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Title

The effects of CO_2 and nutrient fertilization on the growth and temperature response of the mangrove *Avicennia germinans*

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Running headline

CO_2 AND NUTRIENT EFFECT ON MANGROVES

Abstract

In order to understand plant responses to both the widespread phenomenon of increased nutrient inputs to coastal zones and the concurrent rise in atmospheric CO_2 concentrations, CO_2-nutrient interactions need to be considered. In addition to its potential stimulating effect on photosynthesis and growth, elevated CO_2 affects the temperature response of photosynthesis. The scarcity of experiments testing how elevated CO_2 affects the temperature response of tropical trees hinders our ability to model future primary productivity. In a glasshouse study we examined the effects of elevated CO_2 (800 ppm) and nutrient availability on seedlings of the widespread mangrove *Avicennia germinans*. We assessed photosynthetic performance, the temperature response of photosynthesis, seedling growth and biomass allocation. We found large synergistic gains in both growth (42%) and photosynthesis (115%) when seedlings grown under elevated CO_2 were supplied with elevated nutrient concentrations relative to their ambient growing conditions. Growth was significantly enhanced under elevated CO_2 only under high nutrient conditions, mainly in above ground tissues. Under low nutrient conditions and elevated CO_2, root volume was more than double that of seedlings grown under ambient CO2 levels. Elevated CO_2 significantly increased the temperature optimum for photosynthesis by ca. 4°C. Rising CO_2 concentrations are likely to have a significant positive effect on the growth rate of *A. germinans* over the next century, especially in areas where nutrient availability is high.

Key Words

Climate Change, CO_2, Eutrophication, Mangrove, Nitrogen, Phosphorous, Photosynthesis, RUBISCO, Temperature-Response, Tropics

Introduction:

Current increases in the concentration of CO_2 in the Earth's atmosphere are thought to have an overall positive effect on plant growth and productivity (Drake et al. 1997). However, due to factors interacting with CO_2, such as nutrient and water availability and temperature, measured growth responses to elevated CO_2 have often been variable (Körner 2006; van der Sleen et al. 2015). In particular, progressive nitrogen limitation tends to reduce the long-term growth stimulation by elevated CO_2 (Luo et al. 2004; Norby et al. 2010; Reich et al. 2006), and thus under nutrient limiting conditions, the stimulating effects of elevated CO_2 on plant growth are often significantly reduced relative to nutrient replete conditions (Oren et al. 2001). The handful of experiments studying the effects of elevated CO_2 (700–800 ppm) on mangrove seedlings have shown responses in growth and productivity, with a growth enhancement from 12% to up to 47% under elevated CO_2 conditions (Ball et al. 1997; Farnsworth et al. 1996; McKee and Rooth 2008; Reef et al. 2015). Mangroves develop along tropical coastlines, where nutrients frequently are in low supply. In many mangrove forests, nitrogen and sometimes phosphorous have been shown to limit growth (Reef et al. 2010b) and saline conditions may be expected to limit responses to elevated CO_2 (Ball et al. 1997). Thus, to better understand the response of mangroves to elevated CO_2 conditions, CO_2-nutrient interactions need to be considered.

In addition to its potential stimulating effect on photosynthesis and growth, elevated CO_2 affects the temperature response of photosynthesis in C3 plants. Since current mangrove distributions are strongly influenced by temperature (Duke et al. 1998; Hutchison et al. 2014; Quisthoudt et al. 2013; Woodroffe and Grindrod 1991), quantifying the effects of elevated CO_2 on the temperature response of mangroves is key to determining the fate of mangroves in the face of atmospheric and climate change.

Photosynthesis is one of the most temperature sensitive processes in plants (Berry and Bjorkman 1980). The carbon fixing enzyme RUBISCO catalyses both carboxylation (and subsequently photosynthesis) and oxygenation (photorespiration) with CO_2 and O_2 as competing substrates. As temperatures rise, the specificity of RUBISCO for CO_2 decreases and CO_2 solubility decreases to a greater extent than that of O_2. Hence, the ratio between photorespiration and photosynthesis increases with increasing temperature (Bernacchi et al. 2001; Jordan and Ogren 1984), significantly reducing carbon assimilation rates and requiring higher CO_2 concentrations to attain similar levels of carbon assimilation. Based on theoretical models of photosynthesis, elevated CO_2 concentrations could have a strong effect on the temperature response of photosynthesis (Farquhar et al. 1980; Lloyd and Farquhar 2008), but experimental evidence for this is not well documented for tropical trees. A number of recent models predict a significant shift in mangrove distributions, for example the loss of mangrove forests from regions of high temperature and a reduction in productivity based on an anticipated rise in global temperature (Beaumont et al. 2011; Koch et al. 2015; Osland et al. 2013), but these predictions are based on the climatic niche of present day mangroves growing under current CO_2 concentrations. The scarcity of experiments testing how elevated CO_2 affects the temperature relationships of tropical trees hinders our ability to model how elevated CO_2 will affect primary productivity in these systems into the future (Cernusak et al. 2013).

Mangrove forests contribute a large proportion of the primary productivity on tropical coasts, which is important for carbon sequestration and support of both marine and terrestrial food webs (Duarte et al. 2013). Members of the genus *Avicennia* are dominant within higher latitude forests and are documented to have expanded their range in recent decades on three continents (Saintilan et al. 2014). Additionally, in the core of the mangrove distribution (tropical latitudes) they have an important role as they colonize

sediments and are tolerant of disturbance (Fromard et al. 2004). In this study we examined the effects of elevated CO_2 and nutrient availability on the mangrove *Avicennia germinans* (L.) L.. We assessed the photosynthetic performance, the temperature response of photosynthesis, seedling growth and biomass allocation.

Methods:

Avicennia germinans propagules were collected in July 2014 at Galeta Point, Panama (9°24'N, 79°51'W) and transferred to the Santa Cruz Experimental Field Facility, Smithsonian Tropical Research Institute, Gamboa, Panama (9°07'N, 79°42'W) where they were planted in individual 1.6 L tree pots (Short One Treepot™, 10x10x23 cm. Stuewe and Sons, Tangent, Oregon) filled with a mixture (50% / 50%) of local topsoil and sand. The plants (propagules) were randomly assigned to one of two naturally illuminated glasshouses (n=34 pots per glasshouse), one with similar to ambient (ca. 400 ppm) CO_2 concentrations and one with an elevated (800 ppm) CO_2 concentration.

Elevated CO_2 was maintained by releasing CO_2 gas from a high-pressure cylinder in brief pulses to maintain CO_2 concentrations between 790 and 810 ppm. The glasshouses were equipped with split air conditioning units programmed to turn on when ambient air temperature exceeded 30°C. Air temperature and relative humidity were recorded in the two glasshouses every 15 min using a data logger (CR10X; Campbell Scientific, Logan, Utah, USA). The conditions in each of the two glasshouses during the experiment are summarised in Table 1.

Seedlings were watered twice weekly with 300 ml salt solution that saturated the pots. Two nutrient treatments were implemented in each glasshouse, a low nutrient treatment (n=17 in each glasshouse) and a high nutrient treatment (n=17 in each

glasshouse). The solution low in nutrients contained 0.06 mM KNO_3, 0.04 mM $Ca(NO_3)_2$, 0.01 mM $NH_4H_2PO_4$, 0.01 mM $(NH_4)_2HPO_4$, 0.01 mM $MgSO_4$, 2.5 µM H_3BO_3, 0.2 µM $MnSO_4$, 0.2 µM $ZnSO_4$, 0.05 µM $CuSO_4$, 0.05 µM H_2MoO_4, 2 µM $C_{10}H_{12}FeN_2NaO_8$ (ethylenediaminetetraacetic acid iron (III)-sodium salt), which is similar to the nutrient concentrations in mangrove porewater where they are not exposed to anthropogenic eutrophication (Alongi et al. 1993; Chen and Twilley 1999). The concentrations in the high nutrient solution were 5 times those of the low nutrient solution. Ocean salt (Instant Ocean, Blacksburg, VA, USA) was added to both nutrient solutions to a concentration of 20 g L^{-1}. Instant ocean aquarium salt does not contain nitrogen and phosphorus. Once a week the plants received a rinse of fresh water (10 ml) from a spray bottle to simulate a rain event washing the salt from their leaves.

Two plants died during the experimental period. After three months of growth (October 6, 2014), photosynthetic temperature response curves were assessed for four randomly selected plants from each of the four treatments over the period of a week. All plants were harvested on the 14th of October 2014.

Photosynthetic temperature response curves:

Photosynthetic gas exchange was measured on intact leaves of known area enclosed in a Walz gas-exchange cuvette with Peltier temperature control (GWK 3M Walz, Effeltrich, Germany) connected to a LI-6252 infrared gas analyser (Li-Cor, Lincoln NE, USA) under constant illumination of 1000 µmol m^{-2} s^{-1} from a red/blue LED light array. The CO_2 concentration of the air entering the chamber was set to 400 ppm for the seedlings grown at ambient CO_2 concentrations and to 800 ppm for the seedlings grown at elevated CO_2 concentrations. Following the enclosure of the leaf into the chamber, the chamber temperature was reduced to 20°C for ~60 min. The temperature was then increased in 5°C increments (every 20–30 min, when a stable reading was established)

up to 40–50°C. The youngest fully expanded leaves were studied. Leaf temperature was measured using a copper-constantan thermocouple attached to the bottom surface of the leaf.

Temperature response data were fitted to the equation from Battaglia, Beadle & Loghhead (1996; Eq. 1) using the *nlsfit* function in R (Team 2014). The equation describes the photosynthetic rate (P) at a given temperature (T) as a parabolic relationship, with P_{opt} and T_{opt} being the maximal photosynthetic rate, and the temperature at which P_{opt} is achieved, respectively. Analysis of variance was used to detect differences in the parameters P_{opt} (measured here as photosynthetic capacity, A_{max}), T_{opt} and the high-temperature CO_2 compensation point (where net CO_2 exchange is zero) among treatments.

$$P(T)=P_{opt}-b(T-T_{opt})^2 \qquad \text{Eq. 1}$$

Transpiration rate was calculated from the water vapour difference between the air leaving the chamber and the incoming air. Stomatal conductance at each temperature was calculated from the rate of transpiration divided by the leaf-air vapour pressure difference (VPD) in the air leaving the chamber relative to the incoming air. Intrinsic water use efficiency was calculated as the carbon assimilation rate divided by the stomatal conductance.

Plant growth parameters and elemental composition:

Plant growth (stem length, no. of nodes and no. of leaves, no. of branches along the main stem) was monitored throughout the experiment. Leaf temperatures were measured for three leaves per seedlings one week prior to harvest on two cloudless days using a laser

infrared thermometer. The measurements were repeated on all seedlings five times during the day (08:00, 10:00, 13:00, 16:00 and 20:00). Following the harvest, plants were divided into leaves, stem and roots. Leaves were kept in a sealed bag with moist paper towel in order to maintain hydration status. Leaf area was measured using a LI-3100C leaf area meter (Li-Cor Corp. Lincoln, NE, USA). Washed roots free of soil were photographed against a dark background and analysed using the IJ Rhizo root analysis package (Pierret et al. 2013). The entire root system was measured for each seedling. Plant material was then washed in distilled water to remove external salt, patted dry and weighed after which it was dried at 70°C for 5 days and reweighed.

Samples for leaf nutrient concentrations and isotopic composition were taken from finely ground leaves and roots from ten randomly selected plants from each treatment. All leaves from each plant were pooled before grinding. The isotopic composition of the added CO_2 in the 800 ppm treatment differed slightly from that in ambient air. The correction for this was previously determined for this system by growing two C4 plants (*Saccharum spontaneum* and *Portulaca oleracea*) in the chambers. A correction factor of 2‰ was used in foliar $\delta^{13}C$ values of seedlings from the 800 ppm treatment (Cernusak et al. 2011a).

Phosphorous (P) concentrations were determined using a colourimetric assay as described in (Reef et al. 2010a). Leaf isotope values for $\delta^{13}C$ and $\delta^{15}N$ were measured from pooled samples of green leaves for ten seedlings from each treatment. Samples were measured in an elemental-analyser isotope ratio mass spectrometer (EA-IRMS, Sercon System, Griffith University; analytical errors of 0.1‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$). Nitrogen is expressed relative to atmospheric nitrogen and carbon relative to Vienna Pee-Dee Belemnite.

We used ANOVA to test for differences in growth parameters among the treatments. Root/Shoot ratios were *logit* transformed prior to analysis. Partial correlation analysis was used to test the relationship between specific leaf area (SLA) and growth. Climate data for Galeta Point was downloaded from the Smithsonian Physical Monitoring Program climate station at the Galeta Marine Laboratory.

Results:

Effects of CO_2 and nutrients on foliar physiology

Using a two-way ANOVA we found significant effects of both CO_2 concentration and nutrient treatment on photosynthetic capacity, A_{max} (ANOVA, $F_{(1,11)} = 8.5$, $p = 0.014$, and $F_{(1,11)} = 5.6$, $p = 0.04$ respectively, Figs 1A-D, Table 2), where A_{max} increases with increased CO_2 concentration and with nutrient enrichment, but more so when both elevated CO_2 and elevated nutrients were provided (Table 2).

Elevated CO_2 significantly increased the temperature optimum for photosynthesis by ca. 4°C (ANOVA, $F_{(1,12)} = 17.3$, $p = 0.001$, Figs 1A-D, Table 2). Despite the shift in the temperature optimum, the high-temperature CO_2 compensation point, i.e. the temperature at which net CO_2 exchange is zero, did not change significantly and was on average 41.8 (±3)°C. The range of temperatures at which photosynthesis was near maximum (≥80% of A_{max}) spanned 13°C and shifted to higher temperatures with elevated CO_2 (Table 2).

Transpiration rate (E), stomatal water vapour conductance (Gs) and intrinsic water use efficiency (WUEi) are presented for leaf temperatures of 25°C. Elevated CO_2 resulted in a significant reduction in stomatal conductance and transpiration relative to the ambient

CO_2 treatment (ANOVA, $F_{(1,11)} = 5.7$, $p = 0.04$ and $F_{(1,10)} = 13.5$, $p = 0.004$, Figs 2A and 2B respectively), which contributed to a significant increase in water use efficiency ($F_{(1,10)} = 22.1$, $p < 0.001$ Fig. 2C), most notably under the high nutrient regime ($p = 0.03$). The foliar $\delta^{13}C$ of leaves was significantly less negative in the elevated CO_2 treatment indicating that water use efficiency for the duration of the experiment was higher in this treatment ($F_{(1,35)} = 42.4$, $p < 0.001$, Fig. 2D)

There were no significant differences in leaf temperatures among the CO_2 and nutrient treatments (Fig. 3). On sunny days, leaf temperatures of ambient CO_2 grown plants were found to be at the high range, and sometimes exceeded the optimal temperature threshold for photosynthesis (defined here as the temperature range at which 80% of maximum photosynthetic rates can be achieved, Table 2). For plants growing under elevated CO_2 conditions, leaf temperatures were well within the optimal range for photosynthesis (Fig. 3). Neither the CO_2 nor the nutrient treatment significantly affected leaf water content, which was on average (±SD) 71.3% (±2.2%) of the fresh weight.

Effects of CO_2 and nutrients on growth and biomass allocation

Seedling growth (total biomass accumulated) was significantly enhanced under elevated CO_2 but only under high nutrient conditions (ANOVA $F_{(1,62)} = 9.2$, $p = 0.003$, Fig. 4A). In the high nutrient treatment, the rise in CO_2 concentrations from 400 to 800 ppm resulted in a 44% increase in biomass. Growth enhancement in the high nutrient treatment occurred mainly in above ground tissues (Fig. 4B), resulting in significantly lower root/shoot biomass ratios, with a more pronounced decrease in elevated CO_2 grown plants (ANOVA $F_{(1,62)} = 9.8$, $p = 0.003$). However, despite a lower overall allocation to roots vs. shoots, root biomass under elevated CO_2 was significantly greater

for the high relative to the low nutrient treatment (ANOVA $F_{(1,62)} = 6.5$, $p = 0.013$, Fig. 4A). The increased allocation of biomass to shoots was associated with a significant increase in leaf area: for the high nutrient treatment elevated CO_2 resulted in a 55% increase in leaf area and for the elevated CO_2 concentration, high nutrient conditions resulted in a 71% increase in leaf area (ANOVA $F_{(1,62)} = 13.9$, $p < 0.001$ and $F_{(1,62)} = 18.9$, $p < 0.001$ respectively Fig. 4C),

In contrast, in the low nutrient treatment, elevated CO_2 did not lead to significant biomass gains (Tukey HSD, p = 0.96). Increasing nutrient concentrations five-fold alone did not lead to significant biomass gains at ambient CO_2 levels.

Using partial correlation (while controlling for nutrient treatment and CO_2 concentration) we found specific leaf area (SLA) to be negatively correlated with relative growth rate, RGR (R = -0.47, $p < 0.001$, Fig. 4D) and thus higher growth rates were associated with lower SLA values. The slope of this relationship was independent of nutrient treatment or CO_2 concentration ($p > 0.05$).

Consistent with the stimulation of biomass growth, seedlings in the high nutrient – elevated CO_2 treatment had longer stems and more leaves than seedlings from other treatments (ANOVA, $F_{(1,62)} = 4.7$, $p = 0.03$ and $F_{(1,62)} = 7.0$, $p = 0.01$ respectively, Table 3). Notwithstanding the difference in size, we did not observe changes to growth allocation patterns in these stems (e.g. branching rates and internode lengths did not differ among treatments, Table 3).

Root structure was significantly influenced by the CO_2 and nutrient treatments. Roots were significantly longer in elevated CO_2 grown seedlings relative to ambient CO_2 ($F_{(1,37)} = 9.5$, $p = 0.004$). Under low nutrient conditions and elevated CO_2, root volume was

more than double that of seedlings grown under ambient CO_2 levels ($F_{(1,37)} = 5.8$, $p = 0.02$, Table 3). Mean root diameter was also affected, with a higher frequency of fine roots in the ambient CO_2/low nutrient and high CO_2/high nutrient treatments ($F_{(1,37)} = 28.4$, $p < 0.001$, Table 3). We identified three major root types in our seedlings: fine roots with diameters < 2 mm, lateral roots (d = 2–4 mm), and pneumatophores, which developed in a few seedlings (d > 4 mm). Fine roots made up on average 76% of the total root length. The fine root ratio (fine roots/total root biomass) was higher in the low nutrient treatment under ambient CO_2 conditions, as was the total fine root length. Under elevated CO_2 conditions, the effect of nutrients on fine root production was reversed, with a significant decrease in the fine root ratio in the low nutrient treatment. However, total fine root length remained higher in elevated CO_2 than under ambient CO_2 conditions for both nutrient treatments. Roots from elevated CO_2 grown seedlings also had a higher concentrations of carbon, regardless of nutrient treatment ($F_{(1,36)} = 15.5$, $p < 0.001$, Table 4).

Effects of CO_2 and nutrients on plant nutrient content

Phosphorous (P) concentrations in plant tissues were significantly affected by the CO_2 treatment. Elevated CO_2 seedlings had significantly higher concentration of P in their root tissues, relative to ambient CO_2 grown seedlings (ANOVA $F_{(1,36)} = 11.5$, $p = 0.002$, Table 4). In leaves, we found the opposite, lower P concentrations in seedlings from the elevated CO_2 treatment relative to ambient CO_2 ($F_{(1,35)} = 5.1$, $p = 0.03$, Table 4). The nutrient treatment had no significant effect on tissue P concentrations.

The exhaustion of maternal nutrient reserves as the seedlings matured led to a significant loss of foliage in low nutrient grown seedlings where leaf mortality rates were more than double those of the high nutrient grown seedlings (ANOVA, $F_{(1,62)} = 4.8$,

p = 0.03, Table 3). However, N or P concentrations in leaves of the low nutrient plants were not significantly lower than those in plants from the high nutrient treatment (Table 4). Differences in elemental composition between the nutrient treatments were detected in the roots, with higher %N, lower C:N and higher N:P in the high nutrient plants ($F_{(1,36)}$ = 24.8, p < 0.001, $F_{(1,36)}$ = 7.2, p = 0.01 and $F_{(1,36)}$ = 21, p < 0.001 respectively, Table 4).

Discussion:

We found large synergistic gains in both photosynthesis and growth in *Avicennia germinans* seedlings when seedlings grown under elevated CO_2 were supplied with elevated nutrient concentrations. In the high nutrient-elevated CO_2 treatment, photosynthesis was enhanced on average by 75% relative to the high nutrient ambient CO_2 grown seedlings, and 115% when compared with the low nutrient ambient CO_2 grown seedlings. Growth was enhanced by 42% in the elevated CO_2/high nutrient treatment relative to ambient CO_2/high nutrient seedlings. As has been observed in other species, growth was less sensitive than photosynthesis to elevated CO_2 (Kirschbaum 2011). Despite significant differences in water use efficiency among the nutrient and CO_2 treatments, plant water use efficiency was not associated with growth or productivity. This is consistent with growing evidence that indicates mangrove growth is not limited by water availability at moderate salinities (Reef et al. 2012).

Elevated CO_2 had a significant effect on the temperature dependence of light saturated photosynthesis as is predicted by theoretical models (Farquhar et al. 1980; Lloyd and Farquhar 2008). The optimal temperature for carbon fixation increased from 24.5°C at CO_2 concentrations of 400 ppm to 28.3°C in plants that were grown and measured at

800 ppm CO_2, an increase of nearly 4°C, which is higher than the predicted increase in mean global temperature for 2100 for moderate emissions scenarios (IPCC 2013).

T_{max}, the temperature at which net assimilation is zero, was not significantly affected by elevated CO_2 concentrations, remaining on average 41.8°C. Irreversible damage in tropical tree leaves has been shown to occur at temperatures >50 ˚C (Krause et al. 2010; Krause et al. 2014)

Despite differences in transpiration rates of 74% among the different CO_2 and nutrient treatments, leaf temperatures measured during the experiment were not significantly higher in the elevated CO_2 grown seedlings. This could be due to the fact that transpiration plays a relatively small role in leaf temperature regulation compared to the important influence of air temperature and irradiance (Miller 1972) especially in mangroves, where non-evaporative cooling strategies (e.g. leaf orientation, pubescence and salt excretion) are adaptations that maintain high water use efficiencies in these species (reviewed in (Reef and Lovelock 2014b).

The photosynthesis temperature response measured for *A. germinans* was of similar shape to the temperature response measured for the congeneric *Avicennia marina* (Ball et al. 1988) , and while T_{opt} of *A. germinans* was 3°C lower than that of its Australian counterpart, the high temperature CO_2 compensation point was similar to that of *A. marina*. Evidence from field measurements suggests that photosynthesis in *Bruguiera parviflora* from northern Queensland was strongly depressed at leaf temperatures > 34°C (Cheeseman et al. 1991). Also in northern Queensland, assimilation rates in *Rhizophora stylosa* decreased linearly as temperatures increased from 27–40°C and was at nearly the CO_2 compensation point at 39.5°C (Andrews and Muller 1985). However, in both these studies, the effect of temperature on carbon assimilation rates was

confounded by coinciding changes in light levels, humidity and differences in leaf angles. The CO_2 compensation point (T_{max}) for *A. germinans* in our study was on average 41.8±3°C, and while we found a significant increase in T_{opt} with elevated CO_2, we do not find a corresponding increase in T_{max} and our results do not support an increase in the high temperature threshold for this species under elevated CO_2 conditions.

The optimal temperature for photosynthesis under ambient CO_2 conditions was lower than the T_{leaf} measured for the seedlings throughout the day (Fig. 1). T_{opt} was also lower than the mean temperature in the glasshouse (Table 1) and lower than the mean daily atmospheric temperature recorded at Punta Galeta, where the plant material was collected, in the years 2002–2015 between 07:00 and 16:00 (27.8°C ±2). However, the temperature range of near optimal photosynthetic performance of the seedlings was very broad (approx. 13°C, Table 2) and the leaf temperatures measured in the glasshouse during growth were within this range (Fig. 3). Nonetheless, a T_{leaf} that is on average higher than T_{opt} suggests an incomplete acclimation to the mean growing temperature. It is possible that broad response of photosynthesis to temperature in *A. germinans* reflects its broad latitudinal distribution. Despite the low levels of gene flow among *A. germinans* populations (Ceron-Souza et al. 2012), a relatively low T_{opt} could be a conserved trait. There is growing evidence that not all plant species are capable of complete photosynthetic thermal acclimation to growth temperature (e.g. Dillaway and Kruger 2010). Our findings for *A. germinans* support this possibility. Relatively low T_{opt} compared to mean daily temperature, may also indicate acclimation of photosynthesis to early morning conditions when the majority of photosynthetic carbon gain in this species occurs (Smith et al. 1989). The mean temperature in the early morning (06:00-09:00) at Punta Galeta was (26.7±1.9). In mangroves midday depressions in photosynthesis are common (Andrews and Muller 1985; Bjorkman et al. 1988; Cheeseman et al. 1991), with some field studies showing a peak in photosynthesis

before 0800 AM and a cessation of photosynthesis by 1100 AM (Cheeseman et al. 1991). An incomplete acclimation to high ambient temperatures could be one of the causes of these depressions. Photosynthesis temperature response in three Australian mangrove species (*Bruguiera gymnorrhiza, Rhizophora apiculata and Avicennia marina*), measured under ambient (unspecified) CO_2 concentrations showed a broad temperature optima (25-30°C), which was significantly lower than leaf temperatures measured on sun exposed leaves as early as 0825 AM (Ball et al. 1988). In the Ball et al. study (1988) it was shown that leaf angle in mangroves is optimised to reduce leaf temperatures rather than maximise light capture, resulting in lower rates of photosynthesis. Irrespective of the underlying pressure that leads to selection for the broad temperature optima of photosynthesis and the cause of incomplete acclimation to the mean growing temperature, the increase in T_{opt} with increasing CO_2 concentrations could result in improved photosynthetic performance and growth rates for this species within the tropics as CO_2 concentrations continue to increase.

Low nutrient availability restricted the growth response of the mangrove *A. germinans* to elevated CO_2 despite significant improvements to photosynthesis and water use efficiency. Elevated CO_2 stimulated growth mainly above ground (increasing leaf area), although significant increases in below ground biomass were also detected relative to ambient CO_2 concentrations. Leaf SLA decreased as seedling growth rates increased. The enhancements observed in plant performance are consistent with previous studies conducted in greenhouses with mangrove seedlings (Ball et al. 1997; Farnsworth et al. 1996; McKee and Rooth 2008; Reef et al. 2015) and other plant species (Ainsworth and Long 2005; Winter et al. 2001a; Winter et al. 2001b), but also with a historical assessment that indicated SLA has already decreased in response to rises in CO_2 over the period since industrialization (Reef and Lovelock 2014a).

The combination of elevated CO_2 and elevated nutrients resulted in significantly higher leaf areas but no significant differences in the nitrogen and carbon concentration of leaves. An analysis of 16 FACE experiments worldwide found no effects of elevated CO_2 on foliar nitrogen concentrations in woody plants (Nowak et al. 2004). However, due to the increase in leaf area, an increase in nitrogen uptake did occur at the whole-plant level. Elevated CO_2 led to a reduction in foliar phosphorus concentrations (Table 4), a phenomenon which has been observed previously in *A. germinans* (Reef et al. 2015) and could be due to reduced transpiration rates (Fig. 4B), possibly involving subsequent lower translocation rates of P to the shoot via the xylem stream, as has been suggested for other tropical trees (Cernusak et al. 2011b). This is further supported by the increase in P concentrations (and small increases in %N) in the roots of the elevated CO_2 seedlings (Table 4). The reduction in foliar phosphorous concentrations under elevated CO_2 was overcome to some extent (although not significantly so) in the high nutrient treatment. Elevated CO_2 induced reduction in whole seedling transpiration rates, could thus have a significant effect on growth rates in mangrove forests where P is the limiting nutrient for growth such as in forests that are hydrologically isolated from regular tidal inundation (Feller et al. 2003).

Elevated CO_2 had a significant effect on roots, increasing root length and biomass and also the carbon concentration in the roots, but did not increase allocation of biomass to roots (except under high nutrient levels) as has been shown in other woody species (Hättenschwiler and Körner 1997). Root morphology was influenced in a complex interaction between elevated CO_2 and nutrient availability as root systems under elevated CO_2 and high nutrient conditions tended to have a lower proportion of biomass allocated to roots, but roots had a higher proportion of fine roots (Table 3). The increase in fine root production we observed for *A. germinans* under elevated CO_2 conditions is consistent with allocation models based on findings from other tree species (Dybzinski

et al. 2015) and is suggested to be driven by the use of carbon exudates to prime microbial populations to enhance N release for plant growth (Phillips et al. 2011). Root development is influenced by complex interactions among nutrient and water demands of the shoot (Poorter et al. 2012) and carbohydrate availability (Eveland and Jackson 2012). Reduction in transpiration in seedlings grown under elevated CO_2 (and increased WUE) reduces the demand for water, which may be balanced by an increase in nutrient demand due to higher growth rates (Chapin 1980), leading to little overall change in allocation to roots under low nutrient conditions (Fig. 4). As the rate of root development in mangroves is an important determinant of seedling establishment success in the soft sediment of tidal flats (Balke et al. 2011) the rapid elongation of roots under elevated CO_2 may increase survivorship of seedlings. Potential changes under elevated CO_2 in allocation to root biomass, or alterations to root morphology and elemental composition, which may influence decomposition, are important in mangrove forests as these factors are likely to influence capacity for carbon sequestration in these habitats and their responses to sea level rise (Krauss et al. 2014).

Mangroves in a changing environment

Rising CO_2 concentrations are likely to have a significant positive effect on the growth rate of the widespread mangrove *Avicennia germinans* over the next century, especially in areas where nutrients availability is high. For a congenitor in the Pacific Ocean, there is evidence that primary production has already been influenced by elevated CO_2 (Reef and Lovelock 2014a). Increased nutrient loading in coastal areas is widespread and synergistic interactions with elevated CO_2 are likely to result in overall increases in mangrove biomass, C sequestration and below ground C storage. Elevated CO_2 concentrations will affect the temperature response of photosynthesis in this species

more so than the predicted rise in mean global temperature over this period, possibly mitigating growth inhibition by future high temperature anomalies.

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Figure Legends:

Figure 1. Measured carbon assimilation rates (A) for attached, intact *Avicennia germinans* leaves as a function of leaf temperature in seedlings grown under (top) ambient (400 ppm) and (bottom) elevated (800 ppm) CO_2 concentrations subjected to low (left) or high (right) nutrient treatments. The measurements were made under saturating light conditions of 1000 μmol m^{-2} s^{-1}. Points are the mean (±SE) values for four seedlings, fitted lines are derived from the quadratic relationship described in Eq. 1. Dotted vertical lines denote the calculated temperature optimum for each treatment.

Figure 2. Mean (±SE) A) stomatal water vapour conductance (Gs) B) transpiration (E) and C) intrinsic water use efficiency (WUEi) of attached, intact leaves of four seedlings from each treatment at a leaf temperature of 25°C, irradiance of 1000 μmol m^{-2} s^{-1} and CO_2 concentrations of 400 ppm for seedlings from the low and high nutrient treatments grown at ambient CO_2 levels (open bars), or 800 ppm for seedlings grown at the elevated CO_2 concentration (filled bars). D) Foliar $\delta^{13}C$ values for N = 10 seedlings from each treatment measured at the end of the experiment. Different letters denote significant differences among treatments ($p < 0.05$).

Figure 3. Mean (±SE) leaf temperature measured in seedlings grown under ambient (open circles) and elevated (closed circles) CO_2 concentrations using a laser infrared thermometer at different time points on two cloudless days. Diamond symbols are the mean air temperature in the glasshouses at each time point. The optimal temperature range for photosynthesis (see Table 2) at 400 ppm and 800 ppm CO_2 is represented by the area bound by the horizontal dotted lines and the shaded area, respectively. N=33 seedlings for each point.

Figure 4. The mean (±SE) A) final above ground (AG) and below ground (BG) biomass, B) root/shoot biomass ratio, and C) total leaf area of seedlings grown under ambient (400 ppm, open bars) or elevated (800 ppm, filled bars) CO_2 concentrations and subject to either a low or high nutrient treatment. N = 16-17 seedlings per treatment. "*" denotes significant differences among treatments ($p < 0.05$). Panel D shows the relationship between relative growth rate (RGR) and mean specific leaf area (SLA) for each seedling. The fitted linear regression is of the form $SLA = -885RGR + 78.9$ ($R^2 = 0.22$, $p < 0.001$). Open and filled circles represent seedlings grown under ambient or elevated CO_2 concentrations respectively.

Table 1. CO_2, temperature and humidity conditions in the two glasshouses between the 22nd of June and the 13th of October 2014. Measurements were taken every 5 minutes throughout the day.

Parameter measured	Ambient CO_2 glasshouse	Elevated CO_2 glasshouse
Mean air temperature (°C) ± SD	28.6 ± 8.9	28.2 ± 3.4
Mean relative humidity (%) ± SD	67 ± 20	68 ± 22
Mean [CO_2] (ppm) ± SD	423 ± 17	827 ± 27

Table 2. Mean (SD) values describing the temperature response of photosynthesis in *Avicennia germinans* seedling grown at ambient (ca. 400 ppm) and elevated (ca. 800 ppm) CO_2 concentrations and under two nutrient regimes (low and high). A_{max} is the maximal carbon assimilation rate at light saturation and T_{opt} is the temperature at which A_{max} is achieved. T_{max} is the temperature at which the upper CO_2 compensation point occurs, above which net CO_2 loss occurs. Values were calculated from the quadratic relationship fit to the temperature series from each seedling (Eq. 1). *N* = 4 seedlings per treatment. Different letters indicate significant differences among the treatments ($p < 0.05$).

Parameter	*CO_2 ppm* / *Nutrients*	400 Low	400 High	800 Low	800 High
A_{max} (µmol C m^{-2} s^{-1})		7.5 (1.5)[a]	9.4 (1.4)[b]	10.3 (4.4)[c]	16.1 (3.6)[d]
T_{opt} (°C)		24.9 (1.6)[a]	24.1 (2.9)[a]	28.7 (1.8)[b]	27.8 (0.6)[b]
T_{max} (°C)		39.4 (0.6)[a]	41.6 (5.5)[a]	43.8 (2.2)[a]	42.2 (1.9)[a]
$T_{80\% Amax}$ (°C)		19.0 - 31.9	17.2 - 30.9	22.4 – 35.3	21.8 – 34.4

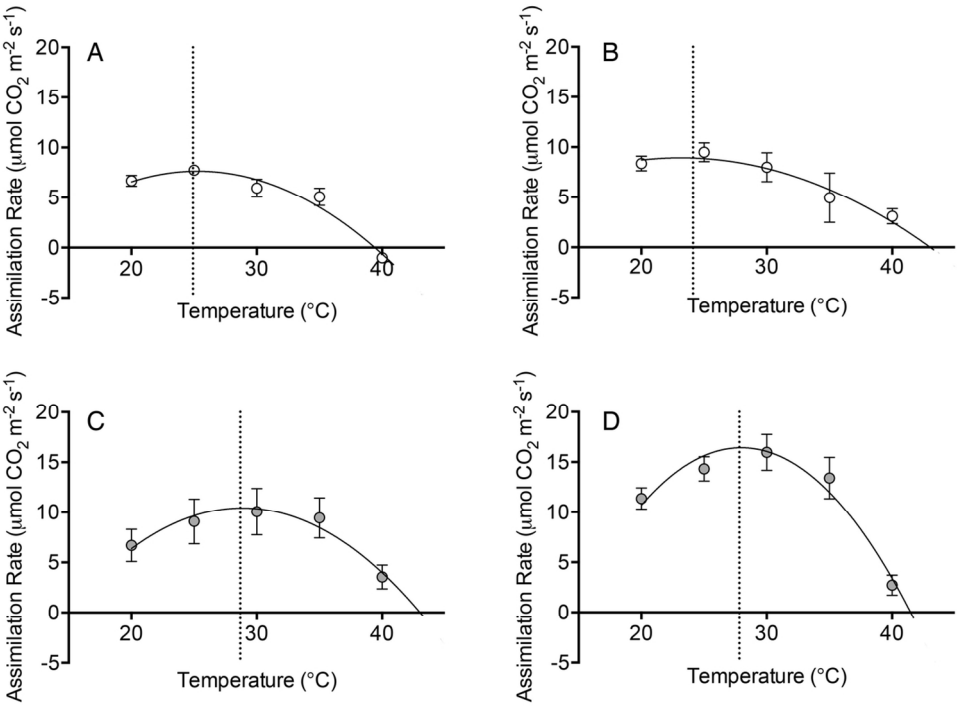
Table 3. Mean (SD) values describing the morphological response of *Avicennia germinans* seedlings to ambient (ca. 400 ppm) and elevated (ca. 800 ppm) CO_2 concentrations and two nutrient regimes (low and high). N = 17 seedlings per treatment for above ground measurements and N = 10 per treatment for root analysis. Different letters indicate significant differences among the treatments ($p < 0.05$).

Parameter	*CO_2 ppm* *Nutrients*	400 Low	400 High	800 Low	800 High
Stem Length (cm)		17.4 (5.8)[a]	17.6 (4.6)[a]	14.6 (4.8)[a]	20.2 (5.0)[b]
Internode Length (cm)		3.2 (0.8)[a]	3.1 (0.8)[a]	2.9 (0.7)[a]	3.0 (0.7)[a]
Leaves per seedling		9.8 (4.5)[a]	11.2 (5.8)[a]	8.5 (3.7)[a]	12.9 (3.5)[b]
Branching rate (cm^{-1})		0.10 (0.07)[a]	0.10 (0.09)[a]	0.09 (0.05)[a]	0.08 (0.04)[a]
Leaf mortality rate (day^{-1})		0.03 (0.03)[a]	0.01 (0.02)[b]	0.03 (0.03)[a]	0.01 (0.02)[b]
Root Length (cm)		864.8 (307.7)[a]	654.6 (249.2)[a]	1065.2 (446.9)[b]	1242.9 (585.7)[b]
Root Volume (cm^3)		2.1 (1.5)[a]	3.7 (1.9)[a]	4.5 (2.0)[b]	3.4 (1.7)[a]
Mean Root Diameter (mm)		0.56 (0.13)[a]	0.90 (0.12)[b]	0.80 (0.06)[b]	0.66 (0.17)[a]
Fine Root Length Ratio		0.85 (0.08)[a]	0.68 (0.08)[b]	0.73 (0.05)[b]	0.80 (0.1)[a]

Table 4. Mean (SD) values describing the elemental composition of roots and leaves of *Avicennia germinans* seedlings grown at ambient (ca. 400 ppm) and elevated (ca. 800 ppm) CO_2 concentrations and two nutrient regimes (low and high). N = 10 seedlings per treatment for above ground measurements and N=10 per treatment for root analysis. Different letters indicate significant differences among the treatments ($p < 0.05$).

Parameter	*CO_2 ppm* / *Nutrients*	400 Low	400 High	800 Low	800 High
Leaves:					
%C		39.5 (0.9)[a]	39.5 (0.5)[a]	39.5 (0.7)[a]	39.7 (0.6)[a]
%N		3.8 (0.7)[a]	3.5 (0.6)[a]	3.7 (0.7)[a]	3.7 (0.4)[a]
%P		0.51 (0.60)[a]	0.51 (0.70)[a]	0.44 (0.8)[b]	0.47 (0.11)[b]
C:N		10.7 (1.8)[a]	11.5 (1.9)[a]	11.1 (2.4)[a]	10.9 (1.2)[a]
C:P		78.3 (12.6)[a]	78.3 (11.3)[a]	92.1 (17.1)[b]	88.7 (19.6)[b]
N:P		7.45 (1.6)[a]	6.86 (2.0)[a]	8.41 (2.3)[a]	7.87 (2.1)[a]
Roots:					
%C		31.1 (2.2)[a]	31.3 (2.1)[a]	33.7 (2.1)[b]	33.9 (1.8)[b]
%N		1.0 (0.04)[a]	1.2 (0.1)[b]	1.1 (0.1)[a]	1.3 (0.1)[c]
%P		0.50 (0.05)[a]	0.50 (0.08)[a]	0.62 (0.14)[a]	0.58 (0.1)[a]
C:N		30.3 (2.6)[a]	26.7 (2.1)[b]	30.5 (2.7)[a]	26.7 (2.7)[b]
C:P		63.1 (5.2)[a]	65.0 (13.0)[a]	56.2 (11.2)[a]	60.0 (9.6)[a]
N:P		2.0 (2.0)[a]	2.44 (0.7)[b]	1.77 (0.38)[a]	2.24 (4.1)[b]

763



Measured carbon assimilation rates (A) for attached, intact Avicennia germinans leaves as a function of leaf temperature in seedlings grown under (top) ambient (400 ppm) and (bottom) elevated (800 ppm) CO2 concentrations subjected to low (left) or high (right) nutrient treatments. The measurements were made under saturating light conditions of 1000 µmol m-2 s-1. Points are the mean (±SE) values for four seedlings, fitted lines are derived from the quadratic relationship described in Eq. 1. Dotted vertical lines denote the calculated temperature optimum for each treatment.
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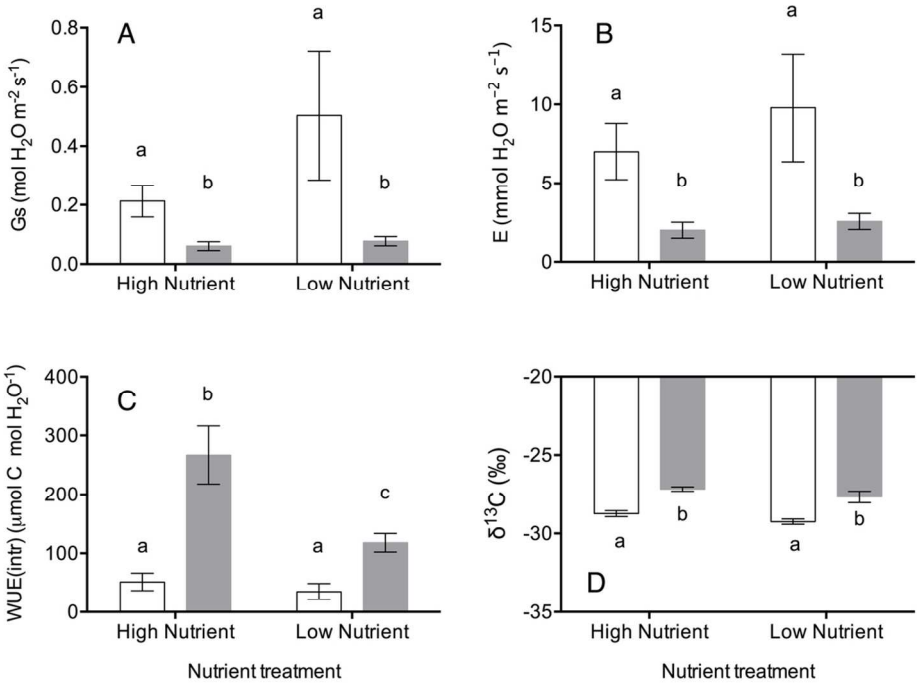

Mean (±SE) A) stomatal water vapour conductance (Gs) B) transpiration (E) and C) intrinsic water use efficiency (WUEi) of attached, intact leaves of four seedlings from each treatment at a leaf temperature of 25°C, irradiance of 1000 µmol m-2 s-1 and CO2 concentrations of 400 ppm for seedlings from the low and high nutrient treatments grown at ambient CO2 levels (open bars), or 800 ppm for seedlings grown at the elevated CO2 concentration (filled bars). D) Foliar δ13C values for N = 10 seedlings from each treatment measured at the end of the experiment. Different letters denote significant differences among treatments ($p < 0.05$).

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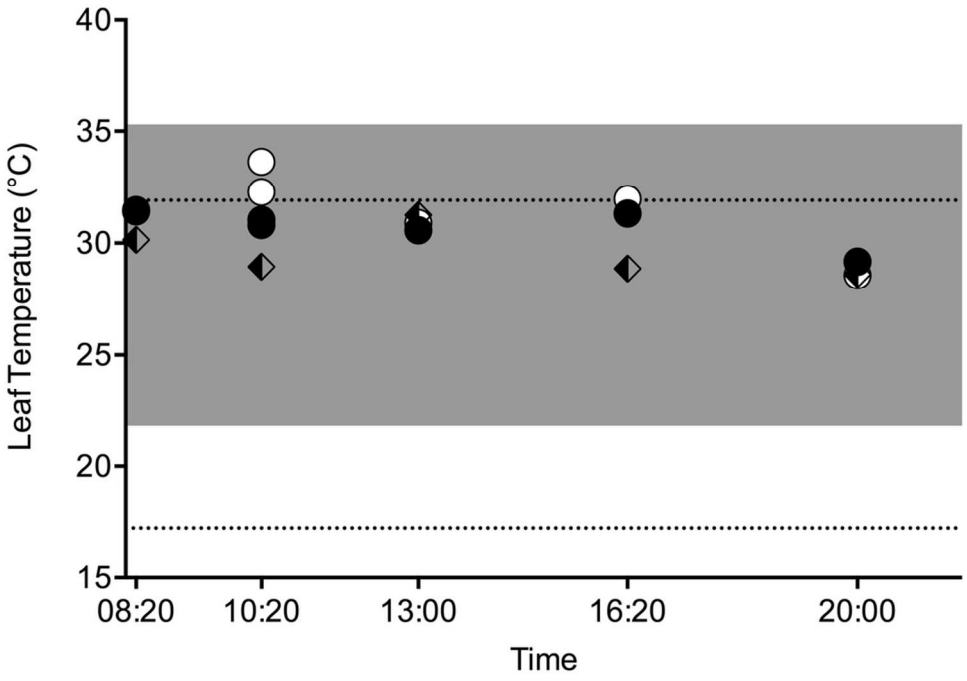

Mean (±SE) leaf temperature measured in seedlings grown under ambient (open circles) and elevated (closed circles) CO2 concentrations using a laser infrared thermometer at different time points on two cloudless days. Diamond symbols are the mean air temperature in the glasshouses at each time point. The optimal temperature range for photosynthesis (see Table 2) at 400 ppm and 800 ppm CO2 is represented by the area bound by the horizontal dotted lines and the shaded area, respectively. N=33 seedlings for each point.
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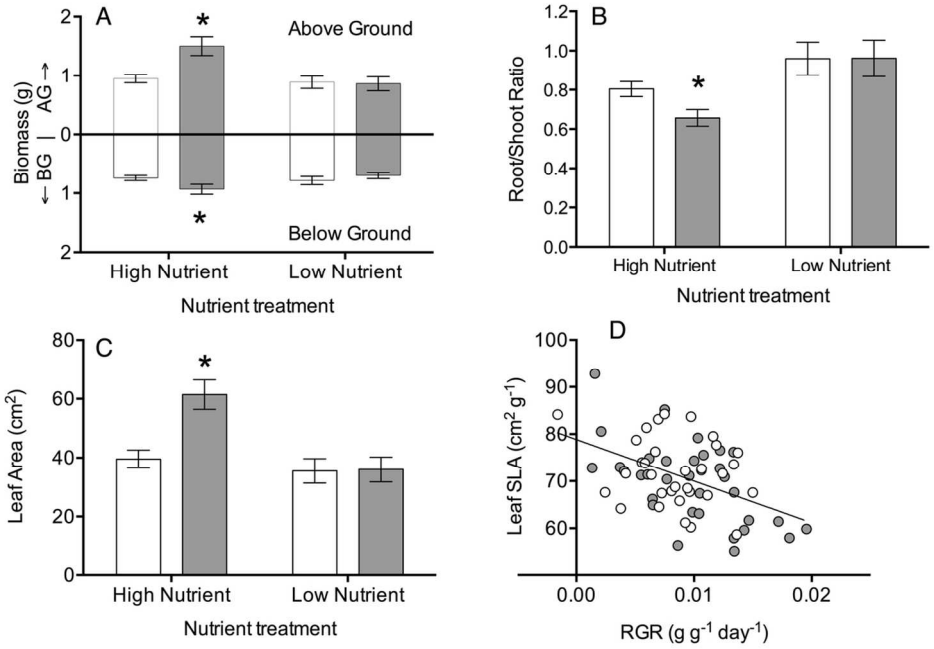

The mean (±SE) A) final above ground (AG) and below ground (BG) biomass, B) root/shoot biomass ratio, and C) total leaf area of seedlings grown under ambient (400 ppm, open bars) or elevated (800 ppm, filled bars) CO2 concentrations and subject to either a low or high nutrient treatment. N = 16-17 seedlings per treatment. "*" denotes significant differences among treatments ($p < 0.05$). Panel D shows the relationship between relative growth rate (RGR) and mean specific leaf area (SLA) for each seedling. The fitted linear regression is of the form SLA = -885RGR +78.9 ($R2 = 0.22$, $p < 0.001$). Open and filled circles represent seedlings grown under ambient or elevated CO2 concentrations respectively.
118x81mm (300 x 300 DPI)