RESULTS

Descriptive statistics and significance of ANOVA and post-hoc tests are shown for all measurements for each combination of treatments in Table 1.

Table 1. Mean and standard deviation (in parentheses) of measured gas exchange rates, biomass and functional traits for each combination of CO2 level and waterlogging treatments. Significant differences as determined by two-way ANOVA are denoted by the letters N, C, W or I (N = no significant effect of either treatment, C = significant effect of CO2 level, W = significant effect of waterlogging treatment, I = significant interaction between CO2 level and waterlogging treatment. Where interactions were found, waterlogging treatments in which significant differences between aCO2 and eCO2 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.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Control** | | **Waterlogged** | | **Recovery** | | **Significant**  **difference** | **Post-hoc** |
|  | *e*CO2 | *a*CO2 | *e*CO2 | *a*CO2 | *e*CO2 | *a*CO2 |  |  |
| ***Acacia floribunda*** |  |  |  |  |  |  |  |  |
| Photosynthetic rate (*A*, μmol CO2 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) | N |  |
| 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) | N |  |
| 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) | N |  |
| ***Casuarina cunninghamiana*** |  |  |  |  |  |  |  |  |
| Photosynthetic rate (*A*, μmol CO2 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) | N |  |
| 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) | I,C | w |
| Dry root biomass (g) | 5.79 (3.1) | 10.88 (3.67) |  |  | 6.31 (2.07) | 7.05 (2.75) | I,C | c |
| Dry fine root biomass (g) | 1.66 (1.23) | 4.11 (1.96) |  |  | 1.95 (0.73) | 2.61 (1.31) | I\*,C | c |
| Dry shoot biomass (g) | 10.44 (3.75) | 17.19 (5.66) |  |  | 11.97 (3.28) | 10.55 (3) | I |  |
| 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) | C |  |
| 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) | N |  |
| 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 CO2 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) | N |  |
| 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) | N |  |
| 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 |  |

*Gas exchange and water use efficiency*

Effects of CO2 level and waterlogging on gas exchange were species specific, and some interaction effects were significant, but 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). Photosynthetic rate in *E. camaldulensis* was significantly greater in recovery treatment plants than control plants (p = 0.008, Fig. 1c). No significant interactions were found between CO2 level and waterlogging status for photosynthetic rate, although waterlogged *A. floribunda* exhibit a only small difference in mean photosynthetic rate between CO2 treatments (20.9 and 22.6 μmol CO2 m⁻² s⁻¹, respectively, Fig. 1a).

Neither CO2 level nor waterlogging status significantly influenced stomatal conductance of *A. floribunda* or *C. cunninghamiana*, alone or interactively. Recovering *E. camaldulensis* plants had higher rates of stomatal conductance than waterlogged plants (p = 0.059) and control plants (0.0002); waterlogged plants higher stomatal conductance than control plants (0.042); no CO2 effect was found for *E. camaldulensis*.

A significant interaction effect was identified for transpiration rate of *A. floribunda* (p = 0.075, Fig. 1d); no differences were significant upon post-hoc analysis, however. Elevated CO2 significantly increased transpiration rate in *C. cunninghamiana* (p = 0.009, Fig. 1e), but not *E. camaldulensis* (Fig. 1f). eCO2 stimulation of transpiration in waterlogged *C. cunninghamiana* also appears diminished, despite non-significance of the interaction term (Fig. 1e). Control *E. camaldulensis* plants transpired less than waterlogged plants (p = 0.019) and recovery plants (p = 0.0005).

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.

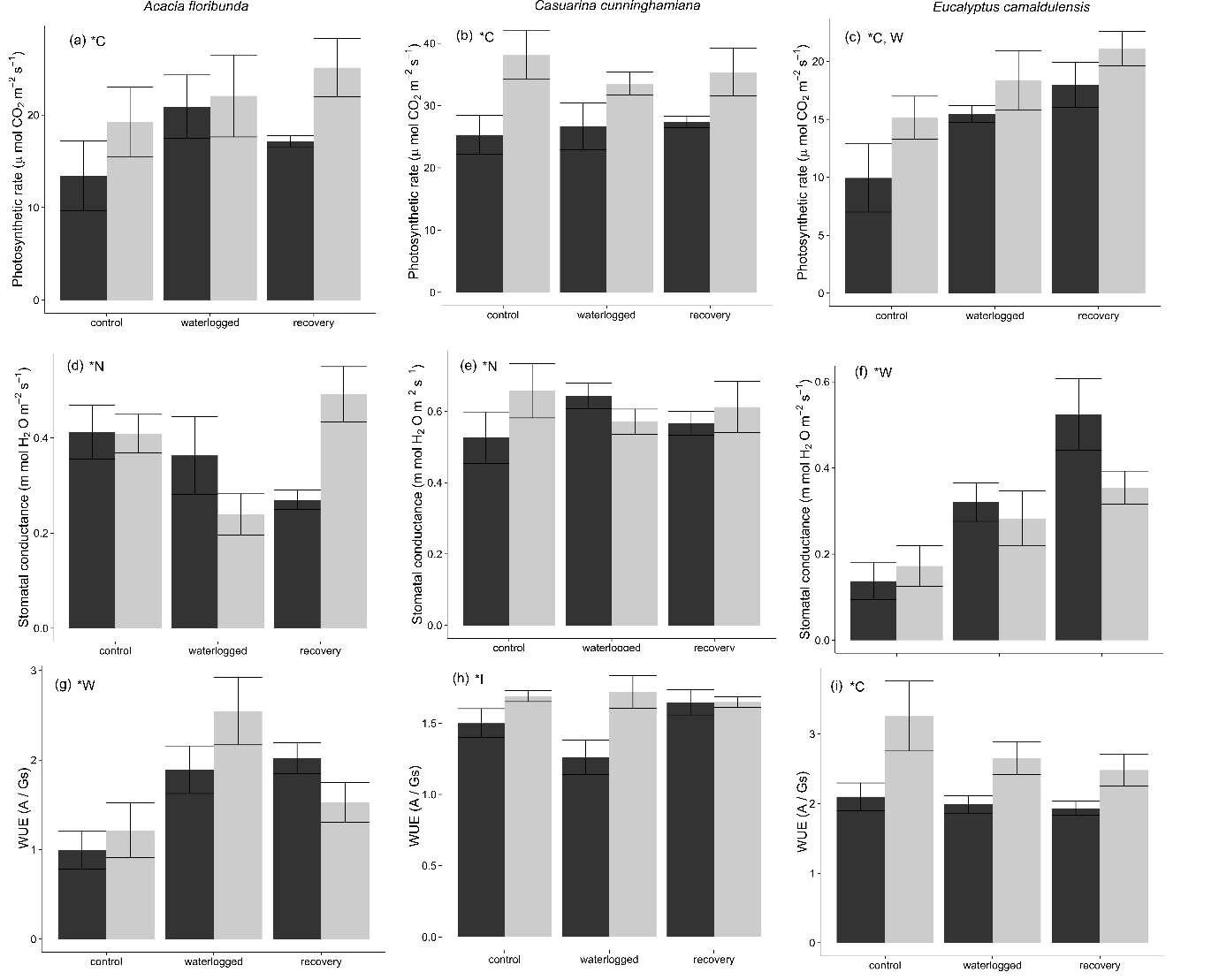


Figure 1. Gas exchange measurements under each combination of waterlogging and CO2 level treatments. Dark shaded columns represent measurements under ambient atmospheric CO2 concentration (390 ppm), light shaded columns represent measurements under elevated atmospheric CO2 concentration (550 ppm). Error bars represent the standardised mean error. \* - letters denote statistical significance of differences between treatment combinations (N = no significant difference, C = significant difference between CO2 level treatments, W = significant difference between waterlogging treatments).

*Biomass production*

Waterlogging status and CO2 level interacted strongly for one species: eCO2 stimulation 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 *E. camaldulensis* total root biomass (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 higher 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).

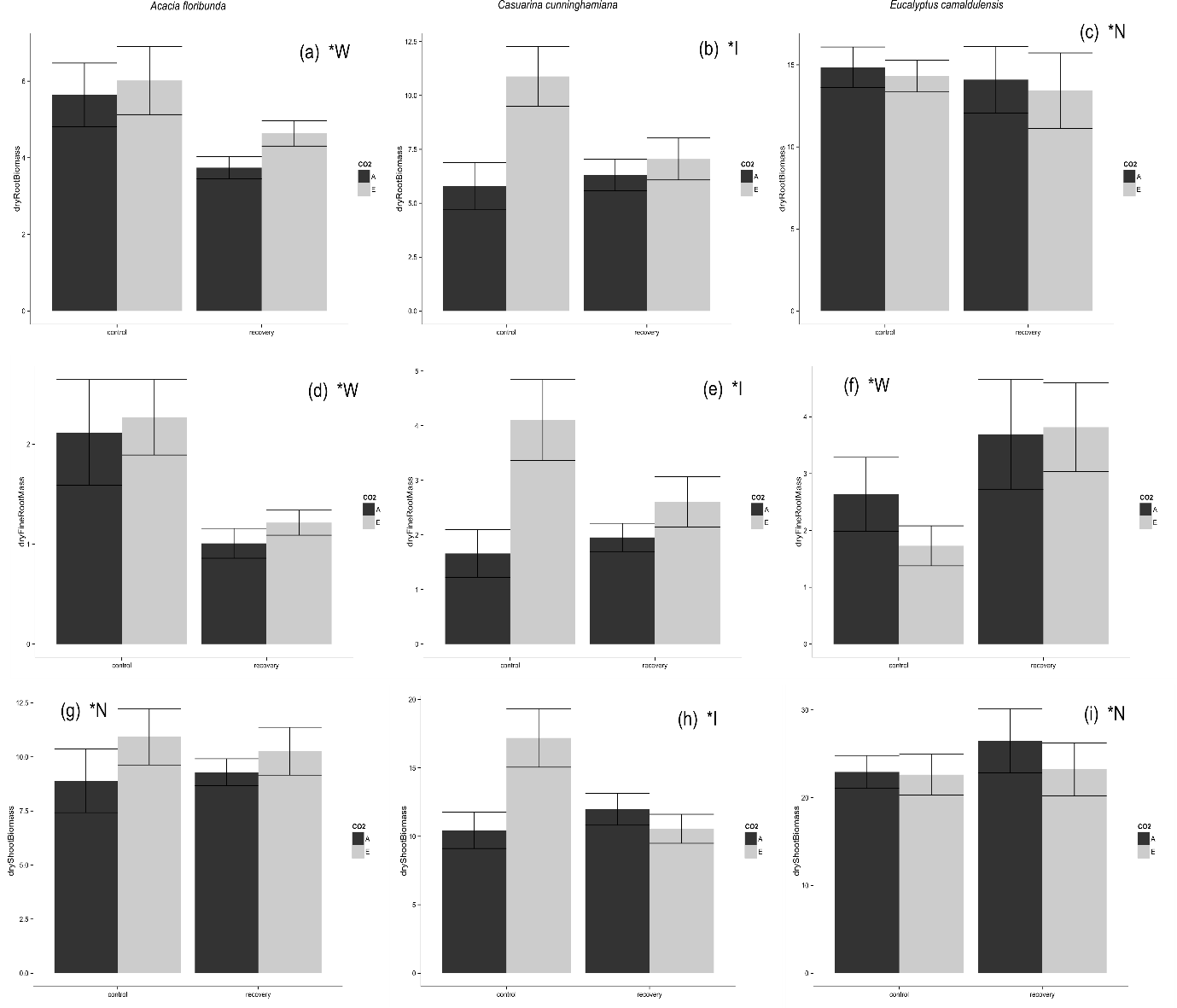


Figure 2. Biomass measurements under each combination of waterlogging and CO2 level treatments. Dark shaded columns represent measurements under ambient atmospheric CO2 concentration (390 ppm), light shaded columns represent measurements under elevated atmospheric CO2 concentration (550 ppm). Error bars represent the standardised mean error. \* - letters denote statistical significance of differences between treatment combinations (N = no significant difference, C = significant difference between CO2 level treatments, W = significant difference between waterlogging treatments).

*Functional traits & biomass allocation*

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

Fine root DMC 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 significant interaction effect was also present for *A. floribunda* (p = 0.067), but no differences were significant upon post-hoc analysis. Waterlogging status also affected *E. camaldulensis* fine root DMC (Fig. 3b): control plants had higher fine root DMC than waterlogged plants (p = 0.018), and recovery plants (p = 0.053) (marginally significant). eCO2 was associated with significantly increased fine root DMC 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).

Root mass fraction was decreased by waterlogging for all species, but no significant CO2 or interaction effects were found. 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 *C. cunninghamiana* 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. RMF of *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.

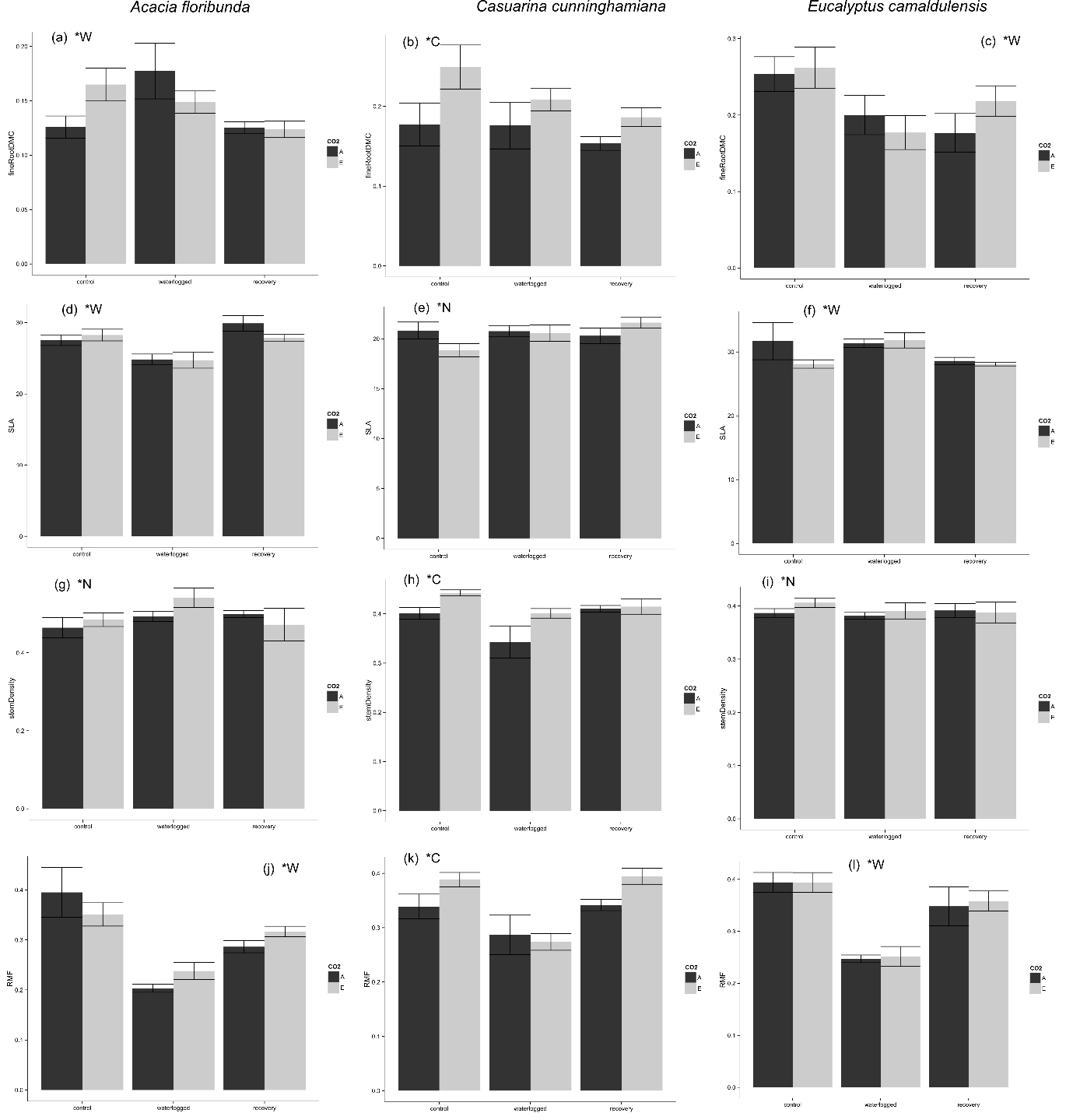
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Figure 3. Functional trait measurements under each combination of waterlogging and CO2 level treatments. Dark shaded columns represent measurements under ambient atmospheric CO2 concentration (390 ppm), light shaded columns represent measurements under elevated atmospheric CO2 concentration (550 ppm). Error bars represent the standardised mean error. \* - letters denote statistical significance of differences between treatment combinations (N = no significant difference, C = significant difference between CO2 level treatments, W = significant difference between waterlogging treatments).