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RESEARCH ARTICLE

CO$_2$-fertilisation enhances resilience to browsing in the recruitment phase of an encroaching savanna tree

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Abstract

1. CO$_2$-fertilisation is implicated in the widespread and significant woody encroachment of savannas due to CO$_2$-stimulated increases in below-ground reserves that enhance sapling regrowth after fire. However, the effect of CO$_2$ concentration ([CO$_2$]) on tree responses to the other major disturbance in savannas, herbivory, is poorly understood. Herbivory responses cannot be predicted from fire responses, as herbivore effects occur earlier during establishment and are moderated by plant palatability and defence rather than below-ground carbon accumulation.

2. The relationship between herbivory and [CO$_2$] is explored here using a widespread, strongly encroaching savanna tree, *Vachellia karroo*. Using greenhouse-grown seedlings under past—through to predicted future—[CO$_2$] (180–1000 ppm) and field-grown seedlings under ambient [CO$_2$], we assessed plant survival, growth, defence and palatability.

3. Increasing [CO$_2$] improves the tolerance of greenhouse-grown seedlings to herbivory by stimulating growth and allowing a critical size threshold associated with survival to be reached earlier, thereby decreasing the probability of fatal herbivory during the vulnerable recruitment phase. Elevated [CO$_2$] also decreases the time taken to reach a second size threshold linked to accelerated recovery of field-grown seedlings following herbivory. Seedling growth responses to increasing [CO$_2$] are nonlinear, suggesting that historic growth and survival enhancements are smaller than those predicted for the future. Increasing [CO$_2$] is associated with greater resistance to herbivores (more branched shoot architecture) but not leaf palatability (C:N ratio) or defence (leaf tannins and spine density).

4. Increasing *V. karroo* densities already constitute a major land management problem in southern African savannas. However, encroachment by this species, and likely other savanna tree species, may be greatly exacerbated under future [CO$_2$], as tolerance to herbivory at the recruitment stage is further enhanced.

1 | INTRODUCTION

In savannas, increases in atmospheric CO_2 concentration ($[CO_2]$) since the last glacial maximum have promoted woody plant growth to a greater extent than that of the other major life-form, C_4 grasses (Ainsworth & Long, 2005; Hovenden & Williams, 2010). A result of this CO_2-induced change in community dynamics, in combination with other factors such as altered fire and precipitation regimes and land-use change (Buitenwerf et al., 2012; García Criado et al., 2020; Venter et al., 2018), is significant increases in tree densities in savannas globally. These increases, known as 'woody encroachment', have been particularly great over recent decades and may threaten the very existence of this mixed tree–grass biome (Buitenwerf et al., 2012; Stevens et al., 2017; Venter et al., 2018; Ward et al., 2014). As well as increasing the ability of trees to compete for resources against the C_4 grass layer, rising $[CO_2]$ also promotes tolerance to and recovery from fire (Bond & Midgley, 2000; Hoffmann et al., 2000; Kgope et al., 2010). However, the interaction between $[CO_2]$ and the other major disturbance that controls savanna vegetation, herbivory, is poorly understood.

Whilst fire and herbivory both consume biomass, their effects on plant survival under elevated $[CO_2]$ may differ. Unlike fire, herbivores selectively consume biomass and respond to its palatability and defences, both of which may be altered by $[CO_2]$. Fire and herbivory may have critical effects at different plant life stages, with herbivory likely causing mortality at earlier demographic stages than fire (Goheen et al., 2004; Wakeling et al., 2011). Encroachment has also been observed in drier sites where fire is less frequent (Stevens et al., 2017), suggesting that an alternative mechanism may be operating. A better understanding of the role of herbivory, and how it interacts with increasing $[CO_2]$, could explain woody encroachment particularly in more arid savannas, and help to project future structural shifts in these ecosystems that are critical for biodiversity, water use and carbon sequestration.

Herbivory is a significant disturbance to savanna vegetation, particularly in Africa, where large populations of wild browsers and grazers remain intact (e.g. Fornara & du Toit, 2007; Moncrieff et al., 2011). For savanna trees, the effect of herbivory depends heavily on plant size and life stage. Mature savanna trees rarely suffer fatal effects of herbivory (elephant-induced mortality being an exception; Barnes, 2001; Baxter & Getz, 2005), because they have sufficient resources to regrow lost biomass and/or they hold their biomass beyond the reach of browsers (i.e. they have escaped the 'browsing trap' sensu Staver & Bond, 2014). In contrast, seedlings can neither escape nor tolerate herbivory, and so herbivory during this life stage is a significant cause of mortality (De Steven, 1991). As herbivory occurs from germination (in contrast to dry-season fire exposure which occurs usually from the age of 6 months; Archibald

et al., 2021), trees are most vulnerable to the fatal effects of herbivory during the first few months of their life cycle.

Herbivory on seedlings is critical to successful tree establishment and creates a demographic bottleneck whereby few individuals are able to escape the seedling stage and reach maturity (Horvitz & Schemske, 2002; Sankaran et al., 2005). For example, large mammalian herbivores cause a 30% decrease in tree abundance in African savannas (Staver et al., 2021). Invertebrate herbivores also significantly impact savanna trees and have been found to be responsible for as much as 60% of herbivory damage experienced by savanna tree seedlings (Shaw et al., 2002). Seedling mortality through herbivory is thus not dissimilar to the 37%–90% mortality attributed to fire in annually burned savannas (Gignoux et al., 2009; Setterfield, 2002). Any factor that promotes seedling survival through this significant herbivory could therefore relax the demographic bottleneck, and allow large numbers of tree individuals to establish.

Improved photosynthetic capacity is predicted to significantly benefit savanna trees, which have the C_3 photosynthetic pathway, and not their C_4 grass competitors, whose photosynthesis is less responsive to increased atmospheric $[CO_2]$ (Kgope et al., 2010; Quirk et al., 2019; but see Reich et al., 2018). Enhanced tree productivity can result directly from CO_2-induced increases in carbon assimilation (Kgope et al., 2010; Quirk et al., 2019) and indirectly through improved water-use efficiency (Souza et al., 2019). In these seasonally dry environments, reduced stomatal conductance under elevated $[CO_2]$ can lessen the effects of drought and improves tree performance (Polley et al., 1999). This greater productivity can promote seedling survival through herbivory by increasing below-ground storage reserves which can be used to recover lost biomass and decrease the time taken to reach critical tolerance-related size thresholds (Hoffmann et al., 2000; Kgope et al., 2010).

Elevated $[CO_2]$ may reduce the likelihood or extent of herbivory in savanna tree seedlings, by reducing biomass palatability, owing to higher leaf C:N and lower nutrient densities. Greater carbon availability may also allow more investment in chemical (e.g. carbon-based secondary metabolites) or physical defences (e.g. spines; Robinson et al., 2012, and references therein). These effects can decrease herbivory and directly impact on the fitness of herbivores (Stiling & Cornelissen, 2007; Robinson et al., 2012; but see Zavala et al., 2008). The effect of $[CO_2]$ on shoot branching, a structural defence associated with browsing resistance and leaf protection (Charles-Dominique et al., 2016), has not been studied. Therefore, understanding the past and predicting the future role of herbivory in determining savanna tree densities require unpicking complex interactions between $[CO_2]$, plant growth, defence and palatability (Ehleringer et al., 2002).

Here, we explore the interaction between herbivory and $[CO_2]$ on savanna tree seedling success. Using *Vachellia karroo*, a species

associated with rapid and widespread savanna encroachment in southern Africa (Trollope, 1980), we explore the different ways in which increases in [CO_2] (both historic and projected future increases) influence the likelihood of herbivory and the ability of seedlings to tolerate it. We combine the assessment of seedling growth, defence and palatability under a range of [CO_2] (from glacial to those predicted for the turn of the 21st century), with data relating to seedling size, regrowth and mortality under current atmospheric conditions. Plant size thresholds of disturbance tolerance have been previously described for savanna tree species (Archibald et al., 2021), and so we hypothesise that CO_2-stimulated growth increases the rate at which *V. karroo* seedlings reach a size threshold that allows them to survive herbivory. We hypothesise that increasing [CO_2] promotes belowground growth which aids recovery from browsing events, and decreases the likelihood of herbivory through reduced palatability and increased plant defences. By establishing interactions between [CO_2] and herbivory, we may better explain the recent increases in woody thickening in savannas seen worldwide.

2 | MATERIALS AND METHODS

2.1 | Study species

Vachellia karroo Hayne (Fabaceae; previously *Acacia karroo*), commonly known as sweet thorn, is a nitrogen (N)-fixing tree that is widely distributed across southern Africa. It occurs in more arid savannas, with a mean annual rainfall of 519 mm ($\pm$5.7 mm) across its African range (calculated from occurrence data available from the Global Biodiversity Information Facility web portal, GBIF.org (26 February 2020; download doi: https://doi.org/10.15468/dl.lwnvjl) and Worldclim variable BIO12 (Fick & Hijmans, 2017)). It is a prominent encroaching species in southern Africa (O'Connor et al., 2014), with encroachment by this species having considerable economic implications (e.g. reduced forage production; du Toit, 1972; Moore et al., 1985).

V. karroo foliage is palatable and consumed by domestic and wild species (Teague, 1989), despite employing chemical (condensed tannins) and physical defences (spines; Sebata, 2016). In a supplementary dataset, we found >95% of *V. karroo* seedlings had suffered herbivory from a combination of both invertebrate and vertebrate herbivores over 15 months in the field (Figure S1).

This study brings together data generated from five experiments on *V. karroo* that each addresses elements of the relationships between atmospheric [CO_2], plant size, likelihood of—and tolerance to—herbivory (Figure 1 outlines these relationships and experiments which focus on each). We acknowledge up-front the limitations of combining and analysing data of multiple experiments that vary in structure and ecological context. However, this aggregation of data allows a more holistic exploration, and we account for experimental variation in the statistical treatment of the data as much as possible. Three experiments (numbered 1–3) manipulated atmospheric [CO_2] and assessed seedling growth, defence and palatability traits.
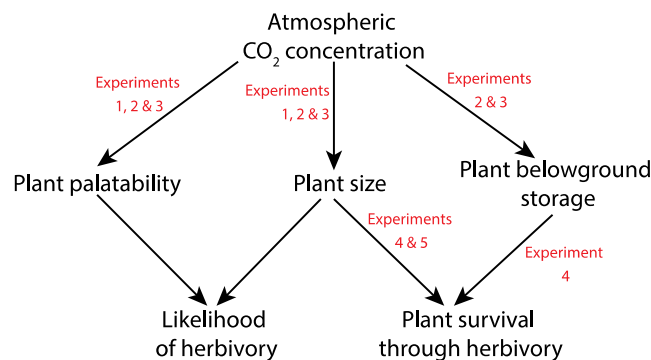
FIGURE 1 The contribution of five experiments presented here to relationships between atmospheric CO_2 concentration, plant traits (size, storage and palatability) and the likelihood to and tolerance of herbivory in the savanna encroaching tree species, *Vachellia karroo*.

Another two experiments (4 and 5) determined the relationship between seedling size and tolerance of herbivory at ambient [CO_2], and included both greenhouse and field experiments. The unit of replication in all experiments is the individual *V. karroo* plant.

2.2 | [CO_2] experiments

Kgope et al. (2010) (Experiment 1) presented data on the effects of [CO_2] on above-ground and below-ground biomass in two savanna tree species (*V. karroo and V. nilotica*). They also measured but did not present data on leaf C:N, condensed tannins and shoot branching. These data for *V. karroo* are presented here, and the data for the above-ground and below-ground response to [CO_2] were also included in the current analyses. *V. karroo* plants were grown from seed (from Hluhluwe-iMfolozi Game Reserve, Kwa-Zulu-Natal, South Africa, –28.22, 31.95; permits not required for collection), and after germination, were grown in sterile sand inoculated with field collected nodules and watered with 100 ml of Rorison-nutrient solution three times a week (see Table S1 for solution components). The growth conditions and CO_2 treatments are described in the Supporting Information. Plants were harvested 25 weeks after application of [CO_2] treatments (180, 280, 380, 550, 700, and 1000 ppm; $n = 3$–4 for each [CO_2]). C:N of leaf material was measured using a 628 Series analyser (LECO). Condensed tannins were extracted from 0.2-g fresh leaf material (following Hagerman & Butler, 1994) and quantified using an assay specific for proanthocyanidins (Porter et al., 1986). Purified *Sorghum* tannin was used as a standard (Hagerman & Butler, 1994). Shoot branching was measured as the number of shoot tips.

CO_2 effects on growth were further investigated in two experiments in which *V. karroo* plants were grown at different [CO_2] in open-top chambers (OTCs; see Supplementary Information for OTC design and performance). Experiment 2 grew *V. karroo* seedlings for 10 weeks in small (110 mm diameter × 110 mm height) OTCs at [CO_2] of 180, 280 or 400 ppm ($n = 7$ OTCs at each

[CO$_2$]). Experiment 3 grew seedlings for 25 weeks in large (2.8 m diameter × 3 m height) OTCs at [CO$_2$] of 400, 600 and 800 ppm (with three chambers per [CO$_2$] with a minimum of three plants per chamber). In both experiments, seeds collected from a semi-arid savanna (Smaldeel Region, Eastern Cape, South Africa) were germinated and transplanted into pots filled with topsoil from a nearby savanna site where *V. karroo* naturally occurs. Experiment 2 used cylindrical pots (104 × 300 mm) which had custom-made 'mini-chambers' inserted over their open ends, whilst Experiment 3 used 10-L pots placed in OTC chambers that were regularly moved to randomise any spatial effects. In both experiments, plants were regularly watered. After 10 (Experiment 2) or 25 (Experiment 3) weeks, plants were harvested, separated into biomass compartments (above-ground, below-ground and spines) and oven-dried to a constant mass. In Experiment 3, the above-ground biomass of each individual was also estimated nondestructively at two additional time points (week 14 and 18) by taking photos of the plants from five different angles (by rotating the plant pot by 72° prior to each photo) as well as immediately before the destructive harvest in week 25. These images were calibrated and analysed using the Image-J Software (version 1.51) to calculate an area of biomass. The significant relationship between average photographed area and above-ground biomass from the 25-week plants (Figure S2; $R^2 = 0.91$) was used to estimate above-ground biomass for plants at weeks 14 and 18. Control plants from Experiment 3, grown at 400 ppm, were used to contribute to developing a relationship between stem basal diameter (SBD) and biomass (Figure S3). The number of shoot branches of plants from Experiment 3 was also measured.

2.3 | Seedling growth and survival after defoliation

To investigate how *V. karroo* seedling size relates to survival and recovery after herbivory, two experiments at ambient [CO$_2$] assessed seedlings responses to simulated herbivory in the greenhouse (Experiment 4) and field (Experiment 5). Herbivory was simulated by defoliation, but we acknowledge that the herbivory process involves damage beyond leaf removal (i.e. stem damage, trampling). However, defoliation was sufficient to cause size-dependent mortality in individuals in these experiments. Therefore, herbivory may cause mortality in larger individuals than those subject to defoliation alone.

In Experiment 4, replicate seedlings were grown (see Supplementary Information for details) in a greenhouse to an age of 6, 12, 16 and 30 weeks and then subject to no-, single- or duplicate-defoliations ($n \geq 7$ for each treatment). Repeat defoliations were conducted after plants had been allowed to recover for 4 weeks. The above-ground and below-ground biomass of seedlings at the time of defoliation was calculated from allometric relationships based on SBD. These relationships were determined using the dried above-ground and below-ground biomass of non-defoliated plants. Survival (at 42 weeks) was expressed as a function of above-ground or below-ground biomass for defoliated plants.

Experiment 5 assessed the relationship between seedling size and herbivory tolerance in a field setting in the Eastern Cape (see Supplementary Information for details). Seedlings, with measured SBD between 2 and 30 mm, were tagged and subjected one of three randomly allocated treatments: 50% defoliation, 100% defoliation or no defoliation ($n = 25$ for each). After 3 months, regrown above-ground biomass was harvested, oven-dried and weighed. Again, SBD was used to derive above-ground and below-ground biomass at the time of the defoliation (Figure S3), using allometric relationships developed on non-defoliated plants. These data were supplemented by biomass and SBD data taken from 142 *V. karroo* individuals excavated from a local piece of land that was being cleared. Individuals with an SBD of <15 mm were selected for excavation. Below-ground biomass of these individuals was carefully excavated, but some loss of fine roots was unavoidable. It was not possible to determine whether these excavated plants were new seedlings or resprouts, although the SBD range selected means individuals were likely to be new seedlings. Whether individuals were resprouts or not would likely impact on biomass allocation patterns (with higher root allocation predicted for resprouting individuals).

2.4 | Statistics and models

To estimate biomass for plants at the point of defoliation non-destructively, allometric relationships were developed between SBD and above-ground and below-ground biomass in seedlings grown at ambient [CO$_2$]. Using data from Experiments 3 (400 ppm treatment only), 4 and 5, linear mixed effect models ('lme' function in the nlme package; Pinheiro et al., 2020) were fit to SBD and biomass data in the R language and platform (R Core Team, 2020). To account for variation in the data arising from the different methodologies used, 'Experiment' was included as a random effect. All variables were log-transformed prior to model-fitting, except above-ground biomass which was power-transformed ('boxcox' function in the MASS package; Venables & Ripley, 2002) as log-transformation did not sufficiently improve normality. The fit of these models was assessed ('r.squaredGLMM' function in the MuMin package; Bartoń, 2020) by generating marginal R^2 values (i.e. those associated with fixed effects only).

The influence of initial seedling biomass (before simulated herbivory; estimated from SBD) on survival and regrowth at ambient [CO$_2$] was determined through the identification of size-related thresholds. The first threshold related to a minimum plant size associated with survival after defoliation. A logistical model was fit to the biomass (both above-ground and below-ground biomass) and binary survival data (from Experiment 4), using the 'SSlogis' function (*Stats* package; R Core Team, 2020). The 'xmid' parameter (the biomass value at the inflection point of the curve) extracted from the model represents a size threshold associated with survival. A second size-related threshold was identified through visualising the relationship between initial plant biomass and the amount of regrown biomass following defoliation (Experiment 5), and recognising the

slope of this relationship changes abruptly at a certain initial biomass threshold. Above this threshold, the rate of above-ground biomass regrowth after defoliation is much higher, presumably because of greater stored resources and an enhanced ability to rapidly acquire more. This threshold in initial above-ground and below-ground biomass was quantified by fitting a broken-line regression to the data and estimating a break point ('segmented' function in the segmented package; Muggeo, 2017). Thresholds were estimated separately for the two defoliation treatments (50 and 100% defoliation), but did not differ (95% confidence intervals for threshold estimates overlapped) and so the data were analysed together.

The effect of $[CO_2]$ on *V. karroo* growth rate was assessed using data from Experiments 1, 2 and 3. A linear mixed effects model was fit to the data (as done above), with the log-transformed above-ground or below-ground biomass as the response variable, and $[CO_2]$ (treated as continuous data) and plant age as the explanatory variables. To account for differences in the data arising from the different methodologies used, 'Experiment' was included as a random effect. Analysis of Covariance (ANCOVA) was used in addition to mixed effects models to quantify the effect of 'Experiment' on biomass values, and establish $[CO_2]$ and plant age effects on biomass when this was accounted for. An interaction between the explanatory variables was tested and included if significant. Coefficients extracted from these models were used to model predictions of survival and regrowth at the different $[CO_2]$ used here.
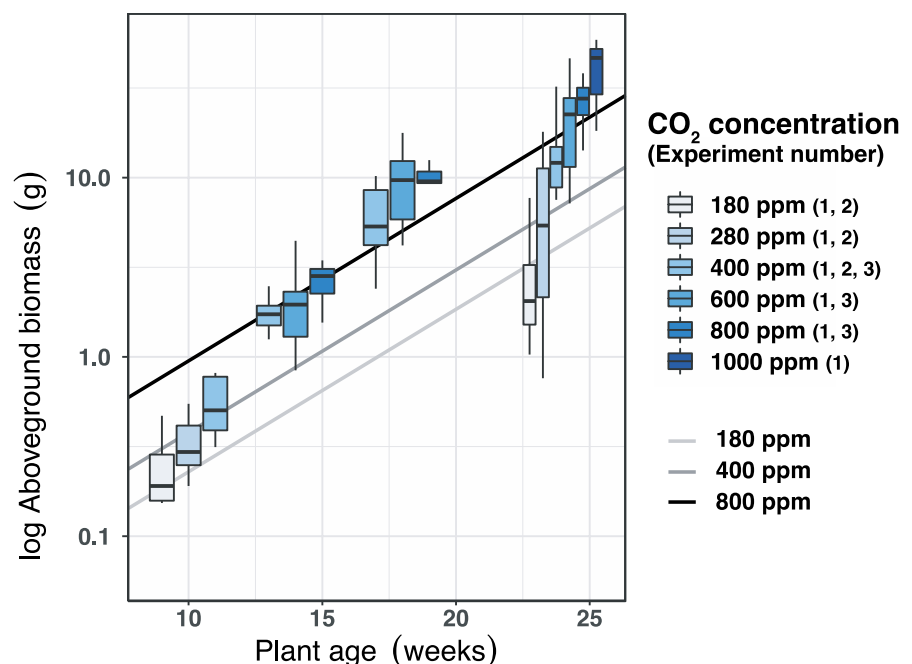
The relationships between $[CO_2]$ and plant anti-herbivore defences (spine mass, leaf C:N, leaf tannin concentration and shoot branching) were investigated. To examine how investment in spines (Experiments 2 and 3) and branching (Experiments 1 and 3) related to $[CO_2]$, data were analysed by linear mixed effects models with 'Experiment' as a random effect. ANCOVA was used to quantify the effect of 'Experiment' on spine and branching values. To account for CO_2-induced changes in above-ground biomass on spine biomass,

the same model was fit but with the ratio of seedling mass to above-ground biomass as the response ratio. The relationships between $[CO_2]$ and both leaf C:N and tannins (Experiment 1) were determined using linear regression.

3 | RESULTS

There was a significant positive effect of $[CO_2]$ on *V. karroo* above-ground biomass production ($t = 7.74$, df = 133, $p < 0.001$; Figure 2), but the interaction between $[CO_2]$ and plant age was not significant indicating that the same CO_2-fertilisation effect was evident in plants of all ages in the experiment (i.e. up to 25 weeks of age). Together, plant age and $[CO_2]$ well explained above-ground biomass (marginal [m] $R^2 = 0.79$). Relative to 180 ppm, growth at 400 ppm resulted in a 53% increase in weekly growth rate (0.052 vs. 0.080 g week^{-1}). However, future predicted $[CO_2]$s are associated with even greater increases in growth rate: relative to 400 ppm, weekly growth at 600 (0.120 g), 800 (0.179 g) and 1000 ppm (0.267 g) $[CO_2]$ was 50%, 124% and 230% higher respectively. Experimental differences did influence biomass values (ANCOVA; $p = 0.003$), but $[CO_2]$ and plant age positively affected biomass when 'Experiment' was accounted for ($p < 0.001$ for both).

Similar to above-ground trends, below-ground biomass increased with $[CO_2]$, but our experiments included limited assessment of below-ground growth over time at $[CO_2]$ other than 400 ppm. Consequently, the effects of $[CO_2]$ were limited to week 10 and 25, the duration at which some of our experiments (1, 2 and 3) were harvested. At week 10 (Experiment 2), below-ground biomass increased with $[CO_2]$ concentration ($t = 4.13$, df = 18, $p < 0.001$). A more comprehensive comparison at week 25 (Experiments 1 and 3) showed a similar effect with $[CO_2]$ having a positive effect on below-ground biomass ($t = 4.69$, df = 58, $p < 0.001$), regardless of the effect

FIGURE 2 The growth of the encroaching savanna tree species, *Vachellia karroo*, is fertilised by increasing CO_2 concentration. Lines representing the relationships between above-ground biomass and plant age at 180, 400 and 800 ppm CO_2 are extracted from a linear mixed effect model that accounts for experimental differences. 'Experiment' influenced above-ground biomass (ANCOVA; $p = 0.003$; experiment number is indicated in the key), but plant age and $[CO_2]$ significantly contributed to values ($p < 0.001$ for both) when experimental differences were accounted for.

of experimental differences (ANCOVA: $p < 0.001$). At 25 weeks, plants grown in 1000 ppm [CO_2] had 31.90 g of below-ground biomass, in comparison to 12.57 g at 400 ppm and 8.94 g at 180 ppm. [CO_2] was a better predictor of below-ground biomass at 10 weeks ($mR^2 = 0.40$) than at 25 weeks ($mR^2 = 0.18$).

Growth rate and plant size are important for seedling tolerance of herbivory. Under 400 ppm [CO_2], the survival of *V. karroo* plants through herbivory is significantly improved once they reach an SBD (stem basal diameter) threshold of 2.11 mm (± 0.05 mm). As

SBD was strongly correlated with both above-ground ($t = 17.55$, df $= 94$, $p < 0.001$, $mR^2 = 0.74$) and below-ground ($t = 19.18$, df $= 106$, $p < 0.001$, $mR^2 = 0.72$) biomass (Figure S3), thresholds in above-ground (Figure 3a) and below-ground biomass (Figure 3b) could be determined. This threshold SBD size is associated with above-ground and below-ground biomass values of 0.51 g (± 0.04 g) and 0.76 g (± 0.03 g) respectively. Therefore, CO_2-induced growth enhancement will reduce the time taken for *V. karroo* individuals to reach this survival threshold, and therefore the period when they are susceptible
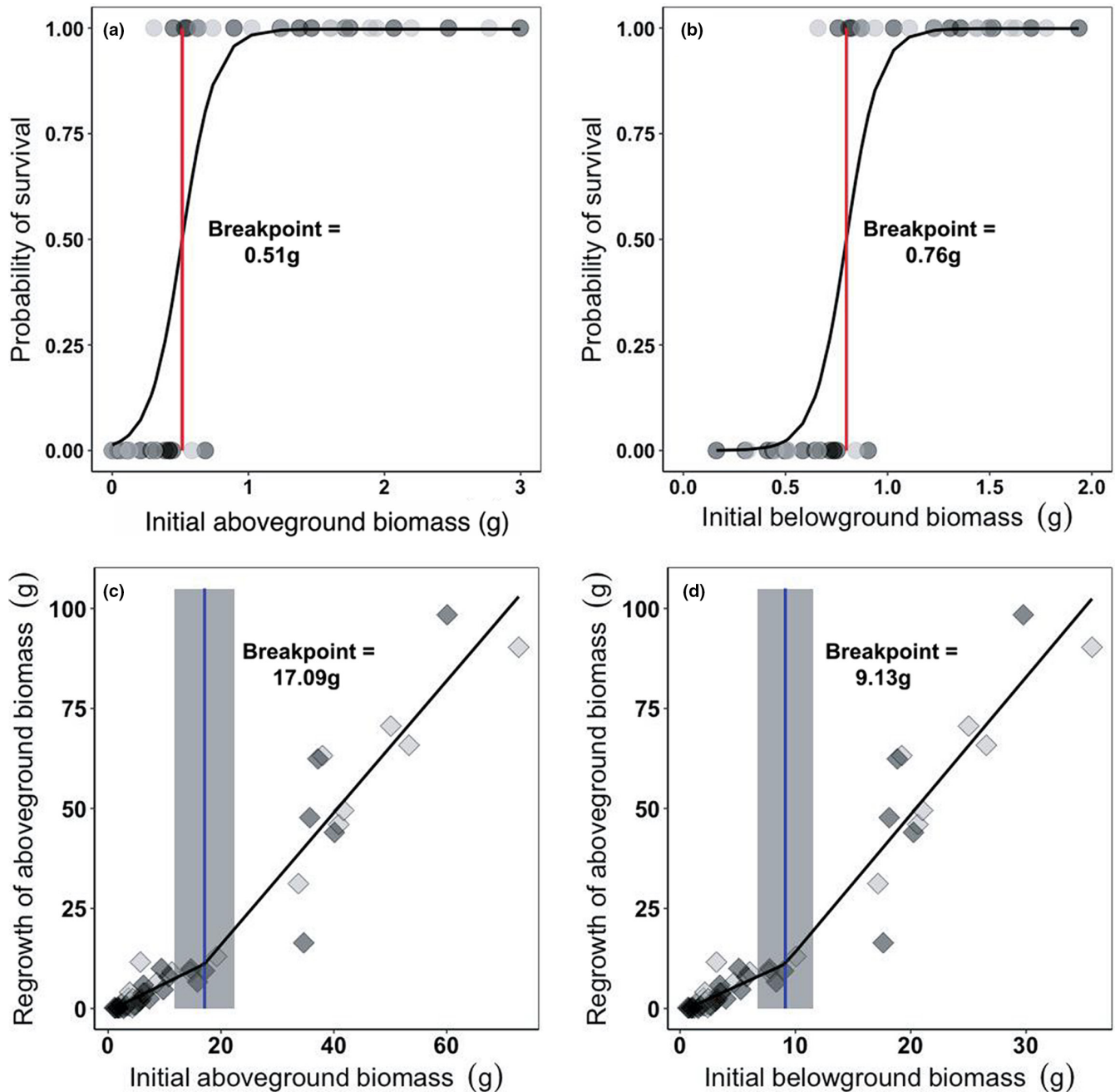
FIGURE 3 Survival of *Vachellia karroo* seedlings through herbivory and regrowth after herbivory is size dependent. Points represent individuals with differing above-ground (a and c) or below-ground (b and d) biomass. Seedlings were subject to a single (dark grey) or duplicate (light grey) defoliation events. The red lines in (a) and (b) are identified breakpoints associated with survival (using data from Experiment 4). The blue lines in (c) and (d) are identified breakpoints (shared area represents the 95% confidence interval) above which the slope of the relationship is much higher (using data from Experiment 5).

to fatal herbivory (Figure 4). Historic [CO₂] increases have already shortened the time to reach this survival threshold: the average time taken to reach the above-ground biomass threshold of 0.51 g decreased by 2 weeks when plants were grown in 400 ppm rather than 180 ppm [CO₂] (10 vs. 12.1 weeks; Figure 4). Future increases in [CO₂] are predicted to shorten this period of vulnerability further: growth at 600 and 800 ppm [CO₂] allowed individuals to reach the
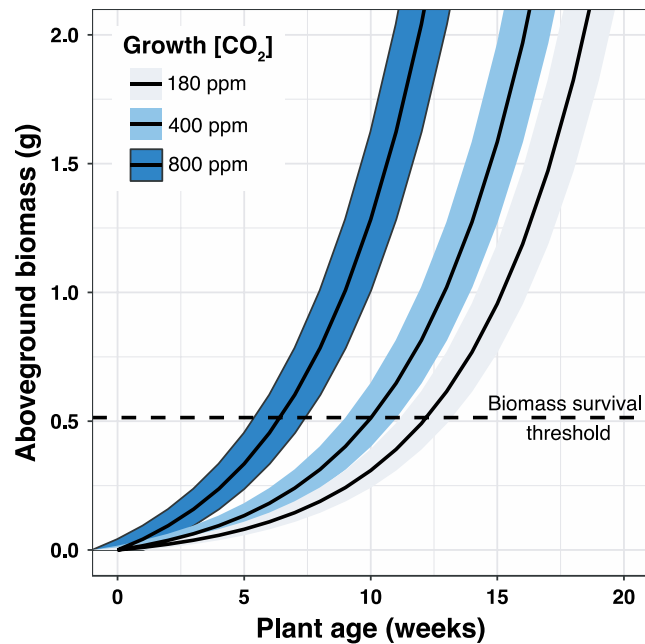
FIGURE 4 Increasing CO₂ concentration decreases the time taken for *Vachellia karroo* seedlings to reach critical size thresholds associated with survival through herbivory. Growth curves are extracted from models based on plant growth under different [CO₂] (presented in Figure 2). The dashed line indicates the above-ground biomass associated with survival. The solid lines represent growth at 180, 400 and 800 ppm CO₂. Shaded areas indicate standard error.

survival threshold 2.0 and 3.9 weeks quicker in comparison to growth at 400 ppm (Figure 4). Growth at 1000 ppm [CO₂] resulted in individuals reaching this survival size threshold just over 1 month (4.2 weeks).

A second, larger size-related threshold was evident in the regrowth of seedlings in the field following simulated herbivory. Almost all seedlings survived, but a biphasic response in regrowth was evident (Figure 3c,d). Above the size threshold, regrowth occurred at a much greater rate (+28 and +38% for above-ground and below-ground biomass respectively), presumably related to enhanced resource acquisition. This threshold is estimated at 17.09 g (±5.34 g; Figure 3c) for above-ground biomass and 9.13 g (±2.42 g; Figure 3d) for below-ground biomass. Enhanced growth under elevated [CO₂] caused this second threshold to also be reached in a shorter period of time (27.2 weeks for 180 ppm vs. 19.3 weeks for 1000 ppm).

Whilst spine biomass was marginally influenced by [CO₂] ($p = 0.08$), with greater investment in spines associated with increasing [CO₂] ($mR^2 = 0.07$), the increase in spine mass scaled allometrically with above-ground biomass such that ratio of spine to above-ground biomass was unchanged by [CO₂] ($p > 0.05$; $mR^2 = 0.01$; Figure 5). ANCOVA revealed that 'Experiment' had no effect on spine biomass or spine: above-ground biomass ($p > 0.05$). Shoot branching, a proxy of herbivore resistance, was positively associated with [CO₂] ($p = 0.003$; $mR^2 = 0.10$), with the number of branches doubling from 180 to 400 ppm (0.83 vs. 1.96), and doubling again from 400 to 800 ppm (1.96 vs. 4.03; Figure 5). 'Experiment' had no effect on branching ($p > 0.05$). Neither leaf C:N ($F_{1,17} = 0.002$, $R^2 = 0.05$, $p > 0.05$) nor condensed tannins ($F_{1,22} = 0.55$, $R^2 = 0.02$, $p > 0.05$) were altered by [CO₂] (Figure S4).

4 | DISCUSSION

Using a common savanna tree species whose encroachment dynamics are increasingly challenging grassland management in southern
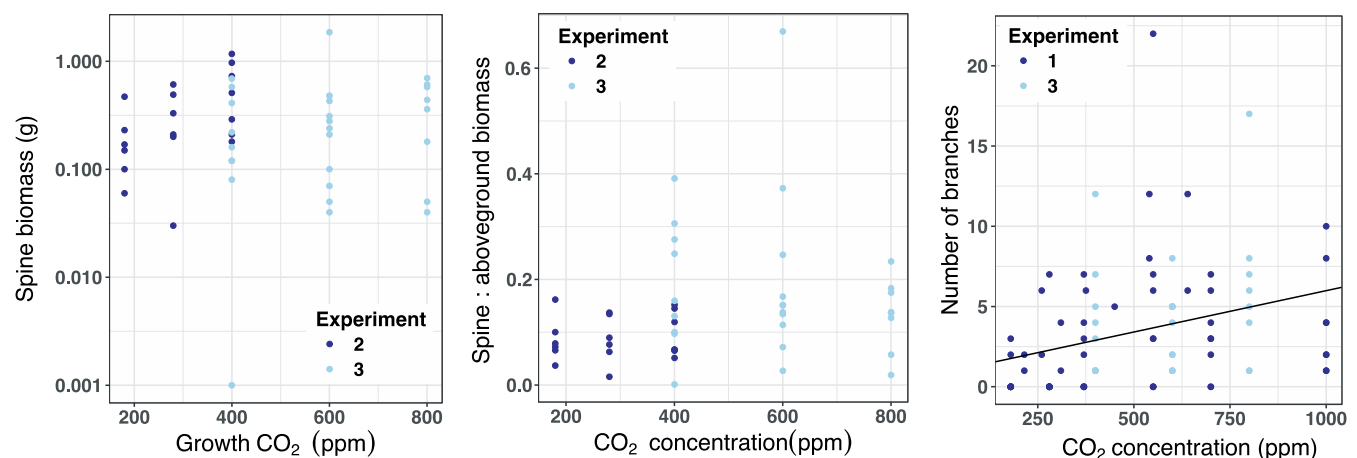
FIGURE 5 Shoot branching (a proxy of herbivore resistance; right panel) increases with CO₂ concentration ([CO₂]) in the tree *Vachellia karroo*. However, both spine biomass (left) and spine: above-ground biomass (centre) are unrelated to [CO₂]. Lines represent significant relations extracted from linear mixed effect models that account for experimental differences. 'Experiment' did not significantly influence any of these three plant defence traits (ANCOVA; $p > 0.05$), whilst [CO₂] significantly contributed to values of branching ($p = 0.006$) when experimental differences were accounted for.

Africa (du Toit, 1972; O'Connor et al., 2014), we explored $[CO_2]$ impacts on herbivory tolerance, to elucidate how these may explain past and predict future levels of encroachment. We demonstrate that CO_2-stimulated growth influences whether *V. karroo* seedlings survive herbivory and determines the rate at which they regrow after herbivory events. This is based on evidence that plant herbivory responses are strongly size dependent, that CO_2-fertilisation increases growth rate and that these responses can be used to model how increasing $[CO_2]$ decreases the growth period required for plants to attain these important size thresholds. Whilst we test CO_2-effects on herbivory responses in *V. karroo*, we expect similar responses in other *Vachellia* species, such as *V. nilotica*, *V. exuvialis* and *V. sieberiana*, which encroach in savannas across the broad rainfall gradient of this biome (Turpie et al., 2019). Other N-fixing encroaching species, such as *Dichrostachys* species, may also be expected to respond to increasing $[CO_2]$ similarly, as their growth fertilisation may not be constrained by nitrogen limitation (Terrer et al., 2019). The growth and herbivory responses of non-N-fixing encroachers, such as *Terminalia* species, to future $[CO_2]$ increases are unclear but may be more limited by N-availability.

Although found at a lower mean annual rainfall (519 mm) than that associated with peak herbivory in Africa (~650 mm; Archibald & Hempson, 2016), herbivory is a significant disturbance to *V. karroo* (Figure S1; Goheen et al., 2004; Shaw et al., 2002). This pressure has likely resulted in the evolution of tolerance of herbivory, as found here with individuals rapidly reaching survival size thresholds. Indeed, Archibald et al. (2021) found that savanna tree species from drier systems (like *V. karroo*) were tolerant of herbivory at younger ages than those from higher rainfall systems. This higher early survival was associated with lower rainfall particularly in palatable species, suggesting that herbivory, and not just aridity, is a selective force on this trait (Archibald et al., 2021).

Our results suggest that historic increases in $[CO_2]$ have enhanced the survival and recovery of *V. karroo* seedlings in the face of high herbivory pressure, and thus contributed to increased *V. karroo* densities in savannas. However, factors other than $[CO_2]$ that are not addressed here, such as altered fire, herbivory and rainfall regimes and land-use change (Buitenwerf et al., 2012; García Criado et al., 2020; Kgope et al., 2010; Quirk et al., 2019; Venter et al., 2018), have certainly contributed to the phenomenon of savanna woody plant encroachment seen worldwide (Stevens et al., 2017). Enhancements in photosynthetic ability and water-use efficiency concurrent with historic increases in $[CO_2]$ are predicted to result in a 53% increase in weekly growth rate of above-ground biomass (180 to 400 ppm; Quirk et al., 2019). A result of this historic CO_2-fertilisation effect is a decrease in the time required for plants to attain the size where they survive herbivory by 21%, and thus a lower chance of fatal herbivory. In addition to above-ground effects, $[CO_2]$ influences *V. karroo* seedling survival via the effect it has on below-ground biomass and stored reserves which support resprouting. When $[CO_2]$ is increased, both below-ground biomass and the concentration of stored carbohydrates are enhanced (Kgope et al., 2010). The size of savanna

tree lignotubers and stored carbohydrate provision determine the regrowth of plants following fire in the field (Bond & Keeley, 2005; Wigley et al., 2009). We anticipate that similar mechanisms will underpin the size–regrowth relationships shown here. An assumption we make here is that the size threshold for survival remains the same across multiple $[CO_2]$s. However, biomass allocation patterns may be altered by elevated $[CO_2]$ in *V. karroo* (e.g. increased root: shoot ratio; Raubenheimer & Ripley, 2022), potentially impacting on vulnerability to browsing. Further work should establish how survival size thresholds vary with $[CO_2]$.

Future increases in $[CO_2]$ are predicted to have even greater effects on *V. karroo* growth, and therefore herbivore tolerance, than historic ones. Compared to 400 ppm, growth at 800 ppm further enhanced growth rate (by an additional 124%) and decreased the period of vulnerability to fatal herbivory (by an additional 40%). This nonlinear response suggests that seedling above-ground growth does not saturate within this range of $[CO_2]$. Kgope et al. (2010) found that the maximum rate of carbon assimilation ($Amax$) in *V. karroo* increases across the range of $[CO_2]$ studied here: from ~8.6 µmol CO_2 m^{-2} s^{-1} at 180 ppm to ~29.5 µmol CO_2 m^{-2} s^{-1} at 1000 ppm. However, the greatest increase in $Amax$ is associated with historic increases in $[CO_2]$ (115% increase from 180 to 400 ppm vs. 60% increase from 400 to 1000 ppm; Kgope et al., 2010). This mismatch in the findings for $Amax$ and the biomass results here are likely because of the multiplicative nature of photo assimilates being used to produce new leaves for further carbon assimilation (Harper, 1989). Future increases in $[CO_2]$ occur on a background of already high $[CO_2]$ (the highest for at least the last 650,000 years; Royer, 2006) and therefore leaf biomass production. In *V. karroo*, a greater investment of biomass above-ground, and therefore in photosynthetic tissue, with increasing $[CO_2]$, further supports this hypothesis (Kgope et al., 2010; Quirk et al., 2019). In addition, given the young age of the seedlings studied here, the value of each leaf in terms of its exported assimilates is predicted to close to its maximum (high photosynthetic rates and high investment in new leaves rather than storage or defence; Harper, 1989).

Whilst the ability to tolerate herbivory changes with $[CO_2]$ in *V. karroo* seedlings, the ability to resist it may not. This species may rely upon rapid growth to escape browsing rather than allocation to defence as N-fixation reduces the cost of lost leaf biomass. The only defence trait significantly associated with $[CO_2]$ is shoot branching, whereby plants grown in higher $[CO_2]$ had a more branched architecture which may protect leaf biomass from the reach of browsers (Charles-Dominique et al., 2016). CO_2-fertilisation of growth is linked to decreased plant palatability, because of increased C:N and reduced nutrient concentrations (Luo et al., 2006). However, we found no difference in leaf C:N with increasing $[CO_2]$—a result not uncommon for N-fixing species as nitrogen fixation and carbon assimilation are both stimulated by increased $[CO_2]$ (Feng et al., 2004; Rogers et al., 2009). The two anti-herbivore defences measured— spines and leaf tannins—similarly did not change with $[CO_2]$. Whilst spine biomass increased, this change was proportional to CO_2-induced increases in biomass, meaning that seedlings were no better

defended (Quirk et al., 2019). The response of tannin accumulation to [CO_2] appears to be highly variable across species (Penuelas & Estiarte, 1998). Together, these results suggest that palatability and defences are unrelated to [CO_2] meaning that the increase in survivorship and growth is not complicated by changes in herbivory resistance.

Our growth estimates are based on experiments using natural unfertilised savanna soils (Experiment 1 being the exception, in which plants were grown in sand with nutrients added at a concentration comparable to field levels) that were not water limited. As the availability of moisture and nutrients influence CO_2-fertilisation (Hoffmann et al., 2000; Polley et al., 1999; Polley et al., 2002; Terrer et al., 2019), our growth conditions and herbivore treatments produced a particular set of growth rates and size thresholds. Therefore, we do not advocate for generalisations about the magnitude of thresholds or responses but emphasise that similar patterns might underpin all seedling responses to herbivory under increasing [CO_2]. By using savanna soils, plants experienced nutrient availabilities comparable to the field, and therefore, their short-term growth responses to [CO_2] should be comparable also. How long-term responses in the field can be projected from elevated CO_2 pot experiments is less clear, but initial gains in tree growth may plateau as soil nutrients are depleted. The non-limiting water supply replicated the flush of germination that occurs at the onset of the rainy season in savannas; however, drought is a frequent disturbance and a major factor in *Vachellia* seedling survival and growth (Kraaij & Ward, 2006; Wilson & Witkowski, 1998). Although drought can lessen the effect of CO_2-fertilisation (Sage & Coleman, 2001), increased [CO_2] improves plant water use and seedling survivorship of drought (Polley et al., 1999; Polley et al., 2002).

Our results arise from statistically testing data combined from five ecological experiments, and so are complicated by differences in those experiments. We have taken steps to greatly reduce the influence of such variation (e.g. by establishing and accounting for the effect of 'Experiment' on plant traits), but acknowledge it may still have an effect, although likely small. Another important caveat to our experimental approach is that *V. karroo* seedlings were grown in the absence of C_4 grasses, which compete for light, nutrients and water, and fuel surface fires, in natural savanna settings (Cramer et al., 2007; February et al., 2013; Vadigi & Ward, 2014). Interactions with grasses can have serious negative implications for seedling survival (Morrison et al., 2019) and the ability for seedling growth to respond to [CO_2] (Manea & Leishman, 2015). Therefore, grasses may mitigate the positive CO_2 effect on seedling herbivory responses. However, in more arid savannas, where *V. karroo* is found, tree–grass interactions are considered more facilitative rather than competitive (Dohn et al., 2013; Wakeling et al., 2015). In addition, increased CO_2 may fertilise C_4 grass biomass as well as tree biomass in savannas (Raubenheimer et al., 2021), but the magnitude of this response is likely much lower (Sage & Kubien, 2007). Regardless, the interaction of grass competition on woody plant seedling responses to CO_2-fertilisation is not understood and warrants further investigation.

Alterations to ecosystem services because of savanna woody plant encroachment, such as reduced forage for livestock and groundwater recharge, threaten the provision of food, water and livelihoods for hundreds of millions of rural and urban people (Ryan et al., 2016). *V. karroo* constitutes a major management problem in both its native range, where it is rapidly increasing in density (du Toit, 1972; O'Connor et al., 2014), and its introduced range, where it is considered a serious invader (e.g. FNCW, 2001). Our results suggest that *V. karroo* (and likely other encroaching savanna tree species) will become an even greater land management challenge under future [CO_2]. How the effect of increasing CO_2 compares to other factors, such as fire suppression and overgrazing, in driving encroachment is unclear and requires further investigation. However, our results also suggest that management interventions like browsing can be effective in controlling future woody thickening if tree seedlings are targeted at a size when herbivory is likely to result in mortality.

AUTHOR CONTRIBUTIONS

Brad S. Ripley conceived the hypothesis and designed the methodology. Sarah L. Raubenheimer, Lavinia Perumal, Maurice Anderson, Emma Mostert, Barney S. Kgope, Guy F. Midgley and Kimberley J. Simpson contributed to methodology, conducted experiments and analysed data. BSR and KJS wrote the manuscript with all authors contributing.

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CONFLICTS OF INTEREST

None.

DATA AVAILABILITY STATEMENT

All plant-trait data are available from the Dryad Digital Repository https://doi.org/10.5061/dryad.hmgqnk9m8 (Ripley et al., 2022).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.