

Growth responses of African savanna trees implicate atmospheric [CO₂] as a driver of past and current changes in savanna tree cover

BARNEY S. KGOPE^{1,2,3} WILLIAM J. BOND³ AND GUY F. MIDGLEY^{1*}

¹*Global Change and Biodiversity Programme, South African National Biodiversity Institute, Private Bag X7, Claremont, Cape Town, 7735, South Africa (Email: midgley@sanbi.org)*, ²*National Business Initiative, Climate Change and Energy, Auckland Park*, and ³*Botany Department, University of Cape Town, Private Bag, Rondebosch, South Africa*

Abstract Atmospheric CO₂ has more than doubled since the last glacial maximum (LGM) and could double again within this century, largely due to anthropogenic activity. It has been suggested that low [CO₂] contributed to reduced tree cover in savanna and grassland biomes at LGM, and that increasing [CO₂] over the last century promoted increases in woody plants in these ecosystems over the past few decades. Despite the implications of this idea for understanding global carbon cycle dynamics and key global role of the savanna biome, there are still very few experimental studies quantifying the effects of CO₂ on tree growth and demography in savannas and grasslands. In this paper we present photosynthetic, growth and carbon allocation responses of African savanna trees (*Acacia karroo* and *Acacia nilotica*) and a C₄ grass, *Themeda triandra*, exposed to a gradient of CO₂ concentrations from 180 (typical of LGM) to 1000 μmol mol⁻¹ in open-top chambers in a glasshouse as a first empirical test of this idea. Photosynthesis, total stem length, total stem diameter, shoot dry weight and root dry weight of the acacias increased significantly across the CO₂ gradient, saturating at higher CO₂ concentrations. After clipping to simulate fire, plants showed an even greater response in total stem length, total stem diameter and shoot dry weight, signalling the importance of re-sprouting following disturbances such as fire or herbivory in savanna systems. Root starch (per unit root mass and total root starch per plant) increased steeply along the CO₂ gradient, explaining the re-sprouting response. In contrast to the strong response of tree seedlings to the CO₂ gradient, grass productivity showed little variation, even at low CO₂ concentrations. These results suggest that CO₂ has significant direct effects on tree recruitment in grassy ecosystems, influencing the ability of trees to recover from fire damage and herbivory. Fire and herbivore regimes that were effective in controlling tree increases in grassy ecosystems could thus be much less effective in a CO₂-rich world, but field-based tests are needed to confirm this suggestion.

Key words: elevated CO₂, fire, last glacial maximum, root, savanna, starch.

INTRODUCTION

Anthropogenic activities have driven atmospheric CO₂ concentrations to levels exceeding anything plants have had to deal with for at least the last 650 000 years and probably the last 25 million years (Royer 2006). The effects of CO₂ enrichment on plants and ecosystems have been the focus of intense research effort (Poorter & Navas 2003; Ainsworth & Long 2004; Nowak *et al.* 2004). Yet recent reviews question both the relevance of many studies in predicting the ecological effects of CO₂ and the longer-term importance of elevated CO₂ in altering plant biomass production (Korner 2006; Millard *et al.* 2007). Free air CO₂ enrichment studies in temperate forests have shown an initial growth spurt but this is generally not sustained,

especially once the canopy closes and other limitations on forest growth, particularly nutrients, become more important (Nowak *et al.* 2004). In grassland studies, elevated CO₂ has resulted in increased above-ground productivity, which is largely attributed to CO₂-induced reduction in water use resulting in effectively moister soils (Morgan *et al.* 2001), rather than direct effects of CO₂ on plant growth (Korner 2006).

There are, as yet, very few studies on the effects of elevated CO₂ on mixed tree–grass ecosystems (Nowak *et al.* 2004) with the successional potential to grow into woodlands or forests. The grass component in such ecosystems is often maintained by the effects of fire and browsing, which reduces the dominance of trees (Bond 2005). Here we ask what effects CO₂ fertilization may have in a system that is subject to fire and herbivory as major ecological disturbances. Might atmospheric CO₂ level exert control over woody plant success in these ecosystems?

*Corresponding author.

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Savanna ecosystems cover about 20% of the terrestrial land surface. Grass cover, dominated by C₄ species, is more or less continuous but tree cover is very variable in space and time (Scholes & Archer 1997). Savannas are far from steady-state systems. Both fire and herbivory exclusion experiments have resulted in large increases in woody biomass, sometimes resulting in complete biome shifts from wooded grassland to closed forests (Trapnell 1959; Tilman *et al.* 2001; Briggs *et al.* 2005). Over the last century, there has been a trend of increasing woody biomass in many savanna regions (Polley *et al.* 2002; Ward 2005a). This has occurred over a wide rainfall range from semi-arid to humid savannas. Woody plant thickening is generally attributed to changes in land use practice, particularly grazing and fire use (Scholes & Archer 1997), and to episodes of high or low rainfall (Sankaran *et al.* 2005). The phenomenon may also have been influenced by increasing atmospheric CO₂ (Idso 1992; Polley 1997; Polley *et al.* 1999).

Two main mechanisms have been suggested. The first is CO₂-induced reduction in water use. Polley *et al.* (1999) summarized the idea thus: 'on grasslands, a decline in stomatal conductance that reduces transpiration rate will increase soil water availability during intervals between rainfall events. Woody or other plants that were previously excluded by low water availability may be favoured as a result'. Although stomatal responses to increased CO₂ were first thought to be restricted to C₃ plants, it has since been shown that both C₃ and C₄ grasses reduce transpiration under elevated CO₂ (Ward *et al.* 1999; Morgan *et al.* 2001; Stock *et al.* 2005). CO₂-induced changes in soil moisture probably account for the large increases in a woody shrub in one of the very few field experiments testing the effects of CO₂ on woody plant thickening. Morgan *et al.* (2007) reported an approximately 40-fold increase in biomass of the shrub, *Artemisia frigida*, in semi-arid short grass prairies subjected to 5 years of elevated CO₂.

The second mechanism invokes direct effects of CO₂ on plant growth and allocation. Bond and Midgley (2000) suggested that woody plants would be favoured by increased rates of re-sprouting after injury in elevated CO₂. Fire is a very frequent disturbance in savannas, which are estimated to account for more than 80% of the global area burnt annually (Mouillot & Field 2005). To establish in frequently burnt grasslands, woody seedlings must rapidly acquire the ability to re-sprout. Once established, saplings then have the formidable challenge of emerging above the flame zone, the fire trap, between successive top-killing fires before they can grow into relatively fire-proof adult trees. Saplings re-sprout after repeated fires for decades before either dying, or finally emerging above the lethal fire trap. The probability of escape is influenced by both the frequency and intensity of fires, and

sapling growth rates to escape height. Bond and Midgley (2000) suggested that re-sprouting and post-burn re-growth would be sensitive to atmospheric CO₂. They noted that seedlings and saplings grow in open, well-lit, conditions relative to closed canopy of forests and may therefore be expected to benefit most from CO₂ fertilization. After a burn, soils are enriched by nutrients from ash and a nitrogen mineralization flush. Because top-kill reduces above-ground, but not below-ground structures, the carbon balance of juvenile forms is far from the equilibrium expected in undisturbed plants. Therefore, under low CO₂ concentrations, re-growth of woody plants after injury such as fire is likely to be carbon limited due to a slow rate of accumulation of below-ground starch reserves. These reserves are critical to support re-sprouting (Bond & Midgley 2000; Hoffmann *et al.* 2000; Schutz *et al.* 2009). Thus, in moist savannas subject to frequent fires, rising CO₂ may promote faster seedling acquisition of sprouting ability and faster sapling growth rates to fire-proof sizes.

Bond and Midgley (2000) suggested that at low CO₂, such as during the last glacial, saplings may not have grown fast enough to escape the fire trap and trees would be eliminated from savannas. At ambient and elevated CO₂, growth rates would be enhanced, increasing escape probabilities, and contributing to increases in woody cover. These predictions were tested with simulation models by coupling simulated CO₂ growth responses of grasses and trees from a dynamic global vegetation model (Woodward *et al.* 2001) with a detailed demographic model modelling fire effects on savanna tree demography (Higgins *et al.* 2000). Simulated tree populations went extinct at last glacial maximum (LGM) CO₂, survived at low densities at pre-industrial CO₂, and showed large increases at ambient CO₂ (Bond *et al.* 2003). The simulations were consistent with the paleoecological (Scott 1999) and recent historical record. However experimental evidence for the assumed CO₂ effects on re-sprouting and stem growth is lacking. Demonstration of such a response would implicate atmospheric CO₂ level as a key controller of the ability of woody species to achieve escape height above the grass-fuelled flame zone.

Here we report the results of an open-top chamber experiment conducted in a glasshouse in which two African acacia species and a common savanna grass were exposed to a gradient of CO₂ concentrations representing LGM, pre-industrial, ambient and supra-ambient conditions. We were particularly interested in tree growth at below-ambient CO₂ to determine whether changes in growth responses were consistent with possible CO₂ effects on woody plant thickening observed over the last century. We focus on root starch accumulation, re-sprouting vigour and stem growth rates of two dominant woody species, and above-ground growth of a common C₄ grass. The experiment

was conducted with plants established from seed. We simulated fire effects on seedling establishment by clipping the plants after the first growing season. The re-sprouting response was observed over a second growing season. Thus the experiment simulates CO₂ effects over the seedling establishment phase but also provides insights into CO₂-induced changes in growth rates likely to affect sapling growth rates. Grass competition is often a major factor limiting woody seedling establishment although, in some studies, grass facilitates establishment (O'Connor 1995). We did not simulate grass competition in the experiment (but see Polley *et al.* 2002).

METHODS

Plant cultures

Seeds of *Acacia karroo* Hayne and *Acacia nilotica* (L.) Willd were obtained from a mesic savanna area in Hluhluwe game reserve in Kwa-Zulu-Natal, South Africa. Both species are common in South African savannas and both are important native invasive species responsible for woody thickening. Within the source area, *A. karroo* is the more fire-tolerant species occurring in frequently burnt mesic savannas whereas *A. nilotica* is more common in heavily grazed savannas (Bond *et al.* 2001). Seeds were pre-germinated in seedling trays, followed by transfer to long plant pots (diameter of 10 cm and length of 50 cm) with filter/sterile sand. We note that pot experiments may produce artefactual growth responses (Arp 1991; Thomas & Strain 1991).

Both acacias are nitrogen-fixing legumes. Plants were inoculated with field-collected nodules and then transferred to open-top chambers. Plants were exposed to the various CO₂ treatments 1 week after germination. Together with the two tree species, we also included a C₄ grass, *Themeda triandra*, in the experiment. *Themeda* is the dominant grass species in many frequently burnt grasslands and savannas in South Africa, including the Hluhluwe area from which the two acacias were sourced. Grasses were established from tillers of *Themeda* obtained from a C₄ grassland in southern Kwa-Zulu-Natal, South Africa (Stock *et al.* 2005).

The experiment was conducted in a polycarbonate greenhouse that transmits 85–95% of incident photosynthetic photon flux density at Kirstenbosch, Cape Town in South Africa. Plants were watered twice daily with water from a reservoir and three times a week with full-strength Rorison-nutrient solution (Booth *et al.* 1993). Watering was not adjusted in the second year to account for plant size; however, we note that leaf N was not lower in the second season, nor was it diluted in high CO₂.

CO₂ concentration treatments

We employed a response surface design comprising CO₂ regimes ranging between 180 and 1000 $\mu\text{mol mol}^{-1}$ (180, 280, 370, 550, 700 and 1000 $\mu\text{mol mol}^{-1}$). The CO₂ regimes

Table 1. Mean open-top chamber [CO₂] with standard errors and target [CO₂] over a representative period between August 2001 and May 2002

Target [CO ₂]	Average [CO ₂]
180–200	150 ± 0.31
250–280	240 ± 0.46
370–380	387 ± 0.78
550	517 ± 1.07
700	709 ± 1.10
1000	995 ± 3.20

represent last glacial (180 $\mu\text{mol mol}^{-1}$), pre-industrial (280 $\mu\text{mol mol}^{-1}$), ambient (370 $\mu\text{mol mol}^{-1}$) and elevated (550, 700 and 1000 $\mu\text{mol mol}^{-1}$). Four replicate plants per species were grown in separate pots in open-top chambers. These chambers were rotated every 2 weeks to counter any greenhouse positional effects. Sub-ambient CO₂ levels were obtained by bubbling ambient air through a column of 120 L of 1.25 M solution of industrial grade sodium hydroxide (NaOH) (CJ Petrow Chemicals, South Africa) in sealed 200-L plastic drums. The air was bubbled through the solution by means of a powerful SCL V3 blower (Effepizeta, Italy). The NaOH solution (scrubber) reduced CO₂ in the air to below 150 $\mu\text{mol mol}^{-1}$. The CO₂ scrubber would gradually deteriorate over a period of a week and was renewed every 7 days. Following the scrubbing of air by the NaOH solution, pure CO₂ from gas cylinders (Afrox, PTY, Ltd, South Africa) was bled back into the air stream just before entering the open-top chambers. The CO₂ flow rate was controlled by float metering valves (model DK800, Krohne, Germany), and each chamber was individually calibrated to achieve the desired CO₂ level. CO₂ concentration of each chamber was monitored daily using the Li-820 infra-red gas analyser (Licor, Nebraska, USA). Table 1 shows the mean chamber [CO₂] measured over a representative period (August 2001–May 2002).

Gas exchange measurements

Leaf gas exchange measurements were performed on three plants of each tree species per CO₂ treatment during the first growing season. Twelve months later, following re-sprouting, we measured a smaller subset of chambers, namely 180, 370, 700 and 1000 $\mu\text{mol mol}^{-1}$. Photosynthesis as a function of light (light response curves) was measured on recent fully expanded leaves from each CO₂ regime using a Li-6400 portable photosynthesis system, fitted with the red blue light source and the CO₂ control unit (Li-cor). Leaf temperature was maintained at 28°C by means of a thermocouple attached to the bottom of the leaf. Leaf-to-air vapour pressure deficit was maintained below 2 kPa. Measurements were made from 09.00 hours in the morning to 15.00 hours in the afternoon. Water use efficiency (WUE) was calculated as the ratio of photosynthetic rate (A_{max})/stomatal conductance (g_s) to give $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$.

Plant growth measurements

Total stem length (TSL) and total stem diameter (TSD) at the base of the shoot (approximately 4 cm from soil surface)

were measured on 6-month-old plants (four individuals per species) of *A. karroo* Hayne and *A. nilotica* (L.) Willd. Following these measurements all plants (trees and the grass) were clipped at approximately 2 cm from the soil surface to simulate fire. Above-ground clippings were dried at 65°C to constant dry weight in a draft oven and weighed. Total stem length and TSD measurements were repeated 12 months later immediately after gas exchange measurements. Subsequently, plants were harvested and separated into shoots and roots. Roots were carefully washed free of sand to minimize loss of fine root material before oven drying. Shoots and roots were put in separate brown paper bags and oven dried at 65°C to constant dry weight.

Foliar and root carbohydrate analysis

Root non-structural carbohydrate (sucrose and starch) concentrations were analysed following a slightly modified phenol sulphuric acid protocol (Buisse & Merckx 1993) and samples were extracted in triplicate. Oven-dried milled samples were suspended in 10-mL volume of 80% ethanol (80:20, v : v, ethanol : water). Following centrifuging the supernatants were adjusted to 25 mL in volumetric flasks for spectrophotometric determination of total sugars (Buisse & Merckx 1993). For starch analysis, residues were hydrolysed for 3 h in 5 mL of 3.6% HCL at 100°C, centrifuged and adjusted to 25 mL with 80% ethanol in the volumetric flasks before spectrophotometrically determining the resultant starch content.

Statistical analysis

The experiment used six chambers in a gradient design with four replicate plants per species in each chamber. We did not have the resources to replicate chambers for a given CO₂ regime but the replicates within chambers provide an indication of the variability in plant response for a given treatment. As plants were grown in a series of CO₂ regimes (180 and 1000 µmol mol⁻¹) and each [CO₂] had more than one γ -value, we used a regression with replication and the curve fitting procedure (Unistat 5.6 for Windows, England) to test for the relationship between CO₂ and the measured physiological and growth parameters as described in Sokal and Rohlf (2001). We expected an asymptotic response of tree growth rates to increasing CO₂ based on simulations using a global vegetation model (Bond *et al.* 2003). We thus fitted a hyperbolic regression model to accommodate both curvilinear and linear responses. Plots of residuals were checked for normality and homoscedasticity and there were no apparent violations of assumptions. Curve fitting was carried out on individual points but for purposes of clarity we plotted means with standard errors for each measured parameter per CO₂ treatment (Anderson *et al.* 2001).

RESULTS

Carbon uptake in response to CO₂ treatment

Carbon assimilation (A_{\max}) of both tree species showed a steep increase with increasing [CO₂] saturating at

high [CO₂] during the first growing season (S1), but we noted increasing photosynthetic down-regulation along the [CO₂] gradient in the second season (S2) (Fig. 1; Table 2). The increase in A_{\max} corresponded to a gradual (*A. karroo*) and steep (*A. nilotica*) decrease in g_s triggering a linear increase in instantaneous WUE of both species for both seasons (Table 2).

Carbon allocation to above- and below-ground growth in response to CO₂ treatment

Total stem length in the first growing season increased significantly along the CO₂ gradient for both tree species. The increase in TSL was non-linear along the CO₂ gradient and saturating over elevated [CO₂] (Fig. 2; Table 3). However, growth in TSL over sub-ambient CO₂ was variable in magnitude between the two tree species with *A. karroo* increasing by approximately 180% and *A. nilotica* by approximately 27% on average from sub-ambient to ambient (Fig. 2; Table 3).

Following clipping to simulate fire, both tree species re-sprouted vigorously with the largest responses from sub-ambient to ambient [CO₂] and saturation over elevated [CO₂]. Similarly, TSL increased significantly along the CO₂ gradient (Table 3) with TSL of *A. karroo* and *A. nilotica* increasing by approximately 221% and approximately 150% respectively from sub-ambient to ambient CO₂ (Fig. 2; Table 3). Increase in TSD as a result of CO₂ fertilization exhibited a similar growth pattern to TSL for both tree species in both seasons (Table 3).

Shoot dry weight (SDW) of both tree species showed a non-linear but strong positive response to the increase in CO₂ fertilization (Fig. 3; Table 3). At the end of the first growing season, SDW had increased by 529% in *A. karroo* and 110% on average in *A. nilotica* under ambient relative to sub-ambient CO₂ treatments. A further increase in [CO₂] from ambient to elevated CO₂ significantly increased SDW of *A. nilotica* by 86%. The re-sprouting response after clipping (simulating fire) was strongly influenced by CO₂ concentration with *A. karroo* SDW of re-sprouted shoot material increasing by 366% from sub-ambient to ambient [CO₂] and *A. nilotica* increasing by 133% on average. The two tree species differed in their re-sprouting response from ambient to elevated CO₂ with SDW of the re-sprouts for *A. karroo* decreasing slightly whereas *A. nilotica* continued to increase.

Root dry weight of both tree species responded strongly across the CO₂ gradient with the biggest change occurring between sub-ambient and ambient [CO₂] for *A. karroo* (292%) and *A. nilotica* (91%) (Fig. 4; Table 3). Starch accumulation in the roots exhibited significantly positive responses to increasing CO₂ but differed for the two tree species (Fig. 5a;

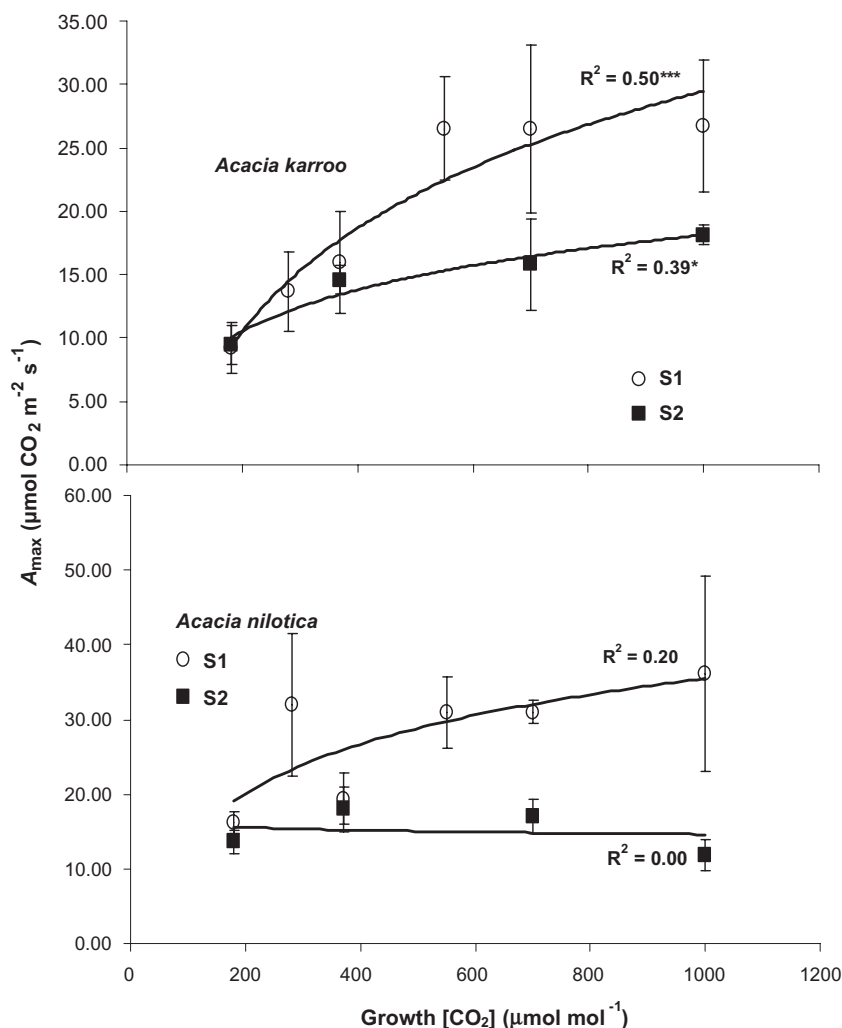


Fig. 1. Photosynthesis as a function of light (A_{\max}) measured in plants grown in chambers across a gradient of CO₂. Circles (○) denote the first growth season (S1) after which plants were clipped and squares (■) denote the second growth season (S2) in which re-sprouting material was measured. Table 2 lists regression models used. Symbols represent * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ and each point with error bars denotes a mean of four measurements.

Table 3). *Acacia karroo* showed a relatively linear increase while *A. nilotica* exhibited a stronger asymptotic response with the increase in [CO₂]. The different responses suggest a larger below-ground sink in *A. karroo* compared with *A. nilotica*. *Acacia nilotica* showed the greatest starch accumulation from below-ambient to ambient CO₂ while *A. karroo* continued to respond strongly to above-ambient CO₂ (Fig. 5a). The total amount of root starch (root dry weight × starch concentration) gives an estimate of the magnitude of the carbon resource available for re-sprouting. It is clear that total starch in roots was significantly enhanced by increasing CO₂ in both species (Fig. 5b). *Acacia karroo* allocated more carbon to roots at below-ambient CO₂ and more to shoots with increasing CO₂ (Fig. 6; Table 3). In contrast, the root : shoot ratio of

A. nilotica was low under sub-ambient CO₂ but increased significantly with increasing CO₂ concentrations (Fig. 6; Table 3).

Grass responses

The C₄ grass, *T. triandra*, was grown alongside the two *Acacia* species in the experiment, in separate pots. Cumulative above-ground biomass production, the source of fuels for savanna fires, showed a flat response curve across the CO₂ gradient (Fig. 7; Table 3). Under sub-ambient [CO₂] *T. triandra* had more than twofold greater above-ground biomass than *A. karroo* but this was reversed under elevated [CO₂] (Fig. 7).

Table 2. Regression models for responses of leaf level gas exchange of *Acacia karroo* and *Acacia nilotica* across the CO₂ treatments

Species	Season	F-stats	P-value	a	b	R ²
Photosynthetic rate (A_{\max}) ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)						
<i>A. karroo</i>	S1	16.16	0.00	47.81	633.41	0.50
<i>A. karroo</i>	S2	6.91	0.02	21.42	211.20	0.39
<i>A. nilotica</i>	S1	3.90	0.07	42.93	233.17	0.20
<i>A. nilotica</i>	S2	0.00	0.98	14.79	1.26	0.00
Stomatal conductance (g_s) ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)						
<i>A. karroo</i>	S1	3.53	0.08	0.11	-99.33	0.18
<i>A. karroo</i>	S2	1.75	0.21	0.25	-72.70	0.14
<i>A. nilotica</i>	S1	2.57	0.13	0.21	-103.86	0.14
<i>A. nilotica</i>	S2	393.51	0.00	0.09	-167.56	0.97
Water use efficiency (A_{\max}/g_s) ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$)						
<i>A. karroo</i>	S1	26.65	0.00	3.E + 15	1.E + 16	0.62
<i>A. karroo</i>	S2	18.19	0.00	133.98	723.98	0.62
<i>A. nilotica</i>	S1	9.50	0.01	525.19	2066.13	0.37
<i>A. nilotica</i>	S2	9.28	0.01	1.82E + 16	4.17E + 16	0.46

The regression model tested for each parameter was hyperbolic ($y = ax/(b + x)$). *P*-values test for slopes of the response curve significantly different from zero and were obtained from non-linear regression models (Unistat 4.5) using actual values for each trait. S1, first growing season; S2, second growing season after clipping.

Table 3. Regression models fitted to above- and below-ground growth response of *Acacia karroo* and *Acacia nilotica* across the CO₂ treatments

Species	Season	F-stats	P-value	a	b	R ²
Total stem length (cm)						
<i>A. karroo</i>	S1	11.32	0.00	108.16	278.47	0.34
<i>A. karroo</i>	S2	5.55	0.03	413.81	281.43	0.17
<i>A. nilotica</i>	S1	13.67	0.00	78.50	85.62	0.38
<i>A. nilotica</i>	S2	6.90	0.02	347.11	223.75	0.25
Total stem diameter (mm)						
<i>A. karroo</i>	S1	13.90	0.00	12.87	281.63	0.39
<i>A. karroo</i>	S2	10.54	0.00	34.49	360.42	0.32
<i>A. nilotica</i>	S1	3.87	0.06	8.22	68.40	0.15
<i>A. nilotica</i>	S2	11.73	0.00	29.88	440.22	0.36
Shoot dry weight (g)						
<i>A. karroo</i>	S1	13.58	0.00	96.85	1676.17	0.38
<i>A. karroo</i>	S2	9.87	0.00	109.22	1061.44	0.31
<i>A. nilotica</i>	S1	3.46	0.08	28.38	341.17	0.14
<i>A. nilotica</i>	S2	9.42	0.01	54.97	915.36	0.31
<i>T. triandra</i>	S1 + S2	0.56	0.46	28.86	30.05	0.02
Root dry weight (g)						
<i>A. karroo</i>	S2	10.41	0.00	87.53	619.86	0.32
<i>A. nilotica</i>	S2	7.63	0.01	67.78	890.58	0.26
Root : shoot ratio						
<i>A. karroo</i>	S2	4.72	0.04	1.03	-91.79	0.18
<i>A. nilotica</i>	S2	7.75	0.01	2.46	472.41	0.26
Root starch ($\mu\text{g mg}^{-1}$ dry weight)						
<i>A. karroo</i>	S2	44.73	0.00	450.46	174.73	0.74
<i>A. nilotica</i>	S2	38.45	0.00	572.41	470.07	0.71

Cumulative above-ground biomass for the trees and *Themeda triandra*, a C₄ grass, are also shown. The regression model for each parameter was hyperbolic ($y = ax/(b + x)$). *P*-values test for slopes of the response curve significantly different from zero and were obtained from non-linear regression models (Unistat 4.5) using actual values for each trait.

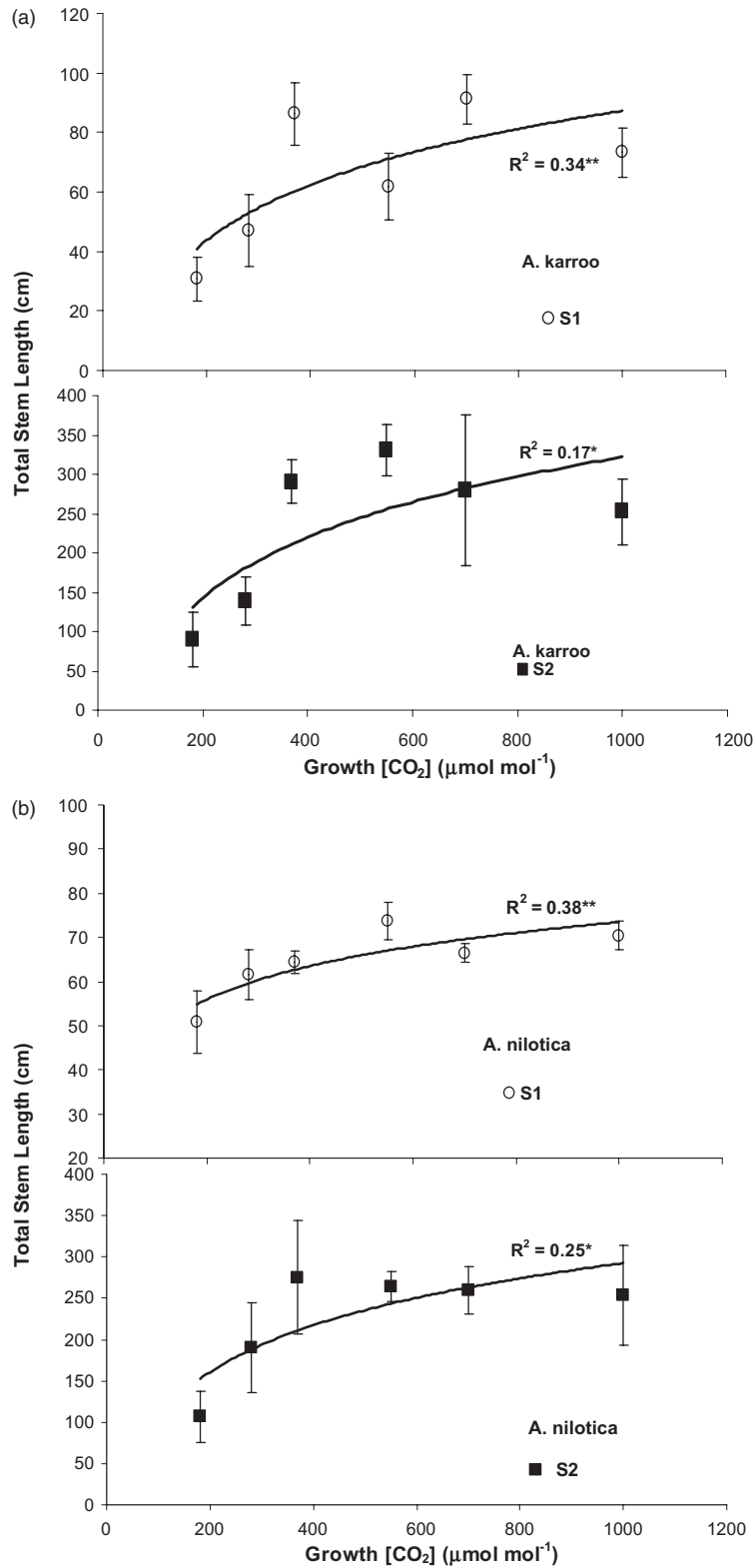


Fig. 2. Total stem length of *Acacia karroo* (a) and *Acacia nilotica* (b) across the CO₂ treatments. Circles (○) denote stem length after the first growth season (S1) and squares (■) denote stems that re-sprouted after clipping at the end of the second growth season (S2). Table 3 lists regression models used. Symbols represent **P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.001 and each point with error bars denotes a mean of four measurements.

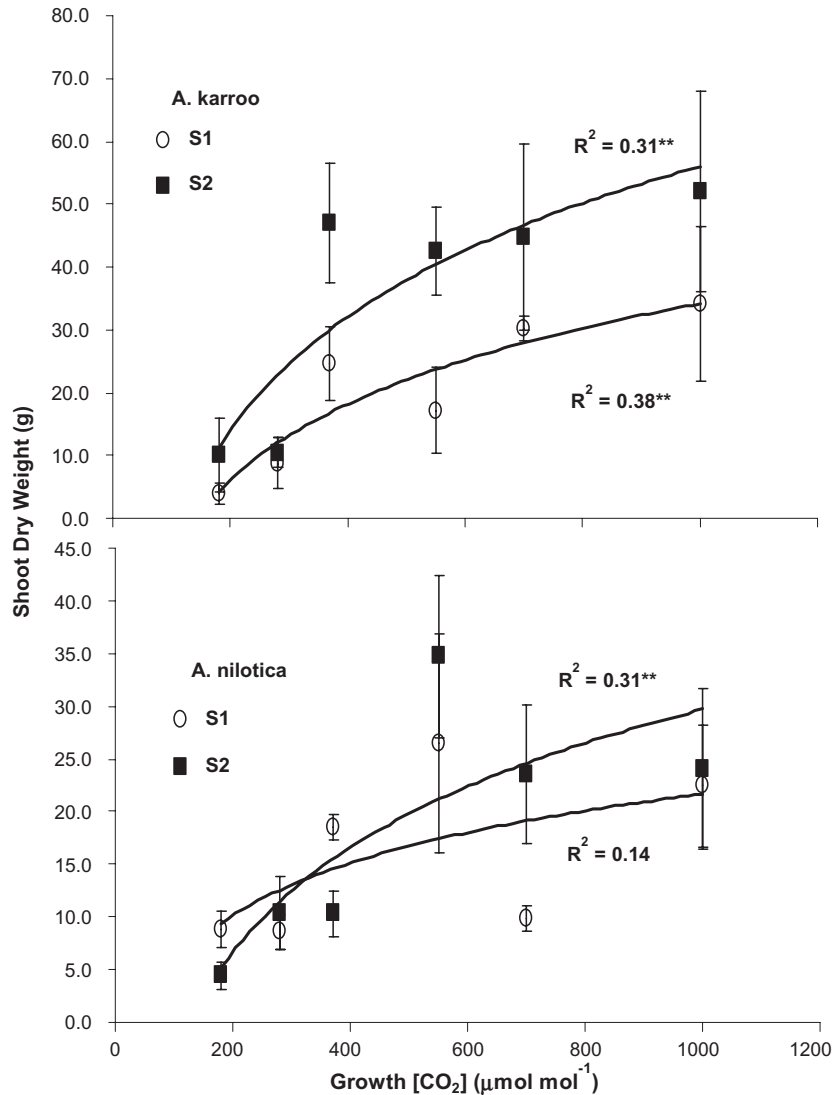


Fig. 3. Shoot dry weight of *Acacia karroo* and *Acacia nilotica* across the CO₂ treatments. Circles (○) denote stem length after the first growth season (S1) and squares (■) denote stems that re-sprouted after clipping at the end of the second growth season (S2). Table 3 lists regression models used. Symbols represent * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ and each point with error bars denotes a mean of four measurements.

DISCUSSION

The goal of this study was to determine whether changes in African savanna tree growth in response to a change in [CO₂] are large enough to help explain woody plant thickening observed over the last century (Bond & Midgley 2000). We were particularly interested in tree growth at below-ambient CO₂ and below-ground carbon investment, hence our emphasis on response variables that help seedlings cope with fire and herbivory. Re-sprouting vigour and stem growth rates are of particular importance in frequently burnt savannas. We found that photosynthesis and the growth measures showed an asymptotic response with the increase in [CO₂] with the biggest response occur-

ring under ambient relative to sub-ambient [CO₂]. The asymptotic growth response of these species implies that future tree responses to anticipated increases in atmospheric CO₂ are likely to diminish relative to the past. This result is consistent with earlier reports on the response of C₃ and C₄ annuals cultured across a series of CO₂ regimes (Polley *et al.* 1993; Sage 1995; Anderson *et al.* 2001; Ward 2005b; Cunniff *et al.* 2008).

Our results indicate no pot effects in the first growing season as the cutting treatments to simulate fire were followed by much larger shoot growth increments in the second growing season even despite photosynthetic down-regulation (Figs 1–3). Down-regulation of photosynthesis under elevated CO₂ has

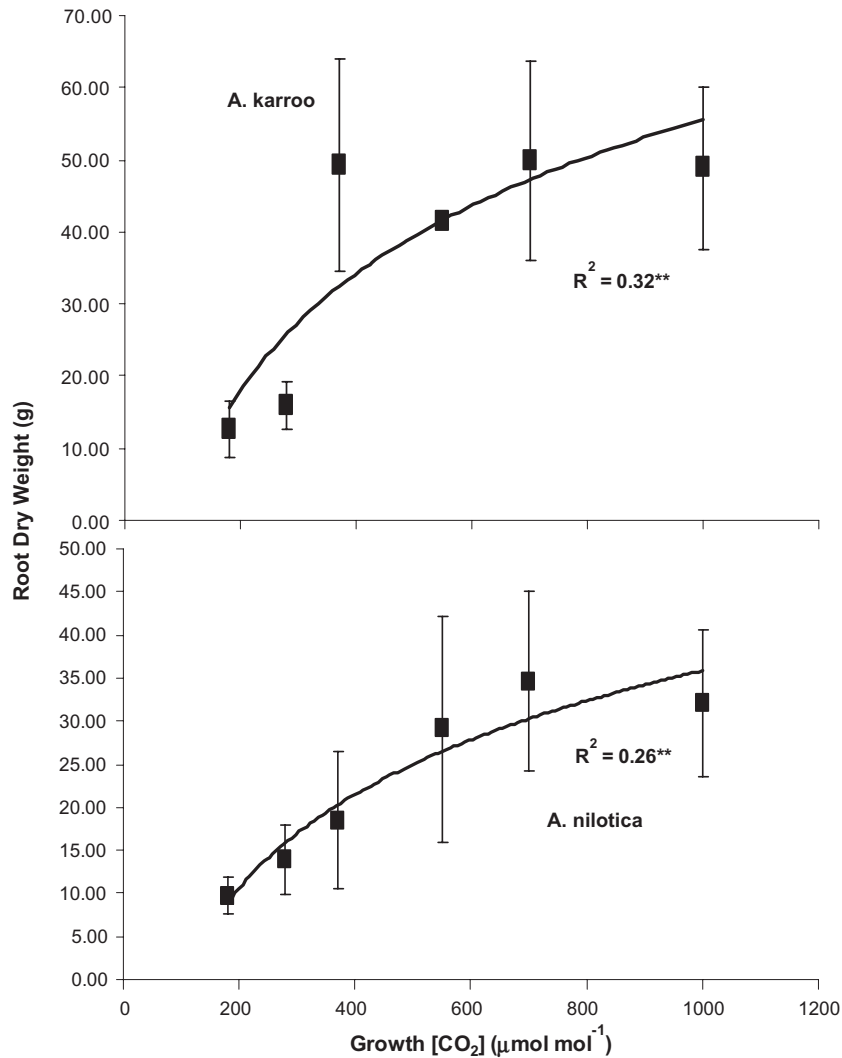


Fig. 4. Root dry weight of *Acacia karroo* and *Acacia nilotica* across the CO₂ treatments. Root dry weight was measured after final harvest (S2). Table 3 provides description of regression models used. Symbols represent * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ and each point with error bars denotes a mean of four measurements.

been linked with an imbalance between source and sink activity, nutrient limitations and the dilution of leaf protein content by carbohydrate accumulation (Stitt 1991; Gunderson & Wullschleger 1994; Saxe *et al.* 1998). The down-regulation we observed may reflect sink limitation, which developed over several months, imposed by limited soil volume especially for saplings exposed to ambient and elevated CO₂, and are not expected to occur under field conditions. Nevertheless, vigorous re-sprouting (re-growth) was recorded in the ambient and high-CO₂-grown plants even in the second season after down-regulation was observed, due to their larger below-ground carbon reserves. This supports the contention that the ecological effects of elevated CO₂ on re-sprouting ability remained despite down-regulation effects that may well be an artefact of the limited pot volume.

Our findings corroborate earlier reports on the effects of elevated CO₂ on soil water content and increased seedling establishment (Morgan *et al.* 2001; Polley *et al.* 2002). The acacias had higher A_{max} , reduced g_s and significantly higher WUE with increasing CO₂ despite the well-watered mesic experimental conditions. However we also show that increased CO₂ altered the growth rate, carbohydrate reserves and structural and chemical defences of the seedlings – in short, their carbon allocation constraints. Changes in [CO₂] from pre-industrial times to the present have effectively produced acacia ‘super seedlings’ in relation to their growth potential over the past several million years. Compared with a century ago, tree seedlings should be much more resistant to fire because of increased root reserves. Where fires once killed seedlings, they are unlikely to do so today resulting in much

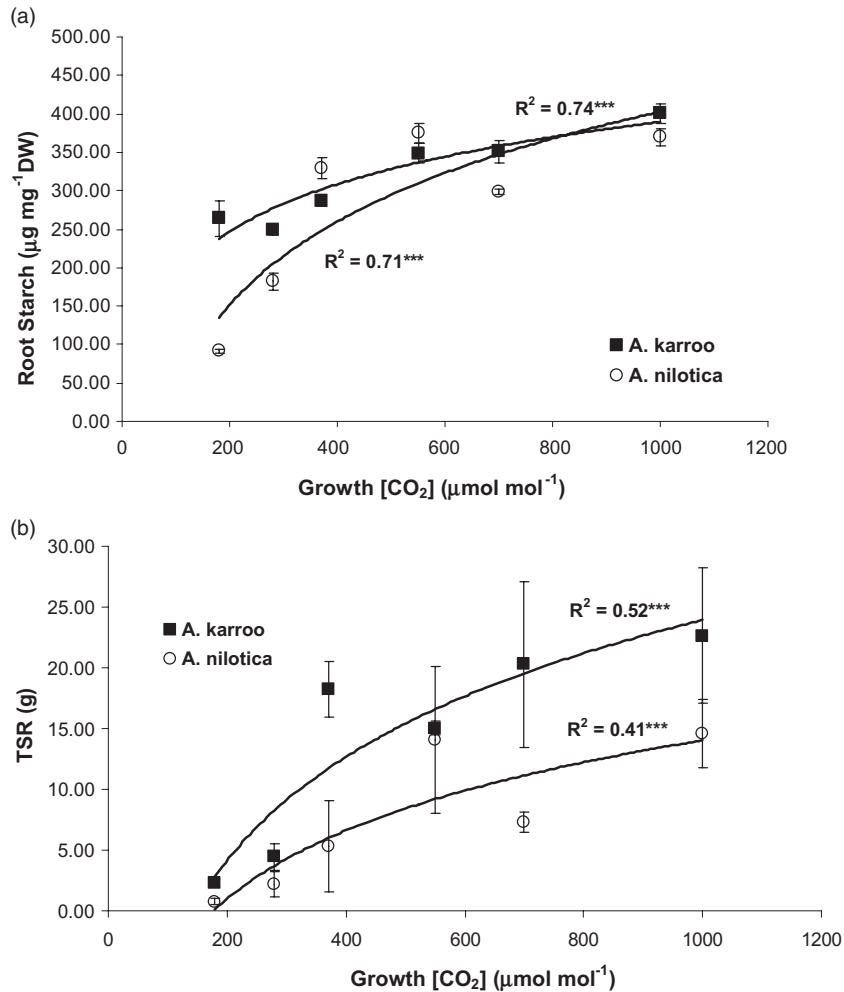


Fig. 5. Below-ground (root) starch concentration of *Acacia karroo* and *Acacia nilotica* across the CO₂ treatments. (a) Measurements were made at final harvest (S2). Table 3 provides description of regression models used. Symbols represent * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ and each point with error bars denotes a mean of four measurements. DW, dry weight. (b) Total starch in roots (TSR) available for re-sprouting in *A. karroo* and *A. nilotica* across the CO₂ treatments. Measurements were made at final harvest (S2). Symbols represent * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ and each point with error bars denotes a mean of four measurements.

higher seedling recruitment rates. The rate of sapling release to adult height classes will also be greatly enhanced because they are able to grow out of the fire trap more rapidly. They should also be better defended against mammal browsers and insect herbivores. Both structural (spines) and chemical (tannins) defences showed significant increases with increasing [CO₂] (Kgope *et al.*, in prep., 2009).

Our results provide experimental support for suggestions and simulation studies predicting that reductions in [CO₂] alone could have led to loss of tree cover in grassy environments in the last glacial (Bond *et al.* 2003; Harrison & Prentice 2003). While C₄ grass productivity was little affected by glacial CO₂ relative to ambient in this experiment, tree seedlings showed slow growth rates, reduced sprouting ability and less effective

defences. If similar responses occurred under field conditions, then low CO₂ would have tipped the tree/grass balance in favour of grasses. In contrast, the large increases in [CO₂] from industrial emissions over the last century would now favour trees at the expense of grasses.

Our results demonstrate that CO₂ levels typical of the LGM limited the growth of trees through reduced carbon uptake despite enhanced stomatal conductance and carboxylation efficiency. However, enhanced carbon uptake under elevated [CO₂] appears to be partially due to their larger carbon sinks in the form of multi-stems, increased stem diameters, more branches, larger roots and below-ground storage. Juveniles of many woody plants in open ecosystems store carbohydrate reserves in the roots that subsidize

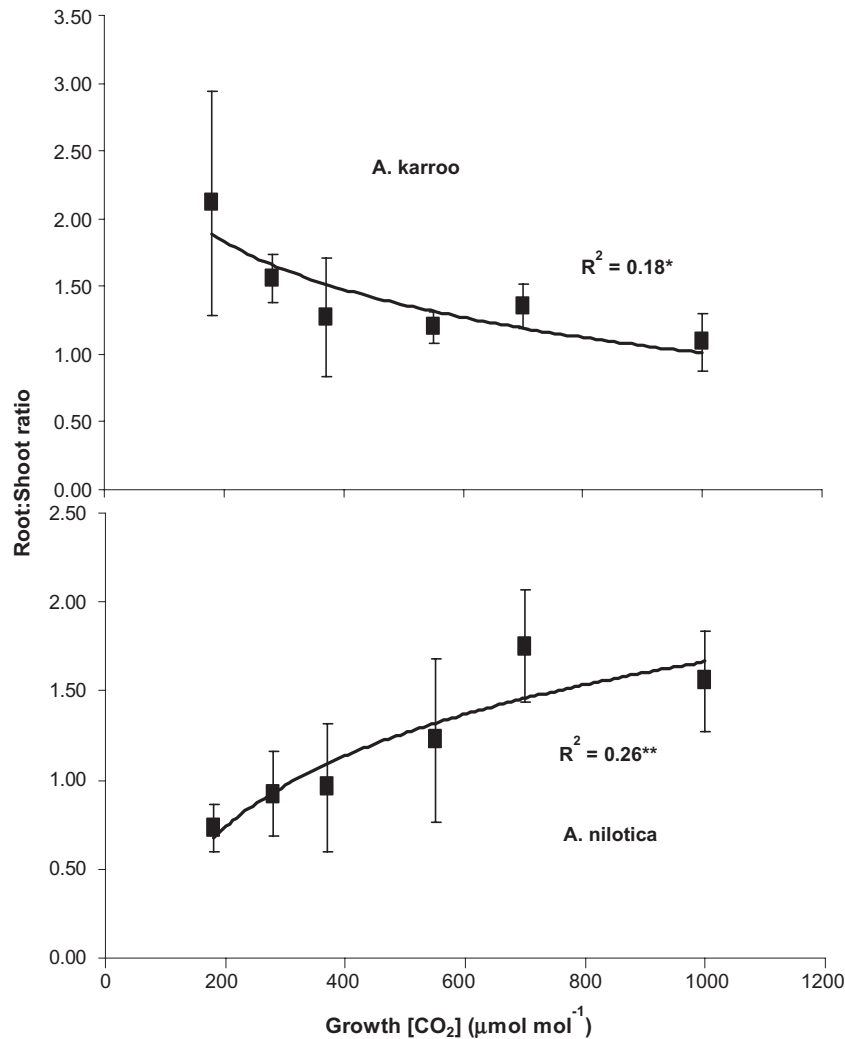


Fig. 6. Root : shoot ratio of *Acacia karroo* and *Acacia nilotica* across the CO₂ treatments. Measurements were made at final harvest (S2). Table 3 provides description of regression models used. Symbols represent * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ and each point with error bars denotes a mean of four measurements.

re-sprouting after fire or browse damage (Hoffmann & Franco 2003; Hoffmann *et al.* 2004). Others, particularly shrubs, spread vegetatively by clonal root-suckering. These large below-ground carbon sinks will help enhance tree and shrub growth responses to increasing [CO₂] (Hoffmann *et al.* 2000). However, the divergent changes in root : shoot ratio in the two tree species investigated highlight our poor knowledge about controls of carbon allocation patterns in savanna trees in response to CO₂ availability, and warrant further attention especially due to the role of this response in broader issues of biogeochemistry.

It is important to note that CO₂ sensitivity of these African savanna trees is the greatest in the region between sub-ambient and current ambient atmospheric [CO₂] diminishing as CO₂ rises into the future.

The latter finding is consistent with DGVM simulations (Bond *et al.* 2003) and palaeo-records, which indicate that trees disappeared from current savanna sites in South Africa during the LGM (Scott 1999), re-appeared in the Holocene, and have rapidly increased over the last half century. Even if the effects of CO₂ on sapling growth are transient, they can have marked ecosystem effects by promoting seedling establishment and sapling escape from the flame zone. Increased recruitment of adult trees can change open savannas to woodlands with large implications for carbon sequestration and the services provided by African savanna ecosystems. There is a pressing need for field experiments under competitive conditions to further validate the importance of CO₂ in tree invasions of grassy ecosystems (Midgley *et al.* 2007).

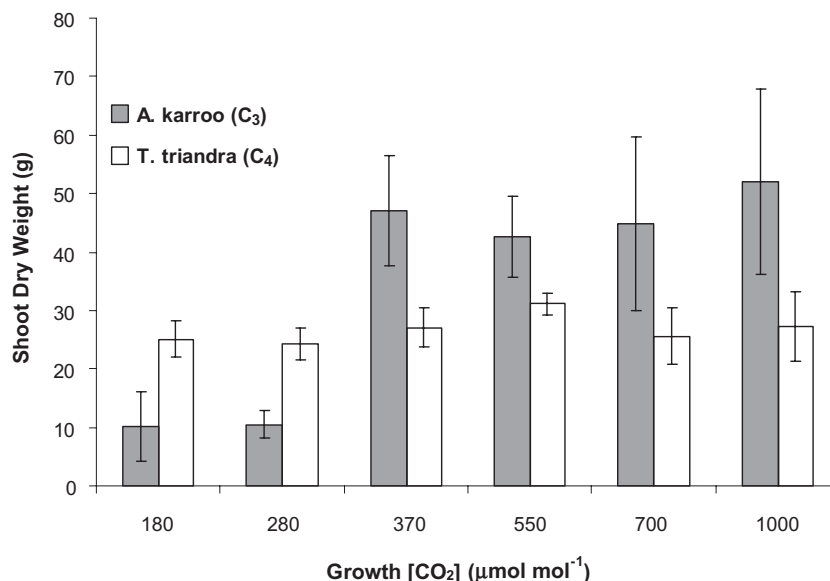


Fig. 7. Dry matter accumulation of *Acacia karroo* (C₃, tree; shaded bars) and *Themeda triandra* (C₄ grass; open bars) across the CO₂ treatments at final harvest.

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