Physiological responses to low CO₂ over prolonged drought as primers for forest–grassland transitions

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Savannahs, open grasslands with scattered C₃ trees, expanded between 24 and 9 million years ago in low latitudes at the expense of forests. Fire, herbivory, drought and the susceptibility of trees to declining atmospheric CO₂ concentrations ([CO₂]a) are proposed as key drivers of this transition. The role of disturbance is well studied, but physiological arguments are mostly derived from models and palaeorecords, without direct experimental evidence. In replicated comparative experimental trials, we examined the physiological effects of [CO₂]a and prolonged drought in a broadleaf forest tree, a savannah tree and a C₄ grass. We show that the forest tree was more disadvantaged than either the savannah tree or the C₄ grass by the low [CO₂]a and increasing aridity. Our experiments provide insights into the role of the intrinsic physiological susceptibility of trees in priming the disturbance-driven transition from forest to savannah in the conditions of the early Miocene.
of growth [CO₂], at all watering levels (Fig. 1; see Extended Data Table 1 for the P values and details of the statistical analysis). In the grass, E_plan, was higher at 200 ppm (‘elevated’) than at 800 ppm (‘ambient’) [CO₂], under both well-watered and drought conditions (Fig. 1). All species responded to drought with an immediate decline in E_plan, which was steeper in the savannah species V. karroo and E. curvula than in C. africana. Under well-watered conditions, leaf relative water content (RWC) consistently scaled with growth [CO₂], but the differences were small and were significant in leaf relative water content (RWC) consistently scaled with growth [CO₂], under both well-watered and drought conditions (Fig. 1). All species responded to drought with an immediate drop, especially at ambient and sub-ambient [CO₂], respectively. In C. africana, the response of gₚ to drought was significant only at elevated [CO₂]. Re-watering led to the recovery of Aₚ and gₚ to initial levels in all species and [CO₂], except for Aₚ in C. africana and gₚ in V. karroo, which remained low at sub-ambient [CO₂].

Photosynthetic capacity. In E. curvula, all the photosynthetic parameters (Rₚₘₚ, Y(CO₂)ₗₗ, GAₚₚ and LCP, defined below; Fig. 4) derived from curves measuring the response of leaf-level CO₂ assimilation (A) to increasing photosynthetic photon flux density (PPFD) (A–PPFD curves) (Extended Data Fig. 3) were independent of growth [CO₂]. The trees grown at elevated [CO₂] had higher respiration in the light (Rₚₘₚ) and light-saturated gross assimilation rate (GAₚₚ) than at sub-ambient [CO₂] (Fig. 4). V. karroo grown at elevated [CO₂] had higher initial quantum yield for CO₂ fixation (Y(CO₂)ₗₗ) for all watering levels.

All species responded to drought with a concerted downregulation of photosynthetic parameters. However, only C. africana reduced Rₚₘₚ and was able to maintain constant Y(CO₂)ₗₗ despite drought, resulting in a progressive reduction in the light compensation point (LCP) as drought progressed. In contrast, drought decreased Y(CO₂)ₗₗ in E. curvula, which negatively impinged on the LCP. In all species and at all [CO₂] levels, GAₚₚ decreased with drought. The steepest decrease was for V. karroo, which, at 40% watering, reduced GAₚₚ to an average across [CO₂] levels of 12% of the initial values, compared with 44% and 53% for E. curvula and C. africana, respectively.

In C. africana, all the photosynthetic parameters (CE, Vₘₚ, I and Aₚₘₚ defined below; Fig. 5 and Extended Data Fig. 4) derived from curves measuring the response of A to [CO₂] in the sub-stomatal cavity (C) (A–C curves, measured under a PPFD of 1,500 μmol m⁻² s⁻¹, were independent of growth [CO₂]. V. karroo had higher carboxylation efficiency (CE) and CO₂-saturated assimilation rate (Aₚₘₚ) when grown at elevated [CO₂], (Fig. 5). In E. curvula, CE and maximal phosphoenolpyruvate carboxylation rate (Vₘₚ) were lower in plants grown under elevated than sub-ambient [CO₂]. E. curvula sharply reduced the CO₂ compensation point (I) in response to drought under elevated [CO₂], but not under any other [CO₂]. In the two savannah species and at all [CO₂], levels,
Fig. 2 | Leaf and pre-dawn water potential. Responses of $\Psi_{\text{vp}}$ (top) and $\Psi_{\text{pd}}$ (bottom) to decreasing levels of water supply and for three levels of growth [CO$_2$]$_a$, in C. africana (left), V. karroo (centre) and E. curvula (right). The plants were grown in controlled-environment chambers at 200 ppm (Sub), 400 ppm (Amb) or 800 ppm (Ele) [CO$_2$]$_a$, and they were subjected to progressive month-long decreasing watering levels of 80%, 60%, 50%, 40% and 30% of pot capacity followed by re-watering back to 80% (shades of grey). The values are means ± s.e. (n = 4). The letters indicate horizontal significant differences (P < 0.05) across watering levels within a given [CO$_2$]$_a$, not across CO$_2$ levels. The symbols indicate differences (P < 0.05) between sub-ambient and elevated ($\dagger$), sub-ambient and ambient ($\ddagger$) or elevated and ambient ($\ddagger\ddagger$); when shown in the lower left corner, they apply to all waterings. The statistical details and P values are given in Extended Data Table 1.

Fig. 3 | Leaf-level gas exchange under operational growth conditions. Responses of $g_s$ (top) and $A_{\text{sat}}$ (bottom) to decreasing levels of water supply and for three levels of growth [CO$_2$]$_a$, in C. africana (left), V. karroo (centre) and E. curvula (right). The plants were grown in controlled-environment chambers at 200 ppm (Sub), 400 ppm (Amb) or 800 ppm (Ele) [CO$_2$]$_a$, and they were subjected to progressive month-long decreasing watering levels of 80%, 60%, 50%, 40% and 30% of pot capacity followed by re-watering back to 80% (shades of grey). The values are means ± s.e. (n = 4). The different letters indicate horizontal significant differences (P < 0.05) across watering levels within a given [CO$_2$]$_a$, not across CO$_2$ levels. The symbols indicate differences (P < 0.05) between sub-ambient and elevated ($\dagger$), sub-ambient and ambient ($\ddagger$) or elevated and ambient ($\ddagger\ddagger$); when shown in the upper left corner, they apply to all waterings. The statistical details and P values are given in Extended Data Table 1.

CE decreased with drought. The maximal carboxylation rate for Rubisco ($V_{\text{max}}$) and $V_{\text{max}}$ decreased in all species and at all [CO$_2$]$_a$ levels to values that, at 40% watering, were 25%, 39% and 46% of the initial values in V. karroo, C. africana and E. curvula, respectively. $A_{\text{sat}}$ followed the same decreasing trend and promptly declined at 60% watering in V. karroo at all [CO$_2$]$_a$ levels, while significant
Re-watering resulted in the recovery of all photosynthetic parameters to initial levels in \textit{V. karroo}, and all parameters except \( Y_{\text{CO}_2} \)\(_{\text{LL}} \) and LCP in \textit{E. curvula}. However, in \textit{C. africana}, \( R_{\text{light}} \), \( \text{GAsat} \), LCP and \( V_{\text{cmax}} \) did not recover (Figs. 4 and 5).

**Leaf and root starch content.** In \textit{E. curvula}, the starch content in both leaves and roots was independent of growth \([\text{CO}_2]_a\) (Fig. 6). In \textit{V. karroo}, root starch content was not affected by growth \([\text{CO}_2]_a\), differences were delayed until 50% watering in the other two species. \( F \) increased with drought only in \textit{V. karroo}. The response of the photochemical integrity of photosystem II as indicated by \( F_v/F_m \) (the ratio between variable fluorescence and maximum fluorescence) is shown in Extended Data Fig. 6. \textit{E. curvula} maintained \( F_v/F_m \) throughout the experiment, while \( F_v/F_m \) followed the general trend of \( A_{\text{w}} \) in the trees: dropping at drought onset in \textit{V. karroo} and later in \textit{C. africana}.
but leaf starch content decreased faster in response to drought in plants grown under sub-ambient [CO2], than at any other [CO2]. C. africana under elevated [CO2] had higher starch content in both roots and leaves than did plants grown under sub-ambient [CO2] for most watering levels. In the trees, leaf starch content decreased with drought, rapidly in V. karroo (significant decreases occurred at 60–50% watering) and gradually in C. africana (significant effects were delayed until 30% watering). Conversely, in the C4 grass E. curvula, leaf starch content did not decrease with drought. In the savannah tree and grass, leaf starch content increased at lower watering levels at sub-ambient and ambient [CO2].

Root starch content slowly decreased with drought in V. karroo, but it was independent of watering in the other species. After re-watering, leaf starch content recovered to initial values in the trees, but it became significantly lower than at any other period in the grass. Root starch remained low in V. karroo.

### Effect of [CO2] over prolonged drought

To compare the effect of growth [CO2] over a season-long drought across species, we computed the difference between the natural logarithms of values at either sub-ambient or elevated [CO2], and those at ambient [CO2], for each variable. The change in the natural logarithms of variables is independent of the initial value and unit of the variable, and therefore allows for direct comparison across species (see the note to Table 1 for more details). We tested whether the response differed between trees and the C4 grass (H1) or between the two trees (H2).

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**Fig. 5 | Photosynthetic parameters derived from A–C curves.** Photosynthetic parameters derived from A–C response curves for C. africana (left), V. karroo (centre) and E. curvula (right) grown at [CO2], of 200 ppm (circles and solid line), 400 ppm (squares and dashed line) and 800 ppm (triangles and dotted line). The A–C curves (Extended Data Fig. 4) were measured on randomly selected plants during each month-long water-level interval and were used to derive the following parameters: CE (the initial slope of a fitted hyperbola); Vcmax for the C3 trees and Vpmax for the C4 grass, which were mechanistically derived; Γ (the y-intercept; that is, C, when A = 0); and A_sat (the asymptote, reflecting the potential for electron transport). The values are means ± s.e. (n = 4). The letters indicate significant differences (P < 0.05) across watering levels and apply to all [CO2]. The symbols indicate vertical differences (P < 0.05) between sub-ambient and elevated (§), sub-ambient and ambient (†) or elevated and ambient (‡); when shown at the left, they apply to all waterings. The statistical details and P values are given in Extended Data Table 1. No values indicates that there were no viable (trees) or measurable (grass) leaves. In trees leaves were dropped in grasses leaves were rolled.
Our experimental design combined three [CO2]a levels spanning the likely range of values expected for past and future scenarios (200 ppm (Sub), 400 ppm (Amb) or 800 ppm (Ele) [CO2]a, and they were subjected to progressive month-long decreasing watering levels of 80%, 60%, 50%, 40% and 30% of pot capacity followed by re-watering back to 80% (shades of grey). The values are means ± s.e. (n = 4). The letters indicate significant differences (P < 0.05) across watering levels within a given [CO2]a, not across CO2 levels. The symbols indicate differences (P < 0.05) between sub-ambient and elevated (%), sub-ambient and ambient (†) or elevated and ambient (‡); when shown in the upper left corner, they apply to all waterings.

The statistical details and P values are given in Extended Data Table 1. No values indicate that there were no viable (trees) or measurable (grass) leaves. In trees leaves were dropped in grasses leaves were rolled.

Fig. 6 | Starch content. Starch content per dry weight in leaves (top) and roots (bottom) in response to decreasing levels of water supply and for three levels of growth [CO2]a in C. africana (left), V. karroo (centre) and E. curvula (right). The plants were grown in controlled-environment chambers at 200 ppm (Sub), 400 ppm (Amb) or 800 ppm (Ele) [CO2]a, and they were subjected to progressive month-long decreasing watering levels of 80%, 60%, 50%, 40% and 30% of pot capacity followed by re-watering back to 80% (shades of grey). The values are means ± s.e. (n = 4). The letters indicate significant differences (P < 0.05) across watering levels within a given [CO2]a, not across CO2 levels. The symbols indicate differences (P < 0.05) between sub-ambient and elevated (%), sub-ambient and ambient (†) or elevated and ambient (‡); when shown in the upper left corner, they apply to all waterings.

The statistical details and P values are given in Extended Data Table 1. No values indicate that there were no viable (trees) or measurable (grass) leaves. In trees leaves were dropped in grasses leaves were rolled.

Growth at sub-ambient [CO2]a decreased gs, A, GA, and leaf starch more in the trees than in the grass and decreased gs more in the grass than in the trees (Table 1). Comparing the savannah species, sub-ambient [CO2]a decreased all photosynthetic indicators more in the savannah tree, V. karroo, than in the savannah grass, E. curvula. The trees responded to sub-ambient [CO2]a by decreasing A, and LCP, but these decreases were larger in C. africana than in V. karroo. Interestingly, this was accompanied by a larger decrease in gs in V. karroo than in C. africana, indicating higher non-stomatal limitation in C. africana. The response accompanied by an increase in Ci in C. africana [Extended Data Fig. 5] to elevated [CO2]a was similar between the trees and the grass, but A and GA more in V. karroo than in C. africana and E. curvula.

Discussion

Our first hypothesis (H1) stated that during a prolonged drought, sub-ambient [CO2]a would reduce assimilation more in trees than in grasses, and this was supported by the experimental evidence: sub-ambient [CO2]a reduced A and GA more in trees than in the C4 grass E. curvula; however, contrary to what we thought, over the course of drought, sub-ambient [CO2]a increased gs more in E. curvula than in the trees (Table 1). The response to drought was found to be time-dependent. In C4 grasses, short-term dips in leaf water potential between days25 or around midday25 cause rapid declines in assimilation that are mainly due to non-stomatal limitation26. Here we found that, after an initial reduction, E. curvula stabilized A and gs, while photosynthesis continued to decline in the trees. We reason that E. curvula may take days to acclimate to drought; when water potential falls fast, non-stomatal limitations would reduce assimilation to the point where avoidance becomes advantageous. Indeed, from 60% watering onwards, E. curvula rolled its leaves in the evening of the day after watering, and increasingly earlier at lower watering levels, then promptly unfurled after watering. In contrast, the trees started dropping their leaves at 50% watering, and by 30% watering they were almost completely defoliated.

Both leaf shedding and leaf rolling effectively reduce canopy evapotranspiration, conferring protection against hydraulic damage27. While leaf shedding is ubiquitous, rolling is more common in spontaneous and cultivated grasses and cereals30–32. Rolled leaves remain in situ, meaning that essential resources (such as nitrogen and phosphorous) are retained in live tissue rather than relocated or lost through leaf senescence. Unfurling operates over hourly timescales, allowing rapid exploitation of post- or mid-drought soil water pulses. Conversely, recovery after leaf shedding requires growing new leaves, imposing a lag time of weeks or months. Redmann19 proposed that rolling amphistomatous leaves would be selected for in habitats where diurnal water supply and demand fluctuate widely. These mechanisms explained a rapid 90% increase in assimilation in a C4 savannah grass following a mid-drought rainfall event compared with only a 22–26% increase in oak trees34, conferring C4...
grasses a growth advantage over trees relying on regrowth\textsuperscript{19}. This advantage would be more pronounced under sub-ambient \([\text{CO}_2]_{\text{a}}\), as the photorespiratory and evaporative losses of \(\text{C}_3\) trees accelerate but not for longer periods. 

Our second hypothesis (H\textsubscript{2}), that the savannah tree \(V.\\text{karroo}\) would be less disadvantaged at sub-ambient \([\text{CO}_2]_{\text{a}}\) than the forest tree \(C.\text{africana}\), was also supported by our experimental evidence (Table 1). However, the timing of the response to drought differed between the trees. \(V.\text{karroo}\) rapidly closed its stomata (Fig. 3) and four of the eight photosynthetic parameters (\(G_{\text{sat}},\) \(\text{LCP},\) \(\text{Ce},\) and \(\text{A}_{\text{cmax}}\) for the trees), \(Y_{\text{op}}\) and \(\text{root starch content}\) of both organs. However, we found that leaf starch concentration in \(C.\text{africana}\) did not recover LRW at elevated \([\text{CO}_2]_{\text{a}}\) (Figs. 1 and 3) and four of the eight photosynthetic parameters (\(G_{\text{sat}},\) \(\text{V}_{\text{max}},\) \(\text{R}_{\text{light}}\) and \(\text{LCP}\)) (Figs. 4 and 5), further supporting H\textsubscript{2}.

By delaying leaf senescence, \(C.\text{africana}\) may have traded its capacity to remobilize nutrients from leaves to storage organs, and this, in turn, may have affected its capacity to recover, particularly under sub-ambient \([\text{CO}_2]_{\text{a}}\). \(C.\text{africana}\) maintained dehydrated leaves on the tree for some time before shedding. Contrasting strategies of rapid versus delayed leaf shedding were also observed in closely related savannah and forest eucalyptus species\textsuperscript{31,39}, potentially underpinned by the advantage of shading out competitors\textsuperscript{31,39}. Furthermore, \(C.\text{africana}\) reduced leaf respiration, which resulted in maintaining quantum yield and in a substantial reduction of the LCP (Fig. 4). This is critical under low light\textsuperscript{49} because it helps saplings maintain a net positive carbon balance in the shady forest understory\textsuperscript{41}. Overall, this adaptive shade strategy may be successful during mild, short droughts\textsuperscript{5}, but not for longer periods.

| Table 1 | Effects of \([\text{CO}_2]_{\text{a}}\) over seasonal drought |
|---|---|---|
| Mean under ambient \([\text{CO}_2]_{\text{a}}\) | Effect of low \([\text{CO}_2]_{\text{a}}\) | Effect of high \([\text{CO}_2]_{\text{a}}\) |
| \(\text{C. africana}\) | \(V.\text{karroo}\) | \(E.\text{curvula}\) | \(\text{C. africana}\) | \(V.\text{karroo}\) | \(E.\text{curvula}\) | \(\text{C. africana}\) | \(V.\text{karroo}\) | \(E.\text{curvula}\) |
| \(\ln(p_{\text{g}})\) | \(-3.12\) | \(-2.66\) | \(-2.75\) | \(0.10\) | \(-0.48\) | \(0.64\) | \(0.002\) | \(0.026\) | \(0.003\) | \(-0.51\) | \(-0.37\) | \(0.35\) | \(0.35\) | \(0.60\) | \(0.61\) |
| \(\ln(A_{\text{cmax}})\) | \(1.38\) | \(1.36\) | \(2.12\) | \(-1.24\) | \(-0.68\) | \(0.12\) | \(<0.001\) | \(0.041\) | \(<0.001\) | \(0.14\) | \(0.80\) | \(0.21\) | \(0.14\) | \(<0.001\) | \(<0.001\) |
| \(\ln(\text{leaf starch content})\) | \(2.47\) | \(1.02\) | \(3.97\) | \(-1.01\) | \(-0.86\) | \(0.04\) | \(0.023\) | \(0.74\) | \(0.01\) | \(0.06\) | \(0.55\) | \(0.07\) | \(0.57\) | \(0.32\) | \(0.15\) |
| \(\ln(\text{root starch content})\) | \(1.79\) | \(1.50\) | \(1.55\) | \(-0.28\) | \(0.21\) | \(-0.17\) | \(-\) | \(0.28\) | \(0.01\) | \(0.04\) | \(-0.19\) | \(0.56\) | \(0.95\) | \(0.57\) |

Bold fonts indicate \(P<0.05\). The values are In-transformed \(p_{\text{g}}, A_{\text{cmax}}, \text{leaf starch content}, \text{root starch content}, Y_{\text{op}}, \Psi_v, \text{and indicators of leaf photosynthetic capacity, which are }\rho_{\text{g}}, Y_{\text{curvula}}, G_{\text{sat}}, \text{LCP, CE, V}_{\text{max}}, \text{and }\text{A}_{\text{cmax}}.\) The natural logarithms of variables over 80%, 60%, 50% and 40% watering were subjected to a generalized mixed linear model (ReML, Genstat 18.2, VSNI) with the effect of species nested within the life form (tree versus grass), \([\text{CO}_2]_{\text{a}}\) and their interaction as fixed factors, and plant, watering level and their interaction as random factors. The effect of \([\text{CO}_2]_{\text{a}}\) was calculated as \(\ln(p_{\text{g}}) - \ln(p_{\text{g}})\) where \(p_{\text{g}}\) and \(p_{\text{g}}^*\) are the predicted estimates of any generic variable \(v\) under either high or low \([\text{CO}_2]_{\text{a}}\) and ambient \([\text{CO}_2]_{\text{a}}\), respectively (recall that\( \delta(\text{ln}(v)) = \frac{L}{2}\)). The effect is dimensionless and can be directly compared across species and variables. Three scenarios were statistically tested: the effect of \([\text{CO}_2]_{\text{a}}\) is the same in the trees and the \(\text{C}_4\) grass \(E.\text{curvula}\), (consistent with a previously described isohydric behaviour\textsuperscript{37}), the effect of \([\text{CO}_2]_{\text{a}}\) is the same in the savannah species \(V.\text{karroo}\), and 40% watering was significantly higher than at higher watering levels, but such an increase was not observed in \(C.\text{africana}\) (Fig. 6). This change in leaf starch content was accompanied by more negative \(\Psi\) in the savannah tree and grass than in the forest tree \(C.\text{africana}\) (Fig. 2). Starch hydrolysis may play a central role in cavitation repair and osmotic adjustment following drought injury\textsuperscript{40-41}. Concentrating starch in leaves could thus have increased the operational safety margins against hydraulic failure for the savannah species under drought. Our observations are consistent with reports showing the accumulation of non-structural carbohydrates in \(V.\text{karroo}\) leaves under drought conditions\textsuperscript{37}. In montane grasslands under ambient \([\text{CO}_2]_{\text{a}}\), the proportion of recent assimilate allocated to root storage increased during drought\textsuperscript{42}. In this view, starch probably functioned as insurance against drought at the expense of growth, rather than a sink for carbon overspill\textsuperscript{42-51}. Finally, our data do not support the suggestion that leaves under high \([\text{CO}_2]_{\text{a}}\) accumulate starch to levels that inhibit photosynthetic performance\textsuperscript{14}. Starch content increased with \([\text{CO}_2]_{\text{a}}\) only within \(C.\text{africana}\) roots, but this was associated with an increase in \(A_{\text{vop}}\).

Physiological responses contribute to ecosystem shifts from forests to savannahs and vice versa. Our results show that forest trees were disadvantaged by low \([\text{CO}_2]_{\text{a}}\) and increasing aridity, conditions that occurred during the early Miocene. Uncertainties in the lower bound of Miocene \([\text{CO}_2]_{\text{a}}\) would probably affect the magnitude of plant responses, and across a lower gradient in \([\text{CO}_2]_{\text{a}}\), the magnitude of treatment effects could be diminished\textsuperscript{44}. Trees carry a carbon tax on large woody structures, both structural and for maintenance, which is avoided by herbaceous grasses. Low \([\text{CO}_2]_{\text{a}}\) and
drought could result in the death of branches and trees through carbon starvation or hydraulic failure, but generally these occur under exceptional levels of stress. It has been shown that drought and low [CO₂] are not sufficient by themselves to tip the transitional balance to open ecosystems, but they would probably increase the intrinsic susceptibility of trees to the effects of disturbance. This may have played a role in the expansion of grassy savannahs at the expense of forests under low [CO₂] some 20 million years ago. Under low [CO₂], trees grow slower and allocate less biomass to both physical (thorns) and chemical defences, accelerating canopy opening by browsers. While forest grasses seldom produce enough biomass to burn, opening environments would accumulate more grass biomass, fuelling deeper penetration of fires. The competition for water and nutrients would intensify, and slow-growing trees would be less likely to grow tall enough to escape destruction by fire (escaping the ‘fire trap’). This, in turn, would reduce sapling recruitment to mature trees, conditioning the ecosystems to entrain positive feedbacks involving fires. It has been suggested that during early stages of forest–grassland transition, local factors including soil properties, rainfall intensity and topography were critical in shaping the competition between grasses and tree saplings.

In these opening habitats, quick-responding trees such as *V. karroo* would progressively displace forest trees such as *C. africana*. The shift in the proportion of *C*. and *C*. grasses could have been driven by contrasting responses of *C*. and *C*. grasses to drought, ultimately giving these emerging ecosystems the shape of modern savannahs. Differences in the timing of the response suggest that *C*. grasses evolved different mechanisms of avoidance and tolerance of drought than *C*. grasses (in addition to those described above, these mechanisms include faster-responding stomata, enhanced water delivery to leaves and low stomatal conductance).

Similar interactions between physiology and disturbance may operate at increasing [CO₂]. Ecosystem models predict that elevated [CO₂] will shift areas that could currently support multiple stable biomes into tree-dominated areas. Higher C₃ assimilation under both physical (thorns) and chemical defences, accelerating canopies opening by browsers. While forest grasses seldom produce enough biomass to burn, opening environments would accumulate more grass biomass, fuelling deeper penetration of fires. The competition for water and nutrients would intensify, and slow-growing trees would be less likely to grow tall enough to escape destruction by fire (escaping the ‘fire trap’). This, in turn, would reduce sapling recruitment to mature trees, conditioning the ecosystems to entrain positive feedbacks involving fires. It has been suggested that during early stages of forest–grassland transition, local factors including soil properties, rainfall intensity and topography were critical in shaping the competition between grasses and tree saplings.

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Leaf and root starch concentration. During the experimental phase, leaves and roots were collected from each plant every two weeks. Roots were extracted from two 2-cm-diameter soil cores taken to a depth of 8 cm and avoiding the main tap root of the trees. A total of 432 samples were microwaved, dried and ground in a mixer mill. Starch concentration was analysed with a highly specific enzymatic method optimized for plant samples that avoids errors of acid hydrolysing methods. Starch was hydrolysed with α-amylase (Bacillus licheniformis E-BLAAM, Megazyme) and then with high-purity amyloglucosidase (Aspergillus niger E-AMGD, Megazyme). The resulting glucose was assayed through a coupled enzymatic reaction of α-dianisidine (PGO kit, Sigma), spectrophotometrically quantified. An internal reference comprising a mix of roots and leaves of all species was analysed in parallel with n = 6 to quantify dry day effect, but the data was not significant.

Leaf gas exchange and fluorometry. Instantaneous leaf gas exchange was measured on fully expanded leaves at midday, after ~12 photoperiod hours since watering, with an infrared gas analyser fitted with a 6 cm² cuvette and a red–blue LED light source (LII6400XT fitted with 6400-02B, LI-COR Biosciences). For *E. curvula*, three to four leaf blades were aligned, avoiding gaps and overlaps, to fill the entire leaf chamber. For the other two species, whenever leaves did not fill the chamber, leaf area was measured using scaled digital images processed in ImageJ (Fiji version 2014 for 32-bit Windows) (NIH (Extended Data Fig. 1). To minimize environmental perturbations and error arising from CO₂ leakage from the infrared gas analyser, the leaf chamber was positioned inside the growth cabinet and supplied air cabinet at [CO₂]c 0.02%, depending on growth conditions. The flow rate was 235 μmol s⁻¹ and PPFD and temperature were set to match the growth conditions. After the readings stabilized, gas exchange was averaged for 10 s and recorded as a single point measurement, for a total of 222 data points. The same leaves were used for measurements of chlorophyll fluorescence on dark-adapted leaves (F₀/F₅) at least four hours after the end of the photoperiod using a Junior PAM (Heinz Walz GmbH) and the factory settings.

Responses of net leaf A to C, and to PPFD (A–C, and A–PPFD response curves) were measured at the bench for each watering interval (n = 4, ~160 A–response curves) on the same leaves (for trees) or similar leaves (for grasses) to those used for the in-cabinet point measurements, between 3 and 72 hours after watering. For the A–PPFD curves, reference [CO₂] was set to 200, 400 or 800 ppm, according to experimental treatment, with ten PPFD increments between 1,500 and 0 μmol m⁻² s⁻¹, with 5–7 min between increments. For the A–C curves, PPFD was 1,500,500 μmol m⁻² s⁻¹, and reference [CO₂] was incremented between 2 and 1,200 ppm, with 2–3 min between interactions.

By combining these curves with empirical modelling, which does not require any assumptions, the following enzyme- and light-limited photosynthetic parameters were derived: Rₚₑₐ₅, Y(CO₂), GAₑₜₑ, Cₑₐₑ, Aₑₑ, the empirical curvature of the A–PPFD and A–C curves (m and α respectively) and F (that is, C, at which A is zero). Two parameters were derived mechanistically: Vₚₑₐ₅ for *E. curvula* and Vₚₑₐ₅ for the *C. plants*. The following assumptions were made for the enzyme-limited C₃ model, (mesophyll conductance to CO₂ diffusion) gₘₜₑₐ = 0.2 mol m⁻² s⁻¹ and (Rubisco Michaelis-Menten constant for CO₂) Kᵣₐₑₜₑ(1 + Oₜₑₐₑ/Kₒₑₐₑ) (O₂ concentration at the photosynthetic rate/ the fraction of O₂ evolution in the bundle sheath) = 550 μmol (ref. 23); and for the enzyme-limited C₄ model, (the fraction of O₂ evolution in the bundle sheath) = 0.5 mol m⁻² s⁻¹ (the reciprocal Rubisco specificity) γₑₑ = 0.000193, (fraction of light respiration in the mesophyll) Rₒₑₑₐₑₐₑ = 0.5; Kₗₑₑ = 650 μmol, Kₐₑₑ = 450,000 μmol, (Phosphoenolpyruvate carboxylase Michaelis-Menten constant for PEP) Kₑₑ = 80 μmol (all from ref. 23) and (bundle sheath conductance for CO₂ diffusion) gₑₑ = 0.0015 mol m⁻² s⁻¹ (ref. 23).

Plant–water relations. We calculated Eₛₑₑₑ from ~70 gravimetric measurements. We took g from the instantaneous gas exchange measurements described previously. We measured Yₑₑₑ and Yₛₑₑₑ in 469 samples using a Scholander pressure chamber (PMS Instrument Company, Model 1000) on leaves cut the day or night before. Instantaneous gas exchange. BWC was calculated for 238 samples as: BWC = 100 × sample weight – dry weight. Turgidity was achieved by submerging cut leaves in distilled water within a sealed environment for 3–4 h (ref. 23). The leaves were then oven-dried for 48 h at 60 °C.

Statistical analysis. The effects of drought and [CO₂], in individual species were analysed with a repeated measures ANOVA with the 10 point measurements in SAS (v.8; SAS Institute). The modelled main effects were watering level, [CO₂] and their interaction; plant number was the random factor. The details and P values are presented in Extended Data Table 1. Contrasts were performed with Fisher’s Partial Least-Squares Difference (PLSD) differences and deemed significant at α = 0.05.

To test the effect of [CO₂] across species and over the seasonal drought, the natural logarithms of variables were subjected to a generalized mixed linear model (REML, Genstat 18.2, VSN; Table 1). [CO₂] species nested within the life form (trees versus grass) and their interaction were fixed factors, while plant and watering level with their interaction were random factors.

**Methods**

**Plants and growth conditions.** Seedlings of *V. karroo* were obtained from the Desert Legume Program (Tucson, AZ, USA), accession number 900474, collected outside the Mountain Zebra National Park, northwest of Cradock (Eastern Cape, South Africa); those of *C. africana* were obtained from Silverhill Seeds (Cape Town, South Africa); and a clone of *E. curvula* was obtained from the Germplasm Resources Information Network (United States Department of Agriculture, Washington, DC, USA), accession number PI-155434. The plants were grown in 2.5 dm³ pots filled with a loam soil that has a gradual decline in soil water potential when dried (Extended Data Fig. 2). The plants were randomly distributed among growth chambers within a large-air circulation growth chamber. Plants were watered at an automatic control of 200, 400 or 800 ppm, rotated weekly within and monthly between cabinets. The temperature was set at 26±1.7 °C, and the relative humidity was 70±5% (day-night).

The trees and grasses were initially grown for 18 and 6 months, respectively, while being watered to a gravimetrically determined 80% of pot capacity three times per week. Subsequently, during the experimental phase, all plants were watered for four weeks each period to 80%, 60%, 50%, 40% and 30% of pot capacity, followed by re-watering at 80%. The oldest leaves of *E. curvula* (about a third at the beginning, reducing to zero as drought progressed) were clipped every two to three weeks to maintain the initial canopy size. For a schematic representation of the measurement and sampling strategy, see the Supporting Information in Quirk et al. **

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Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. The full dataset is available in the Supplementary Information. Source data are provided with this paper.

Received: 23 September 2020; Accepted: 7 July 2022; Published online: 25 August 2022

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Acknowledgements

We acknowledge funding through an ERC advanced grant (CDREP, no. 322998) awarded to D.J.B. C.B. and N.U. received funding from the European Union’s Horizon 2020 research and innovation programme through an MSCA individual fellowship (grant agreement ID no. 702755) awarded to C.B.

Author contributions

D.J.B, C.B. and J.Q. designed the research. C.B. and J.Q. performed the research. N.U., and D.J.B. are responsible for analytical work. N.U., and C.B. contributed to data analysis. C.B. and J.Q. performed the research. N.U., C.B. and J.Q. designed the research. C.B. and N.U. wrote the paper with contributions from D.J.B.

Competing interests

The authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41477-022-01217-8.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41477-022-01217-8.

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Peer review information *Nature Plants* thanks Guy Midgley and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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Extended Data Fig. 1 | Experiment in progress. A) Seedlings of *Celtis africana* and *Vachellia karroo* during the growth phase before measurements began. B) A seedling of *Vachellia karroo* being weighed to gravimetrically determine watering amount. C) Seedlings inside the double-door growth chambers during the night phase of the photoperiod. D) *Eragrostis curvula* growing in the growth chambers; this picture was also taken during the dark phase of the photoperiod. The tobacco plants in C and D were grown alongside experimental plants throughout the experiment and were regularly monitored for growth and morphological traits to ensure they were reproducing treatment expected differences. E) Example of in-situ leaf area measurement prior to operational gas exchange data collection. The leaf is clamped between a piece of white Perspex mounted on a white card background and it is placed at a fixed distance from the camera lens. Subsequently, images are processed in Image J to determine area as illustrated in F, which displays monochrome images of *Vachellia karroo* leaflets.
Extended Data Fig. 2 | Soil water retention of various substrates. Substrates were loosely filled into pots identical to those used in the experiments, watered to field capacity and left to dry under normal growth cabinet conditions. Soil water potential was measured every 2–3 days using a psychrometer (Psypro with L-51 hygrometers, Wescor Inc., Logan, UT, US) calibrated with five standard NaCl solutions according to the manufacturer’s instructions. Soil samples were extracted from the centre of the pot at a depth of ~10 cm using a weighing spatula. Soil and John Innes No. 3 (green up-triangles) was selected as the experimental substrate because it had the steadiest decrease in soil matric potential as it dried. \( \Psi_m = \Psi_e \cdot (\theta/\theta_s)^b \), where \( \Psi_m \) is soil matric potential, \( \Psi_e = 0.0101 \) and \( b = 3.01 \) are the fitted parameters.
Extended Data Fig. 3 | Response of leaf-level CO$_2$ assimilation (A) to increasing photosynthetic photon flux density (PPFD). A - PPFD curves. Values are means ± 1 SE (n = 4) for Celtis africana (top), Vachellia karroo (middle), and C$_4$ Eragrostis curtula (bottom) plants, measured at five watering levels (80, 60, 50, 40 and 30% of pot capacity) and grown at either 200 ppm (left), 400 ppm (centre) or 800 ppm (right) [CO$_2$]. Blue symbols with solid blue lines represent measurements after re-watering (recovery phase).
Extended Data Fig. 4 | Response of CO₂ assimilation (A) to increasing [CO₂] in the sub-stomatal cavity (Cᵢ), A - Cᵢ curves. Values are means ± 1 SE (n = 4) for Celtis africana (top), Vachellia karroo (middle) and C₄ Eragrostis curvula (bottom) plants, measured at five watering levels (80, 60, 50, 40 and 30% of pot capacity) and grown at either 200 ppm (left), 400 ppm (centre) or 800 ppm (right) [CO₂]. Blue symbols with solid blue lines represent measurements after re-watering (recovery phase).
Extended Data Fig. 5 | [CO₂] in the sub-stomatal cavity (Cᵢ) and its ratio to [CO₂] in the measuring cuvette (Cᵢ/Ca) under operational growing conditions. Values are means ± 1 S.E (n = 4) for the forest broad-leaf tree Celtis africana (left), the savanna tree Vachellia karroo (centre), and the C₄ savanna grass Eragrostis curvula (right), measured at five watering levels (80, 60, 50, 40 and 30% of pot capacity followed by a recovery back to 80%) and grown at either 200 ppm (Low), 400 ppm (Amb) or 800 ppm (Ele) [CO₂]a. Gas exchange was measured in-cabinet with gas-analyser set points for temperature, humidity, [CO₂]a, and light intensity set at cabinet levels.
Extended Data Fig. 6 | Photochemical integrity of photosystem II as indicated by $F_v/F_m$. Values are means ($n = 4$) ± 1 SE for the forest broad-leaf tree *Celtis africana* (left), the savanna tree *Vachellia karroo* (middle), and the C₄ savanna grass *Eragrostis curvula* (right), measured at five watering levels (80, 60, 50, 40 and 30% of pot capacity) followed by a recovery back to 80% and grown at either 200 ppm (Low), 400 ppm (Amb) or 800 ppm (Ele) [CO₂]. $F_v/F_m$ was measured from pulse-amplitude modulated (PAM) chlorophyll fluorometry within the cabinets in the dark.
Extended Data Table 1 | Within species effects of [CO₂] and watering level on leaf water relations, instantaneous gas exchange, indicators of leaf photosynthetic capacity, and starch content

| Species          | Analysis  | CO₂ | WL  | CO₂ × WL |
|------------------|-----------|-----|-----|----------|
|                  |           |     |     |          |
| C. africana      | Mixed     | 2.75 (1.2), 133.7 (1.4), 120.0 (1.4) | 0.3110 | 0.0076 |
|                  | Glimmix/  | 8.64 (4.6), 10.14 (4.6), 4.43 (3.1) | 0.3110 | 0.0076 |
|                  | Gamma     | 0.1035 | 0.0001 | 0.0006 |
|                  | Mixed     | 9.78 (3.2), 32.23 (3.2) | 1.92 (3.2) | 0.0001 |
|                  | Glimmix/  | 12.7 (2.2), 62.8 (2.2) | 3.37 (2.2) | 0.0108 |
|                  | Gamma     | 0.0108 | 0.0001 | 0.0006 |
|                  | Mixed     | 8.96 (3.2), 34.04 (3.2) | 1.51 (3.2) | 0.0001 |
|                  | Glimmix/  | 6.32 (3.2), 63.4 (3.2) | 3.08 (3.2) | 0.0205 |
|                  | Gamma     | 0.0205 | 0.0001 | 0.0006 |
|                  | Mixed     | 6.21 (3.2), 60.21 (3.2) | 2.65 (3.2) | 0.0001 |
|                  | Glimmix/  | 6.04 (3.2), 105.3 (3.2) | 3.8 (3.2) | 0.0267 |
|                  | Gamma     | 0.0267 | 0.0001 | 0.0006 |
|                  | Mixed     | 6.15 (3.2), 5.57 (3.2) | 0.37 (3.2) | 0.0001 |
|                  | Glimmix/  | 6.04 (3.2), 105.3 (3.2) | 3.8 (3.2) | 0.0267 |
|                  | Gamma     | 0.0267 | 0.0001 | 0.0006 |
|                  | Mixed     | 6.15 (3.2), 5.57 (3.2) | 0.37 (3.2) | 0.0001 |
|                  | Glimmix/  | 6.04 (3.2), 105.3 (3.2) | 3.8 (3.2) | 0.0267 |
|                  | Gamma     | 0.0267 | 0.0001 | 0.0006 |
|                  | Mixed     | 6.15 (3.2), 5.57 (3.2) | 0.37 (3.2) | 0.0001 |
|                  | Glimmix/  | 6.04 (3.2), 105.3 (3.2) | 3.8 (3.2) | 0.0267 |
|                  | Gamma     | 0.0267 | 0.0001 | 0.0006 |
|                  | Mixed     | 6.15 (3.2), 5.57 (3.2) | 0.37 (3.2) | 0.0001 |
|                  | Glimmix/  | 6.04 (3.2), 105.3 (3.2) | 3.8 (3.2) | 0.0267 |
|                  | Gamma     | 0.0267 | 0.0001 | 0.0006 |
|                  | Mixed     | 6.15 (3.2), 5.57 (3.2) | 0.37 (3.2) | 0.0001 |
|                  | Glimmix/  | 6.04 (3.2), 105.3 (3.2) | 3.8 (3.2) | 0.0267 |
|                  | Gamma     | 0.0267 | 0.0001 | 0.0006 |
|                  | Mixed     | 6.15 (3.2), 5.57 (3.2) | 0.37 (3.2) | 0.0001 |
|                  | Glimmix/  | 6.04 (3.2), 105.3 (3.2) | 3.8 (3.2) | 0.0267 |
|                  | Gamma     | 0.0267 | 0.0001 | 0.0006 |
|                  | Mixed     | 6.15 (3.2), 5.57 (3.2) | 0.37 (3.2) | 0.0001 |
|                  | Glimmix/  | 6.04 (3.2), 105.3 (3.2) | 3.8 (3.2) | 0.0267 |
|                  | Gamma     | 0.0267 | 0.0001 | 0.0006 |
|                  | Mixed     | 6.15 (3.2), 5.57 (3.2) | 0.37 (3.2) | 0.0001 |
|                  | Glimmix/  | 6.04 (3.2), 105.3 (3.2) | 3.8 (3.2) | 0.0267 |
|                  | Gamma     | 0.0267 | 0.0001 | 0.0006 |
|                  | Mixed     | 6.15 (3.2), 5.57 (3.2) | 0.37 (3.2) | 0.0001 |

Leaf water relations parameters (Eplant (canopy evapotranspiration), RWC (leaf relative water content), ψleaf (midday leaf water potential), and ψpd (predawn leaf water potential)). Instantaneous gas exchange parameters: gs (operational stomata conductance), and Aop (operational photosynthetic rate). Indicators of leaf photosynthetic capacity: Rlight (respiration rate in the light), initial quantum yield for CO₂ fixation (Y(CO₂)LL), light saturated gross assimilation rate (GA,SAT), light compensation point (LCP), carboxylation efficiency (CE), maximal rubisco carboxylation rate (VCMAX), maximal PEP carboxylation rate (VPEP), CO₂ compensation point (Γ), and CO₂-saturated assimilation rate (A,SAT). Starch content: leaf starch concentration, and root starch concentration. Output from repeated-measures ANOVA in SAS (v.9.4; SAS Institute, Cary, NC, USA). Modelled main effects were [CO₂]a, (200 ppm, 400 ppm or 800 ppm), watering level (WL: 80%, 60%, 50%, 40%, 30%, recovery), and their interaction; individual plant was the random effect. Analyses were: PROC MIXED (linear model) for normal data with homogenous variance; PROC GLIMMIX (generalized linear mixed model, dist = normal, logit identity) for normal data with heteroscedasticity; and PROC GLIMMIX (dist = gamma, logit link) for non normal data (absolute values were used in the case of variables with negative values). In all analysis individual comparisons for significant model effects were performed with Fisher’s PLSD differences (LSMEANS or ILINK option of the LSMEANS statement in GLIMMIX). The table shows the F-statistic with numerator and denominator degrees of freedom and P-values. Numbers in cells represent: first number is Fndf,ddf: F value, numerator degrees of freedom, denominator degrees of freedom. Second number is P-value.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- A description of all covariates tested
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- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted. Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection
Gas exchange data were acquired through the embedded software provided by Licor, updated at the time of measurements (Open 6.3, 2015). Images were processed with ImageJ (Fiji version 2014 for 32 bit Windows).

Data analysis
Data were treated using tools previously developed by us and that are freely downloadable (Refs 75 and 76). Statistical analyses were conducted using commercial software (SAS 9.4 and Genstat 18.2).

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | We measured at least 4 replicates per species and treatment combination. No statistical method was used to predetermine sample size. These sample size was the maximum number of plants that we could fit in the cabinets. |
| Data exclusions | No data points were excluded from data analyses. |
| Replication | The effects of watering regime and CO2 levels were previously assessed separately (Refs 26 and 55) using identical setup. In this experiment CO2 levels were replicated n=2, the watering regime was applied independently to each plant three times weekly. All replications were consistent. Additionally, for control purposes, Tobacco plants (Figure S1) whose behavior was previously characterized in detail (Ref 75) were grown alongside our experimental plants throughout the entire experiment. These Tobacco plants were regularly monitored for growth and morphological traits to ensure repeatability of the treatment. Measuring equipment was calibrated against standards and operated using best practices that we have developed previously (Refs 73, 75, 76, 79, 80). |
| Randomization | The plants were randomly allocated between treatments. |
| Blinding | The investigator was fully blinded for starch analysis and for water potential measurements: sampling was conducted independently by a second investigator and samples were identified with a code. For gas exchange and for weighing, blinding was not possible because measurements are performed in-situ on the plants and are concurrent with sampling. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

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| --- | --- |
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| | Eukaryotic cell lines |
| | Palaeontology and archaeology |
| | Animals and other organisms |
| | Human research participants |
| | Clinical data |
| | Dual use research of concern |

| Methods | Involved in the study |
| --- | --- |
| n/a | ChIP-seq |
| | Flow cytometry |
| | MRI-based neuroimaging |