1 RESEARCH ARTICLE

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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 favour different ecological strategies. While plant responses to waterlogging and elevated CO_2 individually are relatively well characterised, few studies have asked how the effects of waterlogging might be mediated by atmospheric CO_2 concentration. We investigated interactive effects between 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 inconsistent effects of atmospheric CO_2 concentration and waterlogging status on growth, gas exchange and functional traits between species, and no evidence for a consistent effect of elevated CO_2 in mediating plant responses to flooding. For one species, *Casuarina cunninghamiana*, elevated CO_2 substantially

26 increased growth, but this effect was entirely removed by waterlogging and

27 there was no recovery following a refractory period. Differential responses to

28 combined waterlogging and elevated CO_2 between species may result in

29 compositional changes to riparian plant communities and associated changes

30 in ecosystem functioning.

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INTRODUCTION

Woody plants play an important role in determining the physical structure of many riparian ecosystems (Gurnell et al. 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 2100 is projected (IPCC, 2013). Flooding is already a dominant abiotic stress and an important determinant of ecological strategy for woody riparian plants (Blom and Voesenek 1996; Lawson et al. 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 (Naiman et al. 1993; Colmer and Voesenek 2009). Woody colonists of inset channel features such as bars and benches may experience repeated cycles of soil waterlogging (Corenblit et al. 2009), restricting root access to oxygen (Voesenek and Bailey-Serres 2015). Maintaining root

62 respiration in low O_2 conditions requires switching to costly anaerobic

63 metabolic pathways (Drew 1997). The resulting reduction in respiration

64 weakens root function, impairing uptake of water and nutrients (Piedade et al.

65 2010; Voesenek and Bailey-Serres 2015) and inducing suberisation (Steudle

66 2000). Stomatal closure may also take place following waterlogging, reducing

67 available CO_2 for photosynthesis (Kozlowski 1984; Else et al. 2009). Root-

68 zone hypoxia damages roots by disrupting aerobic respiration and causing an

69 "energy crisis" (Colmer and Voesenek 2009); reactive oxygen species (ROS)

70 then form as bi-products of anaerobic metabolism (Santosa et al. 2007), and

71 subsequent re-aeration further increases ROS production (Steffens et al.

72 2013). Production of toxic ions by microbes under anoxic soil conditions

73 causes additional stress to roots (Blom and Voesenek 1996). Waterlogging

74 may also impair rhizomicrobial nodule formation and activity, resulting in

75 reduced nutrient uptake (Dawson et al. 1989; Shimono et al. 2012). The

76 degree to which this combination of stressors influences plant growth is

77 ultimately determined by species' ability to mobilise physiological and

78 morphological responses which mitigate damage (Bailey-Serres and

79 Voesenek 2008).

80   As with waterlogging, atmospheric CO_2 concentration is known to affect

81 plant physiology and growth by altering the fundamental economics of carbon,

82 water and macronutrient uptake and use (Poorter and Navas 2003; Wang et

83 al. 2012; Reich et al. 2014). Individual species responses are variable, but

84 photosynthetic CO_2 assimilation in C3 plants tends to increase under elevated

85 CO_2 (eCO_2) (Curtis 1996). Stomatal conductance is ~~also~~ typically reduced

86 (Ainsworth and Rogers 2007), with attendant gains in water use efficiency

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87   (Holtum and Winter 2010; Keenan et al. 2013; van der Sleen et al. 2014).

88   Biomass accumulation in response to eCO_2 may be enhanced (Wang et al.

89   2012), but this depends on the availability of water and macronutrients

90   (Körner 2006; Manea and Leishman 2014; Reich et al. 2014). Increased

91   allocation of biomass to roots occurs under eCO_2 (Nie et al. 2013) and this

92   effect is interactive with environmental stresses such as drought or low soil

93   fertility (Wang and Taub 2010). Increased rates of production and turnover of

94   fine roots under eCO_2 have been shown in the field, which has important

95   implications for nutrient cycling and ecosystem functioning (Pregitzer et al.

96   1995, 2000; Matamala and Schlesinger 2000; Lipson et al. 2014). eCO_2 is

97   also known to affect functional traits indicative of positions along economic

98   spectra (sensu Reich 2014). Reduction in specific leaf area (SLA) under eCO_2

99   may be linked to accumulation of non-structural carbohydrates in leaves

100 (Poorter and Navas 2003; Bader et al. 2010). Alteration of traits reflecting

101 economic trade-offs is of particular significance at the seedling stage, as

102 functional traits of trees are most strongly adapted to the regeneration niche

103 (Poorter 2007).

104   Taken individually, waterlogging and elevated atmospheric CO_2

105 concentration appear likely to exert opposing effects on plant growth. The

106 possibility that eCO_2 may mitigate growth reduction under waterlogging

107 warrants investigation of the interactive effects of these two important

108 environmental variables. Literature describing interactive effects of

109 atmospheric CO_2 concentration and waterlogging or flooding on plant growth

110 is sparse, and findings thus far present an inconsistent picture. eCO_2

111 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 et al. 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 O2 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 et al. 2014).
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 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 and BassiriRad 2003), and for waterlogged plants, generation of
reactive oxygen species 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 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
hypothesised mechanisms by which such an interactive effect might occur: a.)
higher water use efficiency under eCO_2 (Holtum and Winter 2010) facilitates

137 photosynthesis in plants with anoxia-impaired root functionality by lowering

138 the water cost of carbon assimilation; b.) eCO_2 facilitates biomass recovery by

139 increasing the rate of fine root production during the recovery period

140 (Pregitzer et al. 1995).

141 **METHODS**

142 We selected three riparian tree species native to south-eastern

143 Australia for this study. Casuarina cunninghamiana subsp. cunninghamiana

144 and Eucalyptus camaldulensis subsp. camaldulensis dominate many riparian

145 environments in south-eastern Australia; *Acacia floribunda* is also common in

146 this region. Table 1 provides further information on the biology and ecology of

147 these species.

148 **Experimental procedure**

149 We used a fully factorial design comprising two CO_2 treatments

150 (ambient and elevated), and three waterlogging treatments (non-waterlogged

151 control, waterlogged and waterlogged then re-aerated for a refractory period),

152 with 8 replicates per treatment combination per species. We measured plant

153 physiology (photosynthetic rate, A; stomatal conductance, Gs; and

154 instantaneous water use efficiency, WUE) as well as biomass, biomass

155 allocation and tissue density traits indicative of ecological strategy and

156 position along economic spectra (Reich et al. 2014).

157 Plants were grown individually in pots constructed from 90 mm by 700

158 mm (4.3 L capacity) sections of PVC pipe with drilled endcaps, containing a

159 commercially sourced 80/20 mixture of river sand and soil (Australian Native

160 Landscapes, North Ryde, NSW, Australia). The bottom 2 cm of each pot was

161 filled with gravel (~1 cm particle size) to promote free drainage. 2.5 g L^{-1} of

time-release fertiliser 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) within 48 hours. After two 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 randomised 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 and 28°C. 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.

Trolleys were swapped between replicate glasshouses monthly.

186   Waterlogging was initiated after 90 days of plant growth and lasted 24

187   days, in order to simulate a significant flooding event and to allow time for

188   morphological adaptation to manifest. Plants were randomly assigned to

189   "control", "waterlogged" and "recovery" treatments. "Waterlogged" and

190   "recovery" plants were waterlogged by immersion to within 10 cm of the soil

191   surface in 450 L plastic tubs filled with water. The black tubs were covered

192   with white polythene sheeting to reduce heat absorption. Photosynthetic rate

193   and transpiration rate of plants assigned to the "waterlogged" treatment were

194   measured at the end of the waterlogging period, after which they were

195   harvested. Tubs were drained following the waterlogging period, and "control"

196   and "recovered" treatment plants were grown for a further 23 days before

197   measurement and harvesting.

198   Photosynthetic rate (CO_2 assimilation rate), stomatal conductance and

199   transpiration rate of the newest fully developed leaf were measured for four

200   plants per treatment between 9am and 12:30pm using a Li-Cor 6400XT

201   infrared gas analyser (Li-Cor Inc., Lincoln, NE, USA). Photon flux was set to

202   1500 µmol m^{-2} s^{-1} and temperature was held at 28oC. For leaves which did

203   not completely fill the cuvette, leaf area was measured by digital analysis

204   (ImageJ 1.48 for Windows) of a photograph of the leaf taken against a 2x3

205   cm2 plastic backdrop, which corresponded to the area of the cuvette.

206   Photosynthetic rate and transpiration rate were determined by correcting

207   values according to the measured area. Instantaneous water use efficiency

208   was calculated as the ratio of CO_2 assimilation to transpiration rate.

209   Upon harvesting, roots were washed free of soil and the plant was

210   separated into fine (<1 mm diameter) and coarse (>1 mm diameter, excluding

211 dead root biomass) roots, and aboveground biomass. Five mature (but not

212 senescing) leaves of each individual were selected for determination of

213 specific leaf area (SLA). Fresh leaf area was determined using a LI-3100C

214 Area Meter (Li-Cor Inc., Lincoln, NE, USA); SLA was calculated as the ratio of

215 fresh area to dry mass. A 5 cm section of stem was cut from 1 cm above the

216 root-stem junction for analysis of stem density. The fresh volume of the stem

217 section was measured using the water displacement method and stem wood

218 density was calculated as the ratio of oven dry mass to green volume. Root

219 dry matter content was used as a proxy for root tissue density (Birouste et al.

220 2013). Dry matter content of fine roots was calculated as the ratio of oven dry

221 mass to fresh mass. Samples were dried in an oven at 70^oC for 72 hours and

222 a microbalance (Mettler-Toledo, Greifensee, Switzerland) was used to

223 determine the resulting mass. Root mass fraction was calculated as the ratio

224 of root dry biomass to whole plant dry biomass. Stunted plants with a shoot

225 length of < 5 cm were excluded.

226 **Data analysis:** All statistical analyses were performed using the R statistical

227 programming environment (R Core Team 2013). We used two-way analysis of

228 variance (ANOVA) to test for main effects of and interactions between

229 waterlogging and CO_2 treatments on physiology (photosynthetic rate, stomatal

230 conductance, water use efficiency), biomass (shoot, total root and fine root)

231 and biomass allocation (root mass fraction), and functional traits (fine root dry

232 matter content, stem density, SLA). Metrics of biomass (total, root biomass,

233 shoot biomass) were compared only between "control" and "recovered"

234 treatment plants, as plants which received the "waterlogged" treatment were

235 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 log10 or square

root transformed 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).

RESULTS

Descriptive statistics and significance of ANOVA and post-hoc tests are

shown for all measurements for each combination of treatments in Table 2.

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 = 0.074, Fig. 1a; *C. cunninghamiana*, p =

0.002, Fig. 1b; *E. camaldulensis*, p = 0.037, Fig. 1c). Photosynthetic rate in *E.

camaldulensis* was significantly greater in recovery treatment plants than

control plants (p = 0.008). 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, Fig.

1a).

261   CO$_2$ level had no effect on stomatal conductance for any species, and

262   waterlogging status influenced stomatal conductance only in *E.*

263   *camaldulensis*. Control plants had lower stomatal conductance than

264   waterlogged plants (p = 0.042), and recovering plants (p = 0.0002).

265   Waterlogged *E. camaldulensis* also had lower stomatal conductance than

266   recovering plants (0.059).

267   Water use efficiency in *A. floribunda* was higher in control than

268   waterlogged (p = 0.002), and higher in control than recovery (p = 0.04), but

269   not waterlogged and recovery plants (Fig. 1g). WUE increased under elevated

270   CO$_2$ as a main effect for *E. camaldulensis* (p = 0.002, Fig. 1h), and

271   interactively with CO$_2$ level for *C. cunninghamiana* (p = 0.063); WUE was

272   higher under eCO$_2$ for waterlogged plants (p = 0.022, Fig. 1i) but not control

273   or recovery plants.

274   **Biomass production and allocation:** Waterlogging status and CO$_2$ level

275   interacted strongly for one species: eCO$_2$ stimulation of all fractions of

276   biomass production in *C. cunninghamiana* was diminished following recovery

277   from waterlogging.

278   Total root biomass of plants recovering from waterlogging was lower than

279   control plants for *A. floribunda* (p = 0.028, Fig. 2a). A significant interaction

280   effect was identified for *C. cunninghamiana* (p = 0.049): total root biomass

281   was substantially increased under eCO$_2$ for control (p = 0.011) but not

282   recovery plants (Fig. 2b). Neither CO$_2$ level nor waterlogging had an effect on

283   total root biomass for *E. camaldulensis* (Fig. 2c).

284   Fine root biomass of *A. floribunda* was lower in recovery plants than control

285   plants (p = 0.005), with no CO$_2$ effect (Fig. 2d). A marginally significant

286 interaction effect was also present for *C. cunninghamiana* fine root biomass (p

287 = 0.076); post-hoc analysis confirmed that control but not recovery plants had

288 significantly greater fine root biomass under eCO_2 (p = 0.008) (Fig. 2e).

289 Waterlogging stimulated fine root growth in *E. camaldulensis* (p = 0.046) but

290 CO_2 level had no effect (Fig. 2f).

291 Neither CO_2 level nor waterlogging had any effect on shoot biomass for *A.*

292 *floribunda* (Fig. 2g) or *E. camaldulensis* (Fig. 2i). As with total root biomass

293 and fine root biomass, CO_2 level and waterlogging influenced *C.*

294 *cunninghamiana* biomass interactively (p = 0.009): shoot biomass was higher

295 under eCO_2 for control (p = 0.015) but not recovery plants (Fig. 2h).

296 Root mass fraction (RMF) was decreased by waterlogging for all species, but

297 no significant CO_2 or interaction effects were found (Fig. 2j-l). RMF of *A.*

298 *floribunda* was lower in waterlogged than control plants (p < 0.0001), and

299 lower in waterlogged than recovery plants (p < 0.0001). RMF of *A. floribunda*

300 recovery plants was also lower than control plants (p = 0.016). RMF of both *C.*

301 *cunninghamiana* and *E. camaldulensis* was lower in waterlogged than control

302 plants (p < 0.0001), and lower in waterlogged than recovery plants (p <

303 0.0001), but there was no difference between recovery and control plants.

304 **Functional traits:** We found no evidence to suggest that CO_2 mediates

305 functional traits in response to waterlogging status.

306 Fine root dry matter content (fRDMC) was higher in waterlogged *A. floribunda*

307 than recovery plants (p = 0.027), but not different between control and

308 recovery or control and waterlogged plants. A marginally significant interaction

309 effect was also present for *A. floribunda* (p = 0.067), but no differences were

310 significant upon post-hoc analysis. Waterlogging status also affected *E.*

camaldulensis fRDMC (Fig. 3b): control plants had higher fRDMC than

waterlogged plants ($p = 0.018$), and recovery plants ($p = 0.053$) (marginally

significant). eCO_2 was associated with significantly increased fRDMC in *C.*

cunninghamiana ($p = 0.013$, Fig. 3c), but waterlogging status had no effect.

Waterlogged *A. floribunda* had lower SLA than control ($p = 0.001$), and

recovery plants ($p < 0.0001$) (Fig. 3 d). Waterlogged *E. camaldulensis* had

higher SLA than control ($p = 0.0013$) and recovery plants ($p = 0.0006$) (Fig.

3f). Waterlogging status had no effect on *C. cunninghamiana* SLA (Fig. 3e).

CO_2 level had no effect on the SLA of any species.

Stem density in *C. cunninghamiana* was increased under elevated CO_2 ($p =$

0.0177) (Fig. 3h). Stem density was lower in waterlogged *C. cunninghamiana*

than control ($p = 0.0167$) or recovery plants (0.050) Neither CO_2 nor

waterlogging status had any effect on stem density of *A. floribunda* (Fig. 3g)

or *E. camaldulensis* (3i).

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.

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 reactive oxygen species and

metal ions, and morphological adaptations such as formation of adventitious

roots may act as energetic sinks (Colmer and Voesenek 2009). Relationships

336 between photosynthetic rate and biomass responses to waterlogging and CO_2

337 level treatments in this study varied widely between species.

338   For the three species studied here, only for *C. cunninghamiana* was an

339 interactive effect of CO_2 concentration and waterlogging status found.

340 Biomass of shoot, total root and fine root fractions was significantly higher

341 under eCO_2 for control *C. cunninghamiana* plants, but not for plants which

342 were recovering from waterlogging, despite increased rates of CO_2

343 assimilation. No significant interaction effect on root mass fraction was found,

344 but visual inspection of the data (Fig. 2k) indicates that eCO_2 stimulation of

345 RMF was present in control and recovering, but not waterlogged plants. Re-

346 establishment of pre-waterlogging biomass allocation appears to have

347 occurred despite no differences in total biomass. We found no evidence to

348 support the hypothesis that eCO_2 facilitated biomass recovery by increasing

349 the rate of fine root production in *C. cunninghamiana* after waterlogging.

350 Photosynthesis remained higher in recovering plants under eCO_2, indicating

351 that their ability to convert the extra photosynthate produced under eCO_2 into

352 biomass was impaired by waterlogging.

353   No increase in any biomass fraction was associated with increased

354 photosynthetic rate under eCO_2 for either *A. floribunda* or *E. camaldulensis*.

355 *A. floribunda* underwent substantial root mortality in response to waterlogging,

356 although the presence of spongy white aerenchymous adventitious roots

357 indicated a degree of morphological adaptation to anoxia (Evans 2004).

358 Conversely, waterlogging stimulated fine root growth in *E. camaldulensis*. A

359 proliferation of fine aerenchymous roots both below and above the water line

360 was observed in waterlogged and recovered plants, corresponding to

361 increased fine root mass compared with control plants. The strong

362 morphological response of *E. camaldulensis* root systems combined with

363 higher photosynthetic rate in recovering compared with control plants, and

364 higher stomatal conductance in waterlogged plants than control or recovering

365 plants, indicates that *E. camaldulensis* responded favourably to waterlogging

366 in this study. This growth response concurs with the results of previous

367 studies (Sena-Gomes & Kozlowski 1980; Marcar 1993, although see

368 Kogawara et al. 2006). No evidence was found to support the hypothesis that

369 higher water use efficiency under eCO_2 might facilitate photosynthesis where

370 waterlogging had caused stomatal closure. WUE was altered by waterlogging

371 only in *A. floribunda*, and by CO_2 level only in *E. camaldulensis*. WUE was

372 dependent on the combination of waterlogging status and CO_2 level in *C.*

373 *cunninghamiana*, being higher at eCO_2 than aCO_2 for waterlogged plants

374 only. The lack of stomatal response to waterlogging indicates that higher

375 WUE under eCO_2 is not the mechanism maintaining photosynthetic rate under

376 waterlogging for *C. cunninghamiana*.

377   Waterlogging and atmospheric CO_2 level also altered functional traits in

378 a species-specific manner, but no interactive effects were found. Traits of *A.*

379 *floribunda* and *E. camaldulensis* were affected by waterlogging status but not

380 CO_2 level, whereas *C. cunninghamiana* was affected by CO_2. Decreased SLA

381 and increased fine root dry matter content – a proxy for fine root tissue density

382 (Birouste et al. 2013) – in waterlogged *A. floribunda* indicate a shift towards

383 the slower growth – longer lifespan end of their respective economic spectra

384 (Reich 2014), but this shift was not sustained following the refractory period. A

385 corresponding pattern in water use efficiency corroborates this inference.

386 Higher root dry matter content under waterlogging has been linked to the

387 requirement for structural support of air spaces in aerenchymous root tissue

388 (Ryser et al. 2011). Suberization of root hypodermal tissue often occurs under

389 waterlogging as a means of reducing radial oxygen loss (Visser et al. 2000;

390 De Simone et al. 2002) and may also increase root dry matter content. *E.*

391 *camaldulensis* responded in an opposite manner, with higher SLA under

392 waterlogging, and lower root dry matter content under waterlogging and after

393 the refractory period. This species appears to employ an opportunistic 'fast

394 growth' ecological strategy in response to waterlogging, involving proliferation

395 of lower density roots, and lower carbon investment in leaf tissue (Wright et al.

396 2004; Reich 2014). We found no evidence for decreased SLA under eCO_2 as

397 previously described (Poorter and Navas 2003). Previous studies report

398 inconsistent effects of eCO_2 on fine root dry matter content in non-riparian

399 species: eCO_2 had no effect on *Liquidambar styraciflua* or *Pinus strobus*

400 fRDMC (Bauer and Berntson 2001; Iversen et al. 2008), caused a small

401 decrease in *Betula alleghaniensis* (Bauer and Berntson 2001) and increased

402 fRDMC in cotton (Prior et al. 1994). In this study, eCO_2 significantly increased

403 fine root dry matter content in *C. cunninghamiana* irrespective of waterlogging

404 treatment.

405   Analysis of gas exchange, biomass accumulation and functional traits

406 after a refractory period provided an opportunity to determine whether

407 responses to waterlogging persisted or were transitory. We were unable to

408 substantiate the hypothesis that eCO_2 would increase the rate of biomass

409 recovery from waterlogging by increasing the rate of fine root turnover. *C.*

410 *cunninghamiana* was the only species for which eCO_2 altered biomass

411 accumulation, and depression of biomass was observed following the

412 refractory period irrespective of CO_2 level. Although we made no analysis of

413 nodulation rates, nodulation of *C. cunninghamiana* by the nitrogen fixing

414 ascomycete Frankia is known to be highest under well aerated soil conditions

415 (Dawson et al. 1989). Reduced nitrogen uptake due to nodule mortality or

416 impairment could account for the constrained biomass response to eCO_2

417 post-waterlogging (Reich et al. 2006). While eCO_2 did not mitigate growth

418 reduction or mediate changes to functional traits under waterlogging for any

419 species in this glasshouse study, we did observe reduced growth stimulation

420 by eCO_2 in one species. This effect was strong, and evident across all

421 measured biomass fractions. Differential responses to eCO_2 and waterlogging

422 between species in the field could have important ecological consequences.

423 *C. cunninghamiana* is a highly effective agent of 'biogeomorphic succession'

424 in fluvial landscape of south-eastern Australia – that is, it facilitates the

425 creation and stabilisation of fluvial landforms (Erskine and Chalmers 2009).

426 Reduction of eCO_2 biomass stimulation by waterlogging could alter spatial

427 patterns of landform stabilisation by *C. cunninghamiana*. Infrequently

428 waterlogged stands on channel banks might be favoured over stands growing

429 on wetter in-channel features such as bars, benches and islands. Differential

430 responses to combined waterlogging and eCO_2 between species – notably *C.*

431 *cunninghamiana* and *A. floribunda*, which are frequently conspecific – may

432 also result in compositional changes to riparian plant communities and

433 associated changes in ecosystem functioning.

434 **CONCLUSIONS**

435   Waterlogging and atmospheric CO_2 concentration both have significant

436   consequences for physiological processes, growth and functional

437   characteristics of riparian tree seedlings. The relative importance of these

438   environmental factors varies according to species, as do the specific effects of

439   each on plants. This study adds to the small but growing body of literature

440   describing the interactive effects of waterlogging and CO_2 concentration;

441   notably, the outcome for *C. cunninghamiana* concurs with that found for

442   *Taxodium distichum*, a flood tolerant colonist of alluvial riparian areas in the

443   south eastern United States (Megonigal et al. 2005). Blunting of eCO_2

444   biomass stimulation in seedlings by waterlogging has the potential to alter

445   demographics and structural dynamics in many Australian riparian

446   communities especially where *C. cunninghamiana* is a keystone species

447   (Woolfrey and Ladd 2001).

448   **LITERATURE CITED**

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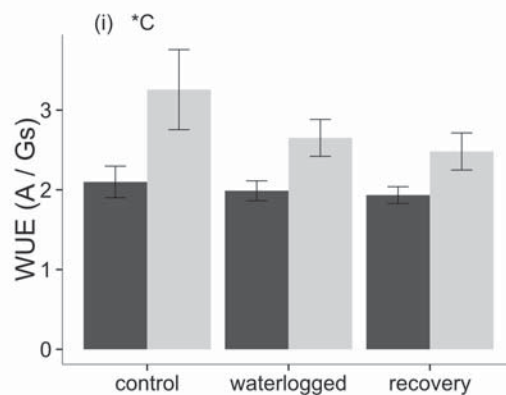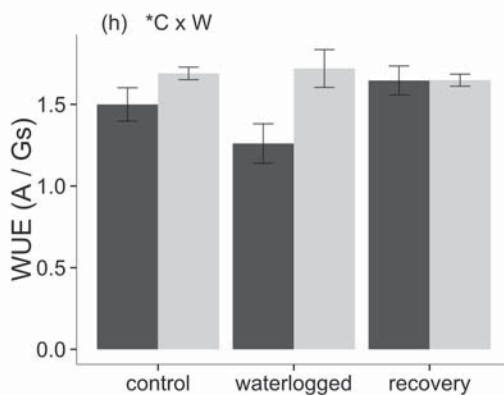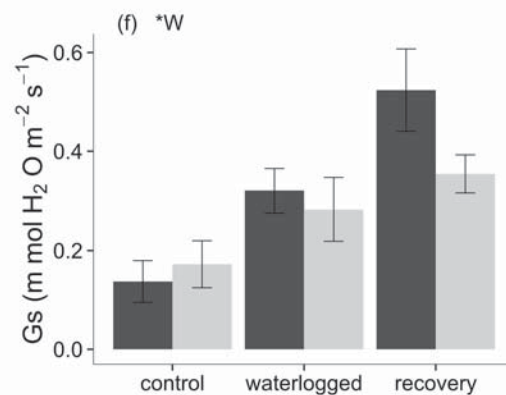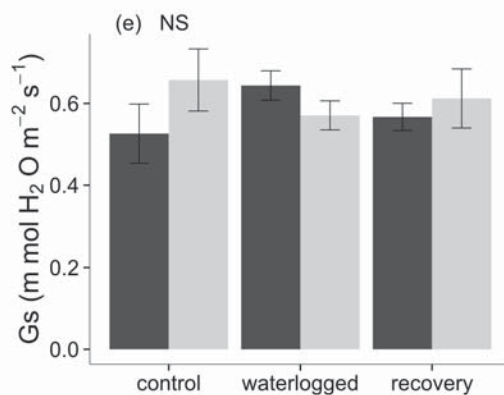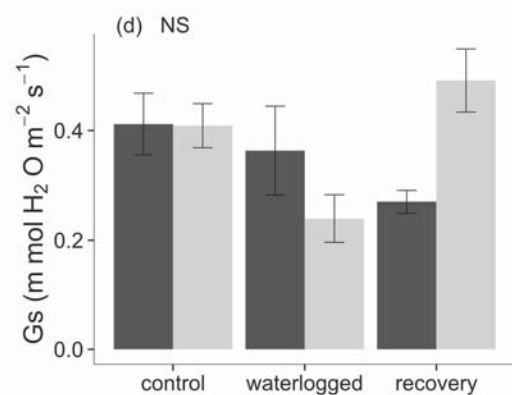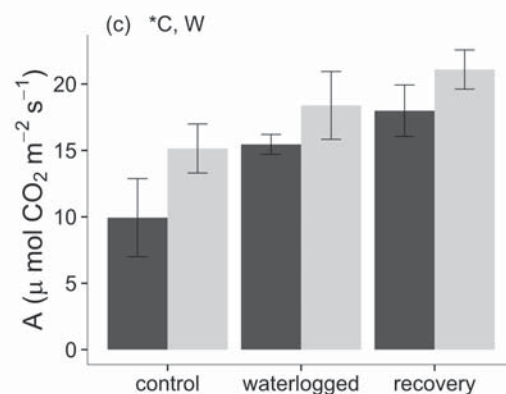
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FIGURE LEGENDS

Figure 1. Gas exchange measurements under each combination of waterlogging and CO_2 level treatments. Dark shaded columns represent measurements under ambient atmospheric CO_2 concentration (390 ppm), light shaded columns represent measurements under elevated atmospheric CO_2 concentration (550 ppm). Error bars represent the standardised 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).
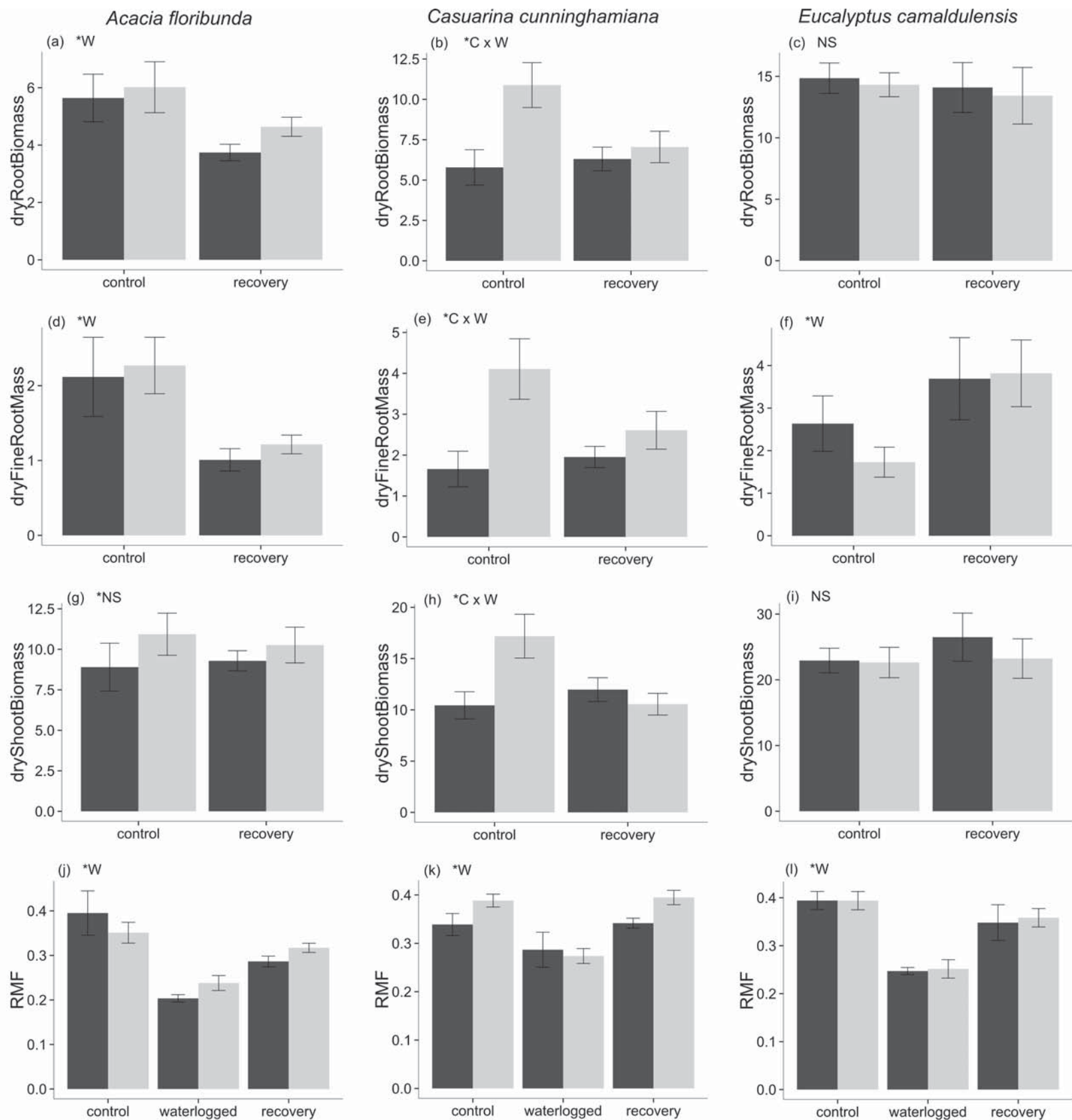
Figure 2. Biomass and root mass fraction (RMF) measurements under each combination of waterlogging and CO_2 level treatments. Dark shaded columns represent measurements under ambient CO_2 concentration (390 ppm), light shaded columns represent measurements under elevated CO_2 concentration (550 ppm). Error bars represent the standardised 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).

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), light shaded columns represent measurements under elevated CO_2 concentration (550 ppm). Error bars represent the standardised mean error. * - letters denote statistical significance of differences between treatment combinations (NS =

655   no significant difference, C = significant difference between CO_2 level

656   treatments, W = significant difference between waterlogging treatments).

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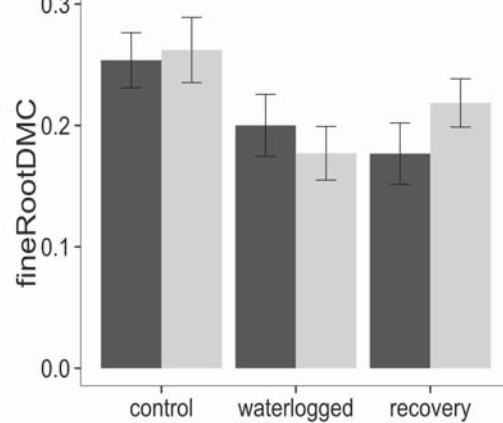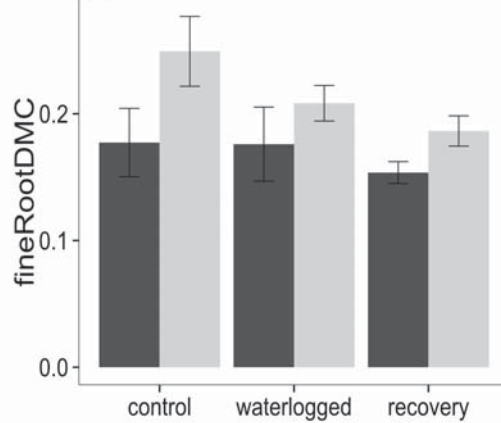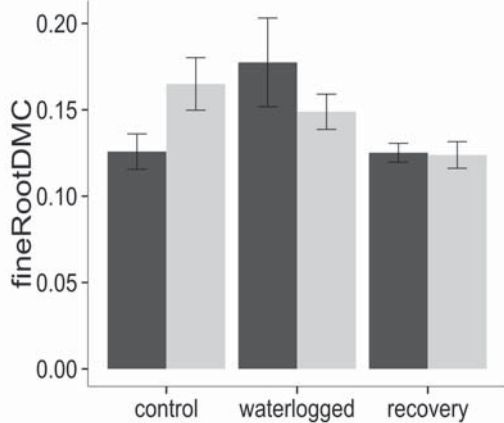*Acacia floribunda*   *Casuarina cunninghamiana*   *Eucalyptus camaldulensis*
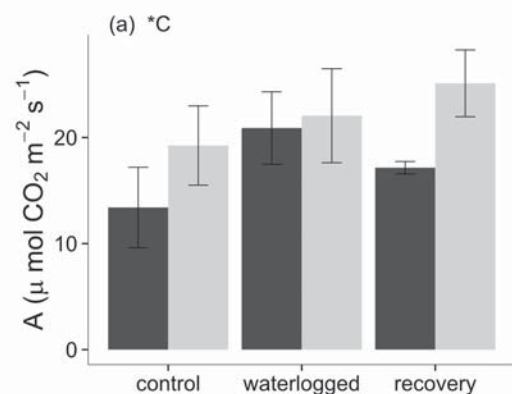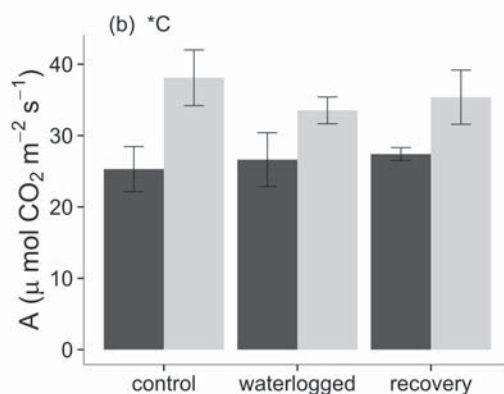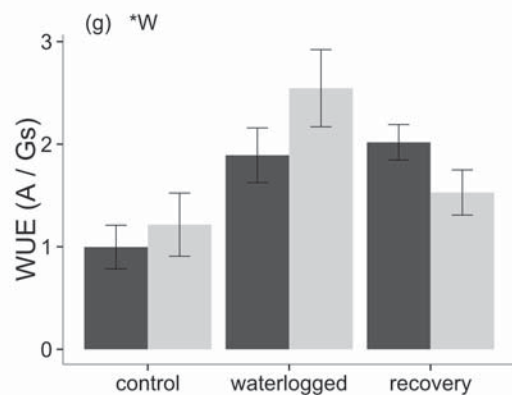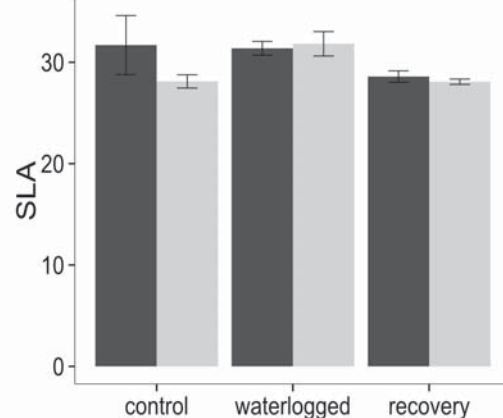
(a) *C   (b) *C   (c) *C, W

(d) NS   (e) NS   (f) *W

(g) *W   (h) *C x W   (i) *C

A (μ mol CO_2 m^{-2} s^{-1})

Gs (m mol H_2O m^{-2} s^{-1})

WUE (A / Gs)

control   waterlogged   recovery

Columns (left to right): *Acacia floribunda*, *Casuarina cunninghamiana*, *Eucalyptus camaldulensis*

(a) *W — dryRootBiomass
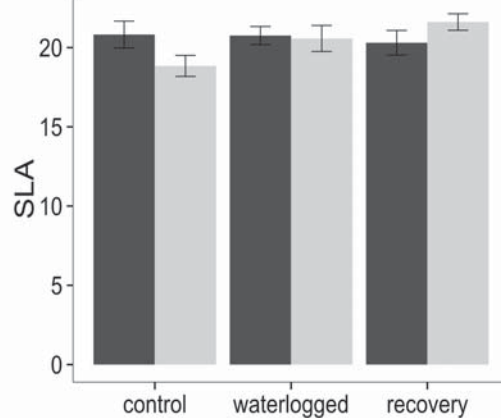(b) *C x W — dryRootBiomass
(c) NS — dryRootBiomass

(d) *W — dryFineRootMass
(e) *C x W — dryFineRootMass
(f) *W — dryFineRootMass

(g) *NS — dryShootBiomass
(h) *C x W — dryShootBiomass
(i) NS — dryShootBiomass

(j) *W — RMF
(k) *W — RMF
(l) *W — RMF

Figure showing fineRootDMC, SLA, and stemDensity for three species (*Acacia floribunda*, *Casuarina cunninghamiana*, *Eucalyptus camaldulensis*) under control, waterlogged, and recovery treatments.
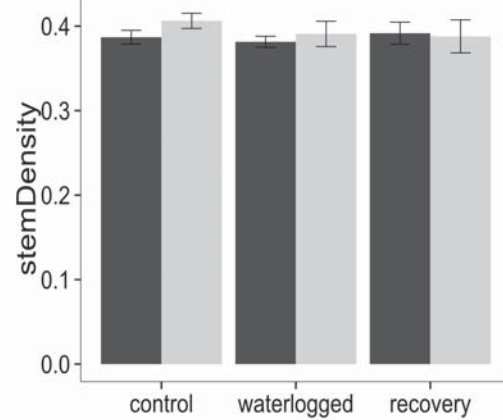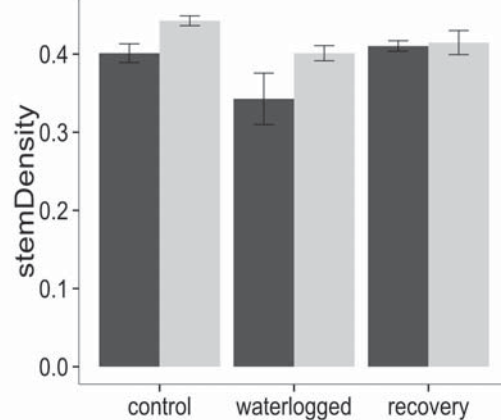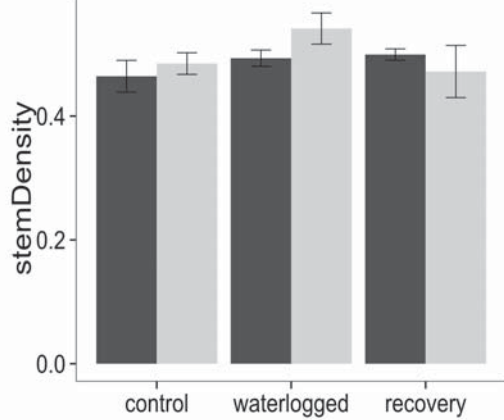
Table 1. Biological and ecological attributes of study species.

	Acacia floribunda	*Casuarina cunninghamiana subsp. cunninghamiana*	*Eucalyptus camaldulensis subsp. camaldulensis*
Family	Fabaceae	Casuarinaceae	Myrtaceae
Distribution	Coastal areas of eastern Australia[1]	Eastern NSW and QLD, Australia. Other subsp. in Gulf of Carpentaria and Papua New Guinea[1]	Inland riparian areas throughout south-eastern Australia. Other subsp. distributed throughout continental Australia[1]
Morphology	Erect or spreading shrub or tree, 3–8 m high[1]. Rooting depth 2 m +[2]	Erect tree, 15–35 m high[1]. Rooting depth to 8 m[2]	Large, spreading tree, 30+ m high[1]. Rooting depth 10 m +[2]
Habitat	Facultative rheophyte. Found in sclerophyll forest, particularly along watercourses and in sandy alluvial soils. Typically on channel banks and raised within-channel features[1]	Obligate rheophyte. Found along permanent watercourses, on substrates ranging from sand to large cobbles. Often found on bars, benches and channel islands[1]	Obligate rheophyte. Found on deep, rich alluvial soils, on banks and flood plains associated with large, permanent water bodies[1]
Community status	Common[1]	Dominant[1]	Dominant[1]
Nitrogen fixing ability	Nodulated with *Rhizobium*[3]	Nodulated with *Frankia*[4]	None
Biogeomorphic effects	Colonist of fresh geomorphic substrates[?]	Ecosystem engineer. Rapid, *en mass* colonisation and stabilisation of fresh geomorphic substrates. Established trees stabilise banks and in-channel features[2]	Ecosystem engineer. Established trees define physical structure of riparian landscapes. Highly effective at mitigation of flooding-induced landform mass failure[2]

[1] Royal Botanic Gardens and Domain Trust (2015), [2] Hubble, Docker & Rutherfurd (2010), [3] Roughley (1987), [4] Dawson *et al.* (1989), [5] J. Lawson personal field observations

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. 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.

	Control		Waterlogged		Recovery		Significant effect	Post-hoc
	eCO_2	aCO_2	eCO_2	aCO_2	eCO_2	aCO_2		
Acacia floribunda								
Photosynthetic rate (A, μmol 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 (cm² 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 (cm² 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 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 (cm² 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 (cm² 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 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 (cm² 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 (cm² 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	