

Table S1. Summary of plant growth parameters.

Growth parameters of eight C₄ grasses grown in control (full sunlight) or shade (16% of natural sunlight) environments. Values are means \pm SE ($n = 3-4$). The ranking (from lowest = a) of subtypes within each single row using multiple-comparison Tukey's *post hoc* test. Values followed by the same letter are not significantly different at the 5% level. Significant percent changes are shown in bold ($p < 0.05$).

Parameter	Treatment	Subtype			C ₄
		NADP-ME	PEP-CK	NAD-ME	
Total DM (g plant ⁻¹)	Control	54.3 \pm 2.7ab	82.1 \pm 3.7b	35.1 \pm 5.9a	57.2 \pm 3.8
	Shade	10.2 \pm 2.2a	6.5 \pm 0.7a	1.6 \pm 0.5a	7.1 \pm 1.3
	% change	-81	-92	-95	-88
Total leaf area (m ² plant ⁻¹)	Control	0.35 \pm 0.03a	0.56 \pm 0.07a	0.36 \pm 0.08a	0.41 \pm 0.03
	Shade	0.16 \pm 0.04a	0.11 \pm 0.01a	0.03 \pm 0.01a	0.12 \pm 0.02
	% change	-53	-81	-92	0.29
Root/shoot DM	Control	0.42 \pm 0.04a	0.32 \pm 0.02a	0.37 \pm 0.06a	0.38 \pm 0.03
	Shade	0.2 \pm 0.01b	0.12 \pm 0a	0.19 \pm 0.02ab	0.17 \pm 0.01
	% change	-53	-62	-49	-54
LMA(g m ⁻²)	Control	41 \pm 3a	34 \pm 1a	37 \pm 2a	38 \pm 2
	Shade	25 \pm 2a	27 \pm 2a	22 \pm 1a	25 \pm 1
	% change	-39	-20	-39	-35
Leaf N _{mass} (mg g ⁻¹)	Control	38 \pm 1a	43 \pm 1ab	48 \pm 2b	42 \pm 1
	Shade	39 \pm 2a	39 \pm 2a	46 \pm 1a	41 \pm 1
	% change	2	-10	-4	-3
Leaf N _{area} (g m ⁻²)	Control	1.56 \pm 0.13a	1.46 \pm 0.03a	1.81 \pm 0.08a	1.59 \pm 0.07
	Shade	0.94 \pm 0.05a	1.03 \pm 0.05a	1.02 \pm 0.03a	0.99 \pm 0.03
	% change	-39	-29	-43	-38
Plant NUE	Control	120 \pm 5b	86 \pm 7ab	72 \pm 6a	99 \pm 5
	Shade	71 \pm 4b	60 \pm 1ab	43 \pm 4a	61 \pm 3
	% change	-41	-31	-39	-38

Table S2. Summary of gas exchange parameters.

Gas exchange parameters of eight C₄ grasses grown in control (full sunlight) or shade (16% of natural sunlight) environments. Values are means \pm SE ($n = 3-4$). The ranking (from lowest = a) of subtypes within each single row using multiple-comparison Tukey's *post hoc* test. Values followed by the same letter are not significantly different at the 5% level. Significant percent changes are shown in bold ($p < 0.05$).

Parameter	Treatment	Subtype			C ₄
		NADP-ME	PEP-CK	NAD-ME	
CO ₂ assimilation at HL, <i>A_h</i> ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	Control	42 \pm 1a	41 \pm 1a	42 \pm 1a	42 \pm 1
	Shade	25 \pm 1b	22 \pm 1ab	16 \pm 1a	22 \pm 1
	% change	-39	-47	-62	-48
CO ₂ assimilation at LL, <i>A_l</i> ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	Control	10 \pm 1a	12 \pm 1a	11 \pm 0a	11 \pm 0
	Shade	10 \pm 0a	9 \pm 0a	8 \pm 1a	9 \pm 0
	% change	0	-26	-30	-16
Conductance at HL, <i>g_{s h}</i> ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	Control	0.33 \pm 0.02a	0.31 \pm 0.01a	0.33 \pm 0.01a	0.32 \pm 0.01
	Shade	0.21 \pm 0.02b	0.17 \pm 0.01ab	0.13 \pm 0.01a	0.18 \pm 0.01
	% change	-35	-43	-60	-45
Conductance at LL, <i>g_{s l}</i> ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	Control	0.07 \pm 0a	0.07 \pm 0a	0.07 \pm 0.01a	0.07 \pm 0
	Shade	0.07 \pm 0.01a	0.05 \pm 0a	0.06 \pm 0.01a	0.06 \pm 0
	% change	5	-28	-14	-10
Dark respiration, <i>R_d</i> ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Control	1.6 \pm 0.1b	1.1 \pm 0.1a	1.5 \pm 0.1ab	1.4 \pm 0.1
	Shade	0.9 \pm 0.1a	1.2 \pm 0.2a	1.1 \pm 0.1a	1.1 \pm 0.1
	% change	-42	5	-29	-25
<i>R_d/A_{GROWTH}</i> (x 100)	Control	3.7 \pm 0.2b	2.7 \pm 0.2a	3.5 \pm 0.2ab	3.3 \pm 0.1
	Shade	9.7 \pm 0.6a	12.8 \pm 1.9a	15.7 \pm 2.2a	12.3 \pm 1
	% change	158	373	341	269
PWUE ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$)	Control	130 \pm 5a	137 \pm 5a	132 \pm 5a	133 \pm 3
	Shade	121 \pm 5a	128 \pm 5a	124 \pm 6a	124 \pm 3
	% change	-7	-6	-6	-7
PNUE ($\mu\text{mol CO}_2 \text{ s}^{-1} \text{ g}^{-1} \text{ N}$)	Control	29 \pm 2a	29 \pm 0a	23 \pm 0a	28 \pm 1
	Shade	29 \pm 1b	23 \pm 1ab	17 \pm 2a	24 \pm 1
	% change	0	-19	-28	-12

Table S3. Summary of carbon isotope discrimination parameters.

Carbon isotope discrimination parameters for eight C₄ grasses grown at control (full sunlight) or shade (16% of natural sunlight) environments. Values are means \pm SE ($n = 3-4$). The ranking (from lowest = a) of subtypes within each single row using multiple-comparison Tukey's *post hoc* test. Values followed by the same letter are not significantly different at the 5% level. Significant percent changes are shown in bold ($p < 0.05$).

Parameter	Treatment	Subtype			C ₄
		NADP-ME	PEP-CK	NAD-ME	
C _i /C _a at HL	Control	0.32 \pm 0.02a	0.3 \pm 0.02a	0.36 \pm 0.02a	0.32 \pm 0.01
	Shade	0.4 \pm 0.02a	0.4 \pm 0.02a	0.43 \pm 0.03a	0.41 \pm 0.01
	% change	28	33	19	27
C _i /C _a at LL	Control	0.35 \pm 0.02a	0.26 \pm 0.04a	0.37 \pm 0.08a	0.33 \pm 0.02
	Shade	0.35 \pm 0.04a	0.24 \pm 0.02a	0.44 \pm 0.04a	0.34 \pm 0.02
	% change	0	-7	19	4
Δ_P (‰) at HL	Control	2.26 \pm 0.17a	2.58 \pm 0.21a	2.91 \pm 0.28a	2.57 \pm 0.13
	Shade	2.41 \pm 0.17a	3.15 \pm 0.29a	3.63 \pm 0.19a	2.98 \pm 0.17
	% change	7	22	25	16
Δ_P (‰) at LL	Control	2.7 \pm 0.48a	4.17 \pm 0.17a	4.66 \pm 0.21a	3.95 \pm 0.23
	Shade	2.45 \pm 0.3a	4.07 \pm 0.35a	4.58 \pm 0.15a	3.69 \pm 0.23
	% change	-9	-2	-2	-3
Leakiness (ϕ_i) at HL	Control	0.13 \pm 0.01a	0.17 \pm 0.03a	0.21 \pm 0.03a	0.17 \pm 0.01
	Shade	0.14 \pm 0.01a	0.23 \pm 0.03ab	0.29 \pm 0.02b	0.22 \pm 0.02
	% change	9	34	39	27
Leakiness (ϕ_h) at LL	Control	0.19 \pm 0.04a	0.27 \pm 0.03a	0.34 \pm 0.02a	0.27 \pm 0.02
	Shade	0.14 \pm 0.03a	0.26 \pm 0.06a	0.32 \pm 0.01a	0.24 \pm 0.02
	% change	-26	-1	-5	-3
Dry matter photosynthetic C-isotope discrimination, Δ_{DM} (‰)	Control	4.8 \pm 0.1a	6.1 \pm 0.3b	6.8 \pm 0.2b	5.5 \pm 0.2
	Shade	5.2 \pm 0.2a	7.5 \pm 0.3b	8.8 \pm 0.2b	6.7 \pm 0.3
	% change	9	22	30	21

Table S4. Summary of biochemical parameters.

Biochemical parameters of eight C₄ grasses grown at control or shade (16% of natural sunlight) environments. Values are means ± SE (*n* = 3-4). The ranking (from lowest = a) of subtypes within each single row using multiple-comparison Tukey's *post hoc* test. Values followed by the same letter are not significantly different at the 5% level. Significant percent changes are shown in bold (*p* < 0.05).

Parameter	Treat	NADP-ME				PEP-CK		NAD-ME		Subtype			Total C ₄
		<i>P. antidotale</i>	<i>C. ciliaris</i>	<i>S. bicolor</i>	<i>Z. mays</i>	<i>M. maximus</i>	<i>C. gayana</i>	<i>P. coloratum</i>	<i>L. fusca</i>	NADP-ME	PEP-CK	NAD-ME	
Rubisco activity ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	Control	33±1a	31±1a	29±1a	31±2a	29±4a	36±4a	37±3a	29±1a	31±1a	32±3a	33±2a	32
	Shade	16±1ab	13±1a	16±2ab	21±2b	11±2a	23±2b	11±1a	15±1ab	16±1a	17±3a	13±1a	16
	% change	-50	-56	-45	-34	-63	-37	-71	-47	-47	-48	-60	-51
Rubisco sites ($\mu\text{mol m}^{-2}$)	Control	6.2±0.1a	5.1±0.2a	5±0.2a	5.7±0.3a	5.5±0.8a	6.3±0.8a	11±1b	6.7±0.2a	5.5±0.2a	5.8±0.5ab	8.5±1b	6.3±0.4
	Shade	2.9±0.2ac	2.2±0.1a	3.1±0.4ac	3.8±0.4bc	2.5±0.2ab	4±0.4c	3.2±0.2ac	3.5±0.2ac	3±0.2a	3.2±0.4a	3.4±0.2a	3.1±0.1
	% change	-52	-56	-39	-34	-54	-37	-71	-47	-47	-44	-61	-51
Rubisco activation (%)	Control	42±4a	41±6a	47±5ab	62±1ab	44±2ab	48±1ab	45±1ab	65±7b	47±3a	46±1a	55±5a	49±2
	Shade	32±6ab	83±5d	44±1bc	58±1cd	36±5ab	36±5ab	41±1bc	25±1a	54±6a	36±3a	33±4a	44±4
	% change	-24	102	-6	-7	-20	-25	-8	-62	15	-22	-40	-9
PEPC activity ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	Control	184±4d	128±6bc	274±13e	193±4d	169±22cd	160±16cd	94±12ab	51±11a	189±13b	165±13ab	69±12a	154±12
	Shade	46±8bc	20±2a	77±8cd	98±6d	48±6bc	65±11cd	27±3ab	19±3a	59±9a	57±7a	23±3a	50±6
	% change	-75	-84	-72	-49	-72	-59	-71	-62	-69	-66	-66	-68
PEPC/Initial Rubisco activity	Control	14±1.6c	10.9±1.9bc	20.1±1.8d	9.6±0.5bc	13.6±1cd	9.3±0.5ac	5.6±0.5ab	2.9±0.3a	13.7±1.3b	11.4±1.1at	4.2±0.7a	11±1
	Shade	8.9±1.1ac	1.8±0.2a	11.8±1.4bc	8.2±0.5ac	14.2±3.8c	8.1±0.5ac	6±0.5ab	5±0.3ab	7.7±1.2a	11.2±2.2a	5.5±0.3a	8±0.9
	% change	-36.18	-83.65	-41.22	-14.7	4.84	-13.15	7.45	73.32	-43.93	-2.48	29.79	-27.22
NADP-ME activity ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	Control	57±2c	47±2b	50±2bc	49±3b	1±0a	1±0a	4±0a	3±1a	51±1b	1±0a	3±0a	28±5
	Shade	23±2b	25±1bc	33±1c	43±3d	0±0a	1±0a	1±0a	1±0a	31±2b	1±0a	1±0a	16±3
	% change	-60	-46	-35	-13	-77	2	-74	-67	-40	-43	-70	-100
NAD-ME activity ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	Control	3±0a	1±0a	2±0a	3±0a	3±1a	3±0a	35±1b	36±3b	2±0a	3±0a	35±2b	10±3
	Shade	1±0a	1±0a	1±0a	2±0a	1±0a	2±0a	15±1b	18±1b	1±0a	1±0a	17±1b	5±1
	% change	-56	-26	-45	-8	-65	-44	-57	-49	-38	-56	-53	-50
PEP-CK activity ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	Control	6±1ab	16±1bc	2±0a	25±1c	46±3d	145±4e	20±3c	20±3c	11±3a	103±20b	20±2a	37±9
	Shade	3±1a	5±1ab	0±0a	13±1b	22±2c	52±3d	9±0ab	5±0a	6±2a	35±6b	7±1a	14±3
	% change	-45	-68	-78	-47	-52	-64	-55	-76	-50	-66	-66	-62
Protein (g m ⁻²)	Control	5.1±0.2ab	4.8±0.4ab	4.2±0.5ab	5.2±0.3ab	5.3±0.7ab	3.5±0.3a	5.8±0.5b	4.4±0.5ab	4.8±0.2a	4.4±0.5a	5±0.4a	4.8±0.2
	Shade	2.3±0.4ab	1.7±0.1a	3.7±0.3b	3.3±0.1b	1.8±0.2a	2.5±0.3ab	2.2±0.1ab	2.8±0.5ab	2.8±0.3a	2.2±0.2a	2.5±0.3a	2.5±0.2
	% change	-54	-64	-12	-37	-67	-27	-62	-37	-42	-51	-50	-47

Table S5. Definitions and units for variable described in the text.

Variable	Definition	Values/Calculation/Units
A	photosynthetic rate	$\mu\text{mol m}^{-2} \text{s}^{-1}$
g_{bs}	bundle-sheath conductance to CO_2 (von Caemmerer, 2000)	$g_{\text{bs}} = 0.003 \text{ mol m}^{-2} \text{s}^{-1}$
ϕ_{h}	leakiness at high light (von Caemmerer et al., 2014)	unitless
ϕ_{l}	leakiness at low light (Ubierna et al., 2013)	unitless
a_{b}	^{13}C fractionation across the boundary layer	2.9 ‰
a	^{13}C fractionation due to diffusion of CO_2 in air	4.4 ‰
a'	weighted fractionation across the boundary layer and stomata in series	$a' = \frac{a_{\text{b}}(C_{\text{a}} - C_{\text{is}}) + a(C_{\text{is}} - C_{\text{i}})}{C_{\text{a}} - C_{\text{i}}}$
a_{i}	fractionation factor associated with the dissolution of CO_2 and diffusion through water	$a_{\text{i}} = s = 1.8 \text{ ‰}$
s	fractionation during leakage of CO_2 out of the bundle-sheath cells (Henderson et al. 1992)	1.8 ‰
f	fractionation associated with photorespiration	11.6 ‰
t	ternary effect of transpiration rate on the carbon isotope discrimination during CO_2 assimilation	$t = \frac{(1+a') \cdot E}{2 \cdot g_{\text{ac}}^{\text{t}}} \text{ ‰}$
E	leaf transpiration rate	$\text{mmol m}^{-2} \text{s}^{-1}$
g_{ac}^{t}	total conductance to CO_2 diffusion including boundary layer and stomatal conductance	$\text{mol m}^{-2} \text{s}^{-1}$
C_{is}	leaf surface CO_2 mole fraction	$\mu\text{mol mol}^{-1}$
b_3	fractionation by Rubisco (Farquhar, 1983)	30 ‰
b_4	combined fractionation of the conversion of CO_2 to HCO_3^- and PEP carboxylation (Farquhar, 1983)	-5.74 ‰
e	fractionation factor associated with respiration (Pengelly et al., 2010)	$e = \delta^{13}\text{C}$ in the CO_2 cylinder – $\delta^{13}\text{C}$ in growth environment
g_{m}	mesophyll conductance to CO_2 (Ubierna et al., 2016)	$g_{\text{m}} = 1.4 \text{ mol m}^{-2} \text{s}^{-1}$ at 28 °C
Δ	Photosynthetic discrimination against ^{13}C	‰
O_{m}	O_2 mole fraction in the mesophyll cells	210000 $\mu\text{mol mol}^{-1}$
O_{s}	O_2 mole fraction in the bundle-sheath cells	$O_{\text{s}} = \frac{\alpha \cdot A}{0.047 \cdot g_{\text{bs}}} + O_{\text{m}}$ $\mu\text{mol mol}^{-1}$
C_{a}	CO_2 mole fraction in the ambient air	Measured
C_{s}	CO_2 mole fraction in the bundle-sheath cells	$C_{\text{s}} = \frac{(\Gamma^* \cdot O_{\text{s}}) \cdot \left(\frac{2}{3} \cdot (A + R_{\text{d}}) + \frac{(1-x) \cdot J_{\text{t}}}{3} \right)}{\frac{(1-x) \cdot J_{\text{t}}}{3} - (A + R_{\text{d}})}$ in $\mu\text{mol mol}^{-1}$
C_{i}	CO_2 mole fraction inside the leaf	$\mu\text{mol mol}^{-1}$
C_{m}	CO_2 mole fraction in the mesophyll cytosol at the sites of CA	$C_{\text{m}} = C_{\text{i}} - \frac{A}{g_{\text{m}}}$ in $\mu\text{mol mol}^{-1}$
R_{d}	non-photorespiratory CO_2 released in the dark (= measured rates of dark respiration)	$\mu\text{mol m}^{-2} \text{s}^{-1}$
R_{m}	Mesophyll mitochondrial respiration rate	$R_{\text{m}} = 0.5 R_{\text{d}}$ in $\mu\text{mol m}^{-2} \text{s}^{-1}$
V_{c}	Rubisco carboxylation rate (von Caemmerer, 2000)	$V_{\text{c}} = \frac{(1-x) \cdot J_{\text{t}}}{3 \cdot \left(1 + \frac{2 \cdot \Gamma^* \cdot O_{\text{s}}}{3 \cdot C_{\text{s}}} \right)}$ in $\mu\text{mol m}^{-2} \text{s}^{-1}$
V_{o}	Rubisco oxygenation rate (von Caemmerer, 2000)	$V_{\text{o}} = \frac{V_{\text{c}} - A - R_{\text{d}}}{0.5}$ $\mu\text{mol m}^{-2} \text{s}^{-1}$
V_{p}	PEP carboxylation rate (von Caemmerer, 2000)	$V_{\text{p}} = \frac{x \cdot J_{\text{t}}}{2}$ $\mu\text{mol m}^{-2} \text{s}^{-1}$
α	fraction of PSII activity in the bundle-sheath cells (Sharwood et al., 2016)	0 for NADP-ME and 0.2 for NAD-ME and PEP-CK species
γ^*	half of the reciprocal of Rubisco specificity (Sharwood et al., 2016)	0.000255, 0.00023 and 0.000233 for NADP-ME, NAD-ME and PEP-CK species, respectively
Γ^* (μbar)	compensation point in the absence of mitochondrial respiration	$\Gamma^* = O_{\text{s}} \cdot \gamma^*$