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Elevated CO₂ alters anatomy, physiology, growth, and reproduction of red mangrove (*Rhizophora mangle* L.)

Abstract Mangroves, woody halophytes restricted to protected tropical coasts, form some of the most productive ecosystems in the world, but their capacity to act as a carbon source or sink under climate change is unknown. Their ability to adjust growth or to function as potential carbon sinks under conditions of rising atmospheric CO_2 during global change may affect global carbon cycling, but as yet has not been investigated experimentally. Halophyte responses to CO_2 doubling may be constrained by the need to use carbon conservatively under water-limited conditions, but data are lacking to issue general predictions. We describe the growth, architecture, biomass allocation, anatomy, and photosynthetic physiology of the predominant neotropical mangrove tree, *Rhizophora mangle* L., grown solitarily in ambient (350 μll^{-1}) and double-ambient (700 μll^{-1}) CO_2 concentrations for over 1 year. Mangrove seedlings exhibited significantly increased biomass, total stem length, branching activity, and total leaf area in elevated CO_2. Enhanced total plant biomass under high CO_2 was associated with higher root:shoot ratios, relative growth rates, and net assimilation rates, but few allometric shifts were attributable to CO_2 treatment independent of plant size. Maximal photosynthetic rates were enhanced among high-CO_2 plants while stomatal conductances were lower, but the magnitude of the treatment difference declined over time, and high-CO_2 seedlings showed a lower P_{max} at 700 μll^{-1} CO_2 than low-CO_2 plants transferred to 700 μll^{-1} CO_2: possible evidence of downregulation. The relative thicknesses of leaf cell layers were not affected by treatment. Stomatal density decreased as epidermal cells enlarged in elevated CO_2. Foliar chlorophyll, nitrogen, and sodium concentrations were lower in high CO_2. Mangroves grown in high CO_2 were reproductive after only 1 year of growth (fully 2 years before they typically reproduce in the field), produced aerial roots, and showed extensive lignification of the main stem; hence, elevated CO_2 appeared to accelerate maturation as well as growth. Data from this long-term study suggest that certain mangrove growth characters will change flexibly as atmospheric CO_2 increases, and accord with responses previously shown in *Rhizophora apiculata*. Such results must be integrated with data from sea-level rise studies to yield predictions of mangrove performance under changing climate.

Key words *Rhizophora mangle* · Growth · Photosynthesis · Reproduction · CO_2

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Introduction

The experimental and theoretical literature addressing the effects of rising atmospheric CO_2 on plant growth and community structure has burgeoned in recent years (reviews and multi-species analyses in Kimball 1983; Cure and Acock 1986; Bazzaz 1990; Drake and Leadley 1991; Hunt et al. 1991; Karieva et al. 1992; Poorter 1993; Solomon and Shugart 1993). While many studies have examined economically important crops, forest trees, and representative C_3 and C_4 species as model systems, to date, less attention has been focused on the potential responses of halophytes and estuarine communities to elevated CO_2 or their capacities for sequestering or releasing CO_2 (Schwarz and Gale 1984; Ball and Munns 1992; Lenssen et al. 1992). Saline conditions may limit the responsiveness of halophytes to CO_2, but the interaction of these factors is difficult to predict, given a paucity of data. The most comprehensive studies involving experimental CO_2 enhancements have been conducted on temperate estuarine marshes (Curtis et al. 1989a,

gules were transported within 24 h to the Harvard climate-controlled CO_2 glasshouse facility, Cambridge, Mass., USA. The glasshouse contains six modules, three of which are maintained at 700 μll^{-1} (double-ambient) atmospheric CO_2, and three at 350 μll^{-1} (ambient) CO_2, constituting three blocks each at two CO_2 levels. During the study, sunlight levels supplemented with metal halide lamps together provided 600–1300 μEm^{-2}s^{-1} photosynthetically active radiation (PAR) at plant height for 12 h a day. Diurnal/nocturnal temperatures were 26° C/19° C, and relative humidity ranged from 75 to 95%.

Single propagules were planted on 9 June 1994 into 6-l plastic pots, each containing a 2:1:1:1 peat:turface (a 2:1 clay):sand:sterile potting soil mixture. Propagules were weighed just prior to planting, and initial in-pot seedling heights were measured. Propagules were assigned to one of six glasshouse modules, in a stratified random design maintaining comparable initial mass distributions among module cohorts. Pots were placed in 10-cm-deep trays which were kept filled with an artificial sea water solution (Instant Ocean, Mentor, Ohio, USA) at a salinity of 35‰; although seedlings were not physically inundated, the soil was constantly saturated. Plants were top-watered daily, and fertilized monthly with 150 ml of 0.25 gl^{-1} Peter's 20:20:20 N-P-K solution. Seedlings were rotated bimonthly among modules within treatments to minimize block effects. No seedlings died: final sample sizes were 10 seedlings per module (30 seedlings per CO_2 treatment). All seedlings were transplanted into 11-l pots in April 1995 to preclude root binding. The experiment lasted from 9 June 1994 to 22 July 1995.

Nondestructive growth analyses

At seven sampling dates following planting (9 June 1994, 26 July 1994, 31 August 1994, 7 October 1994, 21 March 1995, 2 June 1995, and 22 July 1995), all existing seedlings were measured non-destructively for growth analyses. We measured height (±1 mm) of the hypocotyl plus main 'stem' (first vertical stem initiated from the top of the hypocotyl), and number and length of all branches (±1 mm). In March 1995, angles of branch initiation with respect to vertical were measured using a protractor. The proportion (%) of branches without a symmetric pair was recorded in March, as an index of developmental asymmetry. Stem diameter was measured using electronic calipers at three points: (1) the base of the hypocotyl, (2) the base of the first main stem internode, and (3) the middle of the top main stem internode (±0.1 mm); stem volume was calculated subsequently as a truncated cone of these dimensions and stem height. The total number of leaves was counted on each seedling. Emergence of aerial prop roots and reproductive buds was also noted at each date.

Harvest biomass allocation measurements

At three dates following 8 months of seedling growth (26 March 1995, 3 June 1995; and 22 July 1995), 9 seedlings per treatment (3 seedlings per block) were harvested. Seedlings were dissected into leaves, growing stems/branches, hypocotyls, above-ground prop roots, below-ground roots, and reproductive structures. These compartments were wet-weighed immediately, then dried to a constant weight for 1 week at 70° C, and dry-weighed (weight differentials gave an index of tissue water content). Mean wood density (gcm^{-3}) was calculated from stem volume estimates and stem dry mass. The total leaf area was determined at harvest using a Li-Cor 3100 leaf area meter (Li-Cor, Lincoln, Neb., USA). Relative total biomass growth rate for each harvested subpopulation was calculated as:

$$RGR = W_g^{-1} \cdot \Delta W / \Delta t$$

where Wg was the geometric mean of final seedling mass and initial propagule mass, DW the net gain in mass from planting to harvest, and Dt the number of days elapsed between planting and harvest (c.f. Wilson et al. 1986) (Table 1). All allometric ratios, including root:shoot ratios, leaf area ratio, specific leaf weights, pa-

rameters based on relative growth rate, and leaf chemical contents, were examined in relation to total plant biomass. That is, to clarify whether perceived shifts in allometric ratios were attributable to direct CO_2 effects or to CO_2-mediated changes in whole-plant growth rates, treatment populations were compared at the same ontogenetic stage rather than at the same absolute time interval (Evans 1972; Hunt 1990; Coleman et al. 1994).

Physiological measurements

Maximal photosynthetic rates and stomatal conductances of non-harvested seedlings were measured on both 26 March 1995 ($n=21$ seedlings per CO_2 level) and 3 June 1995 ($n=9$ seedlings per CO_2 level) at saturating light levels [>680 μEm^{-2}s^{-1} (Farnsworth and Ellison 1996)], using a Li-Cor 6200 photosynthesis system. Plants were measured in the modules in which they were grown. Relative humidities (mean 76%) were kept constant in the leaf chamber during each measurement; leaf and air temperatures (mean 24.5° C) did not differ. One fully expanded leaf from the top or second leaf pair on the main stem was used, maintaining comparable leaf ages at each sampling date. Because many plants grown for sustained periods in elevated CO_2 exhibit acclimatory downregulation of photosynthesis following an initial photosynthetic enhancement (Sage et al. 1989; Arp 1991; Webber et al. 1994; but see Drake and Leadley 1991), we investigated photosynthetic responsiveness of 6 high- and 6 low-CO_2-grown seedlings to changing CO_2 levels after 1 year of growth (June 1995). Seedlings were first measured in their respective CO_2 treatment environments (under a constant saturating 900 μEm^{-2}s^{-1} PAR), then transferred reciprocally to the opposite CO_2 treatment state and photosynthesis measured. Dark respiration of leaves was recorded on 18 June 1995, 4–6 h after sunset at 25° C on 12 seedlings per treatment level.

Anatomical studies and foliar chemistry

Leaf stomatal densities and stomata:epidermal cell ratios (stomatal indices) were quantified by examining 1-cm^2 foliar peels of clear nail polish under a dissecting microscope ($n=12$ seedlings per treatment, July 1995). At the June harvest, sections of fully expanded first-pair leaves and fine rootlets were preserved in formalin-glacial acetic acid-alcohol (FAA) for subsequent anatomical analyses under a dissecting microscope. Leaf pieces were sectioned free hand; unstained sections in which foliar anatomy was clearly visible were immersed on slides in Cargill Type B immersion oil, and photographed at ×100 magnification using a Nikon F-3 35-mm camera (Nikon, New York, N.Y., USA). From these photographs, we measured the relative thickness of cuticles, and epidermal, hypodermal, palisade, and spongy mesophyll layers (sensu Feller 1996). Tissue discs (0.4 cm^2) were removed from fresh leaves using a hole punch, preweighed, and extracted in 80% acetone solution for spectrophotometric determination of total chlorophyll (Spectronic 20, Bausch and Lomb, Rochester, N.Y., USA; methods of Arnon 1949, correction factors of Porra et al. 1989). Dried leaves from a subsample of 3 seedlings per treatment at each harvest date were ground in liquid nitrogen and analyzed for C:H:N using a Control Equipment 240 elemental analyzer at the University of Massachusetts Microanalytical Laboratory (Exeter Analytical, Lowell, Mass., USA; analytical methods of Ma and Rittner 1979). Because field tidal flooding conditions could not be stimulated in the laboratory it was of interest to know whether experimental conditions produced foliar sodium and nitrogen levels comparable to those of plants in the field. Foliar sodium content (% dry weight) of the above leaf samples was determined by atomic absorption spectrophotometry (Perkin-Elmer 403, Norwalk, Conn., USA; Perkin-Elmer 1982) following digestion of dried leaf material in strong acid with heating. Leaf samples were collected from similarly aged seedlings in Belize (same site as propagule collections), and analyzed for C:H:N and sodium as above ($n=6$ leaves).

b; Drake 1992). In the absence of a rising sea level, salt marshes dominated by *Scirpus olneyi*, *Spartina patens*, and *Distichlis spicata* appear to constitute a net carbon sink under in situ CO_2 enrichment, as canopy photosynthetic assimilation is stimulated (particularly in C_3 species), respiration rates are reduced, and carbon is translocated to below-ground biomass (Drake 1992). Whether this finding can be generalized to woody coastal systems is as yet unknown. Here, we present data on responses at several organismal levels of organization of *Rhizophora mangle* L., a common neotropical mangrove species, to elevated atmospheric CO_2.

Mangrove communities, regarded as a tropical equivalent of temperate marshes in terms of structure and productivity, dominate the majority of protected tropical coasts (Chapman 1976), some 17 million hectares worldwide (Saenger et al. 1983), and are among the most productive systems on earth (Alongi et al. 1992; Twilley et al. 1992). For example, mangroves account for a disproportionate share of estimated tropical forest carbon storage: 1.3% of total storage (Twilley et al. 1992) in only 0.9% of total tropical forest area (Food and Agriculture Organization 1993). The presence, productivity, and stability of mangrove forests influence shoreline geomorphology, production of fisheries for dense resident human populations, and sedimentation and carbon flux to adjacent sea grass and reef communities (Food and Agriculture Organization 1994). Ong (1993), projecting from data on mangrove plantations, hypothesized that most mangrove forests may currently constitute a carbon sink, contingent on stand age, litter export volumes, and rates of conversion of mangroves to other uses. Given the economic and ecological importance of mangroves globally, their response to climate change is of increasing concern (Davis et al. 1994). Recent reviews have offered speculative prognoses regarding mangrove performance under two principle facets of climate change: rising sea level (Woodroffe 1982; Ellison and Stoddart 1991; Ellison 1993; Bacon 1994; Parkinson et al. 1994; Snedaker et al. 1994) and increasing CO_2 (Ball and Munns 1992; Ong 1993; Field 1995; Snedaker 1995). However, integrated hypotheses on responses of mangroves to a sea-level rise, incorporating predictions of their potential compensatory growth in rising CO_2 for example, have not emerged, largely because information on their CO_2 responses is scarce (Field 1995).

Existing in situ physiological studies on the carbon limitation of photosynthesis in C_3 mangroves, together with data on salt marsh halophytes, suggest that growth in these species may be stimulated by enriched CO_2 atmospheres. However, the magnitude of this enhancement will vary with species, soil salinity, and nutrient availability (Ball and Farquhar 1984), as well as the plasticity and maximum capacity for photosynthesis characteristic of a given mangrove species (Cheeseman et al. 1991). Ball and Munns (1992), reviewing unpublished data on responses of *Rhizophora apiculata* to double-ambient CO_2 levels, noted higher photosynthetic rates, water use efficiencies (WUE), and growth rates in seedlings grown

in 'low' salinities (levels unspecified). By contrast, WUE and growth rates were decoupled in 'high' salinities: enhancement of WUE did not translate into higher growth rates. Because this and other experiments indicate that growth of *Rhizophora* species may be in part limited by high salinities and attendant soil physicochemical factors (e.g. Lin and Sternberg 1992; Ball and Passioura 1994), we hypothesized at the outset of the present experiment that at salinities approximating field levels (35‰), photosynthesis and concomitant growth of the mangrove *R. mangle* would show relatively low responsiveness to enhanced CO_2. Our integrated study examining components of growth and biomass partitioning in *R. mangle* grown in ambient and double-ambient CO_2 concentrations, examines interacting morphological and physiological changes from the microscopic to the whole-organism level. We document changes in growth rates, architecture, biomass allocation, reproduction, foliar anatomy and chemistry, photosynthesis, stomatal conductance, and respiration in seedlings monitored for over a year. We pay special attention to the interpretation of potential allometric shifts as products of enhanced whole-plant growth in elevated CO_2. We provide, to our knowledge, the first published data on neotropical mangrove performance in elevated CO_2.

Materials and methods

Study species

Rhizophora mangle L. is the predominant mangrove species fringing protected coasts of the Caribbean mainland and coral cays. Embryos germinate viviparously from cleistogamous flowers on the maternal tree (Lowenfield and Klekowski 1992; Klekowski et al. 1994) in November–January; hypocotyls puncture the fruit wall and elongate up to 30 cm before the seedling is abscised in June–September. The seedling may float and disperse for months prior to stranding on the substrate, rooting, and producing its first leaf pair. Seedlings growing on the seaward carbonate platform of these cays occur in full sun at densities <0.2 m^{-2} and do not interact competitively during the first 5 years of growth; thus, the solitary experimental planting treatments described below reflect natural density conditions. Seedlings at the mean water intertidal perimeter of this cay are flooded twice daily to 2–15 cm by low-amplitude tides of 35‰ salinity. New leaves, growing main stem, and branches are initiated above the persistent hypocotyl. As the plant grows, the hypocotyl lignifies and thickens, but does not elongate. *R. mangle* seedlings and saplings grow via exponential, sylleptic branching (Tomlinson 1986; Ellison and Farnsworth 1996; Farnsworth and Ellison 1996). Branches and leaves are opposite and often initiated symmetrically; several latent, successive pairs of meristem primordia occur in each terminal bud (Gill and Tomlinson 1971). *Rhizophora* spp. produce conspicuous aerial prop roots that aid in anchoring and passive oxygen exchange through lenticels. Mature tree (about 20 years old) do not exceed 10 m in height on Belizean cays. At the collection site in Belize and throughout Central America, reproduction does not occur until saplings are >3 years old (Farnsworth, personal observation).

Collection and planting design

Sixty mature propagules were collected on 7 June 1994, just prior to abscission, from multiple trees on the windward coast of Wee Wee Cay, Belize, Central America (16°46'N, 88°08'W). Propa-
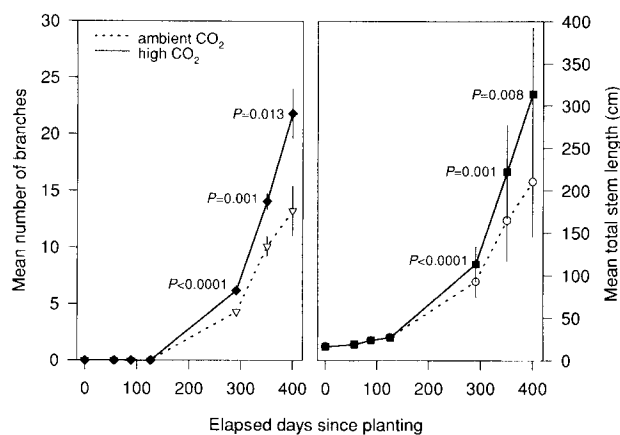
Statistical analyses

All statistical comparisons were done with ANOVA (Systat for Windows v. 5.05), unless otherwise specified, after transformation to eliminate heteroscedasticity of data (if necessary). Non-destructive measures of height growth, and branch and leaf production were analyzed using repeated-measures ANOVA for the 12 seedlings per treatment population maintained until final harvest. Statistical trends for this subsample were identical to trends shown by individual ANOVAs performed at each sampling date for all plants included (due to harvesting, different sample sizes occurred at dates 4–7, precluding a balanced repeated-measures design); thus, results (P values) incorporating all nonharvested plants at each separate date are reported below. Where similar treatment effects were observed at all sampling dates, only the means at the final sampling date are reported. Block and CO_2 level were incorporated into initial fixed-factor models. However, module identity was not a significant covariate in any analysis; thus, populations were pooled across blocks to increase power. ANCOVA was used to explore the dependence of allometric ratios in different treatment populations on plant biomass (interaction term of treatment×plant biomass as covariates).

Results

Nondestructive growth analyses

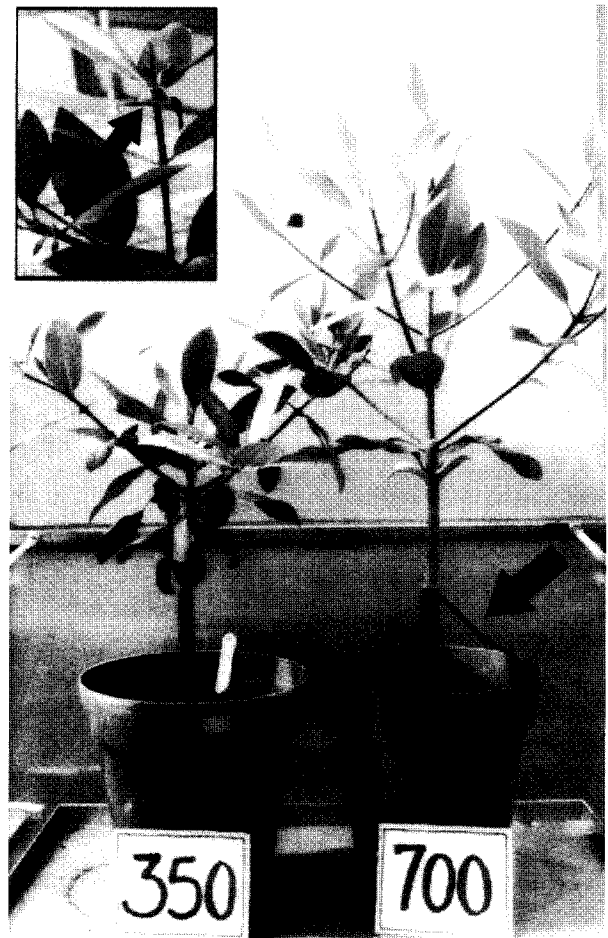Seedlings increased their total stem length 19-fold in the elevated-CO_2 treatment, and 11-fold in the ambient-CO_2 treatment over 400 days, with a significant difference among treatments (Fig. 1). Treatment differences in stem length did not become apparent until more than 270 days into the experiment, but became more pronounced as the experiment continued (Fig. 1). Neither height of the main stem (mean$_{350}$=46.8 cm; mean$_{700}$=49.5 on final sampling date), nor mean branch length (mean$_{350}$=17.6; mean$_{700}$=15.1) differed significantly among treatments at any sampling date. Hence, plant size differences were largely attributable to increased branching activity in ele-

vated CO_2, not to enhanced plant module size. The number of branches produced was significantly higher among high-CO_2 seedlings from day 280 onward, and by 400 days the high-CO_2 mangroves have produced nearly twice as many branches as ambient-CO_2 plants (Fig. 1). Symmetry among branches, expressed as the percent of branches without an opposite pair, did not differ significantly among treatments (mean$_{350}$=19.9%; mean$_{700}$=12.9%). Despite increased branch production, branching angles did not differ between treatments (mean$_{350}$=42.4°; mean$_{700}$=44.4°). Lignification of the main stem began >2 months earlier in elevated-CO_2 plants; hence, estimated main stem volume was nearly doubled in 700 μl^{-1} CO_2 by the final sampling date (mean$_{350}$=81.8 cm^3; mean$_{700}$=150.4; P=0.017).

By day 400, high-CO_2 mangroves had 50% more leaves than low-CO_2 mangroves (mean$_{350}$=77 leaves/plant; mean$_{700}$=112; P=0.005). The number of leaves per branch did not differ among treatments (mean$_{350}$=6 leaves/branch;

Fig. 1 Increase in total stem length (*right panel*) and branch production (*left panel*) of mangrove seedlings grown in 350 μl^{-1} (*dotted line*) and 700 μl^{-1} (*solid line*) CO_2. Shown are means; *error bars* indicate 1 SD. Probability values for differences between treatment populations (obtained by ANOVA) appear next to sampling dates only when P<0.05. Samples sizes for dates 1–7 were 30, 30, 30, 30, 30, 20, and 10 seedlings per treatment, respectively

Fig. 2 Photograph of randomly chosen high-CO_2 mangrove (*right*) and ambient-CO_2 mangrove (*left*) after 250 days of growth. Note appearance of prop roots only on the high-CO_2 mangrove (*arrow*). The inset shows reproductive buds on a 350-day-old seedling grown in elevated CO_2 (*double-headed arrow* points to developing buds)
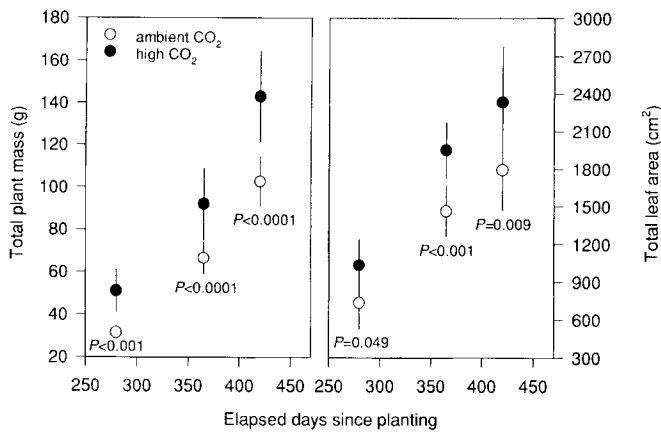
Fig. 3 Increase in total standing leaf area (*right panel*) and total plant dry mass (*left panel*), measured at three harvest dates, of mangrove seedlings grown at ambient CO_2 (*open circles*) and double-ambient CO_2 levels (*closed circles*). Shown are means; *error bars* indicate 1 SD. Probability values for differences between treatment populations (obtained by ANOVA) appear next to sampling dates only when $P<0.05$. Samples sizes are 9 seedlings per treatment at each date

$mean_{700}=5$); thus, standing leaf number differences resulted from branch proliferation among high-CO_2 plants. Area per leaf did not differ among CO_2 treatments ($mean_{350}=24.4$ cm^2; $mean_{700}=25.8$), nor did the rate of leaf turnover, computed as the total number of leaves abscised per seedling by 400 days ($mean_{350}=4.0$ leaves/sapling; $mean_{700}=6.1$).

Most striking among the observed growth differences were the extremely early appearance of aerial prop roots and reproductive buds among the elevated-CO_2 population (Fig. 2). Prop roots were visible on high-CO_2 mangroves by April 1995 (10 months post-planting), whereas none were produced by ambient-CO_2 plants. By the final sampling date, 5 of 9 high-CO_2 mangroves had from 1 to 4 prop roots >1 cm long ($mean_{700}=1.4$ roots/plant). Flower buds emerged on high-CO_2 mangroves in June 1995, less than 1 year post-planting. One-third of high-CO_2 plants produced 1–5 buds/plant by 400 days; no am-

bient-CO_2 plants were reproductive at any time ($mean_{700}=0.9$ buds/plant). Flowering activity was followed on a subset of 3 remaining plants from June 1995–December 1995, and we hand-pollinated all open flowers to promote fruit set. Although 80% of initial buds subsequently opened as flowers, no flowers matured into fruits.

Harvest biomass allocation measurements

Paralleling height growth trends, mean total plant mass of high-CO_2 plants was nearly double that of ambient-CO_2 plants by the final sampling date (Fig. 3). Relative mass growth rates were significantly higher in the high-CO_2 population at all harvest dates (Table 1). From these data, unit leaf rate (ULR: absolute biomass growth supported by a given leaf area) and net assimilation rate (NAR: the relative biomass growth supported by a given leaf area) were computed (Table 1). Both ULR and NAR were significantly enhanced in elevated CO_2 at all dates (Table 1). However, NAR increases in elevated CO_2 were largely explained by allometric changes in plant biomass, while ULR was directly affected by CO_2 treatment independent of CO_2 effects on plant biomass (Table 2).

All mangrove seedlings allocated increasing proportions of biomass to below-ground root production, reflected in increasing root:shoot ratios over time. Above-ground prop roots were small and contributed little to root:shoot ratio estimates. Because high-CO_2 plants attained larger overall sizes and hence higher root:shoot ratios early in the experiment, there were no actual allocational shifts directly attributable to treatment (Table 2). Likewise, no treatment differences in leaf weight ratio (LWR) were observed at any during the experiment (Table 2). Total seedling leaf area was significantly higher among high-CO_2 plants on all dates measured (Fig. 3). Mean area per leaf, however, did not differ significantly between treatments; hence, total area enhancements were attributable to increased leaf numbers. Neither specific leaf area (SLA: leaf area per gram of leaf tissue) nor leaf

Table 1 Summary of growth parameters estimated from initial propagule mass, and total plant mass obtained on subpopulations at three harvest dates (285, 358, and 408 days post-planting). Shown are means for the ambient-CO_2 and high-CO_2 populations (1 SD in parentheses). F and probability values are from ANOVAs on each harvested subpopulation ($n=9$ plants per treatment level per date) [RGR relative growth rate (gg^{-1}day^{-1}); NAR (gm^{-2}day^{-1}) net assimilation rate, the relative biomass gain supported by a given standing leaf area; ULR (gm^{-2} day^{-1}) unit leaf rate, the absolute biomass growth supported by a given standing leaf area]

Harvest date (days)	Growth variable	350 μl^{-1} (×10^{-3})	700 μl^{-1} (×10^{-3})	$F_{1,16}$, P
285	RGR	2.99 (0.67)	4.32 (1.00)	10.782, 0.0047
	NAR	1.61 (0.59)	2.65 (0.58)	14.275, 0.0017
	ULR	1.04 (0.31)	1.46 (0.17)	12.899, 0.0024
358	RGR	4.33 (0.71)	6.36 (0.80)	32.555, <0.0001
	NAR	2.57 (0.36)	4.13 (0.70)	34.915, 0.0002
	ULR	1.26 (0.08)	1.55 (0.17)	22.577, 0.0002
408	RGR	5.90 (0.76)	7.12 (0.84)	10.349, 0.0054
	NAR	5.02 (0.79)	6.55 (0.89)	14.994, 0.0014
	ULR	1.81 (0.18)	2.04 (0.17)	7.695, 0.0136

Table 2 Slopes (±1 SE) of allometric regressions of log-transformed growth parameters measured on seedlings grown in ambient ($350 \, \mu ll^{-1}$) and elevated ($700 \, \mu ll^{-1}$) CO_2. F statistic and probability values (P) are for the interaction term ($CO_2 \times$plant dry biomass) in an ANCOVA incorporating plant size as a covariate, to enable comparisons of functional allocation among plants of the same size (rather than the same age), as CO_2 effects on allometric shifts may operate directly or via enhancement of whole-plant biomass. Coefficients of determination for allometric regression (r^2 values) ranged from 0.508 to 0.940 (*SLA* specific leaf area, *LAR* leaf area ratio, *LWR* leaf weight ratio)

Allometric variables	$350 \, \mu ll^{-1}$	$700 \, \mu ll^{-1}$	F, P, n per treatment group
Root mass×shoot mass (root:shoot)	1.79 (0.12)	1.53 (0.10)	0.037, 0.848, 27
Leaf area×leaf mass (SLA)	0.76 (0.04)	0.77 (0.04)	0.028, 0.868, 27
Laf area×total mass (LAR)	0.69 (0.06)	0.69 (0.05)	0.302, 0.585, 27
Leaf mass×total plant mass (LWR)	0.93 (0.04)	0.92 (0.03)	2.826, 0.099, 27
Reactive growth rate×total mass (RGR)	0.49 (0.06)	0.42 (0.06)	1.218, 0.120, 27
Net assimilation rate×total mass (NAR)	0.79 (0.09)	0.72 (0.06)	1.019, 0.138, 27
Unit leaf rate×total mass (ULR)	0.38 (0.06)	0.26 (0.04)	9.772, 0.003, 27
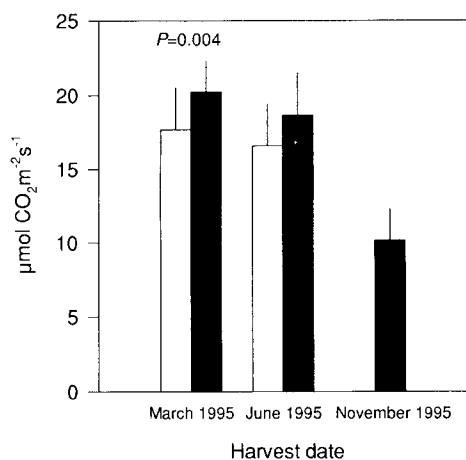
Fig. 4 Mean values (±1 SD) of photosynthetic rate ($\mu mol \, CO_2 \, m^{-2}s^{-1}$) of seedlings grown under ambient CO_2 (*open bars*) and double-ambient CO_2 (*closed bars*), at three sampling dates. Photosynthetic rates were measured on only 3 high-CO_2-grown seedlings in November 1995; on the other two dates, sample sizes were 9 per treatment. Only significant probability levels are shown

Table 3 Photosynthetic rates and differentials for 6 mangrove seedlings per CO_2 level measured in their ambient growth environment and once following temporary transfer to the reciprocal treatment (June 1995). Rows are the initial treatment environment. Shown are (*a*) P_{max} ($\mu molm^{-2}s^{-1}$) measured at saturating photosynthetically active radiation (mean±1 SD), (*b*) the abolute change in photosynthetic rate ($\mu molm^{-2}s^{-1}$) following transfer (mean±1 SD), and (*c*) the percent change from the initial photosynthetic rate (mean±1 SD)

Growth CO_2 level		Measurement CO_2 level	
		$350 \, \mu ll^{-1}$	$700 \, \mu ll^{-1}$
$350 \, \mu ll^{-1}$	a	15.64±1.15	19.12±2.77
	b	–	+3.48±1.69
	c		+21.8±9.3%
$700 \, \mu ll^{-1}$	a	12.89±1.29	15.91±1.83
	b	–3.02±1.26	–
	c	–23.6±9.4%	

area ratio (LAR: the leaf area supported by plant mass) differed significantly among treatments when plants were considered at the same size (Table 2).

Physiological measurements

Maximum photosynthetic rates were significantly higher among mangroves growing in elevated CO_2 in March 1995 relative to controls (Fig. 4). However, by June 1995, photosynthetic capacities had converged among treatments, as high-CO_2 rates declined (Fig. 4). In contrast to photosynthesis, stomatal conductance did not differ significantly among treatments in March 1995 ($mean_{350}=0.36 \, mol \, H_2Om^{-2}s^{-1}$; $mean_{700}=0.38$). However, in June 1995, stomatal conductance was significantly lower among the high-CO_2 plants ($mean_{350}=0.26\pm0.06 \, molH_2Om^{-2}s^{-1}$; $mean_{700}=0.13\pm0.02$; $P<0.0001$), and summer conductance values were lower than in the

spring. The rate of photosynthesis maintained at a given stomatal conductance was examined as the regression of P_{max} on conductance for each plant at each date. At both sampling dates, the slope of this regression was higher among high-CO_2 plants (ratio of $coefficient_{700}$ to $coefficient_{350}=1.48$ in March, 1.72 in June). Despite an apparent decline in photosynthetic capacity from March to June, the responsiveness of the photosynthetic rate to a $350 \, \mu ll^{-1}$ change in CO_2 concentration did not differ significantly among high- and ambient-CO_2 plants (Table 3). On this measurement date, maximal photosynthetic rates among plants grown in high CO_2 were significantly lower than those of ambient-CO_2 seedlings transferred to elevated CO_2 (t-test on P_{max} at $700 \, \mu ll^{-1} \, CO_2$, $P=0.043$). Dark respiration rates were slightly but not significantly lower among elevated-CO_2 mangroves ($mean_{350}=-1.05 \, \mu mol \, CO_2 m^{-2}s^{-1}$; $mean_{700}=-0.97$).

Anatomical studies and foliar chemistry

Total leaf thickness, determined from anatomical sections, did not differ significantly between treatments, and leaves did not exhibit differential allocation to cuticle,
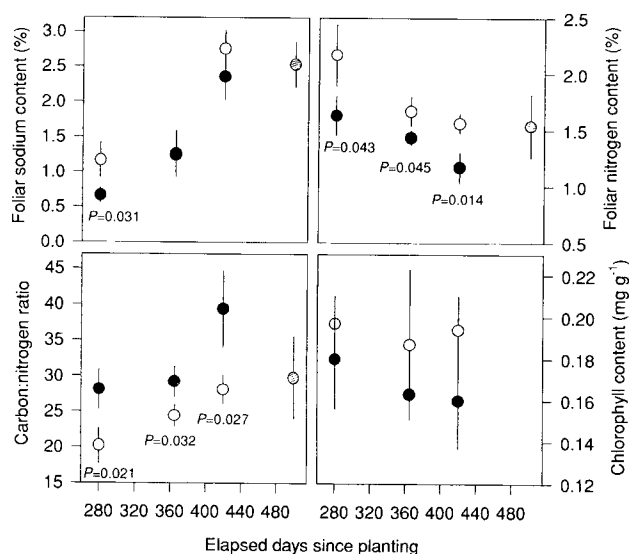
Fig. 5 Foliar chemistry characteristics measured on mangrove seedlings grown in high CO_2 (*closed circles*) and ambient CO_2 (*open circles*), measured at three harvest dates. *Error bars* are 1 SD. Nitrogen, carbon: nitrogen ratios, and sodium were determined on a dry mass basis. Shown on the nitrogen, carbon: nitrogen ratio, and sodium plots are means for leaves collected from field-grown seedlings of comparable age in Belize (*hatched circles*), which did not differ significantly from the ambient-CO_2-treatment population

epidermal layer, hypodermal palisade layer or spongy mesophyll. However, stomatal density was reduced significantly in leaves of high-CO_2 plants by 1 year post-planting (mean$_{350}$=42.4±5.2 stomata mm^{-2}; mean$_{700}$=35.8±5.4; P=0.006). Ratios of stomata to epidermal cells (stomatal indices) did not differ between treatments; thus, changes in stomatal density were attributable to cell expansion rather than production of additional stomata in ambient-CO_2 plants. Gross anatomical features of root and stem compartments were generally unchanged by elevated CO_2. The only aspect of root morphology apparently altered by the CO_2 treatment was tissue concentration of tannins: high-CO_2 root sections were deep red, while low-CO_2 roots showed no such staining (tannin quantities could not be assayed directly in this study). Stem wood density, calculated from stem mass: volume relationships, did not differ between treatments.

Foliar chemistry varied consistently between CO_2 levels on all harvest dates. Carbon:nitrogen ratios increased through time (linear regression of ratio on time since planting, r=0.640, n=18, P=0.019), and were significantly higher in the high-CO_2 population throughout the experiment (Fig. 5). Similarly, foliar chlorophyll contents were diminished in elevated CO_2, although the magnitude of intertreatment differences in chlorophyll varied with sampling date (Fig. 5). Foliar sodium contents also increased through time (linear regression of percent sodium on time, r=0.926, n=18, P<0.0001), and attained levels comparable to leaves of flooded seedlings inundated to 15 cm (Farnsworth et al. 1995). Nitrogen and sodium

contents of ambient-CO_2 leaves did not differ significantly from leaves of seedlings growing on the coast of Belize (Fig. 5). Leaves of high-CO_2 mangroves showed consistently lower sodium contents than ambient-CO_2 mangroves at all dates, but this difference was statistically significant only in March 1995 (Fig. 5). Leaf gravimetric water content, estimated as (wet mass–dry mass)/dry mass of leaves, did not differ between treatments (mean$_{350}$=2.15; mean$_{700}$=2.22).

Discussion

Our study documents multifaceted responses of the dominant Caribbean mangrove species, *R. mangle*, to a doubling of atmospheric CO_2. Contrary to our initial hypothesis, growth, photosynthetic assimilation, and foliar chemistry of *R. mangle* were highly sensitive to CO_2 levels. Importantly, many of these changes were not manifested until >8 months following planting (Fig. 1), and some initial differences diminished in significance after long-term exposure (e.g., photosynthetic rates and foliar sodium, Figs. 4, 5). Lags in CO_2 responses may result from the fact that *R. mangle* begins life as a highly provisioned viviparous seedling (Pannier 1962; Lin and Sternberg 1995), which fuels early growth prior to leaf expansion from maternal carbohydrates stored in the elongate hypocotyl. In the present study, leaves were not initiated until after 1 month post-planting, and photosynthetic autonomy and concomitant responsiveness to CO_2 were likely not attained until >90 days in the treatments. This phenomenon raises two essential points. First, the early establishment period is a time when mangrove seedlings native to fringing or exposed coasts are particularly vulnerable to tidal action and inundation. Separate experiments have demonstrated depression of mangrove growth and photosynthesis during flooding (Naidoo 1985; Farnsworth et al. 1995; Hovendon et al. 1995). Under conditions of rising sea level, elevated CO_2 may not confer an immediate advantage to insensitive young seedlings. Second, long-term studies in excess of 12 months are critical to elucidate the range of mangrove seedling responses to rising CO_2. Typical laboratory studies of mangroves grown for less than 1 year cannot fully characterize complex responses of viviparous seedlings to abiotic factors (e.g., McKee 1993, 1995).

Overall growth and biomass accrual were significantly enhanced in *R. mangle* seedlings after more than 1 year of treatment (Figs. 1, 3). Elevated CO_2 enhanced branching activity rather than height growth (Fig. 1). Multiple bud primordia occur at each meristem (Gill and Tomlinson 1971), and elevated CO_2 may enhance budding rates and shorten the branch plastochron interval by stimulating these primordia simultaneously. Branching angles and meristem symmetry, however, were unchanged by the treatment. These results raise the possibility that branch proliferation under elevated CO_2 may exacerbate biomechanical stresses on the whole seedling imposed by tidal turbulence and drag, especially as the

sea level rises. However, early lignification of the main stem (a developmental phase shift also observed here) may offset this stress.

Most striking among the responses were the exceptionally early production of both prop roots and reproductive buds, noted *only* in the high-CO_2 population (Fig. 2). Prop root production was preceded by early and vigorous lignification of the hypocotyl and main stem. Reproduction occurred fully 2 years before it has ever been observed on *R. mangle* seedlings in the seedling source population in Belize (Ellison and Farnsworth, unpublished data). In the field, *R. mangle* saplings growing in full sun at the tidal fringe typically exceed 1.5 m in stem height before flowering. High-CO_2 seedlings were considerably smaller than this at first flowering. Hence, accelerated maturation of seedlings, rather than attainment of a size threshold, appeared to induce precocious reproduction and accompanying early root production in elevated CO_2. All flowers of the first reproductive cohort were eventually aborted, so initial reproductive effort did not translate into initial higher fitness in *R. mangle*. However, viable propagules *have* been produced after a second flowering flush (November 1995, unpublished data); implications for lifetime fitness are unknown for these perennials. Early reproduction in woody species has also been noted in *Acer pensylvanicum* and *Betula populifolia* (S.L. Miao, personal communication) after >1 year in elevated CO_2. Such accelerated maturation has significant implications for seed production, growth costs, climacteric respiration, carbon sequestration, and senescence of trees and forests exposed to increasing CO_2 over the long term.

When plants were compared at the same size (biomass) rather than the same age (absolute time), it became apparent that the allometry of biomass allocation by mangroves did not shift appreciably between CO_2 treatments. This subtlety of analysis raises critical points for the interpretation of plant biomass partitioning in contrasting CO_2 environment (Coleman et al. 1994), and cautions that a thorough understanding of growth dynamics must inform conclusions from growth analysis. Because patterns of biomass allocation shift over ontogeny due to the nonlinear nature of whole-plant growth, CO_2 enhancements of overall biomass result in apparent differences in allometric ratios when treatment populations are compared at the same time rather than the same size. For example, root:shoot ratios at first harvest were significantly higher among high-CO_2 plants when compared at 270 days ($P=0.016$ for treatment effect, data not shown); hence, we might conclude that CO_2 shifted root:shoot ratios in comparably sized mangroves. However, CO_2 effects were insignificant in an ANCOVA incorporating total plant biomass as a convariate (Table 2), indicating that reductions in the ratio of below-ground roots to shoots did not reflect CO_2-induced functional adjustments of allocation. Rather, these decreases resulted from accelerated plant growth in high CO_2 together with a fixed trajectory to allocation among above- and below-ground compartments over ontogeny. While de-

clining root:shoot ratios have been attributed to pot binding in other studies (Arp 1991), we (1) transplanted mangroves to larger pots after 1 year, (2) saw no morphological evidence of root constraint in harvested seedlings, and (3) observed root:shoot ratios comparable to those of field-grown seedlings. Seedlings had 32–33% of total biomass in fine roots by 400 days, similar to field values for *R. mangle* seedlings observed in Belize (Ellison and Farnsworth 1993), and only slightly lower than *R. mangle* trees measured in Puerto Rico (Golley et al. 1962). A growth-related decrease in root:shoot ratios may limit nutrient uptake over ontogeny, however, potentially exacerbating nitrogen attenuation in elevated-CO_2-grown seedlings.

Above ground, while total leaf area was higher among high-CO_2 plants (principally due to increased branching; Figs. 1, 3), the CO_2 level had no direct effects on observed LAR or SLA independent of its effects on plant biomass (Table 2), and altered few other leaf level characters. Likewise, leaf morphological attributes including thickness, SLA, area per leaf, and leaf cross-sectional anatomy did not differ among the treatments. Only apparent stomatal density significantly decreased in elevated CO_2, primarily due to enhancements of epidermal cell size.

Growth rate parameters, RGR, ULR, and NAR, showed divergent patterns among treatments. The rate of absolute biomass growth sustained by a unit of leaf area, expressed as ULR, was higher (more 'efficient' *sensu* Norby et al. 1992) among high-CO_2 seedlings early on (Table 1), but the magnitude of differences among treatments declined over time and hence the allometry of ULR to plant biomass was shifted by the treatments (Table 2). Our estimates of NAR closely approximate those derived from nondestructive growth measurements on *R. mangle* saplings at Wee Wee Cay (Ellison and Farnsworth 1996), but are low in comparison to other neotropical, upland tree species (reviewed by Ackerly 1996), especially considering the high initial photosynthetic rates we measured (cf. Reich et al. 1992). Relative growth rates measured in this controlled-environment study were only slightly than the RGR of seedlings and saplings measured in sunny sites in Belize (Table 1; Ellison and Farnsworth 1996; Farnsworth and Ellison 1996). The substantial enhancement of RGR we observed, which was comparable in magnitude to a variety of woody C_3 trees (Norby et al. 1992; Poorter 1993), was due to CO_2-induced increases in whole-plant biomass (Fig. 3).

In concert with increased growth rates, leaf photosynthetic rates were generally enhanced in elevated CO_2. In the absence of significantly increased allocation to leaf area in elevated CO_2, enhanced RGR and NAR may be related in part to photosynthetic enhancements. However, both the absolute rates and the magnitude of difference in photosynthesis attributable to treatment declined from March to June (Fig. 4). By November 1995, remaining high-CO_2 seedlings showed even lower photosynthetic rates (mean=10.16 ± 2.12 µmol CO_2m^{-2}s^{-1}; $n=3$). This decline in CO_2 enhancement over time, together with our

findings from the photosynthetic responsiveness study (Table 3), may indicate that photosynthetic acclimation (downregulation) was occurring after long-term exposure to elevated CO_2. Such acclimation has been attributed to downregulation of RuBisCO activity (Sage et al. 1989), buildup of excess foliar starch exceeding sink capacity (Long and Drake 1992), inhibition of RuBP regeneration by sequestration of the P_i pool by phosphorylated intermediates (Stitt 1991), and a shortage of enzymes active in RuBP regeneration (Webber et al. 1994). We are confident that source-sink relationships were not confounded by inadequate pot size, and propose that either (1) starch formation exceeded allocation of carbon to new sinks, and/or (2) attenuation of nitrogen concentrations in tissues may have limited production of RuBisCO as well as other enzymes catalyzing RuBP rgeneration. Lower foliar nitrogen likewise may be implicated in the slight reduction of respiration rates among high-CO_2 seedlings, if enzyme activities in respiratory pathways were diminished (Azcon-Bieto et al. 1994; Wullschleger et al. 1994).

Nitrogen concentrations in leaves were consistently reduced in elevated CO_2 (on both a leaf weight and leaf area basis, as SLA was similar between treatments). This reduction was paralleled by a reduction in foliar chlorophyll over the experiment (Fig. 5). Tissue nitrogen is commonly lower in tree seedlings exposed to elevated CO_2; both salt marsh grasses (Azcon-Bieto et al. 1994) and bottomland species (e.g., Williams et al. 1986) show similar declines in C:N ratios. Reduction in chlorophyll content due to nitrogen attenuation may have contributed to observed declines in photosynthetic rate over time in both treatment populations. However, this reduction in N did not appear to hinder early growth. Nutrient enrichment studies in Belize demonstrate that nitrogen and phosphorus differentially limit *R. mangle* sapling growth in different tidal zones (Feller 1995). Attenuation of photosynthetic rates during long-term exposure to elevated CO_2, whether due to nutrient limitation or other factors, has important implications for the long-term productivity of trees in general, and for the capacity of mangroves to act as carbon sinks in particular. Our preliminary study indicates that long-term studies of trees in a range of ontogenetic phases, crossing CO_2 and nutrient levels, are needed to yield prognoses of forest performance under climate change.

In the complex suite of edaphic and biotic factors that will influence mangrove responsiveness to rising CO_2, salinity and rising sea level may alter plant water relations. Uptake of water must be balanced against accumulation of salts to potentially toxic levels in tissues of halophytes. At the same time, obligate halophytes such as mangroves require some salt to maintain low leaf water potentials and to facilitate turgor-driven leaf expansion and growth (Scholander et al. 1962). Mangroves share convergent mechanisms for salt elimination, coupled with conservative water expenditures through transpiration (Ball and Passioura 1994). If WUE and salt uptake via transpiration are coupled, we might expect lower fo-

liar sodium contents in mangroves grown in high CO_2. Although the seedlings used in our study could not be inundated with salt water due to facility constraints, foliar sodium concentrations by 400 days post-planting were comparable to those of artificially flooded seedlings (Farnsworth et al. 1995) and seedlings in Belize (Fig. 5). Foliar sodium was slightly but not significantly lower in the high-CO_2 set (Fig. 5), and leaf water content and leaf thickness (an index of succulence) were unaffected by treatment. In contrast, by summer 1995, stomatal conductance in high-CO_2 plants was significantly reduced relative to controls, indicating a potential increase in WUE in elevated CO_2 (although we caution that WUE could not be measured directly in this study). Conductance was lower both on a leaf area basis and on a per-stomate basis (taking changes in stomatal density into account). This finding accords with the enhanced growth and WUE, decoupled from foliar sodium, of high-CO_2-grown *R. apiculata* reported by Ball and Munns (1992). Despite extremely conservative water use on the part of *Rhizophora* species (Ball 1988; Passioura et al. 1992), both stomatal conductance and photosynthesis respond plastically to a rise in CO_2, provided adequate is fresh water available to promote growth. Clearly, salt did not limit growth or physiological flexibility in our study, even though salinity was maintained at field levels (35‰). However, while mangroves growing in areas of high humidity and rainfall may exhibit CO_2-related growth enhancements, species in hypersaline areas, whose growth may be more strongly limited by salinity, may not.

The long-term responsiveness of these neotropical fringing mangroves to rising CO_2, and their potential carbon sink capacities will likely depend on a host of local factors including nutrient availability (Feller 1995), rates of sea level rise (Farnsworth et al. 1995), and exposure and sedimentation regimes (Ellison and Farnsworth 1996). A complementary artificial flooding study using seedlings from the same source population (Farnsworth et al. 1995) indicated that compensatory growth and productivity enhancements in elevated CO_2, however dramatic here, may not be sufficient to offset the negative growth effects of a sea-level rise.

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