Seasonality and facilitation drive tree establishment in a semi-arid floodplain savanna

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Abstract A popular hypothesis for tree and grass coexistence in savannas is that tree seedlings are limited by competition from grasses. However, competition may be important in favourable climatic conditions when abiotic stress is low, whereas facilitation may be more important under stressful conditions. Seasonal and inter-annual fluctuations in abiotic conditions may alter the outcome of tree–grass interactions in savanna systems and contribute to coexistence. We investigated interactions between coolibah (*Eucalyptus coolabah*) tree seedlings and perennial C₄ grasses in semi-arid savannas in eastern Australia in contrasting seasonal conditions. In glasshouse and field experiments, we measured survival and growth of tree seedlings with different densities of C₄ grasses across seasons. In warm glasshouse conditions, where water was not limiting, competition from grasses reduced tree seedling growth but did not affect tree survival. In the field, all tree seedlings died in hot dry summer conditions irrespective of grass or shade cover, whereas in winter, facilitation from grasses significantly increased tree seedling survival by ameliorating heat stress and protecting seedlings from herbivory. We demonstrated that interactions between tree seedlings and perennial grasses vary seasonally, and timing of tree germination may determine the importance of facilitation or competition in structuring savanna vegetation because of fluctuations in abiotic stress. Our finding that trees can grow and survive in a dense C₄ grass sward contrasts with the common perception that grass competition limits woody plant recruitment in savannas.

Keywords Australia, tree–grass coexistence, C₄ grass, competition, eucalypt seedlings

Introduction

The coexistence of trees and grasses in savanna ecosystems has intrigued ecologists for decades and produced many theories, which can be broadly grouped into resource or disturbance-based hypotheses (Walter 1971; Sankaran et al. 2004; Bond 2008; February et al. 2013; Ward et al. 2013). Savannas occur across a wide climatic range, often separated from densely forested vegetation by sharp boundaries, suggesting they receive enough rainfall to support a much higher biomass of trees, and are limited by other factors (Sankaran et al. 2004; 2005; Bond 2008). Hence, coexistence seems to be partly driven by resource competition between trees and grasses, especially during tree seedling establishment when competition for resources is strong (Knoop and Walker 1985; Jeltsch et al. 1996; Scholes and Archer 1997; Gignoux et al. 2009; Ward et al. 2013). Identifying the factors that limit tree abundance in savannas has become increasingly important from a management perspective, with increases in tree density in some systems (e.g. Briggs et al. 2005; Eldridge et al. 2011) and decreases in others (Fischer et al. 2009; Allen et al. 2010).

Demographic models of tree–grass coexistence propose that tree abundance is limited by variations in seedling establishment (dependent on rainfall and competition) and limitations in transitions to adult stages (fire and other disturbances; Higgins et al. 2000). Savannas may be governed by variability in environmental conditions, which favours grass or tree dominance at different spatial and temporal scales, and prevents either life-form from becoming dominant across the landscape (Sankaran et al. 2005; Wiegand et al. 2005; 2006). Environmental variability is especially important in semi-arid savannas which are prone to periods of drought interspersed with rainfall periods (Fensham and Holman 1999; Fensham et al. 2005; Sankaran et al. 2005). Favourable conditions promote woody plant establishment and regeneration, while droughts and fires result in woody plant decline (Fensham et al. 2009; Nano and Clarke 2010; Lawes et al. 2011).

Interactions between grasses and trees may be affected by climatic conditions especially during the establishment phase when the two life-forms share the same rooting zone (Scholes and Archer 1997; Ward 2005; Riginos 2009; Ward and Esler 2011). This interplay between biotic and abiotic factors may determine

ecosystem structure, especially in bistable systems such as savannas (Tylianakis et al. 2008; Volder et al. 2013). For example, February et al. (2013) found increased rainfall gave grasses a competitive advantage over tree seedlings. Indeed, many studies have reported grasses can inhibit woody plant recruitment through competition (Davis et al. 1998; Ball et al. 2002; Bloor et al. 2008; Clarke and Knox 2009; Messier et al. 2009). However, facilitation can also be vital for seedling recruitment in severe environments where established plants provide a beneficial microclimate beneath their canopies (Belsky et al. 1989; Callaway et al. 2002; Brooker et al. 2008; Anthelme and Michalet 2009; Bustamante-Sánchez et al. 2011). Newly germinated and young seedlings are susceptible to non-resource based stresses, such as excessive solar radiation and extreme temperatures (Osmond et al. 1987; Ferrar et al. 1989; Niinemets 2010), and protection from these stresses can increase seedling survival. These effects are particularly important in semi-arid and arid environments where light and heat stress in exposed areas are responsible for high seedling mortality (Björkman and Powles 1984; Osmond et al. 1987; Callaway 1995; Gómez-Aparicio et al. 2008). In addition, a number of studies have found facilitative effects of grasses on seedlings by reducing visibility to herbivores (Western and Maitumo 2004; Riginos and Young 2007; Porensky and Veblen 2012). However, neighbouring plants may compete for soil resources below-ground while simultaneously ameliorating non-resource-based stress. Indeed, below-ground competition can be intense in semi-arid and arid environments where soil moisture availability is limited (Pugnaire and Luque 2001; Nano and Clarke 2010).

The overall aim of this study was to investigate the ability of grasses to suppress or facilitate tree seedling establishment under seasonally variable conditions. Semi-arid floodplains dominated by coolibah (*Eucalyptus coolabah* subsp. *coolabah*) trees in inland eastern Australia are a useful model system to investigate the effects of seasonal variability on tree–grass interactions because they undergo a wide range of environmental conditions, from droughts to high rainfall periods and flooding. Tree establishment in these systems can occur *en masse* following flooding and high rainfall periods, which can occur at any time of the year. However, these recruitment events are rare and this has resulted in patches of even-aged stands of trees (with very high stem densities, ~ 2300 ha; Good et al. 2011). Cohorts are generally separated by

several decades (the last successful cohort regenerated 40 years ago) due to the low success rate of regeneration (Good et al. 2012). Tree seedling germination followed by a period of high abiotic stress may reduce tree establishment (Castro et al. 2005), or increase the importance of positive plant–plant interactions (Gómez-Aparicio et al. 2008; Anthelme and Michalet 2009). That is, favourable climatic conditions (in cool/wet seasons or during years of high rainfall) may dramatically improve tree establishment rates or alternatively result in an increase in the importance of competitive interactions among plants (February et al. 2013).

We evaluated the importance of competitive and facilitative interactions between grasses and tree seedlings in different seasons by conducting field and glasshouse experiments. We asked: (1) do grasses facilitate or compete with tree seedlings? (2) Is tree seedling growth and survival affected by seasonal conditions, and does seasonality alter tree-grass interactions? (3) Is above or below-ground competition, for light or soil resources, respectively, more important for tree seedling growth?

Materials and methods

Study system

The floodplains of mid latitudinal inland eastern Australia consist of a continuous C₄ grassy ground layer with discontinuous eucalypt cover of varying density, from treeless grasslands to dense woodlands. The dominant floodplain tree on the alluvial cracking clay soils in this region is *Eucalyptus coolabah* subsp. *coolabah* (hereafter coolibah). Coolibah seeds germinate at a range of temperatures with an optimum temperature of around 30–35 °C (Doran and Boland 1984). The soil surface can reach this temperature on sunny days throughout the year, even in winter months. For this reason, it is likely that seedling emergence is most limited by moisture availability and is generally restricted to high rainfall periods and/or periods immediately following flood recession (which can occur at any time of the year but are more common in summer). We chose three sites of 20–50 ha of grassland (with scattered remnant trees, ~2–5 ha⁻¹), within 5 km of one another (149°13'E, 30°5'S) to conduct the field experiments. Mean annual rainfall of the study area is 597 mm (Wee Waa weather station; Australian Bureau of Meteorology 2012) with a slight peak in

summer (January). Summers are hot (mean minimum of the hottest month is 30°C and the mean monthly maximum is 37°C in January) and winters are mild (mean minimum of the coldest month is 1°C and the mean monthly maximum is 21°C in July). All sites were grazed by livestock in the past but cattle had been excluded for 4 years and the grasslands had recovered and were dominated by native perennial grasses (primarily *Panicum decompositum* and *Paspalidium jubiflorum*).

Field experiments

We implemented three treatments, each replicated four times per site: (1) maximum grass cover (GRASS) with no grass removal; (2) zero grass cover (BARE) with all vegetation clipped and poisoned with glyphosate 2 weeks prior to transplanting of coolabah seedlings and maintained by hand weeding throughout the study, and (3) zero grass cover plus shade (SHADE) with all vegetation treated as in (2), but with 70% shade cloth covering the plot at a height of 50 cm. At each site, a 10 × 10-m experimental grid was established at least 50 m from trees in grassland of relatively homogeneous composition and subdivided into sixteen 2.5 × 2.5-m plots. Treatments were randomly assigned to plots. Within each plot, a central subplot of 0.5 × 0.5 m was marked and coolabah seedlings were transplanted into these subplots on two occasions (summer and winter). Each subplot was surrounded by a buffer of 1 m to minimise disturbance and to ensure the treatments extended >1 m in each direction from transplanted seedlings. Coolabah seed was obtained from commercial seed suppliers who sourced seeds from within 50 km of the study sites. The same seed lot was used for all experiments. Coolabah seeds were germinated and seedlings grown in soils collected from the study region and used in all three experiments. Prior to transplanting, seedlings were grown for 1–2 months to a height of 1–2 cm.

For the summer 2010 seedling transplant experiment, five coolabah seedlings were planted in each subplot, 25–30 cm apart, and watered immediately with 2 L of rainwater. Plots were then watered with 1 L of rainwater every evening (after 1700 hours) for 6 days. For the winter 2010 seedling transplant, seven seedlings were planted in each subplot, 25–30 cm apart, and watered immediately with 1 L of rainwater. Rainfall in the weeks prior to planting and mild winter temperatures meant that the soil was moist and did

not require much watering. We lightly watered all plots (<1 L) every second day for the first week and seedlings received natural rainfall for the remainder of the study. Following transplanting of tree seedlings in summer (March 2010), we recorded seedling survival on days 2, 4, 7 and 14 (after transplant). In the winter experiment (August 2010), we monitored seedling survival and height on days 10, 14, and 27 (after transplant), and then approximately monthly until the end of the experiment in April 2011 (apart from December 2010 when sites were flooded). Seedlings were considered 'dead' if they were missing, had no leaves, or if their leaves were brown and desiccated. Death was recorded as abiotic stress if the leaves were brown and desiccated, and in the winter experiment we recorded evidence of herbivory.

Rainfall and temperature data for the closest stations to the study site were downloaded from the Australian Bureau of Meteorology Climate Data Online service (Australian Bureau of Meteorology, 2012). We recorded soil moisture at all sites each time we monitored seedlings, using a soil moisture probe (ThetaProbe; Delta-T Devices 1998), which indirectly measures volumetric moisture content by measuring electrical conductivity. We took five readings of soil moisture haphazardly throughout sites on each visit. Soil moisture in the summer field experiment averaged approximately 30%, but was still well above wilting point (Stace et al. 1968; McKenzie et al. 2004). In the glasshouse and in the winter field experiment soil moisture was ~35% due to regular watering (in the glasshouse) and mild conditions (in the field). Temperature loggers (iButtons®) were used to record soil surface temperatures and were placed on the soil surface, face down, attached to a small plastic holder and pinned to the ground. They recorded temperatures at 2-h intervals throughout each experiment. In March, temperature loggers were placed in eight subplots (two GRASS, three BARE and three SHADE) and, in August, temperature loggers were placed in 20 plots (six GRASS, seven BARE and seven SHADE). We used a light meter to measure photosynthetically active radiation (PAR) at each seedling between 1130 and 1200 h on a cloudless day, midway through and at the end of the winter experiment, and midway through the glasshouse experiment (see below). Light availability in BARE plots in the field and in CONTROL plots in the glasshouse were considered to be the maximum light availability in each experiment since these treatments were in full sunlight.

Above and below-ground resource manipulations

After 81 days (~3 months) in the winter experiment, we manipulated light availability in GRASS plots in order to investigate the effects of above and below-ground competition on seedling growth. We used wire to hold back the foliage of grasses in some of the GRASS plots so that light availability for seedlings increased (high light) but grass roots were still present. In 'low' and 'high' light treatments, light availability was <500 and $>700 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. In 'low' light treatments mean ($\pm$ SE) light availability was $351 (\pm 31) \mu\text{mol m}^{-2} \text{s}^{-1}$ and in 'high' light treatments it was $1253 (\pm 70) \mu\text{mol m}^{-2} \text{s}^{-1}$. BARE plots were high-light treatments without root competition (first 3 months, $n = 15$ seedlings; second 3 months, $n = 3$ seedlings) and SHADE plots were low-light treatment without root competition (first 3 months, $n = 37$ seedlings; second 3 months, $n = 16$ seedlings). GRASS plots with light availability $>700 \mu\text{mol m}^{-2} \text{s}^{-1}$ were high-light treatments with root competition (first 3 months, $n = 20$ seedlings; second 3 months, $n = 15$ seedlings). GRASS plots with light availability $<500 \mu\text{mol m}^{-2} \text{s}^{-1}$ were low-light treatments with root competition (first 3 months, $n = 42$ seedlings; second 3 months, $n = 3$ seedlings).

Glasshouse experiment

Grass tussocks (*Paspalidium jubiflorum*) were collected from ten locations near the field sites 2 months prior to the experiment using the Whalley method (Whalley and Brown 1973). Round plastic pots (20 cm diameter $\times$ 20 cm deep) were lined with absorbent paper, filled with 2 cm of sand, and filled to 2 cm from the top with field soil. Grass tussocks were separated into smaller parts so that each basal diameter was approximately 2 cm and planted into pots, 5 cm apart, in two densities (2 or 4 tussocks per pot). Grasses were grown and watered regularly for 1 month prior to sowing coolibah seeds to allow them to establish. Pots were placed in a glasshouse maintained at 30°C and 20°C, day and night respectively, to mimic optimal grass growing conditions (summer). A small amount of coolibah seed was added to the centre of the pots, and the resulting seedlings later thinned to one per pot. Soils were kept saturated until seeds germinated thereafter pots were watered every 2 days with 500 mL of water until the end of the experiment.

We used a factorial design with two levels of grass density and two levels of grass clipping. We clipped half of the grasses to 5 cm above the soil surface (SHORT) and the other half were left to grow to a maximum of 30 cm (LONG) at which point they were clipped to reduce shading of neighbouring pots. The treatments were: (1) CONTROL: tree seedlings grown without competition ($n = 26$ seedlings); (2) $2 \times$ GRASS: tree seedlings grown with two grass tussocks with two clipping treatments (LONG: $n = 12$ seedlings, SHORT: $n = 12$ seedlings); and (3) $4 \times$ GRASS: tree seedlings grown with four grass tussocks with two clipping treatments (LONG: $n = 12$ seedlings or SHORT: $n = 12$ seedlings).

Seedling height was measured at week 3, 4 and 5, and after 2 months of growth all grasses and seedlings were harvested, dried and weighed. Seedling biomass was used to calculate Relative Competition Intensity (RCI_{biomass}) (Wilson and Keddy 1986) using the following equation:

$$RCI_{\text{biomass}} = \frac{(\text{Seedling biomass with grass}) - (\text{Seedling biomass without grass})}{(\text{Seedling biomass without grass})}$$

Data analyses

To test the significance of treatment effects on soil surface temperatures, tree seedling growth and survival, seedling death from herbivory and final seedling measurements in the two field experiments, we used a two-factor ANOVA with grass treatment (three levels: BARE, GRASS and SHADE) and site ($n = 3$) as factors. When site $\times$ treatment interactions were not significant ($P > 0.05$), sites were pooled. Bonferroni post hoc comparisons were carried out when significant treatment effects were found. Data were square root or log-transformed where necessary to satisfy ANOVA assumptions of normality and homogeneity of variance. To compare above and below-ground competition in the winter field experiment, we compared tree seedling growth with and without root competition under high and low light availability in the first 3 months separately from the second 3 months, since the latter period coincided with the summer growing season. For each time period, we used a two-factor ANOVA, with light availability (high vs low) and root competition (with and without) as factors. Bonferroni post hoc comparisons were undertaken when

treatment effects were significant ($P < 0.05$). For comparisons of seedling height and Relative Competition Intensity (RCI_{biomass}) in the glasshouse experiment, we used one-way ANOVA. To investigate the importance of above-ground competition in the glasshouse experiment, we compared RCI_{biomass} of the clipped and unclipped grass treatments, using a two-factor ANOVA with clipping and grass density as factors.

Results

Abiotic conditions in the field and glasshouse

Abiotic conditions in the field differed between seasons; daily maximum temperatures were higher in summer ($>10^{\circ}\text{C}$ higher than winter temperatures for most of the first 2 weeks) whereas rainfall was more frequent at the beginning of the winter experiment (Appendix 1). Maximum temperatures in the summer experiment ranged from 24°C to 34°C in the 2 weeks following tree seedling transplant. Daily maxima for the first 2 weeks of the winter experiment ranged from 13°C to 23°C but were $<20^{\circ}\text{C}$ most of the time (Appendix 1). Light availability was much lower ($\sim 40\%$ reduction) and soil moisture was higher in the glasshouse than in the field. As the winter field experiment progressed, daily temperature maxima increased and rainfall was regular, with high daily rainfall on many days in early summer (Appendix 2). The high rainfall resulted in floods and all three experimental sites were inundated for varying amounts of time. Two of the sites were only inundated for 2 weeks and sustained little damage; aerial parts of the grasses were killed but they soon re-sprouted and grew vigorously as soon as the sites dried. The third site was inundated for >2 months and had to be abandoned. When the site eventually dried out, all of the seedlings and herbaceous plants had died.

In both summer and winter, daily maximum soil surface temperatures in BARE plots were consistently higher and more variable than SHADE and GRASS plots, which did not differ from each other (Fig. 1). In the summer experiment, soil surface temperatures in BARE plots exceeded 60°C on 4 days when daily maximum temperatures were around 33°C , and were consistently hotter than in winter.

Tree seedling survival

Tree seedling survival in the glasshouse was 100% over the 2-month duration of the experiment, whereas seedling survival in the field was low. Transplanted tree seedlings did not survive the summer conditions for more than 2 weeks, even with protection from full sun and extreme heat (90% of seedlings were dead within 2 weeks and 100% were dead after 3 weeks; Fig. 2). Seedling survival in winter was higher, with only 7% of tree seedlings dead after 2 weeks (Fig. 3).

Tree seedling survival was consistently higher in GRASS and SHADE than BARE plots throughout the winter experiment and there were no significant site $\times$ treatment interactions (Fig. 3). Differences in the survival of seedlings between SHADE and GRASS treatments were initially significant, with lower survival of SHADE seedlings in the first 3 months; thereafter they became similar. Herbivory of tree seedlings was not recorded in the summer experiment because of rapid mortality that was presumably due to stress (as most seedlings appeared desiccated). In the winter transplant, cause of seedling death varied significantly between treatments but not between sites, the site $\times$ treatment interaction being not significant. The mean percentage of seedling death from herbivory was significantly higher in BARE plots ($37 \pm 6\%$) and SHADE ($30 \pm 5\%$) than GRASS ($5 \pm 3\%$) plots throughout the study. This was mostly driven by the high rate of seedling herbivory in the first month (August) of the experiment.

Tree seedling growth and competition intensity

Seedling growth was not measured during the summer experiment due to rapid seedling mortality. In the first 3 months of the winter experiment, seedling growth was slow and there was no significant difference in seedling height among treatments or among sites (Fig. 4). Between December and January, growth of tree seedlings increased and treatment differences became significant (with no significant site $\times$ treatment interaction). Tree seedlings in GRASS plots were significantly smaller than seedlings in BARE and SHADE plots, which did not differ throughout the study. At the end of the study, SHADE seedlings were significantly larger than GRASS tree seedlings in both height and biomass (Table 1). Only one seedling remained in BARE plots at the end of the study, it being larger than any seedlings in GRASS plots (Table 1). These results were reflected in the glasshouse experiment with mean tree seedling height significantly

greater in BARE pots than in pots with two grass tussocks, and both were greater than seedling height in pots with four grass tussocks (Fig. 5).

Below-ground vs above-ground competition

There were no significant above or below-ground competition effects in the first 3 months of the winter field experiment (Table 2). In the second 3 months of the experiment, root competition effects were significant but there were no significant light or light $\times$ root effects. When light levels were pooled, the presence of roots significantly reduced seedling growth (Table 2). Tree seedlings grown without root competition grew more than twice as much as seedlings grown with root competition (Table 2).

In the glasshouse experiment, reducing above-ground biomass (clipping) did not significantly reduce competition intensity (Fig. 5). Clipped (SHORT) grasses were not significantly different, in terms of competition intensity, to unclipped (LONG) within density treatments (Fig. 5). There was, however, a significant difference between the 4 $\times$ GRASS LONG treatment and the 2 $\times$ GRASS SHORT treatment. Light availability in these treatments differed significantly (mean maximum light availability of 595 ± 64 and $855 \pm 44 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively) but the reduction in light availability did not significantly reduce seedling growth relative to the clipped treatments (Fig. 5).

Discussion

We investigated the effect of season on tree–grass interactions in a semi-arid savanna by comparing the effects of perennial grasses on tree seedling growth and survival in the field over summer and winter, and in the glasshouse under optimal growing conditions. In summer, no tree seedlings survived regardless of grass cover due to very high temperatures and dry conditions immediately after planting, demonstrating the importance of seasonal and abiotic conditions. In cooler, humid winter conditions, grasses and shade significantly increased tree seedling survival compared to seedlings growing in the open (only one survivor), demonstrating that grasses facilitated survival under less stressful conditions. All seedlings survived irrespective of grass cover under optimal glasshouse conditions where temperatures were warm, moisture was not limiting, but solar radiation was reduced. Root competition significantly decreased seedling growth,

with seedlings about twice the size in shade plots compared to grass. In this savanna, conditions benign enough to allow seedling survival without protection from stressors do not occur, even in the coolest, wettest months, and the amelioration of these stressors from neighbouring plants is vital for tree seedling survival – supporting a demographic model of coexistence (Higgins et al. 2000; Sankaran et al. 2005).

Grasses facilitate survival of newly germinated tree seedlings

In the winter experiment, grass and shade cover significantly increased the survival of tree seedlings relative to bare plots, whereas in the summer experiment all seedlings died irrespective of grass cover or shade. Seedling mortality in bare plots was likely mostly due to heat stress; soil surface temperatures exceeded 60°C in summer and reached 40°C in winter in bare plots, whereas temperatures beneath grasses and shade cloth were significantly lower and less variable in both seasons. Plant tissue damage is thought to occur at temperatures around 50–60°C in many species so it is not surprising that seedlings in bare soil did not survive long in summer. Temperature extremes denature proteins and damage cell structure, which can be tolerated in the short term but long-term exposure is fatal (Wang et al. 2003). Hence, shade from adjacent plants can be critical (Flores and Jurado 2003). Newly germinated seedlings are particularly susceptible to damage caused by temperature and solar radiation since they grow close to the soil surface where these factors can be highly variable (Nobel 1984; Osmond et al. 1987; Helgersson 1989). Grass cover provides a favourable microclimate in arid environments by reducing atmospheric aridity at ground level (Anthelme and Michalet 2009). Therefore, the primary mechanism by which grass and shade increased tree seedling survival in our study was through amelioration of heat and light stress and potentially a reduction in soil water loss (but this was not directly measured).

There were also indirect positive effects of grass cover on seedling survival; death from herbivory was lower in grass than shade and control plots. The shade plots were similarly affected by herbivory as bare plots, and although the identity of the herbivores was unclear, they were most likely insects or small vertebrates. Insect herbivory is common in eucalypts and has been responsible for the dieback of mature trees throughout Australia (Landsberg and Wylie 1983; Stone and Bacon 1995). The timing of herbivory in

the first month of the winter transplant experiment suggests that either the young seedlings were more susceptible to herbivory or that herbivores were more common at this time of the year. Herbivory has been implicated in seedling mortality in other savanna systems (e.g. Western and Maitumo 2004; Riginos and Young 2007; Porensky and Veblen 2012). Similar to the current study, Riginos and Young (2007) found that grass cover facilitated *Acacia* saplings by protecting them from herbivory in an African savanna, although in this case the net effect of grasses was negative due to competition. Our results suggest that grass facilitation may be critical for tree establishment in semi-arid savannas, despite negative effects of grass on tree seedling growth.

Plant–plant interactions vary with abiotic stress

We found that grasses reduce the growth of tree seedlings. However, the positive effects of grasses – the amelioration of light and heat stress, and protection from herbivory – outweigh these negative effects by enhancing seedling survival. Other studies have reported competition becoming less important as stress increases (Holmgren et al. 1997; Davis et al. 1999; Pugnaire and Luque 2001). Pugnaire and Luque (2001) found that the change in plant–plant interactions along stress gradients is driven more by the increase in the positive effect of the nurse plant than by the decrease in the intensity of competition. Contrary to these studies, and the stress-gradient hypothesis, we found that facilitation was more important with less abiotic stress, as conditions in summer were too harsh for survival even with grass cover. Measuring net effects of plant–plant interactions (in terms of seedling survival) is vital because the intensity of competitive interactions alone (i.e. the reduction in seedling growth) may not be as relevant in determining community membership. In addition, seasonality interacts with other abiotic and biotic factors in complex ways to shift the relative importance of competition and facilitation in savannas (Veblen 2008).

Below-ground competition between grass and tree seedlings

As expected, below-ground competition for soil resources was the main form of competition between grasses and tree seedlings in this study. Other studies have reported reduced woody seedling success when grown in herbaceous vegetation in semi-arid and arid environments; this is generally attributed to below-

ground competition for soil moisture (Fowler 1986; Harrington 1991; Scholes and Archer 1997; Florentine and Fox 2003; Bloor et al. 2008; van der Waal et al. 2009). It is not surprising, given frequent light saturation in this environment, that below-ground resources are likely to be most limiting. Soil resources were not manipulated in this study so whether nutrients or water were limiting is unknown, but it is likely that water is generally most limiting in the fertile clay soils of our semi-arid study region.

Implications for tree–grass coexistence in savannas

Our results provide support for demographic models of tree–grass coexistence, in which tree abundance is limited by the rarity of conditions suitable for tree recruitment (Higgins et al. 2000). We found that tree seedling survival is dependent on favourable seasonal conditions as well as facilitation from grasses. Similar results have been reported for *Eucalyptus victrix* in semi-arid floodplains in Western Australia, where no seedlings survived following natural, flood-induced germination, irrespective of herbaceous cover (Fox et al. 2004). Other studies have found that periods of high rainfall and seed availability overwhelm the effect of grasses in limiting tree regeneration in savannas (Ward 2005; Kraaij and Ward 2006). These findings are contrary to the common belief that grasses limit tree seedling survival (and hence abundance) in savannas. However, grasses may indirectly limit tree abundance in fire-prone savannas: seedlings growing among a dense grass sward are more likely to be killed by fire (Bond 2008). Fire is rare in our study system, but other disturbances such as drought and flooding may kill tree seedlings and further limit tree abundance.

The climatic conditions required for successful tree recruitment may be rare enough, in space and time, to limit tree cover in this landscape (Wiegand et al. 2006; Moustakas et al. 2010). Winter flooding or unseasonably mild summer conditions as well as adequate grass cover appear to be prerequisites for coolibah recruitment. Our findings support a demographic bottleneck model of savanna stability, in which the rarity of conditions suitable for successful tree recruitment restricts tree cover in the landscape (Higgins et al. 2000; Sankaran et al. 2004).

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Tables

Table 1. Tree seedling responses to treatments after 227 days in the winter field. Values are mean $\pm$ SE for Grass and Shade; *No error available for BARE plots because only one individual remained at the end of the study. Seedlings pooled across all sites; means within rows followed by a different superscript differ significantly (Bonferroni, $P < 0.05$).

Final tree seedling size	Treatment		
	Bare*	Grass	Shade
Height (mm)	200.0	109.7 $\pm$ 11.4 ^a	272.9 $\pm$ 27.9 ^b
Total mass (g)	2.7	0.3 $\pm$ 0.1 ^a	2.2 $\pm$ 0.6 ^b
Above-ground mass (g)	1.1	0.2 $\pm$ 0.1 ^a	1.7 $\pm$ 1.7 ^b
Number of surviving seedlings	1	17	14

Table 2. Seedlings growth (change in seedling height in response to root competition (+ = with; - = without) for the first and second 3-month periods in the winter transplant experiment. Values are mean ($\pm$ SE) (sample size). Means followed by different superscripts were significantly different (Bonferroni, $P < 0.05$). Sample sizes in parentheses (n).

Time period	+ Roots		– Roots		Light levels pooled	
	Low light	High light	Low light	High light	+ Roots	– Roots
First 3 months	22 $\pm$ 2 (42)	30 $\pm$ 3 (20)	24 $\pm$ 3 (37)	24 $\pm$ 4 (15)	25 $\pm$ 2 (62)	24 $\pm$ 2 (52)
Second 3 months	–	–	–	–	83 $\pm$ 10 (18) ^a	172 $\pm$ 23 (19) ^b

Means followed by different superscripts were significantly different (Bonferroni, $P < 0.05$). Sample sizes in parentheses (n).

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Figure Legends

Fig. 1 Maximum daily soil surface temperature (mean $\pm$ SE) for the 2 weeks following seedling transplant in the three treatments (bare, grass and shade) in summer (black) and winter (grey)

Fig. 2 Coolibah tree seedling survival (mean $\pm$ SE) in 'Bare', 'Grass' and 'Shade' treatments from the summer experiment in the 3 weeks after planting

Fig. 3 Coolibah tree seedling survival (mean $\pm$ SE) in 'Bare', 'Grass' and 'Shade' treatments from the winter experiment in the 7 months after planting

Fig. 4 Coolibah tree seedling growth in 'Bare', 'Grass' and 'Shade' treatments from the winter experiment in the 7 months after planting. Values are seedling height (mean $\pm$ SE)

Fig. 5 Relative competition intensity (mean $\pm$ SE), based on coolibah tree seedling growth with two grass tussocks (2 $\times$ Grass) or four grass tussocks (4 $\times$ Grass); grasses were clipped (Short) or unclipped (Long)

Figures

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5