



Riparian and terrestrial grasses display unexpected tolerance to cool-season inundation

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Abstract Vegetation condition has declined along many regulated river systems globally due to alteration of flow regimes via flow regulation. Understanding how plants respond to inundation is critical for managing regulated river flows to improve riparian vegetation condition. We experimentally tested the effects of inundation duration on the survival and growth of six tufted grass species commonly found in riparian zones in south-eastern Australia. We conducted three nursery-based experiments in late winter/early spring, corresponding with natural temperate flow peaks and managed flows, with inundation treatments on: (1) established plants, up to 35 days inundation; (2) seedlings, up to 25 days inundation; and (3) established plants, up to 53 days inundation including additional shaded treatments. Plant survival, height and biomass growth, and onset of

flowering were recorded for established plants, and, for seedlings, survival only. Plant height and biomass growth declined with longer inundation duration across all species, although surprisingly few established plants died. Unexpectedly, grass seedlings were generally tolerant of inundation also, although there was some evidence of increased mortality for the longest treatment (25 days inundation). Shading did not result in increased mortality or reduced height growth of inundated plants. Inundation effects on the onset of flowering were modest and varied between species ranging from earlier to delayed onset. Our results suggest that tufted grasses are tolerant of cool-season inundation. Given that mortality of these species has been observed in the field and experimentally under shorter inundation periods in warmer conditions (late spring and summer), we suggest that seasonal timing of inundation is critical in determining plant responses to inundation.

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Introduction

Temperate river systems typically experience high flows in winter and spring driven by higher rainfall. These high flows are critical for the maintenance and recruitment of riparian vegetation communities

(Weiher and Keddy 1995; Stella et al. 2006; Colmer and Voesenek 2009; Greet et al. 2013a). However, the regulation of rivers through the building of dams and consumptive extraction (e.g. for irrigation) often reduces peak flows and dramatically alters natural flow regimes (Grill et al. 2019). Understanding the impact of altered flow regimes on the plant species composition of riparian zones is critical to the management of regulated waterways.

Inundation presents challenges to plant growth and survival by inhibiting respiration (Bailey-Serres and Voesenek 2008), reducing carbohydrate production (Voesenek et al. 2006), and facilitating the formation of toxic soil compounds (Pezeshki 2001). Reproductive output can also be sensitive to inundation (Warwick and Brock 2003; Greet et al. 2013b). Plants that have adaptations to tolerate or respond to inundation (e.g. development of aerenchyma, modified leaf growth, or use of alternative metabolic pathways), are favoured in inundation-prone habitats such as river banks and floodplains (Blom and Voesenek 1996; Voesenek et al. 2006; Catford and Jansson 2014).

Alteration of natural flow regimes that reduce high flows can disadvantage riparian plants adapted to historic inundation patterns, and facilitate invasion by terrestrial species otherwise unable to establish, grow, or survive in the riparian zone (Bunn and Arthington 2002; Stokes et al. 2010; Catford et al. 2014). Studies in south-eastern Australia have found that exotic plant cover is higher along regulated rivers with reduced flooding (Catford et al. 2011, 2014; Greet et al. 2013a). Environmental flows are increasingly being used to mitigate the impacts of regulation on waterways and river biota (Arthington et al. 2006; Tonkin et al. 2020). In temperate rivers, environmental flows are often released in late winter or spring to mimic naturally elevated flows, and there is evidence to suggest that such flows can be used to control terrestrial exotics and favour native riparian species (Catford et al. 2014; Duong et al. 2019).

Understanding plant growth and survival responses to inundation is critical to informing the use of environmental flows for riparian vegetation management. Many studies have investigated which hydrological attributes are important for determining plant responses to inundation, such as flood duration, depth, timing and frequency as well as water turbidity and water temperature (Poff and Zimmerman 2010; Greet et al. 2011; Main et al. 2022). For example,

a review by Webb et al. (2012) of wetland plant responses to different flow attributes suggested that increasing flood duration can decrease plant establishment and growth and influence reproductive output. Inundation depth has also been shown to affect plant growth and survival by reducing the amount of light that plants receive, thus interacting with inundation duration to reduce plant vigour (Gattringer et al. 2018). Many river systems in south-eastern Australia have high turbidity levels due to erosion, European carp invasion, and land clearing, which may further decrease light availability for plants when submerged (Franklin et al. 2008; Bornette and Puijalon 2011). Dense canopy shade from trees such as willows (*Salix* spp.) can also decrease light availability in the riparian zone and reduce plant abundance (Fletcher et al. 2000). The developmental stage of the plant can also determine its response to inundation, with seedlings typically more sensitive to inundation than mature plants (Denton and Ganf 1994).

Many catchments in south eastern Australia are heavily regulated for irrigation and flood mitigation and receive environmental flows. The effectiveness of these flows to promote native riparian species and control terrestrial species, is the focus of a range of monitoring programs (Victorian Environmental Flows Monitoring and Assessment Program: VEFMAP, Tonkin et al. 2020). Past field observations as part of VEFMAP have noted that flooding events can kill terrestrial exotic grass species (Jones and Thomas 2018). Nonetheless, effective management of river flows in this region and elsewhere requires a better understanding of thresholds for mortality and condition decline for common riparian and terrestrial species.

In this study, we conducted three separate nursery-based inundation experiments to assess the tolerance to cool-season inundation and shading of six tufted grass species of varying flood-tolerances (native and exotic origin) commonly found within riparian zones of south-eastern Australia. The experiments were conducted in late winter and early spring because this is a time when environmental flows are commonly released to mimic natural flows. Mortality and growth responses of seedlings and established plants to inundation were assessed, as well as the flowering onset of established plants. We hypothesised: (1) that plant survival, growth, and flowering would be negatively affected by inundation, with responses to inundation

varying between species according to their inundation tolerance; (2) that seedlings would be more sensitive to inundation than established plants; and (3) that inundation in combination with reduced light conditions (via shading) would further reduce growth and increase mortality.

Methods

Study species

Selection of tufted grass species was based on (hypothesised) inundation tolerances to determine a broad range of responses, and native and exotic species that are desirable or undesirable respectively to managers (Table 1).

Of the three exotic species, *Bromus catharticus* (prairie grass) is considered inundation-intolerant (Casanova and Brock 2000). Conversely, *Phalaris aquatica* (Toowoomba canary grass) is inundation-tolerant (Striker and Ploschuk 2018) and has been found to increase above-ground biomass production when waterlogged (Ploschuk et al. 2017). It is able to do so due to the development of aerenchyma (Colmer and Voesenek 2009). *L. multiflorum* (Italian ryegrass), an annual species, is less-studied; we considered it likely to be moderately inundation-intolerant like its congener *Lolium perenne* (perennial ryegrass) (McFarlane et al. 2003).

There is less literature understanding the effects of inundation on the three native species selected. *Lachnagrostis filiformis* (common blown grass) is considered inundation-tolerant since it grows close to the water and recruits after floods. *Poa labillardierei* (common tussock grass) is considered moderately

flood-tolerant and is often planted on riverbanks at a wide range of bank elevations in restoration projects (Vivian et al. 2020). *Rytidosperma caespitosum* (common wallaby grass) is considered flood-intolerant and typically occurs on floodplains (Vivian et al. 2020). All six grass species were expected to be comparably shade-tolerant and their responses to shading similar given their known distributions in open agricultural land or woodlands rather than forest regions.

Inundation experiments

We conducted three broadly concurrent inundation experiments and used multiple lines of evidence to detect responses of grasses to treatments. Each experiment subjected a different set of grasses to multiple treatments with data collected at regular intervals throughout and after treatment completion. These are summarised as follows:

Experiment 1: Grass inundation assessing mortality, growth, biomass and flowering.

Experiment 2: Seedling inundation assessing mortality.

Experiment 3: Grass inundation and shade assessing mortality, growth, biomass and flowering.

Experiment 1: Inundation of established grasses

We sourced seeds of each study species either commercially, or from sites on the Campaspe River, Victoria, Australia—a heavily regulated river subject to environmental flow management (Supplementary Information, Table S1; Fig. S1). The seeds were sown in seedling trays filled with seed raising mix. We placed the trays in a glasshouse (irrigated three times

Table 1 Details of the six study grass species

Species	Common name	Origin	Life history	Typical flowering period ^a	Hypothesised inundation tolerance
<i>Rytidosperma caespitosum</i>	Common wallaby grass	Native	Perennial	Sep–Dec	Intolerant
<i>Bromus catharticus</i>	Prairie grass	Exotic	Perennial	Aug–Jan	Intolerant
<i>Poa labillardierei</i>	Common tussock grass	Native	Perennial	Oct–Feb	Moderate
<i>Lolium multiflorum</i>	Italian ryegrass	Exotic	Annual	Sep–Dec	Moderate
<i>Lachnagrostis filiformis</i>	Common blown grass	Native	Annual	Sep–Jan	Tolerant
<i>Phalaris aquatica</i>	Toowoomba canary grass	Exotic	Perennial	Nov–Jan	Tolerant

^aData source: <https://vicflora.rbg.vic.gov.au>

a day for 5 min) on a heated bench (~ 22 °C) for 1 month to allow for germination.

After 1 month, we potted up 80 individuals per species into individual 1.9L pots. Pots contained a 3–5 cm layer of pine bark at the bottom (to prevent soil loss), and were then filled with a 7:1 sand:topsoil mix. We placed the potted individuals in a polytunnel for 3 months, during which they were watered for 4 min, three times daily via an automated sprinkler system. They were fertilised every 4 weeks using 0.5 g/L fertiliser (N:P:K = 20.0:8.7:16.6).

We conducted the inundation experiment in August–September 2018, commencing on the 8th of August (a time of typically high flows in temperate Australia and when environmental flows are commonly released). Eighteen 785L tanks located outdoors under a transparent pavilion were used for the experiment. Each tank was assigned one of nine inundation duration treatments: 0, 1, 2, 5, 7, 10, 15, 25, or 35 days, based on a priori assumptions of the potential range of inundation tolerances, with 35 days considered likely to kill most plants. Durations were designed to overlap with the approximate durations of delivered environmental flows in south-eastern Australia, i.e. peak = 2–14 days, peak plus ramp up and down = 5–28 days (VEWH 2020). Plants were placed on a platform about halfway up the tank. We filled each tank with tap water such that plants were fully submerged and the water was approximately 40 cm from the soil surface. We released water at the end of the given inundation period, with the level then kept at 3 cm above the drainage holes at bottom of the pots to ensure plants had access to water.

We selected 54 individuals of each species (324 plants in total) of similar size for Experiment 1. Three individuals from each species were randomly assigned to a tank (18 individuals per tank), with each treatment replicated twice ($n=6$ plants per species per treatment). On a weekly basis, we randomly shifted around individual plants within each tank to control for any shading variation within a tank.

We recorded plant survival and heights (defined as the length of the longest green leaf) weekly, for 5 weeks. We measured water quality (temperature, dissolved oxygen, and pH) 1 month following initial inundation for each tank; the results were similar to typical values for the region (Supplementary Information, Table S2). Immediately following the completion of an inundation treatment, we noted

the presence or absence of top kill (whether above-ground biomass died whilst inundated). Plants were kept in the tanks for a further 5-day recovery period and at the completion of the experiment (40 days following the commencement of the inundation treatments), we recorded whether or not the plant remained dead.

Following the 40-day experimental period, we randomly selected three individuals per species from the 0, 7, 25 and 35-day treatments. We separated these plants into above- and below-ground components and dried and weighed them. We monitored the remaining plants on five separate occasions until the following February to check whether the onset of flowering was delayed or flowering reduced; the experimental period comprised the typical flowering period of all species (Table 1).

Experiment 2: Inundation of grass seedlings

Soil seedbank samples were collected over 2 days (6–7th May 2018) from six sites along the Campaspe River (Supplementary Information, Fig. S1; Table S3). We collected six replicate soil seedbank samples from each site with sampling locations chosen where target species occurred (Supplementary Information, Table S3). Sampling locations within a site were 5–100 m apart, with each study site approximately 200–600 m long. At each sampling location we took five subsamples of 5 cm depth and diameter using a bulb planter and pooled them.

Seedbank samples were dried at 30 °C for 2 weeks. After samples were dried, we combined the samples from each site and then separated them into six sub-samples of equal weight. We then spread each sub-sample evenly in seedling trays (approx. 30 cm \times 20 cm \times 5 cm) half-filled with the same soil mix used in Experiment 1.

We left the trays in a large polytunnel for 10 weeks to allow seedlings to germinate and grow (subject to an automated watering regime of 4 min, three times daily). Any grass seedlings that germinated and reached a height > 5 cm were identified to at least genus level. We recorded the abundance of each species, along with other grasses that were found to be common, for each subsample. We placed colour-coded toothpicks beside each individual of a species of interest. We then placed each tray in a plastic tub (approx. 40 cm \times 40 cm \times 25 cm), and subjected them

to one of six inundation durations: 0, 1, 2, 5, 10 or 25 days based on assumptions of inundation tolerance (lower than mature plants). We filled the tubs with tap water and kept them full for the duration of the treatment. At the end of the treatment period, the tubs were drained and remained in the polytunnel. Following a 1-week recovery period, we recorded the number of individuals of each target species that survived.

Experiment 3: Inundation of established grasses in shade

We then quantified the effect of the interaction between shading (to reflect reduced light conditions associated with dense canopy shade, turbidity, or deep floodwaters) and inundation on plant mortality and growth. We applied two treatments (shaded-wet and shaded-dry) to eight round plastic tubs (four replicate tubs for each treatment), in addition to a light-dry control (unshaded). ‘Wet’ (inundated) plants were placed in tubs that were filled with water to approximately the same depth above the soil as in Experiment 1. ‘Dry’ plants were not inundated, but could access water through shallow water maintained at the base of the tub, as per the control tanks in Experiment 1. We shaded the tubs with commercial shade cloth that reduced light availability by approximately 80%. We measured this light reduction and verified it at different light intensities (full sun and cloud cover) using an instantaneous light meter. Treatments were applied for 53 days, with plant heights measured at 0, 11, 21, 46 and 53 days. The longer inundation periods were opportunistically determined based on the observations of high survival rates in Experiment 1 that commenced 3 weeks prior. The tubs were positioned outdoors, directly adjacent to the tanks used for Experiment 1.

Experiment 3 was started 3 weeks after Experiments 1 and 2. We split a total of 144 plants into nine groups of 16, with eight treatment groups and one group allocated as a control. Within each group of 16 plants, there were only two *L. filiformis* and two *L. multiflorum* individuals; otherwise there were three individuals per study species in each group. Following the experiment, we dried a subset of six individuals per species from each treatment and recorded their root and shoot biomass (four individuals for *L. filiformis* and *L. multiflorum*). As per Experiment 1, we monitored flowering onset for the remaining plants.

Statistical analysis

Statistical analyses were conducted for Experiments 1 and 3 to evaluate the relative effects of treatments on relative growth and biomass of established plants. All analyses were conducted in R (Version 3.6.0, R Core Team 2013). No analyses were conducted for assessment of treatment effects on flowering in Experiments 1 and 3 because most species either did not flower or showed no apparent differences in onset responses. Likewise, no analyses were used for Experiment 2, where there was almost no seedling mortality, which precluded the calculation of treatment effects or use of survival analyses. In both cases, qualitative assessments of treatment effects on flowering and seedling survival are presented.

For Experiment 1, we built linear mixed effect models for the relative height growth of each species, where inundation duration treatment, week since commencement, and their interaction were modelled as potential predictors (fixed effects) for relative height growth, and tank and plant included as nested random effects. Both fixed effect variables were input as factors as opposed to numerical variables due to the expected non-linear responses and likely occurrence of thresholds. This also enabled direct evaluation of treatments at specific time periods. We defined relative height growth as the difference in plant height from the beginning of the experiment for each plant. We ran mixed effects models using the *lme* function in the *nlme* package (Pinheiro et al. 2018). Model performance is indicated by conditional and marginal coefficients determined using the *r.squaredGLMM* function in the *MuMIn* package (Barton 2020). Additionally, likelihood ratio tests were performed on weaker models to verify model improvement over the null model. Models had a significantly better fit than the null model in all cases and so the test outputs are provided in repository outputs only. We performed Tukey’s tests to compare mean relative height growth between different treatments by week, for each species, using the *lsmeans* function in the *emmeans* package (Lenth 2020). Post-hoc tests were conducted using the *lsmeans* function to determine significance of effects, with a significance threshold of $p < 0.05$.

We analysed the data from Experiment 3 similarly using linear mixed effects models for relative height growth, but with binary fixed effects for inundation and shading. Linear mixed models for biomass

analysis using plants from Experiments 1 and 3 included inundation and shade treatments (inundation duration days: 0, 7, 25, 35, shaded-dry 53, and shaded-wet 53) as a categorical fixed effect. We included nested random effects (plant ID in tanks) and an interaction between treatment and week since commencement.

Results

Experiment 1: Inundation of established grasses

In general, plant growth (height) declined with increasing duration of inundation for all six species as leaves progressively senesced from the tips (Fig. 1). All models used to evaluate treatment effects on plants over time performed well with moderate to good model fit for all species (DF = 225, Table 2). For most species, the growth was not significantly lower ($p > 0.05$) between treatments until weeks four or five, but all species showed a significant negative effect of longer inundation treatments compared to shorter treatments (see Supplementary Information, Tables S4–S9). *R. caespitosum* was affected by inundation earlier than all other species with lower growth in the 25-day treatment by week three compared to the 0, 1, 2 and 5-day treatments (and 35-days compared to 1-day). By week four, plant height growth for *R. caespitosum* was lower in both the 25 and 35-day treatments than the 0–7-day treatments. *P. labillardierei* height growth in the 25 and 35-day treatments was lower in week four than plants in the 0, 1, 5, and 7-day (35-days only) treatments. *L. filiformis* growth was only lower for plants in the 35-day treatment than the 2-day treatment in weeks four and five (also 25 c.f. 2-days after 5 weeks). *P. aquatica* responses were weak, with growth in the 35-day treatment only lower than the 2-day treatment after 5 weeks. *L. multiflorum* responses were similar to *P. aquatica* with lower growth in the 35-day treatment than the 1 and 7-day treatments after 5 weeks. For *B. catharticus*, growth in the 25-day treatment was lower than the 0–10-day treatments after week four and likewise for the 25 and 35-day treatments in most cases after 5 weeks.

Only three plants out of 324 in Experiment 1 died from inundation. These were all *B. catharticus* individuals, in response to the longest inundation period (35 days). *B. catharticus* heights were also the most

negatively affected by inundation (Fig. 1), but this species also had low growth in the 0, 1 and 2-day inundation treatments.

Experiment 2: Inundation of grass seedlings

Ten grass species (Table S10) were identified in our soil seedbank samples, including five of the six target species. *L. filiformis* was the most abundant, followed by *Lolium* spp. Only eight grass seedlings from a total of 272 identified died during the experiment; three seedlings died following very brief treatments, while five died in the longest, 25-day treatment (Table 3). The small increase in mortality at the longest treatment (five of eight dead seedlings) suggests that some species may have been approaching a survival threshold at 25 days of inundation (see Supplementary Information, Table S10).

Experiment 3: Inundation of established grasses in shade

Grasses in the third experiment showed reduced height growth when inundated and shaded (Fig. 2), with similar results to the unshaded treatments in Experiment 1. Also similar to Experiment 1, *B. catharticus* was most sensitive to inundation, with 10 out of 12 inundated plants dying after 53 days inundation. Only one other individual plant died during the experiment, a *P. aquatica* individual, in the shaded-wet treatment.

The wet versus dry comparisons for shaded treatments showed large differences due to both the negative impacts of inundation and positive impacts of shading (when dry) on height growth. All models used to evaluate treatment effects on grasses over time performed well with good model fit for all six species ($R^2 > 83$, $df = 56$, Table 4). Shaded-dry treatment plants grew taller and faster than other treatments as leaves elongated in search of light; they had greater height growth ($p < 0.05$) than shaded-wet treatment plants after 3 weeks (*P. labillardierei* after 6.5 weeks) and greater height growth than light-dry treatments after 6.5 weeks for all species (see Supplementary Information, Tables S11–S16). Shaded-wet treatment grasses had significantly less height growth than light-dry treatment grasses after 6.5 and 7.5 weeks for *R. caespitosum* and after 7.5 weeks for *B. catharticus*, with

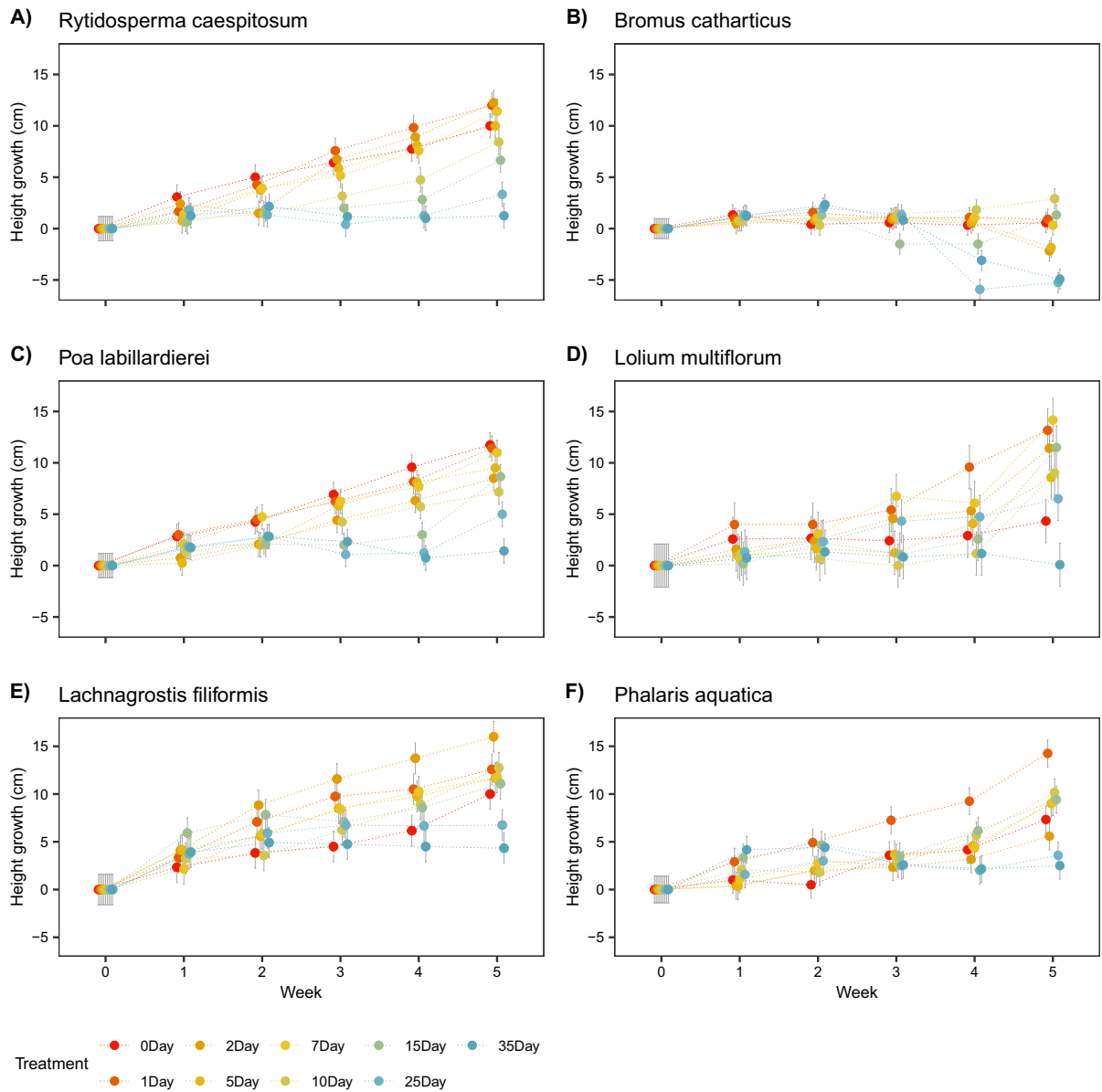


Fig. 1 Modelled predictions of relative height growth of plants subjected to one of nine different inundation duration treatments (from Experiment 1: Inundation of established plants). Each panel represents a different species, with $n=54$

plants for each species across all treatments. Error bars indicate the modelled mean \pm standard error. Values are offset along the x-axis to improve visualisation

no effects for other species (Supplementary Information, Tables S11–S16). Control grasses (light-dry), were relatively slow growing for most species

(Fig. 2), but height growth rates were similar to control grasses (0-day) in Experiment 1.

Table 2 Experiment 1 model goodness of fit indicated by the conditional and marginal coefficient of determination (R^2) for each species

Species model	R^2 marginal	R^2 conditional
<i>Rytidosperma caespitosum</i>	0.68	0.83
<i>Bromus catharticus</i>	0.40	0.55
<i>Poa labillardierei</i>	0.64	0.81
<i>Lolium multiflorum</i>	0.40	0.69
<i>Lachnagrostis filiformis</i>	0.56	0.80
<i>Phalaris aquatica</i>	0.50	0.74

Table 3 Abundance of seedlings (taller than 5 cm at beginning of experiment) of target species in seedbank samples (pooled by flood duration treatment), before and after inundation (from Experiment 2: Inundation of seedlings)

Treatment (day)	Number of grass seedlings present	Number of seedlings that died
0	50	2
1	49	1
2	48	0
5	37	0
10	40	0
25	48	5
Total	272	8

An expanded table by species is provided in Supplementary Information (Table S10)

Biomass assessment from Experiments 1 and 3

Overall, root and shoot biomass tended to decline with increased duration of inundation, with root and shoot biomass consistently lower for the longest inundation treatments (35-day and shaded-wet treatments) (Fig. 3). Root and shoot biomass were also often lower in the 35-day treatment than the shaded-wet, 53-day treatment. However, there were modest and mostly non-significant differences between biomass values under different treatments for each species, likely due to the low replication of samples (Supplementary Information, Table S17). *R. caespitosum*, *B. catharticus* and *L. multiflorum* had clear but non-significant declines in root biomass, with final biomass being much lower for all treatments (even zero days inundation), than the grasses prior

to treatment. Biomass models had moderate fits with low to high marginal R^2 (0.07–0.83) but very high conditional R^2 influenced by the low degrees of freedom ($R^2 > 0.94$, $df = 2$, Table 5).

Flowering assessment for Experiments 1 and 3

In Experiment 1, no clear effects of inundation on flowering were apparent, except for *B. catharticus*. Even for *B. catharticus*, which commenced flowering earlier than other species, only the longest inundation treatment of 35 days delayed or prevented flowering (only 1 of 6 plants flowered c.f. almost all *B. catharticus* plants flowering in the other treatments). No other species flowered during the inundation treatment period (35 days). Following the experimental period (post 40 days), the onset of flowering of *L. filiformis*, *L. multiflorum*, and *R. caespitosum* was relatively unimpacted by prior inundation duration. Both *P. labillardierei* and *P. aquatica* were slow to flower, with only one plant of each flowering during the study period.

Of the grasses in Experiment 3, none flowered while inundated, while *B. catharticus* plants commenced flowering, if not inundated. *L. multiflorum* and *R. caespitosum* plants began flowering soon after the experimental period ended, but they took longer (~3 weeks) when previously inundated. In contrast, *P. aquatica* plants flowered at a greater rate when inundated (4 of 6 plants flowered in the inundated treatment c.f. only 1 plant in the not inundated treatment). Timing or rate of flowering was relatively unimpacted by inundation for *L. filiformis*, and no *P. labillardierei* plants flowered during the experiment. A summary of the flowering data is provided in Supplementary Information, Tables S18 and S19.

Discussion

As expected, we recorded reduced plant height and biomass growth with increasing inundation duration across all species, as well as inundation-induced delays in flowering onset for some species (results summarised in Table 6). However, the low levels of plant mortality in the experiments were unexpected. We hypothesised that complete submergence for the applied durations would result in higher plant mortality, particularly for the longer inundation treatments

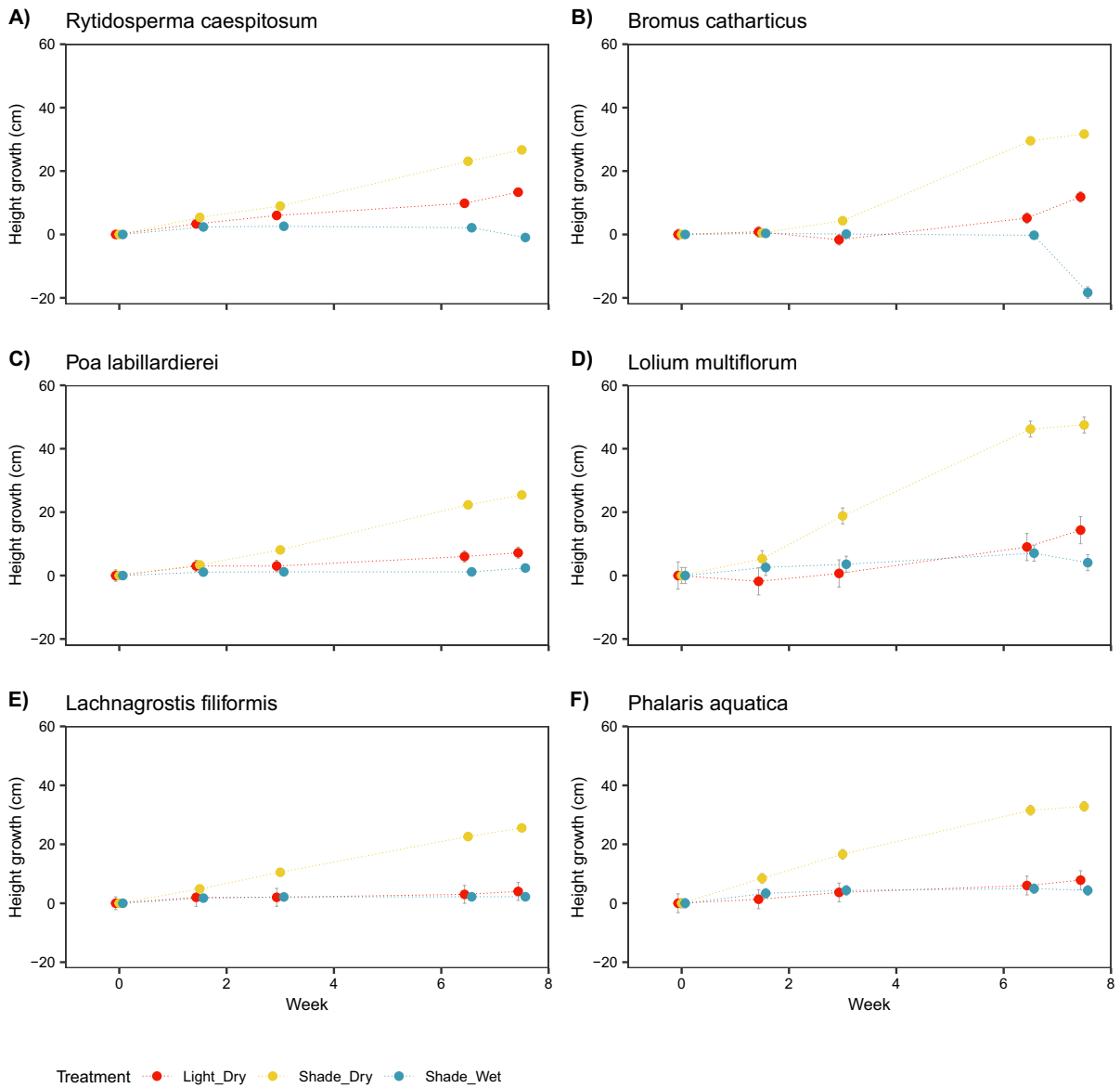


Fig. 2 Modelled predictions of relative height growth of plants subjected to one of three different inundation and shade treatments (from Experiment 3). Each panel represents a different species, with $n=26-27$ plants for each species across all

treatments. Error bars indicate the modelled mean \pm standard error. Values are offset along the x-axis to improve visualisation

for inundation-intolerant species. Even the grass seedlings exhibited low mortality rates. Also contrary to our expectations, shading did not typically augment the effects of inundation. Our results suggest that for our study species, cool-season inundation may reduce plant growth, but it does not typically cause significant mortality in mature or immature plants at inundation durations of up to 35 days.

Our findings are in contrast with our expectations based on prior field observations and expectations of managed flow influences. Field observations have noted that in some cases spring flows of 1–2 weeks in duration have caused mortality in some of the study species (namely, *B. catharticus* and *Lolium* spp.; Jones and Thomas 2018). Furthermore, in a similar experiment to ours except conducted in summer, no

Table 4 Experiment 3 model goodness of fit indicated by the conditional and marginal coefficient of determination (R^2) for each species

Species model	R^2 marginal	R^2 conditional
<i>Rytidosperma caespitosum</i>	0.96	0.97
<i>Bromus catharticus</i>	0.95	0.97
<i>Poa labillardierei</i>	0.91	0.94
<i>Lolium multiflorum</i>	0.85	0.93
<i>Lachnagrostis filiformis</i>	0.89	0.91
<i>Phalaris aquatica</i>	0.83	0.91

B. catharticus plants survived inundation of 2 weeks or more, while some *R. caespitosum* and *L. perenne* plants died after 2 weeks and most plants died after four or more weeks of inundation (Vivian et al. 2020). Our results, together with this previous research suggests that additional factors in association with inundation are important for determining plant inundation responses.

The shading experiment tested whether reduced light from inundation by turbid water or in locations with dense tree canopy was one of these factors. High shading or turbid floods will often inhibit

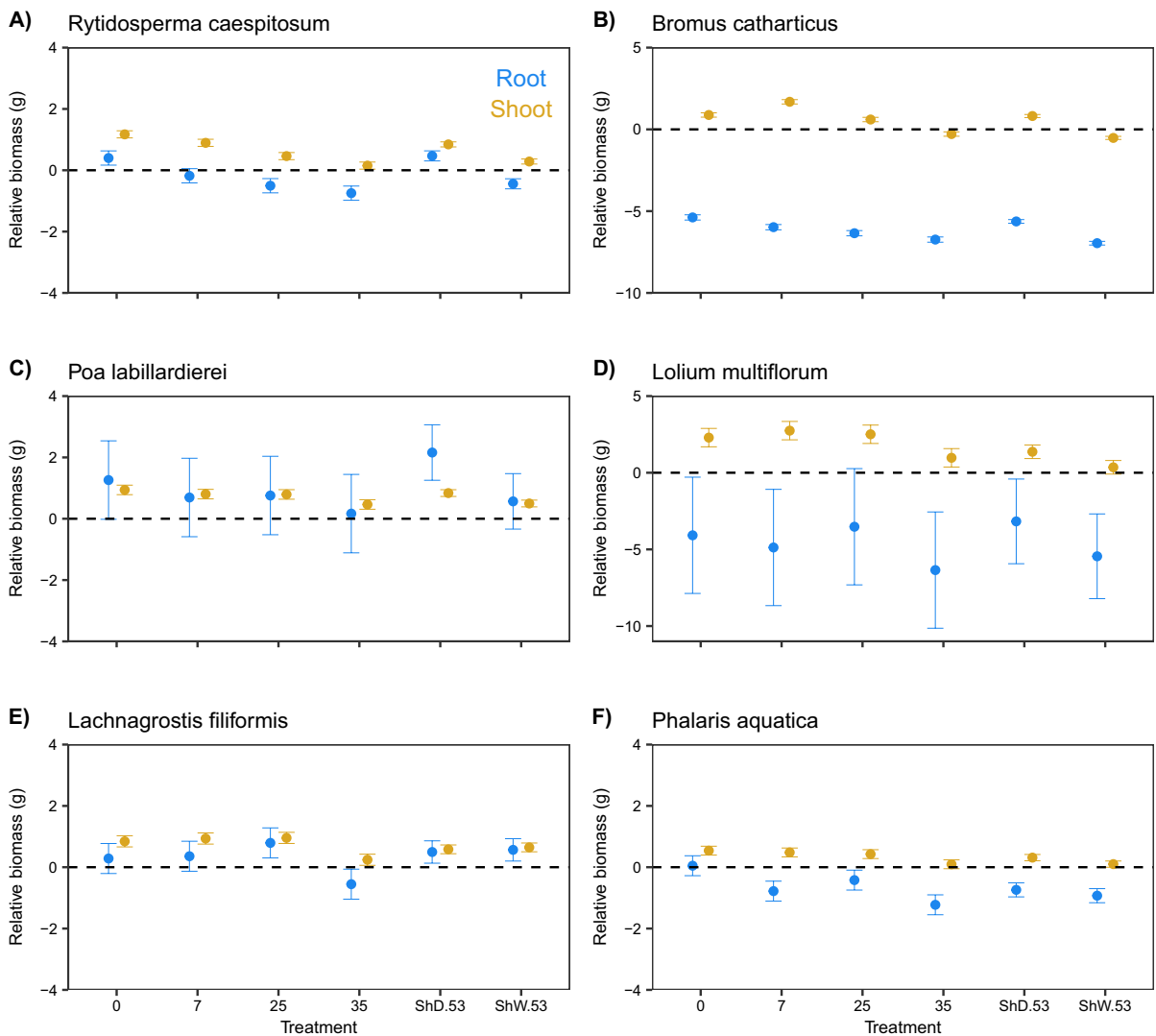


Fig. 3 Modelled predictions of relative root (blue) and shoot (gold) biomass for a subset of plants subjected to various treatments: 0, 7, 25, and 35 days inundated with no shading (Exper-

iment 1), and 53 days shaded, both inundated and dry (Experiment 3). Error bars indicate the modelled mean \pm the standard error

Table 5 Biomass model goodness of fit indicated by the conditional and marginal coefficient of determination (R^2) for each species

Species model	Root biomass		Shoot biomass	
	R^2 marginal	R^2 conditional	R^2 marginal	R^2 conditional
<i>Rytidosperma caespitosum</i>	0.58	0.99	0.75	0.99
<i>Bromus catharticus</i>	0.30	0.99	0.32	0.99
<i>Poa labillardierei</i>	0.18	0.94	0.31	0.99
<i>Lolium multiflorum</i>	0.07	0.95	0.63	0.97
<i>Lachnagrostis filiformis</i>	0.33	0.95	0.47	0.95
<i>Phalaris aquatica</i>	0.83	0.99	0.92	0.99

Table 6 Summary of responses of each species to experimental treatments in this study

Species	Inundation treatment		Inundation + shade cf. Inundation	Biomass ↓	Flowering
	Mortality	Growth ↓			
<i>Rytidosperma caespitosum</i>	None	21 days*	None	None	Delayed
<i>Bromus catharticus</i>	35 days	28 days*	None	None	Delayed
<i>Poa labillardierei</i>	None	28 days*	None	None	NA
<i>Lolium multiflorum</i>	None	35 days*	None	None	Delayed
<i>Lachnagrostis filiformis</i>	None	28 days*	None	None	NA
<i>Phalaris aquatica</i>	None	35 days*	None	35 days*	Accelerated

The number of days indicates the earliest inundation duration that resulted in a significant* or notable result

photosynthesis; the lack of light can interact with inundation to severely reduce cellular oxygen (Bailey-Serres and Voeselek 2008). For example, Visser et al. (2016) found that for *Solanum dulcamara* (bittersweet nightshade), shade and inundation interacted to reduce biomass. However, reducing light availability does not have consistent effects across species. Vervuren et al. (2003) found that, by experimentally increasing sediment load and water depth, only some of their study species were sensitive to lack of light, whilst for others its reduction had little effect. Our study species were all relatively shade tolerant—shading had little to no effect in combination with inundation to further reduce growth or survival. However, by using a shade cloth to replicate turbid waters, we acknowledge our experiment did not account for how sediments in floodwaters deposit on leaves, directly reducing their light capture ability. This effect could be further tested using a sediment addition experiment.

Given the negative impacts of inundation on plant growth, we assumed that this reduction in plant vigor would delay or prevent flowering, with greater impacts for less inundation-tolerant species (Warwick and Brock 2003; Greet et al. 2013b). Our study suggests this may be the case for our three terrestrial species (*B. catharticus*, *L. multiflorum*, and *R.*

caespitosum). The contrasting response of *P. aquatica*, with flowering being promoted by inundation, suggests a continuum of reproduction responses that may contribute to species composition changes with alterations to flow regimes. Nonetheless, the effects of cool-season inundation on the flowering of the study species we observed were often negligible or modest.

A likely explanation for the lack of mortality (and modesty of other responses observed) is the seasonal timing of inundation in our experiment. Warmer water temperature accelerates plant respiration, the use of carbohydrates, and hence biomass loss during flooding (van Eck et al. 2005). In inundation experiments similar to those carried out in this study, van Eck et al. (2006) found that several herbaceous plant species survived inundation longer in winter than in summer. Additionally, Vivian et al. (2020) found that the two flood intolerant grass species we studied (*Bromus catharticus* and *Rytidosperma caespitosum*) suffered significant mortality following only 2 weeks of inundation in summer, while Main et al. (2022) found plants of these species died faster and at higher proportions when inundated in artificially warmed water than in ambient water temperatures in late spring. Thus, winter inundation is likely to be less important in determining the distribution of riparian species than summer inundation (van Eck et al. 2004).

Our inundation experiments coupled with the findings of others suggest that there is an increasing impact of inundation from winter to summer on plant growth and survival (Vivian et al. 2020; Main et al. 2022), as well as reproduction (Warwick and Brock 2003; Greet et al. 2013b). Combined, these results have important implications for environmental flow management, as cool-season flows (such as winter or early spring high-flows) may be ineffective for controlling exotic terrestrial species, unless they occur for longer durations than those tested here (5 weeks) and even the efficacy of those longer events remains untested. While a short-duration summer flow release may be more effective at killing undesirable terrestrial plants within the river channel, these flows may have negative impacts on native riparian vegetation and other native biota that are not adapted to high summer flows (Greet et al. 2013b; Vivian et al. 2020). These seasonal and temperature influences suggest that locally hotter and drier climates with more frequent extreme heat and rainfall events resulting from climate change (CSIRO and Bureau of Meteorology 2021) will increase negative impacts of flows on vegetation, although they may also decrease flows. There was no clear evidence that exotic species were more sensitive to inundation than native species from the small pool of species we tested.

There are of course several other factors contributing to plant mortality associated with inundation in waterways that we did not test. For instance, plants can be sensitive to other flow regime components such as depth of inundation (Magee and Kentula 2005), which can interact with inundation duration to reduce plant growth or survival (Fraser and Karnezis 2005; Gattringer et al. 2018). Conditions leading up to an inundation event may also play a role. For example, the frequency at which past inundations occurred can influence wetland community composition and how plant species allocate their biomass, with implications for plant responses to inundation (Smith and Brock 2007). Furthermore, in addition to shading, turbid floodwaters may cause stress to plants via scour or smothering (Catford and Jansson 2014), which our experiments did not test. We also did not test the influence of water quality (e.g. eutrophication) or biotic (e.g. grazing, carp bioturbation) effects on plant inundation tolerances that may be important (Meeson et al. 2002; Driver et al. 2005; O'Hare et al. 2018).

Conclusions

For our study grass species, cool-season inundation had negative effects on height and biomass growth with increasing inundation duration. Shading the grasses, in combination with inundation, had no additional effect on plant growth. Importantly, plant mortality was almost absent in all experiments, even for seedlings, suggesting that short inundation durations in cool-season conditions may have modest impacts on riparian community composition. We suggest that other factors interacting with inundation, and seasonal timing in particular, are likely to be important for determining plant responses to inundation. How these factors interact with the functional and ecological traits of individual species would be valuable to investigate in future research.

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Author contributions VK led the experimental study and writing the manuscript. CSJ and JG contributed to formulating the ideas, conducting the study and analysis, and writing the manuscript. SAM contributed to the study evaluation and writing the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated and analysed during the current study are available in the OSF link <https://osf.io/j62fd/>.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

Ethical approval Ethical approval was not applicable to this study.

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References

- Arthington AH, Bunn SE, Poff NL, Naiman RJ (2006) The challenge of providing environmental flow rules to sustain river ecosystems. *Ecol Appl* 16:1311–1318
- Bailey-Serres J, Voeselek LACJ (2008) Flooding stress: acclimations and genetic diversity. *Annu Rev Plant Biol* 59:313–339
- Barton K (2020) MuMIn: multi-model inference. R package version 1.43.17. <https://CRAN.R-project.org/package=MuMIn>
- Blom CWPM, Voeselek LACJ (1996) Flooding: the survival strategies of plants. *Trends Ecol Evol* 11:290–295
- Bornette G, Puijalón S (2011) Response of aquatic plants to abiotic factors: a review. *Aquat Sci* 73:1–14
- Bunn SE, Arthington AH (2002) Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environ Manag* 30:492–507
- Casanova MT, Brock MA (2000) How do depth, duration and frequency of flooding influence the establishment of wetland plant communities? *Plant Ecol* 147:237–250
- Catford JA, Jansson R (2014) Drowned, buried and carried away: effects of plant traits on the distribution of native and alien species in riparian ecosystems. *New Phytol* 204:19–36
- Catford JA, Downes BJ, Gippel CJ, Vesk PA (2011) Flow regulation reduces native plant cover and facilitates exotic invasion in riparian wetlands. *J Appl Ecol* 48:432–442
- Catford JA, Morris WK, Vesk PA, Gippel CJ, Downes BJ (2014) Species and environmental characteristics point to flow regulation and drought as drivers of riparian plant invasion. *Divers Distrib* 20:1084–1096
- Colmer TD, Voeselek LACJ (2009) Flooding tolerance: suites of plant traits in variable environments. *Funct Plant Biol* 36:665–681
- CSIRO and Bureau of Meteorology (2021) Climate Change in Australia website. <http://www.climatechangeinaustralia.gov.au/>
- Denton M, Ganf GG (1994) Response of juvenile *Melaleuca halimatum* to flooding: management implications for a seasonal wetland, Bool Lagoon, South Australia. *Aust J Mar Freshw Res* 45:1395–1408
- Driver PD, Closs GP, Koen T (2005) The effects of size and density of carp (*Cyprinus carpio* L.) on water quality in an experimental pond. *Arch Hydrobiol* 163:117–131
- Duong A, Greet J, Walsh CJ, Sammonds MJ (2019) Managed flooding can augment the benefits of natural flooding for native wetland vegetation. *Restor Ecol* 27(1):38–45
- Fletcher DE, Wilkins SD, McArthur JV, Meffe GK (2000) Influence of riparian alteration on canopy coverage and macrophyte abundance in Southeastern USA blackwater streams. *Ecol Eng* 15:S67–S78
- Franklin P, Dunbar M, Whitehead P (2008) Flow controls on lowland river macrophytes: a review. *Sci Total Environ* 400:369–378
- Fraser LH, Karnezis JP (2005) A comparative assessment of seedling survival and biomass accumulation for fourteen wetland plant species grown under minor water-depth differences. *Wetlands* 25:520–530
- Gattringer JP, Ludewig K, Harvolk-Schoning S, Donath TW, Otte A (2018) Interaction between depth and duration matters: flooding tolerance of 12 floodplain meadow species. *Plant Ecol* 219:973–984
- Greet J, Webb JA, Cousens RD (2011) The importance of seasonal flow timing for riparian vegetation dynamics: a systematic review using causal criteria analysis. *Freshw Biol* 56:1231–1247
- Greet J, Cousens RD, Webb JA (2013a) More exotic and fewer native plant species: riverine vegetation patterns associated with altered seasonal flow patterns. *River Res Appl* 29:686–706
- Greet J, Cousens RD, Webb JA (2013b) Seasonal timing of inundation affects riparian plant growth and flowering: implications for riparian vegetation composition. *Plant Ecol* 214:87–101
- Grill G, Lehner B, Thieme M, Geenen B, Tickner D, Antonelli F, Babu S, Borrelli P, Cheng L, Crochetiere H, Ehalt Macedo H (2019) Mapping the world's free-flowing rivers. *Nature* 569(7755):215–221
- Jones CS, Thomas FT (2018) Regular intervention monitoring providing valuable insights into vegetation responses to environmental flows on rivers. In: Vietz GJ, Rutherford ID (eds) 9th Australian Stream Management Conference, Hobart, Tasmania
- Lenth RV (2020) emmeans: estimated marginal means, aka Least-Squares Means
- Magee TK, Kentula ME (2005) Response of wetland plant species to hydrologic conditions. *Wetl Ecol Manag* 13:163–181
- Main AC, Greet J, Vivian LM, Jones CS (2022) Warmer water temperatures exacerbate the negative impacts of inundation on herbaceous riparian plants. *Freshw Biol* 67:1162–1173
- McFarlane NM, Ciavarella TA, Smith KF (2003) The effects of waterlogging on growth, photosynthesis and biomass allocation in perennial ryegrass (*Lolium perenne* L.) genotypes with contrasting root development. *J Agric Sci* 141:241–248
- Meeson N, Robertson AI, Jansen A (2002) The effects of flooding and livestock on post-dispersal seed predation river red gum habitats. *J Appl Ecol* 39:247–258
- O'Hare MT, Baattrup-Pedersen A, Baumgarte I, Freeman A, Gunn IDM, Sinclair R, Wade AJ, Bowes MJ (2018)

- Responses of aquatic plants to eutrophication in rivers: a revised conceptual model. *Front Plant Sci* 9:451
- Pezeshki SR (2001) Wetland plant responses to soil flooding. *Environ Exp Bot* 46:299–312
- Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2018) `nlme`: linear and nonlinear mixed effects models
- Ploschuk RA, Grimoldi AA, Ploschuk EL, Striker GG (2017) Growth during recovery evidences the waterlogging tolerance of forage grasses. *Crop Pasture Sci* 68:574–582
- Poff NL, Zimmerman JKH (2010) Ecological responses to altered flow regimes: a literature review to inform the science and management of environmental flows. *Freshw Biol* 55:194–205
- R Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Smith RGB, Brock MA (2007) The ups and downs of life on the edge: the influence of water level fluctuations on biomass allocation in two contrasting aquatic plants. *Plant Ecol* 188:103–116
- Stella JC, Battles JJ, Orr BK, McBride JR (2006) Synchrony of seed dispersal, hydrology and local climate in a semi-arid river reach in California. *Ecosystems* 9:1200–1214
- Stokes K, Ward K, Colloff M (2010) Alterations in flood frequency increase exotic and native species richness of understorey vegetation in a temperate floodplain eucalypt forest. *Plant Ecol* 211:219–233
- Striker GG, Ploschuk RA (2018) Recovery from short-term complete submergence in temperate pasture grasses. *Crop Pasture Sci* 69:745–753
- Tonkin Z, Jones C, Clunie P, Vivian L, Amtstaetter F, Jones M Lyon J (2020) Victorian environmental flows monitoring and assessment program. Stage 6 Synthesis Report 2016–2020. Technical Report Series No. 316, Department of Environment, Land, Water and Planning, Heidelberg
- van Eck WHJM, van de Steeg HM, Blom CWPM, de Kroon H (2004) Is tolerance to summer flooding correlated with distribution patterns in river floodplains? A comparative study of 20 terrestrial grassland species. *Oikos* 107:393–405
- van Eck WHJM, Lenssen JPM, Rengelink RHJ, Blom CWPM, de Kroon H (2005) Water temperature instead of acclimation stage and oxygen concentration determines responses to winter floods. *Aquat Bot* 81:253–264
- van Eck WHJM, Lenssen JPM, van de Steeg HM, Blom CWPM, de Kroon H (2006) Seasonal dependent effects of flooding on plant species survival and zonation: a comparative study of 10 terrestrial grassland species. *Hydrobiologia* 565:59–69
- Vervuren PJA, Blom CWPM, de Kroon H (2003) Extreme flooding events on the Rhine and the survival and distribution of riparian plant species. *J Ecol* 91:135–146
- VEWH (2020) Seasonal Watering Plan 2019–20. Victorian Environmental Water Holder
- Visser EJW, Zhang Q, De Gruyter F, Martens S, Huber H (2016) Shade affects responses to drought and flooding—acclimation to multiple stresses in bittersweet (*Solanum dulcamara* L.). *Plant Biol* 18:112–119
- Vivian LM, Greet J, Jones CS (2020) Responses of grasses to experimental submergence in summer: implications for the management of unseasonal flows in regulated rivers. *Aquat Ecol* 54:985–999
- Voesenek LACJ, Colmer TD, Pierik R, Millenaar FF, Peeters AJM (2006) How plants cope with complete submergence. *New Phytol* 170:213–226
- Warwick NWM, Brock MA (2003) Plant reproduction in temporary wetlands: the effects of seasonal timing, depth, and duration of flooding. *Aquat Bot* 77:153–167
- Webb JA, Wallis EM, Stewardson MJ (2012) A systematic review of published evidence linking wetland plants to water regime components. *Aquat Bot* 103:1–14
- Weiherr E, Keddy PA (1995) The assembly of experimental wetland plant-communities. *Oikos* 73:323–335

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