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.

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 & Voesenek 2009). Relationships between photosynthetic rate and biomass responses to waterlogging and CO2 level treatments in this study were species specific.

Of the three species studied here, only growth of C. cunninghamiana was interactively affected by CO2 concentration and waterlogging status. Biomass of shoot, total root and fine root components was significantly higher under eCO2 for control C. cunninghamiana plants, but not 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 X) 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 absent differences in total biomass. 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 biomass attended 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 than 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 (Sena-Gomes & Kozlowski 1980; Marcar 1993), 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 interactively dependent on 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 life, end of their respective economic spectra (Reich 2014), but this shift was not sustained following the refractory period. A corresponding pattern in water use efficiency corroborates this inference. Higher root dry matter content under waterlogging has been linked to the requirement for structural support of air spaces in aerenchymous root tissue (Ryser, Gill & Byrne 2011). Suberization of root hypodermal tissue often occurs under waterlogging as a means of reducing radial oxygen loss (Visser *et al.* 2000; De Simone *et al.* 2002) and may increase 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 (Wright *et al.* 2004; Reich 2014). We found no evidence for decreased SLA under eCO2 as previously described (Poorter & Navas 2003). Previous studies report inconsistent effects of eCO2 on fine root dry matter content: eCO2 had no effect on *Liquidambar styraciflua or Pinus strobus fRDMC* (Bauer & Berntson 2001; Iversen, Ledford & Norby 2008), caused a small decrease in *Betula alleghaniensis* (Bauer & Berntson 2001) and increased fRDMC in cotton (Prior *et al.* 1994). In this study, eCO2 consistently increased fine root dry matter content in C. cunninghamiana.

Analysis of gas exchange, biomass accumulation and functional traits after a refractory period provided the opportunity to determine whether responses to flooding 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 by the nitrogen fixing ascomycete *Frankia* is known to be highest C. cunninghamiana under well aerated soil conditions (Dawson, Kowalski & Dart 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). Blunting of eCO2 biomass stimulation in seedlings by waterlogging has the potential to alter demographics and structural dynamics in many Australian riparian communities, where C. cunninghamiana is a keystone species (Woolfrey & Ladd 2001).

CONCLUSIONS

Waterlogging and atmospheric CO2 concentration alone have significant consequences for physiological processes, growth and functional characteristics of tree seedlings. The relative importance of these environmental factors vary according to species, as do the specific effects of each on plants. This study adds to the small 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, Vann & Wolf 2005).

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