

# Growth, leaf photosynthesis and canopy light use efficiency under differing irradiance and soil N supplies in the forage grass *Brachiaria decumbens* Stapf.

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## Abstract

Irradiance and soil nitrogen effects on growth, net photosynthesis and radiation use efficiency (RUE) of *Brachiaria decumbens* were investigated in fertilized and non-fertilized stands. Three levels of photosynthetic photon flux (PPF: S0 = 100%, S1 = 50% and S2 = 30%) and two N supplies, with (N+) and without (N-), were used. Forage biomass and nutrient accumulation, specific leaf area (SLA), leaf area index (LAI), fractional intercepted photosynthetic photon flux (fPPF), leaf photosynthetic response to light and efficiency of radiation use at leaf (A/Q) and canopy (RUE) levels were measured. Shade effects were mostly independent of soil N. Final yield was decreased by 34% (S1) and 57% (S2). Shade increased SLA (25–46%), so maximum LAI (2.4–3.3) was similar among light regimes. In N- stands, reductions in leaf biomass (14%), SLA (17%) and LAI (27%) were recorded, although forage yield was similar between soil N conditions. Under shade, peaks of A were comparable to those at full light, so A/Q was higher around midday. Derived parameters of the A-PPF curves were similar between S0 and S2. A maximum fPPF = 0.8 (S0N+, S1N+) was recorded at LAI = 3–4. Under limited sunlight, relatively high RUE (1.6–2.8 g MJ<sup>-1</sup>) were observed over both soil N conditions. We concluded that *B. decumbens* had a high

plasticity to shade, thus explaining its success under silvopastoral systems.

**Keywords:** signalgrass, shade, biomass accumulation, apparent quantum yield, radiation use efficiency

## Introduction

In Venezuela, natural savanna and forest ecosystems north of the Orinoco river are under continuous replacement by agroecosystems such as annual crops and cultivated pastures. African C<sub>4</sub> grasses like *Brachiaria spp.*, with a high growth potential under local climate and soil conditions, are commonly used to provide high-quality forage in well-drained savannas and partially deforested lands.

Nowadays, many natural forests and savanna-forest mixtures are managed as silvopastoral systems, where trees are present as isolated individuals or as patches of different densities. African forage grasses introduced in these lowland tropical environments, where trees are either naturally present or intentionally sown to expand plantation agriculture or to recover degraded land, can be exposed to a large reduction in available photosynthetically active radiation. The concomitant reduction in plant productivity has important implications in terms of selecting valuable grass species with high tolerance to shade (Humphreys, 1991, 1994; Wilson and Ludlow, 1991).

*Brachiaria decumbens* Stapf. (Signalgrass) is widely used throughout the Neotropics (Argel and Keller-Grein, 1998; Pizarro *et al.*, 1998), Australia and SouthEast Asia (Stür *et al.*, 1998). In Venezuela, approximately 1.6–2.7 × 10<sup>6</sup> ha have been sown with this high-quality forage grass, representing approximately half of the existing land (including savanna and seasonal tropical

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forests) converted to improved pastures (Pizarro *et al.*, 1998).

Differences in solar radiation interception caused by either natural or artificial shade affect the microclimate where pasture grows. Under such conditions, light intensity and air temperature are directly reduced, while improved soil moisture and related organic matter mineralization processes may contribute to enhance nutrient uptake and hence forage quality (Cruz *et al.*, 1995; Ludwig *et al.*, 2004).

Several traits at different scales can be modified under the reduced solar radiation level at which plants are acclimatized. Thus, phenotypic plasticity allows for the adjustment of various physiological, anatomical and morphological characters so plants can compete more efficiently and survive under restricted light conditions (Givnish, 1988). At the leaf level, acclimation to shade implies less supporting tissue and a lower number of mesophyll cells per unit area, so they are thinner [i.e. have a higher specific leaf area (SLA)] and consequently tend to have a lower photosynthetic capacity than sun-acclimated leaves. Shade-acclimated leaves compensate for this reduction in available light by increasing chlorophyll concentration per unit of leaf mass and allocating relatively more of this pigment into the light-harvesting complexes of the chloroplast, resulting in a lower chl<sub>a</sub>/chl<sub>b</sub> ratio (Pearcy and Sims, 1994; Lambers *et al.*, 2008). Typical acclimation of *B. decumbens* to shade is expressed by a large increase in SLA and carbon allocation to leaf biomass (Baruch and Guenni, 2007; Guenni *et al.*, 2008), although total chlorophyll content per unit leaf dry mass and the chl<sub>a</sub>/chl<sub>b</sub> ratio do not vary accordingly with decreasing light intensity (Baruch and Guenni, 2007).

At the plant level, *B. decumbens* was the only species that maintained active growth under 40% of full light intensity and monthly defoliation, when compared with *B. brizantha* and *B. dictyoneura* (Baruch and Guenni, 2007). In addition, plants of *B. decumbens*, cultivated at 57% light and low soil nitrogen, produced the same total biomass as those grown at full light (Guenni *et al.*, 2008). Here, the proportion of leaf N showed a steady increase as irradiance intensity decreased to as low as 29% under artificial shade. Reduced radiation affected more the growth of young plants of *B. decumbens* than soil nitrogen availability. On the contrary, after regrowth, older plants demanded more nutrients, so biomass accumulation was more controlled by the interaction of shade × N fertilization (Guenni *et al.*, 2008).

Regarding the effects of shade on pasture dry matter accumulation and nutritive value, the literature reports only few high-yielding tropical forage grasses with the potential to grow and persist when grazed under shade. Among them, *B. decumbens*, *B. brizantha*,

*B. humidicola* and *Panicum maximum* (Guinea grass) showed intermediate tolerance to shade (Shelton *et al.*, 1987). An increase in N and K concentration in the forage biomass was reported for *B. decumbens* and *B. brizantha* when grown under native legume trees (Carvalho, 1997). Under coconut plantations in the tropics, *B. decumbens* cv. Basilisk showed a higher dry matter yield than *B. humidicola* (Kaligis and Sumolang, 1991; Rika *et al.*, 1991), whereas no improvement in biomass production was observed in *B. decumbens* and other C<sub>4</sub> grasses growing at the same environment (Smith and Whiteman, 1983).

We studied the acclimation potential of *B. decumbens* to grow under differing light regimes and soil nitrogen availability, in order to quantify several plant traits responsible for its persistence in silvopastoral systems. The study area consisted of a semi-evergreen forest which has been partially cleared to establish paddocks of *P. maximum*, *Digitaria swazilandensis*, *B. decumbens*, *B. mutica* and *B. arrecta*. These grasses are currently cultivated under different densities of tree canopies dominated by 'samán' [*Samanea saman* (Jacq.) Merrill], and paddocks are rotationally grazed by cattle (Hernández and Guenni, 2008).

## Materials and methods

The experiment was conducted at 'La Antonia' experimental station of the Veterinary Sciences Faculty, Universidad Central de Venezuela (10°22'28"N, 68°40'45"W; 110 m asl) during the growing season (April–December) of 2006.

Meteorological data were obtained from a nearby station (Naranjal Agroclimatological Station-DANAC Foundation; 10°21'45"N, 68°39'00"W; 107 m asl). Mean annual rainfall and potential evapotranspiration are 1480 and 1720 mm respectively (1988–2006). Normally, the rainy period extends from April to December, with almost 90% of total rainfall whereas the dry period (January–March) usually has ≤ 50 mm month<sup>-1</sup>. Fluctuations between the minimum and maximum mean temperature are usually within the range 20–31°C.

The soil profile has textures varying from sandy-loamy (0.0–0.2 m) to sandy-clay-loamy (0.2–0.4 m) with organic matter decreasing from 1.9 to 0.9%. Soil pH is 4.9–6.1, and electrical conductivity ranges from 0.04 to 0.12 dS m<sup>-1</sup>, whereas P, K, Ca and Mg concentrations are 7, 4, 136 and 90 mg kg<sup>-1</sup> respectively.

A relatively flat treeless and fenced area (50 × 15 m) with a 4-year-old stand of *B. decumbens* (>90% cover) was selected for this study. Immediately after a uniform cut, three regimes of shade were imposed by the use of polyethylene (black) shade cloths of two different openings (2 × 3 and 4.5 × 7 mm ovals; TRICAL<sup>®</sup> de Venezuela C.A.,

Maracay, Aragua, Venezuela). The cloths were mounted on metal frames 0.9 m above ground and were oriented east to west to reduce shading to adjacent plots. They had also a 1.10 m overhang at their distal ends to prevent direct access of sunlight at sunrise and sunset. No lateral overhanging was set to allow easy wind circulation, thus preventing excessive heating beneath the cloths. Daily integrals of photosynthetic photon flux (PPF) at pasture height resulted in the following light regimes: 100% light (S0), 50% (S1) and 30% (S2).

Treatments ( $n = 3$ ) were arranged under a split-plot design, with three light regimes as main plots (12 m long  $\times$  1.8 m wide) and two levels of nitrogen (N) fertilization: with N (N+) and without N (N-) as sub-plots (5.5 m long  $\times$  1.8 m wide). Main plots were separated 2 m from each other. For a 6-month period prior the beginning of the experiment, all plots were mown every 6–8 weeks to 0.05–0.1 m high. At the beginning of that 6-month period, a complete formula of N:P:K (12:24:12) fertilizer was applied on each plot at a rate of 5:10:5 kg ha<sup>-1</sup>. Afterwards, with each uniformity cut, N (as urea) was added to N+ stands at a rate of 70 kg ha<sup>-1</sup>. Once the experiment started, the amount of N to be added to each N+ plot (50 kg ha<sup>-1</sup>) was fractioned in three equal doses applied at days 0, 14 and 28 to ensure high N availability for the N+ stands throughout the measured regrowth period (RP).

All plant and soil measurements were recorded weekly during the 6 weeks of the RP, which was established at the end of the rainy season (26 October–7 December 2006). On day 0 of RP, the pasture was mown to a height of 0.05–0.1 m, and the harvested material removed from the plots. Gravimetric soil water content (0–0.1 m) was only measured in the fertilized sub-plots of S0, S1 and S2. At weekly intervals, a total of three paired readings measurements of incident radiation (PPF<sub>i</sub>, above sward canopy) and transmitted radiation (PPF<sub>t</sub>, at sward cut level) were made within each sub-plot. A 'line quantum sensor' (model LI-191SB; LICOR Inc., Lincoln, NE, USA) was used under a clear sky day to calculate the PPF<sub>t</sub>/PPF<sub>i</sub> ratio or fractional incident radiation around midday. Continuous daily measurements of light within the canopy were not possible, so the PPF<sub>t</sub>/PPF<sub>i</sub> ratio obtained at a particular sampling date was taken as the mean light transmittance ( $T$ ) for the whole week. Three light-related parameters (Guenni *et al.*, 2005) were estimated along RP: (i) the light extinction coefficient within the leaf canopy ( $k$ ), derived from the relationship  $T = a \exp^{(-k)LAI}$  and representing an index of light interception efficiency, (ii) the fractional intercepted photosynthetic photon flux (fPPF), where  $fPPF = 0.96(1 - T)$  (the value 0.96 accounts for the proportion of light reflected by soil type, according to

Sinoquet and Cruz, 1993) and (iii) the radiation use efficiency (RUE): the slope ( $b$ ) between accumulated above-ground biomass ( $Y$ ) and the amount of cumulative fPPF ( $X$ ). RUE was obtained by the equation  $Y = a + bX$ . Total amount of intercepted PPF (MJ m<sup>-2</sup>) across harvests was calculated from the absolute incident short wave radiation ( $R_a$ ) values obtained from meteorological data, so  $X = 0.48R_a fPPF$ . The constant 0.48 is used to convert  $R_a$  into PPF values (Sinclair and Muchow, 1999).

Once PPF<sub>t</sub>/PPF<sub>i</sub> was recorded, a new area of 0.25  $\times$  0.5 m<sup>2</sup> each was selected every week for dry matter yield determination. All plant material 0.05–0.1 m above ground was harvested and weighed. Afterwards, a sample was randomly selected and separated into leaf (leaf lamina), stem (stem + leaf sheath) and dead material. A subsample of fresh green leaves was selected at each harvest to measure leaf area with a leaf area metre (model CI-202; CID Bio-Science, Inc., Camas, WA, USA), before all plant components were oven-dried at 60°C. The SLA (cm<sup>2</sup> g<sup>-1</sup>) and leaf area index (LAI: SLA  $\times$  total green leaf weight per unit ground) were then calculated from leaf area (LA) and dry weight measurements.

Plant samples were also dried and ground (0.2 mm mesh) to determine the concentration of total N (Kjeldahl digestion) and K (spectrophotometric atomic absorption technique). Determinations of N and K fractions in plant material could only be performed on S0 and S1. Total N and K contents in the forage biomass (g m<sup>-2</sup>) were calculated as forage dry weight  $\times$  nutrient concentration. Possible changes in the N nutritional level of the pasture induced by the interaction shade  $\times$  fertilization were investigated by initially plotting the relationship between total N uptake by the aerial biomass ( $y$ , kg ha<sup>-1</sup>) and forage dry matter accumulation ( $x$ , t ha<sup>-1</sup>) along RP. The resulting curves for the different light  $\times$  soil N combinations (in this case only S0 and S1 were included in the analysis) were compared against the theoretical curve (i.e. the critical evolution of N uptake to achieve maximum crop growth rate) predicted for a C<sub>4</sub> grass stand during vegetative growth: N uptake (kg ha<sup>-1</sup>) =  $10\alpha (DM)^{1-\beta}$ , where  $\alpha = 3.6$ ,  $\beta = 0.34$  and DM is shoot dry weight in t ha<sup>-1</sup> (Cruz, 1995, 1997). The coefficient  $10\alpha$  corresponds to the crop N uptake when N is expressed in kg ha<sup>-1</sup> and depends on the steady-state N supply of the crop. The value of  $1 - \beta$  is the ratio between the relative crop N uptake rate and crop relative growth rate (Lemaire and Gastal, 2009). A high degree of similarity between the resulting curve obtained from experimental data and that predicted by the previous model implies an optimal N nutrition level for the grass stand being studied (Cruz, 1997; Guenni *et al.*, 2005).

Daily cycles of leaf water potential ( $\Psi_l$ ) and net  $\text{CO}_2$  exchange ( $A$ ) could only be carried out on fertilized sub-plots. Young fully expanded leaves per plot ( $n = 3-4$ ) were selected, and  $\Psi_l$  and  $A$  were measured at days 21 and 34 with a pressure chamber (model 3005; SoilMoisture Equipment Corp., Goleta, CA, USA) and a portable IRGA (model Lci, ADC BioScientific Ltd., Hoddesdon (Herts), UK) coupled to a leaf chamber (model LCA2, ADC BioScientific Ltd.) respectively. Net photosynthesis measurements on fertilized plots were also carried out at day 42 of a second RP. Although all treatments could not be compared, yet by measuring  $A$  on fertilized S0, S1 and S2 stands, potential shade effects were maximized and negative effects of soil N deficiency were minimized. With paired data of  $A$  and PPF at leaf level, daily variations in the instantaneous light use efficiency ( $A/\text{PPF}$ ) were calculated. Additionally, to fully contrast low and high irradiance effects on net  $\text{CO}_2$  exchange, the photosynthetic light response of individual leaves was assessed on fertilized S0 and S2 stands. The photosynthesis ( $A$ )–light curves were obtained from a 40-day-old pasture and during a clear day, between 11:00 and 13:00 h. Recently expanded leaves ( $n = 3$ ) were chosen in each case. Different PPF intensities reaching the leaf were obtained by increasing the number of layers of a particular shade cloth over the leaf chamber (Dias-Filho, 2002). The light response curves were analysed with the classical four-parameter non-rectangular hyperbola (Marshall and Biscoe, 1980; Charles-Edwards, 1981, 1982). The leaf photosynthetic parameters are summarized in Lambers *et al.* (2008, p. 27). The mathematical model has been recently applied to compare the performance of sun and shade-acclimatized leaves of tropical and temperate forage grasses (Dias-Filho, 2002; Peri *et al.*, 2007). The shape of the light response curve is based on the following equation:

$$A = \frac{\theta \cdot I + A_{\max} - \sqrt{\{(\theta \cdot I + A_{\max})^2 - 4 \cdot \theta \cdot I \cdot A_{\max}\}}}{2\theta} - R_d$$

where  $A_{\max}$  is the light-saturated rate of gross photosynthesis at ambient  $\text{CO}_2$ ,  $\theta$  is the apparent quantum yield based on incident PPF ( $I$ ),  $\theta$  is an index of curve convexity, with a 0–1 range ( $\theta = 0$  results in a rectangular hyperbola,  $\theta \ll 1$  implies a non-rectangular hyperbola) and finally  $R_d$  refers to the leaf dark respiration. For curve-fitting purposes, initial  $A_{\max}$  values (i.e. 36.0 and 29.4  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for S0 and S2 respectively) were provided by the  $A$  vs. PPF relationship obtained from the field. The input value of  $\theta$  was calculated as the slope of the curve between 100 and 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of PPF. As  $R_d$  was not measured in

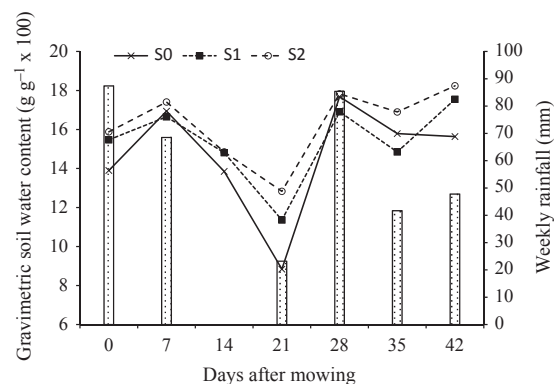
this study, initial values of 3.5 and 2.4  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for S0 and S2, respectively, were taken from the data reported by Dias-Filho (2002) on two related species: *B. brizantha* and *B. humidicola*.

Field data were initially analysed according to the SAS' ANOVA procedure for a split-plot design. Light intensity and soil nitrogen were the main and secondary factors respectively. Subsequently, at each sampling date, data were analysed in a two-way ANOVA (SAS Institute, 1989). Mean comparisons among treatments were carried out by the Duncan's test at  $P = 0.05$ . The UNIVARIATE procedure (SAS Institute, 1989) was used for testing data normality. Under non-normality conditions, data were  $\log_{10}(x + 1)$  or arcsine transformed. Otherwise, data were analysed by the Friedman's two-way non-parametric ANOVA (SAS Institute, 1989).

The fitting of the net leaf photosynthesis ( $A$ ) vs. incident PPF ( $I$ ) data to the corresponding non-rectangular hyperbola (previous equation) was performed using the NLME method of R statistical package (R Development Core Team, 2008). Differences in light responses between fertilized S0 and S2 were analysed by non-parametric statistic (Wilcoxon rank sum test). Statistical comparisons of both data sets were made on the fitted parameters: light compensation point,  $\theta$ , and  $R_d$ . The SAS-NLIN procedure was used to fit fPPF vs. time data to a second-order polynomial equation.

## Results

Throughout the experiment, maximum variation in daily temperature was within the range 21.2–36.8°C, with a total rainfall of 267 mm distributed according to Figure 1.



**Figure 1** Total weekly rainfall (bars) and soil water content at 0–0.1 m (lines) by the time of harvesting in stands of *Brachiaria decumbens* under different photosynthetic photon flux intensities. S0: 100%, S1: 50% and S2: 30%. Soil water data are means of six replicates over fertilized (N+) conditions.

Gravimetric soil water content (0–0.1 m) on fertilized plots showed the same trend among light regimes (Figure 1), decreasing from 17% (day 7) to nearly 12% (day 21), because of no rainfall on week 2. Minimal soil water contents were recorded 21 d after mowing, varying from 8% (S0) to 14% (S2) ( $P = 0.05$ ). From day 21 onwards, soil water contents were higher owing to increased rainfall, so final values at the end of the experiment were in the range 16% (S0) to 18% (S2).

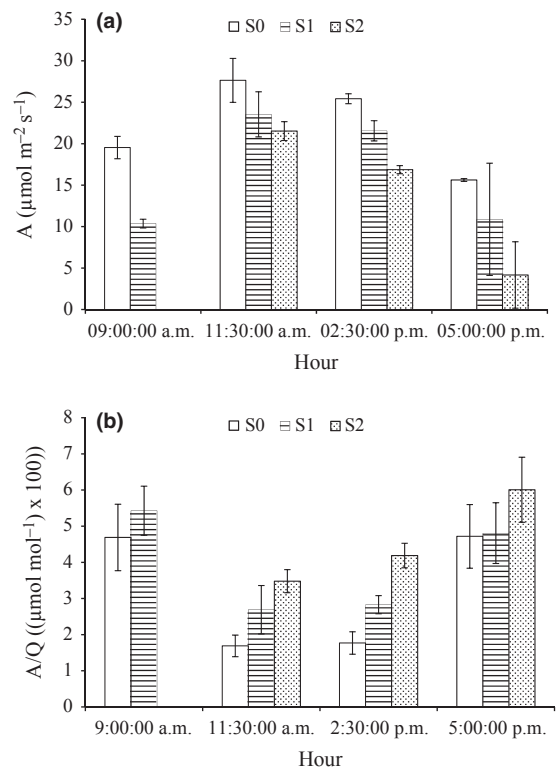
Leaf water potential ( $\Psi_l$ ) varied according to incident solar radiation during the day, with a negative peak ( $-1.12 \pm 0.11$  MPa) from midday to mid-afternoon (*ca.* 12:00–15:00 h) and higher (less negative) values ( $-0.62 \pm 0.16$  MPa) early in the morning and late afternoon. After 21 d of regrowth, midday  $\Psi_l$  was slightly lower ( $-1.24 \pm 0.02$  MPa) under S0 than S1 ( $-1.12 \pm 0.02$  MPa; data not shown). Such differences were maintained at day 34. However, in all cases,  $\Psi_l$  variations throughout the day did not exceed 0.5 MPa.

### Leaf photosynthetic response to light intensity

After 21 and 35 d of RP, maximum leaf photosynthesis under full sunlight and fertilized conditions was  $33.1 \pm 3.2 \mu\text{mol m}^{-2} \text{s}^{-1}$  soon after sunrise. High  $A$  values above  $25 \mu\text{mol m}^{-2} \text{s}^{-1}$  were maintained until mid-afternoon, with a decrease in  $10.2 \pm 0.8 \mu\text{mol m}^{-2} \text{s}^{-1}$  at 16:00 h (data not shown). In contrast, a peak of  $25.2 \pm 0.7 \mu\text{mol m}^{-2} \text{s}^{-1}$  was recorded for S1 around midday, with minimum values in the range  $4\text{--}11 \mu\text{mol m}^{-2} \text{s}^{-1}$  at early morning and late afternoon. Although leaf net photosynthesis was almost always higher under S0,  $A$  values became much closer to those recorded under S1 around noon. This resulted in comparable or even higher instantaneous light use efficiencies ( $A/\text{PPF}$ ) at 50% sunlight reduction.

The response of individual leaves to light intensity was also studied when  $A$  was measured after 40 d of a subsequent RP under similar soil conditions (N+). A greater decrease in  $A$  was observed in S1 and S2 when compared with S0, although the corresponding decrease in net  $\text{CO}_2$  exchange was not proportional to the reduction in light intensity (Figure 2a). During hours of more evaporative demand,  $A/\text{PPF}$  was however higher in leaves under shade (Figure 2b). For the rest of the day, relatively low reductions in  $A$  under S1 and S2 induced similar net  $\text{CO}_2$  assimilations per unit of incident radiation among treatments.

When analysing the  $A$ -PPF curves (Figure 3), maximum recorded values of net photosynthesis ( $A$ ) were  $27.4$  (S2) and  $32.5$  (S0)  $\mu\text{mol m}^{-2} \text{s}^{-1}$  over a PPF range of  $900\text{--}1900 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The light

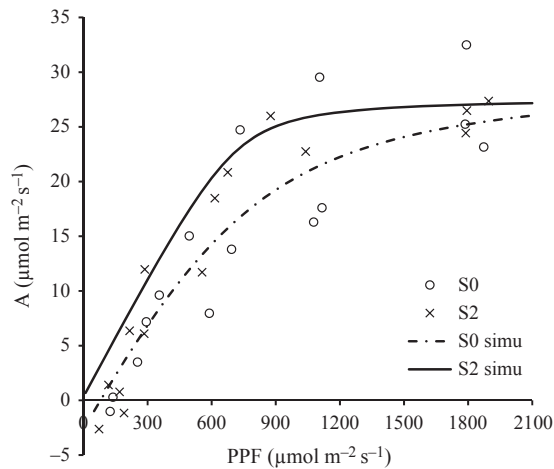


**Figure 2** Daily changes in (a) net photosynthesis ( $A$ ) and (b) instantaneous light use efficiency ( $A/Q$ ) of *Brachiaria decumbens* leaves under different photosynthetic photon flux intensities. S0: 100%, S1: 50% and S2: 30%. Data are means of 9–12 replicates (3–4 leaves per block), and vertical lines on bars are standard deviations of means.

compensation point varied from 13–25 (S0) to 12–31 (S2)  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The apparent quantum yield ( $\Phi$ ) ranges for S0 and S2 were  $22.8 \times 10^{-3}$ – $43.3 \times 10^{-3}$  and  $28.7 \times 10^{-3}$ – $47.0 \times 10^{-3} \text{ mol CO}_2 (\text{mol photon})^{-1}$  respectively. The curve shape factor ( $\Theta$ ) varied between  $0.80 \pm 0.17$  (S0) and  $0.97 \pm 0.03$  (S2). Estimated  $R_d$  values were within the ranges  $1.64\text{--}3.39$  (S2) and  $1.93\text{--}3.97$  (S0)  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . No differences were found between S0 and S2 for any of the evaluated parameters.

### Forage yield, dry matter partitioning, LAI and SLA variations

Until day 21 of RP, total above-ground biomass accumulation was similar ( $\pm 50 \text{ g m}^{-2}$ ) across light and soil N treatments. Once rainfall started, a sustained increase in forage dry weight was observed in all grass stands, although differences among treatments were more marked during the last 2 weeks of RP. SON+



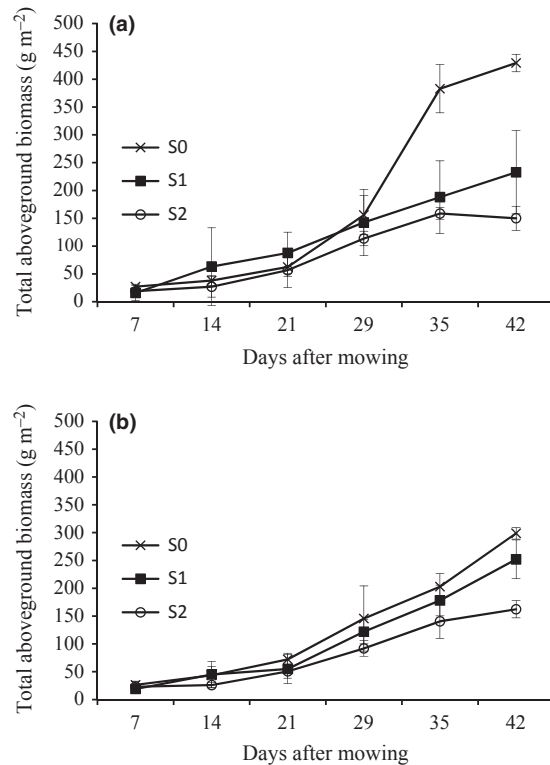
**Figure 3** Photosynthetic response ( $A$ ) to light of leaves of *Brachyaria decumbens* grown under fertilized conditions (+N) and acclimatized to two irradiance intensities: S0 (100% sunlight) and S2 (30% sunlight). The two lines represent simulated values obtained from the theoretical light response curve (Lambers *et al.*, 2008; see Materials and methods).

stands showed the highest ( $429 \text{ g m}^{-2}$ ) and S0N– the lowest dry matter yield ( $162 \text{ g m}^{-2}$ ; Figure 4).

The pattern of leaf and stem biomass accumulation with time was comparable and followed the same trend as for total aerial biomass (data not shown), with a sharp increase in the biomass of both plant components after week 3 of regrowth. Relative contributions of both plant components to total forage yield were not affected by light intensity or N fertilization, so a similar leaf/stem ratio over all treatment combinations was observed, with mean values over light  $\times$  soil N conditions decreasing mainly with sward age: from  $4 \pm 2.0$  (day 14) to  $1.3 \pm 0.2$  (day 42).

At day 35 of RP, the interaction between main effects (i.e. light intensity  $\times$  soil N) on total above-ground biomass was not significant ( $P = 0.31$ ), whereas main effects were significant for light intensity only ( $P = 0.02$ ). As PPF was reduced, a general decrease in the accumulation of above-ground biomass was observed. The highest percentage reduction in total, leaf and stem dry weight was recorded at S2: 47–49% of the biomass yielded at S0 (Table 1). Regardless of soil N condition, shade induced a reduction in dead biomass, with 70–80% decrease at S1 and S2 (Table 1).

Over all light conditions, at day 35, shoot biomass and the dry weight of all plant components were similar between N– and N+ stands (Table 2). At the end of RP (day 42), the light  $\times$  soil N interaction effect was again not significant for total biomass ( $P = 0.23$ ).



**Figure 4** Dry matter yields of fertilized (a) and unfertilized (b) stands of *Brachyaria decumbens* under different photosynthetic photon flux intensities. S0: 100%, S1: 50% and S2: 30%. Data are means of three replicates, and bars are standard deviations of means.

In this case, light intensity was the only main effect to be significant ( $P = 0.01$ ). Dry matter yields ( $\text{g m}^{-2}$ ) from S2 to S0 were within the ranges 156–364, 91–174, 60–167 and 6–23 for total above-ground, leaf, stem and dead biomass respectively (Table 1). Although in the N– plots leaf dry weight was reduced by 14%, final total dry weights were similar for both soil N conditions (Table 2). An increase in dead material biomass (although no significant) may have accounted for leaf dry matter reductions.

Leaf area index increase was proportional to that recorded for leaf dry weight. Accumulated LAI at day 35 ranged from 2.2 (S2) to 3.0 (S0) (Table 1). By the end of the experiment, leaf area production was only influenced by soil N ( $P = 0.04$ ), with LAI varying from 2.4 (N–) to 3.3 (N+) (Table 2). On the other hand, for the last two harvests, variations in SLA were strongly influenced by either PPF or soil N ( $0.0003 < P < 0.05$ ), with no significant interaction between main effects. Consequently, over the two soil N conditions, a clear increase of SLA (i.e. 39–46%) was observed with

**Table 1** Total above-ground, leaf, stem and dead biomass, leaf to stem ratio (L:S), leaf area index (LAI), specific leaf area (SLA) and fractional intercepted radiation (fPPF) of plots of *Brachiaria decumbens* under different PPF intensities. Values are given for the last two harvests (days 35 and 42) of the regrowth period.

PPF intensity	Total, g m <sup>-2</sup>	Leaf, g m <sup>-2</sup>	Stem, g m <sup>-2</sup>	Dead, g m <sup>-2</sup>	L:S, g g <sup>-1</sup>	LAI, m <sup>2</sup> m <sup>-2</sup>	SLA, cm <sup>2</sup> g <sup>-1</sup>	fPPF
Day 35								
S0 (100%)	292.9 <sup>a</sup>	148.2 <sup>a</sup>	127.1 <sup>a</sup>	17.4 <sup>a</sup>	1.2 <sup>a</sup>	3.0 <sup>a</sup>	200.3 <sup>c</sup>	0.79 <sup>a</sup>
S1 (50%)	183.1 <sup>b</sup>	101.7 <sup>b</sup>	76.1 <sup>b</sup>	5.4 <sup>b</sup>	1.4 <sup>a</sup>	2.5 <sup>ab</sup>	243.0 <sup>b</sup>	0.68 <sup>b</sup>
S2 (30%)	149.5 <sup>b</sup>	79.2 <sup>b</sup>	64.7 <sup>b</sup>	3.8 <sup>b</sup>	1.3 <sup>a</sup>	2.2 <sup>b</sup>	278.4 <sup>a</sup>	0.58 <sup>c</sup>
% variation*	-49	-47	-49	-78	+8	-30	+39	-27
Day 42								
S0 (100%)	364.0 <sup>a</sup>	174.2 <sup>a</sup>	166.5 <sup>a</sup>	22.8 <sup>a</sup>	1.1 <sup>a</sup>	3.3 <sup>a</sup>	184.1 <sup>b</sup>	0.76 <sup>a</sup>
S1 (50%)	242.4 <sup>b</sup>	128.1 <sup>b</sup>	108.0 <sup>b</sup>	6.4 <sup>b</sup>	1.2 <sup>a</sup>	2.7 <sup>a</sup>	230.3 <sup>a</sup>	0.64 <sup>b</sup>
S2 (30%)	156.2 <sup>c</sup>	90.7 <sup>c</sup>	60.0 <sup>b</sup>	5.5 <sup>b</sup>	1.6 <sup>a</sup>	2.4 <sup>a</sup>	267.8 <sup>a</sup>	0.50 <sup>c</sup>
% variation*	-57	-48	-64	-76	+46	-27	+46	-34

Significant differences ( $P = 0.05$ ) among PPF intensities are denoted by different letters within each column. \*Maximum variation in each plant trait with respect to S0 is also given.

**Table 2** Total above-ground, leaf, stem and dead biomass, leaf to stem ratio (L:S), leaf area index (LAI), specific leaf area (SLA) and fractional intercepted radiation (fPPF) under fertilized (N+) and non-fertilized (N-) plots of *Brachiaria decumbens*. Values are given for the last two harvests (days 35 and 42) of the regrowth period.

Soil N condition	Total, g m <sup>-2</sup>	Leaf, g m <sup>-2</sup>	Stem, g m <sup>-2</sup>	Dead, g m <sup>-2</sup>	L:S, g g <sup>-1</sup>	LAI, m <sup>2</sup> m <sup>-2</sup>	SLA, cm <sup>2</sup> g <sup>-1</sup>	fPPF
Day 35								
N+	243.1 <sup>a</sup>	129.8 <sup>a</sup>	104.4 <sup>a</sup>	8.8 <sup>a</sup>	1.4 <sup>a</sup>	3.2 <sup>a</sup>	257.8 <sup>a</sup>	0.74 <sup>a</sup>
N-	173.9 <sup>a</sup>	89.5 <sup>a</sup>	74.1 <sup>a</sup>	8.9 <sup>a</sup>	1.2 <sup>a</sup>	2.0 <sup>a</sup>	223.4 <sup>b</sup>	0.63 <sup>a</sup>
Day 42								
N+	270.7 <sup>a</sup>	141.1 <sup>a</sup>	119.8 <sup>a</sup>	9.7 <sup>b</sup>	1.3 <sup>a</sup>	3.3 <sup>a</sup>	247.7 <sup>a</sup>	0.64 <sup>a</sup>
N-	237.7 <sup>a</sup>	120.8 <sup>b</sup>	103.2 <sup>a</sup>	13.4 <sup>b</sup>	1.3 <sup>a</sup>	2.4 <sup>b</sup>	207.1 <sup>b</sup>	0.62 <sup>a</sup>

Significant differences ( $P = 0.05$ ) between soil N conditions are denoted by different letters within each column.

decreasing light intensity, with final values (day 42) within the range 184.1 (S0)–267.8 (S2) cm<sup>2</sup> g<sup>-1</sup> (Table 1). At the end of the experiment, SLA was observed to be higher at the fertilized (247.7 cm<sup>2</sup> g<sup>-1</sup>) than unfertilized (207.1 cm<sup>2</sup> g<sup>-1</sup>) stands, representing an increment of around 20% (Table 2).

### Nutrient content and accumulation in the forage biomass

Similar N and K concentrations in forage tissue were observed for S0 and S1 along RP (data not shown), and this was independent of soil condition. A general increase in nutrient concentration was observed from days 7 to 21 of regrowth, with a subsequent gradual decrease in both elements. This pattern was more obvious for K than N. The mean fractions of these

elements decreased from a maximum of  $3.27 \pm 0.32\%$  (day 21) to a minimum of  $1.71 \pm 0.32\%$  (day 42) and from  $2.28 \pm 0.38\%$  (day 21) to  $1.44 \pm 0.29\%$  (day 42) for K and N respectively.

Over the two light regimes, forage N concentration during the last 2 weeks of regrowth were higher ( $P = 0.05$ ) under fertilized plots than unfertilized plots; mean values ranged from  $1.38 \pm 0.03\%$  (N-) to  $2.03 \pm 0.002\%$  (N+) and from  $1.24 \pm 0.28\%$  (N-) to  $1.64 \pm 0.10\%$  (N+) at days 35 and 42 respectively. Forage K concentration was similar between soil N conditions at day 35 ( $2.22 \pm 0.17\%$ ) and higher ( $P = 0.05$ ) under N+ stands ( $1.88 \pm 0.23\%$ ) than N- stands ( $1.44 \pm 0.09\%$ ) at day 42.

When compared with the theoretical curve for a C<sub>4</sub> grass, in all treatments the N nutritional level of the pasture sward was lower than expected. However, the

related curves for S1N+ and especially S0N+ were closer to the predicted pattern of N accumulation in the forage biomass (Figure 5).

### Efficiency of solar radiation interception and use

Mean  $k$  values (i.e. decrease in fractional incident PPF per LAI unit) varied from 0.25 (S1N+) to 0.49 (S0N–), with no differences among irradiances and between soil N conditions. The highest proportion of fractional intercepted PPF ( $S$ ) was recorded at days 35 and 42, without any significant interaction between light intensity and soil N. At day 35,  $S$  increased ( $P = 0.05$ ) from 0.58 (S2) to 0.79 (S0). At final harvest (day 42),  $S$  values ranged in the increasing order ( $P = 0.05$ ): 0.50 (S2) < 0.64 (S1) < 0.76 (S0). At both days, no differences were found between soil N conditions, with  $S$  ranging between 0.62 and 0.74. Maximum  $S$  values (i.e. 0.70–0.80) were reached around LAI = 3.5 (S1N+, S0N+; Figure 6).

Radiation use efficiency values among the different grass stands are shown in Table 3. In this case, the interaction light intensity  $\times$  soil N condition was highly significant ( $P = 0.01$ ), so light intensity effects on RUE were dependant on soil fertility, and differences caused by soil N conditions were relative to light intensity. On fertilized (N+) stands, RUE showed a clear decrease with decreasing light intensity, with a maximum value of 4.1 g MJ<sup>-1</sup> at S0 and a minimum of 1.6 g MJ<sup>-1</sup> at S2. At N– conditions, light intensity did not modify RUE, which had a mean of 2.5  $\pm$  0.3 g MJ<sup>-1</sup> over light treatments. In S0 stands, RUE was reduced from 4.1 (N+) to 2.7 g MJ<sup>-1</sup> (N–) (34% decrease), whereas the opposite effect was observed at S2 stands, with an increase of 38%

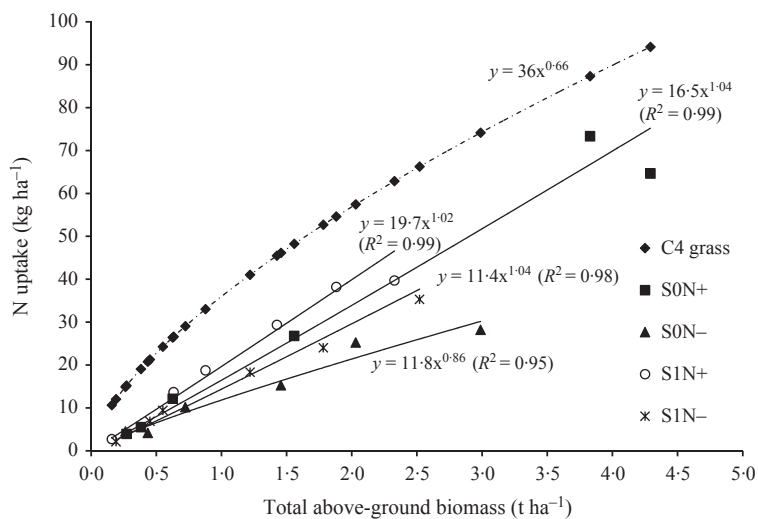
(1.6–2.2 g MJ<sup>-1</sup>) in RUE from N+ to N– condition. At 50% light reduction (S1), RUE did not differ between N+ and N– stands and had a mean value of 2.5  $\pm$  0.4 g MJ<sup>-1</sup> (Table 3).

## Discussion

### Responses at leaf level

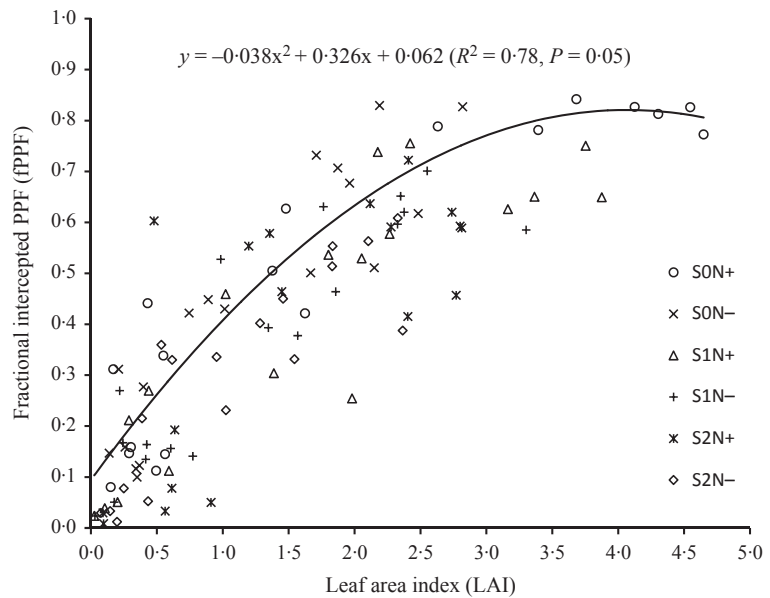
Plant acclimation under restricted irradiance implies a substantial carbon investment in leaf area expansion for light capture. Leaves of *B. decumbens* acclimated to artificial shade showed several traits that differed from those that grew under full daylight. Shade-induced changes in biomass allocation (measured by the leaf to stem ratio) were less important than those observed in SLA, as reported previously in other tropical grasses (Baruch and Guenni, 2007; Paciullo *et al.*, 2007; Guenni *et al.*, 2008; Hernández and Guenni, 2008).

Increased SLA as irradiance decreases is common in shade-acclimated leaves (Humphreys, 1994; Cruz *et al.*, 1999; Walters, 2005; Peri *et al.*, 2007). This response has been morphologically associated with thinner, longer and wider leaves (De Castro *et al.*, 1999; Peri *et al.*, 2007; Guenni *et al.*, 2008) and anatomically with thinner epidermis, and smaller cell size and lower number of cells in the mesophyll (Walters, 2005; Peri *et al.*, 2007). Thinner leaves may imply a lower construction cost of leaf area, which compensates for a reduction in light availability, although with the consequent cost in water loss. However, no apparent water stress was observed in shaded leaves throughout the experiment, and this may have been assisted by a reduction in stomatal density with shade (Walters, 2005).



**Figure 5** Nitrogen uptake (kg ha<sup>-1</sup>) by stands of *Brachiaria decumbens* as a function of total above-ground biomass accumulation (t ha<sup>-1</sup>) along a regrowth period of 42 days. S0: 100% photosynthetic photon flux (PPF), S1: 50% PPF, N+: nitrogen-fertilized plots and N–: non-fertilized plots. The optimal relationship (dashed line) for a C<sub>4</sub> plant (Lemaire and Gastal, 2009) is also shown for comparison.





**Figure 6** Fractional intercepted photosynthetic photon flux (fPPF) of stands of *Brachiaria decumbens* as a function of leaf area index (LAI) over all light and soil N treatments. Data were fitted with a quadratic function.

**Table 3** Variation in radiation use efficiency (RUE,  $\text{g MJ}^{-1}$ ) in *Brachiaria decumbens* under different photosynthetic photon flux (PPF) intensities and soil N conditions (+N: with fertilization, -N: without fertilization).

Soil N condition	PPF intensity		
	S0 (100%)	S1 (50%)	S2 (30%)
N+	4.09 <sup>aA</sup>	2.16 <sup>bA</sup>	1.59 <sup>bB</sup>
N-	2.68 <sup>aB</sup>	2.76 <sup>aA</sup>	2.18 <sup>aA</sup>

Different small and capital letters denote significant differences ( $P = 0.05$ ) among light intensities within soil N conditions and between soil N conditions at a particular light intensity respectively.

Assuming that leaf anatomical structure and hence  $\text{CO}_2$  mesophyll conductance were not highly modified in this  $\text{C}_4$  grass under decreased irradiance (Sage and McKown, 2006), differences in instantaneous light use efficiency of leaves from contrasting irradiance environments may be rather caused by changes in the photosynthetic apparatus activity.

Although the measured leaf N levels in fertilized stands may still indicate suboptimal N nutrition (Cruz, 1997), the regression line for S1 (50% reduction in sunlight) was slightly closer to the theoretical curve than that of S0 (full sunlight). Additionally, leaf N concentration was comparable between S0 and S1. Consequently, a rather similar net  $\text{CO}_2$  exchange and equivalent or even higher A/PPF ratios around midday may be associated with shade-acclimated leaves having more leaf N being invested in the light-harvesting

complexes, so the apparent quantum yield ( $\Phi$ ) can be maximized (Terashima and Hikosaka, 1995; Lambers *et al.*, 2008). In glasshouse conditions, the potential efficiency of chloroplast photosystem II (PSII) of *B. decumbens* remained unaltered at 70% PPF, although it was markedly affected at 40% PPF (Baruch and Guenni, 2007). Although the potential efficiency of PSII was not measured in this study, a high plasticity in the light-harvesting system functioning is postulated, and this may be supported by the fact that even at 70% reduction of sunlight (S2),  $\Phi$  (derived from the light response curve) was not reduced. Only the shape of this curve was altered by PPF reduction, with the photosynthetic rate of 30% PPF (S2) leaves being apparently higher at low irradiance (Figure 3), probably because of their lower respiration rates (Lambers *et al.*, 2008), although not confirmed with the present results. Likewise, the transition from the light-limited part to the light-saturated level was relatively less gradual in 'shade-leaves' ( $\Phi = 0.97$ ) than 'sun-leaves' ( $\Phi = 0.80$ ) (Lambers *et al.*, 2008). In addition, net  $\text{CO}_2$  exchange of shaded leaves was saturated at  $900\text{--}1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ , in comparison with  $>1500 \mu\text{mol m}^{-2} \text{s}^{-1}$  for full sunlight leaves, which may show a stronger carboxylation limitation under severe shade (30% sunlight). At similar PPF intensities, equivalent results were obtained by Dias-Filho (2002) in two related species (*B. humidicola* and *B. brizantha*): light-saturated leaf photosynthesis (Amax) tended to be higher in sun-acclimated leaves, and  $\Phi$  was unaffected by the light regime imposed during growth, the last being apparently a common response (Terashima and Hikosaka, 1995). Reductions in the

light compensation point are expected to be a consequence of lower  $R_d$ , so at least upper leaves in the canopy can sustain a positive C gain at low irradiance (Lambers *et al.*, 2008). Although in this field study  $R_d$  was not recorded, the fitted curves of Figure 3 suggested no changes in this parameter from 100 to 30% sunlight. On the contrary, dark respiration was found by Dias-Filho (2002), as well as by Ludlow and Wilson (1971) in *P. maximum*, to be lower at 30% sunlight. Additionally, in this study, the light compensation point was apparently not modified at low irradiance, with predicted values within the range 12–31  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . These potential values are lower than those (37–70  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) reported by Dias-Filho (2002) for *B. humidicola* and *B. brizantha*. For further assessment of the potential for leaf photosynthetic acclimation to low light intensities, direct measurements of these parameters are then required.

### Responses at canopy and stand level

To maximize light capture in herbaceous stands growing under shade, LAI may be increased, maintained and/or leaf arrangement and inclination modified with height, so the canopy photosynthesis/respiration ratio is optimized (Givnish, 1988; Wilson and Ludlow, 1991).

One way of having higher LAI is by more biomass allocation to leaves. Here, both components of forage yield were equally affected by shade, so the leaf:stem ratio was not altered. Baruch and Guenni (2007) reported similar results in three *Brachiaria* species, including *B. decumbens*.

Maximum LAIs measured here are comparable with those reported previously for several *Brachiaris* under full sunlight (Guenni *et al.*, 2005) or in silvopastoral systems (Hernández and Guenni, 2008). Acclimation capacity of *B. decumbens* to restricted sunlight was evident, as LA expansion was not affected even at 70% PPF reduction at the field. Comparable LAIs under contrasting irradiances may be the result of compensatory effects between tiller density and LA/tiller, with fewer tillers at low irradiance having larger LA. Although tiller density was not measured in this study, this similar LAI response was partially attributed to a trade-off between carbon partitioning to leaf biomass and the metabolic cost for leaf area construction. Here, leaf biomass reduction under decreasing light intensity was compensated by approximately 45% increase in SLA. Assimilates required for expanding laminae seemed to be produced at equivalent rates under all light regimes, confirming early reports on small or moderate effects of shade on leaf area (Squire, 1990). However, in silvopastoral systems, other limiting factors like water stress can alter the

effect of tree canopies on grass LA development (Hernández and Guenni, 2008).

As shade had relatively lesser effects on leaf area expansion than on biomass accumulation, it was expected that the observed higher level of N nutrition in fertilized stands would reflect a greater increase in final LAI and hence dry matter yield. This was particularly the case at full sunlight, where added N induced an improvement of N uptake per unit of stand biomass, thus allowing a higher shoot N concentration and a larger LA accumulation along the RP. Accordingly, total forage biomass accumulation was the highest in this case. Because the observed effects of irradiance and soil N on dry matter yield were independent, total accumulated above-ground biomass was then proportional to either the amount of sunlight intercepted during regrowth or the level of N availability.

Under non-limiting situations, biomass accumulation is largely determined by the interaction between fractional intercepted PPF and canopy RUE (Charles-Edwards, 1982; Squire, 1990; Stöckle and Kemanian, 2009). Light interception is highly dependent on LAI and  $k$ , the latter being a function of leaf insertion angle and leaf distribution with height. The  $k$  values obtained in this study (i.e. 0.3–0.5) correspond in general to canopies with more erect leaves (Nobel *et al.*, 1993; Terashima and Hikosaka, 1995). As a result, relatively similar patterns of light extinction within canopies exposed to contrasting radiation intensities and soil N conditions may rather suggest little plasticity in canopy structure (i.e. low variation in LA distribution and/or leaf arrangement with height). Therefore, in this case, the degree of light interception appears to be only a function of the amount of LA accumulated during regrowth, because a common and significant quadratic correlation ( $R^2 = 0.78$ ,  $P = 0.05$ ) was obtained when  $S$  was regressed against LAI over all treatments (Figure 6). Regardless of soil N condition, maximum  $S$  values were always associated with full sun or 50% PPF regimes.  $S$  values in this study were equivalent to those (0.6–0.8) found in five *Brachiaria* species after 35 d of regrowth at the field (Guenni *et al.*, 2005). An  $S$  value around 0.8 has been considered a critical fraction for maximum N accumulation in stands of different cereals and grain legumes (Giunta *et al.*, 2009).

Variation in dry matter yield was also closely related to differences in RUE among grass stands. Sun-exposed and fertilized stands (SON+) with the highest accumulated biomass (429  $\text{g m}^{-2}$ ) showed the maximum calculated RUE (4.1  $\text{g MJ}^{-1}$ ), which was well above the range obtained for other grass stands (1.9–2.9  $\text{g MJ}^{-1}$ ) and related values reported elsewhere (Guenni *et al.*, 2005; Hernández and Guenni, 2008; Giunta *et al.*, 2009; Stöckle and Kemanian, 2009), but

similar to the maximum estimated for  $C_4$  grasses (Squire, 1990; Cruz, 1996). Under 30% PPFD and fertilized stands (S2N+), the lowest RUE ( $1.6 \text{ g MJ}^{-1}$ ) was observed, with the corresponding lowest dry matter yield of  $150 \text{ g m}^{-2}$ . The narrower range of variation in RUE among the other grass stands may point out a rather positive effect of shade on N nutrition and an effective compensatory response of this grass to restricted radiation.

The observed high radiation use efficiency for fertilized stands is expected as RUE is highly dependent on leaf N (Sinclair and Horie, 1989; Cruz, 1995; Cruz *et al.*, 1999). Mean forage N concentrations along the RP were within the range 1.7–2.5%, which are close to the critical value ( $>2\%$ ) for  $C_4$  plants to maximize yield (Lemaire *et al.*, 1989; Greenwood *et al.*, 1990). As mentioned previously, when compared with the theoretical pattern for a  $C_4$  plant, the observed N uptake by SON+ stands showed a lower N accumulation per unit of accumulated forage biomass. Nonetheless, a fractional radiation interception and RUE close to the potential may rather indicate non-limiting conditions for canopy photosynthesis and hence biomass production in the full sunlight and fertilized plots. Therefore, as suggested elsewhere (Guenni *et al.*, 2005), maximum forage yields in *B. decumbens* may be achieved with relatively lower soil N requirements.

### Integrated responses to optimize light capture and use under shade

Maximum efficiency of light and N use under restricted irradiance is expected to be achieved if every component (i.e. leaf) of the whole grass stand photosynthesizes at its potential rate (Givnish, 1988; Terashima and Hikosaka, 1995). Plants of *B. decumbens* compensated efficiently for low irradiance by primarily increasing SLA and minimizing the cost of tissue construction. Secondly, and not less interestingly, under restricted light environments, recently expanded leaves were capable of maintaining their functional integrity for photosynthesis, so light use efficiency was maintained and even increased when compared with those developed under full sunlight. Therefore, acclimation responses including anatomical and/or biochemical adjustments to shade are expected to be less pronounced in  $C_4$  plants (Sage and McKown, 2006).

Although 30% PPF did not induce a lower dark respiration and light compensation point, as it would be expected for shade-acclimatized leaves, an improved daily C balance under shade may have been achieved by the observed tendency of having the same light-saturated rate of photosynthesis at lower irradiance.

Light penetration into the canopy under shade was facilitated by maintaining a trend for upright leaves

and thus positive C balances even at lower leaf strata. Consequently, fractional intercepted light should be proportional to the amount of leaf biomass and hence LA production. Leaf dry weight accumulation was in turn a function of soil N nutrition and total intercepted radiation during regrowth. Overall, total intercepted PPF and hence forage biomass production was affected mostly under severe light restrictions (i.e. 70%), which such reductions in irradiance being likely to occur at least during part of the year in most tropical silvopastoral systems (Cruz *et al.*, 1999; Hernández and Guenni, 2008).

In conclusion, although none of the discussed plant traits could improve forage biomass under decreased irradiance, the persistence in *B. decumbens* of relatively high and similar RUE values over N– and N+ conditions and 30–50% sunlight seems to be an important strategy for maximizing light conversion into biomass and hence persistence under shade. *B. decumbens* can be considered a sun-shade generalist (according to Sage and McKown, 2006), being successful in both open pastures and partially shaded habitats of tropical silvopastoral systems.

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### References

- ARGEL P.J. and KELLER-GREIN G. (1998) Experiencia regional con *Brachiaria*: Región de América Tropical – Tierras bajas húmedas. In: Miles J.W., Maass B.L. and do Valle C.B. (eds) *Brachiaria: Biología, Agronomía y Mejoramiento*, pp. 226–246. Cali, Colombia; Campo Grande, Brasil: CIAT – EMBRAPA/CNPQC.
- BARUCH Z. and GUENNI O. (2007) Irradiance and defoliation effects in three species of the forage grass *Brachiaria*. *Tropical Grasslands*, **41**, 269–276.
- CARVALHO M. (1997) Asociación de pasturas con árboles en la región centro sur del Brasil. *Agroforestería en las Américas*, **4**, 5–8.
- CHARLES-EDWARDS D.A. (1981) *The mathematics of photosynthesis and productivity*. London, UK: Academic Press.

- CHARLES-EDWARDS D.A. (1982) *Physiological determinants of crop growth*. North Ryde, NSW, Australia: Academic Press.
- CRUZ P. (1995) Use of the RUE concept for analyzing growth of pure and mixed tropical forage crops. In: Sinoquet H. and Cruz P. (eds) *Ecophysiology of tropical intercropping*, pp. 319–330. Paris, France: INRA.
- CRUZ P. (1996) Growth and nitrogen nutrition of a *Dichanthium aristatum* pasture under shading. *Tropical Grasslands*, **30**, 407–413.
- CRUZ P. (1997) Effect of shade on the growth and mineral nutrition of a C<sub>4</sub> perennial grass under field conditions. *Plant and Soil*, **188**, 227–237.
- CRUZ P., TOURNEBIZE C., GAUDICHAU C., HAEGELIN A. and MUNIER-JOLAIN N.M. (1995) Effect of shade on growth, nitrogen content and CO<sub>2</sub> leaf assimilation in a tropical perennial grass. In: Sinoquet H. and Cruz P. (eds) *Ecophysiology of Tropical Intercropping*, pp. 284–293. Paris, France: INRA.
- CRUZ P., SIERRA J., WILSON J.R., DULORMNE M.A. and TOURNEBIZE R. (1999) Effects of shade on the growth and mineral nutrition of tropical grasses in silvopastoral systems. *Annals of Arid Zone*, **38**, 335–361.
- DE CASTRO C.R.T., GARCÍA R., CARVALHO M.M. and COUTO L. (1999) Produção forrageira de gramíneas cultivadas sob luminosidade reduzida. *Revista Brasileira de Zootecnia*, **28**, 919–927.
- DIAS-FILHO M.B. (2002) Photosynthetic light response of the C<sub>4</sub> grasses *Brachiaria brizantha* and *B. humidicola* under shade. *Scientia Agricola*, **59**, 65–68.
- GIUNTA F., PRUNEDDU G. and MOTZO R. (2009) Radiation interception and biomass nitrogen accumulation in different cereal and grain legume species. *Field Crops Research*, **110**, 76–84.
- GIVNISH T.J. (1988) Adaptation to sun and shade: a whole-plant perspective. *Australian Journal of Plant Physiology*, **15**, 63–92.
- GREENWOOD D.J., LEMAIRE G., GOSSE G., CRUZ P., DRAYCOTT A. and NEETESON J.J. (1990) Decline in percentage N of C<sub>3</sub> and C<sub>4</sub> crops with increasing plant mass. *Annals of Botany*, **66**, 425–436.
- GUENNI O., GIL J.L. and GUEDEZ Y. (2005) Growth, forage yield and light interception and use by stands of five *Brachiaria* species in a tropical environment. *Tropical Grasslands*, **39**, 42–53.
- GUENNI O., SEITER S. and FIGUEROA R. (2008) Growth responses of three *Brachiaria* species to light intensity and nitrogen supply. *Tropical Grasslands*, **42**, 75–87.
- HERNÁNDEZ M. and GUENNI O. (2008) Producción de biomasa y calidad nutricional del estrato graminoide en un sistema silvopastoril dominado por samán (*Samanea saman* (Jacq) Merr). *Zootecnia Tropical*, **26**, 439–453.
- HUMPHREYS L.R. (1991) *Tropical pasture utilization*. Cambridge, UK: Cambridge University Press.
- HUMPHREYS L.R. (1994) *Tropical pastures: their role in sustainable agriculture*. New York, USA: Longman Scientific and Technical/John Wiley and Sons, Inc.
- KALIGIS D.A. and SUMOLANG C. (1991) Forages species for coconut plantations in north Sulawesi. In: Shelton H.M. and Stür W.W. (eds) *Forages for plantation crops. Proceedings of a Workshop, Sanur Beach, Bali, 27–29 June 1990*, pp. 45–48.
- LAMBERS H., CHAPIN F.S. III and PONS T.L. (2008) *Plant physiological ecology*, 2nd edn. New York, USA: Springer.
- LEMAIRE G. and GASTAL F. (2009) Quantifying crop responses to nitrogen deficiency and avenues to improve nitrogen use efficiency. In: Sadras V. and Calderini D. (eds) *Crop physiology: applications for genetic improvement and agronomy*, pp. 171–211. San Diego, CA, USA: Academic Press/Elsevier Inc.
- LEMAIRE G., GASTAL F. and SALETTE J. (1989) Analysis of the effect of N nutrition on dry matter yield of a sward by reference to potential yield and optimum N content. *Proceeding of the XVI International Grassland Congress, Nice, 1989*, pp. 179–180.
- LUDLOW M.M. and WILSON G.L. (1971) Photosynthesis of tropical pasture plants. II. Temperature and illuminance history. *Australian Journal of Biological Sciences*, **24**, 1065–1075.
- LUDWIG F., DE KROON H., BERENDSE F. and PRINS H.H.T. (2004) The influence of savanna trees on nutrient, water and light availability and the understory vegetation. *Plant Ecology*, **170**, 93–105.
- MARSHALL B. and BISCOE P.V. (1980) A model for C<sub>3</sub> leaves describing the dependence of net photosynthesis on irradiance. *Journal of Experimental Botany*, **31**, 29–39.
- NOBEL P.S., FORSETH I.N. and LONG S.P. (1993) Canopy structure and light interception. In: Hall D.O., Scurlock J.M.O., Bolhàr-Nordenkamp H.R., Leegood R.C. and Long S.P. (eds) *Photosynthesis and production in a changing environment: a field and laboratory manual*, pp. 79–90. London, UK: Chapman & Hall.
- PACIULLO D.S.C., DE CARVALHO C.A.B., AROEIRA L.J.M., MORENZ M.J.F., LOPES F.C.F. and ROSSIELLO R.O.P. (2007) Morfofisiologia e valor nutritivo do capim-braquiária sob sombreamento natural e a sol pleno. *Pesquisa Agropecuária Brasileira, Brasília*, **42**, 573–579.
- PEARCY R.W. and SIMS D.A. (1994) Photosynthetic acclimation to changing light environments: scaling from the leaf to the whole plant. In: Caldwell M.M. and Pearcy R.W. (eds) *Exploitation of environmental heterogeneity by plants: ecophysiological processes*, pp. 145–174. San Diego, CA, USA: Academic Press.
- PERI P.L., MOOT D.J., JARVIS P., MCNEIL D.L. and LUCAS R.J. (2007) Morphological, anatomical, and physiological changes of Orchardgrass leaves grown under fluctuating light regimes. *Agronomy Journal*, **99**, 1502–1513.
- PIZARRO E.A., DO VALLE C.B., KELER-GREIN G., SCHULTZE-KRAFT R. and ZIMMER A.H. (1998) Experiencia regional con *Brachiaria*: Región de América Tropical – Sabanas. In: Miles J.W., Maass B.L. and do Valle C.B. (eds) *Brachiaria: Biología, Agronomía y Mejoramiento*, pp. 247–269. Cali, Colombia; Campo Grande, Brasil: CIAT/EMBRAPA/CNPGC.
- R DEVELOPMENT CORE TEAM (2008) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>

- RIKA I.K., MENDRA I.K., GUSLI O.M. and NURJAYA M. G. (1991) New forage species for coconut plantations in Bali. In: Shelton H.M. and Stür W.W. (eds) *Forages for plantation crops. Proceedings of a Workshop, Sanur Beach, Bali, 27–29 June 1990*, pp. 41–44.
- SAGE R.F. and MCKOWN A.D. (2006) Is  $C_4$  photosynthesis less phenotypically plastic than  $C_3$  photosynthesis? *Journal of Experimental Botany*, **57**, 303–317.
- SAS INSTITUTE (1989) *SAS/STATM. Guide for personal computers*. Cary, NC, USA: SAS Institute Inc.
- SHELTON H.M., HUMPHREYS L.R. and BATELLO C. (1987) Pastures in plantations of Asia and the Pacific: performance and prospects. *Tropical Grasslands*, **21**, 159–168.
- SINCLAIR T.R. and HORIE T. (1989) Leaf nitrogen, photosynthesis, and crop radiation use efficiency: a review. *Crop Science*, **29**, 90–98.
- SINCLAIR T.R. and MUCHOW R.C. (1999) Radiation use efficiency. *Advances in Agronomy*, **65**, 215–265.
- SINOQUET H. and CRUZ P. (1993) Analysis of light interception and use in pure and mixed stands of *Digitaria decumbens* and *Arachis pintoi*. *Acta Ecológica*, **14**, 327–339.
- SMITH M.A. and WHITEMAN P.C. (1983) Evaluation of tropical grasses in increasing shade under coconut canopies. *Experimental Agriculture*, **19**, 153–161.
- SQUIRE G.R. (1990) *The physiology of tropical crop production*. Wallingford, UK: CAB International.
- STÖCKLE C.O. and KEMANIAN A.R. (2009) Crop radiation capture and use efficiency: a framework for crop growth analysis. In: Sadras V. and Calderini D. (eds) *Crop physiology: applications for genetic improvement and agronomy*, pp. 145–170. San Diego, CA, USA: Academic Press/Elsevier Inc.
- STÜR W.W., HOPKINSON J.M. and CHEN C.P. (1998) Experiencia regional con *Brachiaria*: Asia, el Pacífico Sur y Australia. In: Miles J.W., Maass B.L. and do Valle C. B. (eds) *Brachiaria: Biología, Agronomía y Mejoramiento*, pp. 282–296. Cali, Colombia; Campo Grande, Brasil: CIAT/EMBRAPA/CNPQC.
- TERASHIMA I. and HIKOSAKA K. (1995) Comparative ecophysiology of leaf and canopy photosynthesis. *Plant, Cell and Environment*, **18**, 1111–1128.
- WALTERS R.G. (2005) Towards an understanding of photosynthetic acclimation. *Journal of Experimental Botany*, **56**, 435–447.
- WILSON J.R. and LUDLOW M.M. (1991) The environment and potential growth of herbage under plantations. In: Shelton H.M. and Stür W.W. (eds) *Forages for Plantations Crops. ACIAR Proceedings No. 32, Canberra, Australia, 1991*, pp. 10–24.