C_4 savanna grasses fail to maintain assimilation in drying soil under low CO_2 compared with C_3 trees despite lower leaf water demand

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Figure S1. Soil water retention of various substrates. Substrates were loosely filled into pots identical to those used in the experiments, watered to field capacity and left to dry without a plant under normal growth cabinet conditions (see main text). Soil water potential was measured at regular intervals (every 2–3 days) using a psychrometer (Psypro with L-51 hygrometers, Wescor Inc., Logan, USA) calibrated with five standard NaCl solutions according to the manufacturer's instructions. Soil samples were extracted from the centre of the pot at a depth of ~10 cm using a weighing spatula. We used soil and John Innes No. 3 (green up-triangles) as the experimental substrate because it has the steadiest decrease in soil matric potential as soil dries. $\Psi_m = \Psi_e\ (\theta/\theta_s)^{-b}$, where Ψ_e = 0.0101 and b = 3.01.

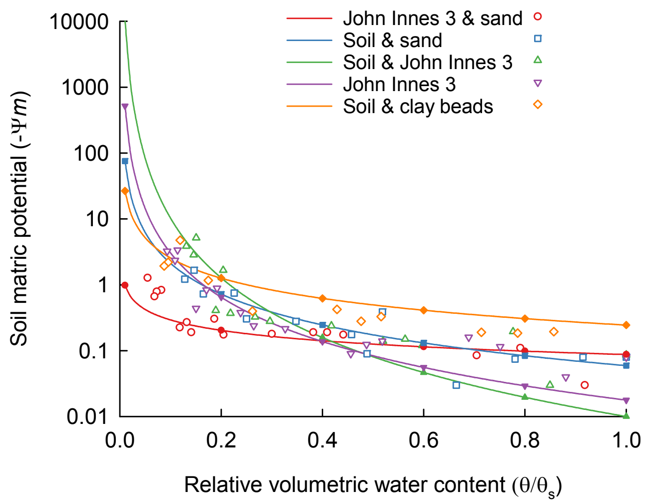

Figure S2. Dependence of assimilation on g_S. Symbols show in-cabinet gas exchange measurements under operational growing conditions for C_3 trees (**a**) and C_4 grasses (**b**).

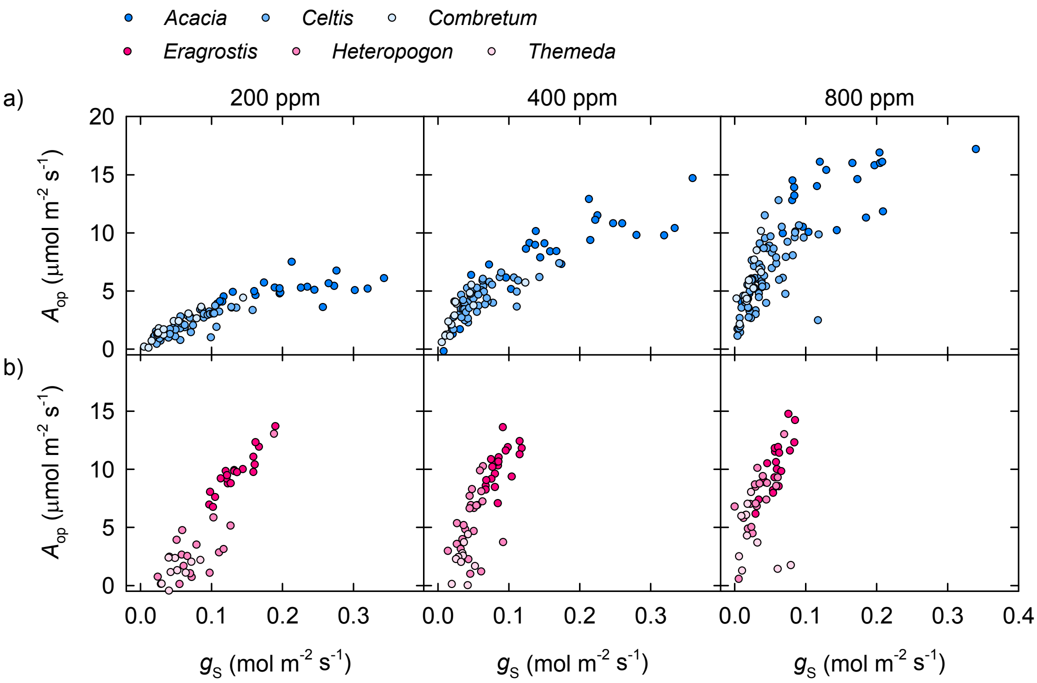

Figure S3. Leaf-level water demand relative to supply. We formulated the relationship between leaf water demand and supply as the ratio of the soil-to-leaf diurnal water potential gradient ($\Delta\Psi$) to the leaf-to-boundary layer water vapour mole fraction gradient (D_S) within **a)** the controlled-environment growth chambers (D_S) and **b)** the leaf chamber during operational gas exchange measurement (D_{Sop}). Daytime D_S for the controlled-environment growth chambers at 26 °C and 70% relative humidity was 10.08 mmol H_2O mol^{-1} air. Boxplots show median values and interquartile range with whiskers showing 10th and 90th percentiles (n=4–10) for C_3 tree species (*Vachellia karroo*, *Celtis africana* and *Combretum apiculatum*) and C_4 grass species (*Eragrostis curvula*, *Heteropogon contortus* and *Themeda triandra*) grown at 200 ppm (left), 400 ppm (centre) or 800 ppm (right) $[CO_2]_a$. Boxes sharing the same letter range do not have significantly different mean values at α = 0.05 (two-way ANOVAs testing for effects of species (nested within photosynthetic type), $[CO_2]_a$ and their interaction with post-hoc Tukey pairwise comparisons). Statistics for main two-way ANOVA effects are given in Table S2.

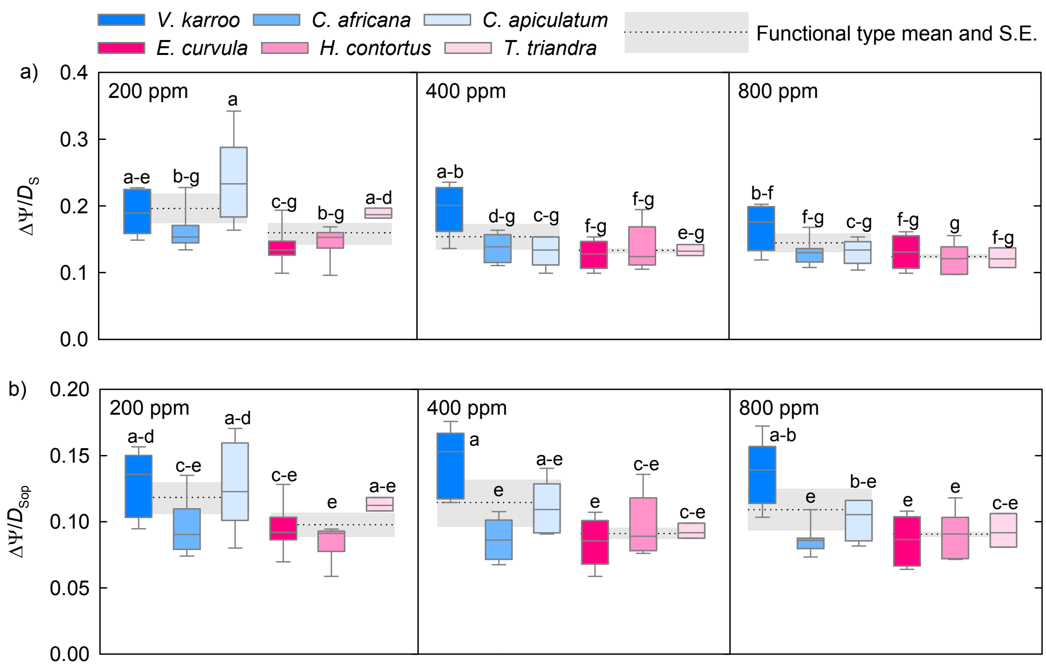

Figure S4. Response of g_S to soil water potential. Symbols show in-cabinet gas exchange measurements of g_S and corresponding values of Ψ_{soil} under operational growing conditions for C_3 trees (**a**) and C_4 grasses (**b**) grown at 200 ppm (left), 400 ppm (centre), and 800 ppm $[CO_2]_a$ (right).

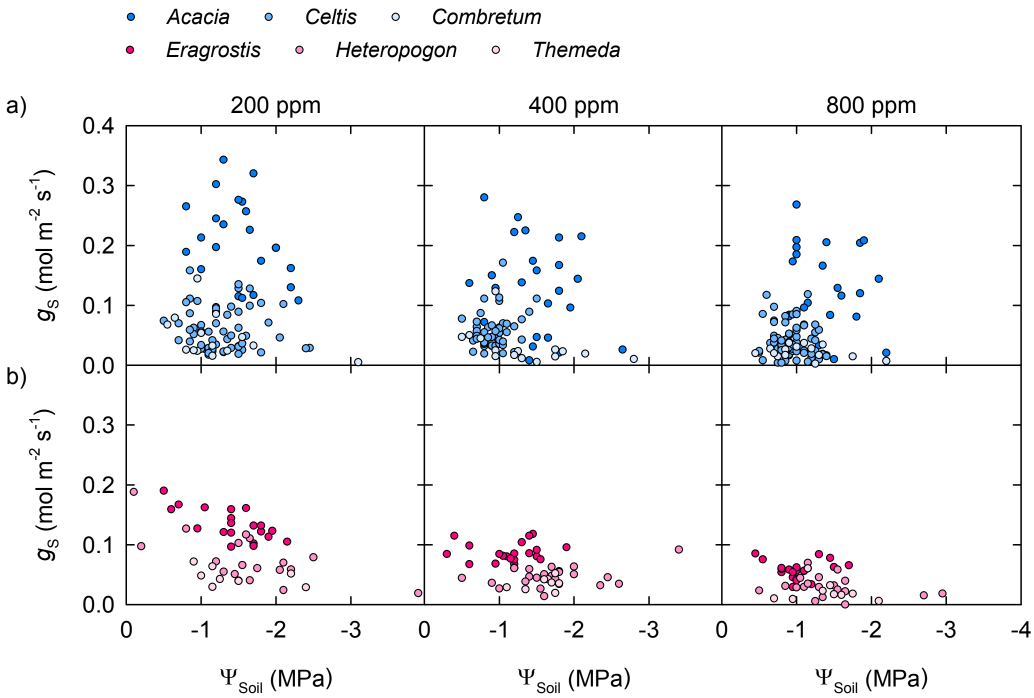

Figure S5. Response of assimilation to soil water potential. Symbols show in-cabinet gas exchange measurements of assimilation and corresponding values of Ψ_{soil} under operational conditions for (**a**) C_3 trees and (**b**) C_4 grasses grown at 200 ppm (left), 400 ppm (centre), and 800 ppm $[CO_2]_a$ (right).

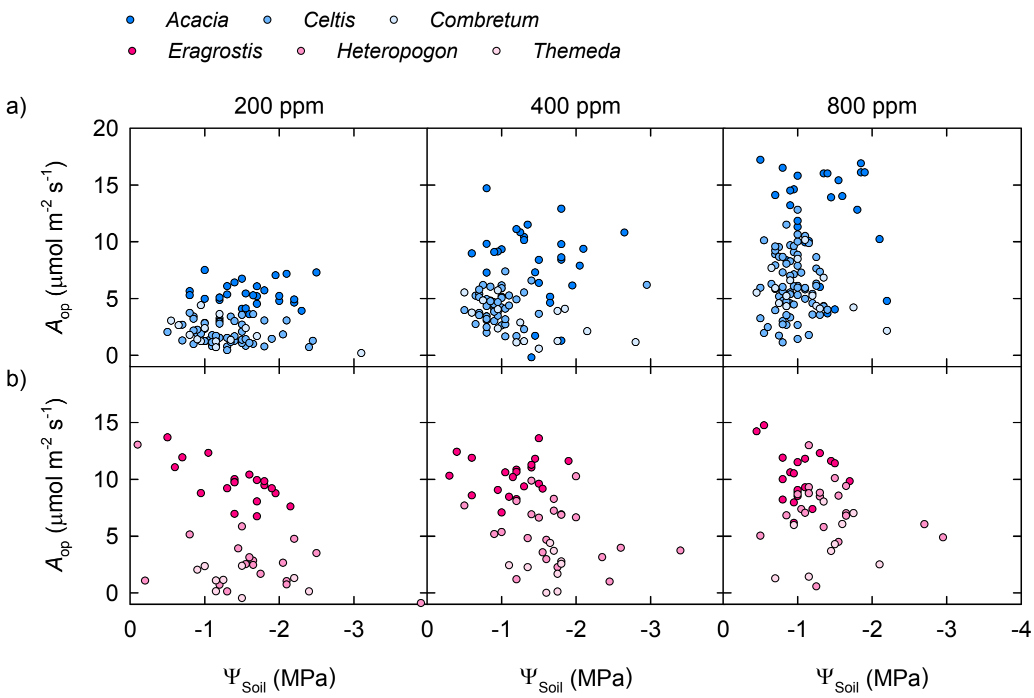

Figure S6. Photochemical integrity of photosystem II as indicated by F_V/F_M. Mean (*n* = 4) F_V/F_M for the savanna tree *Vachellia karroo* (**left**), the forest broad-leaf tree *Celtis africana* (**centre**), and C_4 savanna grass *Eragrostis curvula* (**right**) growing at 200 ppm, 400 ppm or 800 ppm $[CO_2]_a$ in controlled-environment growth chambers. F_V/F_M was measured from pulse-amplitude modulated (PAM) chlorophyll fluorometry within the cabinets in the dark. Error bars are ± 1 S.E.

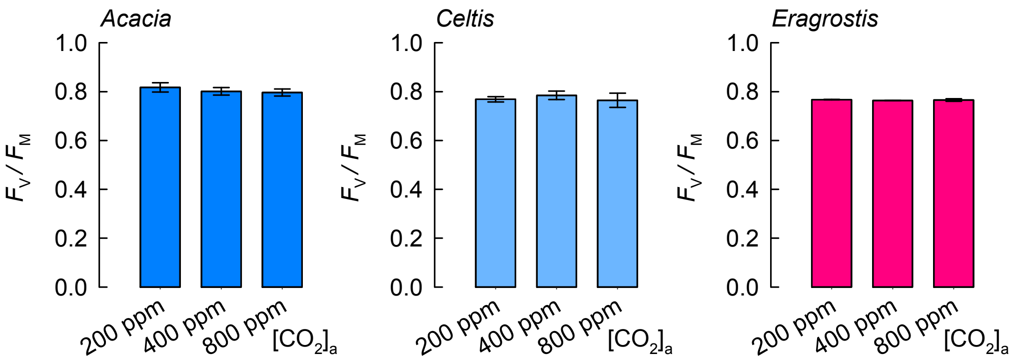

Table S1. Species means of in-cabinet observational measurements. Mean values ± 1 S.D. in parenthesis (*n* = 4–10) for gas exchange and water relations measured under operational conditions. ANOVA results for the in-cabinet measurements are listed in Table S2. Values sharing the same superscript letter within each species are not statistically different at α = 0.05.

			Growth CO_2 concentration			Growth CO_2 concentration			Growth CO_2 concentration		
	Symbol	**Units**	**200 ppm**	**400 ppm**	**800 ppm**	**200 ppm**	**400 ppm**	**800 ppm**	**200 ppm**	**400 ppm**	**800 ppm**
			Vachellia karroo			***Celtis africana***			***Combretum apiculatum***		
C_3 trees	A_{op}	µmol m^{-2} s^{-1}	5.24 (0.59) ^{c}	8.56 (2.51) ^{b}	13.5 (1.95) ^{a}	1.93 (0.63) ^{c}	4.22 (0.879) ^{b}	6.81 (2.31) ^{a}	1.72 (1.37) ^{b}	2.82 (1.62) ^{b}	5.62 (1.53) ^{a}
	g_{Sop}	mol m^{-2} s^{-1}	0.219 (0.11) ^{a}	0.159 (0.11) ^{a}	0.157 (0.09) ^{a}	0.054 (0.02) ^{a}	0.053 (0.015) ^{a}	0.045 (0.024) ^{a}	0.037 (0.02) ^{a}	0.024 (0.025) ^{a}	0.021 (0.008) ^{a}
	Ψ_{PD}	MPa	-1.62 (0.41) ^{a}	-1.38 (0.42) ^{a}	-1.30 (0.39) ^{a}	-1.20 (0.26) ^{b}	-0.938 (0.12) ^{a}	-0.960 (0.10) ^{a}	-1.41 (0.79) ^{a}	-1.35 (0.43) ^{a}	-1.09 (0.11) ^{a}
	Ψ_{MD}	MPa	-3.55 (0.32) ^{b}	-3.33 (0.31) ^{ab}	-3.02 (0.41) ^{a}	-2.84 (0.29) ^{b}	-2.31 (0.24) ^{a}	-2.29 (0.17) ^{a}	-3.39 (0.44) ^{b}	-2.92 (0.27) ^{ab}	-2.47 (0.15) ^{a}
	$\Delta\Psi$	MPa	1.92 (0.34) ^{a}	1.94 (0.36) ^{a}	1.71 (0.33) ^{a}	1.63 (0.29) ^{a}	1.37 (0.21) ^{b}	1.32 (0.18) ^{b}	2.14 (0.30) ^{a}	1.34 (0.23) ^{b}	1.32 (0.19) ^{b}
	WUE	µmol CO_2 / mol H_2O	23.9 (8.84) ^{c}	52.7 (18.5) ^{b}	86.0 (37.9) ^{a}	35.0 (5.16) ^{c}	78.2 (11.1) ^{b}	148 (32.5) ^{a}	41.0 (9.82) ^{c}	110 (39.3) ^{b}	259 (64.3) ^{a}
			Eragrostis curvula			***Heteropogon contortus***			***Themeda triandra***		
C_4 grasses	A_{op}	µmol m^{-2} s^{-1}	9.65 (0.02) ^{a}	10.5 (2.05) ^{a}	10.6 (1.41) ^{a}	2.78 (1.50) ^{b}	5.58 (2.23) ^{a}	7.37 (0.71) ^{a}	1.41 (0.22) ^{b}	2.43 (0.41) ^{ab}	4.39 (1.57) ^{a}
	g_{Sop}	mol m^{-2} s^{-1}	0.137 (0.02) ^{a}	0.092 (0.02) ^{b}	0.062 (0.01) ^{c}	0.073 (0.03) ^{a}	0.045 (0.02) ^{b}	0.031 (0.01) ^{b}	0.051 (0.01) ^{a}	0.036 (0.01) ^{a}	0.021 (0.01) ^{b}
	Ψ_{PD}	MPa	-1.59 (0.21) ^{b}	-1.12 (0.18) ^{a}	-1.05 (0.19) ^{a}	-1.64 (0.69) ^{a}	-1.62 (0.50) ^{a}	-1.40 (0.36) ^{a}	-1.45 (0.30) ^{a}	-1.61 (0.09) ^{a}	-1.29 (0.19) ^{a}
	Ψ_{MD}	MPa	-2.82 (0.09) ^{b}	-2.48 (0.12) ^{a}	-2.44 (0.08) ^{a}	-3.12 (0.55) ^{a}	-3.01 (0.45) ^{a}	-2.54 (0.36) ^{a}	-3.35 (0.36) ^{b}	-2.96 (0.18) ^{ab}	-2.52 (0.33) ^{a}
	$\Delta\Psi$	MPa	1.39 (0.27) ^{a}	1.28 (0.21) ^{a}	1.32 (0.25) ^{a}	1.48 (0.26) ^{a}	1.39 (0.31) ^{a}	1.14 (0.30) ^{a}	1.90 (0.08) ^{a}	1.34 (0.08) ^{b}	1.23 (0.15) ^{b}
	WUE	µmol CO_2 / mol H_2O	70.2 (5.45) ^{c}	113 (22.2) ^{b}	170 (22.1) ^{a}	34.5 (13.9) ^{c}	121 (57.5) ^{b}	236 (50.2) ^{a}	27.8 (7.16) ^{b}	66.4 (20.7) ^{b}	207 (94.9) ^{a}

$\boldsymbol{A_{op}}$, operational assimilation; $\boldsymbol{g_{Sop}}$, operational stomatal conductance; $\boldsymbol{\Psi_{PD}}$, pre-dawn leaf water potential (a proxy for soil water potential); $\boldsymbol{\Psi_{MD}}$, midday leaf water potential; $\boldsymbol{\Delta\Psi}$, soil-to-leaf hydraulic gradient ($\Delta\Psi = \Psi_{PD} - \Psi_{MD}$); **WUE**, operational water use efficiency.

Table S2. Output from ANOVA tests on in-cabinet observational measurements. Results of two-way ANOVAs conducted using a general linear model framework, GLM testing for effects of species nested within photosynthesis type (C_3 trees: *Vachellia karroo*, *Celtis africana*, and *Combretum apiculatum*. C_4 grasses: *Eragrostis curvula*, *Heteropogon contortus*, and *Themeda triandra*.), $[CO_2]_a$, (200 ppm, 400 ppm, or 800 ppm) and the interaction of species and $[CO_2]_a$ on observations. Assumptions of homogeneity of variance for the GLM were satisfied by transforming datasets as indicated.

		Species (nested within photosynthesis type, PT)			**$[CO_2]_a$**			**Spp.(nested in PT) × $[CO_2]_a$**		
Symbol	**Units**	**d.f.**	***F***	***P***	**d.f.**	***F***	***P***	**d.f.**	***F***	***P***
A_{op} [a]	$\mu mol\ m^{-2}\ s^{-1}$	**4, 110**	**84.9**	**<0.0001**	**2, 110**	**84.8**	**<0.0001**	**8, 110**	**3.23**	**0.002**
g_{Sop} [b]	$mol\ m^{-2}\ s^{-1}$	**4, 110**	**18.5**	**<0.0001**	**2, 110**	**18.5**	**<0.0001**	8, 110	0.60	0.777
Ψ_{PD} [b]	MPa	**4, 110**	**6.93**	**<0.0001**	**2, 110**	**6.46**	**0.002**	8, 110	0.94	0.487
Ψ_{MD} [b]	MPa	**4, 110**	**27.6**	**<0.0001**	**2, 110**	**38.9**	**<0.0001**	8, 110	1.77	0.090
$\Delta\Psi$ [b]	MPa	**4, 110**	**7.30**	**<0.0001**	**2, 110**	**20.6**	**<0.0001**	**8, 110**	**2.57**	**0.013**
WUE [b]	$\mu mol\ CO_2$ / $mol\ H_2O$	**4, 110**	**24.2**	**<0.0001**	**2, 110**	**293**	**<0.0001**	**8, 110**	**5.45**	**<0.0001**
$\Delta\Psi/D_S$	$mmol\ mol^{-1}$	**4, 110**	**7.64**	**<0.0001**	**2, 110**	**24.0**	**<0.0001**	**8, 110**	**3.84**	**0.001**
$\Delta\Psi/D_{Sop}$	$mmol\ mol^{-1}$	**4, 110**	**21.7**	**<0.0001**	2, 110	1.78	0.173	8, 110	1.51	0.161

Data transformations: [a]square root; [b]natural log.

Table S3. Biomass of selected species. Mean (± 1 S.E.). Values sharing the same letter within each biomass category are not statistically different at $\alpha = 0.05$ (two-way ANOVA testing for effects of species, $[CO_2]_a$ and their interaction with post-hoc Tukey comparisons (Table S4). Biomass was only available for *Vachellia karroo*, *Celtis africana* and *Combretum apiculatum*.

	V. karroo (C_3)			*C. africana* (C_3)			*E. curvula* (C_4)		
	200 ppm	400 ppm	800 ppm	200 ppm	400 ppm	800 ppm	200 ppm	400 ppm	800 ppm
Aboveground (g)	25.3 (2.19) ^{c}	44.9 (4.90) ^{bc}	60.5 (6.04) ^{b}	12.1 (2.85) ^{d}	24.6 (5.60) ^{cd}	32.4 (7.04) ^{c}	129 (2.34) ^{a}	146 (5.56) ^{a}	153 (3.46) ^{a}
Roots (g)	24.1 (2.63) ^{ab}	26.9 (2.47) ^{ab}	39.3 (3.49) ^{a}	17.9 (3.40) ^{b}	40.7 (9.15) ^{ab}	45.8 (11.5) ^{a}	26.2 (3.27) ^{ab}	27.9 (2.25) ^{ab}	28.3 (3.18) ^{ab}

Table S4. Output from ANOVA tests on biomass of selected species. Results of two-way ANOVAs testing for effects of species (*Vachellia karroo*, *Celtis africana* or *Eragrostis curvula*), $[CO_2]_a$, (200 ppm, 400 ppm, or 800 ppm) and the interaction of species and $[CO_2]_a$. Assumptions of homogeneity of variance for the model were satisfied by transforming datasets as indicated.

		Species			**$[CO_2]_a$**			**Spp. × $[CO_2]_a$**		
Biomass	**Units**	**d.f.**	***F***	***P***	**d.f.**	***F***	***P***	**d.f.**	***F***	***P***
Aboveground ^{a}	g	**2, 41**	**108.3**	**<0.0001**	**2, 41**	**14.97**	**<0.0001**	4, 41	1.82	0.143
Roots ^{a}	g	2, 41	0.10	0.901	**2, 41**	**5.97**	**0.005**	4, 41	1.76	0.155
Total ^{a}	g	**2, 41**	**104.7**	**<0.0001**	**2, 41**	**14.64**	**<0.0001**	4, 41	0.63	0.642

Data transformations: ^{a}natural log.