

Chapter 1

Interactive effects of
waterlogging and atmospheric
CO₂ concentration on gas
exchange, growth and
functional traits of Australian
riparian tree seedlings

Abstract

The ability to survive and thrive in repeatedly waterlogged soils is characteristic of plants adapted to riparian habitats. Rising atmospheric CO₂ 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 CO₂ individually are relatively well characterised, few studies have asked how the effects of waterlogging might be mediated by atmospheric CO₂ concentration. We investigated interactive effects between elevated (550 ppm) atmospheric CO₂ 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 CO₂ 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 CO₂ concentration and waterlogging status on growth, gas exchange and functional traits between species, and no evidence for a consistent effect of elevated CO₂ in mediating plant responses to flooding. For one species, *Casuarina cunninghamiana*, elevated CO₂ 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 CO₂ between species may result in compositional changes to riparian plant communities and associated changes in ecosystem functioning.

Keywords

Climate change, elevated CO₂, flooding, plant functional traits, riparian, waterlogging

1.1 Introduction

Woody plants play an important role in determining the physical structure of many riparian ecosystems (Lawson et al., 2015), 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 CO₂ has also risen substantially over the past century, and a doubling of pre-industrial levels by 2100 is projected (IPCC et al., 2014). 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 CO₂ on plants at multiple scales, little is known about how the effects of flooding might be mediated by atmospheric CO₂ 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 and Voesenek, 2009; Naiman et al., 1993). 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 respiration in low O₂ 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 et al., 2010; Voesenek and Bailey-Serres, 2015) and inducing suberisation (Steudle, 2000). Stomatal closure may also take place following waterlogging, reducing available CO₂ for photosynthesis (Else et al., 2009; Kozlowski, 1984). Root-zone hypoxia damages roots by disrupting aerobic respiration

and causing an energy crisis (Colmer and 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 et al., 2013). Production of toxic ions by microbes under anoxic soil conditions causes additional stress to roots (Blom and Voesenek, 1996). Waterlogging may also impair rhizomicrobial nodule formation and activity, resulting in reduced nutrient uptake (Dawson et al., 1989; Shimono et al., 2012). The degree to which this combination of stressors influences plant growth is ultimately determined by species ability to mobilise physiological and morphological responses which mitigate damage Bailey-Serres2008.

As with waterlogging, atmospheric CO₂ concentration is known to affect plant physiology and growth by altering the fundamental economics of carbon, water and macronutrient uptake and use (Poorter and Navas, 2003; Reich et al., 2014; Wang et al., 2012). Individual species responses are variable, but photosynthetic CO₂ assimilation in C₃ plants tends to increase under elevated CO₂ (eCO₂) (Curtis, 1996). Stomatal conductance is also typically reduced (Ainsworth and Rogers, 2007), with attendant gains in water use efficiency (Holtum and Winter, 2010; Keenan et al., 2013; van der Sleen et al., 2014). Biomass accumulation in response to eCO₂ may be enhanced (Wang et al., 2012), but this depends on the availability of water and macronutrients (Körner, 2006; Manea and Leishman, 2014; Reich et al., 2014). Increased allocation of biomass to roots occurs under eCO₂ (Nie et al., 2013) and this effect is interactive with environmental stresses such as drought or low soil fertility (Wang and Taub, 2010). Increased rates of production and turnover of fine roots under eCO₂ have been shown in the field, which has important implications for nutrient cycling and ecosystem functioning (Lipson et al., 2014; Matamala and Schlesinger, 2000; Pregitzer et al., 2000, 1995). eCO₂ is also known to affect functional traits indicative of positions along economic spectra (*sensu* Reich 2014). Reduction in specific leaf area (SLA) under eCO₂ may be linked to accumulation of non-structural carbohydrates in leaves (Bader et al., 2010; Poorter and 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 CO₂ concentration appear likely to exert opposing effects on plant growth. The possibility that eCO₂ may mitigate growth reduction under waterlogging warrants investigation of the interactive effects of these two important environmental variables. Literature describing interactive effects of atmospheric CO₂ concentration and waterlogging or flooding on plant growth is sparse, and findings thus far present an inconsistent picture. eCO₂ 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 eCO₂ 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 eCO₂ 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 eCO₂ (Arenque et al., 2014). Finally, no evidence for an interaction between CO₂ 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 eCO₂ 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 eCO₂ and waterlogging on gas exchange, biomass accumulation and allocation, and functional traits for riparian tree species. In particular, we were interested in whether eCO₂ 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 oc-

cur: a.) higher water use efficiency under eCO₂ (Holtum and Winter, 2010) facilitates photosynthesis in plants with anoxia-impaired root functionality by lowering the water cost of carbon assimilation; b.) eCO₂ facilitates biomass recovery by increasing the rate of fine root production during the recovery period (Pregitzer et al., 1995).

1.2 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.

Experimental procedure

We used a fully factorial design comprising two CO₂ treatments (ambient and elevated), and three waterlogging treatments (non-waterlogged control, waterlogged and waterlogged then re-aerated for a refractory period), with 8 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. 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.

Table 1.1: Biological and ecological attributes of study species.

	<i>Acacia floribunda</i>	<i>Casuarina cunninghamiana</i> subsp. <i>cunninghamiana</i>	<i>Eucalyptus camaldulensis</i> subsp. <i>camaldulensis</i>
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, 38 m high ¹ . Rooting depth 2 m +2	Erect tree, 1535 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

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

CO₂ treatment (380-400 ppm and 530-570 ppm) were used in two replicate glasshouses per level. These CO₂ 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 CO₂ concentration, while the higher range reflects the predicted atmospheric CO₂ concentration in 2050 (IPCC et al., 2014). 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.

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. 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 (CO₂ assimilation rate), stomatal conductance and transpiration rate of the newest fully developed leaf were measured for four plants per treatment between 9am and 12:30pm using a Li-Cor 6400XT infrared gas analyser (Li-Cor Inc., Lincoln, NE, USA). Photon flux was set to 1500 mol 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 2x3 cm² 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 CO₂ 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 specific leaf area (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 et al., 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 hours and a microbalance (Mettler-Toledo, Greifensee, Switzerland) was used to determine the resulting mass. Root mass fraction 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.

Data analysis

All statistical analyses were performed using the R statistical programming environment (R Core Team, 2015). We used two-way analysis of variance (ANOVA) to test for main effects of and interactions between waterlogging and CO₂ treatments on physiology (photosynthetic rate, stomatal conductance, water use efficiency), biomass (shoot, total root and fine root) and biomass allocation (root mass fraction), and functional traits (fine root dry matter content, stem density, SLA). Metrics of biomass (total, root biomass, shoot biomass) were compared only between control and recovered treatment plants, as plants which received the waterlogged treatment were younger at harvest. Post-hoc comparison (Tukeys 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 (?). 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 ($n = 8$).

1.3 Results

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

Gas exchange and water-use efficiency

Effects of CO₂ level and waterlogging on gas exchange were species specific, and although some significant interactions were found between CO₂ and waterlogging, we found no evidence that interactive effects were maintained following recovery from waterlogging.

Elevated CO₂ 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 CO₂ level and waterlogging status for photosynthetic rate, although waterlogged *A. floribunda* exhibited only a small difference in mean photosynthetic rate between CO₂ treatments (20.9 and 22.6 mol CO₂ m⁻² s⁻¹, respectively, Fig. 1a).

CO₂ 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 ($p = 0.042$), and recovering plants ($p = 0.0002$). Waterlogged *E. camaldulensis* also had lower stomatal conductance than recovering plants (0.059).

Water use efficiency in *A. floribunda* was higher in control than waterlogged ($p = 0.002$), and higher in control than recovery ($p = 0.04$), but not waterlogged and recovery plants (Fig. 1g). WUE increased under elevated CO₂ as a main effect for *E. camaldulensis* ($p = 0.002$, Fig. 1h), and interactively with CO₂ level for *C. cunninghamiana* ($p = 0.063$); WUE was higher under eCO₂ for waterlogged plants ($p = 0.022$, Fig. 1i) but not control or recovery plants.

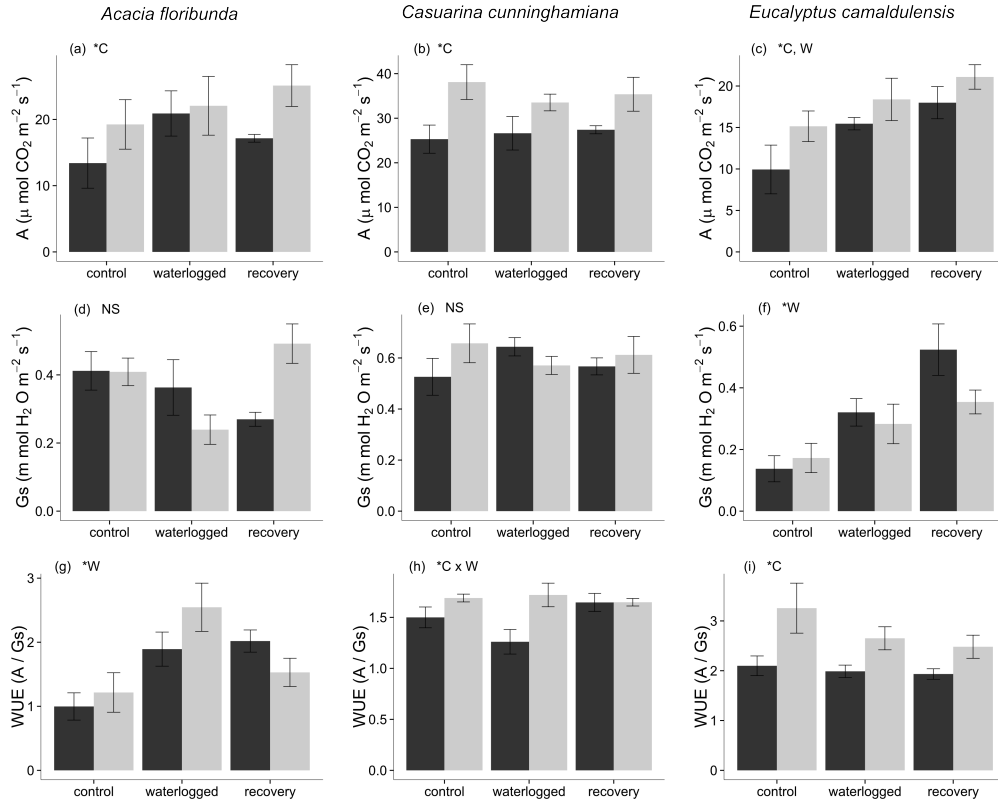


Figure 1.1: Gas exchange measurements under each combination of waterlogging and CO₂ level treatments. Dark shaded columns represent measurements under ambient atmospheric CO₂ concentration (390 ppm), light shaded columns represent measurements under elevated atmospheric CO₂ concentration (550 ppm). Error bars represent the standardised mean error. * - letters denote statistical significance of differences between treatment combinations (NS = no significant difference, C = significant difference between CO₂ level treatments, W = significant difference between waterlogging treatments).

Biomass production and allocation

Waterlogging status and CO₂ level interacted strongly for one species: eCO₂ 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* ($p = 0.028$, Fig. 2a). A significant interaction effect was identified for *C. cunninghamiana* ($p = 0.049$): total

root biomass was substantially increased under eCO₂ for control ($p = 0.011$) but not recovery plants (Fig. 2b). Neither CO₂ level nor waterlogging had an effect on total root biomass for *E. camaldulensis* (Fig. 2c).

Fine root biomass of *A. floribunda* was lower in recovery plants than control plants ($p = 0.005$), with no CO₂ effect (Fig. 2d). A marginally significant interaction effect was also present for *C. cunninghamiana* fine root biomass ($p = 0.076$); post-hoc analysis confirmed that control but not recovery plants had significantly greater fine root biomass under eCO₂ ($p = 0.008$) (Fig. 2e). Waterlogging stimulated fine root growth in *E. camaldulensis* ($p = 0.046$) but CO₂ level had no effect (Fig. 2f).

Neither CO₂ level nor waterlogging had any effect on shoot biomass for *A. floribunda* (Fig. 2g) or *E. camaldulensis* (Fig. 2i). As with total root biomass and fine root biomass, CO₂ level and waterlogging influenced *C. cunninghamiana* biomass interactively ($p = 0.009$): shoot biomass was higher under eCO₂ for control ($p = 0.015$) but not recovery plants (Fig. 2h).

Root mass fraction (RMF) was decreased by waterlogging for all species, but no significant CO₂ or interaction effects were found (Fig. 2j-l). RMF of *A. floribunda* was lower in waterlogged than control plants ($p < 0.0001$), and lower in waterlogged than recovery plants ($p < 0.0001$). RMF of *A. floribunda* recovery plants was also lower than control plants ($p = 0.016$). RMF of both *C. cunninghamiana* and *E. camaldulensis* was lower in waterlogged than control plants ($p < 0.0001$), and lower in waterlogged than recovery plants ($p < 0.0001$), but there was no difference between recovery and control plants.

Functional traits

We found no evidence to suggest that CO₂ mediates functional traits in response to waterlogging status.

Fine root dry matter content (fRDMC) was higher in waterlogged *A. floribunda* than recovery plants ($p = 0.027$), but not different between control and recovery or control and waterlogged plants. A marginally signifi-

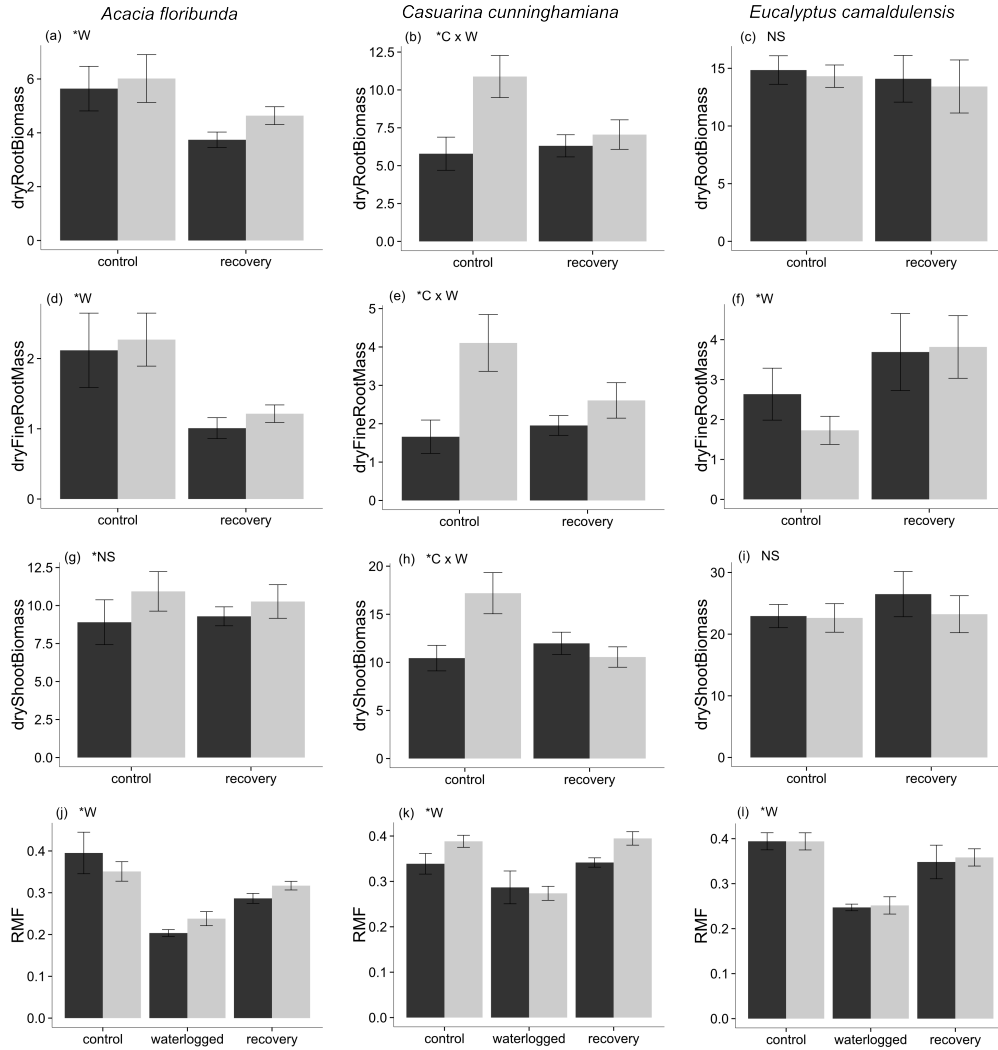


Figure 1.2: Biomass and root mass fraction (RMF) measurements under each combination of waterlogging and CO₂ level treatments. Dark shaded columns represent measurements under ambient CO₂ concentration (390 ppm), light shaded columns represent measurements under elevated CO₂ concentration (550 ppm). Error bars represent the standardised mean error. * - letters denote statistical significance of differences between treatment combinations (NS = no significant difference, C = significant difference between CO₂ level treatments, W = significant difference between waterlogging treatments).

cant interaction effect was also present for *A. floribunda* ($p = 0.067$), but no differences were significant upon post-hoc analysis. Waterlogging sta-

tus 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). eCO₂ 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). CO₂ level had no effect on the SLA of any species.

Stem density in *C. cunninghamiana* was increased under elevated CO₂ ($p = 0.0177$) (Fig. 3h). Stem density was lower in waterlogged *C. cunninghamiana* than control ($p = 0.0167$) or recovery plants (0.050) Neither CO₂ nor waterlogging status had any effect on stem density of *A. floribunda* (Fig. 3g) or *E. camaldulensis* (3i).

1.4 Discussion

We found inconsistent effects of atmospheric CO₂ 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 CO₂ 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 between photosynthetic rate and biomass responses to waterlogging and CO₂ level treatments in this study varied widely between species.

For the three species studied here, only for *C. cunninghamiana* was an interactive effect of CO₂ concentration and waterlogging status found. Biomass of shoot, total root and fine root fractions was significantly higher

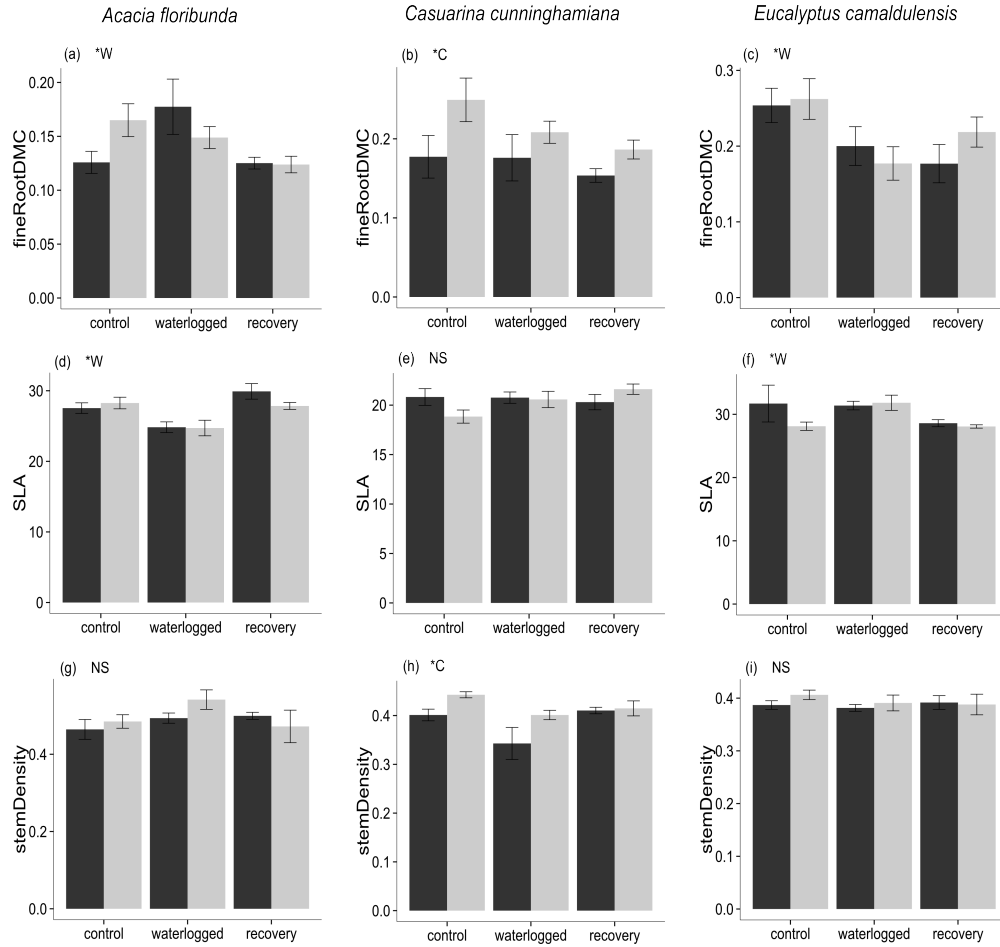


Figure 1.3: Functional trait measurements under each combination of waterlogging and CO₂ level treatments. Dark shaded columns represent measurements under ambient CO₂ concentration (390 ppm), light shaded columns represent measurements under elevated CO₂ concentration (550 ppm). Error bars represent the standardised mean error. * - letters denote statistical significance of differences between treatment combinations (NS = no significant difference, C = significant difference between CO₂ level treatments, W = significant difference between waterlogging treatments).

under eCO₂ for control *C. cunninghamiana* plants, but not for plants which were recovering from waterlogging, despite increased rates of CO₂ assimilation. No significant interaction effect on root mass fraction was found, but visual inspection of the data (Fig. 2k) indicates that eCO₂ 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 eCO₂ facilitated biomass recovery by increasing the rate of fine root production in *C. cunninghamiana* after waterlogging. Photosynthesis remained higher in recovering plants under eCO₂, indicating that their ability to convert the extra photosynthate produced under eCO₂ into biomass was impaired by waterlogging.

No increase in any biomass fraction was associated with increased photosynthetic rate under eCO₂ 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 control or recovering plants, indicates that *E. camaldulensis* responded favourably to waterlogging in this study. This growth response concurs with the results of previous studies (Marcar, 1993; Sena-Gomes and Kozlowski, 1980), although see (Kogawara et al., 2006). No evidence was found to support the hypothesis that higher water use efficiency under eCO₂ might facilitate photosynthesis where waterlogging had caused stomatal closure. WUE was altered by waterlogging only in *A. floribunda*, and by CO₂ level only in *E. camaldulensis*. WUE was dependent on the combination of waterlogging status and CO₂ level in *C. cunninghamiana*, being higher at eCO₂ than aCO₂ for waterlogged plants only. The lack of stomatal response to waterlogging indicates that higher WUE under eCO₂ is not the mechanism maintaining photosynthetic rate under waterlogging for *C. cunninghamiana*.

Waterlogging and atmospheric CO₂ 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 CO₂ level, whereas *C. cunninghamiana* was affected by CO₂. Decreased SLA and increased fine root dry matter content 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 et al., 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 et al., 2011). Suberization of root hypodermal tissue often occurs under waterlogging as a means of reducing radial oxygen loss (De Simone et al., 2002; Visser et al., 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. This 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 et al., 2014; Wright et al., 2004). We found no evidence for decreased SLA under eCO₂ as previously described (Poorter and Navas, 2003). Previous studies report inconsistent effects of eCO₂ on fine root dry matter content in non-riparian species: eCO₂ had no effect on *Liquidambar styraciflua* or *Pinus strobus* fRDMC (Bauer and Berntson, 2001; Iversen et al., 2008), caused a small decrease in *Betula alleghaniensis* (Bauer and Berntson, 2001) and increased fRDMC in cotton (Prior et al., 1994). In this study, eCO₂ significantly increased fine root dry matter content 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 eCO₂ would increase the rate of biomass

recovery from waterlogging by increasing the rate of fine root turnover. *C. cunninghamiana* was the only species for which eCO₂ altered biomass accumulation, and depression of biomass was observed following the refractory period irrespective of CO₂ level. 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 eCO₂ post-waterlogging (Reich et al., 2006). While eCO₂ 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 eCO₂ in one species. This effect was strong, and evident across all measured biomass fractions. Differential responses to eCO₂ 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 stabilisation of fluvial landforms (Erskine et al., 2009). Reduction of eCO₂ biomass stimulation by waterlogging could alter spatial patterns of landform stabilisation by *C. cunninghamiana*. Infrequently waterlogged stands on channel banks might be favoured over stands growing on wetter in-channel features such as bars, benches and islands. Differential responses to combined waterlogging and eCO₂ between species notably *C. cunninghamiana* and *A. floribunda*, which are frequently conspecific may also result in compositional changes to riparian plant communities and associated changes in ecosystem functioning.

1.5 Conclusion

Waterlogging and atmospheric CO₂ 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 CO₂ concentration; 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). Blunting of eCO₂ 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 and Ladd, 2001).

Acknowledgements

We would like to thank Urvashi Lallu, Claire Laws, Samiya Tabassum and Daniel Sloane for their help in the glasshouse, and Anthony Manea, Brian Atwell and Melanie Zeppel for providing advice on study design and implementation.

References

- Ainsworth, E. A. and Rogers, A. (2007). The response of photosynthesis and stomatal conductance to rising [CO₂]: Mechanisms and environmental interactions. *Plant, Cell and Environment*, 30:258–270.
- Arenque, B. C., Grandis, A., Pocius, O., de Souza, A. P., and Buckeridge, M. S. (2014). Responses of *Senna reticulata*, a legume tree from the Amazonian floodplains, to elevated atmospheric CO₂ concentration and waterlogging. *Trees*, 28(4):1021–1034.
- Bader, M. K. F., Siegwolf, R., and Körner, C. (2010). Sustained enhancement of photosynthesis in mature deciduous forest trees after 8 years of free air CO₂ enrichment. *Planta*, 232:1115–1125.
- Bauer, G. a. and Berntson, G. M. (2001). Ammonium and nitrate acquisition by plants in response to elevated CO₂ concentration: the roles of root physiology and architecture. *Tree Physiology*, 21:137–144.
- Birouste, M., Zamora-Ledezma, E., Bossard, C., Pérez-Ramos, I. M., and Roumet, C. (2013). Measurement of fine root tissue density: a comparison of three methods reveals the potential of root dry matter content. *Plant and Soil*, 374(1-2):299–313.
- Blom, C. W. P. M. and Voesenek, L. A. C. J. (1996). Flooding: the survival strategies of plants. *Trends in Ecology & Evolution*, 11(7):290–295.
- Colmer, T. D. and Voesenek, L. A. C. J. (2009). Flooding tolerance: suites of plant traits in variable environments. *Functional Plant Biology*, 36(1986):665–681.

- Corenblit, D., Steiger, J., Gurnell, A. M., Tabacchi, E., and Roques, L. (2009). Control of sediment dynamics by vegetation as a key function driving biogeomorphic succession within fluvial corridors. *Earth Surface Processes and Landforms*, 1810:1790–1810.
- Curtis, P. S. (1996). A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide.
- Dawson, J. O., Kowalski, D. G., and Dart, P. J. (1989). Variation with soil depth, topographic position and host species in the capacity of soils from an Australian locale to nodulate *Casuarina* and *Allocasuarina* seedlings. *Plant and Soil*, 118(1986):1–11.
- De Simone, O., Haase, K., Müller, E., Junk, W. J., Gonsior, G., and Schmidt, W. (2002). Impact of root morphology on metabolism and oxygen distribution in roots and rhizosphere from two Central Amazon floodplain tree species. *Functional Plant Biology*, 29:1025–1035.
- Drew, M. C. (1997). OXYGEN DEFICIENCY AND ROOT METABOLISM: Injury and Acclimation Under Hypoxia and Anoxia. *Annual Review of Plant Physiology and Plant Molecular Biology*, 48:223–250.
- Else, M. a., Janowiak, F., Atkinson, C. J., and Jackson, M. B. (2009). Root signals and stomatal closure in relation to photosynthesis, chlorophyll a fluorescence and adventitious rooting of flooded tomato plants. *Annals of Botany*, 103:313–323.
- Erskine, W., Chalmers, A., Keene, A., Cheetham, M., and Bush, R. (2009). Role of a rheophyte in bench development on a sand-bed river in southeast Australia. *Earth Surface Processes and Landforms*, 953(April):941–953.
- Evans, D. E. (2004). Aerenchyma formation. *New Phytologist*, 161:35–49.
- Gutschick, V. P. and BassiriRad, H. (2003). Extreme events as shaping physiology, ecology, and evolution of plants: toward a unified definition and evaluation of their consequences. *New Phytologist*, 160(1):21–42.
- Hennessy, K., Fawcett, R., Kirono, D., Mpelasoka, M., Jones, D., Bathols, J., Whetton, P., Stafford Smith, M., Howden, M., Mitchell, C., and Plummer,

- N. (2008). An assessment of the impact of climate change on the nature and frequency of exceptional climatic events. Australian Government, Bureau of Meterology. Technical report, Australian Government, Bureau of Meterology, Canberra, Australia.
- Holtum, J. A. M. and Winter, K. (2010). Elevated [CO₂] and forest vegetation: More a water issue than a carbon issue? *Functional Plant Biology*, 37:694–702.
- IPCC, Pachauri, R. K., and Meyer, L. (2014). *Climate Change 2014 Synthesis Report*. IPCC.
- Iversen, C. M., Ledford, J., and Norby, R. J. (2008). CO₂ enrichment increases carbon and nitrogen input from fine roots in a deciduous forest. *New Phytologist*, 179:837–847.
- Keenan, T. F., Hollinger, D. Y., Bohrer, G., Dragoni, D., Munger, J. W., Schmid, H. P., and Richardson, A. D. (2013). Increase in forest water-use efficiency as atmospheric carbon dioxide concentrations rise. *Nature*, 499:324–7.
- Kogawara, S., Yamanoshita, T., Norisada, M., Masumori, M., and Kojima, K. (2006). Photosynthesis and photoassimilate transport during root hypoxia in *Melaleuca cajuputi*, a flood-tolerant species, and in *Eucalyptus camaldulensis*, a moderately flood-tolerant species. *Tree physiology*, 26:1413–1423.
- Körner, C. (2006). Plant CO₂ responses: An issue of definition, time and resource supply. *New Phytologist*, 172:393–411.
- Kozlowski, T. T. (1984). Plant Responses to Flooding of Soil. *BioScience*, 34(3):162–167.
- Lawson, J., Fryirs, K., and Leishman, M. (2015). Hydrological conditions explain variation in wood density in riparian plants of south-eastern Australia. *Journal of Ecology*, 103(4):945–956.
- Lipson, D. a., Kuske, C. R., Gallegos-Graves, L. V., and Oechel, W. C. (2014). Elevated atmospheric CO₂ stimulates soil fungal diversity through increased fine root production in a semiarid shrubland ecosystem. *Global Change Biology*, 20:2555–2565.

- Manea, A. and Leishman, M. R. (2014). Competitive interactions between established grasses and woody plant seedlings under elevated CO₂ levels are mediated by soil water availability. *Oecologia*, 177:499–506.
- Marcar, N. E. (1993). Waterlogging Modifies Growth, Water Use and Ion Concentrations in Seedlings of Salt-treated *Eucalyptus camaldulensis*, *E. tereticornis*, *E. robusta* and *E. globulus*. *Australian Journal of Plant Physiology*, 20:1–13.
- Matamala, R. and Schlesinger, W. H. (2000). Effects of elevated atmospheric CO₂ on fine root production and activity in an intact temperate forest ecosystem. *Global Change Biology*, 6:967–979.
- Megonigal, J., Vann, C., and Wolf, A. (2005). Flooding constraints on tree (*Taxodium distichum*) and herb growth responses to elevated CO₂. *Wetlands*, 25(2):430–438.
- Naiman, R., Decamps, H., and Pollock, M. (1993). The role of riparian corridors in maintaining regional biodiversity. *Ecological Applications*, 3(2):209–212.
- Nie, M., Lu, M., Bell, J., Raut, S., and Pendall, E. (2013). Altered root traits due to elevated CO₂: A meta-analysis. *Global Ecology and Biogeography*, 22:1095–1105.
- Piedade, M. T. F., Ferreira, C. S., Wittmann, A. D. O., Buckeridge, M., and Parolin, P. (2010). *Amazonian Floodplain Forests* - Springer.
- Poorter, H. and Navas, M. L. (2003). Plant growth and competition at elevated CO₂: on winners, losers and functional groups. *New Phytologist*, 157:175–198.
- Poorter, L. (2007). Are species adapted to their regeneration niche, adult niche, or both? *The American Naturalist*, 169(4):433–442.
- Pregitzer, K., Zak, D., Maziasz, J., DeForest, J., Curtis, P., and Lussenhop, J. (2000). Interactive effects of atmospheric CO₂ and soil-N availability on fine roots of *populus tremuloides*. *Ecological Applications*, 10(1):18–33.
- Pregitzer, K. S., Zak, D. R., Curtis, P. S., Kubiske, M. E., Teeri, J. a., and Vogel, C. S. (1995). Atmospheric CO₂, soil-nitrogen and Turnover of fine roots. *New Phytologist*, 129:579–585.

- Prior, S., Rogers, H., Runion, G., and Hendrey, G. (1994). Free-air CO₂ enrichment of cotton: vertical and lateral root distribution patterns. *Plant and Soil*, 165:33–44.
- R Core Team (2015). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Reich, P. B., Hobbie, S. E., Lee, T., Ellsworth, D. S., West, J. B., Tilman, D., Knops, J. M. H., Naeem, S., and Trost, J. (2006). Nitrogen limitation constrains sustainability of ecosystem response to CO₂. *Nature*, 440(April):922–925.
- Reich, P. B., Hobbie, S. E., and Lee, T. D. (2014). Plant growth enhancement by elevated CO₂ eliminated by joint water and nitrogen limitation. *Nature Geoscience*, 7(December):1–5.
- Ryser, P., Gill, H. K., and Byrne, C. J. (2011). Constraints of root response to waterlogging in *Alisma triviale*. *Plant and Soil*, 343:247–260.
- Santosa, I. E., Ram, P. C., Boamfa, E. I., Laarhoven, L. J. J., Reuss, J., Jackson, M. B., and Harren, F. J. M. (2007). Patterns of peroxidative ethane emission from submerged rice seedlings indicate that damage from reactive oxygen species takes place during submergence and is not necessarily a post-anoxic phenomenon. *Planta*, 226:193–202.
- Sena-Gomes, A. and Kozłowski, T. T. (1980). Effects of flooding on *Eucalyptus camaldulensis* and *Eucalyptus globulus* seedlings. *Oecologia*, 46:139–142.
- Shimono, H., Konno, T., Sakai, H., and Sameshima, R. (2012). Interactive Effects of Elevated Atmospheric CO₂ and Waterlogging on Vegetative Growth of Soybean (*Glycine max* (L.) Merr.). *Plant Production Science*, 15(January):238–245.
- Steffens, B., Steffen-Heins, A., and Sauter, M. (2013). Reactive oxygen species mediate growth and death in submerged plants. *Frontiers in Plant Science*, 4(June):179.
- Steudle, E. (2000). Water uptake by roots: an integration of views. *Plant Soil*, 226:45–56.

- Stocker, T. F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P. M., and Others (2013). Climate Change 2013. The Physical Science Basis. Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change-Abstract for decision-makers. Technical report, Groupe d’experts intergouvernemental sur l’évolution du climat/Intergovernmental Panel on Climate Change-IPCC, C/O World Meteorological Organization, 7bis Avenue de la Paix, CP 2300 CH-1211 Geneva 2 (Switzerland).
- van der Sleen, P., Groenendijk, P., Vlam, M., Anten, N. P. R., Boom, A., Bongers, F., Pons, T. L., Terburg, G., and Zuidema, P. A. (2014). No growth stimulation of tropical trees by 150 years of CO₂ fertilization but water-use efficiency increased. *Nature Geoscience*, 8(January):24–28.
- Visser, E. J. W., Colmer, T. D., Blom, C. W. P. M., and Voesenek, L. a. C. J. (2000). Changes in growth, porosity, and radial oxygen loss from adventitious roots of selected mono- and dicotyledonous wetland species with contrasting types of aerenchyma. *Plant, Cell and Environment*, 23:1237–1245.
- Voesenek, L. A. C. J. and Bailey-Serres, J. (2015). Flood adaptive traits and processes: an overview. *New Phytologist*, 206(1):57–73.
- Wang, D., Heckathorn, S. A., Wang, X., and Philpott, S. M. (2012). A meta-analysis of plant physiological and growth responses to temperature and elevated CO₂. *Oecologia*, 169(1):1–13.
- Wang, X. and Taub, D. R. (2010). Interactive effects of elevated carbon dioxide and environmental stresses on root mass fraction in plants: A meta-analytical synthesis using pairwise techniques. *Oecologia*, 163(1):1–11.
- Woolfrey, A. and Ladd, P. (2001). Habitat preference and reproductive traits of a major Australian riparian tree species (*Casuarina cunninghamiana*). *Australian Journal of Botany*, 49(6):705–715.
- Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., Cavender-bares, J., Chapin, T., Cornelissen, J. H. C., Diemer, M., Flexas, J., Garnier, E., Groom, P. K., and Gulias, J. (2004). The worldwide leaf economics spectrum. *Nature*, 428:821–827.