

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

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

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

18

19 **Introduction**

20 The coexistence of trees and grasses in savanna ecosystems has intrigued ecologists for decades and
21 produced many theories, which can be broadly grouped into resource or disturbance-based hypotheses
22 (Walter 1971; Sankaran et al. 2004; Bond 2008; February et al. 2013; Ward et al. 2013). Savannas occur
23 across a wide climatic range, often separated from densely forested vegetation by sharp boundaries,
24 suggesting they receive enough rainfall to support a much higher biomass of trees, and are limited by other
25 factors (Sankaran et al. 2004; 2005; Bond 2008). Hence, coexistence seems to be partly driven by resource
26 competition between trees and grasses, especially during tree seedling establishment when competition for
27 resources is strong (Knoop and Walker 1985; Jeltsch et al. 1996; Scholes and Archer 1997; Gignoux et al.
28 2009; Ward et al. 2013). Identifying the factors that limit tree abundance in savannas has become
29 increasingly important from a management perspective, with increases in tree density in some systems (e.g.
30 Briggs et al. 2005; Eldridge et al. 2011) and decreases in others (Fischer et al. 2009; Allen et al. 2010).

31 Demographic models of tree–grass coexistence propose that tree abundance is limited by variations
32 in seedling establishment (dependent on rainfall and competition) and limitations in transitions to adult
33 stages (fire and other disturbances; Higgins et al. 2000). Savannas may be governed by variability in
34 environmental conditions, which favours grass or tree dominance at different spatial and temporal scales,
35 and prevents either life-form from becoming dominant across the landscape (Sankaran et al. 2005; Wiegand
36 et al. 2005; 2006). Environmental variability is especially important in semi-arid savannas which are prone
37 to periods of drought interspersed with rainfall periods (Fensham and Holman 1999; Fensham et al. 2005;
38 Sankaran et al. 2005). Favourable conditions promote woody plant establishment and regeneration, while
39 droughts and fires result in woody plant decline (Fensham et al. 2009; Nano and Clarke 2010; Lawes et al.
40 2011).

41 Interactions between grasses and trees may be affected by climatic conditions especially during the
42 establishment phase when the two life-forms share the same rooting zone (Scholes and Archer 1997; Ward
43 2005; Riginos 2009; Ward and Esler 2011). This interplay between biotic and abiotic factors may determine

44 ecosystem structure, especially in bistable systems such as savannas (Tylianakis et al. 2008; Volder et al.
45 2013). For example, February et al. (2013) found increased rainfall gave grasses a competitive advantage
46 over tree seedlings. Indeed, many studies have reported grasses can inhibit woody plant recruitment through
47 competition (Davis et al. 1998; Ball et al. 2002; Bloor et al. 2008; Clarke and Knox 2009; Messier et al.
48 2009). However, facilitation can also be vital for seedling recruitment in severe environments where
49 established plants provide a beneficial microclimate beneath their canopies (Belsky et al. 1989; Callaway et
50 al. 2002; Brooker et al. 2008; Anthelme and Michalet 2009; Bustamante-Sánchez et al. 2011). Newly
51 germinated and young seedlings are susceptible to non-resource based stresses, such as excessive solar
52 radiation and extreme temperatures (Osmond et al. 1987; Ferrar et al. 1989; Niinemets 2010), and protection
53 from these stresses can increase seedling survival. These effects are particularly important in semi-arid and
54 arid environments where light and heat stress in exposed areas are responsible for high seedling mortality
55 (Björkman and Powles 1984; Osmond et al. 1987; Callaway 1995; Gómez-Aparicio et al. 2008). In addition,
56 a number of studies have found facilitative effects of grasses on seedlings by reducing visibility to
57 herbivores (Western and Maitumo 2004; Riginos and Young 2007; Porensky and Veblen 2012). However,
58 neighbouring plants may compete for soil resources below-ground while simultaneously ameliorating non-
59 resource-based stress. Indeed, below-ground competition can be intense in semi-arid and arid environments
60 where soil moisture availability is limited (Pugnaire and Luque 2001; Nano and Clarke 2010).

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

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

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

81 **Materials and methods**

82 Study system

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

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

99 Field experiments

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

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

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

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

144 Above and below-ground resource manipulations

145 After 81 days (~3 months) in the winter experiment, we manipulated light availability in GRASS plots in
146 order to investigate the effects of above and below-ground competition on seedling growth. We used wire
147 to hold back the foliage of grasses in some of the GRASS plots so that light availability for seedlings
148 increased (high light) but grass roots were still present. In 'low' and 'high' light treatments, light availability
149 was <500 and $>700 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. In 'low' light treatments mean (\pm SE) light availability was
150 $351 (\pm 31) \mu\text{mol m}^{-2} \text{s}^{-1}$ and in 'high' light treatments it was $1253 (\pm 70) \mu\text{mol m}^{-2} \text{s}^{-1}$. BARE plots were
151 high-light treatments without root competition (first 3 months, $n = 15$ seedlings; second 3 months, $n = 3$
152 seedlings) and SHADE plots were low-light treatment without root competition (first 3 months, $n = 37$
153 seedlings; second 3 months, $n = 16$ seedlings). GRASS plots with light availability $>700 \mu\text{mol m}^{-2} \text{s}^{-1}$ were
154 high-light treatments with root competition (first 3 months, $n = 20$ seedlings; second 3 months, $n = 15$
155 seedlings). GRASS plots with light availability $<500 \mu\text{mol m}^{-2} \text{s}^{-1}$ were low-light treatments with root
156 competition (first 3 months, $n = 42$ seedlings; second 3 months, $n = 3$ seedlings).

157 Glasshouse experiment

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

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

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

$$178 \quad RCI_{\text{biomass}} = \frac{(\text{Seedling biomass with grass}) - (\text{Seedling biomass without grass})}{(\text{Seedling biomass without grass})}$$

180 Data analyses

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

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

197 **Results**

198 Abiotic conditions in the field and glasshouse

199 Abiotic conditions in the field differed between seasons; daily maximum temperatures were higher in
200 summer ($>10^{\circ}\text{C}$ higher than winter temperatures for most of the first 2 weeks) whereas rainfall was more
201 frequent at the beginning of the winter experiment (Appendix 1). Maximum temperatures in the summer
202 experiment ranged from 24°C to 34°C in the 2 weeks following tree seedling transplant. Daily maxima for
203 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
204 (Appendix 1). Light availability was much lower ($\sim 40\%$ reduction) and soil moisture was higher in the
205 glasshouse than in the field. As the winter field experiment progressed, daily temperature maxima increased
206 and rainfall was regular, with high daily rainfall on many days in early summer (Appendix 2). The high
207 rainfall resulted in floods and all three experimental sites were inundated for varying amounts of time. Two
208 of the sites were only inundated for 2 weeks and sustained little damage; aerial parts of the grasses were
209 killed but they soon re-sprouted and grew vigorously as soon as the sites dried. The third site was inundated
210 for >2 months and had to be abandoned. When the site eventually dried out, all of the seedlings and
211 herbaceous plants had died.

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

216 Tree seedling survival

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

222 Tree seedling survival was consistently higher in GRASS and SHADE than BARE plots throughout
223 the winter experiment and there were no significant site \times treatment interactions (Fig. 3). Differences in the
224 survival of seedlings between SHADE and GRASS treatments were initially significant, with lower survival
225 of SHADE seedlings in the first 3 months; thereafter they became similar. Herbivory of tree seedlings was
226 not recorded in the summer experiment because of rapid mortality that was presumably due to stress (as
227 most seedlings appeared desiccated). In the winter transplant, cause of seedling death varied significantly
228 between treatments but not between sites, the site \times treatment interaction being not significant. The mean
229 percentage of seedling death from herbivory was significantly higher in BARE plots ($37 \pm 6\%$) and SHADE
230 ($30 \pm 5\%$) than GRASS ($5 \pm 3\%$) plots throughout the study. This was mostly driven by the high rate of
231 seedling herbivory in the first month (August) of the experiment.

232 Tree seedling growth and competition intensity

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

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

244 Below-ground vs above-ground competition

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

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

257 Discussion

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

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

271 Grasses facilitate survival of newly germinated tree seedlings

272 In the winter experiment, grass and shade cover significantly increased the survival of tree seedlings relative
273 to bare plots, whereas in the summer experiment all seedlings died irrespective of grass cover or shade.
274 Seedling mortality in bare plots was likely mostly due to heat stress; soil surface temperatures exceeded
275 60°C in summer and reached 40°C in winter in bare plots, whereas temperatures beneath grasses and shade
276 cloth were significantly lower and less variable in both seasons. Plant tissue damage is thought to occur at
277 temperatures around 50–60°C in many species so it is not surprising that seedlings in bare soil did not
278 survive long in summer. Temperature extremes denature proteins and damage cell structure, which can be
279 tolerated in the short term but long-term exposure is fatal (Wang et al. 2003). Hence, shade from adjacent
280 plants can be critical (Flores and Jurado 2003). Newly germinated seedlings are particularly susceptible to
281 damage caused by temperature and solar radiation since they grow close to the soil surface where these
282 factors can be highly variable (Nobel 1984; Osmond et al. 1987; Helgersson 1989). Grass cover provides a
283 favourable microclimate in arid environments by reducing atmospheric aridity at ground level (Anthelme
284 and Michalet 2009). Therefore, the primary mechanism by which grass and shade increased tree seedling
285 survival in our study was through amelioration of heat and light stress and potentially a reduction in soil
286 water loss (but this was not directly measured).

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

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

300 Plant–plant interactions vary with abiotic stress

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

313 Below-ground competition between grass and tree seedlings

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

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

322 Implications for tree–grass coexistence in savannas

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

334 The climatic conditions required for successful tree recruitment may be rare enough, in space and
335 time, to limit tree cover in this landscape (Wiegand et al. 2006; Moustakas et al. 2010). Winter flooding or
336 unseasonably mild summer conditions as well as adequate grass cover appear to be prerequisites for coolibah
337 recruitment. Our findings support a demographic bottleneck model of savanna stability, in which the rarity
338 of conditions suitable for successful tree recruitment restricts tree cover in the landscape (Higgins et al.
339 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

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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)

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Figures

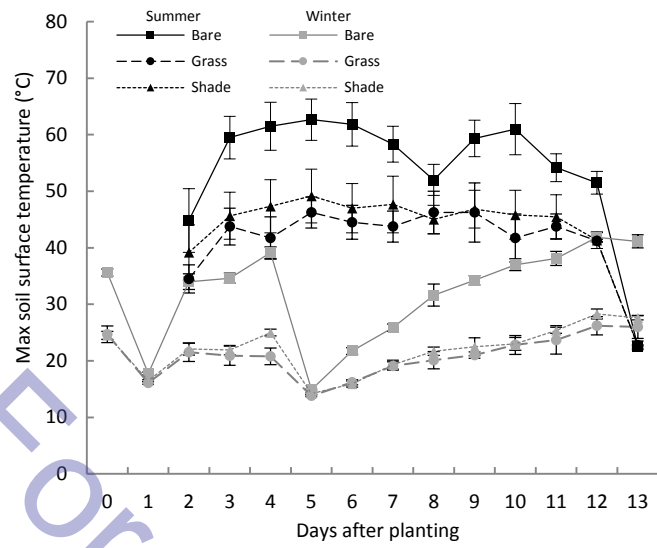


Fig. 1

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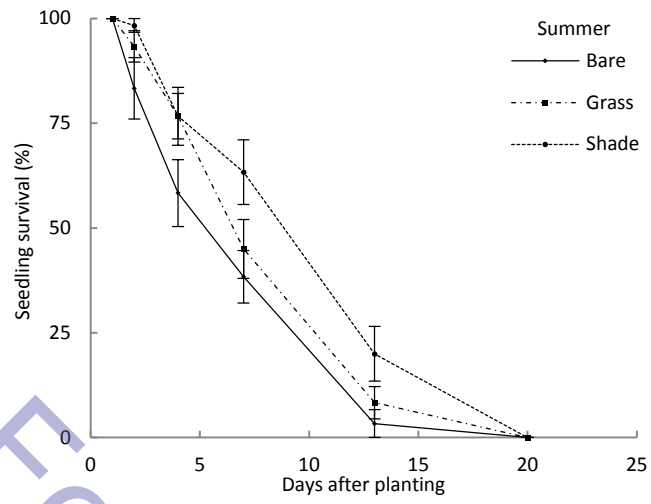


Fig. 2

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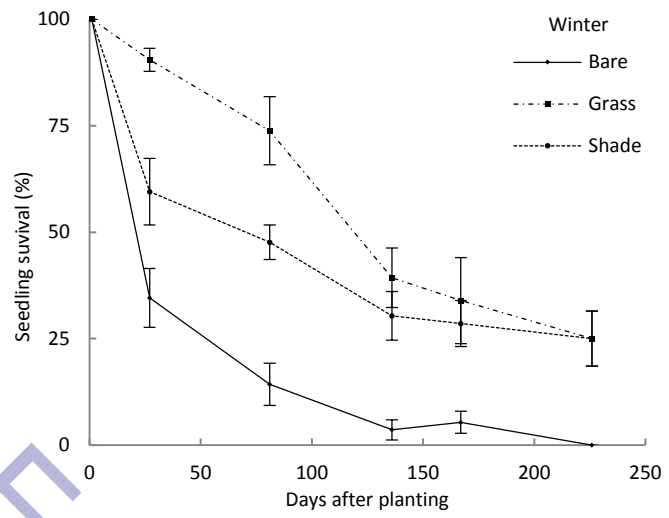


Fig. 3

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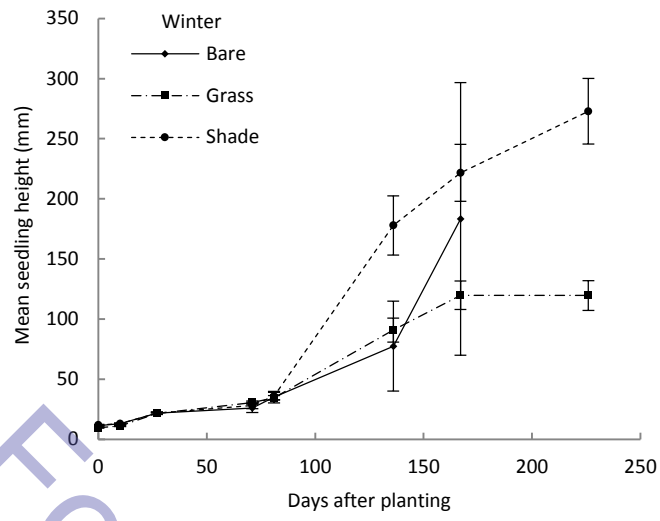


Fig. 4

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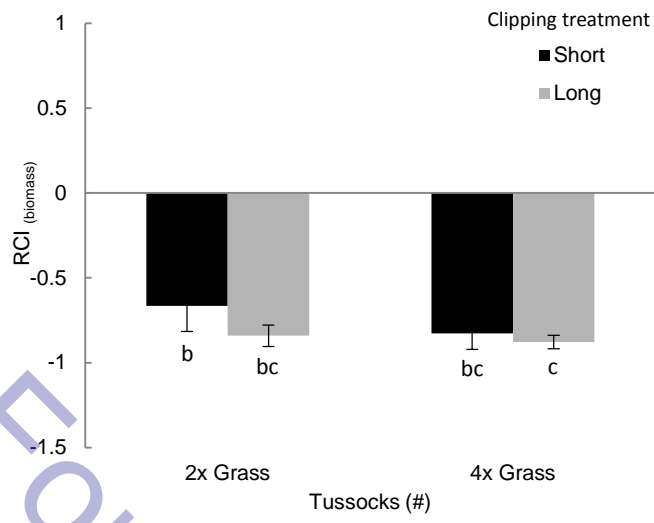


Fig. 5

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