Drought-induced hydraulic limitations constrain leaf gas exchange recovery after precipitation pulses in the C_3 woody legume, *Prosopis velutina*

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Summary

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• The hypothesis that drought intensity constrains the recovery of photosynthesis from drought was tested in the C_3 woody legume *Prosopis velutina*, and the mechanisms underlying this constraint examined.
• Hydraulic status and gas exchange were measured the day before a 39 mm precipitation pulse, and up to 7 d afterwards. The experiment was conducted under rainout shelters, established on contrasting soil textures and with different vegetation cover at the Santa Rita Experimental Range in southeastern Arizona, USA.
• Rates of photosynthesis and stomatal conductance after re-watering, as well as the number of days necessary for photosynthesis to recover after re-watering, were negatively correlated with predawn water potential, a measure of drought intensity ($R^2 = 0.83$, 0.64 and 0.92, respectively).
• Photosynthetic recovery was incomplete when the vascular capacity for water transport had been severely impaired (percentage loss of hydraulic conductance > 80%) during the drought, which largely increased stomatal limitations. However, changes in biochemical capacity or in mesophyll conductance did not explain the observed pattern of photosynthesis recovery. Although the control that hydraulic limitations impose on photosynthesis recovery had been previously inferred, the first empirical test of this concept is reported here.

Introduction

Precipitation is one of the most important factors controlling primary productivity in terrestrial ecosystems, and increases in importance as mean annual precipitation decreases (Huxman *et al.*, 2004a). Accordingly, ecosystems in areas with low mean annual precipitation are predicted to be most susceptible to anticipated changes in rainfall associated with climate warming (de Dios *et al.*, 2007). The rate of photosynthetic carbon assimilation (*A*), a key process related to primary productivity, varies widely over the growing season in arid and semiarid ecosystems, and often responds significantly to changes in resource supply associated with pulsed inputs of growing season precipitation (Sala & Lauenroth, 1982; Williams & Ehleringer, 2000; Huxman *et al.*, 2004b).

Differences in growing conditions, such as soil texture, vegetation cover and atmospheric humidity, along with the high spatial and temporal variance of precipitation in arid and semiarid regions, produce a highly heterogeneous mosaic of water availability which may change dramatically even over very short timescales of hours and days (Reynolds *et al.*, 2004). This variation in water availability results in substantial variation of the photosynthetic gas exchange response following precipitation inputs during the growing season in these environments (Huxman *et al.*, 2004b; Reynolds *et al.*, 2004; Ignace *et al.*, 2007; Patrick *et al.*, 2007; Resco *et al.*, 2008).

Temporal up-scaling of leaf photosynthetic fluxes in these ecosystems is problematic because of the different time lags observed for different components of leaf gas exchange regulation (Tuzet *et al.*, 2003). In response to a precipitation pulse,

stomatal conductance (g_s), A and plant water potential may be temporarily decoupled from each other, although the underlying mechanism has not yet been elucidated (Yan *et al.*, 2000; Tuzet *et al.*, 2003; Resco *et al.*, 2008).

These uncertainties arise partly from our incomplete understanding of how gas exchange recovers from drought after new pulses of precipitation. In recent years, a general model of drought effects on photosynthesis limitations has been proposed, based on the interplay between biochemical, stomatal and mesophyll limitations (Flexas *et al.*, 2006). Stomatal limitations to photosynthesis are regarded as the prevailing limiting factor except when: plants are operating in the asymptotic part of the A/g_s relationship; and g_s drops below 0.05–0.1 mol m^{-2} s^{-1}. Biochemical limitations are generally thought to take over after these thresholds. However, decreases in mesophyll conductance (g_m), the conductance of CO_2 from the substomatal cavity to the site of carboxylation, has been increasingly reported as another dynamic factor limiting photosynthesis (Flexas *et al.*, 2008).

Although photosynthesis responses to drought are relatively well understood, there is a surprising paucity of studies on photosynthesis recovery from drought (Flexas *et al.*, 2006). A and g_s typically increase in response to a biologically significant precipitation pulse, until they reach a 'peak' value, and then return to values comparable to those before the irrigation. Peak response rates of A and g_s (A_p and g_p, respectively) following a precipitation pulse, as well as the time lag (τ) necessary to reach that peak value, are often reported to depend upon the intensity of water stress antecedent to the pulse (Huxman *et al.*, 2004b). However, the mechanisms underlying this relationship are currently being debated. The Flexas *et al.* (2006) model of photosynthesis responses to drought predicts that incomplete recovery of photosynthesis occurs when, as a result of previous drought, biochemical capacity needs to be restored. When g_s drops below 0.05 mol m^{-2} s^{-1}, the concentration of antioxidant compounds in photosynthetic tissues increases while the carboxylation capacity is impaired (Flexas *et al.*, 2006). Hence, incomplete photosynthesis recovery may occur when the plant needs to repair its carboxylation capacity after drought-mediated oxidative stress. Alternatively, stomatal limitations may prevail if the plant has suffered a large percentage loss of hydraulic conductance (PLC) during the drought (Wheeler & Holbrook, 2007). Stomata close when vascular supply diminishes, and incomplete photosynthesis recovery is to be expected when the plant has experienced a large PLC, because xylem refilling under tension is problematic (Clearwater & Goldstein, 2005; Lovisolo *et al.*, 2008). Finally, Galmes *et al.* (2007) observed that incomplete recovery of g_m after re-watering was also important in limiting photosynthesis recovery, because a large resistance to CO_2 diffusion from the substomatal cavity to the chloroplast diminishes the substrate for carboxylation, and g_m is not driven solely by physical diffusion but seems to be controlled by plasma membrane intrinsic proteins (aquaporins) and/or by the activity of carbonic anhydrase (Bernacchi *et al.*, 2002), which, in turn, are highly sensitive to drought (Kaldenhoff *et al.*, 2008).

In this study, we monitored the dynamics of A and g_s in the C_3 woody legume *Prosopis velutina* Woot (mesquite) over a period spanning the day before and up to 7 d after a large precipitation pulse. Mesquite was chosen for this study because of its historic encroachment into grasslands in southwestern North America and its documented impacts on community structure and ecosystem processes (Scholes & Archer, 1997; McClaran *et al.*, 2003; Williams *et al.*, 2006; Yepez *et al.*, 2007; Knapp *et al.*, 2008). Photosynthesis recovery was assessed after two months of imposed drought and at the peak of the summer rainy season on clay loam and sandy loam soils, on different aged seedlings of *P. velutina*, as well as on plants growing on bare ground or with interspecific competition from perennial C_4 grasses. This variety of growing environments allowed us to evaluate drought recovery under contrasting amounts of antecedent water stress. For expediency, 'antecedent' will be used throughout the text to indicate values measured the day before the irrigation input (abbreviated as '$_{D-1}$'), whereas 'peak' denotes the day on which the highest value of A in response to the irrigation pulse was recorded (abbreviated as '$_p$'), unless otherwise noted.

The first goal of this study was to quantify the importance of antecedent conditions on constraining the recovery of A_p, g_p and τ_A (the number of days between the precipitation pulse and A_p after re-watering (Ogle & Reynolds, 2004)). The second goal was to test whether the constraint exerted by antecedent conditions on drought recovery is imposed by biochemical, stomatal or mesophyll limitations in *P. velutina*. The third goal was to test the generality of this relationship in other desert species for which data were available from the literature.

Materials and Methods

Study sites

Field measurements were conducted under experimental rainout shelters installed on sandy loam and clay loam soils at the Santa Rita Experimental Range in southeastern Arizona, USA (English *et al.*, 2005). The rainout shelters and the experimental plots they covered were established in 2002 as part of a global change experiment to investigate mesquite seedling establishment under different seasonal precipitation regimes (50% increase or decrease of the long-term average in summer or winter precipitation), soil textures (sandy loam and clay loam) and grass cover (bare ground, native or invasive C_4 grass). A cohort of 30 seeds was planted yearly on each plot and, because of highly variable treatment-induced patterns of seedling establishment, we were unable to assess seedling physiology across all treatment combinations. Two separate seedling cohorts (1 and 4 yr of age for the present study) were available for intensive physiological measurements. Adequate numbers of

Table 1 Characteristics of the different species and treatments reported in this study, and codes used in figures

Code	Species	Data source	Ecosystem	Soil texture	Ground cover	Age	Measurement
Pv1	*Prosopis velutina*	This study	Sonoran	Sandy loam	*Heteropogon contortus* stand	1 yr	After drought
Pv2	*Prosopis velutina*	This study	Sonoran	Sandy loam	Bare ground	1 yr	After drought
Pv3	*Prosopis velutina*	This study	Sonoran	Sandy loam	Bare ground	4 yr	After drought
Pv4	*Prosopis velutina*	This study	Sonoran	Sandy loam	*H. contortus* stand	1 yr	Rainy season
Pv5	*Prosopis velutina*	This study	Sonoran	Sandy loam	Bare ground	1 yr	Rainy season
Pv6	*Prosopis velutina*	This study	Sonoran	Sandy loam	Bare ground	4 yr	Rainy season
Pv7	*Prosopis velutina*	This study	Sonoran	Clay loam	Bare ground	1 yr	After drought
Pv8	*Prosopis velutina*	This study	Sonoran	Clay loam	Bare ground	4 yr	After drought
At	*Artermisia tridentata*	Loik (2007)	Great Basin	NA	NA	Adult	After drought
Pt	*Prusia tridentata*	Loik (2007)	Great Basin	NA	NA	Adult	After drought
Ps	*Pascopyrum smithii*	Schomp (2007)	Mixed Prairie	NA	Native vegetation	Adult	Rainy season

1- and 4-yr-old seedlings were available in grass-free, bare plots on each of the two soil surfaces, but in plots dominated by the native C_4 grass *Heteropogon contortus* (L.) Beauv only 1-yr-old seedlings were available for measurements and only on the sandy loam surface (Table 1). A detailed description of the demographic patterns is provided by Resco (2008).

The rainout shelters excluded natural precipitation (mean annual precipitation of 394 mm at the sandy loam site and 430 mm at the clay loam site (Fravolini *et al.*, 2005)) from each plot. On June 10, 2006, after 2 months of imposed drought, we applied a 39 mm irrigation pulse to all of the experimental plots on the sandy loam and clay loam surfaces. Another 39 mm pulse was applied to the plots at the sandy loam site on August 1, 2006, at the peak of the summer rainy season, during a period of frequent experimental irrigation. The experiment was conducted on these highly contrasting soil textures, vegetation covers and different parts of the year to ensure large differences in the antecedent stress (Fig. 2, Table 1).

Photosynthetic gas exchange measurements

Spot gas exchange measurements ($n = 3$–5) were performed at 07:30 h the day before the irrigation and 1, 3, 5 and 7 d afterwards with a portable photosynthesis system (LI-6400, Li-Cor Inc., Lincoln, NE, USA). Environmental conditions inside the leaf chamber were set to match early morning conditions. Light intensity, block temperature and CO_2 concentration were 700 µmol m^{-2} s^{-1}, 30°C and 400 ppm, respectively. Leaf-to-air vapor pressure deficit (D) was maintained in the range 1.5–3.5 kPa, depending on environmental conditions. Leaves were marked and the same leaf was measured each day of the pulse period.

To quantify the importance of antecedent water stress for constraining the pulse response, we measured predawn water potential (Ψ_{pd}), an integrated indicator of water availability in the rhizosphere when nocturnal transpiration is negligible, the day before the irrigation pulse. To test whether this constraint originated from limitations in leaf biochemical capacity, CO_2 diffusion through the mesophyll, or through the stomata, we estimated maximal leaf carboxylation capacity (V_{cmax}) and mesophyll conductance (g_m), and measured g_s the day before the watering. A possible role for hydraulic limitations as driver of the pulse response was evaluated by estimating PLC at midday, through a previously established relationship between xylem tension and PLC (see later). Ψ_{pd}, V_{cmax}, g_m and g_s were also monitored 1, 3, 5 and 7 d after the precipitation pulse, to characterize differences in drought recovery across treatments.

Ψ_{pd} and midday water potential (Ψ_{md}) measurements were taken on additional seedlings not used for gas exchange within each experimental plot. They were determined on leaves of 1-yr-old *P. velutina* seedlings using Peltier thermocouple psychrometers (PST-55 Wescor Inc., Logan, UT, USA), and with a Scholander-type pressure chamber (PMS Instruments, Corvallis, OR, USA) on the 4-yr-old seedlings ($n = 3$). No statistical difference in estimated Ψ from these two techniques was found in an independent test (Resco *et al.*, 2008). A slight modification to the original design of the PST-55 was necessary to measure leaves in these soil psychrometers. We enclosed leaves inside an isolated custom-built chamber, and immersed the chamber in a water bath at 25°C until equilibration occurred. Further details are provided by Resco *et al.* (2008) and by the manufacturer's website (http://www.wescor.com).

Response curves relating net photosynthetic rate (A) to variation in the leaf intercellular (C_i) and chloroplast (C_c) concentration of CO_2 were developed the day before the watering between 06:00 and 10:00 h, following Long & Bernacchi (2003) at saturating light (1500 µmol m^{-2} s^{-1}) using the same leaves on which spot gas exchange measurements had been made. Leaves were allowed to acclimate to chamber conditions at a CO_2 concentration (C_a) of 400 ppm, after which gas exchange parameters were recorded. Gas exchange rates were then determined sequentially as C_a was reduced to 300, 200, 100 and 50 ppm, and then as C_a was returned to 400 ppm and then sequentially at 600, 800, 1000, 1200, 1400, 1600, 1800

and 2000 ppm. Each curve was developed within 30–40 min. V_{cmax} was estimated from the Farquhar *et al.* (1980) photosynthesis model, following the assumptions and model-fitting approach of Sharkey *et al.* (2007).

Mesophyll conductance to CO_2 transfer (g_m) was estimated with the 'variable J' method on the same leaves used to develop A/C_i curves. This method compares electron transport rate (J) measured from chlorophyll fluorescence (Genty *et al.*, 1989) with J estimated with the Farquhar *et al.* (1980) model. The difference between both estimates is assumed to be related to g_m; see Harley *et al.* (1992) and Long & Bernacchi (2003) for a detailed description of the method. Four parameters are required to obtain J from chlorophyll fluorescence: (i) the photochemical efficiency of photosystem II (Φ_{PSII}), which was determined using a portable pulse-modulated fluorometer (FMS2, Hansatech Instruments, King's Lynn, UK) immediately after development of each A/C_i curve, and after acclimating each leaf for 10 min at the irradiance value at which the previous A/C_i curve had been measured (Maxwell & Johnson, 2000; Loik & Holl, 2001); (ii) leaf absorptance, which was assumed to be 0.86 for *Prosopis* (Asner *et al.*, 1998); (iii) irradiance (same as in the A/C_i curve); and (iv) the fraction of absorbed irradiance that reaches PSII, which was assumed to be 0.5 for C_3 plants (Ögren & Evans, 1993). Warren (2006) provides a critical analysis on the limitations of using this approach to estimate g_m.

Vulnerability to cavitation

Direct estimates of PLC in the limited population of *Prosopis* seedlings were not possible to attain, because of the destructive nature of the measurement. Hence, we had to estimate PLC from vulnerability to cavitation (VC) curves, which relate PLC to xylem tension (P_x), using the dehydration method (Cochard *et al.*, 1992), where each point of the curve is from an individual plant. Eleven 1-yr-old plants growing on the clay loam site were harvested after the measurements in June, and 16 1-yr-old seedlings from the sandy loam site were harvested after experiment termination in August. Although the growth of new vascular tissue between June and August is possible, *P. velutina* is a ring-porous species, and the new growth of small-diameter vessels will have a minor impact on the VC curve, because water flow scales with the fourth power of conduit diameter (Tyree & Ewers, 1991). Moreover, the VC curves reported here are remarkably similar to those reported for stems of adult *P. velutina* trees growing nearby (Hultine *et al.*, 2006), which indicates that the different collection times likely had a negligible effect on the VC curve. For instance, plants growing at the sandy loam site lost 50% of conductance at *c.* −2.05 MPa in Hultine *et al.* (2006), and at −2.15 MPa in this study; and 75% of conductance at *c.* −4.10 MPa in Hultine *et al.* (2006), and at −3.85 MPa in this study (Fig. 1). Indeed, we also used the VC curve obtained by Hultine *et al.* (2006) to estimate PLC in the 4-yr-old seedlings
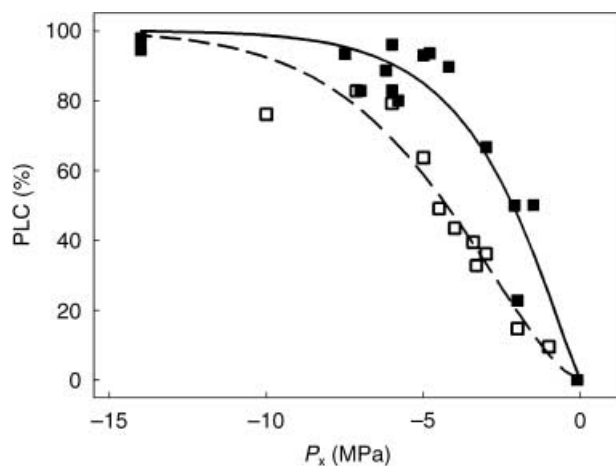
Fig. 1 Relationship between the percentage loss of hydraulic conductivity (PLC) with xylem tension (P_x) in 1-yr-old *Prosopis* seedlings growing on sandy loam (closed squares, continuous line) and clay loam (open squares, dashed line) sites. Different lines result from fitting the Weibull function $y = 100(1 - \exp - (-x/a)^b)$, where a and b are 2.98 and 1.19 for sandy loam seedlings and 5.40 and 1.54 for clay loam seedlings, respectively.

in Fig. 6, but observed no significant difference in the relationship reported between PLC with the drought recovery parameters (not shown).

Plants were harvested under water, at least 10 cm below the root collar, and transported to the laboratory in wet paper towels inside zip bags. In the laboratory, they were allowed to air dry to different stages, from 1 up to 10 d (−0.1 to −14 MPa). The whole plant was kept inside a zip bag the night before measurement, to allow for equilibration of spatial gradients in water potential. Before the conductivity measurement, a segment centered on the root collar was cut under water. Hydraulic conductivity was measured in this segment as the ratio between the flow of deionized water (measured by XYL'EM, Bronkhorst, France (Cruiziat *et al.*, 2002)) and the gravity-induced pressure gradient (10 kPa). Maximum conductivity was estimated after flushing the segment at high pressure (100 kPa, refer to the XYL'EM manual for further details (http://www.bronkhorst.fr/fr/produits/xylem_emboliemetre)). Xylem tension was measured with a Scholander type pressure chamber (PMS Instruments, Corvallis, OR, USA) in the above-ground part of the plant and VC curves were fitted through a Weibull function (Sperry *et al.*, 1998, Fig. 1). PLC was then estimated by substituting Ψ_{md} for P_x in the equations given in Fig. 1. Ψ_{md} measures the minimum water potential throughout the day, and we thus estimated the maximum PLC that might occur.

Water potential in the leaves is necessarily more negative than in the stem for water to flow. Because Ψ_{md} was measured in leaves and not in the stem, we may have consistently overestimated stem PLC. However, this study was performed in rather short mesquite plants (< 40 cm), and spatial gradients

in Ψ from the stem to the leaf will likely be in the order of a few tenths of a kPa (Tyree & Ewers, 1991), a negligible error for the accuracy necessary in this study (Fig. 1).

Data analyses

To quantify the importance of antecedent water stress ($\Psi_{pd.D-1}$) in constraining the pulse response in mesquite and in other desert species, we conducted data searches on Web of Science (http://portal.isiknowledge.com/) for studies that were performed on C_3 plants; that reported the pulse response of spot gas exchange at leaf level for at least the day before the watering and up to the day when A_p and g_p occurred; and that maintained comparable light intensities inside the photosynthesis chamber. Unfortunately, only one study (Gillespie & Loik, 2004; Loik, 2007) matched our criteria. Loik (2007) reported results for two shrubs from the southwestern Great Basin desert – *Purshia tridentata* (Rosaceae) Pürsh and *Artemisia tridentata* (Asteraceae) Nutt. We also incorporated the results from an unpublished thesis (Schomp, 2007), where a pulse response study was conducted in mixed-grass prairie in southeastern Wyoming as a component of the Prairie Heating and CO_2 Enrichment (PHACE) experiment (http://www.phace.us/). Schomp (2007) reported results for the C_3 perennial grass *Pascopyrum smithii* (Poaceae) (Rybd.) A. Love (Table 1).

We examine the relationship between $\Psi_{pd.D-1}$ and A_p, g_p and τ_A through least-squares fitting. Because the different species included in this analysis had different photosynthetic capacities (Table 1), we normalized A_p as percentage photosynthesis recovery:

$$\% \text{ recovery} = 100\ (\hat{A}_{p,s} / \hat{A}_{max,s}) \qquad \text{Eqn 1}$$

where $\hat{A}_{p,s}$ is the average ($n = 3$–5) of the value of A_p for species s; and $\hat{A}_{max,s}$ is the maximum of the mean assimilation rates under optimum conditions (during the peak of the rainy season) for species s: 22 μmol m^{-2} s^{-1} for mesquite (this study), 18 μmol m^{-2} s^{-1} for *A. tridentata* (Gillespie & Loik, 2004), 15 μmol m^{-2} s^{-1} for *P. tridentata* (Gillespie & Loik, 2004) and 20 μmol m^{-2} s^{-1} for *P. smithii* (Schomp, 2007; V. Resco, unpublished).

Then, we evaluate in mesquite whether the relationship between drought recovery and antecedent stress is mediated by antecedent stomatal limitations ($g_{s.D-1}$), antecedent V_{cmax} ($V_{cmax.D-1}$) or antecedent mesophyll conductance ($g_{m.D-1}$), by examining whether these parameters correlate with A_p. To understand why higher-stressed plants did not attain the same A_p as lower-stressed plants, we tested for differences in g_s, V_{cmax} and g_m across treatments when A_p is reached (g_p, $V_{cmax.p}$, and $g_{m.p}$, respectively), through analysis of variance. Finally, because g_s could be influenced by both PLC and leaf-to-air vapor pressure deficit (D), we partitioned the effects of these two through stepwise regression following the model selection

criteria proposed in Crawley (2007). Nonlinear curve fits were just chosen to establish empirical relationships, but without exploring the potentially relevant biological information stored in the parameters. We used R 2.5.0 (R Development Core Team, Vienna, Austria) in all of our statistical analyses.

Results

### Constraints on drought recovery imposed by antecedent drought stress

Because this study was performed on plants growing on different sites and under different plant covers, the underlying assumption is that a comparable degree of hydration across treatments was attained after the application of the 39 mm irrigation pulses, such that variance in A_p does not merely reflect differences in post-irrigation Ψ_{pd}. Indeed, Fig. 2c shows that there is no significant relationship between peak Ψ_{pd} and antecedent Ψ_{pd} ($P = 0.63$). Moreover, the relationship between peak Ψ_{pd} and A_p was not significant ($P = 0.56$, Fig. 2b), indicating that the results from this experiment are not an artifact originating from post-irrigation differences in plant water status.

Antecedent water stress, as indicated by $\Psi_{pd.D-1}$, explained 83, 92 and 64% of the variance in A_p, τ_A and g_p, respectively (Figs 2, 3), suggesting that antecedent conditions exert substantial control on drought recovery.

No differences in antecedent values of V_{cmax} and g_m across treatments were observed (Table 2, Fig. 4). However, we did observe differences in antecedent g_s (Table 2, Fig. 4), such that $g_{s.D-1}$ was lower in the seedlings growing at the sandy loam site in June than in the other treatments (Fig. 4). The control of antecedent stress on drought recovery does not seem to be mediated by nonstomatal limitations, as no significant relationship ($P > 0.3$) was observed between $V_{cmax.D-1}$ or $g_{m.D-1}$ with A_p (Fig. 5b,c) in *P. velutina* seedlings. However, the degree of antecedent g_s was significantly correlated ($P < 0.01$, $R^2 = 0.53$) with photosynthesis recovery from drought (Fig. 5a).

Stomatal limitations in our system may result from either high D and/or PLC. Stomatal conductance responds rather rapidly to variations in D, and no direct mechanistic link is to be expected between gas exchange after re-watering and the D that occurred during drought. However, the effects of increasing PLC after a prolonged period of drought may last for some time, even after re-watering, if complete recovery of hydraulic capacity is not achieved. Indeed, PLC_{D-1} was significantly correlated with A_p ($R^2 = 0.83$, Fig. 6c), τ_A ($R^2 = 0.97$, Fig. 6b) and g_p ($R^2 = 0.82$, Fig. 6a), suggesting that limited hydraulic conductivity caused the observed differences in the dynamics of photosynthesis recovery from drought.

Antecedent water stress also imposed an important constraint on A_p and g_p in *A. tridentata*, *P. tridentata* and *P. smithii*, and no interspecific differences were apparent, since these values
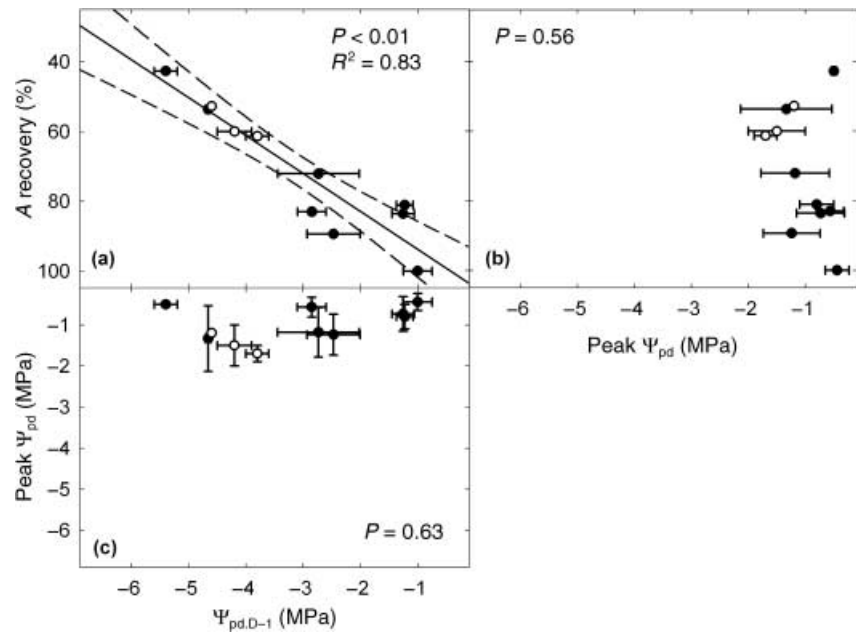
Fig. 2 Recovery of photosynthesis (Eqn 1) as a function of antecedent predawn water potential ($\Psi_{pd.D-1}$) (a) and peak predawn water potential (b). (c) Relationship between antecedent and peak predawn water potential. Closed circles, *Prosopis velutina* values; open circles, data from the literature. Error bars are standard errors. The line and *P* values are the result of least-squares fitting over the *P. velutina* data.
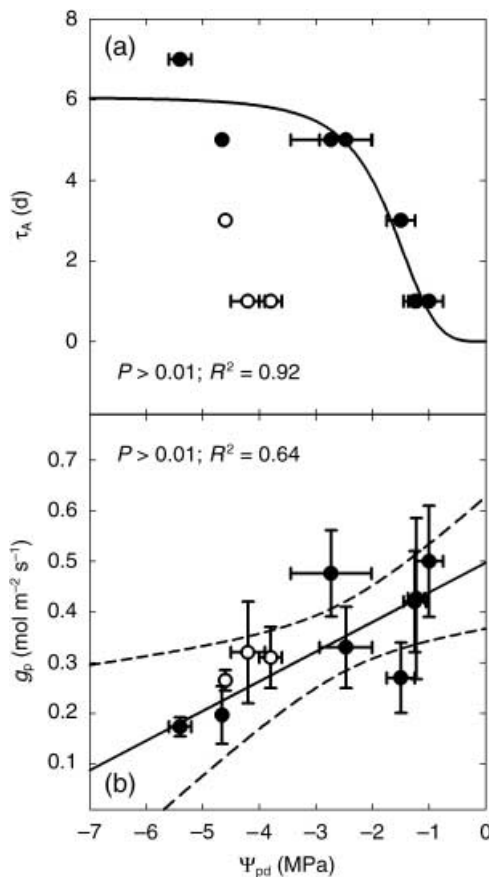
Fig. 3 Time lag (τ_A) (a) and peak stomatal conductance (g_p) (b) as a function of antecedent predawn water potential ($\Psi_{pd.D-1}$). The dashed line indicates the 95% confidence interval of the regression. Closed circles, *Prosopis velutina* values; open circles, data from the literature. Error bars are standard errors. The curve was fitted over the *P. velutina* data only.

Table 2 Analysis of variance testing for significant differences in maximum carboxylation capacity (V_{cmax}), mesophyll conductance (g_m) and stomatal limitations to photosynthesis (l) across eight treatments (Table 1, Fig. 4), the day before the pulse (pre-pulse) and when A_p was reached (post-pulse)

Period	Variables	df	P
Pre-pulse	V_{cmax}	7	0.49
	g_m	7	0.23
	l	7	< 0.05
Post-pulse	V_{cmax}	7	0.55
	g_m	7	0.70
	l	7	< 0.05

fell within the 95% confidence interval for the *Prosopis* data (Figs 2a and 3b). However, τ_A was up to 4 d shorter in these species than in *P. velutina* (Fig. 3a).

Effects of post-irrigation conditions on photosynthetic recovery

We only observed significant differences in g_s across treatments after the application of the 39 mm precipitation pulses, but not in peak values of V_{cmax} and of g_m (Table 2). The effect of peak stomatal limitations on A_p could be the result of the previously reported differences in antecedent PLC, but also of differences in peak D (D_p) across seasons, as D_p was significantly lower in June than in August ($P < 0.01$). To partition the effect of PLC_{D-1} from that of D_p on stomatal conductance, we performed a stepwise regression where we compared a model with PLC_{D-1} as the only independent
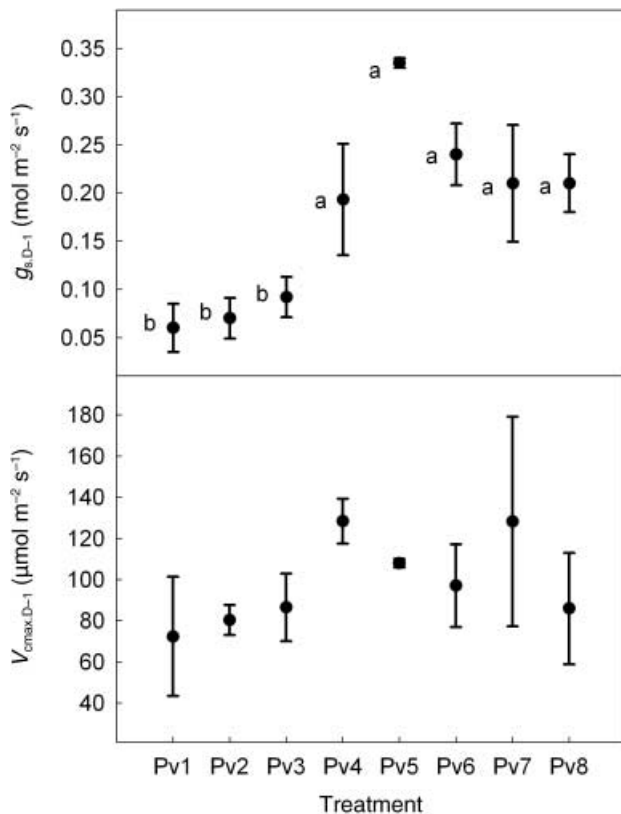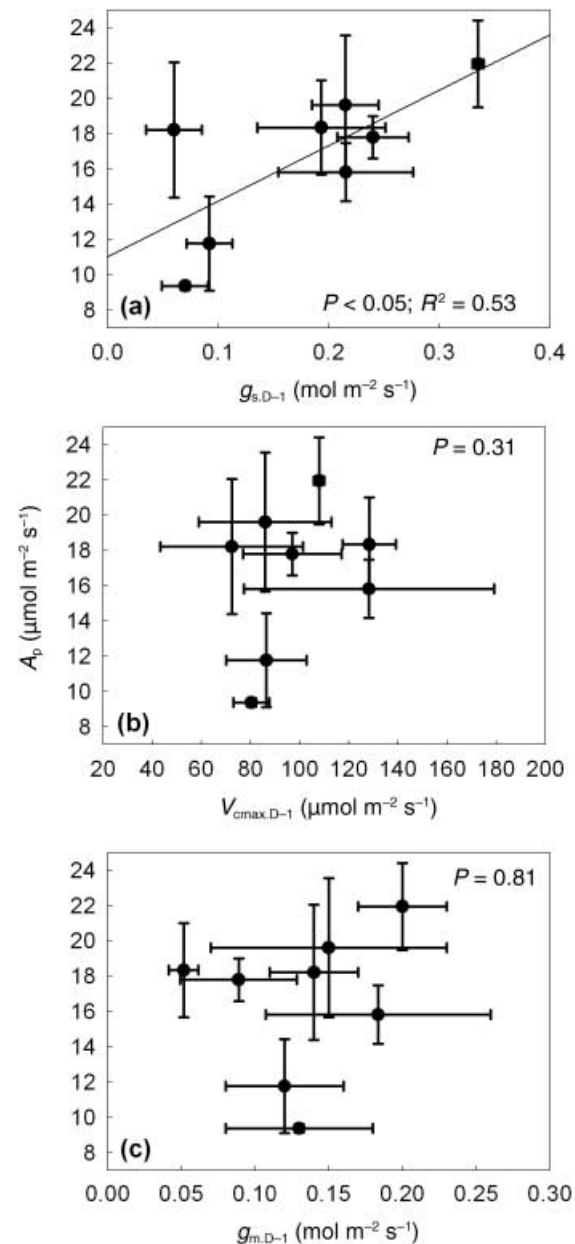
Fig. 4 Stomatal conductance ($g_{s.D-1}$) and maximum carboxylation capacity ($V_{cmax.D-1}$) before the precipitation input. Error bars indicate standard errors. Different letters indicate significant differences at $P < 0.05$ according to an analysis of variance followed by the Tukey honest significant differences test, and absence of letters in V_{cmax} reflects lack of significant differences. The codes for each treatment are explained in Table 1.

variable (Fig. 6b) with another regression model where both D_p and PLC_{D-1} were independent variables. D_p and PLC_{D-1} were not significantly correlated with each other ($P > 0.05$). The inclusion of D_p did not significantly improve the performance of the model ($P = 0.13$), and we thus concluded that the effect of different D on g_p across seasons was overridden by that of PLC_{D-1}.

Discussion

Photosynthesis limitations and recovery from drought

Our results partially support the generality of a recently proposed conceptual model on the changes in photosynthetic limitations with drought (Flexas *et al.*, 2006), which predicts that stomatal limitations prevail except under severe stress (defined as $g_s < 0.05$ mol m^{-2} s^{-1}), when biochemical limitation starts to operate. We failed to observe any statistical difference in V_{cmax} or in g_m across treatments before the irrigation pulse (Table 2), although antecedent stomatal conductance varied from 0.06 to 0.33 mol m^{-2} s^{-1}.

Fig. 5 Maximum (peak) assimilation (A) in response to 39 mm precipitation pulses as a function of antecedent stomatal conductance ($g_{s.D-1}$) (a), maximum carboxylation capacity ($V_{cmax.D-1}$) (b), and mesophyll conductance ($g_{m.D-1}$) (c). Error bars are standard errors.

A prediction from this photosynthesis model is that recovery from drought will be complete and immediate when no reductions in biochemical capacity are experienced, because a relaxation in g_s is often assumed after the water input (Flexas *et al.*, 2006; Galmes *et al.*, 2007). Stomatal aperture is thought to be regulated by changes in epidermis and guard cell water potential, which, in turn, are affected by D as well as by xylem hydraulic conductance (Brodribb *et al.*, 2003;
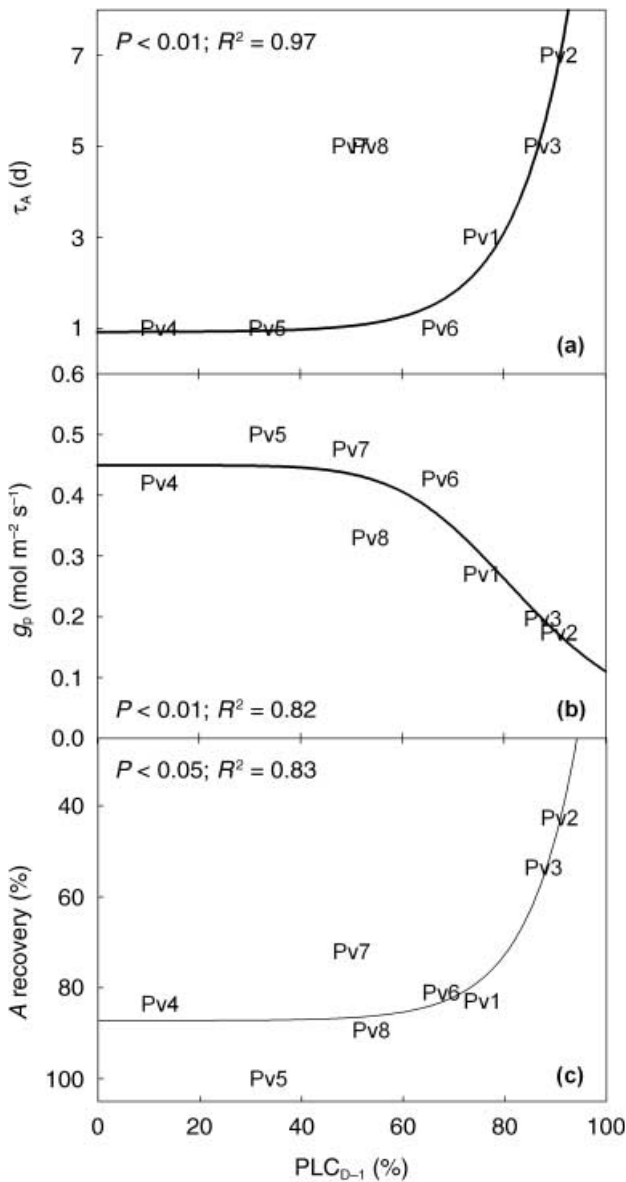
Fig. 6 Relationship between the number of days necessary to reach maximum (peak) assimilation (*A*) after a precipitation pulse (τ_A) (a), peak value of stomatal conductance (g_p) (b) and photosynthesis recovery (c), as a function of antecedent percentage loss of hydraulic conductance (PLC_{D-1}). Species abbreviations are explained in Table 1. Error bars are standard errors. The regression line in (a) was fitted without the values of Pv7 and Pv8.

sary to reach this peak assimilation value is between 1 and 3 d, when the percentage loss of hydraulic conductance antecedent to the pulse is < 80%. However, an incomplete recovery of photosynthesis from drought was observed when antecedent PLC was above 80% (Fig. 6).

Previous studies had suggested thresholds for incomplete drought recovery in terms of a minimum stomatal conductance (Flexas *et al.*, 2006; Galmes *et al.*, 2007). However, we have shown that proposing this threshold as a function of g_s, instead of PLC, may be misleading: complete and timely photosynthetic recovery is to be expected if low $g_{s,D-1}$ is the result of large antecedent *D* (alone or in combination with a low Ψpd, which does not lead to substantial cavitation), but incomplete drought recovery will follow when large PLC_{D-1} is motivating the low antecedent stomatal conductance.

With the goal of predicting the time lag necessary to reach peak gas exchange, one must take into account the relationship between water infiltration and soil texture. A regression model was able to explain 95% of the variation in τ_A as a function of PLC_{D-1} for the 'sandy loam seedlings', whereas the 'clay loam seedlings' fell out of this regression line (Fig. 6a). This is likely because of the longer time required for water infiltration and for water potential to change in finer-textured soils (Hillel, 2004), the observed correlation between the time necessary to reach A_p with the time necessary to reach peak Ψ_{pd} ($P < 0.05$, $R^2 = 0.63$), and differences in rooting depth across soil textures (Resco, 2008).

The accumulation of abscisic acid (ABA) signaling stomatal closure has previously been reported as another mechanism affecting photosynthetic gas exchange recovery from drought (Davies & Zhang, 1991). However, high ABA concentrations are not likely to persist for > 3 d after water stress is relieved (Davies & Zhang, 1991). An ABA-mediated response would not explain either the long τ_A or the low A_p observed 5–7 d after watering in the seedlings with the highest PLC_{D-1} (Fig. 6). Our results support the findings of Fuchs & Livingston (1996), who suggested that woody plants rely more on hydraulic signals, whereas ABA regulation is probably more common in herbaceous plants.

Our results also support a rapidly increasing body of literature showing the dependence of photosynthetic gas exchange on plant hydraulic properties (Brodribb & Feild, 2000; Sperry, 2000; Maherali *et al.*, 2006; Sack & Holbrook, 2006; Brodribb *et al.*, 2007), although previous studies focused mainly on interspecific comparisons between different parameters related to maximum photosynthetic and hydraulic capacities. Moreover, considering the reported paucity of studies on photosynthetic drought recovery (Flexas *et al.*, 2006; Galmes *et al.*, 2007), this may be the first report linking biochemical, mesophyll and stomatal limitation with plant hydraulic architecture. Indeed we provide, to the best of our knowledge, the first study demonstrating that hydraulic limitations determine peak rates of *A*, g_s and τ_A associated with the short-term responses to precipitation pulses.

Buckley, 2005), amongst others. Whereas an increase in relative humidity usually accompanies a large precipitation pulse, and this would alleviate atmospheric stress, our data indicate that photosynthetic gas exchange recovery may be neither complete nor fast when a large proportion of the hydraulic capacity has been lost as a result of drought. For *P. velutina*, photosynthesis limitation in response to a pulse remains at around or below 10–20%, and the time lag neces-

Do antecedent drought-induced hydraulic limitations constrain photosynthetic recovery across species?

We observed that the degree of antecedent water stress explains up to 87% of the variance in the pulse response in *P. velutina*. We failed to find significant differences in the relationship between A_p and g_p with $\Psi_{pd.D-1}$ (Figs 2, 3) as a function of species identity, as values for *A. tridentata*, *P. tridentata* and *P. smithii* fell within the 95% confidence interval. This result may seem surprising at first, as these species are likely to have different VC curves. However, when antecedent drought stress is so severe that Ψ_{pd} drops to the values reported here (–3.8 to –4.6 MPa), PLC values of all four species are likely to converge at rather large values. Indeed, photosynthesis recovery of these three species was around or above 40%, which, according to our model for *Prosopis*, would imply a PLC_{D-1} of 80% or higher. Published VC curves for some of these species, although growing at different sites, support this possibility (Kolb & Sperry, 1999).

Our estimate of percentage photosynthesis recovery is very sensitive to the maximum photosynthetic rates for a given species ($\hat{A}_{max,s}$, Eqn 1). We conducted a sensitivity analysis which indicated that values for the recovery of photosynthesis in *A. tridentata*, *P. tridentata* and *P. smithii* fell within the 95% confidence interval in Figs 2 and 3 when the error in A_{max} was up to 15%.

Galmes *et al.* (2007) and Flexas *et al.* (2006) observed an incomplete photosynthetic recovery from drought when antecedent g_s dropped below 0.15 mol m^{-2} s^{-1} across a range of phylogenetically and functionally diverse plant species. However, they did not observe any reductions in the leaf biochemical capacity until g_s was smaller than 0.05 mol m^{-2} s^{-1}, and stomatal limitations seemed to prevail after re-watering. Hence, it could be hypothesized that hydraulic limitations developed during drought, as PLC_{D-1} rises when $g_{s.D-1}$ drops below 0.15 mol m^{-2} s^{-1}, are a widespread mechanism limiting photosynthetic recovery, at least, until biochemical limitations arise. Unfortunately, the paucity of studies in the literature on this important topic prevent us from developing any further synthetic advancements. It is an important research need to elucidate the link between stem hydraulics and photosynthesis recovery from drought.

Understanding photosynthesis responses to precipitation may prove extremely useful for the temporal up-scaling of leaf fluxes. Ignace *et al.* (2007) showed that in two C$_4$ grasses, the cumulative carbon gain following precipitation pulses was, under some environmental conditions, highly predictable based upon $\Psi_{pd.D-1}$. Although predicting cumulative carbon gain was beyond the scope of this study, our results suggest that by understanding how hydraulics constrain photosynthesis, we may develop simpler quantitative models of leaf-level rates of gas exchange than current approaches (Tuzet *et al.*, 2003; Schymanski *et al.*, 2008), which would require less data input and parameterization, and without compromising accuracy.

Moreover, understanding plant responses to pulses may prove key to develop water-saving land management techniques, and to mitigate effects of anticipated global warming.

Conclusions

In this study, we observed that drought-induced hydraulic limitations strongly constrain photosynthetic recovery after re-watering in *P. velutina*. PLC_{D-1} < 80% seems to be the threshold value after which photosynthesis recovery is incomplete, and this threshold roughly corresponds to $g_{s.D-1}$ < 0.15 mol m^{-2} s^{-1}. Contrary to recent hypotheses, incomplete photosynthesis recovery may occur even without reductions in leaf biochemical capacity. There is a great need for further tests on the mechanisms constraining drought recovery.

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