1　**Title**

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3　The effects of CO_2 and nutrient fertilization on the growth and temperature response of

4　the mangrove *Avicennia germinans*

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28   **Running headline**

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30   CO_2 AND NUTRIENT EFFECT ON MANGROVES

31

32   **Abstract**

33   In order to understand plant responses to both the widespread phenomenon of

34   increased nutrient inputs to coastal zones and the concurrent rise in

35   atmospheric CO_2 concentrations, CO_2-nutrient interactions need to be

36   considered. In addition to its potential stimulating effect on photosynthesis and

37   growth, elevated CO_2 affects the temperature response of photosynthesis. The

38   scarcity of experiments testing how elevated CO_2 affects the temperature

39   response of tropical trees hinders our ability to model future primary

40   productivity. In a glasshouse study we examined the effects of elevated CO_2 (800

41   ppm) and nutrient availability on seedlings of the widespread mangrove

42   *Avicennia germinans*. We assessed photosynthetic performance, the temperature

43   response of photosynthesis, seedling growth and biomass allocation. We found

44   large synergistic gains in both growth (42%) and photosynthesis (115%) when

45   seedlings grown under elevated CO_2 were supplied with elevated nutrient

46   concentrations relative to their ambient growing conditions. Growth was

47   significantly enhanced under elevated CO_2 only under high nutrient conditions,

48   mainly in above ground tissues. Under low nutrient conditions and elevated CO_2,

49   root volume was more than double that of seedlings grown under ambient CO2

50   levels. Elevated CO_2 significantly increased the temperature optimum for

51   photosynthesis by ca. 4°C. Rising CO_2 concentrations are likely to have a

52   significant positive effect on the growth rate of *A. germinans* over the next

53   century, especially in areas where nutrient availability is high.

54   **Key Words**

55

56   Climate Change, CO_2, Eutrophication, Mangrove, Nitrogen, Phosphorous, Photosynthesis,

57   RUBISCO, Temperature-Response, Tropics

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59

60   Introduction:

61

62   Current increases in the concentration of CO_2 in the Earth's atmosphere are thought to

63   have an overall positive effect on plant growth and productivity (Drake et al. 1997).

64   However, due to factors interacting with CO_2, such as nutrient and water availability and

65   temperature, measured growth responses to elevated CO_2 have often been variable

66   (Körner 2006; van der Sleen et al. 2015). In particular, progressive nitrogen limitation

67   tends to reduce the long-term growth stimulation by elevated CO_2 (Luo et al. 2004;

68   Norby et al. 2010; Reich et al. 2006), and thus under nutrient limiting conditions, the

69   stimulating effects of elevated CO_2 on plant growth are often significantly reduced

70   relative to nutrient replete conditions (Oren et al. 2001). The handful of experiments

71   studying the effects of elevated CO_2 (700–800 ppm) on mangrove seedlings have shown

72   responses in growth and productivity, with a growth enhancement from 12% to up to

73   47% under elevated CO_2 conditions (Ball et al. 1997; Farnsworth et al. 1996; McKee and

74   Rooth 2008; Reef et al. 2015). Mangroves develop along tropical coastlines, where

75   nutrients frequently are in low supply. In many mangrove forests, nitrogen and

76   sometimes phosphorous have been shown to limit growth (Reef et al. 2010b) and saline

77   conditions may be expected to limit responses to elevated CO_2 (Ball et al. 1997). Thus, to

78   better understand the response of mangroves to elevated CO_2 conditions, CO_2-nutrient

79   interactions need to be considered.

80

81   In addition to its potential stimulating effect on photosynthesis and growth, elevated

82   CO_2 affects the temperature response of photosynthesis in C3 plants. Since current

83   mangrove distributions are strongly influenced by temperature (Duke et al. 1998;

84   Hutchison et al. 2014; Quisthoudt et al. 2013; Woodroffe and Grindrod 1991),

85   quantifying the effects of elevated CO_2 on the temperature response of mangroves is key

86   to determining the fate of mangroves in the face of atmospheric and climate change.

87   Photosynthesis is one of the most temperature sensitive processes in plants (Berry and

88   Bjorkman 1980). The carbon fixing enzyme RUBISCO catalyses both carboxylation (and

89   subsequently photosynthesis) and oxygenation (photorespiration) with CO_2 and O_2 as

90   competing substrates. As temperatures rise, the specificity of RUBISCO for CO_2

91   decreases and CO_2 solubility decreases to a greater extent than that of O_2. Hence, the

92   ratio between photorespiration and photosynthesis increases with increasing

93   temperature (Bernacchi et al. 2001; Jordan and Ogren 1984), significantly reducing

94   carbon assimilation rates and requiring higher CO_2 concentrations to attain similar

95   levels of carbon assimilation. Based on theoretical models of photosynthesis, elevated

96   CO_2 concentrations could have a strong effect on the temperature response of

97   photosynthesis (Farquhar et al. 1980; Lloyd and Farquhar 2008), but experimental

98   evidence for this is not well documented for tropical trees. A number of recent models

99   predict a significant shift in mangrove distributions, for example the loss of mangrove

100   forests from regions of high temperature and a reduction in productivity based on an

101   anticipated rise in global temperature (Beaumont et al. 2011; Koch et al. 2015; Osland et

102   al. 2013), but these predictions are based on the climatic niche of present day

103   mangroves growing under current CO_2 concentrations. The scarcity of experiments

104   testing how elevated CO_2 affects the temperature relationships of tropical trees hinders

105   our ability to model how elevated CO_2 will affect primary productivity in these systems

106   into the future (Cernusak et al. 2013).

107

108   Mangrove forests contribute a large proportion of the primary productivity on tropical

109   coasts, which is important for carbon sequestration and support of both marine and

110   terrestrial food webs (Duarte et al. 2013). Members of the genus *Avicennia* are dominant

111   within higher latitude forests and are documented to have expanded their range in

112   recent decades on three continents (Saintilan et al. 2014). Additionally, in the core of the

113   mangrove distribution (tropical latitudes) they have an important role as they colonize

114   sediments and are tolerant of disturbance (Fromard et al. 2004). In this study we

115   examined the effects of elevated CO_2 and nutrient availability on the mangrove *Avicennia*

116   *germinans* (L.) L.. We assessed the photosynthetic performance, the temperature

117   response of photosynthesis, seedling growth and biomass allocation.

118

119   Methods:

120

121   *Avicennia germinans* propagules were collected in July 2014 at Galeta Point, Panama

122   (9°24′N, 79°51′W) and transferred to the Santa Cruz Experimental Field Facility,

123   Smithsonian Tropical Research Institute, Gamboa, Panama (9°07′N, 79°42′W) where

124   they were planted in individual 1.6 L tree pots (Short One Treepot™, 10x10x23 cm.

125   Stuewe and Sons, Tangent, Oregon) filled with a mixture (50% / 50%) of local topsoil

126   and sand. The plants (propagules) were randomly assigned to one of two naturally

127   illuminated glasshouses (n=34 pots per glasshouse), one with similar to ambient (ca.

128   400 ppm) CO_2 concentrations and one with an elevated (800 ppm) CO_2 concentration.

129

130   Elevated CO_2 was maintained by releasing CO_2 gas from a high-pressure cylinder in brief

131   pulses to maintain CO_2 concentrations between 790 and 810 ppm. The glasshouses were

132   equipped with split air conditioning units programmed to turn on when ambient air

133   temperature exceeded 30°C. Air temperature and relative humidity were recorded in

134   the two glasshouses every 15 min using a data logger (CR10X; Campbell Scientific,

135   Logan, Utah, USA). The conditions in each of the two glasshouses during the experiment

136   are summarised in Table 1.

137

138   Seedlings were watered twice weekly with 300 ml salt solution that saturated the pots.

139   Two nutrient treatments were implemented in each glasshouse, a low nutrient

140   treatment (n=17 in each glasshouse) and a high nutrient treatment (n=17 in each

141   glasshouse). The solution low in nutrients contained 0.06 mM KNO_3, 0.04 mM $Ca(NO_3)_2$,

142   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

143    $MnSO_4$, 0.2 µM $ZnSO_4$, 0.05 µM $CuSO_4$, 0.05 µM H_2MoO_4, 2 µM $C_{10}H_{12}FeN_2NaO_8$

144   (ethylenediaminetetraacetic acid iron (III)-sodium salt), which is similar to the nutrient

145   concentrations in mangrove porewater where they are not exposed to anthropogenic

146   eutrophication (Alongi et al. 1993; Chen and Twilley 1999). The concentrations in the

147   high nutrient solution were 5 times those of the low nutrient solution. Ocean salt

148   (Instant Ocean, Blacksburg, VA, USA) was added to both nutrient solutions to a

149   concentration of 20 g L^{-1}. Instant ocean aquarium salt does not contain nitrogen and

150   phosphorus. Once a week the plants received a rinse of fresh water (10 ml) from a spray

151   bottle to simulate a rain event washing the salt from their leaves.

152   Two plants died during the experimental period. After three months of growth (October

153   6, 2014), photosynthetic temperature response curves were assessed for four randomly

154   selected plants from each of the four treatments over the period of a week. All plants

155   were harvested on the 14th of October 2014.

156

157   *Photosynthetic temperature response curves:*

158

159   Photosynthetic gas exchange was measured on intact leaves of known area enclosed in a

160   Walz gas-exchange cuvette with Peltier temperature control (GWK 3M Walz, Effeltrich,

161   Germany) connected to a LI-6252 infrared gas analyser (Li-Cor, Lincoln NE, USA) under

162   constant illumination of 1000 µmol m^{-2} s^{-1} from a red/blue LED light array. The CO_2

163   concentration of the air entering the chamber was set to 400 ppm for the seedlings

164   grown at ambient CO_2 concentrations and to 800 ppm for the seedlings grown at

165   elevated CO_2 concentrations. Following the enclosure of the leaf into the chamber, the

166   chamber temperature was reduced to 20°C for ~60 min. The temperature was then

167   increased in 5°C increments (every 20–30 min, when a stable reading was established)

168   up to 40–50°C. The youngest fully expanded leaves were studied. Leaf temperature was

169   measured using a copper-constantan thermocouple attached to the bottom surface of

170   the leaf.

171   Temperature response data were fitted to the equation from Battaglia, Beadle &

172   Loghhead (1996; Eq. 1) using the *nlsfit* function in R (Team 2014). The equation

173   describes the photosynthetic rate (P) at a given temperature (T) as a parabolic

174   relationship, with P_{opt} and T_{opt} being the maximal photosynthetic rate, and the

175   temperature at which P_{opt} is achieved, respectively. Analysis of variance was used to

176   detect differences in the parameters P_{opt} (measured here as photosynthetic capacity,

177   A_{max}), T_{opt} and the high-temperature CO_2 compensation point (where net CO_2 exchange is

178   zero) among treatments.

179

180

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

182

183   Transpiration rate was calculated from the water vapour difference between the air

184   leaving the chamber and the incoming air. Stomatal conductance at each temperature

185   was calculated from the rate of transpiration divided by the leaf-air vapour pressure

186   difference (VPD) in the air leaving the chamber relative to the incoming air. Intrinsic

187   water use efficiency was calculated as the carbon assimilation rate divided by the

188   stomatal conductance.

189

190   *Plant growth parameters and elemental composition:*

191

192   Plant growth (stem length, no. of nodes and no. of leaves, no. of branches along the main

193   stem) was monitored throughout the experiment. Leaf temperatures were measured for

194   three leaves per seedlings one week prior to harvest on two cloudless days using a laser

195   infrared thermometer. The measurements were repeated on all seedlings five times

196   during the day (08:00, 10:00, 13:00, 16:00 and 20:00). Following the harvest, plants

197   were divided into leaves, stem and roots. Leaves were kept in a sealed bag with moist

198   paper towel in order to maintain hydration status. Leaf area was measured using a LI-

199   3100C leaf area meter (Li-Cor Corp. Lincoln, NE, USA). Washed roots free of soil were

200   photographed against a dark background and analysed using the IJ Rhizo root analysis

201   package (Pierret et al. 2013). The entire root system was measured for each seedling.

202   Plant material was then washed in distilled water to remove external salt, patted dry

203   and weighed after which it was dried at 70°C for 5 days and reweighed.

204

205   Samples for leaf nutrient concentrations and isotopic composition were taken from

206   finely ground leaves and roots from ten randomly selected plants from each treatment.

207   All leaves from each plant were pooled before grinding. The isotopic composition of the

208   added CO_2 in the 800 ppm treatment differed slightly from that in ambient air. The

209   correction for this was previously determined for this system by growing two C4 plants

210   (*Saccharum spontaneum* and *Portulaca oleracea*) in the chambers. A correction factor of

211   2‰ was used in foliar $\delta^{13}C$ values of seedlings from the 800 ppm treatment (Cernusak

212   et al. 2011a).

213

214   Phosphorous (P) concentrations were determined using a colourimetric assay as

215   described in (Reef et al. 2010a). Leaf isotope values for $\delta^{13}C$ and $\delta^{15}N$ were measured

216   from pooled samples of green leaves for ten seedlings from each treatment. Samples

217   were measured in an elemental-analyser isotope ratio mass spectrometer (EA-IRMS,

218   Sercon System, Griffith University; analytical errors of 0.1‰ for $\delta^{13}C$ and 0.2‰ for

219    $\delta^{15}N$). Nitrogen is expressed relative to atmospheric nitrogen and carbon relative to

220   Vienna Pee-Dee Belemnite.

221

222 We used ANOVA to test for differences in growth parameters among the treatments.

223 Root/Shoot ratios were *logit* transformed prior to analysis. Partial correlation analysis

224 was used to test the relationship between specific leaf area (SLA) and growth. Climate

225 data for Galeta Point was downloaded from the Smithsonian Physical Monitoring

226 Program climate station at the Galeta Marine Laboratory.

227

228 Results:

229

230 *Effects of CO_2 and nutrients on foliar physiology*

231

232 Using a two-way ANOVA we found significant effects of both CO_2 concentration and

233 nutrient treatment on photosynthetic capacity, A_{max} (ANOVA, $F_{(1,11)}$ = 8.5, p = 0.014, and

234 $F_{(1,11)}$ = 5.6, p = 0.04 respectively, Figs 1A-D, Table 2), where A_{max} increases with

235 increased CO_2 concentration and with nutrient enrichment, but more so when both

236 elevated CO_2 and elevated nutrients were provided (Table 2).

237

238 Elevated CO_2 significantly increased the temperature optimum for photosynthesis by ca.

239 4°C (ANOVA, $F_{(1,12)}$ = 17.3, p = 0.001, Figs 1A-D, Table 2). Despite the shift in the

240 temperature optimum, the high-temperature CO_2 compensation point, i.e. the

241 temperature at which net CO_2 exchange is zero, did not change significantly and was on

242 average 41.8 (±3)°C. The range of temperatures at which photosynthesis was near

243 maximum (≥80% of A_{max}) spanned 13°C and shifted to higher temperatures with

244 elevated CO_2 (Table 2).

245

246 Transpiration rate (E), stomatal water vapour conductance (Gs) and intrinsic water use

247 efficiency (WUEi) are presented for leaf temperatures of 25°C. Elevated CO_2 resulted in a

248 significant reduction in stomatal conductance and transpiration relative to the ambient

249   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

250   respectively), which contributed to a significant increase in water use efficiency ($F_{(1,10)}$ =

251   22.1, p < 0.001 Fig. 2C), most notably under the high nutrient regime (p = 0.03). The

252   foliar $\delta^{13}C$ of leaves was significantly less negative in the elevated CO_2 treatment

253   indicating that water use efficiency for the duration of the experiment was higher in this

254   treatment ($F_{(1,35)}$ = 42.4, p < 0.001, Fig. 2D)

255

256   There were no significant differences in leaf temperatures among the CO_2 and nutrient

257   treatments (Fig. 3). On sunny days, leaf temperatures of ambient CO_2 grown plants were

258   found to be at the high range, and sometimes exceeded the optimal temperature

259   threshold for photosynthesis (defined here as the temperature range at which 80% of

260   maximum photosynthetic rates can be achieved, Table 2). For plants growing under

261   elevated CO_2 conditions, leaf temperatures were well within the optimal range for

262   photosynthesis (Fig. 3). Neither the CO_2 nor the nutrient treatment significantly affected

263   leaf water content, which was on average (±SD) 71.3% (±2.2%) of the fresh weight.

264

265

266   *Effects of CO_2 and nutrients on growth and biomass allocation*

267

268   Seedling growth (total biomass accumulated) was significantly enhanced under elevated

269   CO_2 but only under high nutrient conditions (ANOVA $F_{(1,62)}$ = 9.2, p = 0.003, Fig. 4A). In

270   the high nutrient treatment, the rise in CO_2 concentrations from 400 to 800 ppm

271   resulted in a 44% increase in biomass. Growth enhancement in the high nutrient

272   treatment occurred mainly in above ground tissues (Fig. 4B), resulting in significantly

273   lower root/shoot biomass ratios, with a more pronounced decrease in elevated CO_2

274   grown plants (ANOVA $F_{(1,62)}$ = 9.8, p = 0.003). However, despite a lower overall

275   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

303   more than double that of seedlings grown under ambient CO_2 levels ($F_{(1,37)} = 5.8$, $p =$

304   0.02, Table 3). Mean root diameter was also affected, with a higher frequency of fine

305   roots in the ambient CO_2/low nutrient and high CO_2/high nutrient treatments ($F_{(1,37)} =$

306   28.4, $p < 0.001$, Table 3). We identified three major root types in our seedlings: fine

307   roots with diameters < 2 mm, lateral roots (d = 2–4 mm), and pneumatophores, which

308   developed in a few seedlings (d > 4 mm). Fine roots made up on average 76% of the

309   total root length. The fine root ratio (fine roots/total root biomass) was higher in the

310   low nutrient treatment under ambient CO_2 conditions, as was the total fine root length.

311   Under elevated CO_2 conditions, the effect of nutrients on fine root production was

312   reversed, with a significant decrease in the fine root ratio in the low nutrient treatment.

313   However, total fine root length remained higher in elevated CO_2 than under ambient CO_2

314   conditions for both nutrient treatments. Roots from elevated CO_2 grown seedlings also

315   had a higher concentrations of carbon, regardless of nutrient treatment ($F_{(1,36)} = 15.5$, p

316   < 0.001, Table 4).

317

318   *Effects of CO_2 and nutrients on plant nutrient content*

319

320   Phosphorous (P) concentrations in plant tissues were significantly affected by the CO_2

321   treatment. Elevated CO_2 seedlings had significantly higher concentration of P in their

322   root tissues, relative to ambient CO_2 grown seedlings (ANOVA $F_{(1,36)} = 11.5$, $p = 0.002$,

323   Table 4). In leaves, we found the opposite, lower P concentrations in seedlings from the

324   elevated CO_2 treatment relative to ambient CO_2 ($F_{(1,35)} = 5.1$, $p = 0.03$, Table 4). The

325   nutrient treatment had no significant effect on tissue P concentrations.

326

327   The exhaustion of maternal nutrient reserves as the seedlings matured led to a

328   significant loss of foliage in low nutrient grown seedlings where leaf mortality rates

329   were more than double those of the high nutrient grown seedlings (ANOVA, $F_{(1,62)} = 4.8$,

330     $p = 0.03$, Table 3). However, N or P concentrations in leaves of the low nutrient plants

331   were not significantly lower than those in plants from the high nutrient treatment

332   (Table 4). Differences in elemental composition between the nutrient treatments were

333   detected in the roots, with higher %N, lower C:N and higher N:P in the high nutrient

334   plants ($F_{(1,36)} = 24.8$, $p < 0.001$, $F_{(1,36)} = 7.2$, $p = 0.01$ and $F_{(1,36)} = 21$, $p < 0.001$

335   respectively, Table 4).

336

337   Discussion:

338

339   We found large synergistic gains in both photosynthesis and growth in *Avicennia*

340   *germinans* seedlings when seedlings grown under elevated CO_2 were supplied with

341   elevated nutrient concentrations. In the high nutrient-elevated CO_2 treatment,

342   photosynthesis was enhanced on average by 75% relative to the high nutrient ambient

343   CO_2 grown seedlings, and 115% when compared with the low nutrient ambient CO_2

344   grown seedlings. Growth was enhanced by 42% in the elevated CO_2/high nutrient

345   treatment relative to ambient CO_2/high nutrient seedlings. As has been observed in

346   other species, growth was less sensitive than photosynthesis to elevated CO_2

347   (Kirschbaum 2011). Despite significant differences in water use efficiency among the

348   nutrient and CO_2 treatments, plant water use efficiency was not associated with growth

349   or productivity. This is consistent with growing evidence that indicates mangrove

350   growth is not limited by water availability at moderate salinities (Reef et al. 2012).

351

352   Elevated CO_2 had a significant effect on the temperature dependence of light saturated

353   photosynthesis as is predicted by theoretical models (Farquhar et al. 1980; Lloyd and

354   Farquhar 2008). The optimal temperature for carbon fixation increased from 24.5°C at

355   CO_2 concentrations of 400 ppm to 28.3°C in plants that were grown and measured at

356   800 ppm CO_2, an increase of nearly 4°C, which is higher than the predicted increase in

357   mean global temperature for 2100 for moderate emissions scenarios (IPCC 2013).

358

359   T_{max}, the temperature at which net assimilation is zero, was not significantly affected by

360   elevated CO_2 concentrations, remaining on average 41.8°C. Irreversible damage in

361   tropical tree leaves has been shown to occur at temperatures >50 °C (Krause et al. 2010;

362   Krause et al. 2014)

363

364   Despite differences in transpiration rates of 74% among the different CO_2 and nutrient

365   treatments, leaf temperatures measured during the experiment were not significantly

366   higher in the elevated CO_2 grown seedlings. This could be due to the fact that

367   transpiration plays a relatively small role in leaf temperature regulation compared to

368   the important influence of air temperature and irradiance (Miller 1972) especially in

369   mangroves, where non-evaporative cooling strategies (e.g. leaf orientation, pubescence

370   and salt excretion) are adaptations that maintain high water use efficiencies in these

371   species (reviewed in (Reef and Lovelock 2014b).

372

373   The photosynthesis temperature response measured for *A. germinans* was of similar

374   shape to the temperature response measured for the congeneric *Avicennia marina* (Ball

375   et al. 1988) , and while T_{opt} of *A. germinans* was 3°C lower than that of its Australian

376   counterpart, the high temperature CO_2 compensation point was similar to that of *A.*

377   *marina*. Evidence from field measurements suggests that photosynthesis in *Bruguiera*

378   *parviflora* from northern Queensland was strongly depressed at leaf temperatures >

379   34°C (Cheeseman et al. 1991). Also in northern Queensland, assimilation rates in

380   *Rhizophora stylosa* decreased linearly as temperatures increased from 27–40°C and was

381   at nearly the CO_2 compensation point at 39.5°C (Andrews and Muller 1985). However,

382   in both these studies, the effect of temperature on carbon assimilation rates was

383 confounded by coinciding changes in light levels, humidity and differences in leaf angles.

384 The CO_2 compensation point (T_{max}) for *A. germinans* in our study was on average

385 41.8±3°C, and while we found a significant increase in T_{opt} with elevated CO_2, we do not

386 find a corresponding increase in T_{max} and our results do not support an increase in the

387 high temperature threshold for this species under elevated CO_2 conditions.

388

389 The optimal temperature for photosynthesis under ambient CO_2 conditions was lower

390 than the T_{leaf} measured for the seedlings throughout the day (Fig. 1). T_{opt} was also lower

391 than the mean temperature in the glasshouse (Table 1) and lower than the mean daily

392 atmospheric temperature recorded at Punta Galeta, where the plant material was

393 collected, in the years 2002–2015 between 07:00 and 16:00 (27.8°C ±2). However, the

394 temperature range of near optimal photosynthetic performance of the seedlings was

395 very broad (approx. 13°C, Table 2) and the leaf temperatures measured in the

396 glasshouse during growth were within this range (Fig. 3). Nonetheless, a T_{leaf} that is on

397 average higher than T_{opt} suggests an incomplete acclimation to the mean growing

398 temperature. It is possible that broad response of photosynthesis to temperature in *A.*

399 *germinans* reflects its broad latitudinal distribution. Despite the low levels of gene flow

400 among *A. germinans* populations (Ceron-Souza et al. 2012), a relatively low T_{opt} could be

401 a conserved trait. There is growing evidence that not all plant species are capable of

402 complete photosynthetic thermal acclimation to growth temperature (e.g. Dillaway and

403 Kruger 2010). Our findings for *A. germinans* support this possibility. Relatively low T_{opt}

404 compared to mean daily temperature, may also indicate acclimation of photosynthesis

405 to early morning conditions when the majority of photosynthetic carbon gain in this

406 species occurs (Smith et al. 1989). The mean temperature in the early morning (06:00-

407 09:00) at Punta Galeta was (26.7±1.9). In mangroves midday depressions in

408 photosynthesis are common (Andrews and Muller 1985; Bjorkman et al. 1988;

409 Cheeseman et al. 1991), with some field studies showing a peak in photosynthesis

410   before 0800 AM and a cessation of photosynthesis by 1100 AM (Cheeseman et al. 1991).

411   An incomplete acclimation to high ambient temperatures could be one of the causes of

412   these depressions. Photosynthesis temperature response in three Australian mangrove

413   species (*Bruguiera gymnorrhiza, Rhizophora apiculata and Avicennia marina*), measured

414   under ambient (unspecified) CO_2 concentrations showed a broad temperature optima

415   (25-30°C), which was significantly lower than leaf temperatures measured on sun

416   exposed leaves as early as 0825 AM (Ball et al. 1988). In the Ball et al. study (1988) it

417   was shown that leaf angle in mangroves is optimised to reduce leaf temperatures rather

418   than maximise light capture, resulting in lower rates of photosynthesis. Irrespective of

419   the underlying pressure that leads to selection for the broad temperature optima of

420   photosynthesis and the cause of incomplete acclimation to the mean growing

421   temperature, the increase in T_{opt} with increasing CO_2 concentrations could result in

422   improved photosynthetic performance and growth rates for this species within the

423   tropics as CO_2 concentrations continue to increase.

424

425   Low nutrient availability restricted the growth response of the mangrove *A. germinans*

426   to elevated CO_2 despite significant improvements to photosynthesis and water use

427   efficiency. Elevated CO_2 stimulated growth mainly above ground (increasing leaf area),

428   although significant increases in below ground biomass were also detected relative to

429   ambient CO_2 concentrations. Leaf SLA decreased as seedling growth rates increased. The

430   enhancements observed in plant performance are consistent with previous studies

431   conducted in greenhouses with mangrove seedlings (Ball et al. 1997; Farnsworth et al.

432   1996; McKee and Rooth 2008; Reef et al. 2015) and other plant species (Ainsworth and

433   Long 2005; Winter et al. 2001a; Winter et al. 2001b), but also with a historical

434   assessment that indicated SLA has already decreased in response to rises in CO_2 over the

435   period since industrialization (Reef and Lovelock 2014a).

436

437   The combination of elevated CO_2 and elevated nutrients resulted in significantly higher

438   leaf areas but no significant differences in the nitrogen and carbon concentration of

439   leaves. An analysis of 16 FACE experiments worldwide found no effects of elevated CO_2

440   on foliar nitrogen concentrations in woody plants (Nowak et al. 2004). However, due to

441   the increase in leaf area, an increase in nitrogen uptake did occur at the whole-plant

442   level. Elevated CO_2 led to a reduction in foliar phosphorus concentrations (Table 4), a

443   phenomenon which has been observed previously in *A. germinans* (Reef et al. 2015) and

444   could be due to reduced transpiration rates (Fig. 4B), possibly involving subsequent

445   lower translocation rates of P to the shoot via the xylem stream, as has been suggested

446   for other tropical trees (Cernusak et al. 2011b). This is further supported by the increase

447   in P concentrations (and small increases in %N) in the roots of the elevated CO_2

448   seedlings (Table 4). The reduction in foliar phosphorous concentrations under elevated

449   CO_2 was overcome to some extent (although not significantly so) in the high nutrient

450   treatment. Elevated CO_2 induced reduction in whole seedling transpiration rates, could

451   thus have a significant effect on growth rates in mangrove forests where P is the limiting

452   nutrient for growth such as in forests that are hydrologically isolated from regular tidal

453   inundation (Feller et al. 2003).

454

455   Elevated CO_2 had a significant effect on roots, increasing root length and biomass and

456   also the carbon concentration in the roots, but did not increase allocation of biomass to

457   roots (except under high nutrient levels) as has been shown in other woody species

458   (Hättenschwiler and Körner 1997). Root morphology was influenced in a complex

459   interaction between elevated CO_2 and nutrient availability as root systems under

460   elevated CO_2 and high nutrient conditions tended to have a lower proportion of biomass

461   allocated to roots, but roots had a higher proportion of fine roots (Table 3). The increase

462   in fine root production we observed for *A. germinans* under elevated CO_2 conditions is

463   consistent with allocation models based on findings from other tree species (Dybzinski

464 et al. 2015) and is suggested to be driven by the use of carbon exudates to prime

465 microbial populations to enhance N release for plant growth (Phillips et al. 2011). Root

466 development is influenced by complex interactions among nutrient and water demands

467 of the shoot (Poorter et al. 2012) and carbohydrate availability (Eveland and Jackson

468 2012). Reduction in transpiration in seedlings grown under elevated CO_2 (and increased

469 WUE) reduces the demand for water, which may be balanced by an increase in nutrient

470 demand due to higher growth rates (Chapin 1980), leading to little overall change in

471 allocation to roots under low nutrient conditions (Fig. 4). As the rate of root

472 development in mangroves is an important determinant of seedling establishment

473 success in the soft sediment of tidal flats (Balke et al. 2011) the rapid elongation of roots

474 under elevated CO_2 may increase survivorship of seedlings. Potential changes under

475 elevated CO_2 in allocation to root biomass, or alterations to root morphology and

476 elemental composition, which may influence decomposition, are important in mangrove

477 forests as these factors are likely to influence capacity for carbon sequestration in these

478 habitats and their responses to sea level rise (Krauss et al. 2014).

479

480 *Mangroves in a changing environment*

481

482 Rising CO_2 concentrations are likely to have a significant positive effect on the growth

483 rate of the widespread mangrove *Avicennia germinans* over the next century, especially

484 in areas where nutrients availability is high. For a congenitor in the Pacific Ocean, there

485 is evidence that primary production has already been influenced by elevated CO_2 (Reef

486 and Lovelock 2014a). Increased nutrient loading in coastal areas is widespread and

487 synergistic interactions with elevated CO_2 are likely to result in overall increases in

488 mangrove biomass, C sequestration and below ground C storage. Elevated CO_2

489 concentrations will affect the temperature response of photosynthesis in this species

490   more so than the predicted rise in mean global temperature over this period, possibly

491   mitigating growth inhibition by future high temperature anomalies.

492

493

501

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691   Figure Legends:

692

693   Figure 1. Measured carbon assimilation rates (A) for attached, intact *Avicennia*

694   *germinans* leaves as a function of leaf temperature in seedlings grown under (top)

695   ambient (400 ppm) and (bottom) elevated (800 ppm) CO_2 concentrations subjected to

696   low (left) or high (right) nutrient treatments. The measurements were made under

697   saturating light conditions of 1000 μmol m^{-2} s^{-1}. Points are the mean (±SE) values for

698   four seedlings, fitted lines are derived from the quadratic relationship described in Eq. 1.

699   Dotted vertical lines denote the calculated temperature optimum for each treatment.

700

701   Figure 2. Mean (±SE) A) stomatal water vapour conductance (Gs) B) transpiration (E)

702   and C) intrinsic water use efficiency (WUEi) of attached, intact leaves of four seedlings

703   from each treatment at a leaf temperature of 25°C, irradiance of 1000 μmol m^{-2} s^{-1} and

704   CO_2 concentrations of 400 ppm for seedlings from the low and high nutrient treatments

705   grown at ambient CO_2 levels (open bars), or 800 ppm for seedlings grown at the

706   elevated CO_2 concentration (filled bars). D) Foliar $\delta^{13}C$ values for $N = 10$ seedlings from

707   each treatment measured at the end of the experiment. Different letters denote

708   significant differences among treatments ($p < 0.05$).

709

710   Figure 3. Mean (±SE) leaf temperature measured in seedlings grown under ambient

711   (open circles) and elevated (closed circles) CO_2 concentrations using a laser infrared

712   thermometer at different time points on two cloudless days. Diamond symbols are the

713   mean air temperature in the glasshouses at each time point. The optimal temperature

714   range for photosynthesis (see Table 2) at 400 ppm and 800 ppm CO_2 is represented by

715   the area bound by the horizontal dotted lines and the shaded area, respectively. $N=33$

716   seedlings for each point.

717

718   Figure 4. The mean (±SE) A) final above ground (AG) and below ground (BG) biomass,

719   B) root/shoot biomass ratio, and C) total leaf area of seedlings grown under ambient

720   (400 ppm, open bars) or elevated (800 ppm, filled bars) CO_2 concentrations and subject

721   to either a low or high nutrient treatment. N = 16-17 seedlings per treatment. "*"

722   denotes significant differences among treatments ($p < 0.05$). Panel D shows the

723   relationship between relative growth rate (RGR) and mean specific leaf area (SLA) for

724   each seedling. The fitted linear regression is of the form $SLA = -885RGR + 78.9$ ($R^2 = 0.22$,

725   $p < 0.001$). Open and filled circles represent seedlings grown under ambient or elevated

726   CO_2 concentrations respectively.

727

728

729   Table 1. CO_2, temperature and humidity conditions in the two glasshouses between the

730   22nd of June and the 13th of October 2014. Measurements were taken every 5 minutes

731   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

732

733

734   Table 2. Mean (SD) values describing the temperature response of photosynthesis in

735   *Avicennia germinans* seedling grown at ambient (ca. 400 ppm) and elevated (ca. 800

736   ppm) CO_2 concentrations and under two nutrient regimes (low and high). A_{max} is the

737   maximal carbon assimilation rate at light saturation and T_{opt} is the temperature at which

738   A_{max} is achieved. T_{max} is the temperature at which the upper CO_2 compensation point

739   occurs, above which net CO_2 loss occurs. Values were calculated from the quadratic

740   relationship fit to the temperature series from each seedling (Eq. 1). $N = 4$ seedlings per

741   treatment. Different letters indicate significant differences among the treatments ($p <$

742   0.05).

743

Parameter	CO_2 ppm	400	400	800	800
	Nutrients	Low	High	Low	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

744

745

746   Table 3. Mean (SD) values describing the morphological response of *Avicennia*

747   *germinans* seedlings to ambient (ca. 400 ppm) and elevated (ca. 800 ppm) CO_2

748   concentrations and two nutrient regimes (low and high). $N = 17$ seedlings per treatment

749   for above ground measurements and $N = 10$ per treatment for root analysis. Different

750   letters indicate significant differences among the treatments ($p < 0.05$).

751

	CO₂ ppm	400	400	800	800
Parameter	*Nutrients*	Low	High	Low	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 (cm³)		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]

752

753

754   Table 4. Mean (SD) values describing the elemental composition of roots and leaves of

755   *Avicennia germinans* seedlings grown at ambient (ca. 400 ppm) and elevated (ca. 800

756   ppm) CO_2 concentrations and two nutrient regimes (low and high). $N = 10$ seedlings per

757   treatment for above ground measurements and N=10 per treatment for root analysis.

758   Different letters indicate significant differences among the treatments ($p < 0.05$).

759

760

| 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]

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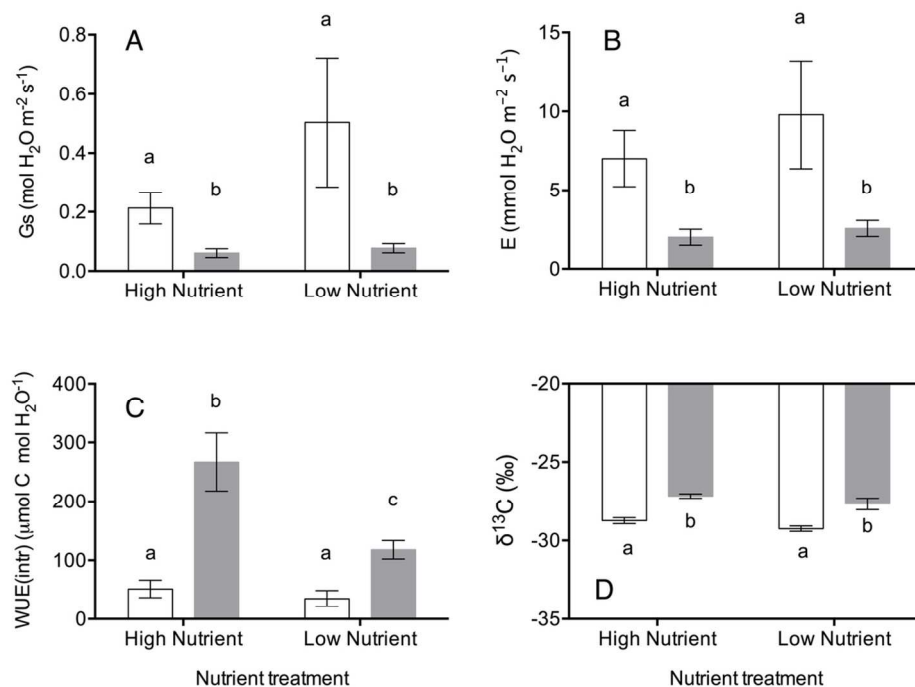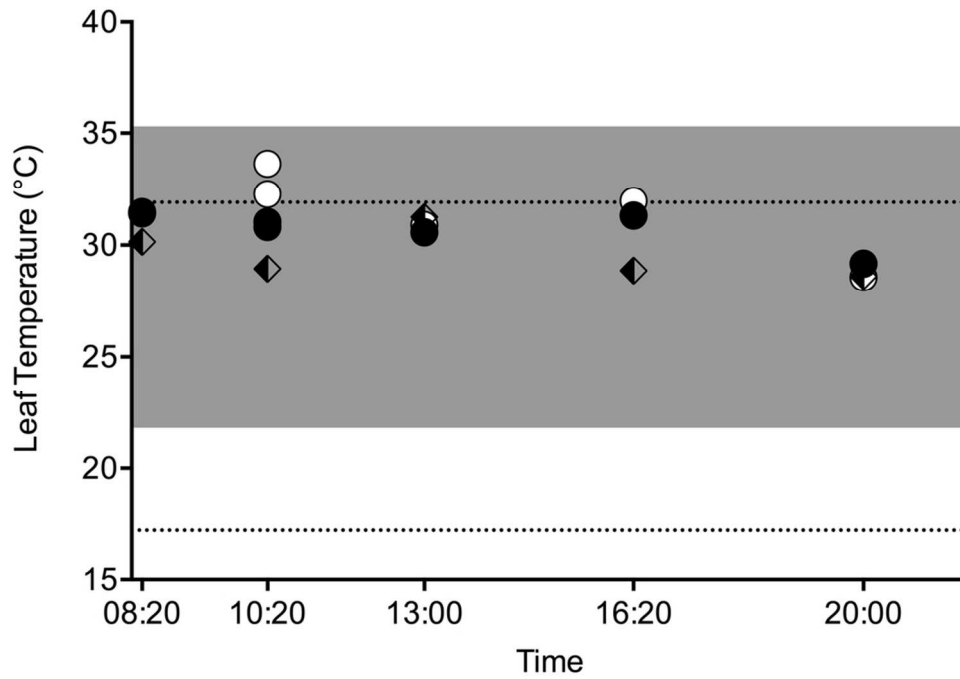47
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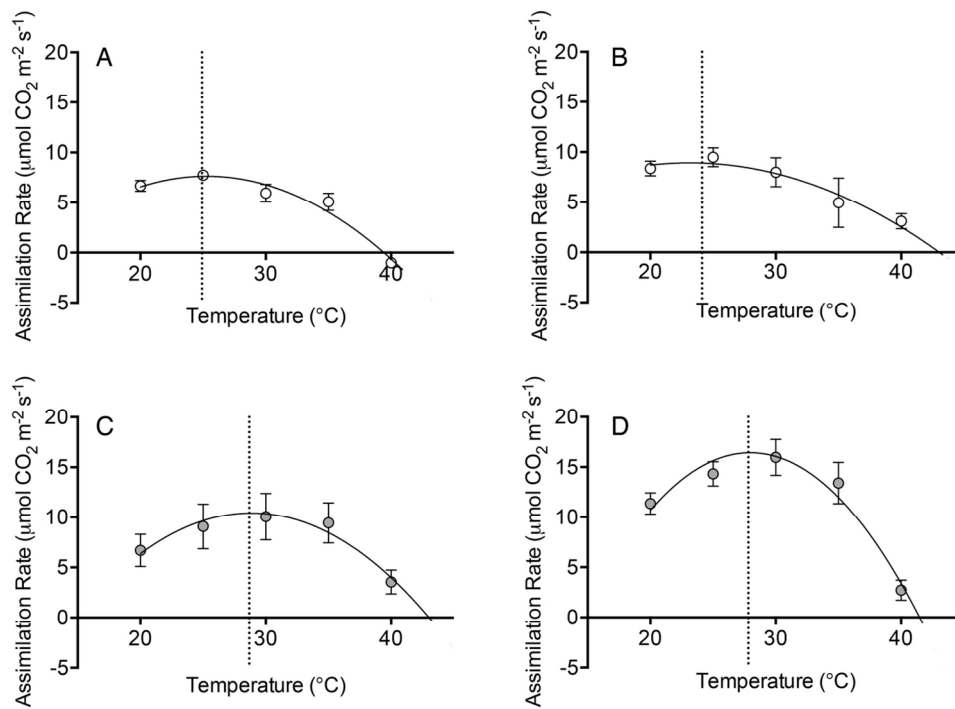
Measured carbon assimilation rates (A) for attached, intact Avicennia germinans leaves as a function of leaf temperature in seedlings grown under (top) ambient (400 ppm) and (bottom) elevated (800 ppm) CO2 concentrations subjected to low (left) or high (right) nutrient treatments. The measurements were 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.
121x89mm (300 x 300 DPI)

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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) 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.
95x67mm (300 x 300 DPI)
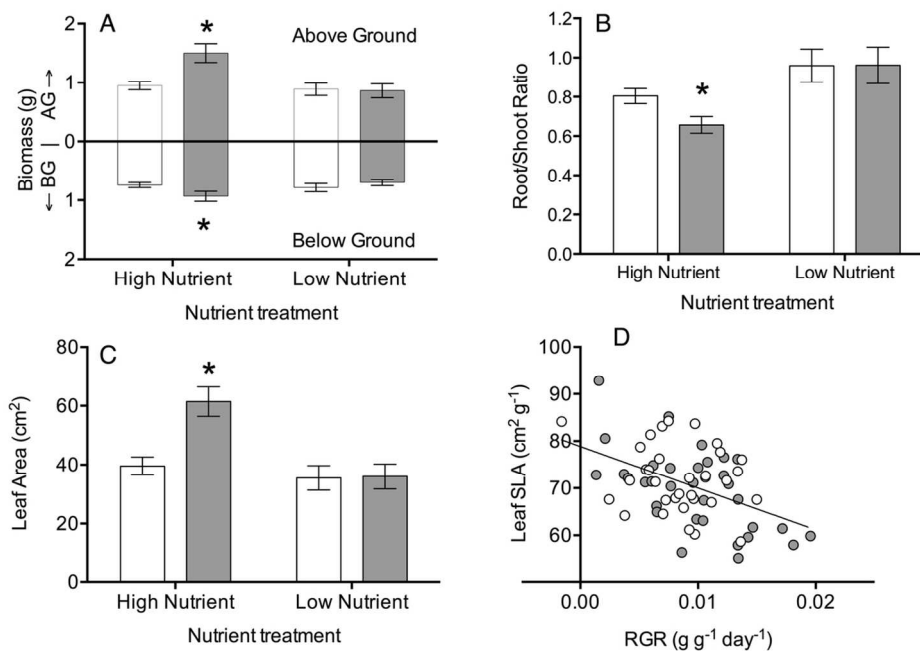
The mean (±SE) A) final above ground (AG) and below ground (BG) biomass, B) root/shoot biomass ratio, and C) total leaf area of seedlings grown under ambient (400 ppm, open bars) or elevated (800 ppm, filled bars) CO_2 concentrations and subject to either a low or high nutrient treatment. N = 16-17 seedlings 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.
118x81mm (300 x 300 DPI)