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Elevated CO₂ enhances resprouting of a tropical savanna tree

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Abstract The savannas (cerrado) of south-central Brazil are currently subjected to frequent anthropogenic burning, causing widespread reduction in tree density. Increasing concentrations of atmospheric CO₂ could reduce the impact of such frequent burning by increasing the availability of nonstructural carbohydrate, which is necessary for resprouting. We tested the hypotheses that elevated CO₂ stimulates resprouting and accelerates replenishment of carbohydrate reserves. Using a factorial experiment, seedlings of a common Brazilian savanna tree, *Keilmeyera coriacea*, were grown at 350 ppm and 700 ppm CO₂ and at two nutrient levels. To simulate burning, the plants were either clipped at 15 weeks or were left unclipped. Among unclipped plants, CO₂ and nutrients both stimulated growth, with no significant interaction between nutrient and CO₂ effects. Among clipped plants, both CO₂ and nutrients stimulated resprouting. However, there was a strong interaction between CO₂ and nutrient effects, with CO₂ having a significant effect only in the presence of high nutrient availability. Under elevated CO₂, carbohydrate reserves remained at higher levels following clipping. Root total nonstructural carbohydrate remained above 36% in all treatments, so carbohydrate reserves did not limit regrowth. These results indicate that under elevated CO₂ this species may be better able to endure the high frequency of anthropogenic burning in the Brazilian savannas.

Key words Savanna · Cerrado · Fire · Elevated CO₂ · Carbohydrate

Introduction

Humans have had an enormous impact on the Brazilian savanna (cerrado) ecosystem. The region originally consisted of 1.8×10⁶ km² of savanna, but already 40% has been converted to pasture and crops with the remaining being transformed at a rate of 1.7% per year (Klink et al. 1995). The remaining areas of intact savanna are typically subjected to burning at intervals of 1–3 years (Coutinho 1990; Mistry 1998), rates that are causing large-scale reductions in tree density (Hoffmann 1999). The current rate of burning exceeds the fire frequency under indigenous occupation, which has been estimated at 3–10 years (Ratter et al. 1973; Eiten 1975; Eiten and Goodland 1979).

The ability of trees to resprout under frequent burning is dependent upon carbohydrate reserves, which are replenished between burns. Replenishment of carbohydrate reserves following burning or cutting of woody plants may require 1–2 years (Bell and Pate 1996; Kays and Canham 1991; Miyanishi and Kellman 1986). Since atmospheric CO₂ concentration is known to have large effects on plant carbohydrate status (Cruz et al. 1997; Bassirad et al. 1996; den Hertog et al. 1996; Read and Morgan 1996; Körner and Miglietta 1994), increasing levels of CO₂ may have important implications for plant responses to fire. Atmospheric CO₂ has been increasing since last century and is expected to reach twice pre-industrial levels by the end of the twenty-first century (Houghton 1997). This increase could reduce the impact of frequent anthropogenic burning on tropical savanna trees by increasing pre-burn carbohydrate reserves and increasing the rate at which carbohydrate reserves are replenished following fire.

However, nutrients reserves can also limit the regeneration of aerial biomass following burning (Miyanishi and Kellman 1986). Nutrient availability of cerrado soils

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is considered critically low, particularly with regard to P and Ca (Lopes and Cox 1977). If nutrients rather than carbohydrate reserves limit resprouting under these conditions, elevated CO₂ may have a limited effect on resprouting.

We hypothesized that CO₂ should increase the capacity of savanna tree seedlings to resprout, and increase the rate at which carbohydrate reserves are replenished following clipping. The effect of CO₂ was tested under two levels of nutrient availability to examine potential interactions between CO₂ and nutrients.

Materials and methods

General methods

The experiments were performed on *Kielmeyera coriacea* Mart. (Guttiferae), a drought-deciduous tree common throughout most of the cerrado savannas of south-central Brazil (Ratter et al. 1996). Seedlings of this species exhibit high survival when subjected to fire while less than 1 year old (Hoffmann 2000). Seeds were obtained from several individuals near Brasilia, D.F., Brazil at 15°52'S, 47°51'W.

Seeds were germinated and grown in six environmentally controlled greenhouse modules in Cambridge, Massachusetts, United States, with 12 h supplemental lighting per day from metal halide lamps. Three of the greenhouse modules were maintained at a CO₂ concentration of 350 ppmv and three at 700 ppmv. Within a week of germination, 216 seedlings were transplanted to 35-cm-deep pots in a 1:2 mixture of sand:perlite and were watered daily. Seedlings were randomly assigned to two nutrient treatments. High-nutrient seedlings were given 50 ml of 1/5 Hoagland's solution and low-nutrient seedlings were given 50 ml of 1/20 Hoagland's solution, weekly. The seedlings were used for two experiments described below, which were run concurrently under the same growing conditions.

Experiment 1: effects of CO₂ and nutrients on growth and allocation

To test for effects of elevated CO₂ and nutrients in the absence of clipping, 144 seedlings were randomly chosen and assigned to a factorial design with the two levels of nutrients, two CO₂ concentrations, and four harvest dates (10, 15, 20, and 25 weeks). At each date, nine randomly chosen individuals were harvested from each nutrient/CO₂ combination. Individuals were separated into leaves, stem, cotyledons, coarse roots and fine roots. Samples were dried at 100°C for 1–2 h, then for at least a week at 60°C.

Experiment 2: effect of CO₂ and nutrients on resprout capacity

Seventy-two seedlings were randomly chosen for a clipping experiment to simulate the effects of fire. These individuals were randomly assigned to nutrient and CO₂ levels and were interdispersed among the seedlings of experiment 1. They were grown in the same manner as above, except they were clipped at ground level when 10 weeks old. They were allowed to resprout, and half were harvested 5 weeks after clipping, with the remaining being harvested 10 weeks after clipping. All material was dried and weighed as above.

Carbohydrate analyses

A subsection of the harvested seedlings were chosen for quantifying root total nonstructural carbohydrate (TNC). To test for treat-

ment effects on TNC, individuals were chosen from the date of clipping and from the two post-clipping dates. At the latter two dates, only clipped individuals were sampled for TNC analysis.

Nonstructural carbohydrates were extracted from 50-mg tissue samples of finely ground taproots with a commercial amylase preparation (0.2% Clarase 40,000) for 24 h at 40°C. Tissue samples were pre-boiled in a small volume of water to stop any endogenous tissue enzyme activity. The Clarase mixture contained amylase, invertase, and maltase activities, so insoluble starch and sucrose were hydrolyzed enzymatically, but other soluble oligo- or polysaccharides were acid hydrolyzed (0.6 kmol m⁻³ HCl) in the extract. Analyses employed potassium ferricyanide in an automated Technicon Autoanalyzer II (Chatterton et al. 1987). Carbohydrate concentrations were expressed on a structural dry weight (g g⁻¹ specific dry weight) basis (dry weight minus TNC) to avoid errors associated with simultaneous changes in carbohydrate content and dry weight (Moser et al. 1982).

Statistical analyses

Statistical analyses were conducted using SYSTAT 5.01. Three-factor ANOVA was used to test the effects of CO₂, nutrients, and harvest date on plant biomass. Variation in original seed size contributed considerably to the overall variation in plant mass. Because seed mass could not be adequately measured without destructively drying the seeds, we used cotyledon area as a measure of seed size. To remove this source of variation, cotyledon area was included as a covariate in this ANOVA. Cotyledon area was unaffected by CO₂ level ($F=1.193$, $P=0.28$) and nutrient level ($F=1.190$, $P=0.28$).

Relative growth rate was calculated using the formula $RGR=(\ln M_2 - \ln M_1)/(t_2 - t_1)$, where M_1 and M_2 are plant dry weights at times t_1 and t_2 , respectively.

Results and discussion

Experiment 1: effects of CO₂ and nutrients on growth and allocation

Total plant biomass was affected by both CO₂ ($F_{1,128}=25.3$, $P<<0.0001$, Fig. 1A) and nutrients ($F_{1,128}=8.9$, $P=0.003$; Fig. 1B), with no interaction between CO₂ and nutrient effects ($F_{1,128}=2.43$, $P=0.12$). Dry weight of leaves, cotyledons, coarse roots, and fine roots were all greater under elevated CO₂ (Table 1). Only stem biomass was unaffected. Root:shoot ratio was greater under elevated CO₂ ($F_{1,128}=5.41$, $P=0.02$; Fig. 1C) and under low nutrients ($F_{1,128}=9.48$, $P=0.003$; Fig. 1D). However, root:shoot ratio increased with plant size through time ($F_{1,128}=159.0$, $P<<0.0001$), so the effect of CO₂ on root:shoot ratio might be explained by accelerated ontogeny under elevated CO₂. To test this, total plant mass, rather than harvest date, was used as an independent variable in the analysis. In this analysis, CO₂ had no effect on root/shoot ratio independently of the effect of accelerated ontogeny ($F_{1,139}=0.34$, $P=0.56$).

After the first harvest, there was little growth in shoot biomass (partial $r^2=0.027$, $P=0.053$), or fine root biomass (partial $r^2=0.029$, $P=0.045$). In contrast, coarse root biomass increased very significantly throughout the experiment (partial $r^2=0.557$, $P<<0.0001$). Since the coarse roots are probably not directly involved in resource uptake, the capacity of the plants to acquire resources did

Fig. 1A-D Effect of CO₂ concentration and nutrient level on growth and allocation of *Kielmeyera coriacea* (mean±SE). Elevated CO₂ caused an overall increase in **A**, biomass ($P < 0.001$) and **C**, root:shoot ratio ($P = 0.02$). High nutrient availability had a positive effect on **B** plant biomass ($P = 0.003$) and a negative effect on root:shoot ratio **D** ($P = 0.003$). Growth more closely fit a linear relationship (partial $r^2 = 0.52$) than an exponential relationship (partial $r^2 = 0.18$). Symbols for comparison of treatments on specific harvest dates: NS (not significant), * $0.05 > P > 0.01$, ** $0.01 > P > 0.001$, *** $P < 0.001$

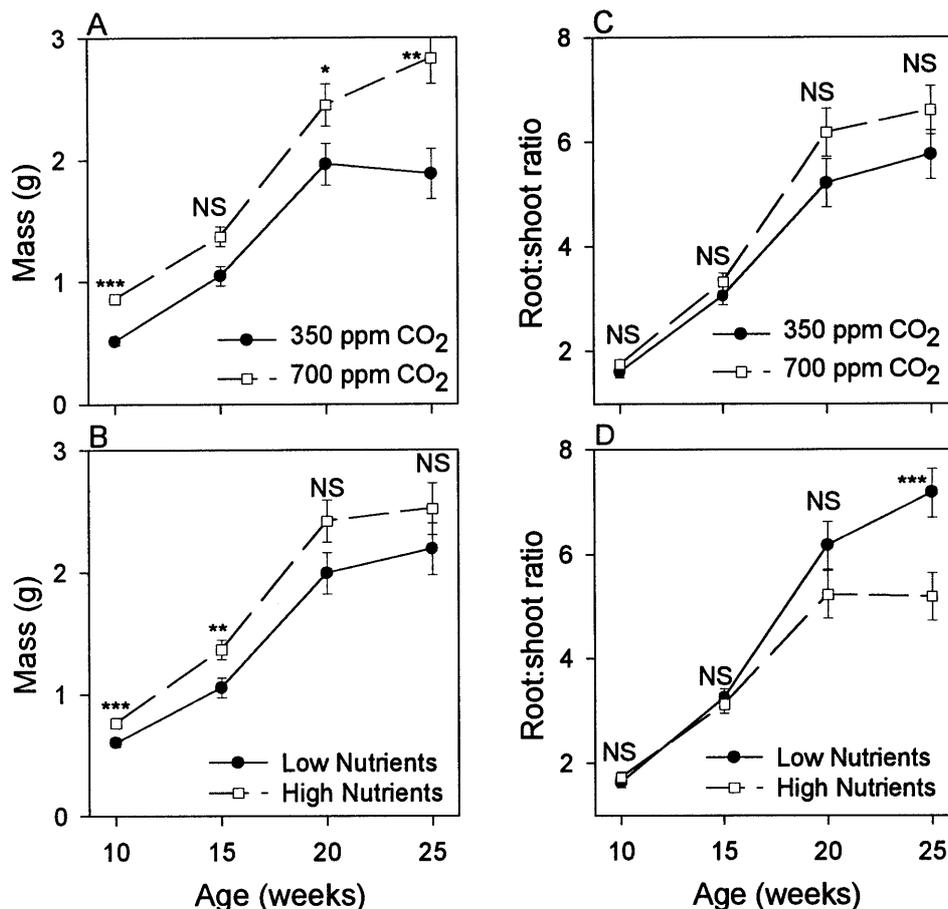


Table 1 Effect of elevated CO₂ and nutrients on dry weight of various plant components of unclipped individuals. The mean ratio (elevated/ambient CO₂) over all four harvest dates is shown

Component	CO ₂ effect	Nutrient effect
Leaves	1.31***	1.75***
Stem	0.99 ^{ns}	1.19***
Cotyledons	1.17***	0.94 ^{ns}
Fine roots	1.29***	1.18*
Coarse roots	1.43***	1.17**
Total	1.39***	1.21***

* $0.05 > P > 0.01$, ** $0.01 > P > 0.001$, *** $P < 0.001$, ^{ns}not significant

not increase appreciably as they grew. Under such allocation patterns, it is expected that growth be linear, rather than exponential. Indeed, plant growth more closely fit a linear trend (partial $r^2 = 0.52$) than an exponential trend (partial $r^2 = 0.18$). Associated with this increasing allocation to coarse root biomass was a sharp decline in relative growth rate through time. Relative growth rate, when pooled over all treatments, declined from 0.016 g g⁻¹ day⁻¹ between weeks 10 and 15, to 0.006 g g⁻¹ day⁻¹ between weeks 15 and 20, to 0.002 g g⁻¹ day⁻¹ between weeks 20 and 25. This illustrates that investment in reserves and coarse root biomass clearly has a large cost in terms of growth; however, this allocation pattern is probably necessary for surviving drought and fire, which are

important risks during the 1st year of life (Hoffmann 2000).

Experiment 2: effect of CO₂ and nutrients on resprouting

Five weeks after clipping, when many individuals had not yet begun to resprout, shoot biomass was not significantly affected by nutrient or CO₂ level. However, 10 weeks after clipping, individuals grown in high CO₂ had produced more shoot biomass than individuals grown in ambient CO₂ ($F_{1,32} = 5.35$, $P = 0.027$). Similarly, individuals grown in high nutrients had produced more above-ground biomass than individuals grown in low nutrients ($F_{1,32} = 7.08$, $P = 0.012$). There was a strong interaction between the effects of nutrients and CO₂. Under low nutrient availability, CO₂ concentration had no effect on resprout biomass ($F_{1,16} = 0.08$, $P = 0.77$, Fig. 2A), whereas under high nutrient availability, CO₂ had a strong effect ($F_{1,16} = 7.38$, $P = 0.015$, Fig. 2B).

The effects of nutrients and CO₂ on resprout biomass might be explained by the larger pre-clipping size of individuals in high nutrients or high CO₂, or may be the direct result of CO₂ and nutrients, independently of plant size. To determine if the enhancement of resprouting was solely the result of increased pre-clipping size, we tested whether CO₂ had an effect on resprout mass after ac-

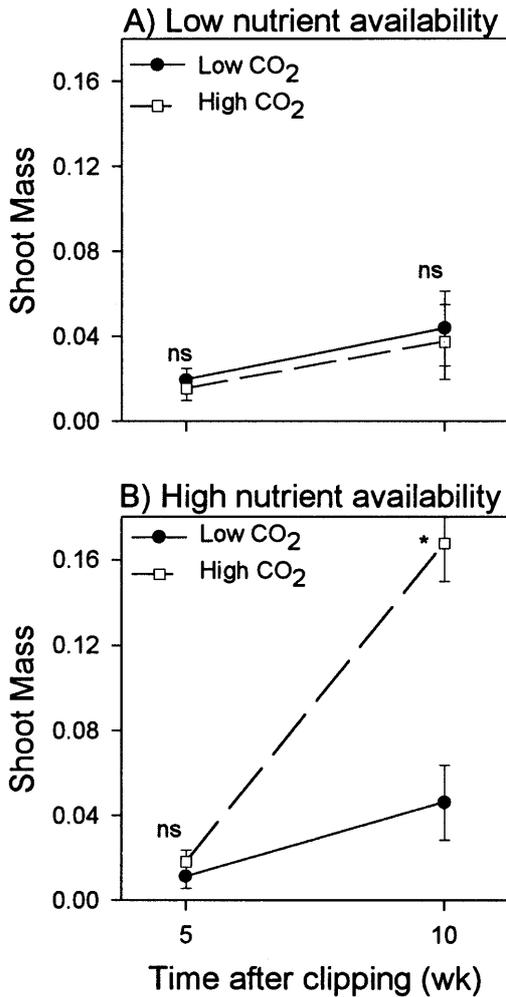


Fig. 2A,B Effect of CO₂ concentration and nutrient level on resprout biomass following clipping (mean±SE). Resprout biomass was stimulated by both CO₂ ($P=0.03$) and nutrients ($P=0.02$)

counting for pre-clipping individual size. When pre-clipping aerial biomass was used as a covariate, CO₂ still had a significant effect on resprout biomass ($F_{1,31}=5.08$, $P=0.03$), but nutrients did not ($F_{1,31}=1.83$, $P=0.19$). Therefore, CO₂ stimulated regrowth independently of the effects of CO₂ on pre-clipping size. Other studies have that found CO₂ stimulates resprouting following defoliation (Fajer et al. 1991; Wilsey et al. 1994, 1997; Pearson and Brooks 1996) and coppicing (Will and Ceulemans 1997), but in these studies, the effect of CO₂ was not greater for clipped than unclipped individuals. In agreement with the current study, Kruger et al. (1998) found the CO₂ effect to be greater for defoliated *Acer saccharum*. Similarly, Hättenschwiler et al. (1997) found significant CO₂ enhancement in coppiced *Quercus ilex* stands but no enhancement after the stands had matured.

Elevated CO₂ resulted in greater root TNC, but this effect was significant only after clipping ($F_{1,64}=4.99$, $P=0.029$; Fig. 3). In contrast, nutrients had an overall negative effect on carbohydrate concentrations, but only prior to clipping ($F_{1,32}=12.36$, $P=0.001$; Fig. 3). There

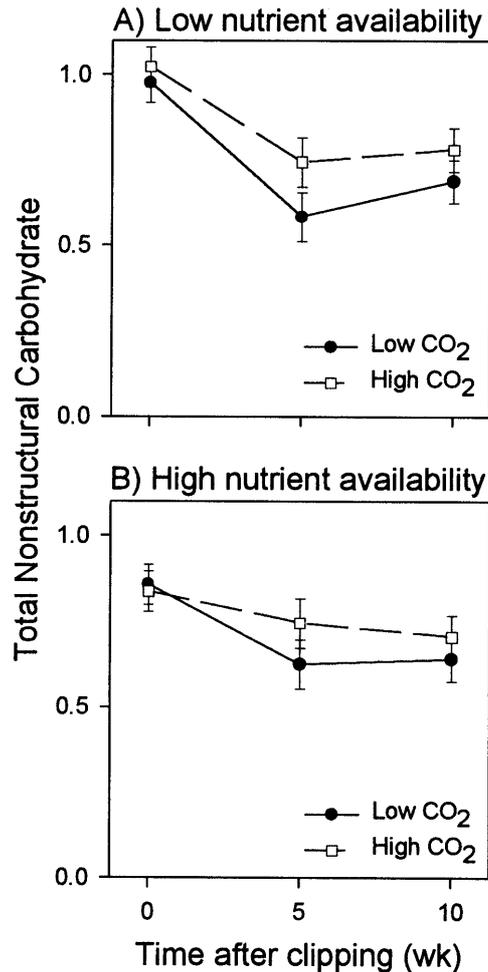


Fig. 3A,B Effects of CO₂ and nutrient concentration on root total non-structural carbohydrate (TNC) (mean±SE), expressed as the fraction of structural dry weight (dry weight–TNC). CO₂ had a positive effect on TNC, but only after clipping ($P=0.03$). Nutrients had a significant negative effect on TNC, but only before clipping ($P=0.001$). There was no significant interaction between CO₂ and nutrient effects

was no significant interaction between nutrient and CO₂ effects on carbohydrate concentrations ($F_{1,96}=0.40$, $P=0.53$).

The enhanced capacity for resprouting under elevated CO₂ cannot be explained by differences in TNC concentration prior to clipping, since pre-clipping TNC concentration in the two CO₂ treatments were indistinguishable. Also, TNC concentrations were extremely high in all treatments even after resprouting. At no time did root TNC concentration fall below 36% in any of the treatments, indicating carbohydrate availability did not limit regrowth. The high levels of TNC following resprouting may indicate a conservative strategy in which the plant quickly reverts to photosynthesis as the source of carbohydrates, thus insuring the plant against future disturbances. Indeed, carbohydrate reserves did not decline between 5 and 10 weeks after clipping (Fig. 3) even though most of the resprout biomass was produced during this

interval (Fig. 2). The active production of new biomass would have acted as an active carbohydrate sink, reducing the downward regulation of photosynthesis caused by carbohydrate accumulation under elevated CO₂.

The high concentration of TNC following clipping may also indicate that regeneration was nutrient limited. This is supported by the strong interaction between nutrient and CO₂ effects. The effect of CO₂ on resprouting was observed only under high nutrient availability. Perhaps elevated CO₂ would not enhance resprouting of this species under natural conditions, since nutrient availability in the soils of the cerrado is considered critically low, particularly for P and Ca (Lopes and Cox 1977). However, the large pulse of nutrients following fire may compensate for the inherently low nutrient status of the soils, permitting enhanced resprouting under elevated CO₂.

There is no evidence that reserves were being more quickly replenished under elevated CO₂, as was hypothesized. Rather, it appears that a lower proportion of the root TNC was mobilized under elevated CO₂. Such a response would be expected if the remobilization of root carbohydrate is affected by the strengths of other carbohydrate sources. Under elevated CO₂, photosynthesis is expected to be higher, so the recovering leaf area would have acted as a stronger carbohydrate source than under ambient CO₂, perhaps reducing the demand on stored carbohydrate earlier during resprouting.

It seems likely that the enhanced growth under elevated CO₂ would continue beyond the early regeneration phase. In forests, elevated CO₂ may enhance tree growth only in early stand development, with little enhancement occurring as the stand matures (Hättenschwiler et al. 1997). In the cerrado, stand development is regularly curtailed by fire and canopy closure is rarely observed, so the vegetation may be maintained continuously in a state that is highly responsive to elevated CO₂.

The effect of elevated CO₂ on production of aerial biomass following fire can have important implications for fire cycles (Sage 1996). Accelerated growth following fire may result in more frequent fires due to increased fuel accumulation. However, in tropical savannas, fire occurrence is more strongly dependent upon grass fuel than woody fuel. Since most tropical savannas are dominated by C₄ grasses, which exhibit smaller responses to CO₂ concentration (Carlson and Bazzaz 1982; Poorter 1993), this effect may not be realized. If elevated CO₂ does not cause an increase in fire frequency, then woody plant populations would certainly benefit from enhanced resprouting. Hoffmann (1999) found that the negative effect of fire on population growth of savanna trees is primarily due to the reduction in plant size, rather than reductions in survival or size-specific reproduction. Under elevated CO₂, enhanced growth following fire will reduce the time required for individuals to regain the pre-burn size, minimizing the negative effect of fire on population growth. In short, the greater growth rates and higher capacities of regeneration under elevated CO₂ are expected to increase the ability of woody plants to withstand the high fire frequencies currently prevalent in moist savannas throughout the tropics.

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