The flooding tolerance of two critical habitat-forming wetland shrubs, *Leptospermum lanigerum* and *Melaleuca squarrosa*, at different life history stages

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Abstract

Understanding the effect of water regime on the different life history stages of woody wetland plants is essential to managing their persistence. The common and widespread myrtaceous shrub species, *Melaleuca squarrosa* and *Leptospermum lanigerum*, provide habitat for two critically endangered fauna within the Yellingbo Nature Conservation Reserve (SE Australia), but are in decline putatively due to altered flooding regimes. We thus tested the effects of flooding depth and duration on their seed germination and seedling establishment, and seedling growth and survival in two separate glasshouse experiments. We also compared the condition of mature plants of both species at an intermittently flooded (reference) site, and two near permanently flooded (impact) sites. Seeds of both species were able to germinate underwater, but early flooding reduced seedling establishment. Seedling growth of both species was greater in waterlogged than in well-drained or inundated conditions, while no seedlings of either species survived >8 weeks submergence. *L. lanigerum* seedlings were generally more flood tolerant than *M. squarrosa* seedlings. Correspondingly, crown condition of mature *M. squarrosa*, but not *L. lanigerum*, was poorer at impact than reference sites. Prolonged flooding in swamp forests is likely to a) limit woody plant recruitment, as flooding reduces seedling establishment, growth and survival, and b) be deleterious to the maintenance of less flood-tolerant species (e.g. *M. squarrosa*). Moist exposed substrate is likely to be best for promoting the recruitment of both study species, and intermittent flooding for maintaining adult *M. squarrosa* plants.

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Flooding tolerance of wetland shrubs

Keywords

Water regime; wetlands; riparian shrubs; Yellingbo Nature Conservation Reserve.

Introduction

Water regime—the spatial and temporal pattern of wetting and drying—is an important driver of wetland systems, including the recruitment and maintenance of wetland and riparian plants (Naiman and Décamps 1997; Poff *et al.* 1997). Components of the water regime, such as the depth and duration of flooding, can be considered abiotic filters that control the presence or absence of a species in wetland environments (Catford and Jansson 2014), resulting in the zonation of plant species according to their tolerances to flooding (Ge *et al.* 2012; van der Valk 1981).

Water regime can act as a filter for plant assembly at different life history stages: germination (e.g. suppression by flooding; Ge *et al.* 2012); recruitment (e.g. creation of zonation patterns along water level gradients; Keddy and Ellis 1985); growth (e.g. reduced growth of flooded seedlings; Greet 2016a); and maintenance (e.g. reduced condition of permanently flooded mature plants; Salter *et al.* 2010). The degree to which woody plant species can tolerate flooding depends on adaptations at each life history stage.

For survival and growth, woody wetland plants often exhibit several physiological and morphological adaptations to survive the reduced availability of oxygen under flooded conditions (Crawford and Braendle 1996). The formation of hypertrophied lenticels, aerenchyma tissue and adventitious roots, which aid oxygen transport under flooded conditions, are thought to be principal mechanisms through which woody plants survive flooded conditions (Kozlowski 1997; Pryor *et al.* 2006). However, flooding can induce an increased shoot-to-root ratio in seedlings, in turn affecting a host of physiological processes (Kozlowski 1997; Smith and Brock 2007) that can retard seedling growth and the ability of a seedling to recover once water levels recede (Argus *et al.* 2014; Denton and Ganf 1994).

Many *Leptospermum* and *Melaleuca* (widespread genera of Australian wetland environments) species are particularly flood tolerant (Myers 1983; Pryor *et al.* 2006).

However, the ability of woody wetlands plants to tolerate flooding is dependent on their level of development (age/life history stage) and the extent of the flooding. For example, for many *Melaleuca* species: seed germination is delayed by flooding; older or taller seedlings typically survive flooding better than younger shorter seedlings, while the survival of both is reduced by longer or deeper flooding; and the growth and condition of mature plants may be reduced by prolonged flooding (Denton and Ganf 1994; Ladiges *et al.* 1981; Raulings *et al.* 2007; Salter *et al.* 2007; Salter *et al.* 2010).

Globally, our knowledge of the ability of woody wetland plants to tolerate changes to water regimes has become more important with increasing water regime alteration of wetland and river systems due to water extraction, damming and draining (Bunn and Arthington 2002; Poff *et al.* 1997). In Australia, it is common for water management practices to replace water regime variability with either constant wetting or extended drying (Brock *et al.* 1999), causing the elimination of some species and promotion of others (van der Valk *et al.* 1994). For example, the decline of river red gum (*Eucalyptus camaldulensis*) floodplain forests in south-eastern Australia is attributed to reduced flooding (Wen *et al.* 2009). Conversely, the common practice in Australia of using wetlands as off-channel storages creates permanently flooded conditions reducing wetland plant diversity (Kingsford 2000).

Yellingbo Nature Conservation Reserve (NCR) in south-eastern Australia is a wetland of ecological significance that has been negatively affected by changes to its water regime. The wetland shrub species *Melaleuca squarrosa* and *Leptospermum lanigerum* provide important foraging and nesting habitat for two critically endangered fauna within the reserve, the Leadbeater's possum (*Gymnobelideus leadbeateri*) and helmeted honeyeater (*Lichenostomus melanops cassidix*) (Harley *et al.* 2005; Pearce and Minchin 2001), but the abundance and distribution of these key plant species is locally declining, putatively in part due to altered water regimes (Harley 2016).

A number of catchment-scale and local anthropogenic impacts including land clearing, diversion of surface flows and construction of dams upstream have impacted water regimes at Yellingbo NCR (Craigie *et al.* 1998). The construction of levee banks and channelisation of creeks upstream of and in the reserve have caused channel incision and erosion, increasing sediment deposition in low-lying areas and subsequently hindering drainage (Fig. 1) (Greet 2016a). The consequence is that some areas of Yellingbo NCR now experience prolonged and deeper inundation (elevated water table), whilst in other areas some of the creeks have become deeply incised and the adjacent floodplain now rarely floods. are rarely flooded (lowered water table). These changes are thought to be associated with decline in the health of woody wetland vegetation and an absence of woody plant recruitment, thus threatening the persistence of endangered fauna within the reserve (Harley 2016).

To determine how *M. squarrosa* and *L. lanigerum* respond to changes in water regime at different life history stages, we conducted both glasshouse experiments and a field survey. In two separate glasshouse experiments, we tested the effect of flooding on seed germination and seedling establishment, and seedling growth and survival, respectively. We predicted that: 1) more seeds would germinate and seedlings establish more successfully in moist than in flooded conditions; and, 2) seedling growth and survival would decrease with increasing depth and duration of flooding. Using field surveys of intermittently-flooded (reference) and near permanently-flooded (impact) areas, we assessed the association between water regime and the condition of mature shrubs and predicted that: 3) crown extent would be greater at intermittently-flooded than at near permanently-flooded sites.

Materials and Methods

Study site and species

Both *M. squarrosa* and *L. lanigerum* are native to south-eastern Australia and are common in low-lying and swampy areas (Walsh and Entwisle 1993). Yellingbo NCR is a 640-ha swampy woodland reserve ~50 km east of Melbourne, Victoria, Australia. It comprises narrow linear sections along several streams, including the Cockatoo Swamp (Fig. 1), a 170-ha floodplain area along the lower reaches of Cockatoo and Macclesfield Creeks. Cockatoo Swamp contains the largest extent of the FFG-listed 'Sedge-rich *E. camphora* Swamp' community anywhere; these swamp forests include *M. squarrosa* and *L. lanigerum* shrub thickets along anastomosing channels and swamp margins (Moser and Greet 2018). This swamp would naturally experience inundation between 3–10 months per year, but currently experiences near permanent flooding due to impeded drainage (McMahon and Franklin 1993). The ecological integrity of the sedge-rich *E. camphora* swamp community, including important midstorey thickets of *M. squarrosa* and *L. lanigerum*, is threatened by increased flooding duration in parts of the Yellingbo NCR (Greet 2016a).

Glasshouse experiments

We conducted two glasshouse experiments at The University of Melbourne, Burnley campus (37° 83'S, 145° 02'E) using seed collected from Yellingbo NCR. Infructescences were collected using long-reach secateurs from a range of heights and aspects of the crown, up to a height of 6 m. Infructescences were kept in paper bags in a drying oven at 40°C for one week following collection to encourage seed release from capsules. Seed were then stored for three months before use in the experiments. No pre-treatment of the seeds was required as seeds of both species germinate readily.

Experiment 1: Effect of flooding on seed germination and seedling establishment

Day 1 – 28: seed germination

To test the effect of flooding on seed germination and seedling establishment, we set up a glasshouse experiment using a randomised block design, with ten replicate 10-L buckets of each of three water regime treatments: well-drained, flooded and flooded-drawdown. For the first 28 days, water levels in the well-drained and flooded treatments were maintained at 10 cm below and 5 cm above the substrate surface respectively, while in the flooded-drawdown treatment water levels were reduced from 5 cm above to 10 cm below the substrate surface at day 17. Buckets for each of the three treatments had holes drilled in the side and were topped up regularly with tap water to maintain appropriate water levels. Glasshouse temperatures were maintained at 22 °C (± 2 °C) throughout the experiment, and lit to provide 13 hours daylight:11 hours night.

For each species, ten seeds were sown into each of 30 forestry tubes (50×50×125 mm) containing ~2 cm pine bark at the base and ~10 cm river sand above, with seeds then covered with 0.2 cm sand. Forestry tubes were randomly allocated to the 30 buckets, such that each bucket contained one forestry tube of each species, and the buckets randomly positioned within the glasshouse. Seed germination was monitored on a ~weekly basis up to Day 28. After 28 days, each tube was inspected for germinated seed, as evidenced by cotyledons emergent from the sand. No seed was noted to have germinated and died by Day 28, when data was recorded.

Day 29 – 56: seedling establishment

After 28 days, seedlings were left to grow with water levels in all treatment groups maintained at 10 cm below the substrate surface to observe the survival (seedling establishment) of the seedlings that had germinated in the first 28 days of the experiment. At the end of the experiment (day 56), the numbers of established seedlings of each species in each replicate bucket were recorded.

Experiment 2: Effect of flooding on seedling growth and survival

This experiment was set up using a randomised block design, with ten replicate buckets in each of four treatments: well-drained, waterlogged, inundated (plants only partially submersed), and submerged (plants wholly submersed). Water levels in each treatment were as follows: well-drained, ~6 cm below the substrate surface; waterlogged, level with the substrate surface; inundated, ~2 cm above the substrate surface; submerged, ~9 cm above the substrate surface. As in the previous experiment, water levels were maintained in 10-L plastic buckets.

Two hundred seedlings of each species were grown in preparation for the experiment, following the procedure described in Experiment 1. Within a shaded glasshouse, planted seeds were kept moist by mist irrigation and on heated mats, maintaining the substrate at a constant temperature of ~18 °C. Seedlings were watered with fertiliser (Peter's Professional, 20:8.7:16.6, N:K:P) twice a week, and at 3.5 weeks were moved to an unshaded glasshouse maintained at 23–25 °C. At eight weeks, seedlings were thinned to one seedling per tube. At 10 weeks, 150 seedlings of similar height of each species were selected. The average height of the *L. lanigerum* and *M. squarrosa* seedlings selected were 3.9 cm and 2.5 cm respectively.

Of the 150 seedlings of each species, 60 were allocated to each of the submerged and inundated treatment groups (six seedlings in each of 10 buckets), and 10 to each of the waterlogged and well-drained treatment groups (with one seedling in each bucket). Additional seedlings were required in the submerged and inundated treatment groups to enable fortnightly assessments of seedling survival in these treatments following removal of seedlings and a one-week recovery period. Competition for resources (e.g. light) between seedlings in the submerged and inundated treatment is considered to have been negligible because plants were contained within separate tubes and tubes distributed evenly within each bucket.

Seedling heights were measured from substrate to the shoot tip at the beginning of the experiment and at fortnightly intervals for 10 weeks. Seedling survival was recorded fortnightly throughout the experiment. For the inundated and submerged treatments, one seedling of each species from each bucket was randomly selected for removal and survival assessed following a one-week recovery period, during which conditions were the same as those in the well-drained group (such a recovery period can be important to accurately assess survival in response to flooding; Denton and Ganf 1994). Survival was assessed using the same seedlings each fortnight in the unflooded (well-drained and waterlogged) treatment groups.

At the conclusion of the experiment, heights, stem diameters and presence of adventitious roots for all seedlings were recorded. Survival was again recorded after a one-week recovery period. Finally, seedlings were harvested, separated into above and below ground biomass components, dried at 40 °C for one week, and weighed using a Sartorius CPA225D analytical balance (precise to 100 μg).

Experiment 3: Field survey

Plant surveys were conducted on the 15–16th June 2015 within the Yellingbo NCR. Informed by surface and ground water data, three sites were selected representing two water regimes; Macclesfield Creek (MC, Fig. 1) is the furthest upstream site and was a reference site (i.e. intermittently flooded; ~3–6 months/year), while Cockatoo Swamp sites 1 & 2 (Fig. 1) were impact sites situated within an area where drainage is impeded and flooding/waterlogging persistent. At each site, twenty shrubs of each species were haphazardly selected from along swamp margins (along the wetted edge of the swamp during winter) and surveyed.

Effect of flooding on plant condition

Shrub condition assessments were based on visual estimates of crown extent. While often used in combination with other indicators, visual assessments of crown extent to quantify plant condition have proven a reliable indicator of condition for a number of woody riparian species (Cunningham *et al.* 2007; Salter *et al.* 2010; Souter *et al.* 2010). Crown extent is an estimate of the amount of crown area with live foliage as a percentage of the total crown, and was visually estimated to the nearest 10%. Thus, when the entire crown has full foliage, it is given a score of 100%, meaning the plant is in optimal condition.

Soil auger holes were dug adjacent to the base of each shrub surveyed to estimate depth to water table (as a relative measure of propensity to flood; groundwater and surface water [flooding] regimes at the site have been shown to be highly correlated; Hart 2015). Holes were dug until saturated soils were reached and depth-to-water-table measurements were made when no further inflow into the auger holes was apparent. In locations where the saturated soil zone was greater than 1 m deep, a depth of >100 cm was recorded. In locations where the trunk was submerged, water depth at the base of the trunk was recorded as a negative depth.

Statistical analyses

For Experiment 1, differences in seed germination and seedling establishment between species and flooding treatments after 28 and 56 days, respectively, were assessed using a varying-intercept, multi-level, logistic regression model (Gelman and Hill 2006). The model had two predictors for individual seeds—species, with two levels, *M. squarrosa* and *L. lanigerum*; and treatment, with three levels, well-drained, flooded, flooded drawdown; their interaction (to assess differences in response to treatments between species)—and tube identifier as an unmodelled, group-level predictor.

For Experiment 2, the difference in survival between the two species in the submerged treatment (the only treatment with reduced survival over time) was assessed using a Cox proportional hazards model (Therneau and Grambsch 2000). Comparisons of plant measurements among treatment groups assessed at week 10 were limited to the well-drained, inundated and waterlogged treatments, and did not include the submerged treatment, because no submerged plants survived to week 10. Seedling height was however compared across all four treatments at week four, before a significant number of seedling deaths. Differences in seedling height, stem width, total biomass, and ratio of above-ground to below-ground biomass ($\log_{10}$-transformed to remove heteroscedacity) were assessed by normal linear regression models with two predictors: species (two levels) and treatment (three levels).

For Experiment 3, we tested for differences in condition between the two species and between reference and impact sites, and also if the variation was explained by water-table depth. For crown extent, these effects were assessed using a regression model with a beta response distribution (as the response variable ranges between 0 and 1: Ferrari and Cribari-Neto 2004), with three predictor variables: species (two levels) and site (three levels: the reference site, Macclesfield Ck, and the two impact sites, Cockatoo Swamp 1 and 2), their interaction, and a continuous variable, depth to water table, and its interaction with species. The model used proportional crown extent minus 0.05 to allow for records of 100% crown extent, because the beta distribution requires values >0 and <1. Depth to water table ranged from -0.24 m (i.e. the tree was inundated to a depth of 0.24 m) to 1 m (water-table depth was >1 m for four trees, so the data is right-censored). To reduce leverage of outlying values, water table depth (m) was $\log_{10}(x+1)$-transformed, which meant that the intercept (zero depth) remained zero in the transformed variable.

Multi-level models and those with non-normal response distributions were calculated with the glmmADMB function in R (Fournier *et al.* 2012; Skaug *et al.* 2016). For all models, residual plots were inspected to confirm assumptions of the analyses were met. For each model we present the mean and 95% confidence intervals (CIs) of the coefficients for each comparison among predictors, and infer significant effects if the 95% CIs do not span zero. We present the results of each analysis graphically, showing both the raw data and the coefficients and CIs of each comparison, which illustrates both the effect size and its precision (Gelman and Hill 2006). In all models, the well-drained flooding effect and the *M. squarrosa* species effect were the intercepts. The coefficient for each flooding treatment was thus compared to the well-drained treatment, and the species coefficient compared *L. lanigerum* to *M. squarrosa*. Any two flooding treatment effects can be interpreted as different from each other if the CIs of their comparisons with the well-drained treatment did not overlap. All data and R code used to conduct analyses are available at https://doi.org/10.17605/osf.io/yajfw.

Results

Experiment 1: Effect of flooding on seed germination and seedling establishment

Percentage germination after 28 days did not differ significantly between well-drained, flooded-drawdown and flooded treatments for either species, and there was no significant interaction between flooding treatment and species (Fig. 2C). However, the mean germination of *L. lanigerum* (79 $\pm$ 15% [standard deviation, sd]) was higher than that of *M. squarrosa* (52 $\pm$ 16%) across all treatment groups (Fig. 2A, C). After 56 days, the difference in seedling establishment between species remained similar to the difference in germination (81 $\pm$ 17% for *L. lanigerum* vs 53 $\pm$ 16% for *M. squarrosa*: higher means because a few additional seeds germinated between 28 and 56 days). However, for both species, seedling establishment after 56 days in the flooded-drawdown treatment (62 ± 25%) was lower than in the well-drained treatment (72 ± 16%), while seedling establishment in the flooded treatment did not differ significantly from the well-drained treatment (Fig. 2B, D).

Experiment 2: Effect of flooding on seedling growth and survival

Only one death was recorded in any of the well-drained, waterlogged and inundation treatments over the 10 weeks of this experiment (that being of a smaller-than-average inundated *L. lanigerum* seedling at week 6; Fig. 3). No seedling of either species survived 10 weeks of submergence, and the survival rate did not differ significantly between species (Fig. 3; Cox model coefficient of *L. lanigerum* with *M. squarrosa* as intercept was -0.73, 95% CIs -1.62, 0.16). However, the first *L. lanigerum* seedling deaths was recorded at six weeks, compared to two *M. squarrosa* seedling deaths recorded at four weeks. And at six weeks, 60% of *M. squarrosa* seedlings had died, compared to 10% of *L. lanigerum* seedlings (Fig. 3).

After four weeks, submerged seedlings of both species were smaller (mean height of *L. lanigerum* 4.8 ± 0.9 cm, and of *M. squarrosa* 3.9 ± 1.0 cm) than those in the other treatments (submerged CI does not overlap CIs of other treatments, Fig. 4D), and this difference was larger for *L. lanigerum* (mean height across other treatments 11.2 ± 1.6 cm) than for *M. squarrosa* seedlings, which tended to be smaller (6.8 ± 1.5 cm) (Fig. 4A, negative submerged x *L. lanigerum* coefficient in Fig. 4D, indicating a larger negative effect of the submerged treatment on *L. lanigerum* than on *M. squarrosa*).

By week 10, seedlings of both species were taller in the waterlogged treatment than in the well-drained treatment (*L. lanigerum* 16.6 ± 2.4 vs 15.3 ± 2.7 cm; *M. squarrosa* 12.2 ± 3 vs 9.9 ± 1.3 cm: Fig. 4B, E), and their stems were thicker in both the waterlogged and inundated treatments than in the well-drained treatment (mean stem diameter for both species in well-drained treatment 1.7 ± 0.2 mm compared to 2.2 ± 0.4 mm in other treatments: Fig. 4C, F).

Total biomass was lower in the inundated treatment than in well-drained or waterlogged treatments, and this was marginally more pronounced for *M. squarrosa* (Fig. 5A, C). There were no significant differences in the ratio of above-ground to below-ground biomass, although there was a tendency for seedlings in the inundated and waterlogged treatments to have less below-ground biomass (Fig. 5B, D). Adventitious roots formed more commonly in the inundated than the waterlogged treatment (not at all in the well-drained treatment) and more commonly in *L. lanigerum* (8 out of 10 seedlings in the waterlogged treatment, and all 10 in the inundated treatment) than in *M. squarrosa* (4 and 6).

Experiment 3: Effect of flooding on plant condition

In the field, depth to water table differed widely between sites and species (Fig. 6), limiting our ability to infer differences in crown extent in response to water table depth. The wide CIs in Fig. 6C show that water table depth was not strongly associated with crown extent.

Differences in crown extent between sites differed between the two species (negative coefficients for both near permanently flooded (impact) sites vs the intermittently flooded (reference) site, and the positive site/species interaction for impact site 2 in Fig. 6C). Crown extent of *M. squarrosa* was lower at both impact sites (53 ± 25% and 34 ± 22% at impact sites 1 and 2, respectively) than at the reference site (69 ± 22%). In contrast, crown extent of *L. lanigerum* did not differ between sites, being 60 ± 22%, 65 ± 25%, and 72± 20% at the reference site and impact sites 1 and 2, respectively (Fig. 6A, B).

Discussion

Our study highlights the importance of water regime as an environmental filter for community assembly in wetland forests. Our results demonstrating negative effects of flooding on seedling establishment, growth and survival, for both species, indicate that recruitment of *M. squarrosa* and *L. lanigerum* is unlikely in areas that are typically flooded, such as the impact areas of our study. Furthermore, such hydrological conditions are likely to threaten the persistence of mature populations of less flood-tolerant wetland species such as *M. squarrosa* (c.f. *L. lanigerum*, which was generally more flood tolerant).

Effect of flooding on seed germination and seedling establishment

Flooding has been found to delay the germination of some wetland species (Ladiges *et al.* 1981; Pierce and King 2007; van der Valk *et al.* 1992) and inhibit the germination of others (Casanova and Brock 2000). Although flooding had no effect on the germination of either *M. squarrosa* or *L. lanigerum* in this study, seedlings were unable to grow past cotyledon stage while flooded, and flooding reduced the seedling establishment of both species. It is likely that young *M. squarrosa* and *L. lanigerum* seedlings are adversely affected by even short periods of submergence, as found for other *Melaleuca* species (Denton and Ganf 1994; Myers 1983). Exposed moist substrate is likely to be most appropriate for the seedling establishment of these woody wetland plant species (Greet 2016a; Robinson *et al.* 2006; Robinson *et al.* 2008).

Effect of flooding on seedling growth and survival

Depth and duration of flooding are considered to be the primary factors that limit the recruitment of woody wetland plants, with young seedlings being particularly vulnerable to flooding (Kozlowski 1997). We identified critical limits of ~eight weeks submergence for young seedlings of both *M. squarrosa* and *L. lanigerum*. Similar limits are known for other species of *Melaleuca*, such as *M. halmaturorum* (5 weeks; Denton and Ganf 1994), and *M. ericifolia* (10 weeks; Salter *et al.* 2007), and for the dominant woody wetland plant at Yellingbo NCR, *Eucalyptus camphora* (4 weeks; Greet 2016a).

Although complete submergence longer than eight weeks was fatal for 20-week-old seedlings, both species were tolerant of shallow flooding (waterlogging and inundation). Such flooding induced morphological changes such as stem hypertrophy (indicating aerenchyma formation) and the development of adventitious roots. The development of aerenchyma and adventitious roots is likely to have allowed waterlogged seedlings to maintain equal biomass and grow taller compared to seedlings in well-drained soils. A similar response has been observed in *M. quinquenervia*, which rapidly forms many adventitious roots on stems and roots, and grows better in saturated soil than in well-drained conditions (Myers 1983; Sena Gomes and Kozlowski 1980). However, inundation did tend to result in reduced relative below-ground biomass and overall biomass compared to the unflooded treatments.

Also consistent with other studies, our results indicate that increases in flooding depth are correlated with increases in the incidence of adventitious root and aerenchyma formation (as indicated by greater stem width) in seedlings of flood-tolerant woody plants (Greet 2016a). Overall, the tendency of *L. lanigerum* seedlings to survive submergence longer, and produce greater numbers of stem-borne adventitious roots while flooded, suggest that they are more flood-tolerant than *M. squarrosa* seedlings.

Effect of flooding on plant condition

Prolonged flooding can negatively affect the condition of mature woody wetland plants. For example, Salter *et al*. (2010) found that *M. ericifolia* trees that had been constantly flooded (>30 years) had reduced crown condition compared to trees that were intermittently flooded. Our surveys suggested a decline in the crown extent of *M. squarrosa* at near permanently flooded sites, while *L. lanigerum* did not show a similar decline in crown extent, thus seemingly less sensitive to prolonged flooding. Correspondingly, in areas of poor drainage, mature populations of *M. squarrosa* are now largely confined to elevated swamp margins, while *L. lanigerum* persists within the swamp, albeit often restricted to raised hummocks.

Considering the results from each life history stage examined, it seems that growth of these wetland shrubs is higher in waterlogged conditions, moderate in either shallowly inundated or well-drained conditions, and lower in submerged or prolonged flooded conditions. This could contribute to the absence of a linear response of plant condition to water table depth observed in our field survey, with condition of the two study species likely to be compromised by either extended dry or flooded conditions.

Management Implications

According to ecological assembly theory, the presence or absence of species is controlled by a combination of abiotic conditions, biotic interactions and availability of propagules acting as filters (Catford and Jansson 2014). Increased extent and duration of flooding in some areas of Yellingbo NCR represent a change to the abiotic conditions at the site which has altered the distribution and caused the decline of some woody species. In particular, prolonged flooding within parts of the Cockatoo Swamp at Yellingbo NCR is unsuitable for the maintenance of *M. squarrosa* (as it is for *E. camphora*; Greet 2015). Furthermore, distinct areas of constantly flooded and rarely flooded conditions created by the lack of variability in the water regime have reduced the spatial extent of the wet-dry ecotone and area suitable for the recruitment of either of the study species (Brock and Casanova 1997).

Management interventions to increase the recruitment of the two study species that provide habitat for critically endangered fauna are urgently needed at Yellingbo NCR (Harley 2016). Hydrological restoration works (e.g. levee removal, de-channelisation, and pumping to drain persistently flooded areas) aimed at reinstating more variable flooding regimes to floodplain areas have the potential to promote the maintenance and regeneration of wetland forest at Yellingbo NCR (Greet 2016a), and are currently being implemented by

Melbourne Water. Specifically, floodplain reengagement (to increase the intermittently wetted floodplain extent) and reduction in areas subject to prolonged flooding at depths likely to submerge young seedlings (e.g. depths ≥10 cm) are necessary to promote the recruitment of the two study species.

Competition from dense understorey vegetation (e.g. *Phragmites australis*), as well as browsing pressure from feral and native species such as sambar deer (*Dama dama*) and black wallabies (*Wallabia bicolor*) have also been identified as important biotic filters limiting the recruitment of these species at Yellingbo NCR (Moser and Greet 2018; Pearce 2000). As *M. squarrosa* and *L. lanigerum* do not form persistent soil seed banks (Greet 2016b), but instead rely on canopy-stored seed, their presence at a site is dependent on the seed production of the extant population or the arrival of propagules into the site through hydrochory. It is therefore essential that the mature populations of these species within the Yellingbo NCR and any potential source populations upstream be maintained.

Land managers are increasingly investigating opportunities to manipulate water regimes in wetlands in order to ameliorate adverse human impacts and promote restoration, and it is essential that their management decisions are robust and science-based. This study demonstrates a strong association between the early life history stages of two woody wetland plant species and two water regime components (depth and duration of flooding). Our findings suggest that intermittent rather than near-permanent flooding and increased exposure of sediments during the early seedling stages may be best for maintaining woody wetland vegetation.

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Figure captions

Fig. 1. Map showing the sections of Yellingbo NCR that run along the Woori Yallock, Cockatoo and Macclesfield Creeks. The poorly-draining areas of the Cockatoo Swamp are illustrated pictorially. Small squares indicate impact sites, ‘Cockatoo Swamp 1 & 2’, and an open black circle indicates the reference site ‘Macclesfield Creek’.

Fig. 2. Percentage (A) seed germination and (B) seedling establishment per pot for each species (*L. lanigerum* circles, *M. squarrosa* crosses) under three experimental treatments (flooded, F; flooded-drawdown, FDD; well-drained, W), after (A) 28 days and (B) 56 days. For each treatment the positions are jittered on the y-axis to reveal overlapping values. Mean (±95% confidence intervals) coefficients for each comparison of the two effects (species, with *M. squarrosa* as intercept, and treatment, with well-drained as intercept) are shown for (D) the 28-day and (E) 56-day germination and establishment models, respectively.

Fig. 3. Survival of seedlings of each species in each of four flooding treatments over 10 weeks. At each interval, survival was recorded using a different group of seedlings for the inundated and submerged treatments. One *L. lanigerum* seedling from the week six seedlings died, however all ten from week eight and ten groups survived, explaining the drop and rise in survival seen in the inundated *L. lanigerum* group.

Fig. 4. Seedling height at (A) week 4 and (B) week 10 for each species (*L. lanigerum* circles, *M. squarrosa* crosses) under the four experimental treatments. C. Stem width at week 10 for the same species and treatments. For each treatment the positions of points in A–C are jittered on the y-axis to reveal overlapping values. D–F. Mean (±95% confidence intervals) coefficients for each comparison of the two effects (species, with *M. squarrosa* as intercept, and treatment, with well-drained as intercept) for seedling height at week 4 and week 10, and stem width at week 10, respectively.

Fig. 5. A. Total biomass and B. ratio of above-ground to below-ground biomass for each species (*L. lanigerum* circles, *M. squarrosa* crosses) under three experimental treatments at week 10 (no seedlings survived 10 weeks in the submerged treatment). C, D. Mean (±95% confidence intervals) coefficients for each comparison of the two effects (species, with *M. squarrosa* as intercept, and treatment, with well-drained as intercept) for total biomass and biomass ratio respectively.

Fig. 6. Percentage crown extent of (A) *L. lanigerum* and (B) *M. squarrosa* plotted against depth to water table at each of the three sites (open circles, reference; grey and black squares, impact 1 and 2, respectively). Points are jittered on the y-axis to reveal overlapping data. C. Mean (±95% confidence intervals) of coefficients for the crown-extent linear models with species (*M. squarrosa* as intercept) and site (reference as intercept) as predictors.

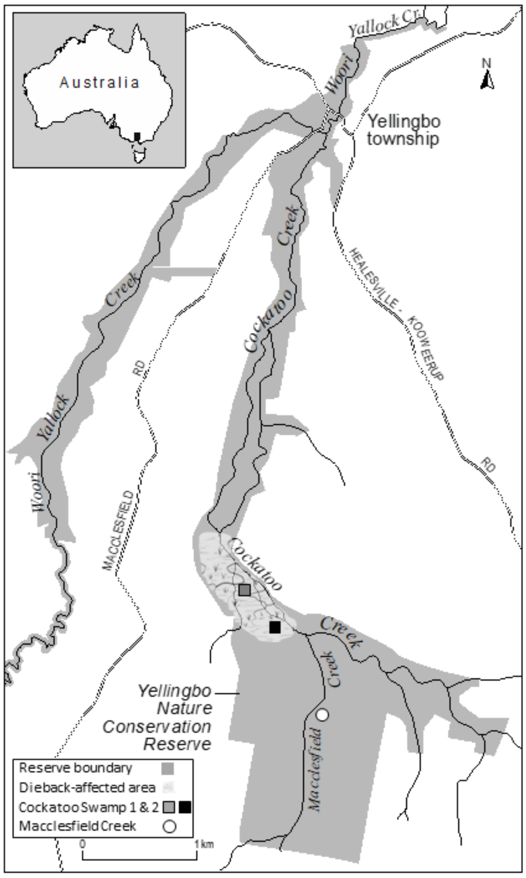
Australia
Yallock Cr
Woori
Yellingbo
township
N
Creek
Cockatoo
HEALESVILLE - KOOWEERUP
RD
Creek
Yallock
RD
Woori
MACCLESFIELD
Cockatoo
Creek
Creek
Macclesfield
Yellingbo
Nature
Conservation
Reserve
Reserve boundary
Dieback-affected area
Cockatoo Swamp 1 & 2
Macclesfield Creek
0
1 km

Fig. 1.

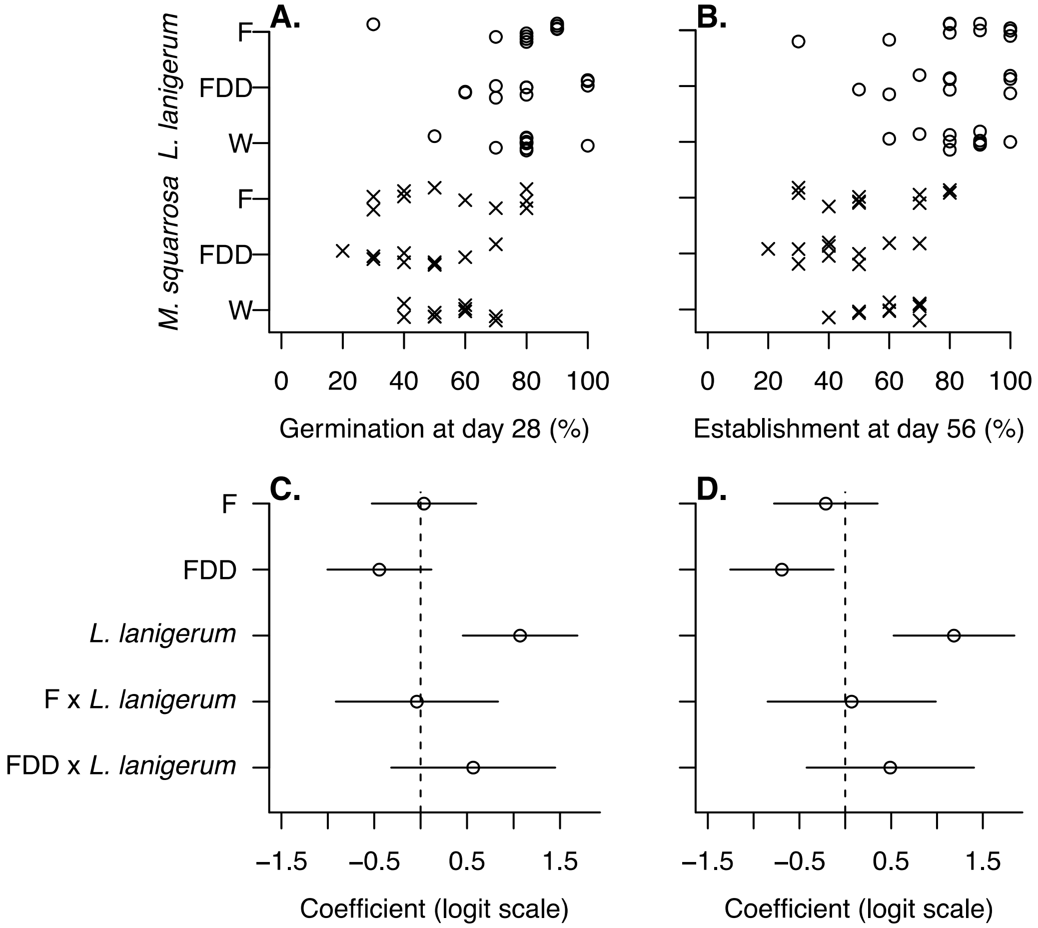
A.
B.
L. lanigerum
M. squarrosa
F
FDD
W
F
FDD
W
0
20
40
60
80
100
Germination at day 28 (%)
Establishment at day 56 (%)
C.
D.
F
FDD
L. lanigerum
F x L. lanigerum
FDD x L. lanigerum
−1.5
−0.5
0.5
1.5
Coefficient (logit scale)

Fig. 2.

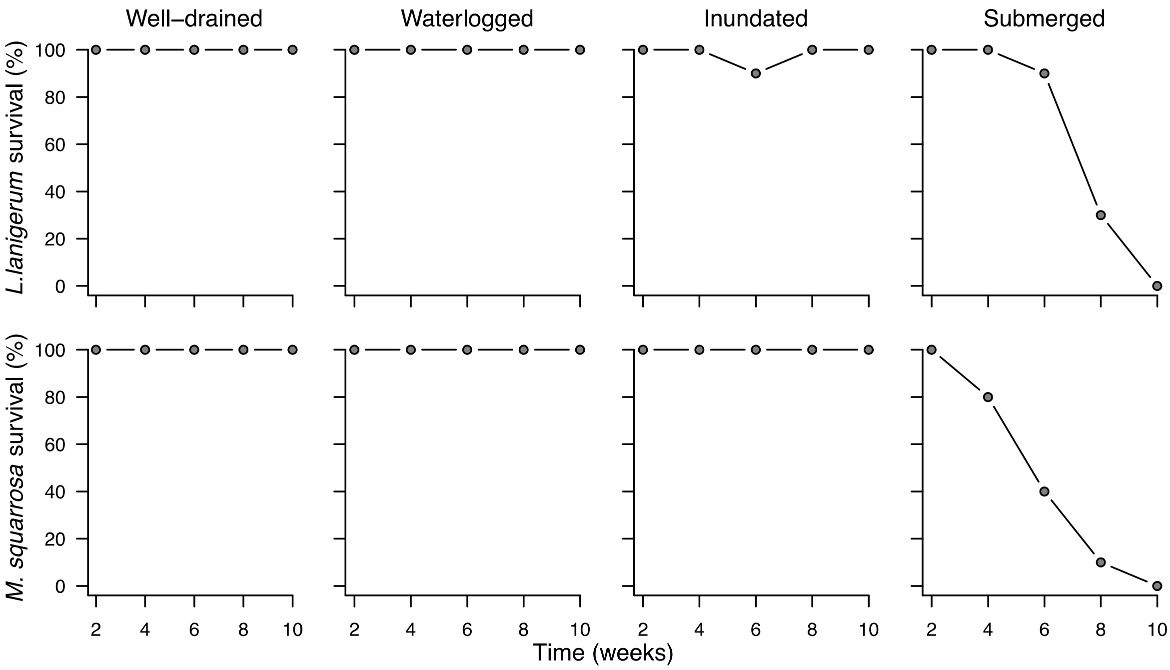
Well−drained
Waterlogged
Inundated
Submerged
L.lanigerum survival (%)
M. squarrosa survival (%)
100
80
60
40
20
0
2
4
6
8
10
Time (weeks)

Fig. 3.

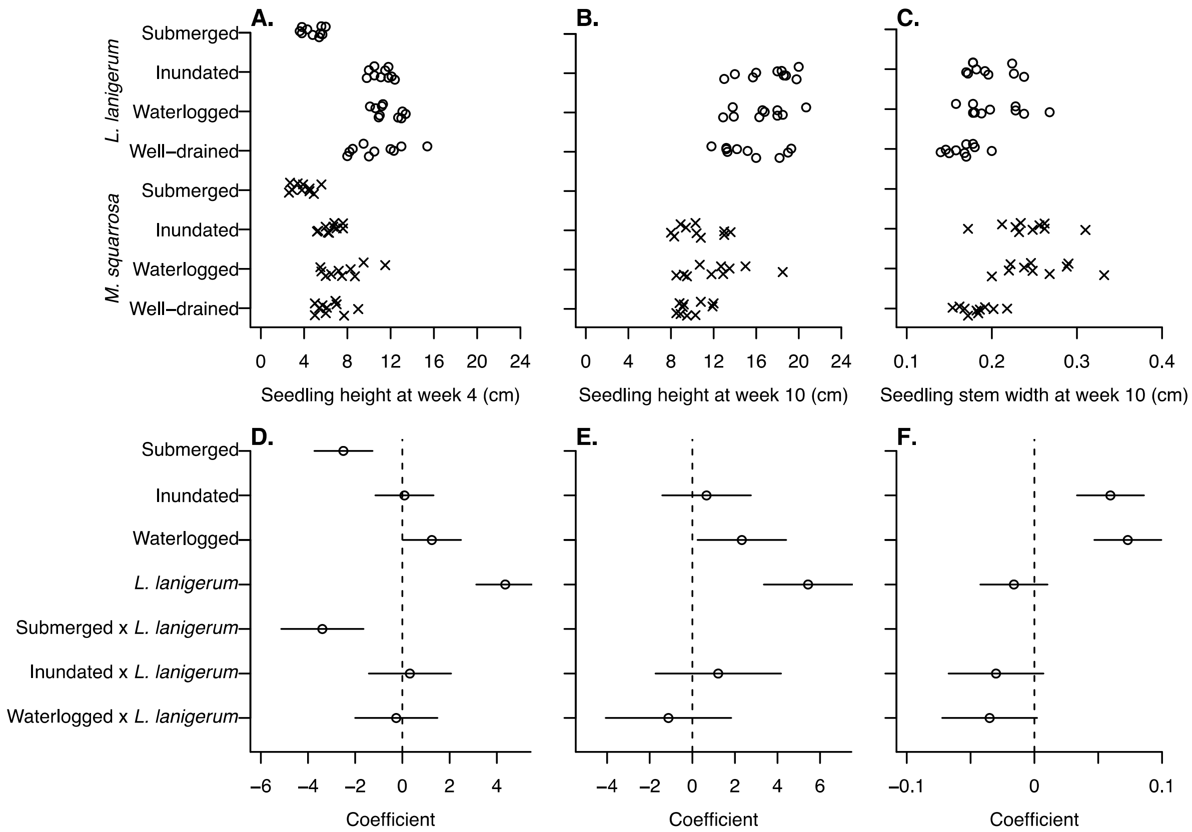
A.
B.
C.
D.
E.
F.
L. lanigerum
M. squarrosa
Submerged
Inundated
Waterlogged
Well–drained
Seedling height at week 4 (cm)
Seedling height at week 10 (cm)
Seedling stem width at week 10 (cm)
L. lanigerum
Submerged x L. lanigerum
Inundated x L. lanigerum
Waterlogged x L. lanigerum
Coefficient

Fig. 4.

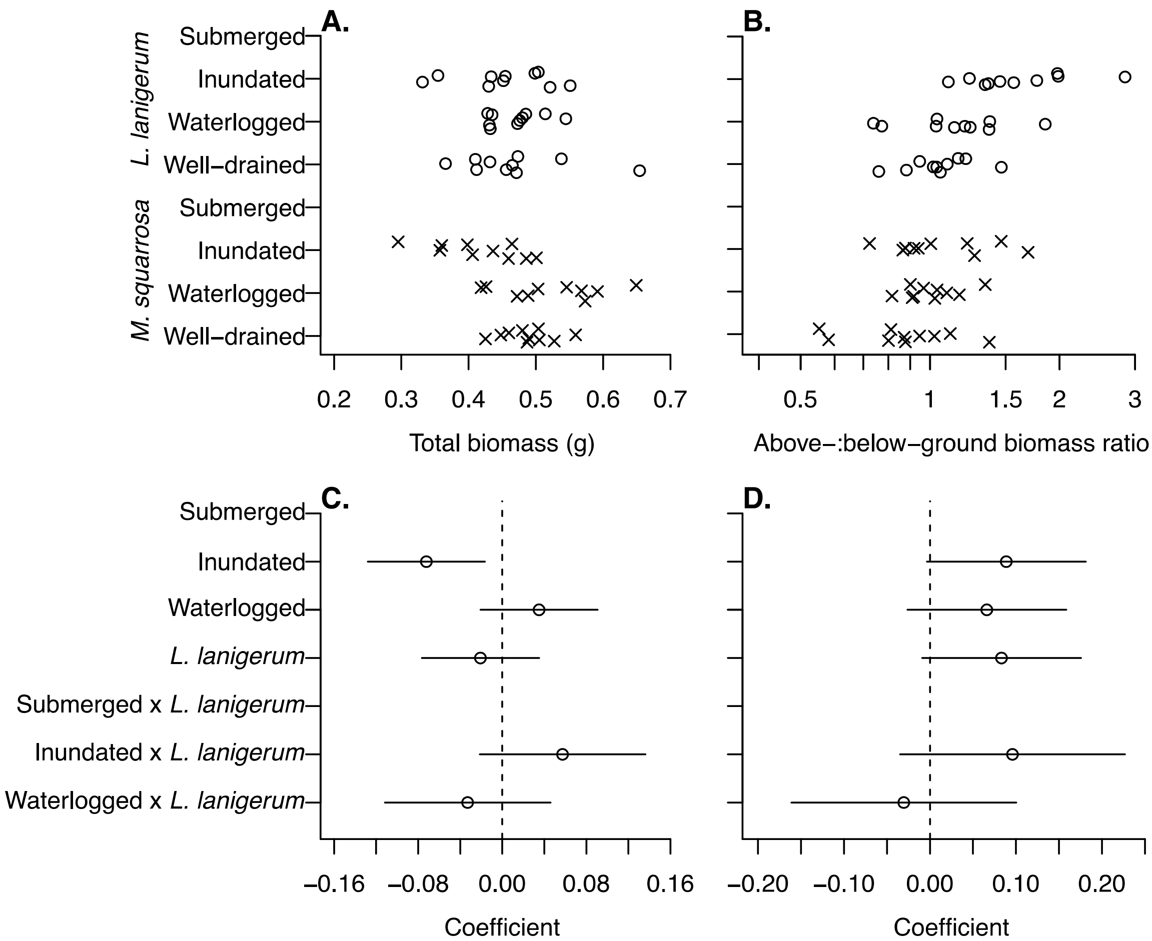
A.
B.
C.
D.
L. lanigerum
M. squarrosa
Submerged
Inundated
Waterlogged
Well–drained
Total biomass (g)
Above–:below–ground biomass ratio
L. lanigerum
Submerged x L. lanigerum
Inundated x L. lanigerum
Waterlogged x L. lanigerum
Coefficient

Fig. 5.

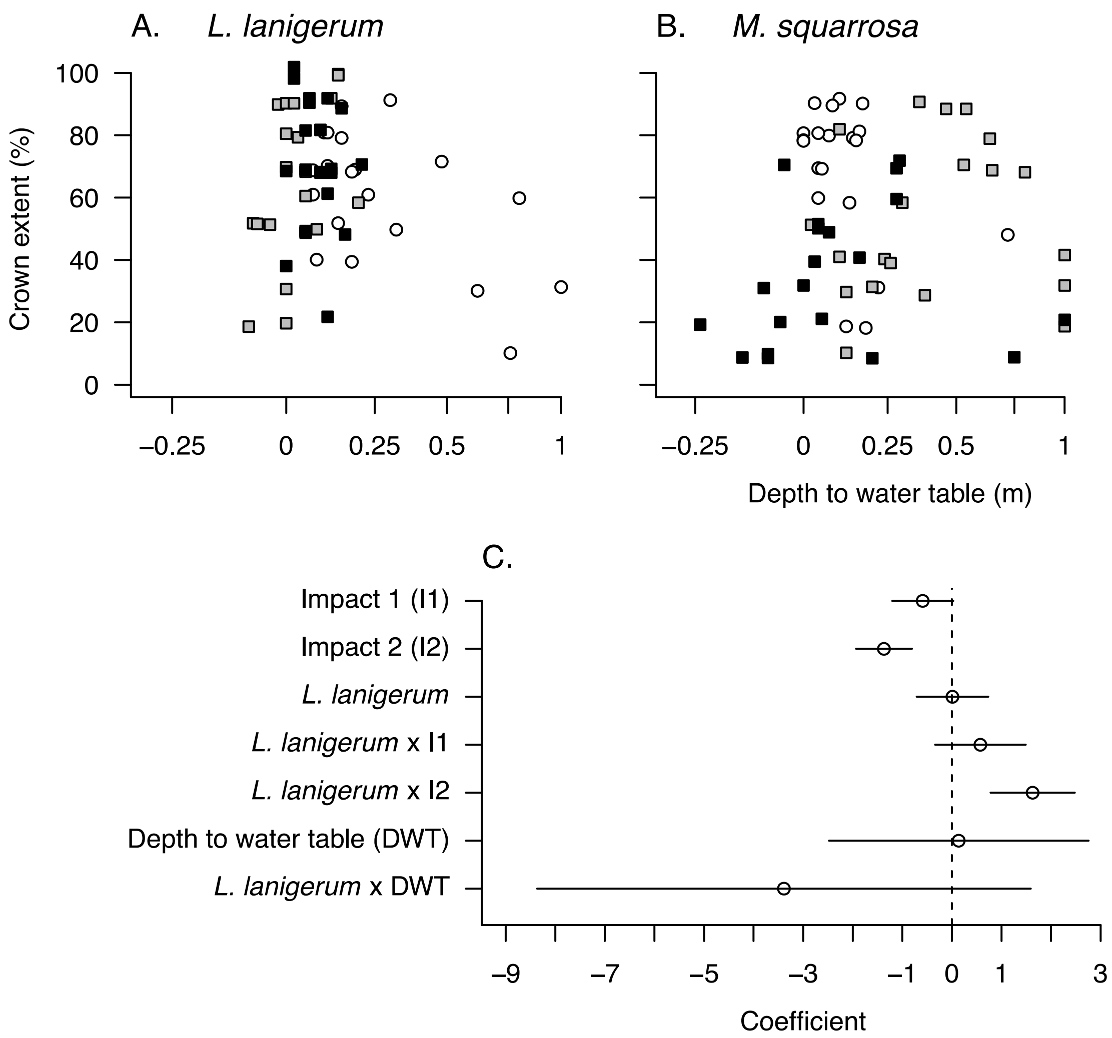
A. L. lanigerum
B. M. squarrosa
Crown extent (%)
100
80
60
40
20
0
–0.25
0
0.25
0.5
1
Depth to water table (m)
C.
Impact 1 (I1)
Impact 2 (I2)
L. lanigerum
L. lanigerum x I1
L. lanigerum x I2
Depth to water table (DWT)
L. lanigerum x DWT
–9
–7
–5
–3
–1
0
1
3
Coefficient

Fig. 6.