Nonstomatal limitations are responsible for drought-induced photosynthetic inhibition in four C_4 grasses

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Summary

• Here, the contribution of stomatal and nonstomatal factors to photosynthetic inhibition under water stress in four tropical C_4 grasses was investigated (*Panicum coloratum*, *Bothriochloa bladhii*, *Cenchrus ciliaris* and *Astrebla lappacea*).

• Plants were grown in well watered soil, and then the effects of soil drying were measured on leaf gas exchange, chlorophyll *a* fluorescence and water relations.

• During the drying cycle, leaf water potential (Ψ_{leaf}) and relative water content (RWC) decreased from *c.* –0.4 to –2.8 MPa and 100–40%, respectively. The CO_2 assimilation rates (*A*) and quantum yield of PSII (Φ_{PSII}) of all four grasses decreased rapidly with declining RWC. High CO_2 concentration (2500 μl l^{-1}) had no effect on *A* or Φ_{PSII} at any stage of the drying cycle. Electron transport capacity and dark respiration rates were unaltered by drought. The CO_2 compensation concentrations of *P. coloratum* and *C. ciliaris* rose sharply when leaf RWC fell below 70%. In *P. coloratum*, 5% CO_2 did not prevent the decline of O_2 evolution rates under water stress.

• We conclude that inhibition of photosynthesis in the four C_4 grasses under water stress is dependent mainly on biochemical limitations.

Key words: C_4 photosynthesis, chl*a* fluorescence, drought, O_2 evolution, stomatal and metabolic inhibition.

Abbreviations

A: CO_2 assimilation rate, C_i: intercellular CO_2 concentration, [CO_2]: CO_2 concentration, Φ_{PSII}: quantum yield of photosystem II of light-adapted leaves, F_v/F_m: photochemical efficiency of dark-adapted leaves, Γ: CO_2 compensation concentration, J_{O_2}: O_2 evolution rate, *g*: stomatal conductance, PEPC: phospho*enol*pyruvate carboxylase, Rubisco: ribulose-1,5-bisphosphate carboxylase/oxygenase, RWC: relative water content, Ψ_{leaf}: leaf water potential.

Introduction

About half of the world grasses fix atmospheric CO_2 via the C_4 photosynthetic pathway (Hattersley, 1992) and C_4 grasslands contribute approximately 20% of global primary productivity (Ehleringer *et al.*, 1997). In Australia, C_4 grasses dominate the vegetation of the vast grasslands and rangelands, which are characterized by frequent droughts (Hattersley, 1992). The Australian C_4 grasslands form the basis of a large, but low-intensity, pastoral industry and significant effort has gone into modelling plant and animal productivity to minimize land degradation in the face of unpredictable rainfall, which is likely to increase under global climate change (McKeon *et al.*, 1990, 1998). Central to predicting the effects of water

availability and climate change on productivity, and for developing effective management strategies of C_4 grasslands, is a sound understanding of the physiological responses of C_4 grasses to drought, particularly the process of CO_2 fixation.

C_4 photosynthesis is characterized by the operation of a CO_2-concentrating mechanism which serves to raise the CO_2 concentration ($[CO_2]$) at the site of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) sufficiently to nearly suppress photorespiration and saturate photosynthesis in air despite the small $[CO_2]$ in the atmosphere (Hatch, 1987). C_4 plants have relatively small stomatal conductance (g), which decreases water loss while maintaining rapid rates of photosynthesis. This is sometimes interpreted as conferring their inherent resistance to drought. Most research into the response of photosynthesis to water stress has been on C_3 plants (Cornic, 1994; Lawlor, 1995; Lawlor & Cornic, 2002). In C_3 species, water stress can reduce CO_2 assimilation rates (A) of leaves through stomatal and nonstomatal factors (Cornic, 1994, 2001; Kramer & Boyer, 1995; Lawlor, 1995; Lawlor & Cornic, 2002). Stomata are very sensitive to the plant's water status and reduced g under soil water deficit represents one of the early indicators of water stress (Cowan, 1981). Small g, without a proportional decrease in photosynthetic potential (A_{pot}, the value under unstressed conditions) causes a concomitant reduction in intercellular $[CO_2]$ (C_i) and hence, A. This stomatal phase of water stress is characterized by the restoration of A to A_{pot} following the removal of stomatal limitation by raising ambient $[CO_2]$, increasing C_i, or rehydration (Vassey & Sharkey, 1989; Cornic, 2001; Lawlor & Cornic, 2002). As water stress progresses, there is evidence that nonstomatal factors become progressively more important (Lawlor, 1995, 2002). This is diagnosed by the inability of high $[CO_2]$ to restore A to A_{pot}, which may be accompanied by reduced RuBP and ATP pools, or sucrose synthesis (Vassey & Sharkey, 1989; Gimenez *et al.*, 1992; Tezara *et al.*, 1999). These nonstomatal effects may be the result of direct drought effect on photosynthetic biochemistry (Lawlor, 2002), or stomatal-related CO_2 deprivation (Vassey & Sharkey, 1989; Meyer & Genty, 1999) or both (Lawlor & Cornic, 2002).

Research on the effect of drought on C_4 photosynthesis has been carried out largely on monocotyledonous grasses, particularly with the two major C_4 crops, maize and sorghum, and to a lesser extent sugarcane. Inhibition of A in maize is mainly due to stomatal closure (Lal & Edwards, 1996; Saccardy *et al.*, 1996; Foyer *et al.*, 1998). Drought had either no effect on the activity of photosynthetic enzymes (Saccardy *et al.*, 1996; Castrillo *et al.*, 2001), or the reductions were too small to account for the photosynthetic inhibition (Lal & Edwards, 1996). However, large changes in the content of metabolites with small A, suggests that biochemical processes are altered (Lawlor & Fock, 1978). Foyer *et al.* (1998) suggested that changes in activities of phosphoenolpyruvate carboxylase (PEPC), sucrose phosphate synthase and nitrate reductase in water stressed maize leaves serve to balance carbon and nitrogen metabolism with the prevailing A. Unlike maize, neither elevated $[CO_2]$ nor re-watering restored A to control values in water-stressed sorghum and sugarcane leaves, indicating that nonstomatal factors (i.e. impaired metabolism) are responsible for photosynthetic inhibition (Contouransel *et al.*, 1996; Du *et al.*, 1996; Massacci *et al.*, 1996). Therefore, there are conflicting opinions about the response of photosynthesis to drought in C_4 crops. Further, little is known about the response of photosynthesis in wild C_4 grasses to water stress. This study was therefore undertaken to investigate the effect of drought on the photosynthesis of wild C_4 grasses, focusing on four tropical species used for pasture. Two species (*Astrebla lappacea* and *Bothriochloa bladhii*) are native to northern Australia and two (*Cenchrus ciliaris* and *Panicum coloratum*) are introduced, and have become widely spread. *Astrebla lappacea* and *P. coloratum* belong to the NAD malic enzyme (NAD-ME) biochemical subtype while *B. bladhii* and *C. ciliaris* are NADP-ME. The main aims of this study were to determine the effects of drought on their photosynthesis and to assess the role of stomatal vs nonstomatal factors in the inhibition of photosynthesis in these grasses under water stress. To this end, leaf gas exchange, chlorophyll *a* (chl*a*) fluorescence, O_2 evolution and water relations were measured at ambient and elevated $[CO_2]$ in greenhouse-grown, potted plants grown without drought, and then exposed to drying soil, contrasted with well-watered plants.

Materials and Methods

Plant culture

Seeds of four tropical C_4 grass species (*A. lappacea* (Lindl.) Domin, *B. bladhii* Kuntze, *C. ciliaris* L. and *P. coloratum* L.), obtained from Grass Seeds Australia and Queensland Agricultural Seeds (Toowoomba, Australia), were germinated in potting soil in 0.5-l pots, in a naturally lit glasshouse (Rothamsted Research, Harpenden, UK) in May–June 2000 (10-h daylength with supplementary illumination to give minimum of *c.* 400 μmol m^{-2} s^{-1} of photosynthetic active radiation (PAR) and an average of *c.* 600 μmol m^{-2} s^{-1}; average day and night temperatures were 25°C and 18°C, respectively. Two seedlings were transplanted into 2-l pots containing soil that had been premixed with slow-release fertilizer, and were watered regularly. There were 20 pots per species. Three weeks after transplantation, watering was withheld from half the pots of each species, while it was continued for the other half. Measurements were made in the subsequent drying cycle, which lasted for approximately 7 d.

Gas exchange measurements

Gas exchange measurements were made on attached, recently expanded leaves of all four species using a six-chamber open

gas exchange system (Lawlor *et al.*, 1989). Conditions in the chambers were 28°C, leaf-to-air vapour pressure deficit (VPD) of 1.0 kPa and PAR photon flux of 1000 μmol quanta m^{-2} s^{-1} supplied by metal-halide photoflood lamps (Wotan, Philips, Holland). The middle section of each leaf was placed in the (2×5 cm^2) chamber, which was covered with black cloth. After 30 min dark-adaptation, dark respiration rates (R_d) and dark-adapted photochemical efficiency (F_v/F_m) were measured. The cloth was then removed and leaves allowed to reach steady-state CO_2 uptake in the light at a chamber [CO_2] of 350 μl l^{-1} for 1.5–2 h, after which A and light-adapted quantum yield of photosystem II (Φ_{PSII}) were measured. Chamber [CO_2] was then raised to 2500 μl l^{-1}, and A and Φ_{PSII} measured again after 1.5–2 h. The high [CO_2] of 2500 μl l^{-1} was chosen to ensure that drought-induced stomatal limitations of photosynthesis are overcome (Lawlor, 1995).

Chlorophyll *a* fluorescence

Chlorophyll *a* fluorescence was measured concurrently with gas exchange using an OS-100 (Opti-Sciences, MA, USA) modulated fluorometer. The optic fibre probe could be removed and replaced in a fixed position over each gas exchange chamber using metal guides. F_v/F_m and Φ_{PSII} were calculated as $F_v = F_m - F_0$ and $(F_m' - F_s)/F_m'$, respectively (Genty *et al.*, 1989) (F_0, fluorescence of a dark-adapted leaf with all PSII reaction centres open; F_m, maximal fluorescence of a dark-adapted leaf with all PSII reaction centres closed following a saturating light pulse; F_s, fluorescence during steady state of photosynthesis; F_m', maximal fluorescence of a light-adapted leaf with all PSII reaction centres closed following a saturating light pulse) (van Kooten & Snel, 1990).

Leaf water relations

Leaf water relations were measured at the end of gas exchange measurements. The leaves were cut and relative water content (RWC) and leaf water potential (Ψ_{leaf}) were determined as described by Ghannoum *et al.*, 2002.

CO_2 compensation concentration

Equilibrium CO_2 compensation concentration (Γ) of detached leaves of *P. coloratum* and *C. ciliaris* was measured on leaf sections enclosed in an illuminated sealed chamber, cooled by a fan, containing a pump that circulated gas through an infrared gas analyser (Tezara *et al.*, 1999). Air temperature, measured with a thermocouple inside the chamber, averaged (mean ± SE) 29.8 ± 0.8°C. Once [CO_2] reached a steady state, the RWC of the leaf sections was determined. In the most severely water-stressed leaves [CO_2] did not reach an equilibrium but increased slowly.

O_2 electrode measurements

The rate of O_2 evolution (J_{O_2}) was measured (Walker, 1987) on leaf sections of *P. coloratum* in an oxygen electrode chamber (LD2/2; Hansatech Instruments, Norfolk, UK). Illumination at the leaf surface in the chamber was 1000 μmol m^{-2} s^{-1}, and temperature was maintained at 27°C. The leaf chamber was flushed for 5 min with 5% CO_2 (50 000 μl l^{-1}) using gas mixing pumps (Wösthoff, Bochum, Germany) before measuring J_{O_2}.

Data analysis

The effects of species and [CO_2] was analysed by two-way analysis of variance (ANOVA) with species and [CO_2] as independent variables and RWC as a covariate. Plotted data were fitted with the function that gave the best fit (linear, polynomial or exponential).

Results

Leaf gas exchange and chl*a* fluorescence

There was a strong, linear relationship between Ψ_{leaf} and RWC for three (*P. coloratum*, *B. bladhii* and *C. ciliaris*) out of the four C_4 grass species (Fig. 1). In *A. lappacea*, RWC changed little as Ψ_{leaf} decreased to −1.5 MPa. Below this value, RWC declined steeply (Fig. 1). However, the Ψ_{leaf} vs RWC relationships did not differ significantly between species ($P_{(species)} > 0.05$). The rest of the data is presented against RWC because it is a better indicator than Ψ_{leaf} for metabolic function (Sinclair & Ludlow, 1985).

A declined substantially and progressively with decreasing RWC, although the pattern varied significantly ($P_{(species)} < 0.001$) between the four species (Fig. 2). *A* became negative around a RWC of 50% in *P. coloratum*, *B. bladhii* and *C. ciliaris* (Fig. 2a–c) and 60% for *A. lappacea* (Fig. 2d). Elevated [CO_2] (2500 μl CO_2 l^{-1}) had no significant effect ($P_{([CO_2])} > 0.05$) on *A* at any point on the *A*/RWC relationship for any of the species (Fig. 2). Dark respiration rates ranged between −0.5 and −4.0 μmol m^{-2} s^{-1}, was similar between species ($P_{(species)} > 0.05$) and the linear fits of R_d against RWC were not significant, except for a slight negative trend in *C. ciliaris* (data not shown). Stomatal conductance, measured at both ambient and elevated [CO_2], declined rapidly with increasing water stress (data not shown). Similar to *A*, *g* showed slightly different sensitivity to drought among the grasses ($P_{(species)} < 0.05$). Nevertheless, the relationship between *A* and *g* can be described by a common relationship for the four grasses, which was distinct for each measurement [CO_2] ($P_{([CO_2])} < 0.001$) (Fig. 3). The relationship between *A* and *g* was best fitted with a polynomial, rather than linear, function with an inflection point around a RWC of 80% ($A \approx 23$ μmol m^{-2} s^{-1} and $g \approx 0.15$ mol m^2 s^{-1} at ambient [CO_2]) (Fig. 3a). This

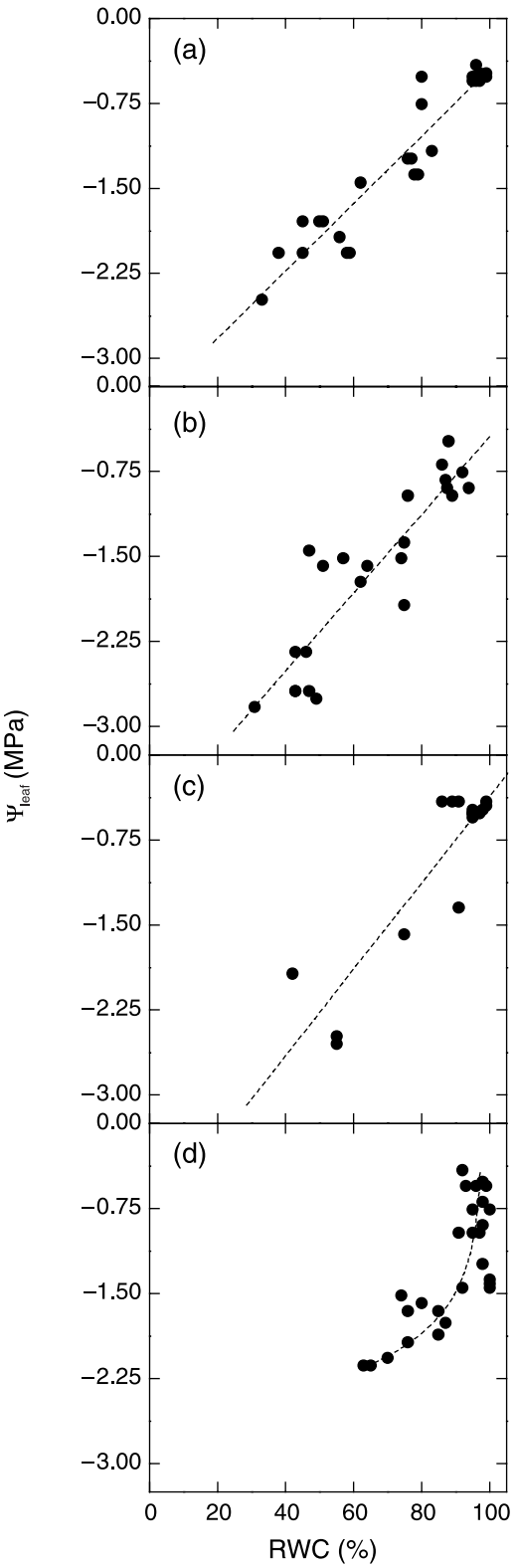

Fig. 1 The relationship between leaf water potential (Ψ_{leaf}) and relative water content (RWC) in four tropical C_4 grasses growing in a drying soil. Each data point is from a different leaf. The lines are best fits for (a) *Panicum coloratum* ($y = -3.4 + 0.030x$, $r^2 = 0.90$), (b) *Bothriochloa bladhii* ($y = -3.9 + 0.034x$, $r^2 = 0.80$), (c) *Cenchrus ciliaris* ($y = -4.2 + 0.038x$, $r^2 = 0.81$) and (d) *Astrebla lappacea* ($y = 98 + 0.296(1 - e^{-x/0.45})$).

coincided with a sharp rise in calculated C_i at both ambient [CO_2] (data not shown).

There was some scatter in Φ_{PSII} data, which is most likely due to variations in photosynthetic capacity and optical characteristics among the different leaves and plants at the various stages of the drying cycles. However, Φ_{PSII} declined with RWC (Fig. 4) for all the species although they did not differ significantly ($P_{(species)} > 0.05$). The relationship between A and Φ_{PSII} remained linear throughout the drying cycle (Fig. 5). The Φ_{PSII} was unaffected by elevated [CO_2] ($P_{([CO_2])} > 0.05$) and F_v/F_m was similar among the C_4 grasses ($P_{(species)} > 0.05$) and insensitive to water stress (Fig. 4).

CO_2 compensation concentration

The CO_2 compensation point, Γ, was measured on two of the four C_4 grasses and ranged between 5 and 12 μl l^{-1} for well-watered leaf sections of *P. coloratum* and *C. ciliaris* (Fig. 6). It was little affected by water stress down to a RWC of 70%, but rose sharply below a RWC of 60% (Fig. 6). It was possible to determine Γ of only a few severely water-stressed leaves because [CO_2] in the chamber did not reach a steady state, but increased steadily.

O_2 evolution rates

In order to test whether very high [CO_2] can restore photosynthetic activity in the water-stressed leaves, J_{O_2} was measured in leaf sections of *P. coloratum* at 5% CO_2. The J_{O_2} ranged between 28 μmol m^{-2} s^{-1} and 42 μmol m^{-2} s^{-1} (Fig. 7), which corresponded well with A measured by gas exchange (Fig. 2a). In the water-stressed leaves, J_{O_2} declined in a curvilinear fashion with RWC. However, small J_{O_2} were still detectable (6–7 μmol m^{-2} s^{-1}) at a RWC of 40% (Fig. 7).

Discussion

The relationship between Ψ_{leaf} and RWC was linear in three and curvilinear in one species. This may be attributed to differences in cell wall elasticity and/or osmotic adjustment among the C_4 grasses (Jones, 1978; Kobayashi & Hori, 2000). In all four C_4 grasses, photosynthesis was very sensitive to water stress, measured as loss of RWC or more negative water potential. Similar results have been reported with C_3 (Stuhlfauth *et al.*, 1990; Ortiz-Lopez *et al.*, 1991; Tezara *et al.*, 1999) and C_4 (Lawlor & Fock, 1978; Du *et al.*, 1996; Lal & Edwards, 1996; Saccardy *et al.*, 1996; Saliendra *et al.*, 1996) species. Importantly, A (and Φ_{PSII}) of both well-watered and water-stressed plants were not significantly enhanced by [CO_2] as high as 2500 μl l^{-1} (0.25%) in the gas exchange chamber. Massacci *et al.* (1996) and Williams *et al.* (2001) reported similar results with sorghum. Because of the presence of a CO_2-concentrating mechanism, photosynthesis operates at near CO_2-saturation in well-watered C_4 plants

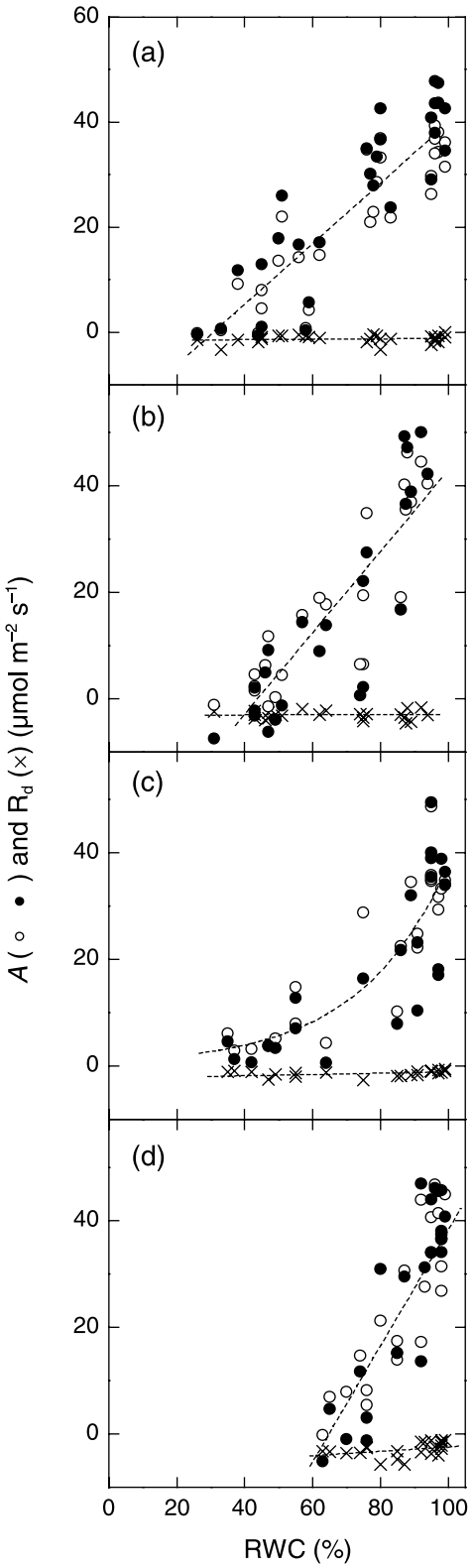

Fig. 2 The CO_2 assimilation (*A*, circles) and dark respiration (R_d, crosses) rates as a function of relative water content (RWC) in (a) *Panicum coloratum*, (b) *Bothriochloa bladhii*, (c) *Cenchrus ciliaris* and (d) *Astrebla lappacea* growing in a drying soil. *A* was measured at 28°C, 1000 μmol quanta m^{-2} s^{-1}, leaf-to-air vapour pressure deficit (VPD) of 1.0 kPa and ambient $[CO_2]$ of either 350 μl l^{-1} (open circles) or 2500 μl l^{-1} (closed circles). Dark respiration (R_d) was measured at ambient $[CO_2]$ after 0.5 h dark adaptation. Each data point represents a different leaf. The lines are linear regression fits of all data points, except for *A* in (c), where data was fitted exponentially. Regression equations of *A* vs RWC are: (a) $y = -18 + 0.58x$, $r^2 = 0.77$; (b) $y = -33 + 0.76x$, $r^2 = 0.76$; (c) $y = 0.86e^{x/26.4}$, $r^2 = 0.75$; (d) $y = -70 + 1.09x$, $r^2 = 0.67$.

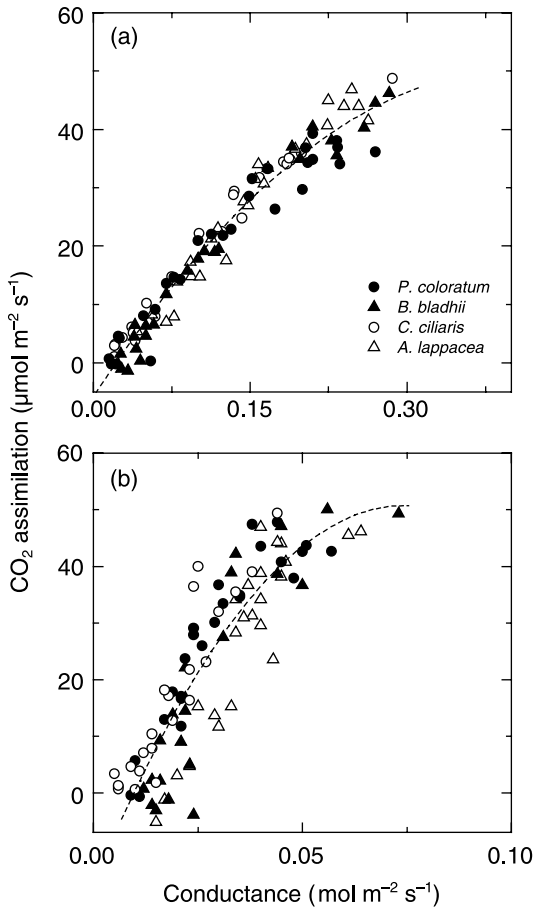

Fig. 3 The relationship between CO_2 assimilation rates (*A*) and stomatal conductance (*g*) in *Panicum coloratum* (closed circles), *Bothriochloa bladhii* (closed triangles), *Cenchrus ciliaris* (open circles) and *Astrebla lappacea* (open triangles) growing in a drying soil. Each data point is from a different leaf. *A* and *g* were measured at 28°C, 1000 μmol quanta m^{-2} s^{-1}, leaf-to-air vapour pressure deficit (VPD) of 1.0 kPa, at a $[CO_2]$ of 350 μl l^{-1} (a) or 2500 μl l^{-1} (b). The lines are polynomial fits for all data points at ambient ($y = -6 + 274x - 335x^2$, $r^2 = 0.96$) and elevated ($y = -6 + 1808x - 12184x^2$, $r^2 = 0.80$) $[CO_2]$.

(Osmond *et al.*, 1982; von Caemmerer, 2000). The A/C_i response curve of C_4 leaves is characterized by a steep initial slope and an abrupt saturation at a C_i around 100–150 μl l^{-1} (von Caemmerer & Furbank, 1999). The operational C_i (which corresponds to ambient $[CO_2]$) depends on a number of environmental factors, such as light and nitrogen supply (Ghannoum *et al.*, 1997; Ghannoum & Conroy, 1998). In

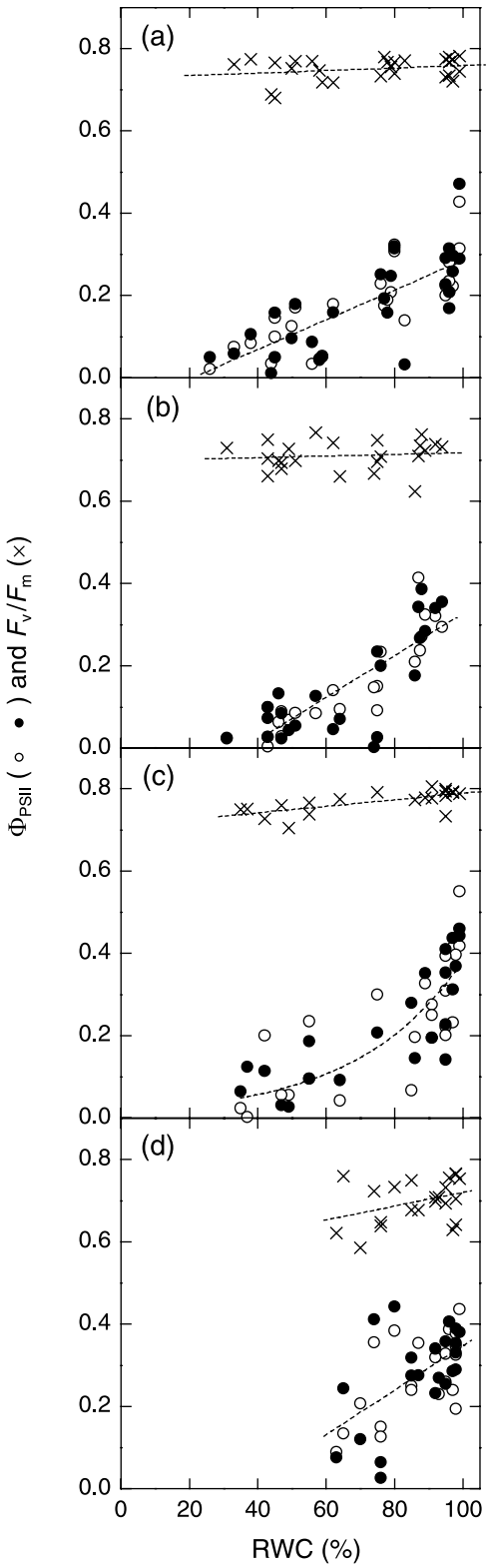

Fig. 4 The quantum yield of PSII (Φ_{PSII}, circles) and photochemical efficiency (F_v/F_m, crosses) as a function of relative water content (RWC) in four tropical C_4 grasses growing in a drying soil. Φ_{PSII} and F_v/F_m were measured concurrently with CO_2 assimilation rates (A) and dark respiration (R_d), respectively. Φ_{PSII} was measured at 28°C, 1000 µmol quanta m^{-2} s^{-1}, leaf-to-air vapour pressure deficit (VPD) of 1.0 kPa and ambient [CO_2] of either 350 µl l^{-1} (open circles) or 2500 µl l^{-1} (closed circles). F_v/F_m was measured at ambient [CO_2] after 0.5 h dark adaptation. The lines are linear regression fits of all data points, except for Φ_{PSII} in (c), where data was fitted exponentially. Regression equations of Φ_{PSII} vs relative water content (RWC) are: (a) $y = -0.008 + 0.004x$, $r^2 = 0.59$; (b) $y = -0.184 + 0.005x$, $r^2 = 0.71$; (c) $y = 0.0.159e^{x/31.5}$, $r^2 = 0.65$; (d) $y = -0.193 + 0.005x$, $r^2 = 0.35$.

this study, gas exchange measurements were made at 1000 µmol quanta m^{-2} s^{-1}, which represented the upper limit where accurate Φ_{PSII} measurements can be made (data not shown). At this light intensity, the operational C_i is expected to be on the saturated part of the A/C_i response curve (Ghannoum *et al.*, 1997), which explains the lack of CO_2-responsiveness of A in the well-watered and mildly water-stressed C_4 grasses in the present study and the similarity of A at elevated [CO_2], despite the much smaller g (Fig. 3).

However, as g decreases with water stress, the operational C_i is expected to progressively move down to the CO_2-responsive region of the A/C_i curve (Long, 1999). Increasing CO_2 supply should then return A to that of the unstressed leaves (i.e. A_{pot}). But this did not occur in our study, indicating inhibition of photosynthetic capacity under drought (Lawlor & Cornic, 2002). It is worth noting that due to the operation of a CO_2-concentrating mechanism, C_4, compared with C_3, photosynthesis is less affected by the initial reduction in g (and hence C_i) (Kawamitsu *et al.*, 1993). Therefore, it is likely that, by the time the reduction in g lowered the operational C_i to the CO_2-sensitive part of the C_4 A/C_i curve, water stress was advanced enough to cause a biochemical (nonstomatal) inhibition of photosynthesis. This inhibition, whether permanent or recoverable on rehydration, was not alleviated by short-term increases in ambient [CO_2]. It has been suggested that the metabolic inhibition of photosynthesis observed under drought is the result of low C_i (due to reduced g) rather than a direct effect of water stress (Vassey & Sharkey, 1989; Meyer & Genty, 1999; Cornic, 2001). However, several lines of evidence suggest that the photosynthetic inhibition observed in our study under moderate to severe water stress was independent of CO_2 supply. First, neither A nor Φ_{PSII} were responsive to high [CO_2] during the early phase of the drying cycle, when the decline in g (and stomatal heterogeneity if present), was still not very large (Meyer & Genty, 1999; Sharkey & Seemann, 1989). If the decline in A or Φ_{PSII} was mainly the result of reduced C_i, then increasing ambient [CO_2] should affect A or Φ_{PSII}, at least in the early, mild stress phase (Lawlor & Cornic, 2002). Second, photosynthetic O_2 evolution was measured in *P. coloratum* at 5% CO_2. This very high [CO_2] has been used to overcome any stomatal limitation by forcing CO_2 to diffuse through the cuticle (Saccardy *et al.*, 1996; Tezara *et al.*, 1999). However, 5% CO_2 did not prevent the decline in J_{O_2}. Third, Γ was measured in *P. coloratum* and *C. ciliaris*. Theoretically, Γ is independent of g and depends

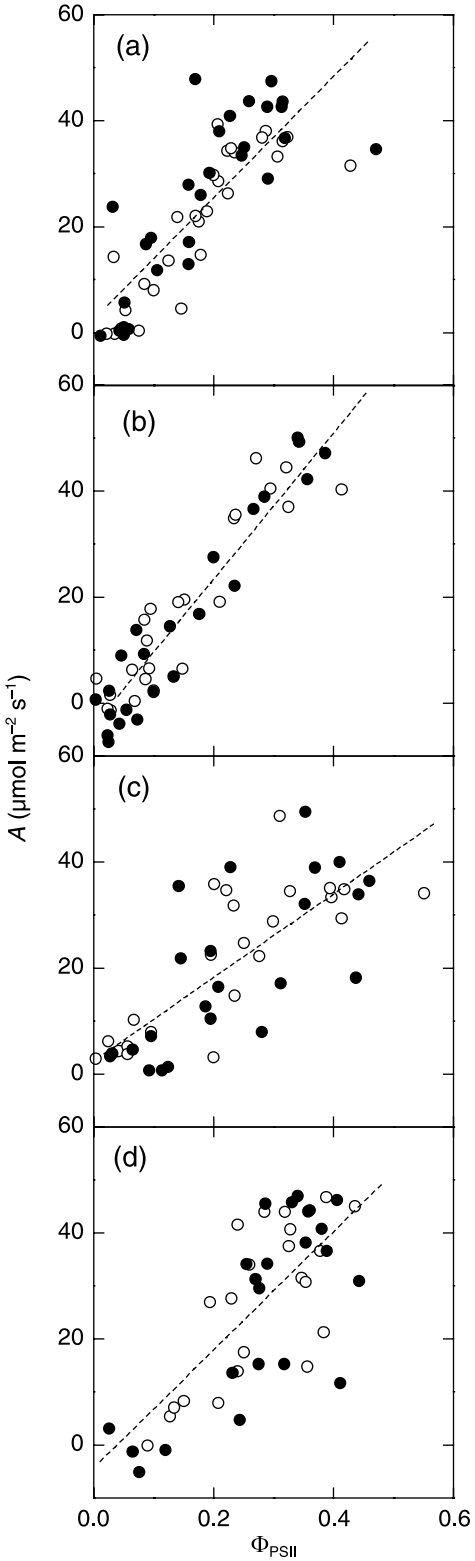

Fig. 5 The relationship between CO_2 assimilation rates (A) and quantum yield of PSII (Φ_{PSII}) in four tropical C_4 grasses growing in a drying soil: (a) *Panicum coloratum*, (b) *Bothriochloa bladhii*, (c) *Cenchrus ciliaris* and (d) *Astrebla lappacea*. A and Φ_{PSII} were measured at 28°C, 1000 μmol quanta m^{-2} s^{-1}, leaf-to-air vapour pressure deficit (VPD) of 1.0 kPa and ambient [CO_2] of either 350 μl l^{-1} (open circles) or 2500 μl l^{-1} (closed circles). The lines are linear regression fits of all data points: (a) $y = 3 + 114x$, $r^2 = 0.67$; (b) $y = -4 + 138x$, $r^2 = 0.88$; (c) $y = 2 + 79x$, $r^2 = 0.57$; (d) $y = -4 + 111x$, $r^2 = 0.53$).

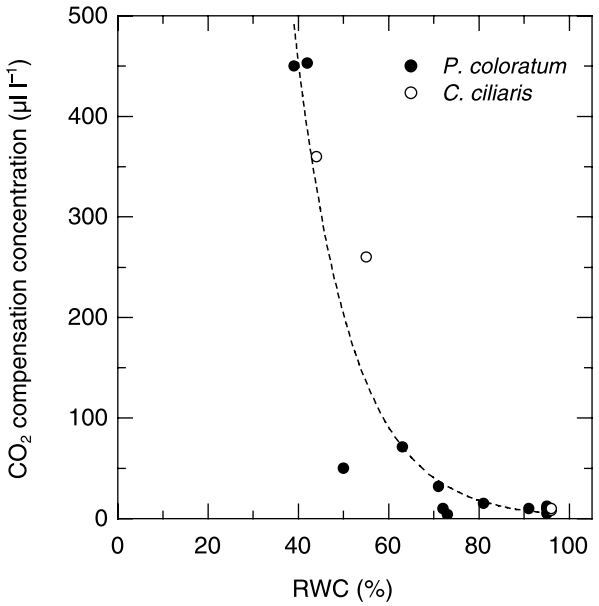

Fig. 6 The equilibrium CO_2 compensation concentration (Γ) of leaves of *Panicum coloratum* (filled circles) and *Cenchrus ciliaris* (open circles), detached from plants growing in a drying soil, as a function of relative water content (RWC). Each data point is from a different leaf. The line is an exponential fit of all data points ($y = 11320e^{-x/12.43}$, $r^2 = 0.89$).

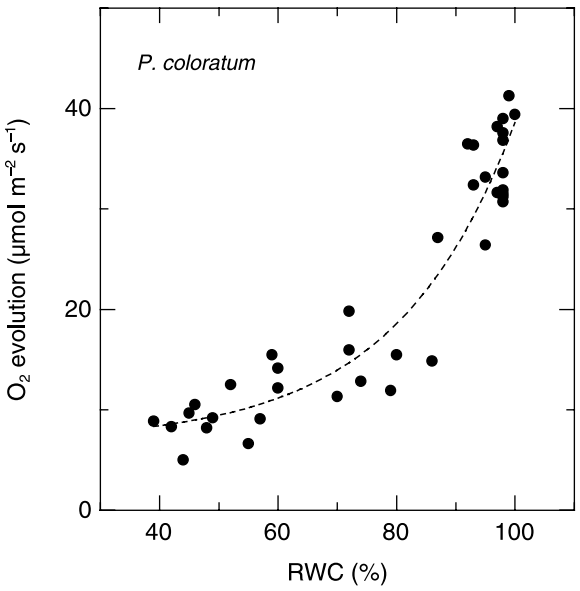

Fig. 7 Rates of O_2 evolution in detached leaves of *Panicum coloratum* as a function of relative water content (RWC). Measurements were made at 5% CO_2. Each data point is from a different leaf. The line is an exponential fit of all data points ($y = 6.8 + 0.22e^{x/20}$, $r^2 = 0.90$).

only on the balance between photosynthesis and respiration (Farquhar *et al.*, 1980). In well-watered C_4 leaves, photorespiration contributes very little to Γ because of the large $[CO_2]$ in the bundle sheath which inhibits the oxygenase reaction of Rubisco, and also because of the rapid and efficient refixation by PEPC of photorespiratory CO_2 (Ghannoum *et al.*, 1998; von Caemmerer & Furbank, 1999). While control leaves had Γ typical of C_4 photosynthesis (Morgan & Brown, 1980; von Caemmerer & Furbank, 1999), it increased dramatically below a RWC of 70–60%. This increase in Γ can only be explained by the inability of stressed leaves to refix respired CO_2, as R_d continued under drought (Lawlor, 1995). Lastly, the decline in leaf water relations was gradual in our study, and photosynthesis was progressively impaired and stopped approximately 7 d after watering was withheld. This rate of drying is similar to that considered as slow in many controlled environment experiments (Lal & Edwards, 1996; Saccardy *et al.*, 1996). Therefore, the photosynthetic inhibition is not attributable to rapid drying, such as usually observed within hours of detaching a leaf (Cornic, 1994; Saccardy *et al.*, 1996). Accordingly, all these results taken together suggest that the decline in A and Φ_{PSII}, under moderate to severe water stress, was independent of ambient or internal $[CO_2]$. This explanation is supported by results from growth experiments with long-term exposure to elevated $[CO_2]$. Seneweera *et al.* (2001) reported that growth at high $[CO_2]$ does not alter the relationship between A and RWC in *P. coloratum* growing in drying soil under controlled environment, indicating that exposure to elevated $[CO_2]$ *per se* has no direct effect on its photosynthetic metabolism. A similar conclusion was reached in a free air CO_2 enrichment (FACE) study with sorghum growing in the field under wet and dry conditions (Williams *et al.*, 2001). In both studies, elevated $[CO_2]$ alleviated the stress effects on A and growth indirectly, by reducing g and thus transpiration by the plant. This resulted in soil water saving, extending the period over which photosynthesis and growth were active (Seneweera *et al.*, 2001; Williams *et al.*, 2001). Further support for our conclusion comes from work on the effect of drought on activities of key C_3 and C_4 cycle enzymes in sorghum and sugarcane (Contouransel *et al.*, 1996; Du *et al.*, 1996). In these studies, it was found that the activity of Rubisco, PEPC, pyruvate, P_i dikinase and NADP-ME decreased under water stress. However, these responses are not always observed. For example, drought had no effect on Rubisco activity in maize (Castrillo *et al.*, 2001) or sugarcane (Saliendra *et al.*, 1996), while PEPC activity increased slightly in maize (Foyer *et al.*, 1998) and sugarcane (Saliendra *et al.*, 1996). Therefore, it appears that water stress affects enzyme activity differently in different C_4 plants, suggesting that other metabolic processes (e.g. ATP synthesis; Tezara *et al.*, 1999) might also be responsible for loss of photosynthetic capacity.

Photochemical efficiency, as measured by dark-adapted F_v/F_m was not significantly affected by water stress in any of the C_4 grasses. The resilience of F_v/F_m to water stress is commonly reported in the literature (Stuhlfauth *et al.*, 1990; Tezara *et al.*, 1999), indicating that electron transport capacity is unaltered by water stress. However, Φ_{PSII} declined concomitantly with A under water stress, suggesting that the activity of the photosynthetic electron chain is finely tuned to that of CO_2 assimilation in C_4 plants, as has been previously observed under various environmental conditions (Genty *et al.*, 1989; Loreto *et al.*, 1995; Lal & Edwards, 1996). Interestingly, small Φ_{PSII} and J_{O_2} were measured when A had dropped to zero and when leaves respired in the light under water stress. Similar results were reported in sorghum (Loreto *et al.*, 1995), and the $A : J_{O_2}$ ratio decreased under water stress in maize (Lal & Edwards, 1996). These results, and those of Tezara *et al.* (1999) for sunflower (a C_3 plant), suggest that electron transport capacity does not reflect CO_2 assimilation faithfully in C_4 or C_3 plants under water stress, and that alternative electron sinks, such as the Mehler reaction, are available. For example, when the rates of O_2 evolution and uptake were measured in a number of C_4 grasses, it was concluded that significant O_2 exchange can be associated with the Mehler reaction in the light (K. Siebke, O. Ghannoum & S. von Caemmerer, unpubl. data).

In conclusion, we demonstrated that the photosynthesis of four tropical, wild C_4 grasses is very sensitive to the leaf water status, as measured by loss of RWC and water potential. The drought-induced inhibition of photosynthesis in our study was independent of ambient $[CO_2]$, suggesting it is of metabolic, rather than stomatal, origin. These results imply that the enhanced growth response of water-stressed C_4 grasses under elevated $[CO_2]$ is unlikely to be caused by the alleviation of the adverse effects of drought on photosynthesis (Ghannoum *et al.*, 2000).

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