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Original article

Responses of growth, nitrogen and carbon partitioning to elevated atmospheric CO_2 concentration in live oak (*Quercus virginiana* Mill.) seedlings in relation to nutrient supply

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Abstract – Live oak (*Quercus virginiana* Mill.) seedlings were exposed at two concentrations of atmospheric carbon dioxide ([CO_2], 370 or 520 $\mu mol \cdot mol^{-1}$) in combination with two soil nitrogen (N) treatments (20 and 90 $\mu mol \cdot mol^{-1}$ total N) in open-top chambers for 6 months. Seedlings were harvested at 5–7 weeks interval. CO_2 treatment had a positive effect on seedling growth. Differences in biomass between elevated and ambient CO_2-treated plants increased over the experimental period. Soil N availability did not significantly affect growth. Nevertheless, growth in elevated [CO_2] in combination with high N levels led to a consistently higher accumulation of total biomass by the end of the experiment (30–40 %). Biomass allocation between plant parts was similar for seedlings in all treatments, but was significantly different between harvests. The N regimes did not result in different relative growth rate (RGR) and net assimilation rate (NAR), while CO_2 treatment had an overall significant effect. Across all [CO_2] and N levels, there was a positive relationship between plant mass and subsequent RGR, and this relationship did not differ between treatments. Overall, specific leaf area (SLA) decreased in CO_2-enriched air. Fine root–foliage mass ratio was increased by elevated [CO_2] and decreased by high N. High CO_2- and high N-treated plants had the greatest height and basal stem diameter. The allometric relationships between shoot and root dry weight and between height and basal stem diameter were not significantly affected by elevated [CO_2]. Leaf N concentrations were reduced by low soil N. Plant N concentrations decreased with time. Elevated [CO_2] increased the C/N ratio of all plant compartments, as a result of decreasing N concentrations. High CO_2-grown plants reduced N concentrations relative to ambient CO_2-grown plants when compared at a common time, but similar when compared at a common size. (© Inra/Elsevier, Paris.)

carbon allocation / carbon dioxide enrichment / growth / nitrogen / *Quercus virginiana*

Résumé – Croissance, répartition de l'azote et du carbone chez des semis de *Quercus virginiana* Mill. en réponse à une concentration élevée de CO_2. Interaction avec l'alimentation en azote. Des semis de *Quercus virginiana* Mill. ont été exposés pendant six mois à deux concentrations en CO_2 atmosphérique (370 $\mu mol\ mol^{-1}$ ou 520 $\mu mol\ mol^{-1}$) en combinaison avec deux traitements d'alimentation en azote (20 et 90 $\mu mol\ mol^{-1}$ N total) du sol dans des chambres à ciel ouvert. Des semis ont été récoltés à intervalle de 5–7 semaines. Le traitement CO_2 a eu un effet positif sur la croissance des semis. Les différences observées dans le

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poids de la biomasse entre les deux traitements CO_2 ont augmenté au cours de la période d'expérimentation. La disponibilité du sol en azote n'a pas affecté la croissance de manière significative. Néanmoins, la croissance en CO_2 élevée, en combinaison avec des niveaux élevés d'azote, amène une accumulation uniformément plus élevée de biomasse totale en fin d'expérience (30–40 %). L'allocation de biomasse entre les différentes parties a été semblable dans tous les traitements, mais était sensiblement différente entre les récoltes. Les régimes azotés n'ont pas entraîné de différence dans les taux de croissance relative (RGR) et les taux d'assimilation nette (NAR), alors que le traitement de CO_2 avait un effet significatif. A travers toutes les concentrations en CO_2 et les niveaux d'apport azoté, il a été mis en évidence une relation positive entre la masse des plantes et RGR, et cette relation n'a pas différé entre les traitements. La surface spécifique de feuille (SLA) a diminué en concentration élevée de CO_2. Le rapport de la masse de racine fine et de la masse de feuillage a été augmenté en forte concentration en CO_2 et a diminué avec les fortes concentrations en azote. Les semis traités avec une forte concentration en azote en CO_2 ont eu la plus grande croissance en hauteur et en diamètre. Les rapports allométriques entre la biomasse de tige et de la racine et entre la croissance en hauteur et en diamètre n'ont pas été sensiblement affectés par une concentration élevée. Les concentrations du feuillage en azote ont été réduites par les basses concentrations en azote du sol. La concentration en azote des semis diminue avec le temps. La concentration élevée en CO_2 a augmenté le rapport C/N de tous les compartiments des semis, en raison de la diminution des concentrations en azote. Les semis soumis à une concentration élevée en CO_2 ont réduit les concentrations en azote comparativement au traitement CO_2 en concentration actuelle, si la comparaison se fait sur une base temporelle, mais sont semblables si l'on compare des semis de hauteurs identiques. (© Inra/Elsevier, Paris.)

azote / croissance / enrichissement en dioxyde de carbone / *Quercus virginiana* / répartition du carbone

1. INTRODUCTION

Atmospheric carbon dioxide concentration [CO_2] is currently increasing at a rate of about 1.5 mmol·mol^{-1} annually [52] as a result of increasing fossil fuel consumption and deforestation. Models of future global change are in general agreement predicting levels reaching 600–800 μmol·mol^{-1} by the end of the next century from present levels ranging from 340–360 μmol·mol^{-1} [14].

Elevated [CO_2] promoted growth stimulation varies with plant species and growth conditions [1, 10]. The impact of increased [CO_2] on plant growth is modified by the nutrient level (e.g. [3, 5, 19]). Ceulemans and Mousseau [10] reported that in short-term (< 6 months) studies of elevated [CO_2] and varying resource availability, whole-plant biomass increased 38 % for conifers (12 species) and 63 % for broadleaved trees (52 species). Growth may be decreased at higher [CO_2] due to nutrient stress [29, 36]. Indeed, enhanced growth may increase plant nutrient requirement, but most temperate and boreal sites are considered to have low nitrogen (N) availability [24]. On the other hand, it has been proposed that plants adjust physiologically to low nutrient availability by reducing growth rate and accumulating a high concentration of C-based secondary metabolites [9] due to increases in carbon (C) relative to N. Numerous studies have shown decreases in N concentrations for plant grown under elevated [CO_2] at various N availabilities (e.g. [12, 29]). Changes in N concentrations and C/N ratios in plant tissues will likely affect plant–herbivore interactions and litter decomposition rates [15, 30].

The immediate effects of CO_2 on leaf photosynthesis can lead to changes in allocation patterns and other properties at whole-plant level (e.g. [21]). Patterns of biomass partitioning and resource allocation to roots and shoots are critical in determining the growth performance of plants. Changing allocation patterns may be one of the most effective means by which plants deal with environmental stresses [11, 41].

There have been no studies of the response of live oak to [CO_2], despite its importance in natural ecosystems in the southeastern United States, often on soils with low N availability. The objectives of the project were to investigate how CO_2 availability alters whole-plant tissue N concentration in live oak seedlings examined both at a common time and size, to examine the effects of increased [CO_2] on C partitioning to assess the production of biomass and its allocation.

This study was performed on seedlings on a 6-month exposure basis to test the null hypothesis that elevated CO_2 and interactions of CO_2 with soil resource limitations (N) would have no effect on biomass productivity and partitioning, and tissue N content. Obviously, experiments on seedlings cannot substitute for forest longer-term experiments, but the physiological mechanism of response to CO_2 of trees during the regeneration phase may still be addressed [10, 35]. Indeed, a small increase in relative growth at the early stage of development may result in a large size difference of individuals in successive years [5].

2. MATERIALS AND METHODS

2.1. Plant material and growth conditions

Acorns of live oak (*Quercus virginiana* Mill.) were collected in late November from three adult (open-pollinated) trees growing in the campus gardens of the University of Florida (29°43' N and 82°12' W; Gainesville, FL, USA). Seeds of each tree were broadcast in individual trays filled with growing medium (mixture of peat, vermiculite, perlite and bark) and moistened regularly. The containers subsequently were placed in a growth chamber (day/night temperature, 25 °C; day/night relative humidity [RH], 80 %; photosynthetic photon flux density (PPFD), 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$; photoperiod, 16 h). Germination took place at ambient [CO_2] in the containers. Seedlings emerged in all trays after 10 days.

After 2 weeks of growth in the trays, 40 seedlings per family were transplanted into black PVC containers (Deepots®; 25 cm height x 5.5 cm averaged internal diameter, 600 cm^3) and maintained in the growth chamber. The tubes were filled with a mixture (v/v) of 90 % sand and 10 % peat; a layer of stones was placed in the base of each tube. Seedlings in the growth chamber were watered daily. While plants were growing in the growth chamber, the first stage of growth was supported by adding commercial slow-release Osmocote (18/18/18, N/P/K); the nutrient additions were given in two pulses of 3 g each, applying the first after 1 week of growth in the tubes and the second after 6 weeks. Soil nutrients in terrestrial systems suggest that N mineralization is sometimes limited to short periods early in the growing season; furthermore, by giving an initial pulse of nutrients, we created a situation in which plant requirements for nutrients were increasing (due to growth) while supply was decreasing (due to uptake) [12], a phenomenon that may occur in natural systems poor in N such as the sandy soil of Florida. Before moving the seedlings to the open-top chambers, the superficial layer of Osmocote was removed from the tubes and the latter flushed repeatedly for 1 week with deionized water in order to remove accumulated salts and nutrients. During the 1st month of growth the seedlings were fumigated twice with a commercial fungicide.

Four months after germination (17 March), the seedlings were moved to six open-top chambers. Each chamber received one of two CO_2 treatments: ambient [CO_2] or 150 $\mu mol \cdot mol^{-1}$ exceeding ambient [CO_2]. The chambers were 4.3 m tall and 4.6 m in diameter, covered with clear polyvinylchloride film and fitted with rain-exclusion tops. Details of the chamber characteristics may be found in Heagle et al. [20]. CO_2, supplied in liquid form that vaporized along the copper supply tubes, was delivered through metering valves to the fan boxes of three chambers. The CO_2 treatment was applied during the 12 h (daytime) the fans were running with delivery being controlled by a solenoid valve connected to a timer. The CO_2 was delivered for about 15 min after the fans were turned off in the evenings in order to maintain higher concentrations in the chambers. [CO_2] was measured continuously in both the ambient and elevated [CO_2] chambers using a manifold system in conjunction with a bank of solenoid valves that would step through the six chamber sample lines every 18 min. Overall mean [CO_2] for these treatments was 370 or 520 $\mu mol \cdot mol^{-1}$ at present or elevated CO_2 concentrations, respectively [25].

Ten days after transferring the plants to the open-top chambers, two different nutrient solution treatments were initiated and seedlings of each family were randomly assigned to a CO_2 × nutrient solution treatment combination. Thus, the two CO_2 treatments were replicated three times, with the two nutrient solution treatments replicated twice within each CO_2 treatment. The seedling containers were assembled in racks and wrapped in aluminum foil to avoid root system overheating, and set in trays constantly containing a layer of nutrient solution to avoid desiccation and minimize nutrient loss, thus limiting nutrient disequilibrium ([22]).

Plants were fertilized every 5 days to saturation with one of the two nutrient solutions obtained by modifying a water soluble Peters fertilizer (Hydro-Sol®, Grace-Sierra Co., Yosemite Drive Milpitas, CA, USA): complete nutrient solution containing high N (90 $\mu mol \cdot mol^{-1}$ NH_4NO_3), or a nutrient solution with low N (20 $\mu mol \cdot mol^{-1}$ NH_4NO_3). Both nutrient solutions contained [in $mmol \cdot mol^{-1}$]: PO_4 (20.6), K (42.2), Ca (37.8), Mg (6), SO_4 (23.5), Fe (0.6), Mn (0.1), Zn (0.03), Cu (0.03), B (0.1) and Mo (0.02), and were adjusted to pH 5.5; every 5 weeks supplementary Peters (S.T.E.M.) micronutrient elements (0.05 $g \cdot L^{-1}$) were added. Deionized water was added to saturation every other day in order to prevent salt accumulation. Plant containers were moved frequently in the chambers to avoid positional effects.

2.2. Growth analysis

Heights and root-collar diameters were measured with a caliper on all the plants from day 4 of exposure and continued at regular intervals. Groups of six different plants were harvested (day 7) from each treatment for growth measurements, at the start of CO_2 and nutrient treatments; harvests continued every 5–7 weeks until

September. Total leaf area of each seedling was measured with an area meter (DT Devices Ltd., Cambridge, England). Seedlings were separated into leaves, stem and roots (for the last harvest, roots were divided in tap roots, > 2 mm, and fine roots, < 2 mm) and dried at 65 °C to constant weight, and dry weight (DW) measurements were made. Leaf area ratio (LAR; $m^2 \cdot g^{-1}$) was calculated as the ratio of total leaf area to plant dry weight; specific leaf area (SLA; $m^2 \cdot g^{-1}$) was calculated as the ratio of total leaf area to leaf dry weight; partitioning of total plant biomass – LWR, SWR and RWR ($g \cdot g^{-1}$) – was determined as the fraction of plant dry weight belonging to leaves (L), stem (S) and roots (R), respectively; and the root–shoot dry weight ratio (RSR; $g \cdot g^{-1}$) and fine root–foliage mass ratio ($g \cdot g^{-1}$) were determined. Relative growth rate (RGR; $g \cdot g^{-1} \cdot day^{-1}$) of seedlings was calculated as $\text{Ln}(W_2) - \text{Ln}(W_1) / (t_2 - t_1)$, in which W is plant mass and t is time. First harvest date RGR was calculated using seed mass for W_1. Net assimilation rate (NAR; $g \cdot m^{-2} \cdot day^{-1}$) of seedlings was calculated as $(W_2 - W_1) [(\text{Ln}(l_1) - \text{Ln}(l_2)] / (l_2 - l_1) (t_2 - t_1)$, in which l is total leaf area at the respective time.

2.3. Carbon and nitrogen analysis

Previously dried plant materials were separated and ground in a Wiley mill fitted with a 20-mesh screen. Total C and N concentrations ($mg \cdot g^{-1}$ DW) were determined by catharometric measurements using an elemental analyser (CHNS 2500, Carlo Erba, Milan, Italy) on 5–9 mg of powder of dried samples.

2.4. Statistical analysis

Three-way analysis of variance (ANOVA) with harvest time, [CO_2] and N availability as the main effects was conducted for all parameters except for those relative to the last harvest date only which were tested by two-way ANOVA. Two- and/or three-way interaction was included in the model. Proportions were transformed using the arcsine of the square root prior to analysis.

The relationships between whole-plant dry biomass and plant age, between RGR and Ln whole-plant biomass and between whole-plant % N and Ln whole-plant biomass were examined using non-linear regression techniques separately for each [CO_2] and nutrient treatment. The relationships between height and basal stem diameter were examined with linear regression analysis using Ln-transformed data in order to linearize the relationship. Allometric relationships between shoots and roots DW were also analyzed. The allometric relationships were calculated by linear regression based on Ln-transformed data [$\text{Ln}(y) = a + k \text{Ln}(x)$] with the previous mentioned variables as y and x and the allometric coefficient as the slope. Analysis of covariance (ANCOVA) was used to test for equality of regression coefficients.

3. RESULTS

3.1. Growth and biomass partitioning

CO_2 treatment had a positive effect on live oak seedlings growth (*figure 1*, *tables I* and *II*). Differences in biomass between elevated and ambient CO_2-treated plants increased during the experimental period and reached a maximum by the end of the study. In particular, roots and total biomass showed a significant interaction between CO_2 treatment and harvest day, respectively, $P < 0.01$ and $P < 0.05$, CO_2 effect increasing over time. CO_2 treatment had a strong effect ($P = 0.01$) on tap roots and fine roots (*table III*). Overall, soil N availability, did not affect growth (all DW) significantly, although the interaction between harvest date and N was significant ($P < 0.05$, $P < 0.1$ for roots), N effect increasing over time. Interaction between CO_2 treatment and N availability was not significant overall. Nevertheless, growth in elevated [CO_2] in combination with high N led to a consistently higher accumulation of total biomass (30–40 % higher than other treatments by the end of the experiment, day 178 of exposure).

Biomass allocation among plant components (foliage, stem and roots) was similar for seedlings in all treatments, but was significantly different ($P \leq 0.0001$) between harvests (data not shown). In all treatments, the proportion of foliage (and roots) biomass declined (or remained constant) and stem biomass increased during the course of the experiment.

The N regimes did not affect RGR, while CO_2 treatment had an overall significant positive effect ($P < 0.05$), particularly in high N and elevated [CO_2] during the first 2 months from exposure, high N and elevated [CO_2] (HE) plants showing higher values than other treatments at the final harvest date (*figure 2*, upper panel, and *table II*). Across all CO_2 and N levels, there was a positive relationship between plant mass and subsequent RGR (*figure 2*, lower panel), and this relationship did not differ between treatments. NAR was only marginally ($P = 0.08$) affected by CO_2 treatment and not influenced by N regime (*figure 3*, *table II*). Nevertheless, NAR was higher initially in HE plants and kept growing (also in high N and ambient [CO_2] [HA] plants) by the end of the experiment whereas in low N and ambient [CO_2] (LA)

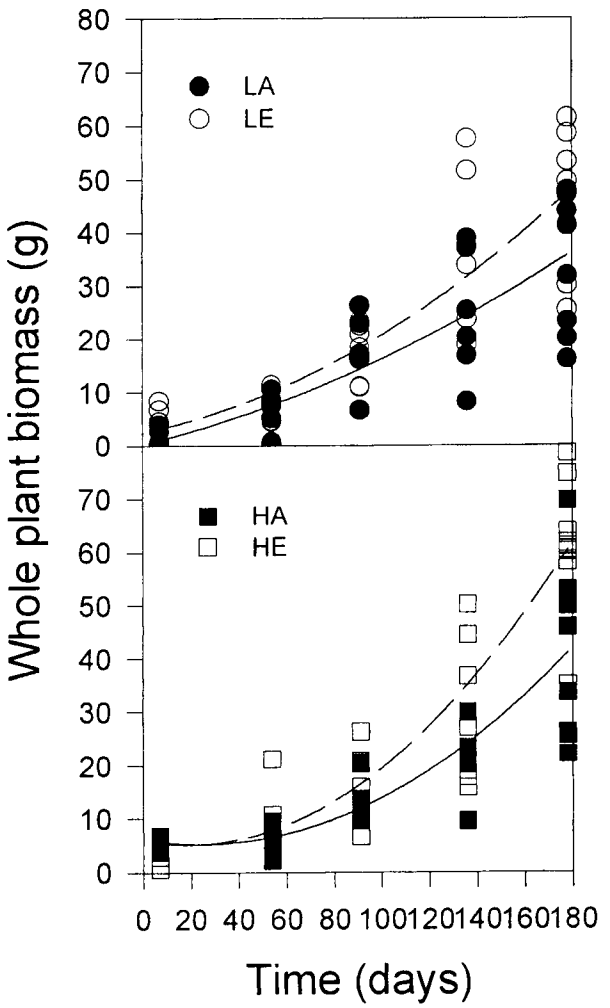

Figure 1. Plant biomass as a function of plant age (days of exposure), [CO_2], nutrient treatment. Each point represents data collected from the destructive harvest of a single individual. Lines correspond to a second-order polynomial equation fit to the raw data ($P < 0.0001$, R^2 0.66, 0.72, 0.71 and 0.84, respectively, for LA (low N and ambient [CO_2]), LE (low N and elevated [CO_2]), HA (high N and ambient [CO_2]) and HE (high N and elevated [CO_2]) plants. Treatments are referred to by symbols in the figure.

and low N and elevated [CO_2] (LE) plants stabilized on pretreatment values after an initial increase.

LAR and LWR decreased ($P \leq 0.0001$) during the experiment but were unaffected by both CO_2 and N levels (*figure 4*, *table II*), although interaction between treatments was significant ($P < 0.01$) for LAR and inspection of *figure 4* suggests that elevated [CO_2] consistently decreased LAR at the last three harvest dates in both N treatments. Similarly, SLA (*figure* 4) decreased ($P \leq 0.0001$) throughout the experiment, and overall CO_2 effect was significant ($P < 0.05$), as well as the interaction between CO_2 and N ($P < 0.001$), and plants in elevated [CO_2] had lower values, particularly by the end of experiment.

SWR, RWR and RSR (*figure 5*, *table II*) were unaffected by both CO_2 and N treatment (although the interaction was significant, $P \leq 0.05$ for RSR and RWR, $P = 0.07$ for SWR). While RSR and RWR remained relatively constant, SWR increased during the experiment. CO_2 and N treatments did not result in significantly different slopes for the relationship between shoot and roots, although high [CO_2] (particularly in conjunction with low N) treatment resulted in moderately lower allometric coefficient (*figure 6*), indicating a preferential shift in dry-matter allocation from above- to below-ground components. Fine root–foliage mass ratio was affected significantly by both CO_2 ($P < 0.05$) and N ($P < 0.01$) treatments; fine root–foliage mass ratio was particularly high in LE plants (*table III*).

There was no large difference in the initial rate of leaf area development between treatments (*figure 7*), but by the end of the experiment the high N treatment in combination with elevated CO_2 showed an increase more rapidly than other treatments. Overall, both treatments had relevant effects (*table II*), respectively $P < 0.05$ for N and $P = 0.06$ for [CO_2] treatment. Leaf area per leaf and number of leaves were unaffected by all treatments (*table II*). Height was largely affected by both treatments ($P < 0.0005$), particularly by the end of experiment (*figure 7*, *table II*). Basal stem diameter was similarly affected ($P = 0.02$, N, and $P \leq 0.0001$, CO_2) (*figure 7*, *table II*). High CO_2- and high N-treated plants showed the greatest heights and basal stem diameters at the final harvest date. There was a tendency in the relationship between height and basal stem diameter (*figure 8*) for a shift towards a higher diameter relative to height in high CO_2-grown plants with respect to ambient CO_2-grown plants.

3.2. Carbon and nitrogen analysis

Leaf N concentrations were significantly ($P \leq 0.0001$) decreased by low N level at all harvests (*tables I* and *II*). They were also significantly ($P < 0.05$) lowered by CO_2 treatment, at both N levels except at the first three harvest dates where leaf N concentrations were not modified by CO_2; the interaction between harvest date and CO_2 treatment was significant ($P < 0.05$). Overall, stem and root N concentrations were significantly ($P < 0.05$)

Table I. Biomass, nitrogen (N), carbon (C) and C/N ratio of live oak seedlings at different harvests when grown at two [CO_2] (ambient, A, and elevated, E, [CO_2]) and two N fertilizations (low, L, and high, H, level of N).

Measurement	Treatment	24 March (7)*	10 May (54)	16 June (91)	31 July (136)	11 September (178)
Foliage DW	LA	1.078(0.204)	1.529(0.553)	4.352(0.693)	4.989(0.914)	5.388(0.573)
	LE	1.569(0.330)	1.609(0.296)	4.656(0.666)	6.296(0.806)	6.034(0.645)
	HA		1.486(0.287)	3.895(0.599)	5.594(1.040)	6.777(0.632)
	HE		2.296(0.528)	4.693(1.131)	7.429(0.999)	9.582(0.811)
Stem DW	LA	0.404(0.055)	1.114(0.494)	4.010(1.276)	7.754(2.150)	10.632(1.578)
	LE	0.626(0.180)	1.040(0.248)	4.418(0.656)	10.378(1.606)	11.675(1.790)
	HA		0.880(0.155)	3.488(1.215)	5.821(0.938)	14.248(2.275)
	HE		1.423(0.481)	4.730(1.090)	11.291(2.682)	18.959(2.163)
Roots DW	LA	2.724(0.497)	2.864(0.827)	7.651(1.632)	11.812(2.090)	19.517(2.346)
	LE	2.233(0.420)	4.437(0.641)	7.863(1.164)	20.498(3.913)	27.777(2.557)
	HA		4.009(0.709)	6.774(0.545)	9.904(1.527)	20.790(2.755)
	HE		6.132(1.466)	7.786(1.058)	13.220(2.412)	32.988(2.396)
Foliage % N	LA	2.290(0.035)	2.338(0.078)	1.835(0.125)	1.907(0.211)	1.373(0.075)
	LE	2.349(0.194)	2.229(0.142)	2.292(0.125)	1.290(0.057)	1.056(0.070)
	HA		2.545(0.110)	2.387(0.164)	2.070(0.156)	1.787(0.104)
	HE		2.402(0.209)	2.086(0.145)	1.881(0.147)	1.412(0.098)
Stem % N	LA	1.774(0.095)	1.674(0.235)	1.015(0.095)	0.629(0.080)	0.468(0.040)
	LE	1.750(0.075)	1.252(0063)	1.226(0.113)	0.447(0.016)	0.386(0.025)
	HA		1.471(0.068)	1.672(0.236)	0.809(0.106)	0.554(0.043)
	HE		1.519(0.087)	0.902(0.067)	0.614(0.038)	0.441(0.020)
Roots % N	LA	1.954(0.088)	1.916(0.438)	0.963(0.130)	0.558(0.055)	0.431(0.027)
	LE	1.758(0.164)	1.382(0.058)	1.225(0.141)	0.374(0.017)	0.364(0.017)
	HA		1.524(0.051)	1.804(0.283)	0.807(0.120)	0.645(0.063)
	HE		1.713(0.138)	1.118(0.090)	0.722(0.102)	0.471(0.017)
Foliage % C	LA	35.711(2.218)	39.144(3.353)	37.568(3.573)	43.501(2.504)	43.341(1.249)
	LE	34.460(2.146)	34.827(1.324)	40.370(2.436)	40.721(2.860)	43.281(1.190)
	HA		35.031(1.081)	51.671(1.468)	52.897(11.42)	41.766(1.426)
	HE		36.414(4.581)	37.850(3.631)	41.989(2.664)	43.299(1.282)
Stem % C	LA	35.589(1.968)	41.031(2.491)	40.446(3.206)	43.648(1.804)	42.888(1.195)
	LE	31.914(0.396)	35.711(2.973)	36.977(2.016)	43.582(1.492)	43.090(1.082)
	HA		33.257(1.162)	46.108(1.617)	41.866(2.081)	41.128(1.254)
	HE		34.177(0.833)	40.423(2.614)	42.388(2.461)	42.633(1.172)
Roots % C	LA	32.296(2.226)	37.448(2.052)	35.728(1.831)	42.382(0.750)	40.516(1.108)
	LE	31.223(1.217)	32.742(0.816)	36.778(1.248)	40.638(1.792)	41.152(1.002)
	HA		32.949(0.692)	42.270(2.122)	39.452(1.887)	39.298(1.217)
	HE		30.242(1.081)	36.837(2.347)	40.191(1.992)	40.777(1.109)
Foliage C/N	LA	15.670(1.182)	16.768(1.332)	20.758(1.964)	23.587(1.667)	31.975(1.059)
	LE	15.469(1.942)	15.719(0.633)	18.163(1.969)	31.529(1.709)	42.214(3.052)
	HA		13.900(0.772)	21.946(1.044)	25.003(3.732)	23.748(0.926)
	HE		15.896(1.806)	18.365(1.676)	22.715(1.497)	31.421(1.446)
Stem C/N	LA	20.509(2.018)	26.851(3.105)	40.363(1.677)	75.182(8.061)	95.737(5.915)
	LE	18.371(0.662)	29.515(3.856)	31.461(2.555)	98.203(4.576)	114.47(6.161)
	HA		22.739(0.721)	30.654(3.666)	55.285(5.115)	78.315(6.491)
	HE		23.009(1.594)	46.043(3.197)	69.595(3.898)	97.628(3.081)
Roots C/N	LA	17.806(2.213)	22.671(2.456)	42.157(6.553)	80.031(7.030)	100.78(4.553)
	LE	18.517(1.933)	23.943(1.197)	34.075(5.642)	110.76(8.218)	125.04(6.555)
	HA		21.724(0.689)	26.659(3.524)	53.202(5.814)	67.669(5.563)
	HE		18.352(1.665)	34.457(3.648)	63.184(9.230)	89.028(2.524)

*On first harvest day nitrogen treatment was not yet applied. Treatment duration (days) in open-top chambers is indicated below harvest dates. Values (g DW and %) are the mean (±SE) at each time interval (n = 6–9).

Table II. Summary of three-way ANOVA (*P* values) for the main effect of harvest date, [CO_2] and nitrogen (N) level and their interaction on all of the measured parameters of live oak seedlings.

Parameter	Date (4)*	CO_2 (1)	N (1)	Day × CO_2 (4)	Day × N (4)	CO_2 × N (1)	Day × CO_2 × N (4)
Foliage DW	0.0001	0.0087	0.6634	0.5547	0.0448	0.4822	0.6773
Stem DW	0.0001	0.0424	0.2285	0.4064	0.0257	0.3441	0.9145
Roots DW	0.0001	0.0004	0.9125	0.0034	0.0976	0.6840	0.5282
Total DW	0.0001	0.0013	0.4371	0.0432	0.0439	0.7625	0.7384
RGR	0.0001	0.0320	0.5893	0.7021	0.4878	0.1871	0.0055
NAR	0.0012	0.0840	0.5663	0.8591	0.2059	0.7097	0.5813
LWR	0.0001	0.5952	0.2289	0.6946	0.1441	0.2148	0.9340
SLA	0.0001	0.0128	0.7988	0.9339	0.6686	0.0002	0.0016
LAR	0.0001	0.1581	0.2589	0.5657	0.3991	0.0010	0.0131
RSR	0.0001	0.9615	0.5422	0.3034	0.2974	0.0508	0.9536
RWR	0.0001	0.9309	0.3030	0.2823	0.0818	0.0334	0.7675
SWR	0.0001	0.7436	0.7002	0.1135	0.2588	0.0692	0.8199
Total leaf area	0.0001	0.0591	0.0478	0.9805	0.1742	0.3685	0.8302
Leaf area per leaf	0.0005	0.8387	0.8993	0.6474	0.9079	0.5498	0.9944
Number of leaves	0.0001	0.2102	0.0898	0.8077	0.2682	0.6380	0.9317
Stem length	0.0001	0.0003	0.0001	0.3841	0.0001	0.0119	0.2883
Stem diameter	0.0001	0.0001	0.0194	0.0123	0.0752	0.1499	0.8702
Foliage % N	0.0001	0.0214	0.0001	0.0348	0.5954	0.7885	0.0120
Stem % N	0.0001	0.0034	0.1227	0.6228	0.7087	0.1932	0.0002
Roots % N	0.0001	0.0278	0.1739	0.9921	0.1026	0.4357	0.0019
Foliage % C	0.0008	0.0778	0.4036	0.4193	0.3078	0.1266	0.1375
Stem % C	0.0001	0.0638	0.6458	0.2603	0.0123	0.6105	0.1990
Roots % C	0.0001	0.1189	0.9265	0.2803	0.0479	0.5683	0.2884
Foliage C/N ratio	0.0001	0.0529	0.0007	0.0001	0.0009	0.0522	0.1508
Stem C/N ratio	0.0001	0.0017	0.0016	0.0102	0.0010	0.7411	0.1989
Roots C/N ratio	0.0001	0.0028	0.0001	0.0023	0.0001	0.4411	0.2809

*Degree of freedom.
DW: dry weight; RGR: relative growth weight; NAR: net assimilation rate; LWR: plant dry mass belonging to leaves; SLA: specific leaf area; LAR: leaf area ratio; RSR: root–shoot dry mass ration; RWR: plant dry mass belonging to roots; SWR: plant dry mass belonging to stem.

decreased by CO_2 treatment but less by low N levels (*tables I* and *II*). Leaf, stem and root N concentrations significantly ($P \leq 0.0001$) decreased with time in all treatments. Whole plant % N as a function of plant size is reported in *Figure 9*; plants of any given size, whether grown at elevated or ambient [CO_2], had similar N concentrations within a given nutrient supply. N availability affected patterns of tissue N concentration as a function of plant size. Both CO_2 and N treatments had small effects on leaf, stem and root C concentrations (*tables I* and *II*). CO_2 enrichment had significant effects on C/N ratios (*tables I* and *II*) of stem and roots ($P < 0.005$) and small but significant ($P = 0.05$) on those of leaves. The C/N ratios of plant material increased for plants grown at elevated [CO_2] compared with ambient conditions. In addition, the greater N supply significantly ($P < 0.005$) decreased the C/N ratios of leaf, stem and roots due to an increase in the N concentration. The effects of CO_2 and N treatment increased with time; the interaction between harvest date and CO_2 or N treatment was significant ($P \leq 0.01$).

CO_2 enrichment had a significant effect ($P < 0.05$) on N concentrations of fine roots (*table III*), measured at the final harvest, with decreases of 10 and 25 % in low N and high N grown plants, respectively; N concentrations of tap root were not affected significantly by CO_2 enrichment. Increasing the N supply significantly increased (20–45 %, $P < 0.005$) the N concentrations of tap and fine roots. No significant differences were found between treatment effects on the C concentrations of tap and fine roots. The decrease of N concentrations resulted in an increase of the C/N ratio ($P < 0.05$) of both tap

Table III. Root biomass components, fine root–foliage mass ratio and nitrogen (N), carbon C concentration and C/N ratio (tap roots and fine roots) in live oak seedlings at the end of experiment when grown at two [CO_2] (ambient, A, and elevated, E, [CO_2]) and two N levels (low, L, and high, H, level of N).

Treatment	Tap root g DW	Fine roots g DW	Fine root-foliage mass ratio	Tap root % N	Tap root % C	Tap root C/N ratio	Fine roots % N	Fine roots % C	Fine roots C/N ratio
LA	12.202(1.982)	7.315(1.061)	1.396(0.144)	0.347(0.029)	39.709(1.218)	118.341(6.640)	0.515(0.038)	41.323(1.144)	83.253(5.887)
LE	15.454(1.539)	12.323(2.202)	1.961(0.175)	0.263(0.008)	40.324(1.081)	154.685(7.650)	0.464(0.036)	41.979(1.092)	95.395(9.628)
HA	13.471(1.494)	7.319(1.387)	1.077(0.144)	0.573(0.092)	38.537(1.318)	77.847(8.920)	0.717(0.047)	40.059(1.296)	57.491(3.365)
HE	19.801(1.858)	10.956(1.565)	1.301(0.199)	0.482(0.065)	40.445(1.086)	91.973(7.256)	0.555(0.041)	41.619(1.092)	77.520(4.155)
Probability									
CO_2	0.0104	0.0099	0.0268	0.1582	0.2961	0.0025	0.0145	0.3592	0.0106
N	0.1205	0.6685	0.0070	0.0009	0.6655	0.0001	0.0012	0.4984	0.0009
CO_2 x N	0.3884	0.6665	0.3250	0.9511	0.5957	0.1582	0.1845	0.7050	0.5176

Values (g DW) are the mean (±SE) at each time interval (n = 8–10). The probability level (P) is also reported (two-way ANOVA).

(15–20 %) and fine roots (15–25 %) at elevated [CO_2]. In addition, increasing the N supply significantly decreased the C/N ratio of both tap (35–40 %) and fine roots (20–30 %) due to an increase ($P < 0.001$) in the N concentration.

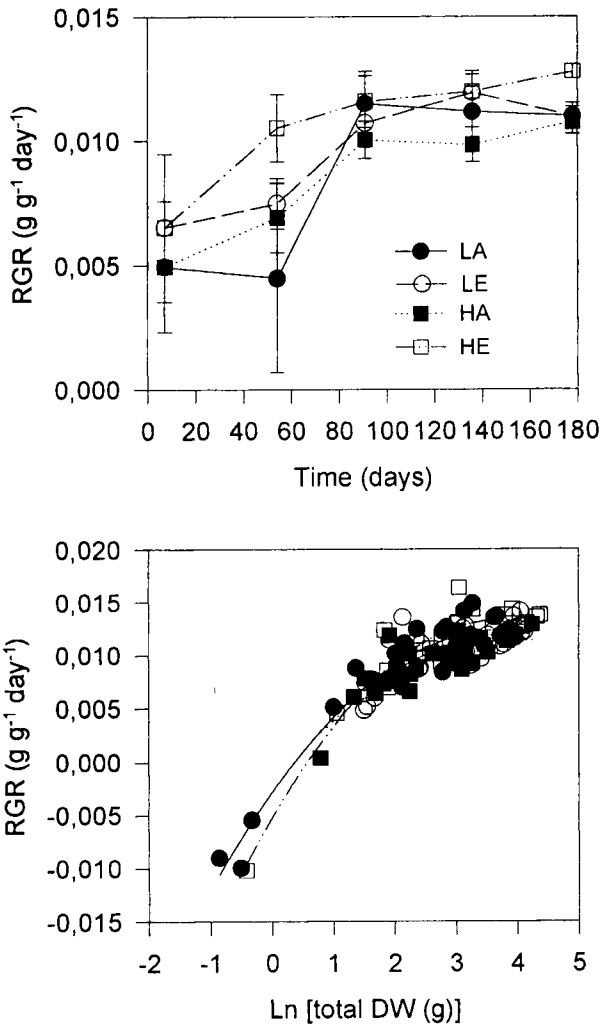

Figure 2. Relative growth rate (RGR) as a function of plant age (days of exposure), [CO_2], nutrient treatment (upper panel). Values are the mean (± SE) at each time interval (n = 6–9). The relationship between RGR and plant size (Ln transformed) is shown in the lower panel. Each point represents data collected from the destructive harvest of a single individual. Lines correspond to a second-order polynomial equation fit to the raw data ($P < 0.0001$, R^2 0.93, 0.56, 0.70 and 0.90, respectively, for LA (low N and ambient [CO_2]), LE (low N and elevated [CO_2]), HA (high N and ambient [CO_2]) and HE (high N and elevated [CO_2]) plants. Treatments are referred to by symbols in the figure. DW: dry weight.

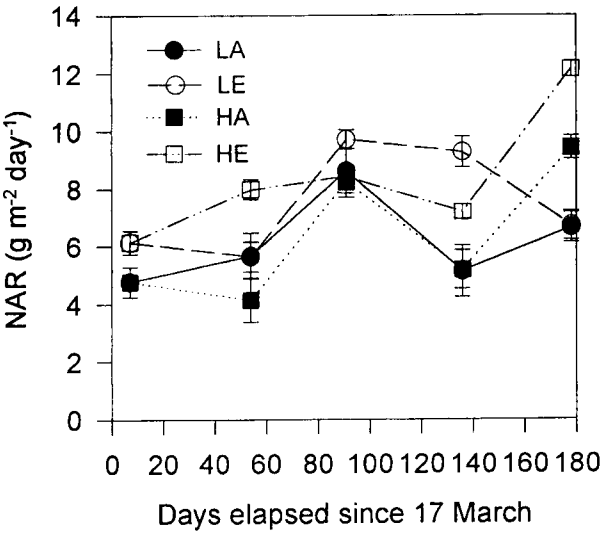

Figure 3. Net assimilation rate (NAR) as a function of plant age (days after treatment), [CO_2], nutrient treatment. Values are the mean (± SE) at each time interval (n = 6–9). Treatments are referred to by symbols in the figure. LA: low N and ambient [CO_2]; LE: low N and elevated [CO_2]; HA: high N and ambient [CO_2]; HE: high N and elevated [CO_2].

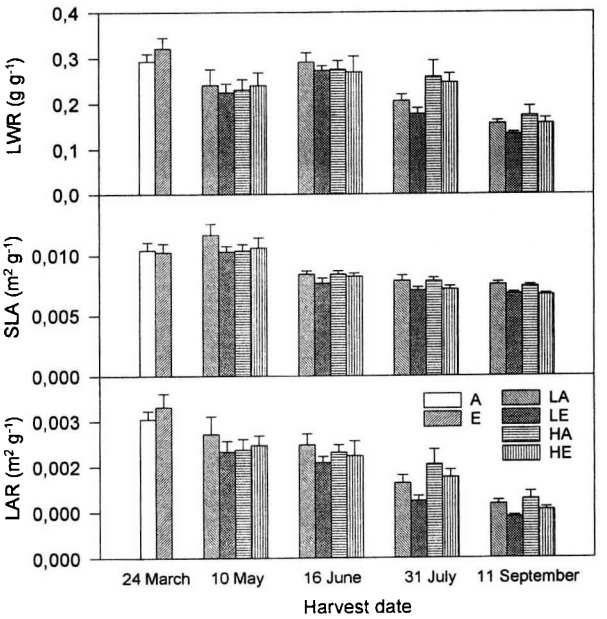

Figure 4. Plant dry mass belonging to leaves (LWR), specific leaf area (SLA) and leaf area ratio (LAR) as a function of plant age (harvest date), [CO_2], nutrient treatment. Values are the mean (± SE) at each time interval (n = 6–9). Treatments are referred to by symbols in the legend. A and E, respectively: ambient and elevated [CO_2], on first harvest day N treatment was not yet applied; LA: low N and ambient [CO_2]; LE: low N and elevated [CO_2]; HA: high N and ambient [CO_2]; HE: high N and elevated [CO_2].

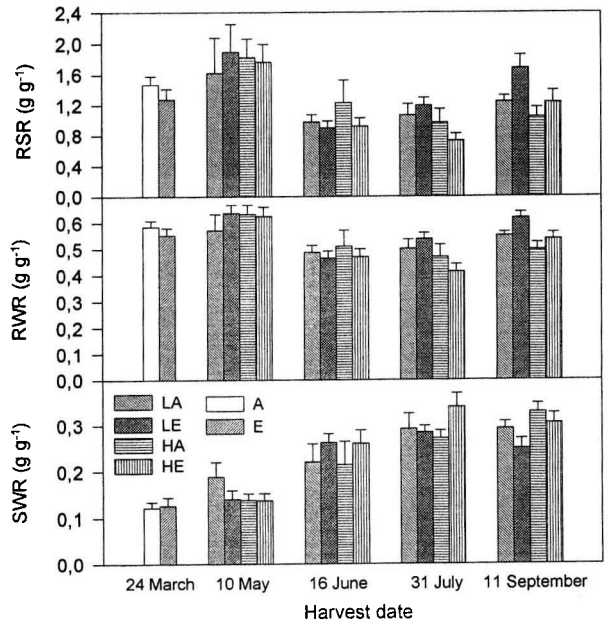

Figure 5. Root–shoot dry mass ratio (RSR), plant dry mass belonging to roots (RWR) and stem (SWR) as a function of plant age (harvest date), [CO_2], nutrient treatment. Values are the mean (±SE) at each time interval (n = 6–9). Treatments are referred to by symbols in the legend. A and E, respectively: ambient and elevated [CO_2], on first harvest day N treatment was not yet applied; LA: low N and ambient [CO_2]; LE: low N and elevated [CO_2]; HA: high N and ambient [CO_2]; HE: high N and elevated [CO_2].

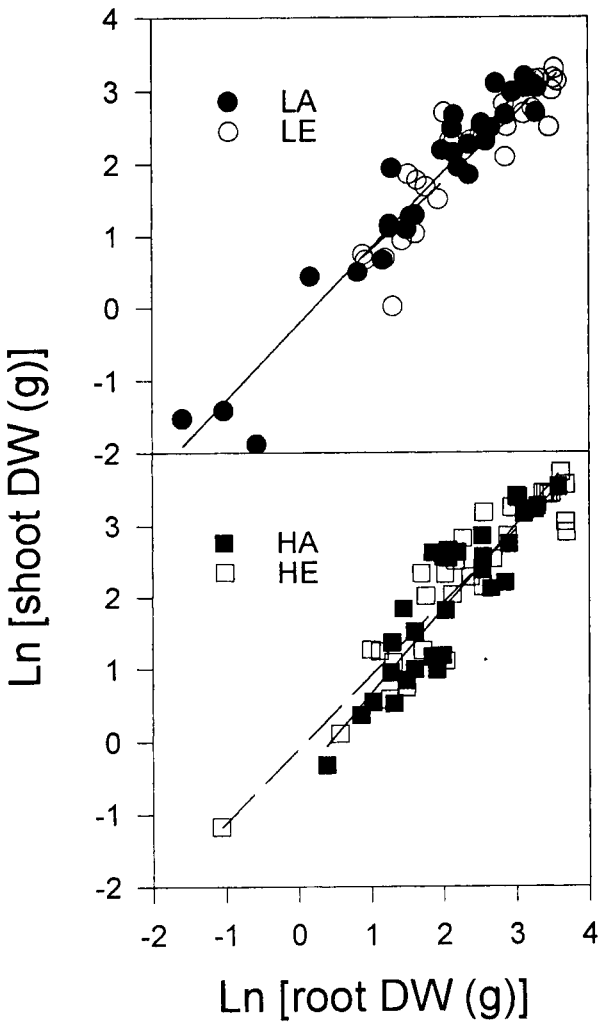

Figure 6. Relationship between shoot and root biomass (Ln transformed). Each point represents data collected from the destructive harvest of a single individual. Lines correspond to a first-order linear equation fit to the raw data ($P < 0.0001$, R^2 0.93, 0.83, 0.80 and 0.88, allometric coefficient, slope, 1.057, 0.945, 1.180 and 1.015 with SE ranging from 0.055 to 0.111, respectively, for LA (low N and ambient [CO_2]), LE (low N and elevated [CO_2]), HA (high N and ambient [CO_2]) and HE (high N and elevated [CO_2]) plants. DW: dry weight. Treatments are referred to by symbols in the figure.

4. DISCUSSION

Live oak seedlings exhibited increased biomass in response to elevated [CO_2] (27–33 %, depending on the specific treatment combination). The responses we observed were in line with responses of many other tree species to elevated [CO_2]. Luxmoore et al. [32], reviewing 58 studies with 73 tree species, found that the growth enhancement most frequently observed was 20–25 % and that the stimulation of growth was more or less equally partitioned to foliage, stem and roots biomass, whereas leaf area increased only marginally. Live oak seedlings responded to elevated [CO_2] by increasing foliage and stem biomass particularly when N availability was high. Conversely, roots (both tap and fine roots) responded positively to elevated [CO_2] irrespective of N availability. Several studies indicate that the responsiveness to CO_2 by woody seedlings is often conditional on the adequate availability of other resources, despite other reports that this is not the case [2, 13, 19, 29, 32, 34, 37, 46].

Greater total leaf area per plant, height and basal stem diameter (with a tendency for relatively more diameter than height growth in high-CO_2) were particularly evident in elevated CO_2- and high N-grown plants with respect to ambient CO_2- and high N-grown plants, while low N-grown plants did not differ regardless of CO_2 treatment. The absence of any large treatment effect on

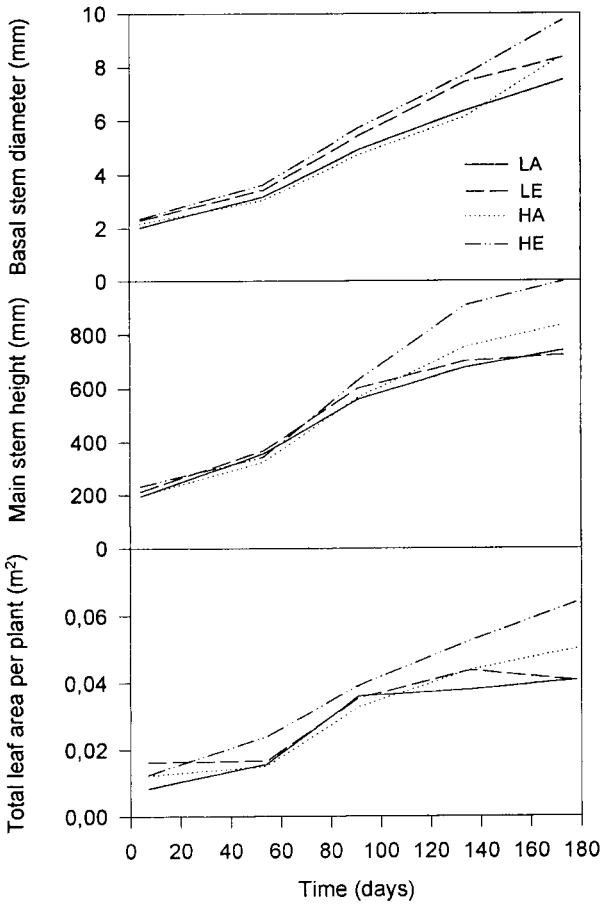

Figure 7. Basal stem diameter, seedling height and total leaf area per plant as a function of plant age (days after treatment), $[CO_2]$, nutrient treatment. Values are the mean (SE, not shown, ranged from 5 to 30 %) at each time interval (n = 6–9 for leaf area and n = 30–8 for diameter and height). Treatments are referred to by symbols in the figure. LA: low N and ambient $[CO_2]$; LE: low N and elevated $[CO_2]$; HA: high N and ambient $[CO_2]$; HE: high N and elevated $[CO_2]$.

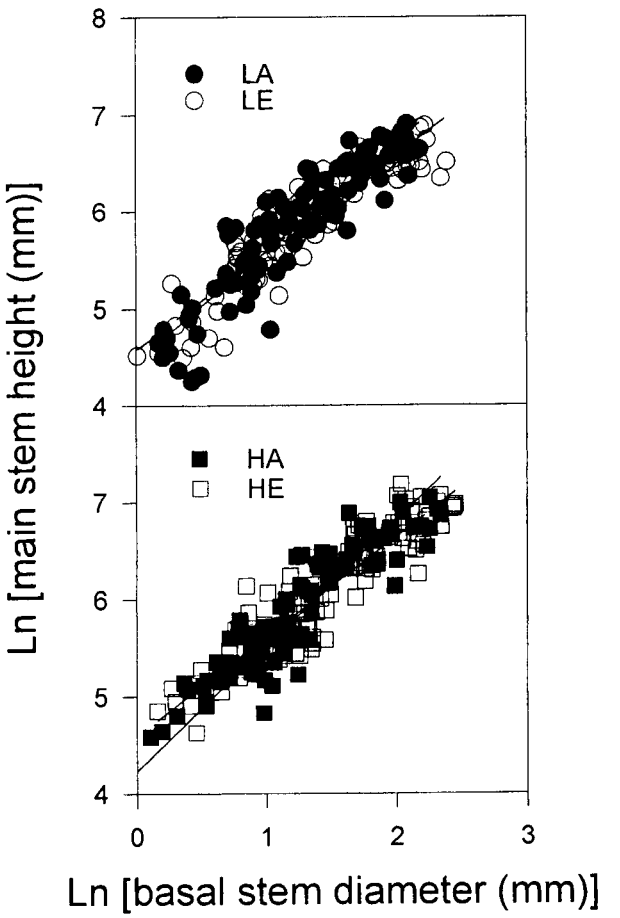

Figure 8. Relationship between seedling main stem height and basal stem diameter (Ln transformed). Each point represents data collected at a different date on a single individual. Lines correspond to a first-order linear equation fit to the raw data ($P < 0.0001$, R^2 0.83–0.84, allometric coefficient, slope, 1.125 1.002, 1.101 and 1.028 with SE ranging from 0.044 to 0.051, respectively, for LA (low N and ambient $[CO_2]$), LE (low N and elevated $[CO_2]$), HA (high N and ambient $[CO_2]$) and HE (high N and elevated $[CO_2]$) plants. Treatments are referred to by symbols in the figure.

number of leaves and leaf area per leaf may partly be related to the duration of the experiment. Contrasting results have been reported in the literature [38, 42, 45].

Partitioning of biomass between plant parts was similar for seedlings in all treatments regardless of differences in total biomass. SLA was significantly reduced in high CO_2-grown plants. Most studies with CO_2 enrichment report decreases in SLA (e.g. [16, 38]), and an increased allocation to roots (cf. [4, 44]). A reduction in SLA with elevated $[CO_2]$ may be the result of changes in leaf anatomy and/or accumulation of carbohydrates [38]. Total plant leaf area increased in response to elevated $[CO_2]$ (and to high N) and LAR (and secondarily LWR) decreased over time in response to elevated $[CO_2]$ in both N treatments, even if the overall CO_2 effect was not significant, suggesting that canopy-level adjustment in C assimilation might occur but that total plant leaf area increased mainly as a result of accelerated ontogeny [48]. With time, it would be expected that the advantage of overall higher RGR at elevated $[CO_2]$ would offset a disadvantage of lower LAR in contributing to an eventually more rapid development of total leaf area in elevated

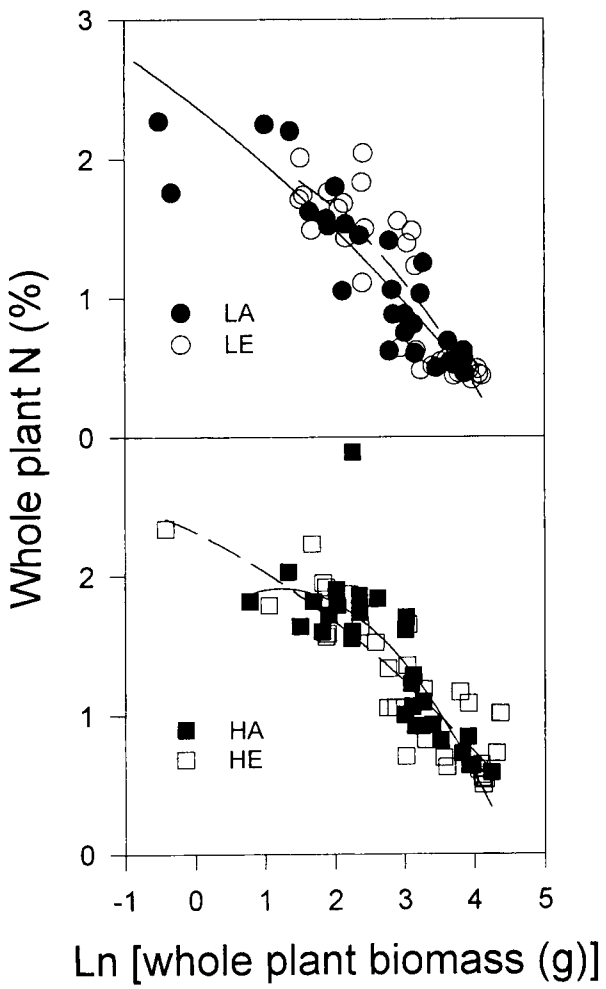

Figure 9. Percent whole plant N as a function of plant size, [CO_2], nutrient treatment. Each point represents data collected from the destructive harvest of a single individual. Lines correspond to a second-order polynomial equation fit to the raw data ($P < 0.0001$, R^2 0.83, 0.78, 0.71 and 0.78, respectively, for LA (low N and ambient [CO_2]), LE (low N and elevated [CO_2]), HA (high N and ambient [CO_2]) and HE (high N and elevated [CO_2]) plants. Treatments are referred to by symbols in the figure.

CO_2- and high N-grown plants with respect to other treatments.

CO_2 treatment increased the fine root–foliage mass ratio while N treatment had the opposite effect. Pregitzer et al. ([40]), studying *Pinus ponderosa*, also observed that N fertilization decreased the fine root–foliage mass ratio but the same authors found that elevated [CO_2] had no effect. The change in allocation might represent a substitution between potential C assimilation and nutrient acquisition [37]. Contrasting results are reported in the literature [28, 37, 40, 47] and this may reflect species-specific responses to CO_2.

RSR response to elevated [CO_2] has been found to be quite variable [44]. In the present experiment, RSR was found to be higher in elevated CO_2-grown plants by the end of the experiment when RWR and SWR tended to increase and decrease, respectively. This, however, was more evident for low N-treated plants [18]. King et al. [27] concluded that *Pinus taeda* and *Pinus ponderosa* had the potential to increase substantially belowground biomass in response to rising [CO_2], and this response is sensitive to N; an allometric analysis indicated that modulation of the secondary root fraction was the main response of the seedlings to altered environmental conditions, although neither species exhibited shifts in C accumulation in response to elevated [CO_2]. In the present experiment the observed shifts in C accumulation were not large, and the moderately lower allometric coefficients in elevated CO_2-grown live oak seedlings (particularly in low N), overall, were weakly indicative of partitioning toward roots. Farrar and Williams [18] found no change in the allometric constant due to elevated [CO_2] for Sitka spruce. However, in *Quercus robur* RSR was decreased at the end of the growing season by elevated [CO_2] [50]. It is not clear if, on a longer period, RSR could be altered by elevated [CO_2] and then if the investment of additional photosynthate into root growth for improved acquisition of nutrients is necessary for elevated CO_2-grown live oak plants in nutrient-rich soil (cf. [39, 48]). Many results support the concept that biomass partitioning in plants is related to C and N substrate levels (e.g. [31, 36, 43]).

RGRs of live oak seedlings exposed to elevated [CO_2] and high N nutrition in the first 2 months of exposure increased in association with the increased NAR of these plants more than other treatments and then converged, although differences were again detected between elevated CO_2- and high N-grown seedlings and other treatments by the end of the experiment (mean RGRs of elevated CO_2- and high N-grown seedlings were at that time approximately 15 % higher than those of other treatments and showed a stimulated NAR). McConnaughay et al. [33] found that doubling the amount of nutrients within a constant soil volume may increase the relative growth response to CO_2. Many experiments have indicated that CO_2-induced growth increases were greatest shortly after seedling emergence, and this was followed by a transition stage where RGRs of CO_2 treatments converged (e.g. [4, 6–8, 23]). On the other hand, Pettersson and McDonald [38], studying birch at optimal nutrition, found that although RGR was only moderately greater at elevated [CO_2], the difference

in RGR persisted and resulted in much larger plants in elevated [CO_2] by the end of the experiment (about 40 days of treatment). Where sink sizes are adequate (eg, large tap roots of live oak seedlings, despite the limited pot volume), C assimilation [49] can be maintained at high rates in elevated [CO_2]. If optimal nutrition is maintained, larger sapling might be attained more rapidly at elevated [CO_2]. Conversely, where nutrient uptake is insufficient for the maintenance of optimal nutrition, the potential for increased dry matter productivity at elevated [CO_2] may not be realised. Across all CO_2 and N treatments there was a positive relationship between plant mass and subsequent RGR.

The reduction in the % N at elevated [CO_2] agrees with previous studies on several tree species (eg, [17], see [10] for a review). Coleman et al. [12] suggested that the decrease in plant % N as a result of exposure to elevated [CO_2] might be a size-dependent phenomenon resulting from accelerated plant growth, rather than increased N use efficiency. According to these authors, an analysis of tissue N concentrations as a function of total plant biomass showed that live oak seedlings of any given size, whether grown under ambient or elevated [CO_2], had similar N concentrations within a given nutrient supply. Nevertheless, plants grown with different N availability showed different patterns of tissue N concentration as a function of plant size. Low N plants had mean N content lower than high N plants, but this was evident at the end of the experiment and overall significant only for leaves. The decline in foliage N content with plant age is consistent with the partial declining stimulation of growth and at later harvests.

The effects of elevated [CO_2] on % C of plant tissue were very small [26]. The effect of CO_2 in stimulating growth and increasing C/N ratios might affect C storage and nutrient cycling in this as in other *Quercus* species [51]. An increase in the C/N ratios, due to a decrease of N content, may led to increases in concentrations of C based compounds such as phenolics [30, 49].

In summary, an early and positive response to elevated [CO_2] rapidly and substantially increased total plant biomass in live oak seedlings, particularly at high soil nutrient conditions. Dry matter allocation might be altered in low nutrient soil conditions but probably not at optimal nutrition, and the form coefficient (height/diameter ratio) might vary considerably. In this context, it may be of considerable relevance to nutrient acquisition that fine root–foliage mass ratio in our study was greater at elevated [CO_2] by the end of the experiment. Elevated [CO_2] increased the C/N ratio of all plant compartments as a result of decreasing N concentrations. High CO_2-grown plants had reduced N concentrations relative to ambient CO_2-grown plants when compared at a common time, but similar when compared at a common size.

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