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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* Donn. ex Sm. and *Leptospermum lanigerum* (Aiton) Sm., provide habitat for two critically endangered fauna within the Yellingbo Nature Conservation Reserve (south-eastern Australia), but are in decline putatively because of the 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, whereas no seedlings of either species survived >8 weeks of submergence. *Leptospermum lanigerum* seedlings were generally more flood tolerant than were *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 (1) limit woody plant recruitment, because flooding reduces seedling establishment, growth and survival, and (2) 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.

Additional keywords: riparian shrubs, water regime, Yellingbo Nature Conservation Reserve.

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Introduction

Water regime, i.e. 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 (van der Valk 1981; Ge *et al*. 2013).

Water regime can act as a filter for plant assembly at different life history stages, including germination (e.g. suppression by flooding; Ge *et al*. 2013), recruitment (e.g. creation of zonation patterns along water level gradients; Keddy and Ellis 1985), growth (e.g. reduced growth of flooded seedlings; Greet 2015) 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 (Denton and Ganf 1994; Argus *et al*. 2015).

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 (Ladiges *et al*. 1981; Denton and Ganf 1994; Raulings *et al*. 2007; Salter *et al*. 2007, 2010).

Globally, our knowledge of the ability of woody wetland plants to tolerate changes to water regimes has become more important with an increasing water-regime alteration of wetland and river systems because of water extraction, damming and draining (Poff *et al*. 1997; Bunn and Arthington 2002). 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*; Pearce and Minchin 2001; Harley *et al*. 2005), but the abundance and distribution of these key plant species is locally declining, putatively in part because of the altered water regimes (Harley 2016).

Several catchment-scale and local anthropogenic impacts including land clearing, diversion of surface flows and construction of dams upstream have affected 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 2015). The consequence is that some areas of Yellingbo NCR now experience prolonged and deeper inundation (elevated watertable), whereas, in other areas, some of the creeks have become deeply incised and the adjacent floodplain is rarely flooded (lowered watertable). 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 an 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 and 10 months per year, but currently experiences near permanent flooding because of impeded drainage (McMahon and Franklin 1993). The ecological integrity of the sedge-rich *E. camphora* swamp community, including important mid-storey thickets of *M. squarrosa* and *L. lanigerum*, is threatened by increased flooding duration in parts of the Yellingbo NCR (Greet 2015).

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, with 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 1 week following collection, to encourage seed release from capsules. Seed were then stored for 3 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

Days 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 10 replicate 10-L buckets of each of the following 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, whereas 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

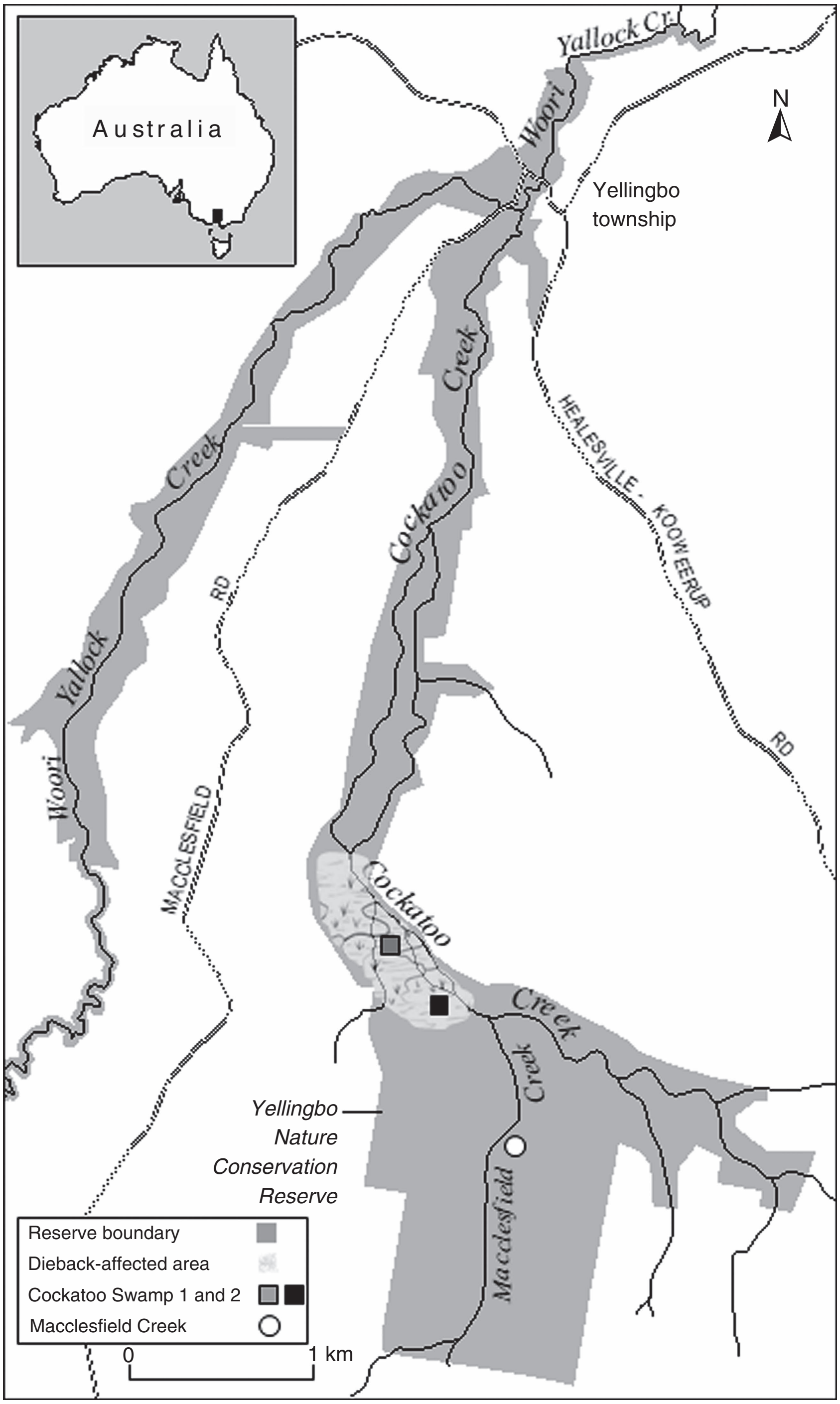

Fig. 1. Map showing the sections of Yellingbo Nature Conservation Reserve that run along the Woori Yallock, Cockatoo and Macclesfield Creeks. The poorly draining areas of the Cockatoo Swamp are illustrated pictorially. Small squares indicate impact sites, ‘Cockatoo Swamp 1 and 2’, and an open black circle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

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 h daylight : 11 h darkness.

For each species, 10 seeds were sown into each of the 30 forestry tubes (50 × 50 × 125 mm) containing ~2 cm of pine bark at the base and ~10 cm of river sand above, with seeds then being covered with 0.2 cm of sand. Forestry tubes were randomly allocated to the 30 buckets, such that each bucket contained one forestry tube of each species, and the buckets were randomly positioned within the glasshouse. Seed germination was monitored on about a weekly basis up to Day 28. After

28 days, each tube was inspected for germinated seeds, as evidenced by cotyledons emergent from the sand. No seed was noted to have germinated and died by Day 28, when data were recorded.

Days 29–56: seedling establishment

After 28 days, seedlings were left to grow, with water levels in all treatment groups being 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 10 replicate buckets in each of the following 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, Everris, Geldermalsen, The Netherlands, 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 8 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 1-week recovery period. Competition for resources (e.g. light) among seedlings in the submerged and inundated treatment is considered to have been negligible because plants were contained within separate tubes and tubes were 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 1-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 1-week recovery period. Finally, seedlings were harvested, separated into above- and belowground biomass components, dried at 40°C for 1 week, and weighed using a Sartorius CPA225D analytical balance (precise to 100 µg, Sartorius, Goettingen, Germany).

Experiment 3: Field survey

Plant surveys were conducted on 15–16 June 2015 within the Yellingbo NCR. Informed by surface and groundwater 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 per year), while Cockatoo Swamp Sites 1 and 2 (Fig. 1) were impact sites situated within an area where drainage is impeded and flooding/waterlogging persistent. At each site, 20 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. Although often used in combination with other indicators, visual assessments of crown extent to quantify plant condition have proven a reliable indicator of condition for several 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 that the plant is in optimal condition.

Soil auger holes were dug adjacent to the base of each shrub surveyed to estimate the depth to watertable (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-watertable 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 among 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: (1) species, with two levels, *M. squarrosa* and *L. lanigerum*, and (2) treatment, with three levels, well drained, flooded and flooded–drawdown; and their interaction (to assess differences in response to treatments between species). Tube identifier was 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. However, seedling height was compared across all four treatments at Week 4, before a significant number of seedling deaths. Differences in seedling height, stem width, total biomass and ratio of aboveground to belowground biomass ($\log_{10}$-transformed to remove heteroscedacity) were assessed by normal linear regression models with the following 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 whether the variation was explained by watertable depth. For crown extent, these effects were assessed using a regression model with a β 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 watertable, and its interaction with species. The model used proportional crown extent minus 0.05 to allow for records of 100% crown extent, because the β distribution requires values >0 and <1. Depth to watertable ranged from –0.24 m (i.e. the tree was inundated to a depth of 0.24 m) to 1 m (watertable depth was >1 m for four trees, so the data are right-censored). To reduce leverage of outlying values, watertable 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 that the 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

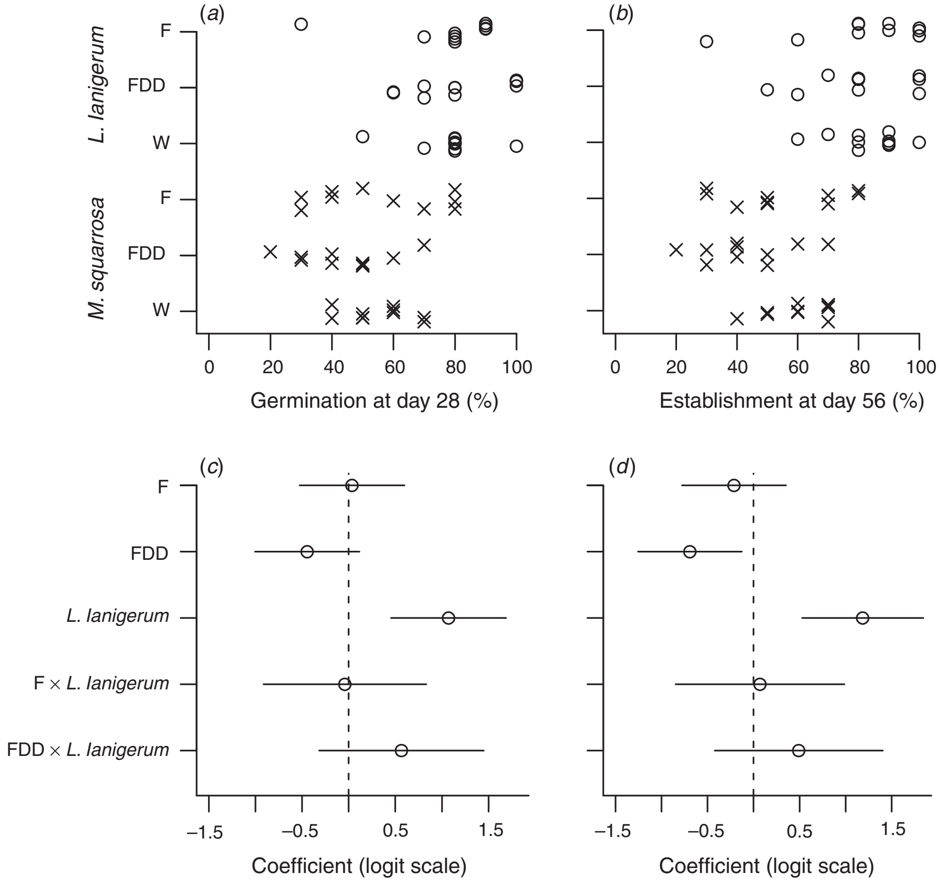

Fig. 2. Percentage (*a*) seed germination and (*b*) seedling establishment per pot for each species (*Leptospermum lanigerum* (○), *Melaleuca squarrosa* (×)) under three experimental treatments (flooded, F; flooded–drawdown, FDD; well drained, W), after (*a*) 28 days and (*b*) 56 days. For each treatment, the positions are jittered on the *y*-axis to show 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 the (*c*) 28-day and (*d*) 56-day germination and establishment models respectively.

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 with the well drained treatment, and the species coefficient compared *L. lanigerum* with *M. squarrosa*. Any two flooding treatment effects were 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 among well drained, flooded–drawdown and flooded treatments for either species, and there was no significant interaction between flooding treatment and species (Fig. 2*c*). However, the germination (mean ± s.d.) of *L. lanigerum* (79 ± 15%) was higher than that of *M. squarrosa* (52 ± 16%) across all treatment groups (Fig. 2*a, c*). After 56 days, the difference in seedling establishment between species remained similar to the difference in germination (81 ± 17% for *L. lanigerum* vs 53 ± 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 that in the well drained treatment (72 ± 16%), whereas seedling establishment in the flooded treatment did not differ significantly from that in the well drained treatment (Fig. 2*b, 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 death was recorded at 6 weeks, compared with two *M. squarrosa* seedling deaths recorded at 4 weeks. And at 6 weeks, 60% of *M. squarrosa* seedlings had died, compared with 10% of *L. lanigerum* seedlings (Fig. 3).

After 4 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 in other treatments, Fig. 4*d*), 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. 4*a*, negative submerged × *L. lanigerum* coefficient in Fig. 4*d*, indicating a larger negative effect of the submerged treatment on *L. lanigerum* than on *M. squarrosa*).

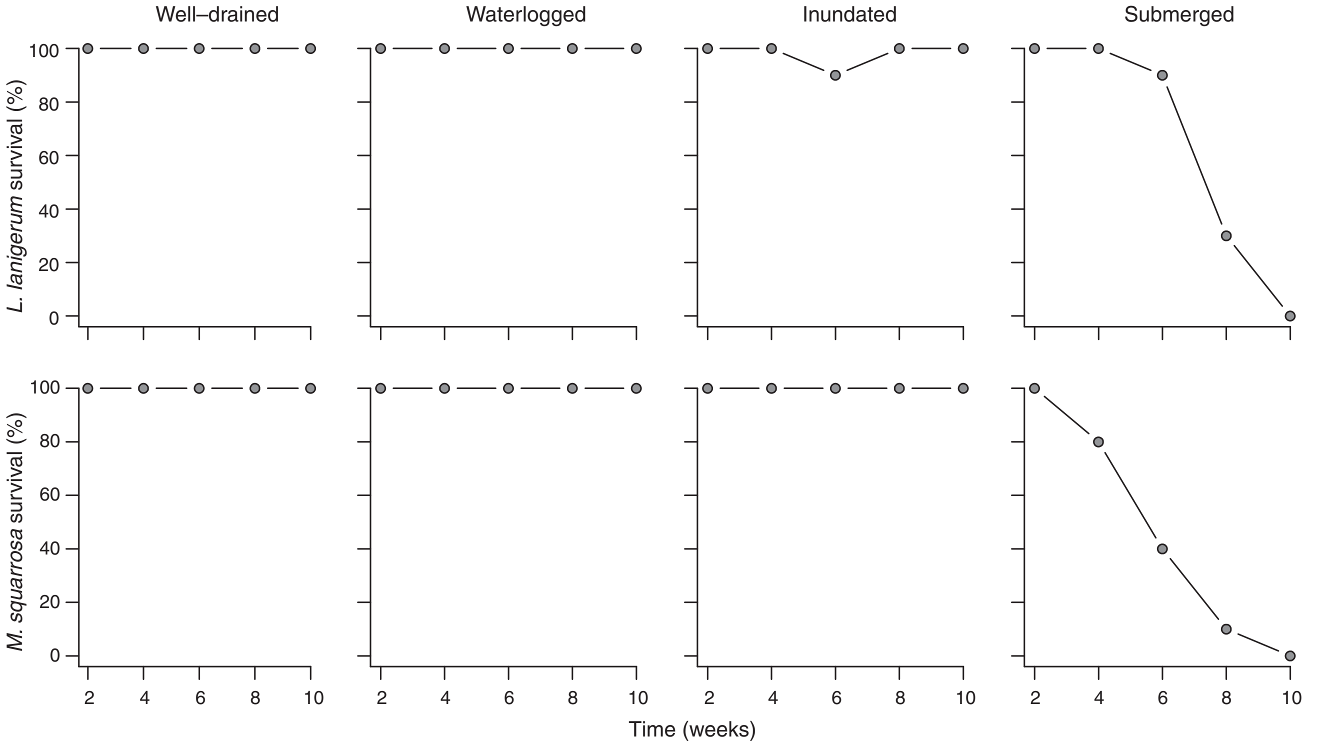

Fig. 3. Survival of seedlings of each species in each of four flooding treatments over 10 weeks. At each interval, survival was recorded using a different group of seedlings for the inundated and submerged treatments. One *Leptospermum lanigerum* seedling from the Week 6 seedlings died; however, all 10 from Week 8 and Week 10 groups survived, explaining the drop and rise in survival seen in the inundated *L. lanigerum* group.

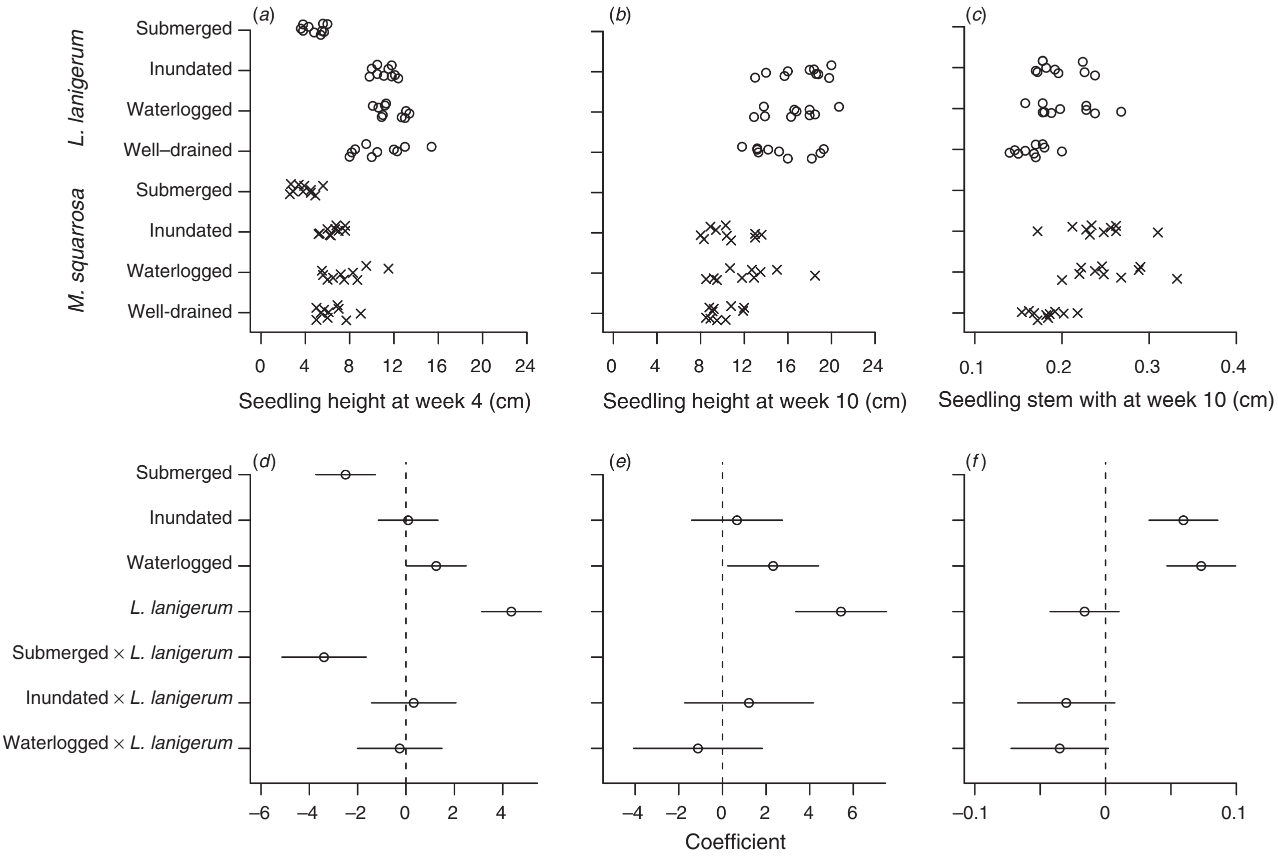

Fig. 4. Seedling height at (*a*) Week 4 and (*b*) Week 10 for each species (*Leptospermum lanigerum* (○), *Melaleuca squarrosa* (×)) under the four experimental treatments. (*c*) Stem width at Week 10 for the same species and treatments. For each treatment, the positions of points in *a–c* are jittered on the *y*-axis to show 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) for seedling height at (*d*) Week 4, (*e*) Week 10, and (*f*) stem width at Week 10.

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. 4*b, 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 with 2.2 ± 0.4 mm in other treatments; Fig. 4*c, 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. 5*a, c*). There were no significant differences in the ratio of aboveground to belowground biomass, although there was a tendency for seedlings in the inundated and waterlogged treatments to have less belowground biomass (Fig. 5*b, 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 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 watertable differed widely among sites and between species (Fig. 6), limiting our ability to infer differences in crown extent in response to watertable depth. The wide CIs in Fig. 6*c* show that watertable depth was not strongly associated with crown extent.

Differences in crown extent among sites differed between the two species (negative coefficients for both near permanently flooded (impact) sites versus the intermittently flooded (reference) site, and the positive site × species interaction for Impact site 2 in Fig. 6*c*). 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 among sites, being 60 ± 22%, 65 ± 25% and 72 ± 20% at the reference site and Impact sites 1 and 2 respectively (Fig. 6*a, b*).

Discussion

Our study has highlighted 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, indicated 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

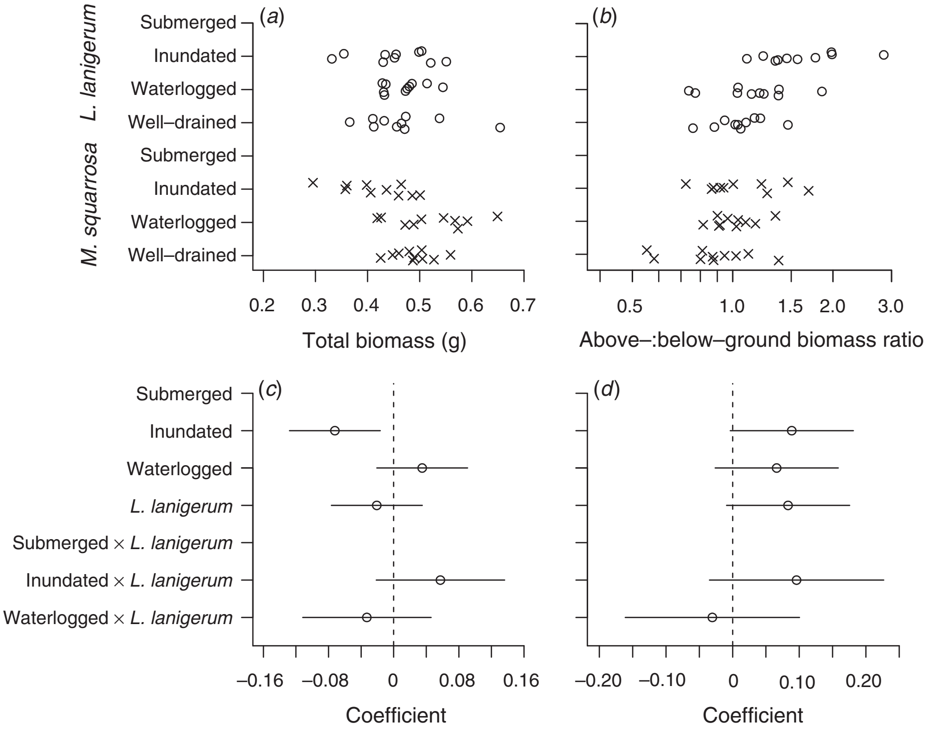

Fig. 5. (*a*) Total biomass and (*b*) ratio of aboveground to belowground biomass for each species (*Leptospermum lanigerum* (○), *Melaleuca squarrosa* (×)) under three experimental treatments at Week 10 (no seedlings survived 10 weeks in the submerged treatment). Mean (±95% confidence intervals) coefficients for each comparison of the two effects (species, with *M. squarrosa* as intercept, and treatment, with well drained as intercept) for (*c*) total biomass and (*d*) biomass ratio.

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; van der Valk *et al*. 1992; Pierce and King 2007) 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 the present 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 (Myers 1983; Denton and Ganf 1994). Exposed moist substrate is likely to be most appropriate for the seedling establishment of these woody wetland plant species (Robinson *et al*. 2006; Robinson *et al*. 2008; Greet 2015).

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 ~8 weeks of 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 2015).

Although complete submergence longer than 8 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 than 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 (Sena Gomes and Kozlowski 1980; Myers 1983). However, inundation did tend to result in reduced relative belowground biomass and overall biomass compared with the unflooded treatments.

Also consistent with other studies, our results indicated that increases in flooding depth are correlated with increases in the incidence of adventitious roots and aerenchyma formation (as indicated by greater stem width) in seedlings of flood-tolerant woody plants (Greet 2015). Overall, the tendency of *L. lanigerum* seedlings to survive submergence longer, and produce greater numbers of stem-borne adventitious roots

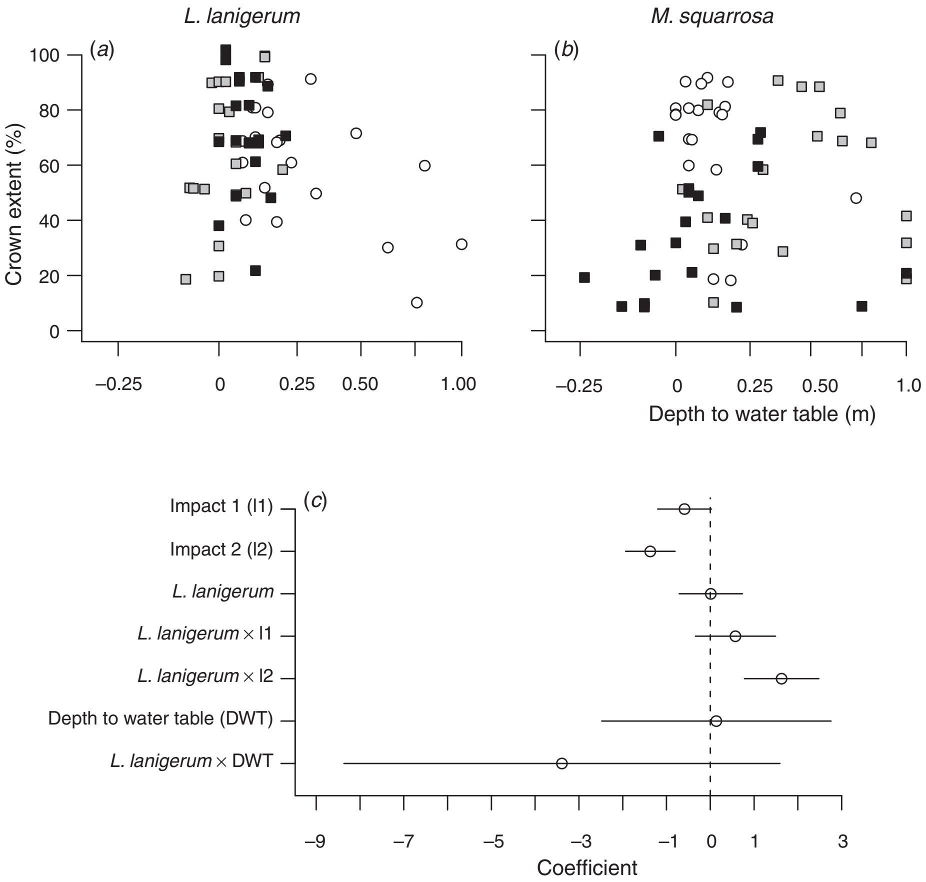

Fig. 6. Percentage crown extent of (*a*) *Leptospermum lanigerum* and (*b*) *Melaleuca squarrosa* plotted against depth-to-watertable at each of the three sites (reference (○), Impacts 1 (▨) and 2 (■)). Points are jittered on the *y*-axis to show overlapping data. (*c*) Mean (±95% confidence intervals) of coefficients for the crown-extent linear models with species (*M. squarrosa* as intercept) and site (reference as intercept) as predictors.

while flooded, suggests that they are more flood-tolerant than are *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 a reduced crown condition compared with trees that were intermittently flooded. Our surveys suggested a decline in the crown extent of *M. squarrosa* at near permanently flooded sites, whereas *L. lanigerum* did not show a similar decline in crown extent, thus being 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, whereas *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 watertable depth observed in our field survey, with the 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 2015), and are currently being implemented by Melbourne Water. Specifically, floodplain re-engagement (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 $\geq$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). Because *M. squarrosa* and *L. lanigerum* do not form persistent soil seed banks (Greet 2016), 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, so as to ameliorate adverse human impacts and promote restoration, and it is essential that their management decisions are robust and science-based. The present study demonstrated 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.

Conflicts of interest

The authors declare no conflicts of interest.

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