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RESEARCH ARTICLE

Interactive effects of waterlogging and atmospheric CO_2 concentration on gas exchange, growth and functional traits of Australian riparian tree seedlings

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Abstract

The ability to survive and thrive in repeatedly waterlogged soils is characteristic of plants adapted to riparian habitats. Rising atmospheric CO_2 has the potential to fundamentally alter plant responses to waterlogging by altering gas exchange rates and stoichiometry, modifying growth, and shifting resource-economic trade-offs to favor different ecological strategies. While plant responses to waterlogging and elevated CO_2 individually are relatively well characterized, few studies have asked how the effects of waterlogging might be mediated by atmospheric CO_2 concentration.

We investigated interactive effects of elevated (550 ppm) atmospheric CO_2 and waterlogging on gas exchange, biomass accumulation and allocation, and functional traits for juveniles of three woody riparian tree species. In particular, we were interested in whether elevated CO_2 mitigated growth reduction under waterlogging, and whether this response was sustained following a refractory "recovery" period during which soils were re-aerated.

We found species-specific effects of atmospheric CO_2 concentration and waterlogging status on growth, gas exchange, and functional traits between species, and no evidence for a general effect of elevated CO_2 in mediating plant responses to flooding. For one specie, *Casuarina cunninghamiana*, elevated CO_2 substantially increased growth, but this effect was entirely removed by waterlogging, and there was no recovery following a refractory period.

Differential responses to combined waterlogging and elevated CO_2 among species may result in compositional changes to riparian plant communities and associated changes in ecosystem functioning.

KEYWORDS

climate change, elevated CO_2, flooding, plant functional traits, riparian, waterlogging

1 | INTRODUCTION

Woody plants play an important role in determining the physical structure of many riparian ecosystems (Gurnell, Bertoldi, & Corenblit, 2012), and understanding the responses of woody riparian plants to environmental stresses is central to river rehabilitation and riparian conservation efforts. Riparian plant communities are often dominated by keystone species, and responses of such species to environmental change may have important consequences for riparian landscapes defined by their presence. Changing climatic conditions over the next century are expected to cause shifts in hydrological patterns (Stocker et al., 2013), with changes to the prevalence and intensity of extreme flooding events predicted for many regions (Hennessy et al., 2008). Atmospheric CO_2 has also risen substantially over the past century, and a doubling of pre-industrial levels by 2,100 is projected (IPCC, 2013). Flooding is already a dominant abiotic stress, and an important determinant of ecological strategy for woody riparian plants (Blom & Voesenek, 1996; Lawson, Fryirs, & Leishman, 2015), but while a significant body of research describes the effects of elevated CO_2 on plants at multiple scales, little is known about how the effects of flooding might be mediated by atmospheric CO_2 concentration.

To thrive near stream channels, plants must navigate a trade-off between ease of access to water and stresses associated with waterlogging or inundation (Colmer & Voesenek, 2009; Naiman, Decamps, & Pollock, 1993). Woody colonists of inset channel features such as bars and benches may experience repeated cycles of soil waterlogging (Corenblit, Steiger, Gurnell, Tabacchi, & Roques, 2009), restricting root access to oxygen (Voesenek & Bailey-Serres, 2015). Maintaining root respiration in low O_2 conditions requires switching to costly anaerobic metabolic pathways (Drew, 1997). The resulting reduction in respiration weakens root function, impairing uptake of water and nutrients, (Piedade, Ferreira, Wittmann, Buckeridge, & Parolin, 2010; Voesenek & Bailey-Serres, 2015) and inducing suberization (Steudle, 2000). Stomatal closure may also take place following waterlogging, reducing available CO_2 for photosynthesis (Else, Janowiak, Atkinson, & Jackson, 2009; Kozlowski, 1984). Root-zone hypoxia damages roots by disrupting aerobic respiration and causing an "energy crisis" (Colmer & Voesenek, 2009); reactive oxygen species (ROS) then form as bi-products of anaerobic metabolism (Santosa et al., 2007), and subsequent re-aeration further increases ROS production (Steffens, Steffen-Heins, & Sauter, 2013). Production of toxic ions by microbes under anoxic soil conditions causes additional stress to roots (Blom & Voesenek, 1996). Waterlogging may also impair rhizomicrobial nodule formation and activity, resulting in reduced nutrient uptake (Dawson, Kowalski, & Dart, 1989; Shimono, Konno, Sakai, & Sameshima, 2012). The degree to which this combination of stressors influences plant growth is ultimately determined by species' ability to mobilize physiological and morphological responses which mitigate damage (Bailey-Serres & Voesenek, 2008).

As with waterlogging, atmospheric CO_2 concentration is known to affect plant physiology and growth by altering the fundamental economics of carbon, water, and macronutrient uptake and use (Poorter & Navas, 2003; Reich, Hobbie, & Lee, 2014; Wang, Heckathorn, Wang, & Philpott, 2012). Individual species responses are variable, but photosynthetic CO_2 assimilation in C3 plants tends to increase under elevated CO_2 (eCO_2) (Curtis, 1996). Stomatal conductance is also typically reduced (Ainsworth & Rogers, 2007), with attendant gains in water use efficiency (Holtum & Winter, 2010; Keenan et al., 2013; Van der Sleen et al., 2014). Biomass accumulation in response to eCO_2 may be enhanced (Wang et al., 2012), but this depends on the availability of water and macronutrients (Körner, 2006; Manea & Leishman, 2014; Reich et al., 2014). Increased allocation of biomass to roots occurs under eCO_2 (Nie, Lu, Bell, Raut, & Pendall, 2013), and this effect is interactive with environmental stresses such as drought or low soil fertility (Wang & Taub, 2010). Increased rates of production and turnover of fine roots under eCO_2 have been shown in the field, which has important implications for nutrient cycling and ecosystem functioning (Lipson, Kuske, Gallegos-Graves, & Oechel, 2014; Matamala & Schlesinger, 2000; Pregitzer et al., 1995, 2000). eCO_2 is also known to affect functional traits indicative of positions along economic spectra (*sensu* Reich, 2014). Reduction in specific leaf area (SLA) under eCO_2 may be linked to accumulation of nonstructural carbohydrates in leaves (Bader, Siegwolf, & Körner, 2010; Poorter & Navas, 2003). Alteration of traits reflecting economic trade-offs is of particular significance at the seedling stage, as functional traits of trees are most strongly adapted to the regeneration niche (Poorter, 2007).

Taken individually, waterlogging and elevated atmospheric CO_2 concentration appear likely to exert opposing effects on plant growth. The possibility that eCO_2 may mitigate growth reduction under waterlogging warrants investigation of the interactive effects of these two important environmental variables. Literature describing interactive effects of atmospheric CO_2 concentration and waterlogging or flooding on plant growth is sparse, and findings thus far present an inconsistent picture. eCO_2 stimulated biomass production in waterlogged (water table at −10 cm) but not inundated (water table at +5 cm) juveniles of the flood-tolerant tree species *Taxodium distichum* (Megonigal, Vann, & Wolf, 2005). Increased photosynthesis under eCO_2 was not reduced by inundation. This effect was attributed to the increased metabolic cost of maintaining roots under low O_2 conditions. In the same study, inundation had no effect on eCO_2 stimulation of photosynthesis or biomass production of the aquatic herbaceous species *Orontium aquaticum*. The opposite response was found for a highly flooding tolerant Amazonian tree: waterlogged *Senna reticulata* grown in open top chambers showed greater increment in biomass under eCO_2 (Arenque, Grandis, Pocius, de Souza, & Buckeridge, 2014). Similarly, eCO_2 was shown to ameliorate the effects of stress due to both salinity and flooding on biomass production in herbaceous saltmarsh plants (Langley et al. 2009). In a follow-up field experiment using open top chambers however, no significant interactions were found between eCO_2 concentration and elevation above sea level, which was strongly correlated with proportion of time spent inundated (Langley et al. 2013). Finally, no evidence for an interaction between CO_2 concentration and waterlogging status was found on growth or stomatal conductance in soybean (Shimono et al., 2012). To our knowledge, no studies have specifically investigated the effects of eCO_2 on recovery from waterlogging. Ability to recover following stress events may be a better indicator of fitness than tolerance of the stress (Gutschick & BassiriRad, 2003), and for waterlogged plants, generation of ROS following re-aeration is likely to be a significant additional stress (Drew, 1997).

The objective of this study was to investigate interactive effects between eCO_2 and waterlogging on gas exchange, biomass accumulation and allocation, and functional traits for seedlings of riparian tree species. In particular, we were interested in whether eCO_2 mitigated growth impairment under waterlogging, and whether this response was sustained following a refractory "recovery" period during which soils were re-aerated. We also investigated two hypothesized mechanisms by which such an interactive effect might occur (a) higher water use efficiency under eCO_2 (Holtum & Winter, 2010) facilitates photosynthesis in plants with anoxia-impaired root functionality by lowering the water cost of carbon assimilation; (b) eCO_2 facilitates biomass recovery by increasing the rate of fine root production during the recovery period (Pregitzer et al., 1995).

2 | STUDY SPECIES AND METHODS

We selected three riparian tree species native to south-eastern Australia for this study. *Casuarina cunninghamiana subsp. cunninghamiana* and *Eucalyptus camaldulensis subsp. camaldulensis* dominate many riparian environments in south-eastern Australia; *Acacia floribunda* is also common in this region. Table 1 provides further information on the biology and ecology of these species.

TABLE 1 Biological and ecological attributes of study species

	Acacia floribunda	*Casuarina cunninghamiana* subsp. *Cunninghamiana*	*Eucalyptus camaldulensis* subsp. *Camaldulensis*
Family	Fabaceae	Casuarinaceae	Myrtaceae
Distribution	Coastal areas of eastern Australia[a]	Eastern NSW and QLD, Australia. Other subsp. in gulf of Carpentaria and Papua New Guinea[a]	Inland riparian areas throughout south-eastern Australia. Other subsp. distributed throughout continental Australia[a]
Morphology	Erect or spreading shrub or tree, 3–8 m high[a]. Rooting depth 2 m +[b]	Erect tree, 15–35 m high[a]. Rooting depth to 8 m[b]	Large, spreading tree, 30+ m high[a]. Rooting depth 10 m +[b]
Habitat	Facultative rheophyte. Found in sclerophyll forest, particularly along watercourses and in sandy alluvial soils. Typically on channel banks and raised within-channel features[a]	Obligate rheophyte. Found along permanent watercourses, on substrates ranging from sand to large cobbles. Often found on bars, benches, and channel islands[a]	Obligate rheophyte. Found on deep, rich alluvial soils, on banks and flood plains associated with large, permanent water bodies[a]
Community status	Common[a]	Dominant[a]	Dominant[a]
Nitrogen fixing ability	Nodulated with *Rhizobium*[c]	Nodulated with *Frankia*[d]	None
Biogeomorphic effects	Colonist of fresh geomorphic substrates[5]	Ecosystem engineer. Rapid, *en mass* colonization and stabilization of fresh geomorphic substrates. Established trees stabilize banks and in-channel features[b]	Ecosystem engineer. Established trees define physical structure of riparian landscapes. Highly effective at mitigation of flooding-induced landform mass failure[b]

[a]Royal Botanic Gardens and Domain Trust (2015),
[b]Hubble, Docker & Rutherfurd (2010),
[c]Roughley (1987),
[d]Dawson *et al.* (1989),
[5]J. Lawson personal field observations.

2.1 | Experimental procedure

We used a fully factorial design comprising two CO_2 treatments (ambient and elevated), and three waterlogging treatments (nonwaterlogged control, waterlogged, and waterlogged then re-aerated for a refractory period), with eight replicates per treatment combination per species. We measured plant physiology (photosynthetic rate, *A*; stomatal conductance, *Gs*; and instantaneous water use efficiency, *WUE*) as well as biomass, biomass allocation, and tissue density traits indicative of ecological strategy and position along economic spectra (Reich et al., 2014).

Plants were grown individually in pots constructed from 90 mm by 700 mm (4.3 L capacity) sections of PVC pipe with drilled endcaps, containing a commercially sourced 80/20 mixture of river sand and soil (Australian Native Landscapes, North Ryde, NSW, Australia). The bottom 2 cm of each pot was filled with gravel (~1 cm particle size) to promote free drainage. A 2.5 g L^{-1} of time-release fertilizer granules (NPK 19.1, 0, 11.9, Yates Australia, Padstow, NSW, Australia) was mixed evenly through the soil medium.

Seeds were obtained from a commercial supplier (Nindethana Seed Service, Albany, WA, Australia) and germinated on moist tissue paper in trays at ~20°C. Following cotyledon emergence, four seedlings were transplanted into each growing pot. Germination was staggered by species to ensure all seedlings were transplanted at the same stage of development (radicle just emerged); all species were transplanted within a 48 hr window. After 2 weeks of growth, plants were thinned to retain a single, medium sized individual.

Plants were grown in glasshouses at Macquarie University, in Sydney, Australia, between June and November, 2014. Pots were supported by wire mesh on trolleys; pot positioning on trolleys was randomized with respect to species, and trolleys were rotated weekly to offset potential microclimatic effects associated with position within each glasshouse. Two levels of CO_2 treatment (380–400 ppm and 530–570 ppm) were used in two replicate glasshouses per level. These CO_2 ranges were monitored and maintained using an automated gas delivery system (Canary Company Pty Ltd, Lane Cove, NSW, Australia). The lower range corresponds to the ambient atmospheric CO_2 concentration, while the higher range reflects the predicted atmospheric CO_2 concentration in 2050 (IPCC, 2013). Temperature was maintained between 16°C and 28°C. Mean air temperatures in the glasshouses ranged between 18°C and 22°C, and there were no significant differences between glasshouses in monthly mean, minimum, or maximum temperatures. As in previous studies of flooding—CO_2 interactions, plants were exposed to ambient light conditions (Arenque et al., 2014; Megonigal et al., 2005; Shimono et al., 2012). Solar exposure ranged between 9 MJ/m^2 in June and 22 MJ/m^2 in November, with an estimated 40% of solar radiation being intercepted by the walls and roof of the glasshouses. Plants were watered by a misting sprinkler system three times daily and provided with supplementary hand watering every 3–4 days to maintain constant soil moisture levels between pots. Relative humidity was not controlled but is likely to have been higher than ambient atmospheric conditions due to the sprinkler system. Trolleys were swapped between replicate glasshouses monthly.

Waterlogging was initiated after 90 days of plant growth and lasted 24 days, in order to simulate a significant flooding event and to allow time for morphological adaptation to manifest. This waterlogging period lies between that of Shimono et al. (2012) (14 days of waterlogging beginning on 14 day old plants) and Arenque et al.

(2014) (45 days of waterlogging beginning on 90-day old plants). Plants were randomly assigned to "control", "waterlogged," and "recovery" treatments. "Waterlogged" and "recovery" plants were waterlogged by immersion to within 10 cm of the soil surface in 450 L plastic tubs filled with water. The black tubs were covered with white polythene sheeting to reduce heat absorption. Photosynthetic rate and transpiration rate of plants assigned to the "waterlogged" treatment were measured at the end of the waterlogging period, after which they were harvested. Tubs were drained following the waterlogging period, and "control" and "recovered" treatment plants were grown for a further 23 days before measurement and harvesting.

Photosynthetic rate (CO_2 assimilation rate), stomatal conductance, and transpiration rate of the newest fully developed leaf were measured for four plants per treatment between 9 a.m. and 12:30 p.m. using a Li-Cor 6400XT infrared gas analyser (Li-Cor Inc., Lincoln, NE, USA). Photon flux was set to 1500 μmol m^{-2} s^{-1}, and temperature was held at 28°C. For leaves which did not completely fill the cuvette, leaf area was measured by digital analysis (ImageJ 1.48 for Windows) of a photograph of the leaf taken against a 2 × 3 cm^2 plastic backdrop, which corresponded to the area of the cuvette. Photosynthetic rate and transpiration rate were determined by correcting values according to the measured area. Instantaneous water use efficiency was calculated as the ratio of CO_2 assimilation to transpiration rate.

Upon harvesting, roots were washed free of soil and the plant was separated into fine (<1-mm diameter) and coarse (>1-mm diameter, excluding dead root biomass) roots, and aboveground biomass. Five mature (but not senescing) leaves of each individual were selected for determination of SLA. Fresh leaf area was determined using a LI-3100C Area Meter (Li-Cor Inc., Lincoln, NE, USA); SLA was calculated as the ratio of fresh area to dry mass. A 5-cm section of stem was cut from 1 cm above the root-stem junction for analysis of stem density. The fresh volume of the stem section was measured using the water displacement method, and stem wood density was calculated as the ratio of oven dry mass to green volume. Root dry matter content was used as a proxy for root tissue density (Birouste, Zamora-Ledezma, Bossard, Pérez-Ramos, & Roumet, 2013). Dry matter content of fine roots was calculated as the ratio of oven dry mass to fresh mass. Samples were dried in an oven at 70°C for 72 hr then weighed on a microbalance (Mettler-Toledo, Greifensee, Switzerland). Root mass fraction (RMF) was calculated as the ratio of root dry biomass to whole plant dry biomass. Stunted plants with a shoot length of <5 cm were excluded (one individual from each of the following treatments: *A. floribunda*, 390 ppm CO_2, "recovered"; *C. cunninghamiana*, 550 ppm CO_2, "control"; *E. camaldulensis*, 500 ppm CO_2, "control").

2.2 | Data analysis

All statistical analyses were performed using the R statistical programming environment (R Core Team, 2013). We used two-way analysis of variance (ANOVA) to test for main effects of and interactions between waterlogging and CO_2 treatments on physiology (photosynthetic rate, stomatal conductance, and water use efficiency), biomass (shoot, total root, and fine root) and biomass allocation (root mass fraction), and functional traits (fine root dry matter content [fRDMC], stem density, and SLA). One observation was omitted as an outlier in analysis of *E. camaldulensis* SLA (390 ppm CO_2, "control" treatment) due to substantially higher SLA than conspecifics. Metrics of biomass (total, root biomass, and shoot biomass) were compared only between "control" and "recovered" treatment plants, as plants which received the "waterlogged" treatment were 23 days (17%) younger at harvest.

Post hoc comparison (Tukey's HSD) was used to determine which combination of treatments were responsible for interaction effects and waterlogging treatment main effects. Type II sums of squares were used where unbalanced analyses resulted from removal of stunted plants from the study, following Lansgrud (2003). Data were log_{10} (root mass fraction, SLA) or square root transformed (total root biomass, fine root biomass, and shoot biomass) where appropriate to satisfy assumptions of normality inherent in ANOVA. Statistical significance was thresholded at alpha = 0.1 for photosynthetic rate, stomatal conductance, and WUE measurements (n = 4) and 0.05 for all other measurements (n = 8).

3 | RESULTS

Descriptive statistics and significance of ANOVA and post hoc tests are shown for all measurements for each combination of treatments in Table 2.

3.1 | Gas exchange and water use efficiency

Effects of CO_2 level and waterlogging on gas exchange were species specific, and although some significant interactions were found between CO_2 and waterlogging, we found no evidence that interactive effects were maintained following recovery from waterlogging.

Elevated CO_2 significantly increased leaf-level photosynthesis for all three species (*A. floribunda*, p = .074, Figure 1a; *C. cunninghamiana*, p = .002, Figure 1b; *E. camaldulensis*, p = .037, Figure 1c). Photosynthetic rate in *E. camaldulensis* was significantly greater in recovery treatment plants than control plants (p = .008). No significant interactions were found between CO_2 level and waterlogging status for photosynthetic rate, although waterlogged *A. floribunda* exhibited only a small difference in mean photosynthetic rate between CO_2 treatments (20.9 and 22.6 μmol CO_2 m^{-2} s^{-1}, respectively, Figure 1a).

CO_2 level had no effect on stomatal conductance for any species, and waterlogging status influenced stomatal conductance only in *E. camaldulensis*. Control plants had lower stomatal conductance than waterlogged plants (p = .042), and recovering plants (p = .0002). Waterlogged *E. camaldulensis* also had lower stomatal conductance than recovering plants (.059).

Water use efficiency in *A. floribunda* was higher in control than waterlogged (p = .002), and higher in control than recovery (p = .04), but not waterlogged and recovery plants (Figure 1g). WUE increased under elevated CO_2 as a main effect for *E. camaldulensis* (p = .002, Figure 1h) and interactively with CO_2 level for *C. cunninghamiana* (p = .063); WUE was higher under eCO_2 for waterlogged plants (p = .022, Figure 1i) but not control or recovery plants.

TABLE 2 Mean and standard deviation (in parentheses) of measured gas exchange rates, biomass, and functional traits for each combination of CO_2 level and waterlogging treatments

	Control		Waterlogged		Recovery		Significant effect	Post hoc
	aCO_2	eCO_2	aCO_2	eCO_2	aCO_2	eCO_2		
Acacia floribunda								
Photosynthetic rate (*A*, μmol CO_2 m^{-2} s^{-1})	13.41 (7.58)	19.25 (7.47)	20.9 (6.83)	22.06 (7.68)	17.15 (1.17)	25.11 (6.3)	C	
Stomatal conductance (*Gs*, mmol m^{-2} s^{-1})	0.41 (0.11)	0.41 (0.07)	0.36 (0.16)	0.24 (0.07)	0.27 (0.04)	0.49 (0.12)	NS	
Water use efficiency (*A/Gs*)	1 (0.43)	1.22 (0.62)	1.89 (0.53)	2.55 (0.65)	2.02 (0.35)	1.53 (0.44)	W	Cw, cr
Dry root biomass (g)	5.64 (2.35)	6.02 (2.51)			3.74 (0.76)	4.64 (0.94)	W	
Dry fine root biomass (g)	2.12 (1.5)	2.27 (1.07)			1.01 (0.39)	1.21 (0.35)	W	
Dry shoot biomass (g)	8.9 (4.17)	10.93 (3.67)			9.29 (1.65)	10.27 (3.13)	Ns	
Root mass fraction	0.4 (0.14)	0.35 (0.07)	0.2 (0.02)	0.24 (0.05)	0.29 (0.03)	0.32 (0.03)	W	Cw, wr, cr
Fine root DMC (%)	0.13 (0.03)	0.16 (0.04)	0.18 (0.07)	0.15 (0.03)	0.13 (0.01)	0.12 (0.02)	W	Wr
SLA (cm^2 g^{-1})	27.54 (2.12)	28.26 (2.33)	24.83 (2.15)	24.72 (3.12)	29.91 (2.91)	27.84 (1.4)	W	Cw, wr
Stem density (cm^2 g^{-1})	0.46 (0.07)	0.48 (0.05)	0.49 (0.04)	0.54 (0.07)	0.5 (0.02)	0.47 (0.12)	NS	
Casuarina cunninghamiana								
Photosynthetic rate (*A*, μmol CO_2 m^{-2} s^{-1})	25.3 (6.32)	38.11 (7.8)	26.63 (7.53)	33.53 (3.75)	27.41 (1.81)	35.38 (7.6)	C	
Stomatal conductance (*Gs*, mmol m^{-2} s^{-1})	0.53 (0.14)	0.66 (0.15)	0.64 (0.07)	0.57 (0.07)	0.57 (0.07)	0.61 (0.14)	NS	
Water use efficiency (*A/Gs*)	1.5 (0.2)	1.69 (0.08)	1.26 (0.24)	1.72 (0.23)	1.65 (0.18)	1.65 (0.07)	C x W, C	W
Dry root biomass (g)	5.79 (3.1)	10.88 (3.67)			6.31 (2.07)	7.05 (2.75)	C x W, C	C
Dry fine root biomass (g)	1.66 (1.23)	4.11 (1.96)			1.95 (0.73)	2.61 (1.31)	C x W*, C	C
Dry shoot biomass (g)	10.44 (3.75)	17.19 (5.66)			11.97 (3.28)	10.55 (3)	C x W	
Root mass fraction	0.34 (0.06)	0.39 (0.04)	0.29 (0.1)	0.27 (0.04)	0.34 (0.03)	0.39 (0.04)	W	
Fine root DMC (%)	0.18 (0.08)	0.25 (0.07)	0.18 (0.08)	0.21 (0.04)	0.15 (0.02)	0.19 (0.03)	C	
SLA (cm^2 g^{-1})	20.82 (2.39)	18.84 (1.76)	20.76 (1.61)	20.57 (2.33)	20.3 (2.19)	21.61 (1.47)	NS	
Stem density (cm^2 g^{-1})	0.4 (0.03)	0.44 (0.02)	0.34 (0.09)	0.4 (0.03)	0.41 (0.02)	0.41 (0.04)	C	
Eucalyptus camaldulensis								
Photosynthetic rate (*A*, μmol CO_2 m^{-2} s^{-1})	9.94 (5.88)	15.46 (1.49)	15.46 (1.49)	18.39 (5.11)	17.99 (3.87)	21.09 (2.95)	C, W	Cr
Stomatal conductance (*Gs*, mmol m^{-2} s^{-1})	0.14 (0.08)	0.17 (0.10)	0.32 (0.09)	0.28 (0.13)	0.52 (0.17)	0.35 (0.08)	W	Cw, wr, cr
Water use efficiency (*A/Gs*)	2.1 (0.4)	3.26 (1)	1.99 (0.25)	2.65 (0.46)	1.93 (0.21)	2.48 (0.47)	C	
Dry root biomass (g)	14.85 (3.5)	14.32 (2.58)			14.09 (5.73)	13.42 (6.51)	NS	
Dry fine root biomass (g)	2.64 (1.84)	1.73 (0.93)			3.69 (2.73)	3.82 (2.22)	W	
Dry shoot biomass (g)	22.93 (5.31)	22.63 (6.13)			26.49 (10.35)	23.23 (8.49)	Ns	
Root mass fraction	0.39 (0.05)	0.39 (0.05)	0.25 (0.02)	0.25 (0.06)	0.35 (0.11)	0.36 (0.05)	W	Cw, rw
Fine root DMC (%)	0.25 (0.06)	0.26 (0.07)	0.2 (0.07)	0.18 (0.07)	0.18 (0.07)	0.22 (0.06)	W	Cw, cr
SLA (cm^2 g^{-1})	31.7 (8.24)	28.11 (1.74)	31.38 (1.8)	31.82 (3.61)	28.59 (1.59)	28.08 (0.74)	W	Cw, wr
Stem density (cm^2 g^{-1})	0.39 (0.02)	0.41 (0.02)	0.38 (0.02)	0.39 (0.04)	0.39 (0.04)	0.39 (0.06)	N	

Significant differences as determined by two-way ANOVA are denoted by the letters NS, C, W, or I (NS = no significant effect of either treatment, C = significant effect of CO_2 level, W = significant effect of waterlogging treatment, C x W = significant interaction between CO_2 level and waterlogging treatment). Where interactions were found, waterlogging treatments in which significant differences between aCO_2 and eCO_2 were determined by post hoc tests are denoted by: c = control, w = waterlogged, r = recovery. Significant differences between waterlogging treatments determined by post hoc tests are denoted using the following script: cw = difference between control and waterlogged measurements, cr = difference between control and recovery measurements, wr = difference between waterlogged and recovery measurements. * - interaction effect was marginally significant, but post hoc analysis confirmed significant differences among treatments. N.B. biomass measurements for waterlogged plants are omitted because these plants were harvested at a younger age than control or recovery plants and are thus not comparable.

3.2 | Biomass production and allocation

Waterlogging status and CO_2 level interacted strongly for one species: eCO_2 stimulation of all fractions of biomass production in *C. cunninghamiana* was diminished following recovery from waterlogging.

Total root biomass of plants recovering from waterlogging was lower than control plants for *A. floribunda* (p = .028, Figure 2a). A significant interaction effect was identified for *C. cunninghamiana* (p = .049): total root biomass was substantially increased under eCO_2 for control (p = .011) but not recovery plants (Figure 2b). Neither CO_2 level nor

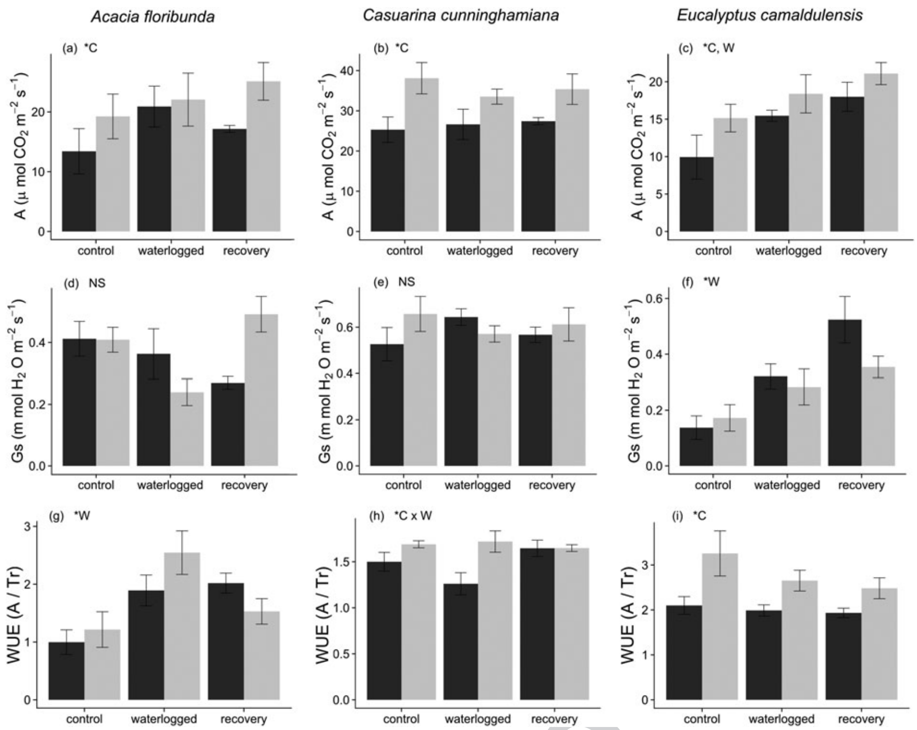

FIGURE 1 Gas exchange measurements under each combination of waterlogging and CO_2 level treatments. Tr = transpiration rate (mmol H_20 m^{-2} s^{-1}) dark shaded columns represent measurements under ambient atmospheric CO_2 concentration (390 ppm), and light shaded columns represent measurements under elevated atmospheric CO_2 concentration (550 ppm). Error bars represent the standardized mean error. *-letters denote statistical significance of differences between treatment combinations (NS = no significant difference; C = significant difference between CO_2 level treatments; W = significant difference between waterlogging treatments)

waterlogging had an effect on total root biomass for *E. camaldulensis* (Figure 2c).

Fine root biomass of *A. floribunda* was lower in recovery plants than control plants (p = .005), with no CO_2 effect (Figure 2d). A marginally significant interaction effect was also present for *C. cunninghamiana* fine root biomass (p = .076); post hoc analysis confirmed that control but not recovery plants had significantly greater fine root biomass under eCO_2 (p = .008) (Figure 2e). Waterlogging stimulated fine root growth in *E. camaldulensis* (p = .046) but CO_2 level had no effect (Figure 2f).

Neither CO_2 level nor waterlogging had any effect on shoot biomass for *A. floribunda* (Figure 2g) or *E. camaldulensis* (Figure 2i). As with total root biomass and fine root biomass, CO_2 level and waterlogging influenced *C. cunninghamiana* biomass interactively (p = .009): shoot biomass was higher under eCO_2 for control (p = .015) but not recovery plants (Figure 2h).

Root mass fraction was decreased by waterlogging for all species, but no significant CO_2 or interaction effects were found (Figure 2j–l). RMF of *A. floribunda* was lower in waterlogged than control plants (p < .0001) and lower in waterlogged than recovery plants (p < .0001). RMF of *A. floribunda* recovery plants was also lower than control plants (p = .016). RMF of both *C. cunninghamiana* and *E. camaldulensis* was lower in waterlogged than control plants (p < .0001), and lower in waterlogged than recovery plants (p < .0001), but there was no difference between recovery and control plants.

3.3 | Functional traits

We found no evidence to suggest that CO_2 mediates functional traits in response to waterlogging status.

Fine root dry matter content was higher in waterlogged *A. floribunda* than recovery plants (p = .027), but not different between control and recovery or control and waterlogged plants. A marginally significant interaction effect was also present for *A. floribunda* (p = .067), but no differences were significant upon post hoc analysis. Waterlogging status also affected *E. camaldulensis* fRDMC (Figure 3b): control plants had higher fRDMC than waterlogged plants (p = .018), and recovery plants (p = .053) (marginally significant). eCO_2 was associated with significantly increased fRDMC in *C. cunninghamiana* (p = .013, Figure 3c), but waterlogging status had no effect.

Waterlogged *A. floribunda* had lower SLA than control (p = .001), and recovery plants (p < .0001) (Figure 3d). Waterlogged *E. camaldulensis* had higher SLA than control (p = .0013) and recovery plants (p = .0006) (Figure 3f). Waterlogging status had no effect on *C. cunninghamiana* SLA (Figure 3e). CO_2 level had no effect on the SLA of any species.

Stem density in *C. cunninghamiana* was increased under elevated CO_2 (p = .0177) (Figure 3h) and was lower in waterlogged than control (p = .0167) or recovery plants (.050). Neither CO_2 nor waterlogging status had any effect on stem density of *A. floribunda* (Figure 3g) or *E. camaldulensis* (3i).

4 | DISCUSSION

We found inconsistent effects of atmospheric CO_2 concentration and waterlogging status on growth, gas exchange and functional traits between species of riparian tree seedlings and no evidence for a consistent effect of elevated CO_2 in mediating plant responses to flooding.

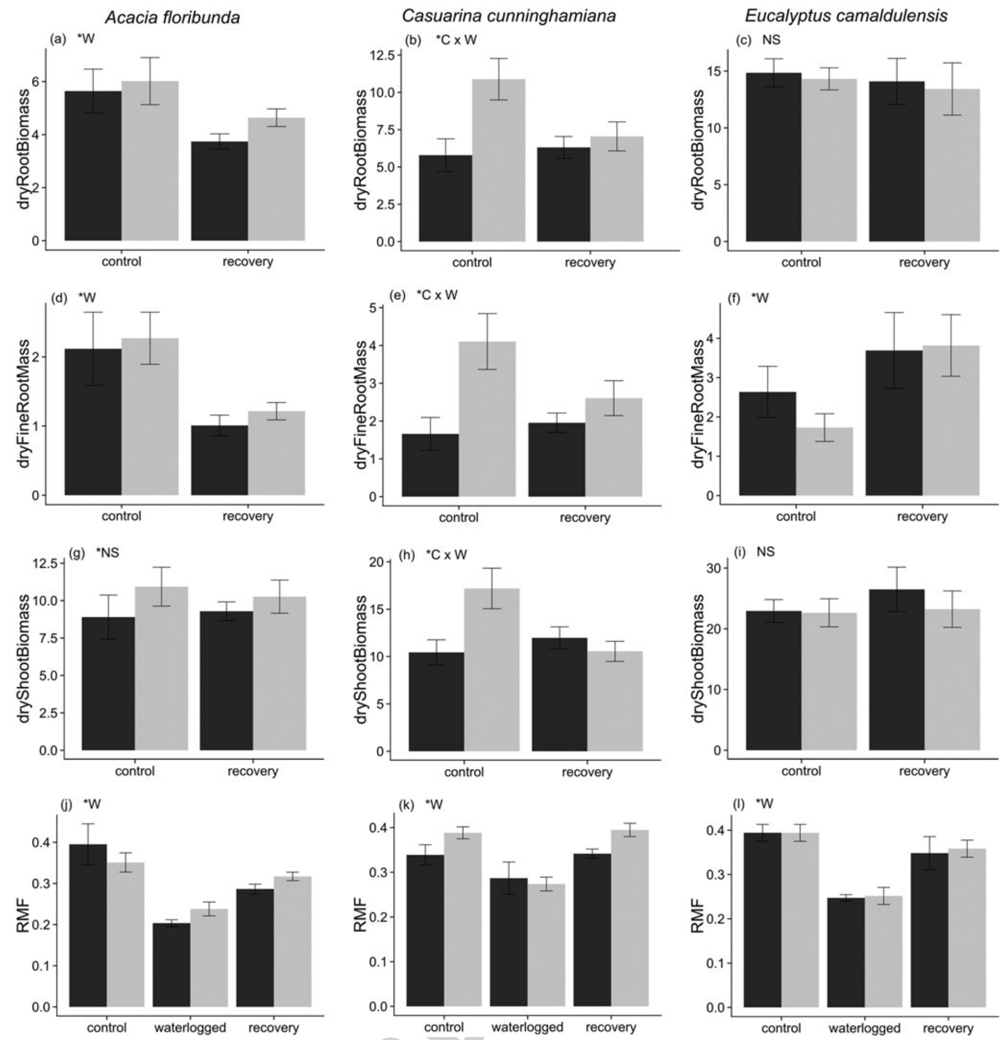

FIGURE 2 Biomass and root mass fraction (RMF) measurements under each combination of waterlogging and CO_2 level treatments. Dark shaded columns represent measurements under ambient CO_2 concentration (390 ppm), and light shaded columns represent measurements under elevated CO_2 concentration (550 ppm). Error bars represent the standardized mean error. *-letters denote statistical significance of differences between treatment combinations (NS = no significant difference; C = significant difference between CO_2 level treatments; W = significant difference between waterlogging treatments)

While photosynthesis is the primary means by which plants accumulate biomass, increases in leaf-level photosynthesis may not necessarily translate to biomass gains. Metabolically, costly responses to waterlogging tolerance, such as anaerobic catabolism, detoxification of ROS and metal ions, and morphological adaptations such as formation of adventitious roots may act as energetic sinks (Colmer & Voesenek, 2009). Relationships between photosynthetic rate and biomass responses to waterlogging and CO_2 level treatments in this study varied widely between species.

For the three species studied here, only for *C. cunninghamiana* was an interactive effect of CO_2 concentration and waterlogging status found. Biomass of shoot, total root, and fine root fractions was significantly higher under eCO_2 for control *C. cunninghamiana* plants, but not for plants which were recovering from waterlogging, despite increased rates of CO_2 assimilation. No significant interaction effect on RMF was found, but visual inspection of the data (Figure 2k) indicates that eCO_2 stimulation of RMF was present in control and recovering but not waterlogged plants. Re-establishment of pre-waterlogging biomass allocation appears to have occurred despite no differences in total biomass. We found no evidence to support the hypothesis that eCO_2 facilitated biomass recovery by increasing the rate of fine root production in *C. cunninghamiana* after waterlogging. Photosynthesis remained higher in recovering plants under eCO_2, indicating that their ability to convert the extra photosynthate produced under eCO_2 into biomass was impaired by waterlogging.

No increase in any biomass fraction was associated with increased photosynthetic rate under eCO_2 for either *A. floribunda* or *E. camaldulensis*. *A. floribunda* underwent substantial root mortality in response to waterlogging, although the presence of spongy white aerenchymous adventitious roots indicated a degree of morphological adaptation to anoxia (Evans, 2004). Conversely, waterlogging stimulated fine root growth in *E. camaldulensis*. A proliferation of fine aerenchymous roots both below and above the water line was observed in waterlogged and recovered plants, corresponding to increased fine root mass compared with control plants. The strong morphological response of *E. camaldulensis* root systems combined with higher photosynthetic rate in recovering compared with control plants, and higher stomatal conductance in waterlogged plants than

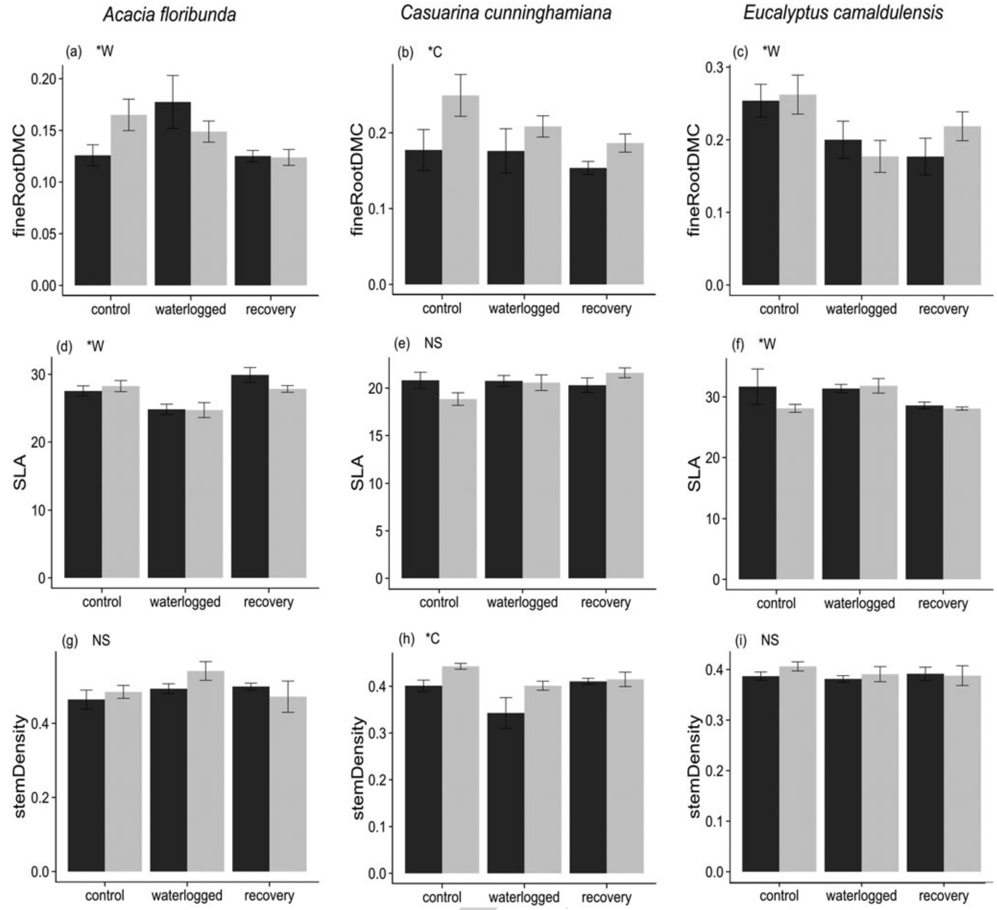

FIGURE 3 Functional trait measurements under each combination of waterlogging and CO_2 level treatments. Dark shaded columns represent measurements under ambient CO_2 concentration (390 ppm), and light shaded columns represent measurements under elevated CO_2 concentration (550 ppm). Error bars represent the standardized mean error. *-letters denote statistical significance of differences between treatment combinations (NS = no significant difference; C = significant difference between CO_2 level treatments; W = significant difference between waterlogging treatments)

control or recovering plants, indicates that *E. camaldulensis* responded favorably to waterlogging in this study. This growth response concurs with the results of previous studies (Marcar, 1993; Sena-Gomes & Kozlowski, 1980, although see Kogawara, Yamanoshita, Norisada, Masumori, & Kojima, 2006). No evidence was found to support the hypothesis that higher WUE under eCO_2 might facilitate photosynthesis where waterlogging had caused stomatal closure. WUE was altered by waterlogging only in *A. floribunda* and by CO_2 level only in *E. camaldulensis*. WUE was dependent on the combination of waterlogging status and CO_2 level in *C. cunninghamiana*, being higher at eCO_2 than aCO_2 for waterlogged plants only. The lack of stomatal response to waterlogging indicates that higher WUE under eCO_2 is not the mechanism maintaining photosynthetic rate under waterlogging for *C. cunninghamiana*.

Waterlogging and atmospheric CO_2 level also altered functional traits in a species-specific manner, but no interactive effects were found. Traits of *A. floribunda* and *E. camaldulensis* were affected by waterlogging status but not CO_2 level, whereas *C. cunninghamiana* was affected by CO_2. Decreased SLA and increased fRDMC—a proxy for fine root tissue density (Birouste et al., 2013)—in waterlogged *A. floribunda* indicate a shift towards the slower growth—longer lifespan end of their respective economic spectra (Reich, 2014), but this shift was not sustained following the refractory period. A corresponding pattern in water use efficiency corroborates this inference. Higher root dry matter content under waterlogging has been linked to the requirement for structural support of air spaces in aerenchymous root tissue (Ryser, Gill, & Byrne, 2011). Suberization of root hypodermal tissue often occurs under waterlogging as a means of reducing radial oxygen loss (De Simone et al., 2002; Visser, Colmer, Blom, & Voesenek, 2000) and may also increase root dry matter content. *E. camaldulensis* responded in an opposite manner, with higher SLA under waterlogging, and lower root dry matter content under waterlogging and after the refractory period. These species appears to employ an opportunistic "fast growth" ecological strategy in response to waterlogging, involving proliferation of lower density roots and lower carbon investment in leaf tissue (Reich, 2014; Wright et al., 2004). We found no evidence for decreased SLA under eCO_2 as previously described (Poorter & Navas, 2003). Previous studies report inconsistent effects of eCO_2 on fRDMC in non-riparian species: eCO_2 had no effect on *Liquidambar styraciflua* or *Pinus strobus* fRDMC (Bauer & Berntson, 2001; Iversen, Ledford, & Norby, 2008), caused a small decrease in *Betula alleghaniensis* (Bauer & Berntson, 2001) and increased fRDMC in cotton (Prior, Rogers, Runion, & Hendrey, 1994). In this study, eCO_2 significantly increased fRDMC in *C. cunninghamiana* irrespective of waterlogging treatment.

Analysis of gas exchange, biomass accumulation, and functional traits after a refractory period provided an opportunity to determine whether responses to waterlogging persisted or were transitory. We were unable to substantiate the hypothesis that eCO_2 would increase the rate of biomass recovery from waterlogging by increasing the rate of fine root turnover. *C. cunninghamiana* was the only specie for which eCO_2 altered biomass accumulation, and suppression of this response to eCO_2 was observed following the recovery period. Although we made no analysis of nodulation rates, nodulation of *C. cunninghamiana* by the nitrogen fixing ascomycete *Frankia* is known to be highest under well aerated soil conditions (Dawson et al., 1989). Reduced nitrogen uptake due to nodule mortality or impairment could account for the constrained biomass response to eCO_2 postwaterlogging (Reich et al., 2006). While eCO_2 did not mitigate growth reduction or mediate changes to functional traits under waterlogging for any species in this glasshouse study, we did observe reduced growth stimulation by eCO_2 in one species. This effect was strong and evident across all measured biomass fractions. Differential responses to eCO_2 and waterlogging between species in the field could have important ecological consequences. *C. cunninghamiana* is a highly effective agent of "biogeomorphic succession" in fluvial landscape of south-eastern Australia—that is, it facilitates the creation and stabilization of fluvial landforms (Erskine & Chalmers, 2009). Reduction of eCO_2 biomass stimulation by waterlogging could alter spatial patterns of landform stabilization by *C. cunninghamiana*. Infrequently waterlogged stands on channel banks might be favored over stands growing on wetter in-channel features such as bars, benches, and islands. Differential responses to combined waterlogging and eCO_2 between species—notably *C. cunninghamiana* and *A. floribunda*, which frequently coexist—may also result in compositional changes to riparian plant communities and associated changes in ecosystem functioning.

An important concern in making such ecological interpretations from manipulative glasshouse experiments is the extent to which field conditions are properly represented by the experimental design. Replication is an obvious issue: an experiment conducted at a single location at a single time point does not necessarily provide the basis for making general inferences about ecology. Irradiance is likely to be the most important uncontrolled environmental factor influencing the experimental results. Stronger irradiance would likely strengthen the stimulating effect of eCO_2 on carbon assimilation and potentially exacerbate differences in growth rates between plants growing in aerated soil and those with metabolic limitations imposed by waterlogging. As the experiment was conducted primarily over the austral winter and spring (June to November), the effect sizes found here may therefore be reduced compared with summer light conditions. Additionally, atmospheric conditions in the glasshouses may have been more consistently humid than field conditions, due to regular watering by the sprinkler system. As relative atmospheric humidity is known to influence stomatal conductance (Kozlowski, 1984), this effect is another relevant point of difference between natural growing conditions and our experimental setup.

In the field, riparian plant communities are exposed to cyclical wetting and drying, with compounding effects on component populations. Seedlings are less likely to be exposed to multiple waterlogging events; however, replicating this aspect of the riparian environment was of minor concern here. Other factors such as constraints by pots on root spread, nutrient supplementation, and the lack of interaction between study individuals and other organisms may limit the scope of inference able to be made from this study, however. While open air field setups such as FACE (free air CO_2 enrichment) experiments (Norby and Zak 2011) would provide a greater degree of realism, the scale of such approaches typically limits the ability to construct manipulative experiments. Glasshouse experiments focused on individual plants grown in pots are able to provide data which would not be feasible to obtain using free air setups, either because the methods are too destructive, manipulations cannot be made, or the extensive funding required to construct infrastructure in the system of interest is not available.

5 | CONCLUSIONS

Waterlogging and atmospheric CO_2 concentration both have significant consequences for physiological processes, growth, and functional characteristics of riparian tree seedlings. The relative importance of these environmental factors varies according to species, as do the specific effects of each on plants. This study adds to the small but growing body of literature describing the interactive effects of waterlogging and CO_2 concentration on woody plants; notably, the outcome for *C. cunninghamiana* concurs with that found for *Taxodium distichum*, a flood tolerant colonist of alluvial riparian areas in the south eastern United States (Megonigal et al., 2005). If it occurs in the field, impairment of eCO_2 biomass stimulation in seedlings by waterlogging has the potential to alter demographics and structural dynamics in many Australian riparian communities, especially where *C. cunninghamiana* is a keystone species (Woolfrey & Ladd, 2001).

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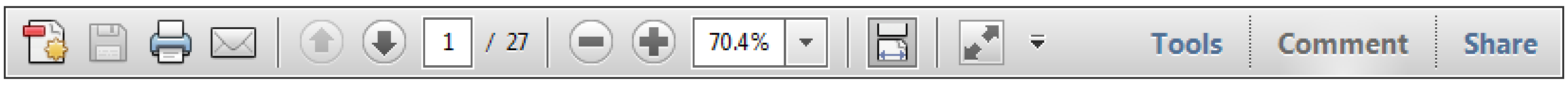
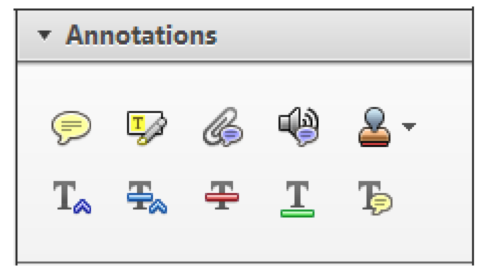
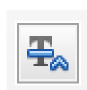
Once you have Acrobat Reader open on your computer, click on the Comment tab at the right of the toolbar:



This will open up a panel down the right side of the document. The majority of tools you will use for annotating your proof will be in the Annotations section, pictured opposite. We've picked out some of these tools below:



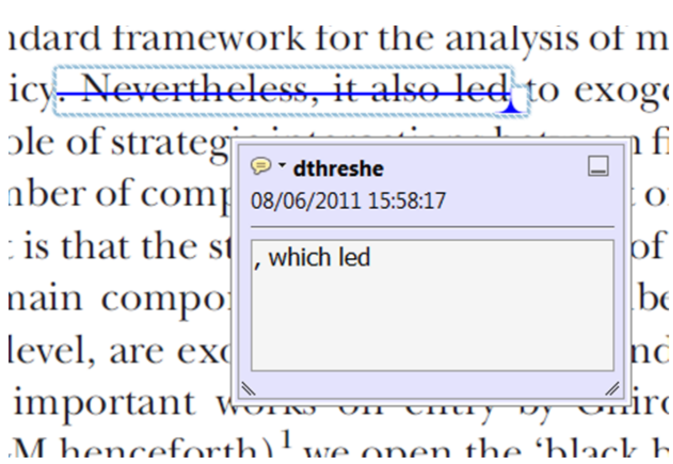
1. Replace (Ins) Tool – for replacing text.



Strikes a line through text and opens up a text box where replacement text can be entered.

How to use it

- Highlight a word or sentence.
- Click on the Replace (Ins) icon in the Annotations section.
- Type the replacement text into the blue box that appears.

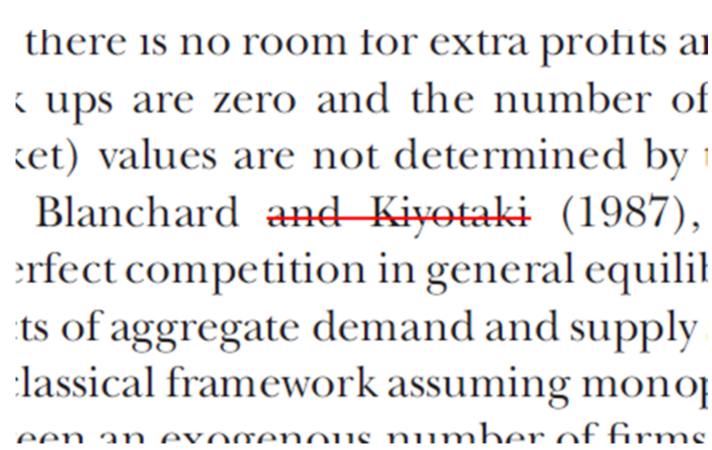


2. Strikethrough (Del) Tool – for deleting text.

Strikes a red line through text that is to be deleted.

How to use it

- Highlight a word or sentence.
- Click on the Strikethrough (Del) icon in the Annotations section.

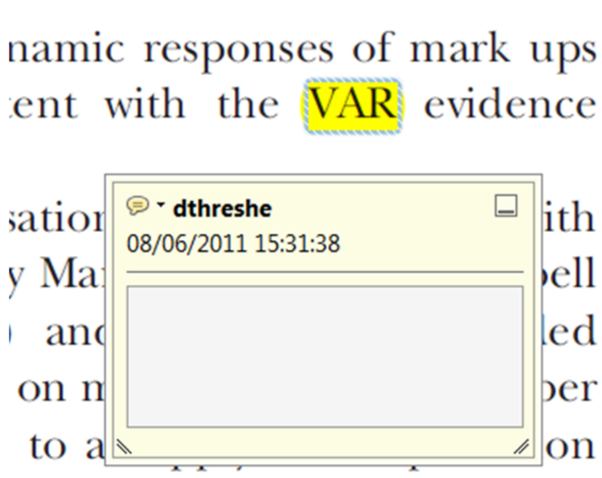


3. Add note to text Tool – for highlighting a section to be changed to bold or italic.

Highlights text in yellow and opens up a text box where comments can be entered.

How to use it

- Highlight the relevant section of text.
- Click on the Add note to text icon in the Annotations section.
- Type instruction on what should be changed regarding the text into the yellow box that appears.

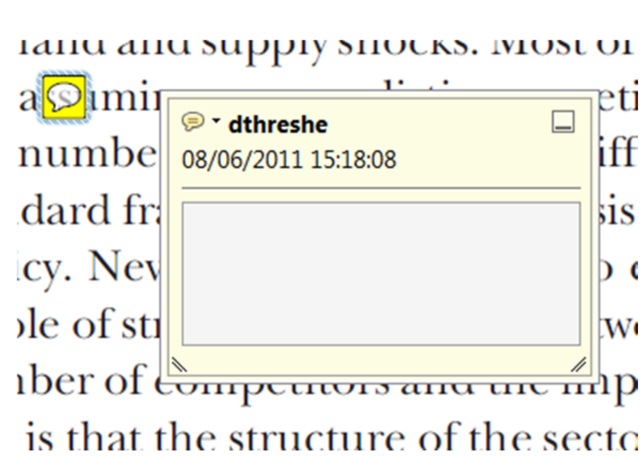


4. Add sticky note Tool – for making notes at specific points in the text.

Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the Add sticky note icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.

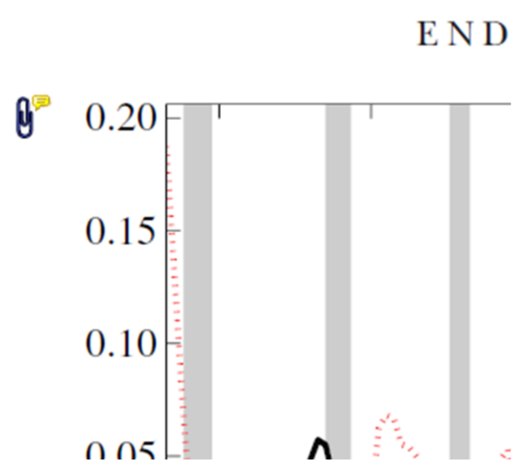


5. Attach File Tool – for inserting large amounts of text or replacement figures.

Inserts an icon linking to the attached file in the appropriate pace in the text.

How to use it

- Click on the Attach File icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.

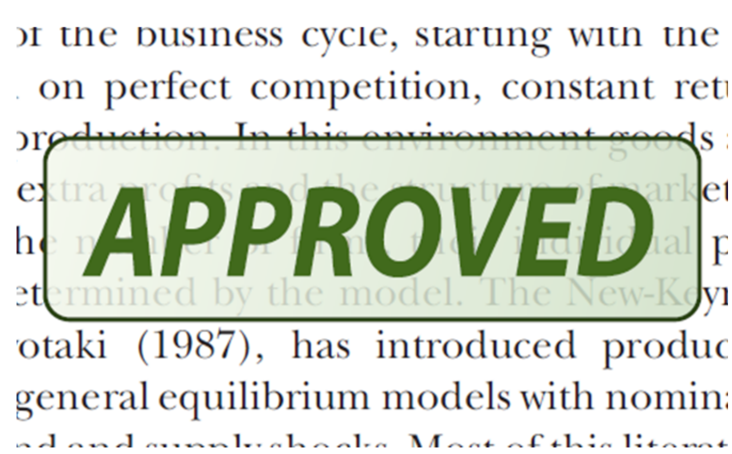


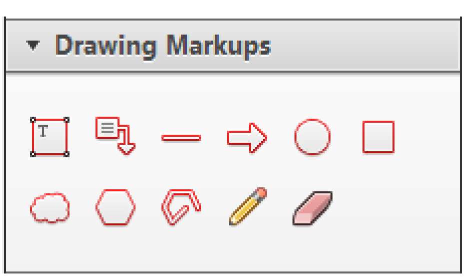
6. Add stamp Tool – for approving a proof if no corrections are required.

Inserts a selected stamp onto an appropriate place in the proof.

How to use it

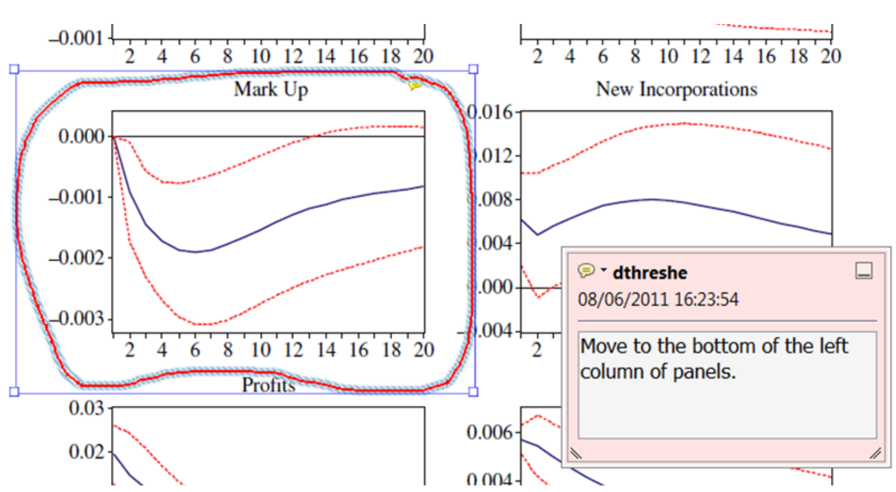
- Click on the Add stamp icon in the Annotations section.
- Select the stamp you want to use. (The Approved stamp is usually available directly in the menu that appears).
- Click on the proof where you'd like the stamp to appear. (Where a proof is to be approved as it is, this would normally be on the first page).





7. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks..



How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.

For further information on how to annotate proofs, click on the Help menu to reveal a list of further options: