Water and nutrients alter herbaceous competitive effects on tree seedlings in a semi-arid savanna

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Summary

1. Globally, both climatic patterns and nitrogen deposition rates show directional changes over time. It is uncertain how woody seedlings, which coexist with herbaceous plants in savannas, respond to concurrent changes in water and nutrient availability.

2. We investigated competition effects between herbaceous vegetation and tree seedlings (*Colophospermum mopane*) under changed water and nutrient (fertilized) conditions in a garden experiment situated in a semi-arid savanna.

3. Herbaceous competition significantly suppressed woody seedling growth. The effect of herbaceous competition on woody seedling growth remained constant with both increasing water and nutrient availability. However, during a wet-season drought, herbaceous competition apparently caused premature leaf senescence in non-irrigated treatments. Fertilization exacerbated negative competition effects during the drought, while irrigation prevented leaf loss of tree seedlings in spite of herbaceous competition and fertilization.

4. Based on a conceptual model, we propose that the vigorous response of herbaceous plants to increased nutrient availability leads to faster depletion of soil water, which increasingly causes water stress in woody seedlings if the interval between watering events is prolonged, e.g. during wet-season droughts.

5. *Synthesis*. Our data support the notion that changes in drought frequency are of greater importance to woody recruitment success than changes in annual rainfall amount. Based on the water and nutrient interactions observed in our experiment, we suggest that the effect of increased nitrogen deposition on woody seedling recruitment is contingent on water availability.

Key-words: climate change, drought, herbaceous, nitrogen deposition, nutrients, semi-arid savanna, tree-grass competition, water, woody seedling

Introduction

The rapid increase in dominance of woody species over herbaceous plants observed in many savannas around the globe (bush encroachment) highlights the sensitivity of woody-herbaceous biomass ratios to changed environmental conditions (Scholes & Archer 1997; Sankaran *et al.* 2005,

2008). The ratio between tree and grass biomass is important because it affects animal production, shapes animal assemblages and determines carbon sequestration capacities (Scholes & Archer 1997; Sankaran *et al.* 2005; Prins & Gordon 2008). The current understanding is that water availability primarily affects the relative proportion of woody to herbaceous vegetation of an area and that soil factors (e.g. soil texture and fertility) and disturbance factors (e.g. fire and herbivory) are of secondary importance (van Langevelde *et al.* 2003;
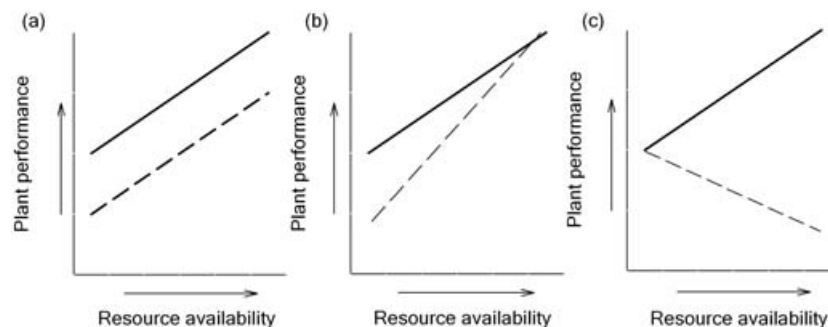
Fig. 1. Hypothetical relationships between the performances of target plants growing in the absence (solid lines) or presence (dashed lines) of competition under increasing resource availability. The difference in performance between plants with and without competition represents the effect of competition on target plants. In (a), the effect of competition on target plants remains constant with increasing resource availability; in (b), the effect of competition diminishes with increasing resource availability; while in (c), the effect of competition increases with increasing resource availability.

Sankaran *et al.* 2005, 2008; de Knegt *et al.* 2008; Groen *et al.* 2008). Moreover, resource availability in savannas is undergoing directional changes. For instance, in an analysis of changes in soil moisture and drought between 1950 and 2000, Sheffield & Wood (2008) found a drying trend, accompanied by an increase in spatial extent of droughts in Africa, while soil moisture increased in Australia over the same period. In addition, artificially enhanced nitrogen deposition, e.g. a three- to fourfold increase in nitrogen deposition in parts of southern Africa (Scholes *et al.* 2003), is also occurring (Bouwman *et al.* 2002; Dentener *et al.* 2006). These trends are likely to continue into the future (Dentener *et al.* 2006; Sheffield & Wood 2008), but the responses of woody vs. herbaceous plants to these changes are still uncertain (Scholes & Archer 1997; House *et al.* 2003; Sankaran *et al.* 2004, 2005). Here we aim to contribute to the understanding of woody recruitment responses to changed water and nutrient availability.

In savannas, woody cover generally increases with increasing mean annual precipitation (Scholes *et al.* 2002; Sankaran *et al.* 2005, 2008), therefore woody cover is expected to track changes in annual rainfall patterns (Sankaran *et al.* 2008). In arid and semi-arid (dry) savannas, seedling recruitment apparently regulates woody cover (Higgins *et al.* 2000; van Wijk & Rodriguez-Iturbe 2002; Sankaran *et al.* 2004), although not in all species (Seymour 2008). Woody seedlings are intolerant of wet season droughts and successful recruitment is dependent on a continuous water supply (Harrington 1991; O'Connor 1995; Wilson & Witkowski 1998; Higgins *et al.* 2000; Kraaij & Ward 2006), which tends to occur more frequently as mean annual precipitation increases. In addition, herbaceous plants, which coexist with woody plants in savannas, compete for water, nutrients and light and can pose an important hurdle to establishing woody seedlings (Scholes & Archer 1997; House *et al.* 2003). However, it is not clear how the strength of competition between herbaceous plants and woody seedlings changes with increasing water availability. We propose two scenarios for explaining the positive water-availability – woody-recruitment pattern: (i) the

effect of herbaceous-species competition remains constant (additive effect) over a water-availability gradient although the net supply of water for plant use increases (Fig. 1a) or (ii) herbaceous plants – seedling competition relaxes with increasing water availability (Fig. 1b). Distinguishing between these competition scenarios has direct implications for the management of dry savannas, where disturbances such as overgrazing, which lowers the competitiveness of the herbaceous layer, has been implicated as a cause of bush encroachment (Harrington 1991; Jeltsch *et al.* 1997; Scholes & Archer 1997).

In contrast to the positive water availability – woody cover relationship, woody cover is negatively related to nutrient (e.g. nitrogen) availability in savannas (Sankaran *et al.* 2008), apparently because performance of woody seedlings in herbaceous swards is adversely affected by soil fertility (Cohn *et al.* 1989; Kraaij & Ward 2006). Woody cover is therefore predicted to be negatively affected by enhanced nitrogen deposition (Sankaran *et al.* 2008). Furthermore, Cohn *et al.* (1989) and Debain *et al.* (2005) demonstrated in competition experiments that the negative effect of fertilization on seedling performance is caused by the intensification of herbaceous competition on seedlings under fertile conditions (Fig. 1c) and not by direct effects of high nutrient availability on seedlings. Little is known, however, about the interactions between water and nutrient availability on the competitiveness of herbaceous swards and establishing woody seedlings. This knowledge gap hampers predictions of woody cover responses to concurrent changes in water and nutrient availability. A possibility is that the increased herbaceous competitiveness under fertile conditions is not caused by the direct pre-empting of nutrients by herbaceous plants, but indirectly by lowering the availability of an intermediary resource, which in turn limits the growth and survival of seedlings (Cohn *et al.* 1989). As water is a likely intermediary resource (Davis *et al.* 1998; Debain *et al.* 2005), it follows that dry conditions are expected to exacerbate negative fertilizer effects, by intensifying competition between herbaceous plants and woody seedlings.

In this paper, we report on the findings of an experiment conducted in a semi-arid savanna where we studied the effect of herbaceous competition on establishing woody seedlings in relation to manipulated water and nutrient conditions. For this purpose, we used the model tree species, *Colophospermum mopane* (Kirk ex Benth.) Kirk ex J. Léonard. *Colophospermum mopane* (Mopane) was chosen because it currently covers more than 500 000 km^2 of southern African lowlands where livestock production, game ranching and conservation are the main forms of land use (Smit 2001; MacGregor & O'Connor 2002). Mopane has also been implicated as a bush encroacher, forming dense monotypic stands, and its proliferation occurs at the expense of herbaceous production and dry season woody foliage retention, thereby negatively affecting both grazer (Smit 2001; MacGregor & O'Connor 2002) and browser populations (Smit 2001), with potential negative impacts on biodiversity.

We expressed competition in terms of the absolute effect of competitors (herbaceous plants) on target plant (woody seedling) performance because the size of seedlings at the end of the growing season is positively correlated with their subsequent survival chances during the dry season (Poorter 2005). We tested the following hypotheses: (i) competition between herbaceous vegetation and woody seedlings relaxes with increasing water availability (Fig. 1b), (ii) competition between herbaceous vegetation and woody seedlings intensifies with increasing nutrient availability (Cohn *et al.* 1989; Kraaij & Ward 2006) (Fig. 1c) and (iii) competition effects of herbaceous plants on establishing woody seedlings are greatest under low water-availability and high nutrient-availability conditions (Davis *et al.* 1998).

Methods

STUDY SITE

The study was conducted in the Timbavati Private Nature Reserve in South Africa in a fenced area (24°15′02″ S, 31°23′36″ E) from where large herbivores (> 5 kg) were excluded. The experiment was laid out in an open, i.e. non-wooded, flat area on a crest. The area had been annually mowed in the past and had, to our knowledge, not been fertilized before.

The soil at the study site is derived from granite (Venter *et al.* 2003). Topsoil samples (0–15 cm depth) of the study site were sent to the Agricultural Research Council's laboratory in Nelspruit, South Africa, for standard analyses according to procedures in the Handbook of Standard Soil Testing Methods for Advisory Purposes (Anonymous 1990). The soil is coarsely textured (88% sand: 2% silt: 10% clay) and poor in macro nutrients (e.g. 4.4–6.4 mg P(Bray 1) kg^{-1} soil, 457 mg Ca kg^{-1} soil, 131 mg K kg^{-1} soil). The topsoil contained total nitrogen amounts of 0.55–0.65 g N kg^{-1} and total carbon values of 5.8–7.0 g C kg^{-1} soil (C/N ratio = 10.4–10.7).

The long term mean rainfall (1983–2004) is about 450 mm (Ingwalala rainfall station 10 km N), of which 78% is received between October and end of March. The mean maximum temperature for the Satara weather station (50 km E) during January (hottest month) is 33.7 °C and the mean minimum temperature for June (coolest month) is 9.4 °C (Venter *et al.* 2003).

EXPERIMENTAL SETUP

We compared the growth response of the focal species (*C. mopane*) in isolation with its response in the presence of herbaceous competitors. The competition series consisted of a single Mopane seedling, herbaceous plants only and a mixture of the two, which allowed us to deduce effects of herbaceous vegetation on seedlings and, conversely, seedling effects on herbaceous swards. In the single-Mopane-seedling treatment, plots were kept clear of herbaceous plants by monthly hoeing, although more frequent hoeing was necessary to keep herbaceous plants out at the start of the experiment. The first hoeing was done in December 2006. The cleared material was removed from the plots.

Experimental plots (*n* = 120) were 1.5 × 1.5 m in size. Treatments followed a two-way factorial design with two water availability levels and two fertilizer levels in a split-plot layout. The experiment was spatially divided into five blocks (7 × 10 m per block), each split in quarters. A buffer area of at least 0.5 m width separated blocks and block quarters. The four water- and nutrient-treatment combinations were randomly allocated among the block quarters with the provision that the two water addition treatments (irrigated-fertilized and irrigated-non-fertilized) were placed adjacent to each other to facilitate the irrigation process. Two single *C. mopane* plots, two herbaceous monoculture plots and two mixtures were randomly allocated to block quarters, which means that all competition-series treatment combinations were replicated 10 times.

PRE-TREATMENT

The study site was ploughed (about 20 cm deep) and tilled in the 2006 dry season to homogenize the soil and to give all treatments the same starting conditions. The natural establishment of herbaceous species in the new growing season was allowed, but as a precaution against poor herbaceous establishment, locally collected seeds of *Urochloa mosambicensis*, a locally dominant grass species, were sown into herbaceous-only and mixture plots (*n* = 80) in November 2006 and supplied with 3 mm (7 L plot^{-1}) water by hand. By December 2006 the herbaceous layer, comprising of different species (see the Results section), reached about 15 cm height and densely covered all plots. In December 2006, a single, year-old *C. mopane* seedling was planted in the centre of plots (*n* = 80). During December 2006 an additional 38 mm (85 L plot^{-1}) water was supplied by hand to all plots (*n* = 120) to facilitate the establishment of both herbaceous and *C. mopane* seedlings.

The Mopane seedlings were earlier propagated from seeds collected in the study area and grown in a nursery in nursery bags filled with a mixture of coarse river sand and commercial potting soil. Seedlings were grouped into five size classes and randomly allocated to the five experimental blocks. At the start of the experiment the seedlings were 0.75 ± 0.02 m tall and had 39 ± 2 leaves per seedling (mean ± SE).

IRRIGATION AND FERTILIZER TREATMENTS

A drip irrigation system was installed using pressure self-regulating drippers with an inline spacing of 300 mm and a supply rate of 1 L h^{-1} dripper^{-1}. Three parallel dripper lines per plot ensured an even distribution of water in the irrigated plots. Low retaining walls surrounded each plot to prevent runoff water spilling over on adjacent plots. In total, 13 irrigation events, each supplying about 15 mm, were supplied from the end of January to early May 2007. A locally

developed water budget model (Sapwat; www.sapwat.org.za) was used to determine when to irrigate. The model predicts the soil water status over time from generalized evapotranspiration data and current rainfall and irrigation data and indicates when to irrigate to avoid water stress in plants. The scheduling of irrigation events in our experiment simulated an increase in the frequency of rainfall events, by irrigating between rainfall events, rather than increasing the intensity of rainfall events. A comparison of dry and wet rainfall seasons (seasons that deviated by more than 10% below and above, respectively, from the long-term mean) for the nearby Satara weather station in the Kruger National Park revealed that the frequency of rainfall events during wet years (52.3 ± 9.6, mean $\pm$ SD) was significantly higher than during dry years (28.5 ± 5.3; $t = 7.402$, d.f. $= 20$, $P < 0.001$), while the median rainfall amount per event was not significantly different between dry and wet years (Mann-Whitney U test, $Z = -0.97$, $P > 0.05$). This suggests that our manipulation of the frequency of rainfall events is more realistic than the manipulation of rainfall intensity would be.

We used a commercial NPK fertilizer. The fertilizer was evenly spread by hand and a total of 22.5 g N m^{-2}, 15 g P m^{-2} and 7.5 g K m^{-2} was supplied on two occasions, two-thirds on 21 December 2006 and the remainder on 12–14 February 2007. Our nitrogen fertilizer amount was comparable to that used by Ludwig *et al.* (2001), and is in line with local fertilizer recommendations for commercial, irrigated summer pastures (Dickinson *et al.* 1990). In the Kruger National Park the annual amount of nitrogen mineralized at a site comparable to our study site, was estimated at $5.8 \text{ g N m}^{-2} \text{ yr}^{-1}$ (Scholes *et al.* 2003), suggesting that we increased local nitrogen availability about four times above background levels by fertilization.

MEASUREMENTS

After the seasonal senescence of the herbaceous layer, at the end of May 2007, plots were harvested with shears, once the species composition had been assessed. The aerial cover of each individual herbaceous species (forbs as a group), was assessed in the inner 1 m^2 plot area according to the Braun-Blanquet scale (Kent & Coker 1994). We clipped the herbaceous material of plants rooted within the inner 1 m^2 plot area at a height of approximately 8 cm above ground level and the total biomass per plot was weighed in the field. The moisture content of sub-samples ($n = 20$) was determined after drying sub-samples at 70 °C for 48 h in a drying oven. Field biomass values were adjusted accordingly. The nomenclature of grass species follows Gibbs Russell *et al.* (1990).

On 5 March 2007, all leaves on Mopane seedlings were counted, the plant height measured and the diameter of stems measured with callipers. Stem diameter was measured about 2 cm above ground level. Because the immature leaves that were still folded might not have matured, immature leaves were omitted from leaf counts. Following a wet season drought during February–March 2007 (Fig. 2a), senescing leaves were counted on 30 March 2007. Senescing leaves were defined as leaves where more than half the leaf area was necrotic (brown and crisp). Studies in both semi-arid grassland (Harrington 1991) and tropical forests (Engelbrecht *et al.* 2006; Engelbrecht *et al.* 2007; Slot & Poorter 2007) showed that visual signs of water stress are good predictors of actual water stress and subsequent survival in woody seedlings. New growing season shoot length was measured on 4 April 2007. In *C. mopane* a distinctive dry season scar indicates the start of new season growth. We summed the length of all new shoots per seedling.

STATISTICAL ANALYSIS

The fixed effects of competition, fertilization and irrigation were analyzed using a full factorial, three-way ANOVA for herbaceous production, and a full factorial, three-way MANOVA for *C. mopane* shoot growth, leaf number, plant height and stem diameter. Competition (present, absent), water (irrigated, non-irrigated) and nutrients (fertilized, non-fertilized) were treated as fixed factors. Block was included as a random factor. Untransformed herbaceous biomass data followed a normal distribution (Kolmogorov-Smirnov, $P > 0.05$) and had equal variances (Levene's test of equality of error variances, $P > 0.05$). All *C. mopane* variables were logarithmically transformed to meet the ANOVA assumptions. A significant competition × resource interaction implies that the competition effect of the one growth form on the other was affected by the amount of resources (e.g. either Fig. 1b or Fig. 1c); an insignificant competition × resource interaction is depicted in Fig. 1a. Tukey's multiple comparison tests were used to detect differences between subgroups.

The proportion of leaves surviving the drought was calculated from the total number of leaves before the drought occurred minus senesced leaves per seedling. Leaf proportion data were arcsine-transformed and subjected to a full factorial, three-way ANOVA similar to the procedures described for herbaceous production.

All tests were carried out in SPSS version 12.

Results

WATER AVAILABILITY

Non-irrigated plots received a total of 390 mm water (878 L plot^{-1}) during the study period (November 2006 to end May 2007), of which 335 mm (754 L plot^{-1}) was received as rain. The difference was supplied during the establishing phase in 2006 (about 40 mm) and in March 2007 (15 mm or 34 L plot^{-1}) to avert seedling mortalities after extremely dry conditions during February and March 2007 (Fig. 2a). Irrigated treatments received in total 575 mm water (1294 L plot^{-1}). Compared to long-term data for the study area, the water treatments were realistic and represented average and above-average rainfall conditions, respectively (Fig. 2b).

HERBACEOUS SPECIES COMPOSITION

The herbaceous cover of plots was dominated by *Urochloa mosambicensis* (median cover class = '26–50%'), while forbs as a group (median = '6–25%'), *Melinis repens* (median = '6–25%') and *Brachiaria deflexa* (median = '6–25%') were also important.

HERBACEOUS PRODUCTION

Above-ground herbaceous biomass significantly increased with fertilization ($P < 0.001$) and irrigation ($P < 0.001$) (Table 1, Fig. 3), and these two factors significantly interacted ($P < 0.05$) (Table 1, Fig. 3). Irrigated-only plots had on average 44% higher herbaceous biomass than controls and fertilized-only plots 64% higher biomass than controls, but differences between irrigated and fertilized biomass were not significant (Tukey, $P > 0.05$). The mean biomass of plots both
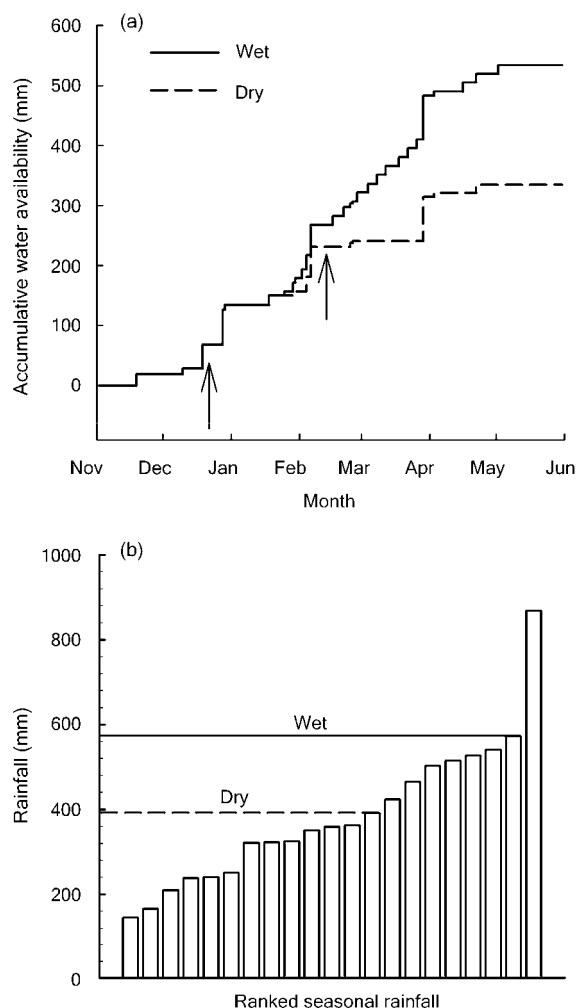
Fig. 2. (a) Accumulative water availability (mm) for irrigated (wet) and not irrigated (dry) treatments from November 2006 to the end of May 2007. Arrows indicate the timing of fertilization. (b) The water received in controls and irrigation treatments in relation to the ranked seasonal rainfall (November to end May; 1983 to 2004) for the Ingwalala rainfall station (10 km N), showing that treatment values are realistic for this system.

irrigated and fertilized was significantly (Tukey, $P < 0.001$) higher than irrigated-only and fertilized-only treatments and the mean biomass was increased by 175% relative to controls.

Interestingly, the presence of a single *C. mopane* seedling per plot significantly suppressed herbaceous above-ground biomass ($P < 0.01$; Table 1). The herbaceous biomass in the mixed plots was on average 21% lower than the biomass of herbaceous plants alone (Fig. 3). The nutrient × competition and water × competition interaction effects were not significant, indicating that neither fertilization nor irrigation modified the competition effect that *C. mopane* seedlings exercized on herbaceous biomass (Table 1).

COLOPHOSPERMUM MOPANE SEEDLING GROWTH

The above-ground growth of *C. mopane* seedlings (represented by shoot length, leaf number, stem diameter and plant height) was significantly increased by irrigation (MANOVA, Wilk's Lambda, $P < 0.001$) and decreased by herbaceous competition (Wilk's Lambda, $P < 0.001$), but not significantly affected by fertilization (Table 1). Compared to the non-irrigated seedlings, irrigated seedlings had on average more leaves (+21%, $P < 0.1$), longer shoot lengths (+35%, $P < 0.05$), thicker stems (+21%, $P < 0.05$) and were taller (+16%, $P < 0.05$) (Table 1, Fig. 3 & Appendix S1). Fertilization significantly increased seedling leaf number (+23%, $P < 0.05$), but the increases in shoot length (+21%) and stem diameter (+1%) were not statistically significant. A significant water × nutrient interaction effect on seedling height ($P < 0.01$) indicated that nutrients mediated the effect of water on seedling height (Table 1, Appendix S1). Seedlings, both fertilized and irrigated, were taller than seedlings that were only fertilized (Tukey, $P < 0.05$), which in turn could not be statistically distinguished from either controls or watered-only seedlings (Tukey, $P \geq 0.05$).

Herbaceous swards suppressed *C. mopane* growth. Seedling shoot growth (−51%; Appendix S1), leaf number (−53%; Fig 3), plant height (−11%; Appendix S1) and stem diameter (−12%; Appendix S1) were all consistently suppressed by the presence of herbaceous vegetation ($P < 0.01$; Table 1). The insignificant competition × water and competition × nutrients interaction terms (Table 1) indicate that neither water nor nutrient availability altered the suppressing effect of herbaceous vegetation on seedling growth. Thus we fail to reject the null hypotheses 1 and 2.

COLOPHOSPERMUM MOPANE DROUGHT RESPONSE

The proportion of *C. mopane* leaves that survived the mid-season drought, an indicator of water stress (Engelbrecht *et al.* 2007), varied significantly among the different combinations of competition, water and nutrient treatments (Fig. 3). Considering first the main effects, the surviving leaf proportion was highly significantly affected by herbaceous competition ($F_{1,61} = 108.1$, $P < 0.001$) and irrigation ($F_{1,61} = 223.9$, $P < 0.001$), but not fertilization. Block (random factor) was also not significant ($P > 0.05$). Significant two-way interactions were found for competition × water ($F_{1,61} = 110.9$, $P < 0.001$) and competition × nutrients ($F_{1,61} = 9.9$, $P < 0.01$), but not for the water × nutrient interaction ($P > 0.05$). A significant ($F_{1,61} = 6.4$, $P < 0.05$) three-way interaction was found for competition × water × nutrients. Treatments that received water had the highest proportion of surviving leaves and were not significantly different from each other (Tukey, $P > 0.05$). Leaf survival of single-control seedlings was lower (Tukey, $P < 0.05$) than that of single seedlings receiving both irrigation and fertilizer, but was higher than in the two dry-mixture treatments (Tukey, $P < 0.001$; Fig. 3). In turn, fertilized dry-mixture seedlings had lower surviving leaf proportions than unfertilized, dry-mixture seedlings (Tukey, $P < 0.01$; Fig. 3b). We therefore conclude the following. (i) Wet season leaf senescence was largely prevented either by irrigating mixtures or by eliminating grass competition (Fig. 3a). (ii) Under dry conditions, more leaves died when *C.*

Table 1. Analysis of variance tests for herbaceous above-ground biomass and *Colophospermun mopane* shoot growth, number of leaves, plant height and stem diameter as affected by nutrients (two levels), water (two levels) and competition (presence–absence). Absence of competition refers to situations where herbaceous vegetation and seedlings occurred as monocultures or singly, respectively. Presence of competition refers to mixtures of herbaceous vegetation and seedlings. Block was included as a random factor. Herbaceous above-ground biomass responses were tested with an ANOVA and *C. mopane* responses with a MANOVA. The degrees of freedom, *F*-value and significance are indicated per factor and factor interactions

	ANOVA		MANOVA						
		Herbaceous biomass	Multivariate test			Shoot length	Leaf number	Plant height	Stem diameter
Source	d.f.	*F*-value	*F*-value		d.f.	*F*-value	*F*-value	*F*-value	*F*-value
Corrected Model					11	8.635 ***	8.240 ***	5.181 ***	5.640 ***
Intercept	1	281.558 ***†	30523.339(4)‡ ***		1	26770.254 ***	8879.738 ***	118188.866 ***	15919.821 ***
Nutrient (N)	1	55.335 ***	1.472(4) NS		1	2.068 NS	4.574 *	0.853 NS	0.035 NS
Water (W)	1	34.131 ***	6.251(4) ***		1	6.110 *	3.399 NS	9.755 **	25.337 ***
Competition (C)	1	9.444 **	17.987(4) ***		1	62.491 ***	58.991 ***	6.117 *	12.212 **
N × W	1	6.849 *	2.351(4) NS		1	0.001 NS	0.034 NS	9.043 **	2.047 NS
N × C	1	1.795 NS	1.694(4) NS		1	0.643 NS	0.198 NS	1.784 NS	2.611 NS
W × C	1	0.254 NS	0.951(4) NS		1	2.113 NS	0.013 NS	0.129 NS	0.003 NS
N × W × C	1	1.798 NS	0.625(4) NS		1	0.751 NS	0.040 NS	0.354 NS	0.593 NS
Block	4	2.609 *	2.561(16) **		4	3.972 **	4.026 **	5.880 ***	3.343 *
Error	61				59				
R^2 (adjusted)						0.55	0.53	0.40	0.42

†Significance level; ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; NS = not significant.
‡Values in brackets are degrees of freedom for Multivariate test.

mopane seedlings were fertilized (Fig. 3b). (iii) This negative effect of fertilization was reversed when water was supplied in addition to fertilizer (Fig. 3c). Thus the *C. mopane* seedling drought response supports hypothesis 3.

Discussion

RESPONSE DIFFERENCES TO WATER AND NUTRIENT ADDITION BETWEEN HERBACEOUS SWARDS AND WOODY SEEDLINGS

Herbaceous above-ground biomass was highly significantly affected by both fertilization and irrigation, which confirms that herbaceous production in African savannas is strongly co-limited by water and nutrients (Penning de Vries & Djitèye 1982; Donaldson *et al.* 1984; Snyman 2002; Fynn & O'Connor 2005). The large response of the herbaceous layer to fertilization accentuates that the low natural concentrations of the nutrients added, N, P and K, put severe constraints on herbaceous production in the study area. The interaction between water and nutrients, where the relative effect of nutrients on herbaceous biomass production was amplified when irrigated (Fig. 3c), is in agreement with other studies in semi-arid areas (Penning de Vries & Djitéye 1982; Snyman 2002), showing that the magnitude of a fertilizer response increases as water availability improves.

The above-ground growth response of *C. mopane* seedlings to increasing resource availability was more subdued than the marked response of the herbaceous layer exposed to similar resource levels. Moreover, the responses of the two growth forms to resource additions were that *C. mopane* growth was relatively more affected by water than nutrients (leaf number is an exception), while the herbaceous vegetation responded relatively stronger to fertilization compared to irrigation. Grasses invest a higher fraction of biomass to nutrient-demanding resource-capturing tissue (e.g. photosynthetic active leaves), which feeds back to faster relative growth rates (Poorter & Nagel 2000), while trees allocate more biomass towards woody structure, which is carbon rich, but nutrient poor (Bond *et al.* 2003). In addition, the C4 photosynthetic pathway followed by grasses in the study area offers higher water and nitrogen use efficiencies, compared to the less efficient C3 photosynthetic pathway followed by *C. mopane* (Ehleringer & Monson 1993; Turekian *et al.* 1998). Thus for a unit increase in a limiting resource, more biomass is produced in C4 plants than in C3 plants, which presents a competitive advantage for C4 grasses.

COMPETITION EFFECTS OF WOODY SEEDLINGS ON HERBACEOUS PLANTS

The significant suppression of the herbaceous production associated with single juvenile *C. mopane* seedlings is surprising and demonstrates the competitive ability of *C. mopane*, even at a young stage. A *C. mopane* thinning experiment, involving mostly mature trees, showed a strong negative correlation between *C. mopane* density and herbaceous production in an arid savanna (Smit & Rethman 2000), apparently because the shallow rooting pattern of *C. mopane* (about 60% of *C. mopane* root mass was found in the top 40 cm soil layer) increases opportunities for below-ground interaction with herbaceous roots (Smit & Rethman 1998). The tendency of *C. mopane* to form dense monotypic stands (MacGregor & O'Connor 2002), where grasses are largely excluded (Smit 2001,
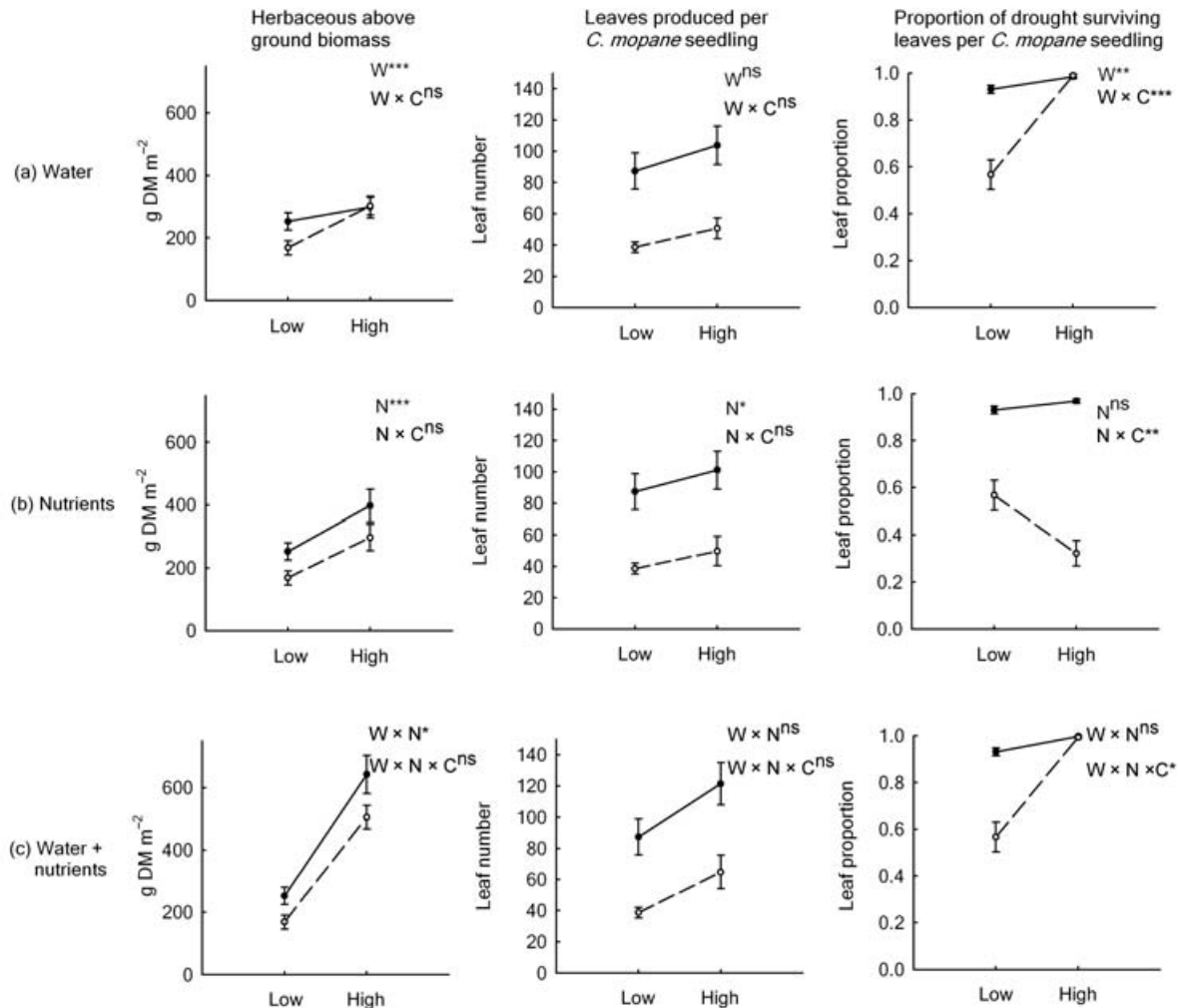
Fig. 3. The responses of target plants growing in the absence of competition (solid circles) and competing plants (open circles) as affected by (a) irrigation, (b) fertilization and (c) irrigation plus fertilization. The first column depicts herbaceous above-ground production (dry mass), the middle column leaf number per *Colophaspermum mopane* seedling and the last column the proportion of leaves per *C. mopane* seedling that survived a mid-season drought. The 'low' values (*x*-axis) in columns represent the same control values, and 'high' the response to resource enrichment. Means ± SE are given. Statistics are also given for the main effects of irrigation (W), fertilization (N) and their interactions with competition (C). Superscripts denote: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns, not significant.

2004), suggests that *C. mopane* facilitate the establishment of its own seedlings by suppressing the competing herbaceous vegetation in a positive feedback loop.

Our results further suggest that seedlings already contribute to this process by suppressing neighbouring herbaceous plants. The suppression of herbaceous production by mature woody plants are widely reported for other systems (references in Scholes & Archer 1997; Smit 2004), but we are unaware of other studies demonstrating self facilitation by juvenile woody plants.

HERBACEOUS COMPETITION EFFECT ON WOODY SEEDLINGS AS AFFECTED BY WATER AND NUTRIENT AVAILABILITY

On account of the growth data, hypothesis 1, which states that the competition between herbaceous vegetation and woody

seedlings relaxes with increasing water availability, was not supported (Fig. 3a). Instead we found that the effect of water availability on competition was additive. However, we have to qualify this statement. First, we only explored average and above-average water availability levels characteristic of the study area (390 vs. 575 mm year^{-1}). It therefore remains uncertain whether the effect of herbaceous competition will remain additive if a larger range in water availability is explored, especially towards the lower end in water availability. For example, the low availability of water during a mid-season drought strongly increased the competitiveness of swards as indexed by the premature leaf senescence of the *C. mopane* seedlings, which might have resulted in mortalities if the drought had persisted for a few more days. As it was, all seedlings recovered. Wet season droughts of more than 30 days, which can occur even in average years, can lead to woody seedling mortality (references in Higgins *et al.* 2000). To

summarize, the strength of herbaceous competition on woody seedlings appears to remain constant with increasing water availability under non-drought conditions, but may intensify under drought conditions. For woody seedlings establishing in herbaceous swards this implies that the temporal distribution of watering events is more important than changes in the total amount received (Wilson & Witkowski 1998).

Moreover, although the *C. mopane* growth data suggest an additive effect of herbaceous competition with increasing nutrient availability (not supporting hypothesis 2; Fig. 1a), the leaf senescence pattern observed in our study contradictorily supports hypothesis 2, i.e. that herbaceous competition effects on seedlings increase under fertile conditions. Also, in support of hypothesis 2, the density of live *C. mopane* germinants (cotyledons still present), which established naturally in herbaceous swards in a concurrent field experiment during November and December 2007, was negatively affected by fertilization in spite of similar seed densities (C. van der Waal, unpublished data). For example, areas fertilized at 6 g N m^{-2} and 30 g N m^{-2} had on average 1.9 and 0.3 *C. mopane* germinants per 100 m^2, respectively, compared to the 9.8 germinants per 100 m^2 of controls. This supports the notion that woody recruitment in fertile areas is constrained by intensified herbaceous competition (Sankaran *et al.* 2008). Davis *et al.* (1998) hypothesized that the suppressing effect of fertilization on establishing seedlings operates indirectly through the pre-empting of soil water resources by vigorous herbaceous growth. However, the nutrient × competition interaction effect in our study only emerged during a wet-season drought, suggesting that the effect of nutrients on the competitiveness of the herbaceous layer is contingent on background water supply rates. This is clearly illustrated in our study by the reversal of the negative fertilizer effect (premature leaf senescence) when additional water was supplied (Fig. 3a,b). We propose the following mechanism to explain these phenomena based on a conceptual model (Fig. 4). Herbaceous growth in fertile conditions is vigorous (e.g. Fig. 3b), which results in increased transpiration rates and thus faster depletion of soil water reserves after a watering event relative to water use in infertile soils (Fig. 4). Fertilized swards wilted at an earlier date than controls under drying conditions (Snyman 2002) and soils of fertilized swards were significantly drier 1 day and 10 days after a watering event (Kraaij & Ward 2006). This, in turn, increases the period (period a–c vs. b–c in Fig. 4) and severity of water stress seedlings are exposed to (Wilson & Witkowski 1998; Poorter 2005). In our conceptual model more frequent watering shortens the period that seedlings experience critical water stress and thus facilitates woody seedling establishment.

COMPARISON OF THE RESPONSE OF *C. MOPANE* AND OTHER AFRICAN WOODY SPECIES TO HERBACEOUS COMPETITION AND RESOURCE AVAILABILITY

In agreement with our results for *C. mopane* seedlings, other studies also found suppressing effects of herbaceous vegetation on the growth and survival of various *Acacia* species
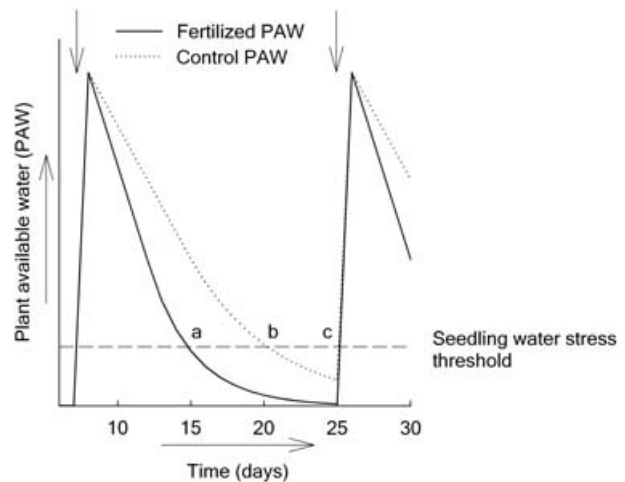

Fig. 4. A conceptual model of the influence of fertilization of herbaceous plants – woody seedling mixtures on plant available water (PAW) over a wet-season drought period, illustrating the water stress consequences for establishing woody seedlings. The solid line indicates PAW for fertilized mixtures and the dotted line PAW for non-fertilized mixtures. Vertical arrows indicate the timing of rainfall events (under higher rainfall conditions more rainfall events would occur over the same time period). The horizontal dashed line shows the water stress threshold for woody seedlings, below which seedlings experience water stress. The illustration shows that seedlings in fertilized mixtures experience water stress for a longer period (distance a–c) and more intensely (lower PAW) than seedlings in non-fertilized mixtures (distance b–c, higher PAW).

(Knoop & Walker 1985; Cramer *et al.* 2007) and *Euclea divinorum* (Sharam *et al.* 2006), although O'Connor (1995) showed that *A. karroo* seedlings were not negatively affected by herbaceous competition in his study.

In terms of resource availability, most studies agree with our results that savanna woody seedlings benefit from an increase in the frequency of watering and are negatively affected by wet-season droughts (Wilson & Witkowski 1998; Higgins *et al.* 2000; Kraaij & Ward 2006; but see Seymour 2008). However, Kraaij & Ward (2006) showed that *A. mellifera* seedling growth in herbaceous swards was lower when fertilized with nitrogen, even when frequently watered. This contradicts our growth data where we found additive effects of water and nutrients on *C. mopane* growth, but agrees with the leaf-loss patterns observed during the wet-season drought for fertilized *C. mopane* seedlings competing with herbaceous plants.

IMPLICATIONS FOR WOODY RECRUITMENT IN DRY SAVANNAS UNDER CHANGED WATER AND NUTRIENT REGIMES

Recently, Sankaran *et al.* (2008) predicted that woody cover will track changes in mean annual precipitation and that woody cover will be negatively affected by increased nitrogen deposition. Our study, and others (e.g. Harrington 1991; Wilson & Witkowski 1998; Higgins *et al.* 2000), emphasized the importance of droughts as an obstacle for woody recruitment in arid and semi-arid areas, suggesting that changes in

drought frequency, intensity and duration might be more important than changes in mean annual rainfall. Therefore predictions of recruitment responses of woody plants to climate change should include drought as a factor, especially because drought characteristics (frequency, intensity and duration) are anticipated to change relatively more than total rainfall amount (Trenberth *et al.* 2003). Furthermore, our data suggest that the effect of increased nitrogen deposition on establishment of woody plants is dependent on water availability; under low water availability (e.g. frequent droughts) recruitment of woody plants will be constrained, while under high, continuous water availability recruitment might be enhanced. If this holds for larger systems as well, we expect that the negative soil fertility – woody cover relationship described by Sankaran *et al.* (2008), is modified by water availability; woody cover declines faster along a decreasing rainfall gradient in fertile areas compared to infertile ones.

Lastly, we found that competition effects of herbaceous vegetation on woody seedlings intensified during a wet-season drought, which occurs frequently in dry systems, even during average rainfall years (Wilson & Witkowski 1998). This suggests that disturbances (e.g. overgrazing) that reduce the competitiveness of the herbaceous layer, might lower wet-season drought constraints on seedling establishment of woody plants.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. The responses of *Colospermum mopane* seedlings growing in the absence of competition and competing with herbaceous plants as affected by additional water, additional nutrients and additional water plus nutrients.

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