

Biomass and leaf-level gas exchange characteristics of three African savanna C₄ grass species under optimum growth conditions

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Abstract

C₄ savanna grass species, *Digitaria eriantha*, *Eragrostis lehmanniana* and *Panicum repens*, were grown under optimum growth conditions with the aim of characterizing their above- and below-ground biomass allocation and the response of their gas exchange to changes in light intensity, CO₂ concentration and leaf-to-air vapour pressure deficit gradient (D_1). *Digitaria eriantha* showed the largest above- and below-ground biomass, high efficiency in carbon gain under light-limiting conditions, high water use efficiency (WUE) and strong stomatal sensitivity to D_1 ($P = 0.002$; $r^2 = 0.5$). *Panicum repens* had a high aboveground biomass and attained high light saturated photosynthetic rates (A_{sat} , $47 \mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance, (g_{sat} , $0.25 \text{ mol m}^{-2} \text{ s}^{-1}$) at relatively high WUE. *Eragrostis lehmanniana* had almost half the biomass of other species, and had similar A_{sat} and g_{sat} but were attained at lower WUE than the other species. This species also showed the weakest stomatal response to D_1 ($P = 0.19$, $r^2 = 0.1$). The potential ecological significance of the contrasting patterns of biomass allocation and variations in gas exchange parameters among the species are discussed.

Key words: above- and below-ground biomass, C₄ grasses, *Digitaria eriantha*, *Eragrostis lehmanniana*, gas exchange characteristics, leaf-to-air vapour pressure deficit, Okavango Delta, *Panicum repens*

Résumé

On a fait pousser des espèces herbeuses de savane de type C₄, *Digitaria eriantha*, *Eragrostis lehmanniana* et *Panicum repens*, dans des conditions optimales dans le but de caractériser l'allocation de leur biomasse aérienne et racinaire et la réponse de leur échange gazeux à des changements d'intensité de la lumière, de concentrations de CO₂, et à un gradient déficitaire (D_1) de pression de vapeur feuille-air. *D. eriantha* montrait la plus grande biomasse aérienne et racinaire, une grande efficacité de l'assimilation de carbone dans des conditions de luminosité limitée, une grande efficacité d'utilisation de l'eau (WUE) et une forte sensibilité des stomates à D_1 ($P = 0,002$; $r^2 = 0,5$). *P. repens* avait une grande biomasse aérienne et atteignait des taux photosynthétiques élevés en lumière saturée (A_{sat} , $47 \mu\text{mol m}^{-2} \text{ s}^{-1}$), et une conductance stomatique (g_{sat} , $0.25 \text{ mol m}^{-2} \text{ s}^{-1}$) à une WUE relativement élevée. *E. lehmanniana* avait une biomasse qui était presque la moitié de celle des autres espèces et avait un A_{sat} et un g_{sat} similaires mais qui étaient atteints à une WUE plus basse que les autres espèces. Cette espèce montrait aussi la plus faible réponse stomatique à D_1 ($P = 0,19$, $r^2 = 0,1$). L'on discute de la signification écologique potentielle de ces schémas contrastés d'allocations de biomasse et des variations des paramètres des échanges gazeux entre les espèces.

Introduction

The main constraints on plant and ecosystem productivity in the seasonally dry tropical savanna-wetland mosaic of

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the Okavango Delta in north-western Botswana are low soil water availability during the dry season, periodic flooding, generally low soil fertility, fire and grazing. The wet season (November–April) in this environment, when over 90% of the 400 mm annual rain falls, coincides with high leaf-to-air vapour pressure deficit (D_i ; >4.5 kPa) and leaf temperatures (T_l) may exceed 35°C.

A main factor that is supposed to regulate the adaptation of plants to environmental constraints is plasticity in the pattern of resource allocation (Lambers & Poorter, 1992). Plants that originate from infertile soils frequently display greater allocation of biomass to fine roots, and to increase the absorbing area (Baruch, 1994), invest less biomass to produce root length and produce fine roots with greater specific root length (SRL) (Lambers & Poorter, 1992). For grasses in the Okavango Delta, below ground accumulation of carbon and nutrient reserves would also allow for quick recovery of photosynthetic biomass after fires (Baruch, Ludlow & Davis, 1985). Previous studies have reported contrasting patterns in below- and above-ground biomass allocation in tropical and temperate grasses (Veenendaal, Shushu & Scurlock, 1993; Baruch, 1994; Ryser & Lambers, 1995), which suggest amplitude for resource partitioning.

C₄ species constitute a high proportion of the grass flora in the Okavango Delta. C₄ photosynthesis involves at least three different biochemical pathways that differ in the mechanisms used to transport CO₂ to the bundle sheath cells and also to regenerate phosphoenol pyruvate (Hatch, 1987). These biochemical pathways are NAD-malic enzyme species (NAD-ME), NADP-malic enzyme species (NADP-ME) and PEP carboxykinase species (PCK). Plants that use C₄ metabolism possess a CO₂ concentrating mechanism that enables them to have high nitrogen use efficiency, high water use efficiency, preference of high light intensities and high optimum leaf temperatures for CO₂ uptake. Such adaptations make these plants particularly adapted for survival in the infertile and seasonally dry tropical ecosystems that often experience high vapour pressure deficits.

The aim of this work was to quantify the biomass allocation and to elucidate important information about the ecophysiology of little known, but commonly found, C₄ grass species under optimum growth conditions. In the Okavango Delta, these species are found in different habitats: *Digitaria eriantha* Steud occurs in a wide-range of drier often partially shaded habitats in woodlands and islands and, *Eragrostis lehmanniana* Nees dominates dry, open and

disturbed habitats, while *Panicum repens* L. dominates moist seasonally flooded areas of floodplains (Gibbs Russel *et al.*, 1990). This was the first study to investigate gas exchange characteristics of C₄ grasses from the Okavango Delta. Growing these plants under similar growth conditions (optimum water and nitrogen levels, high air temperature and high vapour pressure deficit) that mimic a period of high productivity with plant material sourced from their natural environment could provide insights into their potential responsiveness in natural conditions.

Materials and methods

Growth conditions

Vegetative cuttings of a tillering perennial, *Digitaria eriantha* Steud (NADP-ME), a tufted perennial *Eragrostis lehmanniana* Nees (NAD-ME), and a rhizomatous perennial *Panicum repens* L. (PCK), were collected from a floodplain in the Okavango Delta. *Digitaria eriantha* is native to Southern Africa. It is regarded as drought tolerant, and considered to have low shade tolerance, but high fire tolerance (Bogdan, 1977; Gibbs Russel *et al.*, 1990). When collected from the field *D. eriantha* was growing under or very near to trees. *Eragrostis lehmanniana* is a seed-banking species and following fire, it re-germinates when soil water content becomes favourable. It dominates disturbed well-drained sandy soils (Gibbs Russel *et al.*, 1990). When it was collected, *E. lehmanniana* was growing in open areas. Tropical and north Africa and the Mediterranean are the native range of *P. repens* but is now widespread throughout the tropics (Gibbs Russel *et al.*, 1990). It favours open sunny conditions on moist, often sandy soil, and can withstand occasional flooding, but its rhizomes can stand prolonged dry periods. *Panicum repens* was collected in the middle of a seasonal floodplain. Common characteristic among these species is that they are grazed extensively by herbivores (Gibbs Russel *et al.*, 1990).

The plants were then planted into large bags (three cuttings per bag, 19 × 7.5 cm) to provide for ample rooting space, and kept outside a shade house to allow full exposure to light and ambient air temperatures which ranged from 35 to 39°C. The bags were filled with sandy soil from Lake Ngami and watered daily. After 5 weeks, the cuttings were transferred (one vegetative cutting of one species per bag) into new bags filled with uniform river sand. After transplanting, each plant received a fertilizer application. The fertilizer was applied as 10 ml liquid plant

food (Supranure, containing 11% N, 7.3% P and 3.7% K) diluted in 5 l of tap water. Each bag was given 200 ml of this solution. Fertilizer was re-applied after 3.5 weeks before the start of the photosynthesis measurements.

Harvest

After 8 weeks of growth and photosynthesis measurements had been completed, plants were harvested. The soil of roots of all individual plants was carefully rinsed with water, to minimize root damage or loss. Subsequently, each plant was separated into roots and shoots, and in the case of *P. repens*, also rhizomes. Subsamples of five leaves per shoot were taken to determine leaf area with a leaf area meter (model 3100 area meter; Li-Cor Inc., Lincoln, NE, U.S.A.). Subsamples of the roots were taken to determine SRL, using a ruler. All plant samples (roots and shoots) were oven-dried for 48 h at 80°C to determine dry weight. Total biomass, shoot–root ratio (SRR), leaf area ratio (LAR) and specific leaf area (SLA) were determined according to Beadle (1993). SRL was determined by dividing the length of the root sample by the dry weight.

Photosynthesis characteristics

Steady-state leaf gas exchange measurements were made using an open gas exchange system (LI-6400; Li-Cor Inc.) on youngest and fully expanded leaves from at least four randomly selected plants or separate tillers of each species. Measurements were made between 08.00 and 10.00 hours, to avoid midday stomatal depression. The leaf temperature inside the 6-cm² leaf chamber was not controlled, but did not exceed 40°C, the temperature threshold above which C₄ net photosynthetic assimilation may become inhibited (Lawlor, 2001). During the gas exchange measurements, all plants experienced similar leaf temperature and leaf-to-air vapour pressure deficit (D_l) values (c. 37°C and 4.3 kPa respectively). Leaf areas were calculated using leaf dimensions of the enclosed leaf sections in the chamber (Anderson *et al.*, 2001). Measurements were recorded only after the photosynthetic rate and stomatal conductance were considered constant and at equilibrium with the ambient conditions within the gas exchange cuvette.

During measurements both the leaf-to-air vapour pressure deficit, D_l , and leaf temperature, T_l , in the chamber did not always exactly match the ambient conditions because these two parameters are affected by leaf transpiration rate

(Beale, Morison & Long, 1999); T_l typically exceeded air temperature at time of measurements by not more than 1–2°C. The red/blue LED light source inside the chamber allowed for automatic changes of light intensity (I) of 2000, 1500, 1000, 500, 200, 100, 50, 20 and 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Apparent light use efficiency (ϕ) was estimated as the slope of the first four points in the linear part of the light response curve. The qualification, apparent, is used as the estimate is based on incident and not absorbed photon flux (Lawlor, 2001). Light-saturated photosynthetic rate, A_{sat} , light-saturated stomatal conductance, g_{sat} , the ratio of ambient to internal CO₂ concentration ($[\text{CO}_2]$), C_i/C_a ratio, and water use efficiency, WUE, were obtained at high photon irradiance ($>1500 \mu\text{mol m}^{-2} \text{s}^{-1}$) and at an ambient $[\text{CO}_2]$ of 380 $\mu\text{mol mol}^{-1}$. WUE was determined as $A_{\text{sat}}/g_{\text{sat}}$.

CO₂ response curves were then determined at high photon irradiance (2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and at different chamber $[\text{CO}_2]$ in the sequence ambient, 380, 300, 200, 100, 50, 400, 400, 600, 700 and 800 $\mu\text{mol mol}^{-1}$. The different CO₂ concentrations were obtained by means of a portable CO₂/air mixture tanks and automatically controlled by a CO₂ injector. From each CO₂ response curve, we determined the CO₂ compensation point, Γ , the initial slope, α , and the light and CO₂ saturation rate of photosynthesis (A_{pot}). Γ was determined by extrapolating the linear portion of the CO₂ response curve to intercept the x-axis, and carboxylation efficiency (α ; the initial slope of the CO₂ response curve) was determined as the slope of the first four points in the linear part of the CO₂ response curve (Polley *et al.*, 1992). Gas phase limitation to photosynthesis, L_g , was estimated from the CO₂ response curves as $[(A_{\text{pot}} - A)/A_{\text{pot}}] \times 100$, (Farquhar & Sharkey, 1982; Ripley *et al.*, 2007), where A is net photosynthesis at ambient chamber $[\text{CO}_2]$, 380 $\mu\text{mol mol}^{-1}$. To test the significance of differences among the species in growth parameters and gas exchange parameters, data were analysed with univariate analysis of variance (ANOVA), using Tukey's HSD test. Statistical analyses were performed using the SPSS statistical package (SPSS 11.0 for Windows; SPSS Inc., Chicago, IL, USA).

Results

Plant growth

Digitaria eriantha and *P. repens* had almost twice as much biomass, dry weight, as that of *E. lehmanniana*. SLA realized

Table 1 C₄ photosynthetic subtypes and mean values and standard deviations of biomass allocation characteristics of the three savanna grass species

Species	C ₄ subtype	n	Biomass (g)	SRR (g g ⁻¹)	SRL (cm g ⁻¹)	LAR (cm ² g ⁻¹)	SLA (cm ² g ⁻¹)
<i>Digitaria eriantha</i>	NADP-ME	8	39.4 ^b	3.8 ^b	326 ^b	90 ^c	245 ^b
<i>Eragrostis lehmanniana</i>	NAD-ME	11	21.2 ^a	3.7 ^b	211 ^a	36 ^a	191 ^a
<i>Panicum repens</i>	PCK	12	40.0 ^b	1.8 ^a	170 ^a	63 ^b	191 ^a

SRR, shoot-root ratio; SRL, specific root length; LAR, leaf area ratio; SLA, specific leaf area.

Different superscripts in each column indicate significant differences at $P = 0.05$; Tukey HSD test.

with this biomass was highest in *D. eriantha* and similar between the other two species (Table 1). As was the case with biomass, the ratio of leaf area to total plant weight (LAR) of *D. eriantha* and *P. repens* was significantly higher ($P < 0.05$) than that of *E. lehmanniana*. Root length per unit root biomass (SRL) of *D. eriantha* was significantly higher ($P < 0.05$) than that of the other two species. *Digitaria eriantha* had similar SRR with *E. lehmanniana* that were almost double that of *P. repens* (Table 1).

Photosynthetic characteristics

Typical of C₄ species, net photosynthesis in all the species was not light saturated (Fig. 1). *Digitaria eriantha* had highest mean apparent light use efficiency [ϕ : 0.067 mol (CO₂) mol⁻¹ (incident photon)] which was significantly higher ($P = 0.021$) than that of *P. repens* [0.056 mol (CO₂) mol⁻¹ (incident photon); Table 2].

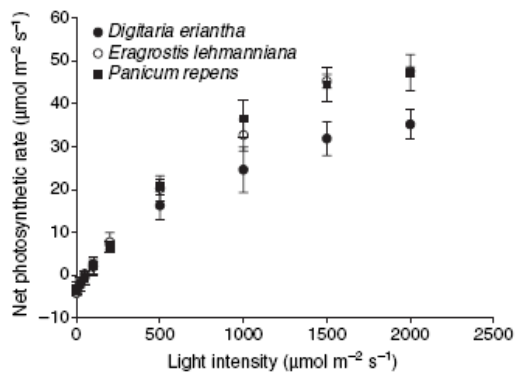


Fig 1 Mean values and standard deviations of the response of net CO₂ assimilation to photon irradiance in leaves of three C₄ grasses. Each datum point is a mean of at least four measurements. For clarity of the figure, only standard deviations for A are shown

Eragrostis lehmanniana and *P. repens* displayed similar mean A_{sat} and mean g_{sat} values (47 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 0.27 mol m⁻² s⁻¹ respectively) that were significantly higher ($P < 0.05$) than in *D. eriantha* (Table 3). Mean WUE values were similar between *D. eriantha* and *P. repens* (c. 190 $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$) and were significantly higher ($P < 0.05$) than those of *E. lehmanniana* (Table 3). The rank order of the species remained the same even when WUE was determined as A/E (net photosynthesis/transpiration) (data not shown). *Digitaria eriantha* and *P. repens* displayed similar mean C_i/C_a ratio (0.11 and 0.12 respectively) that were significantly lower ($P < 0.05$) than that of *E. lehmanniana* (0.18; Table 3). Consistent with these observations, there were similar slopes of the relationship between A_{sat} and g_{sat} in *D. eriantha* and *P. repens* (Fig. 2). This relationship was linear and close to proportional in *D. eriantha* and *P. repens*, in contrast to that of *E. lehmanniana* which was nonlinear. In *E. lehmanniana*, A_{sat} increased with g_{sat} until values of c. 0.3 mol m⁻² s⁻¹, above which a further stomatal opening did not lead to any evident further increase in A_{sat} .

Despite considerable scatter in the data, g_{sat} and C_i/C_a ratio decreased with an increase in D_i in all the species (Fig. 3). The strongest and significant linear correlations between g_{sat} versus D_i and C_i/C_a ratio versus D_i relationships were found in *P. repens* ($P < 0.05$, $r^2 = 0.5$ and 0.6 respectively). In contrast, both relationships were weak and nonsignificant ($P > 0.05$, $r^2 = 0.1$) in *E. lehmanniana*. In all the species, the relationship between A_{sat} and D_i followed a similar pattern to that of g_{sat} versus D_i (data not shown).

Consistent with C₄ photosynthesis, CO₂ assimilation rates increased rapidly, in response to increasing internal CO₂ concentrations, C_i , up to C_i values of 50–80 $\mu\text{mol mol}^{-1}$, before becoming saturated at high chamber [CO₂] (Fig. 4). Also, typical of C₄ plants, CO₂ compensation points of all species were low, c. 6 $\mu\text{mol m}^{-2} \text{s}^{-1}$, with no significant differences ($P > 0.05$) between the species

Table 2 Mean values and standard deviations of carboxylation efficiency (CE), CO₂ compensation point (Γ), light use efficiency (ϕ), dark respiration (R_d), gas phase limitation to photosynthesis (L_g) and leaf-to-air vapour pressure deficit (D) for the three grass species

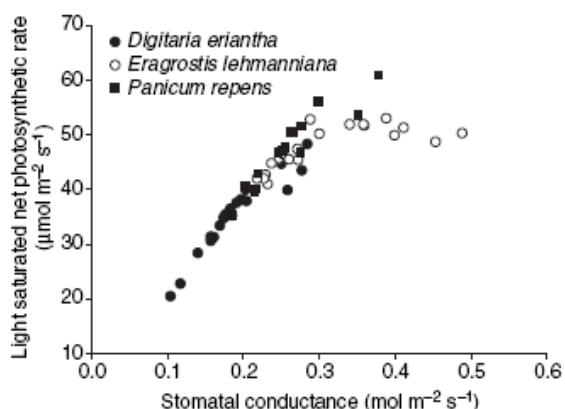
	CE [$\mu\text{mol m}^{-2} \text{s}^{-1}$ ($\mu\text{mol mol}^{-1})^{-1}$]	Γ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	ϕ (mol mol ⁻¹)	L_g (%)
<i>Digitaria eriantha</i>	2.19 (0.84) ^a	6.96 (1.19) ^a	0.067 (0.01) ^b	22.14 (4.44) ^b
<i>Eragrostis lehmanniana</i>	1.66 (0.33) ^a	5.98 (3.34) ^a	0.063 (0.01) ^{ab}	14.44 (3.06) ^a
<i>Panicum repens</i>	2.59 (1.08) ^a	6.91 (1.31) ^a	0.056 (0.02) ^a	17.47 (2.39) ^{ab}

Different superscripts in each column indicate significant differences at $P = 0.05$; Tukey HSD test.

Table 3 Mean values and standard deviations of light saturated net photosynthetic rate (A_{sat}), light and CO₂ saturated net photosynthetic rate (A_{pot}), light saturated stomatal conductance (g_{sat}), the ratio between ambient and intercellular CO₂ (C_i/C_a) and water use efficiency (WUE) for the three species at saturating light (1600–2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and at ambient [CO₂] of 380 $\mu\text{mol mol}^{-1}$

	A_{sat} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	A_{pot} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	g_{sat} (mol m ⁻² s ⁻¹)	C_i/C_a	WUE ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$)
<i>Digitaria eriantha</i>	35.28 (6.76) ^a	41.23 (4.37) ^a	0.19 (0.05) ^a	0.11 (0.04) ^a	190.3 (14.2) ^b
<i>Eragrostis lehmanniana</i>	47.47 (4.04) ^b	52.33 (2.25) ^b	0.31 (0.08) ^b	0.18 (0.05) ^b	161.8 (28.6) ^a
<i>Panicum repens</i>	47.33 (6.86) ^b	51.30 (8.07) ^b	0.26 (0.05) ^b	0.12 (0.03) ^a	184.2 (12.9) ^b

Different superscripts in each column indicate significant differences at $P = 0.05$; Tukey HSD test.

**Fig 2** Relationship between light saturated net CO₂ assimilation and stomatal conductance of leaves of C₄ species. Each point represents an individual measurement at saturating light (1600–2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and at ambient [CO₂] of 380 $\mu\text{mol mol}^{-1}$

(Table 2). Initial slopes of the CO₂ response curves (α), showed no significant differences ($P = 0.306$) between the species, ranging from 1.7 to 2.6 $\mu\text{mol m}^{-2} \text{s}^{-1}$ ($\mu\text{mol mol}^{-1})^{-1}$ (Table 2).

In *D. eriantha* and *P. repens*, raising chamber C_a values above ambient [CO₂] led to a relatively small increase in C_i , up to 120 $\mu\text{mol mol}^{-1}$ (Fig. 4). In contrast, at high C_a , C_i of *E. lehmanniana* increased to almost 400 $\mu\text{mol mol}^{-1}$. Similar to A_{sat} , highest mean light and CO₂ saturated photo-

synthetic rates (A_{pot}) values were found in *E. lehmanniana* and *P. repens* (c. 52 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and were significantly higher ($P < 0.05$) than that found in *D. eriantha* (41 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Mean gas phase limitation (L_g) values, which indicate the magnitude of increase of photosynthesis at ambient CO₂ concentration relative to A_{pot} , were highest in *D. eriantha* (22%) while *E. lehmanniana* had lowest values (14%; Table 2).

Discussion

The aim of this study was to characterize plant biomass and leaf-level gas exchange of commonly found C₄ grasses with the aim of improving our knowledge in predicting their field performance in natural environments. In the long term, our objective was to identify representative species that may be used to gather information that would be useful when constructing the carbon budget in the ecosystems of the Okavango Delta.

Although caution is necessary, when comparing allocation patterns in plants when only single harvests are taken (Lambers & Poorter, 1992), the long growth period and the similar order of magnitude of the final biomass of the plants at the end of the experiment suggest that some differences can still be considered to reflect true differences allocation patterns. For instance, the larger biomass in *D. eriantha* was associated with high SRL. Increased SRL

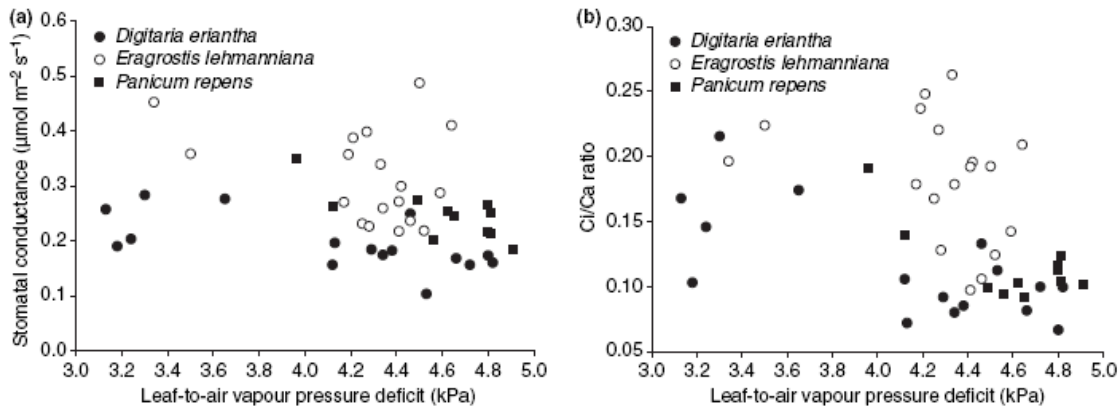


Fig 3 The response of stomatal conductance (a) and C_i/C_a ratio (b) to leaf to air vapour pressure deficit of the three species. Each point represents an individual measurement at saturating light ($1600\text{--}2000 \mu\text{mol m}^{-2} \text{s}^{-1}$) and at ambient $[\text{CO}_2]$ of $380 \mu\text{mol mol}^{-1}$

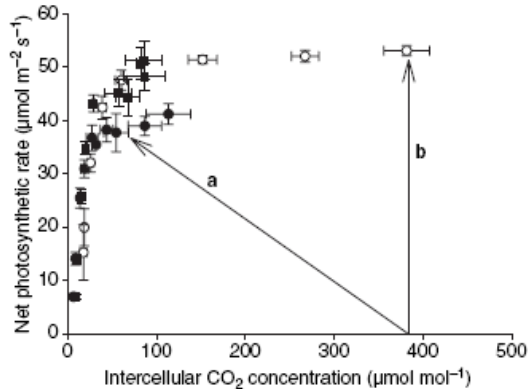


Fig 4 Mean values and standard errors of the response of net CO_2 assimilation to CO_2 in leaves of three C_4 grasses. Each datum point is a mean of at least four measurements. The vertical line, denoted *b*, represents photosynthetic rate at ambient $[\text{CO}_2]$ if resistance to diffusion was zero and that denoted *a*, represents the limitation on net photosynthesis imposed by CO_2 diffusion. Line *a* has a slope set by the stomatal conductance and both lines intercept the C_i axis at ambient $[\text{CO}_2]$. Symbols are the same as those in Fig. 1

results from longer root length (and hence larger root surface area) per unit carbon invested (Eissenstat, 1991), and indicates potential for high rates of root growth in favourable soil conditions. Further, the high LAR displayed by *D. eriantha*, which was probably due to its high SLA, suggests a physiological trait to optimize photon harvesting. The advantage of a high SLA is a higher photosynthetic rate per unit leaf weight (Roderick & Cochrane,

2002). Indeed, our results showed that despite lowest area based photosynthetic rates in *D. eriantha*, mass based photosynthetic rates between the species were similar. Taken together, the above- and below-ground allocation of biomass in *D. eriantha* suggests high potential of this species to exploit the seasonal pulses in nutrient and water availability and to tolerate different levels of shading that it encounters under natural conditions, hence its occurrence in a wide range of habitats within the Okavango Delta and throughout Southern Africa (Gibbs Russel *et al.*, 1990).

The low SRR found in *P. repens* indicates a strong priority for shoot growth. A trait particularly important in wetlands where under more stable moisture conditions shoot competition may be more important. The high productivity of *P. repens* makes it an important grazing resource for game and domestic livestock (Gibbs Russel *et al.*, 1990; Van Bommel *et al.*, 2006). However, the small root allocation in this species could imply low capacity to tolerate dry conditions and also reduced capacity for soil water and nutrient uptake when growing under natural conditions.

The range of mean A_{sat} and g_{sat} , $35\text{--}48 \mu\text{mol m}^{-2} \text{s}^{-1}$, $0.19\text{--}0.31 \text{mol m}^{-2} \text{s}^{-1}$, respectively, observed in this study was comparable to that reported for other C_4 species grown under optimum conditions (25 and $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $0.2\text{--}0.4 \text{mol m}^{-2} \text{s}^{-1}$ respectively) (Lawlor, 2001). The positive curvilinear relationship between g_{sat} and A_{sat} found in *E. lehmanniana* implies more stress on g_{sat} than on photosynthetic capacity (Hetherington & Woodward, 2003), while the close to proportional and almost linear relationship in *D. eriantha* and *P. repens*, if maintained would result in a constant C_i

(Jones, 1987). However, this relationship did not pass through the origin; therefore C_i could not be constant.

The range of mean C_i/C_a values of these species, 0.11–0.18, was on the lower side of those previously reported at moderate vapour pressure deficits and well-watered C_4 species (0.2–0.4; Lawlor, 2001). Such low C_i/C_a values were probably a reflection of both the direct response of stomata to high D_i and also the relatively high photosynthetic capacity (Condon *et al.*, 2002). The C_i/C_a ratio is determined by the balance between the supply of CO_2 to the leaf interior and the demand for CO_2 (Farquhar & Sharkey, 1982). The low C_i/C_a ratio and high WUE displayed by *D. eriantha* and *P. repens* indicate that carbon gain occurred at minimal loss of water. However, our results suggest that the low C_i/C_a ratio in *P. repens* might have been due to high photosynthetic capacity as indicated by its high A_{sat} , in contrast, the low C_i/C_a ratio of *D. eriantha* might have been a result of lower stomatal conductance, as indicated by the low A_{sat} .

The slope of the relationship between g_{sat} and D_i has been used in C_4 species to indicate stomatal sensitivity to D_i (Bunce, 1983; Kawamitsu, Yoda & Agata, 1993; Maroco, Pereira & Chaves, 1997). The lower sensitivity of g_{sat} to D_i and the weaker response of A_{sat} to D_i displayed by *E. lehmanniana* suggest better adaptation to seasonally dry environments where high D_i is associated with the wet season. High stomatal sensitivity, as found in *P. repens* and *D. eriantha* might be ecologically disadvantageous, since plants might miss the opportunity for photosynthesis when there is available soil water to meet the transpirational demand. A study on the three Sahelian C_4 grass species attributed pronounced stomatal sensitivity to drought resistance, while a lack of stomatal sensitivity was associated with drought escaping species (Maroco *et al.*, 1997).

Well-established theory suggests that, on average, NADP-ME species have higher ϕ than NAD-ME species with PCK species being intermediate (Ehleringer & Pearcy, 1983). This pattern was not apparent in this study and may have been masked by the use of only one species per C_4 subtype. However, the high ϕ found in *D. eriantha* indicates higher efficiency of leaf photosynthesis in light-limiting conditions (Ehleringer & Pearcy, 1983). Functionally, this may be a useful trait as this species is sometimes found in savanna communities with higher leaf area indices (Gibbs Russel *et al.*, 1990). Because of the presence of a CO_2 -concentrating mechanism, photorespiration is suppressed and photosynthesis

operates at near CO_2 -saturation in well-watered C_4 plants (Von Caemmerer, 2000). Consistent with this theory, our results showed Γ of $<10 \mu\text{mol mol}^{-1}$ and a relatively small increase, 14–22%, in photosynthesis at short-term exposure to high $[CO_2]$.

In conclusion, substantial differences in above- and below-ground biomass allocation may potentially contribute to their different abilities to extract water and nutrients, and probably reflect the adaptations to the different habitats they dominate. *Digitaria eriantha* produced high above- and below-ground biomass, showed high WUE and high adaptation to low light conditions. If this pattern holds in the field, it would suggest that both morphological (biomass accumulation) and physiological (gas exchange characteristics) play a role in facilitating this species relative abundance in a wide range of habitats within the savanna ecosystem. Gas exchange parameters in *E. lehmanniana* showed the weakest response to high evaporation demand, indicative of better adaptation to seasonally dry environments and consistent with the dry, open and disturbed habitats dominated by this species. The high above ground biomass allocation of *P. repens* may be indicative of a mechanism to cope with herbivory. On the other hand, high stomatal sensitivity suggests a physiological basis for this species greater abundance in wet areas of the Okavango Delta.

The experimental design of this study prevented the detection of plant–plant interactions which would be found under natural conditions. Moreover, as these plants were grown under ambient $[CO_2]$ and exposed only for short periods to high CO_2 chamber environment, it was not possible to compare our results to other C_4 grasses grown under high $[CO_2]$ (e.g. Roumet & Roy, 1996). A more complete comparative assessment of the morphological and leaf-level photosynthetic characteristics of these species awaits the incorporation of species-specific leaf nitrogen content. We suggest that the adaptive traits observed here could be tested over a larger number of species and growing them over a longer period of time, combined with manipulation of water and/or fertilization regimes, to allow for a further identification of evolutionary pressures that best reflect selection for functionally adaptive morphological and physiological characteristics. Taken together, our results make a significant contribution to the understanding needed to predict the spatio-temporal distribution of these species and may also be useful in determining gross primary productivity of this savanna-wetland mosaic.

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