Furthermore, a decrease in g_i may be responsible for decreased photosynthesis under drought (Flexas *et al.*, 2002) and rewatering (Table 3). In an experiment by Huxman *et al.* (2004), following a pulse of precipitation, two grass species showed substantial increases in g_s up to values that were three times higher than pre-pulse values, followed by a substantial increase in A . For the Great Basin Desert shrub species of the USA, Loik (2007) showed that both g_s and A largely increased, particularly the latter, and their findings were confirmed by the current experiment (Table 3). However, Galmés *et al.* (2007b) found that the recovery of photosynthesis 24 h after rewatering ranged from only $<10\%$ to 70% , not up to the control level. Ignace *et al.* (2007) further reported that the photosynthetic recovery status of previous drought after a water pulse may depend on the temporal variation of antecedent soil moisture.

Plants in drying conditions may use different water use strategies to cope with fluctuation in water status; there may be a trade-off between physiological activity and biomass accumulation, such as the maintenance of photosynthesis as a cost of plant growth decline (Xu *et al.*, 2007). A current major concern is that plant leaf photosynthetic activity may not always be associated with plant productivity (Long *et al.*, 2006), implying that the trade-off may occur. Different sensitivities to water stress were found between

plant growth and photosynthesis: the former may be more sensitive (Acevedo *et al.*, 1971; Hsiao, 1973). Lizana *et al.* (2006) reported that crop varieties lacking plasticity to g_s , photosynthetic rate, and resistance to photoinhibition can be compensated by an enhanced tendency for a morphological response, such as a rapid decrease in plant RGR. The present results showed that plant RGR and leaf photosynthetic potential were markedly stimulated by pre-drought following rewatering, depending on drought intensity. However, the light capture seemed to encounter a limitation from previous drought, and more heat dissipates (e.g. higher NPQ) (Table 3). This result might highlight the central role of the trade-off between physiological activity and biomass accumulation in plant growth, survival, and resource use processing. Furthermore, plant acclimation and tolerance to environmental stresses may also be associated with gene expression and molecular mechanisms in relation to signal transduction (Foyer *et al.*, 1997), which should be investigated in the future.

In arid and semi-arid areas, persistent water limitation during periods of otherwise favourable metabolic conditions can maintain a reference state of minimal biological activity, such as maintaining a low gas exchange level (Potts *et al.*, 2006a). Following wetting, however, plants immediately show a high rate of biological activity, including photosynthetic capacity and new organ growth, which can be considered as alternative functional states that may overcompensate for the limitation to plant growth and metabolic activity due to previous drought, in which processing may negate the constraints of dry conditions to NPP. Thus, plants exposed to well-watered conditions after a long-term drought may be able to increase both positive metabolic activity and growth rate. This current result may be significant because the synchrony of decreasing precipitation and rising temperature may lead to high potential evapotranspiration and low water availability, not only in the Northern Chinese steppe ecosystem (Wang and Gao, 2003; Cheng *et al.*, 2006) but also in many of the semi-arid regions of the world, such as the Patagonian region of Argentina (Paruelo and Sala, 1995), the western European grassland (De Boeck *et al.*, 2007), and the Chihuahuan Desert of the USA (Patrick *et al.*, 2007). The world is facing drought that is likely to intensify; thus, in order to improve ecosystem management, the focus should be on recharging the soil profile with casual rainfall pulse events that may overcompensate the limitations resulting from previous drought.

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