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Effects of elevated CO_2 concentrations on photosynthesis, growth and reproduction of branches of the tropical canopy tree species, *Luehea seemannii* Tr. & Planch.

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ABSTRACT

Mature trees have already experienced substantial increases in CO_2 concentrations during their lifetimes, and will experience continuing increases in the future. Small open-top chambers were used to enclose branchlets that were at a height of between 20 and 25 m in the canopy of the tree species *Luehea seemannii* Tr. & Planch. in a tropical forest in Panamá. Elevated concentrations of CO_2 increased the rate of photosynthetic carbon fixation and decreased stomatal conductance of leaves, but did not influence the growth of leaf area per chamber, the production of flower buds and fruit nor the concentration of nonstructural carbohydrates within leaves. The production of flower buds was highly correlated with the leaf area produced in the second flush of leaves, indicating that the branchlets of mature trees of *Luehea seemannii* are autonomous to a considerable extent. Elevated levels of CO_2 did increase the concentration of nonstructural carbohydrates in woody stem tissue. Elevated CO_2 concentration also they increased the ratio of leaf area to total biomass of branchlets, and tended to reduce individual fruit weight. These data suggest that the biomass allocation patterns of mature trees may change under future elevated levels of CO_2. Although there were no effects on growth during the experiment, the possibility of increased growth in the season following CO_2 enrichment due to increased carbohydrate concentrations in woody tissue cannot be excluded.

Key-words: Luehea seemannii Tr. & Planch.; branch autonomy; branch-bag; carbohydrate; elevated CO_2; forest canopy; growth; photosynthesis.

INTRODUCTION

Trees are long-lived organisms. Tropical trees that began life in the late eighteenth century have already been exposed to considerable increases in carbon dioxide concentrations ($[CO_2]$) during their lifetimes, and will be exposed to continuing increases in the decades to come. Understanding how these mature canopy trees respond to elevated $[CO_2]$, with respect to their rate of photosynthetic carbon gain, growth and reproductive output, is important because mature trees are responsible for forest carbon sequestration and provide the seed bank for forest regeneration. Few studies have attempted to investigate the effects of elevated $[CO_2]$ in mature forest stands because of the difficulties involved in exposing whole trees to such concentrations and also accessing the canopy. The development of open-top chambers permitted the study of the influence of elevated $[CO_2]$ *in situ* (Drake *et al*. 1989), but limited studies to vegetation types where the plants under study were similarly scaled, for example, intertidal communities (e.g. Drake *et al*. 1989), herbaceous communities (e.g. Potvin & Vasseur 1997), young trees (e.g. Norby, O'Neill & Luxmoore 1986; Idso, Kimball & Allen 1991; Lovelock *et al*. 1998), and woody communities of low stature (e.g. Cipollini, Drake & Whigham 1993). The development of free-air CO_2 enrichment (FACE) technology (Hendrey 1992) has increased the scale at which the influence of elevated $[CO_2]$ on vegetation can be studied (e.g. Ellsworth *et al*. 1995 in loblolly pine forest). However, a FACE system is not yet constructed within any tropical forest. One solution which can be used to study the response of mature trees to elevated $[CO_2]$ is to enclose branches of trees in branch-bags (Barton, Lee & Jarvis 1993; Dufrene, Pontailler & Saugier 1993; Teskey 1995; Teskey 1997; Lee & Jarvis 1996), and even small parts of leaves of mature trees in smaller chambers (Körner & Würth 1996).

Studies exposing only parts of trees to elevated $[CO_2]$ often meet with criticism because any responses to the elevated concentrations are influenced by source-sink carbohydrate relations within plants (Körner, Pelaez-Riedl & van Bel 1995; Rogers *et al*. 1996). That is, plant productivity enhancements under elevated $[CO_2]$ are thought to be largely determined by the capacity of plants to use the carbon fixed in photosynthesis (Drake, González-Meler & Long 1997). This can be limited by the number and activity of carbon sinks, including storage (Farrar & Williams 1991; Rogers *et al*. 1996), or by the ability to transport carbon to the sinks (Körner *et al*. 1995). Whether due to insufficient carbohydrate sink size or to carbohydrate

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transport limitations, exposure to elevated [CO_2] during growth has been shown to sometimes lead to decreased photosynthetic capacity and also to little increase in growth (Ceulemans & Mousseau 1994). It is suggested that when a small portion of a canopy is enclosed, potentially altering the source of carbohydrates, but not the sink, which in mature trees could be infinitely large, the results obtained will not be applicable to the situation where a whole tree is exposed to elevated [CO_2]. For example, in the studies of Liu & Teskey (1995), Teskey (1997), and Wang & Kellomäki (1997) there was no reduction in photosynthetic capacity of the conifers grown under elevated [CO_2], indicating no regulatory effects of carbohydrates on photosynthesis. These results could be an artifact of the unlimited sink for carbohydrates provided by the rest of the tree, and may not occur if the whole tree was exposed to elevated [CO_2]. But other experiments have provided evidence that leaf photosynthetic capacity of branches grown under elevated [CO_2] can be reduced (Barton *et al*. 1993; Marek & Kalina 1996). This suggests that the rest of the tree is not an infinite sink for the products of photosynthesis within branches, and that branches may be a reasonable surrogate for studying the response of trees to elevated [CO_2].

The applicability of results obtained by the exposure of branches to elevated [CO_2], to the understanding of the influence of [CO_2] on the physiology of trees depends upon the physiological autonomy of the branches under study (Sprugel, Hinckley & Schaap 1991). Branch autonomy is influenced by source-sink relationships (Sprugel *et al*. 1991). Branches are likely not to be autonomous when the sinks or sources of carbohydrates outside the branch are strong; that is when there is a high level of apical dominance or carbon is translocated from the trunk wood to the developing leaves or roots. Branches are probably relatively autonomous during the growing season, when they largely fuel their own growth (Sprugel *et al*. 1991). Models of branch development in trees have indicated that as trees mature, the branches probably become more autonomous because the proportion of carbon required to provide structural support declines relative to the amount of foliage (Ford, Avery & Ford 1990). Therefore, studies of branch response to elevated [CO_2] are more likely to be representative of the whole tree during the middle part of the growing season and in trees with many orders of branching.

Here we report the results from a study exposing seventh-order branchlets of mature trees of *Luehea seemannii* Tr. & Planch. to elevated [CO_2] in a forest canopy in Panamá. We tested whether leaf growth, leaf photosynthetic rates, and carbohydrate concentrations were modified by growth under elevated [CO_2] and also examined the effect of these elevated [CO_2] on flower and fruit production and carbohydrate storage in woody tissue.

MATERIALS AND METHODS

Branchlets of 25–30 m tall *Luehea seemannii* (Tiliaceae) trees were placed in small open-top chambers suspended in the canopy within Parque Natural Metropolitano, on the Pacific coast of the Republic of Panamá. *Luehea seemannii* is a common 'building phase' tree species (Condit, Hubbell & Foster 1996). The forest is a semi-deciduous tropical dry forest that is approximately 80 years old. The rainfall is 1·8 m $year^{-1}$ and highly seasonal, mostly falling in the wet season (May–December). Access to the canopy of the forest was provided by a construction crane.

Chamber design

The branch chamber design was based on the open-top chambers commonly used in experiments examining the influence of elevated [CO_2]. This design was chosen instead of the branch bags commonly used in other experiments, where branches are more or less completely enclosed (e.g. Barton *et al*. 1993; Teskey 1995; Teskey 1997) because of: (1) the horizontally spreading foliage of *L. seemannii*, and (2) the difficulty in maintaining ambient temperatures within branch bags under the high temperature and solar radiation conditions in Panamá without the use of air conditioning. The open-top chamber design was advantageous because these chambers were easier to deploy high in the canopy than branch bags, they were light-weight, inexpensive and led to only small increases in foliage temperature.

The open-top chambers were 44 cm in diameter. The chambers were made from two circular transparent plastic trays placed together to form a small space between them (Fig. 1). Air was forced into the space between the trays using a ventilator suspended on a nearby branch and connected to the chambers by 10-cm-diameter flexible plastic tubing. Air passed out of the space between the trays through holes drilled into the top tray which acted as a diffuser. The walls of the open-top chambers were a 30-cm-high mylar screen placed around the trays. Drain holes were drilled in the lower tray to prevent rainwater collecting in the chambers. The chambers were suspended using nylon cord from aluminium poles that were attached to major branches. They were stabilized using 2-cm-diameter rigid plastic tubing attached to adjacent small branches. This arrangement allowed the chambers to move freely with the branches of the tree during periods of high wind-speeds.

The chambers were arranged in pairs. Pure CO_2 was injected into the ventilation stream of one chamber of each pair at a flow rate of 0·6 L min^{-1} using a needle valve and flow meters. The pure CO_2 was piped to the ventilators in the canopy using 0·7 mm diameter plastic tubing. The other chamber of each pair received ambient air in the ventilation stream. The [CO_2] within the chambers was monitored weekly using a portable infrared gas analyser (CI-301PS; CID Inc., Vancouver, WA, USA) set to record [CO_2] every 5 s. The CO_2 concentrations in the ambient CO_2 chambers was approximately 360 μmol mol^{-1}, and ranged between 340 and 450 μmol mol^{-1} depending on the time of day and ambient weather conditions. The mean [CO_2] within the elevated CO_2 open-top chambers was calculated from 4200 observations of [CO_2], each of 5 s duration, over the experiment. The mean [CO_2] was

Figure 1. The open-top chambers used to enhance the concentration of CO_2 around branches of *Luehea seemannii*. Chambers were deployed and accessed by a construction crane in the forest canopy in Parque Natural Metropolitano, Republic of Panamá.

754 μmol mol^{-1}, and for 85% of the time it was between 600 and 900 μmol mol^{-1} (Fig. 2a). Excursions in the [CO_2] away from the mean were less than 1 min in duration (Fig. 2b). Daytime leaf temperatures were approximately 1 °C higher inside the chambers than the temperature of leaves outside the chambers, and night-time leaf temperatures were approximately 1 °C lower, because of the continuous ventilation stream.

Experimental design

On each of the five trees, three seventh-order branchlets, on the eastern side of the canopy, with similar exposure to light were selected. Two branchlets were placed in chambers, while the third was a control to assess the influence of the chambers. Thus there were three treatments: (1) with chamber at ambient [CO_2]; (2) with chamber at elevated[CO_2]; and (3) without chamber at ambient [CO_2].

Luehea seemannii is deciduous. It has an initial leaf flush at the beginning of the wet season (May–June) on wood formed the previous year, followed by a second leaf flush with the production of new wood (August–September) prior to flower and fruit production at the end of the wet season (November–December). It flowers and fruits in the dry season, and then loses all its foliage. In order to test the chambers, and to assess the durability of the equipment and the variability among trees and branches, an initial experiment, using three trees, was conducted from June 1995 to January 1996. In May 1996, prior to the initial leaf flush (first week in June), chambers were deployed in five trees. The chambers on one of the five trees were lost due to a severe wind storm in August 1996 and thus the data presented are for four trees only. The experiment continued for 39 weeks (until March 5).

The leaf area development during the experiment was monitored at approximately 2 week intervals by measuring leaf length of all leaves and converting this to leaf area using a relationship between leaf length and area. The presence and abundance of reproductive structures (flower buds and fruits) were also noted. Net photosynthesis and stomatal conductance of five leaves per branchlet were measured *in situ* between 0700 and 1230 h for one day each month of the experiment using a portable open gas exchange system (LI-COR 6400; LI-COR Inc., Lincoln, NB, USA). The environment within the leaf cuvette was controlled to be similar to ambient conditions. One leaf per

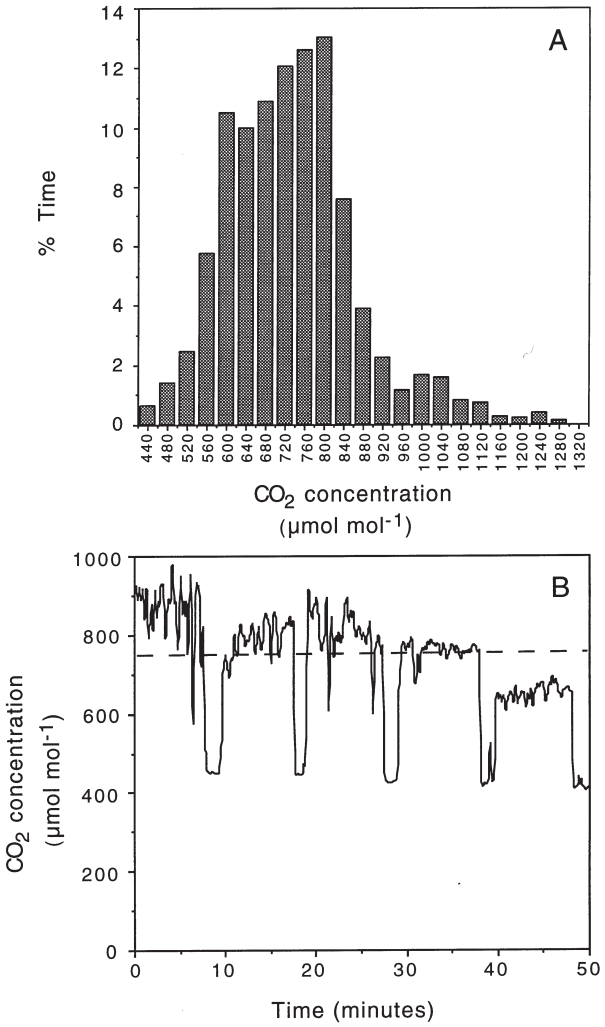

Figure 2. Frequency distribution of CO_2 concentration within open-top branch chambers obtained from multiple 5 s recordings taken once a week for the 39 weeks of the experiment (A), and variation in CO_2 concentration in the open-top chambers measured in the five elevated CO_2 chambers on one morning during the experiment (B). The time course shows consecutive measurements of the CO_2 concentration within each high CO_2 chamber. For approximately 3 min between each high CO_2 chamber the CO_2 concentration of the ambient air was recorded while the crane gondola was repositioned.

branchlet was harvested in the early morning and late evening once a month for carbohydrate analysis and to determine the specific leaf area ($cm^2\ g^{-1}$). The leaves were submerged in liquid N_2, freeze dried and stored at –20 °C for further analysis.

On 5 March 1997, all branches were harvested in the morning. The branchlets were divided into stems and leaves of either the first or second flush, and fruits. the stems were placed in a microwave oven on full power for 1 min in order to stop any enzymic activity and the leaf area was measured using a leaf area meter (LI-3100; LI-COR Inc.). All of the material was then placed in a drying oven at 60 °C for 2 weeks and weighed.

Total nonstructural carbohydrate concentrations of leaf and stem material were assessed by colorimetric assay of the soluble and insoluble carbohydrates from 0·1 g of dry tissue ground to a powder in a commercial coffee grinder. Soluble carbohydrates were extracted in 10 mL of boiling water for 10 min. The suspension was centrifuged for 10 min at 2500 r.p.m. and 0·5 mL of the supernatant was diluted to 10 mL for analysis of carbohydrates (see below). The pellet was resuspended in 5 mL of 1 M HCl and boiled for 1 h to hydrolyse the insoluble carbohydrates. After centrifugation for 10 min at 2500 r.p.m. the supernatant was made up to 10 mL with distilled water and 0·5 mL of this solution was diluted to 10 mL. Carbohydrates were assayed using the method described by Sturgeon (1990), with glucose (Sigma Chemical Co., St Louis, MO, USA) as a standard. The assay consists of rapidly injecting 2·5 mL of concentrated H_2SO_4 into a mixture of 0·5 mL of the leaf extract and 0·5 mL of a 5% phenol solution. After the solutions had attained room temperature their absorbance was measured at 490 nm in a spectrophotometer (model UV-2100 U; Shimadzu, Kyoto, Japan). At one time point (week 11) the leaf carbohydrates were also determined using the enzymatic procedure described in Lovelock *et al*. (1997). The simpler colorimetric assay was found to be insufficiently accurate to detect daily changes in carbohydrate concentrations and so, for measurement of the total nonstructural carbohydrates in leaf tissue, the measures for the morning and evening samples were pooled. The data presented are therefore the means of eight leaves per CO_2 treatment. For woody stem tissue, the total nonstructural carbohydrates were determined from five random sections of stem for each branchlet for both woody stems bearing the initial or the second leaf flush. Thus the values of total nonstructural carbohydrates for stems are the means of 20 measures.

Data analysis

Growth of leaf area and changes in specific leaf area and leaf carbohydrate concentrations over time were analysed using repeated measures analysis where treatment, either (1) with chamber at ambient [CO_2]; (2) with chamber at elevated [CO_2]; or (3) without chamber at ambient [CO_2], was considered a fixed effect. Because of the high variability among branches within treatments, the analysis of the effects of the treatments on both reproductive and final harvest data was by analysis of covariance (ANCOVA). Treatments were considered a fixed effect and the continuous variable (e.g. leaf area of second flush, or number of flower buds) was considered a random effect. Woody tissue carbohydrate concentrations were analysed by ANOVA. Here, the fixed effects were the treatments, and whether the woody stem tissue was associated with the first or second leaf flush.

RESULTS

Exposure to elevated [CO_2] during the growing season, or placement of branchlets in chambers did not influence the leaf area growth of branchlets (Fig. 3a, Table 1). Variability

among leaf area growth of branchlets was high. No differences were evident among the treatments in the growth of leaf area of either the first or second leaf flush, or the timing of leaf flushes (Fig. 3b). The ratio of leaf area to biomass (weight of leaves + woody tissue) of the second flush was also influenced by the treatments ($F_{2,10}$ = 8·104, P = 0·0119, Table 1). Exposure to elevated [CO_2] increased the ratio of leaf area to branchlet biomass (P = 0·0122).

Figure 4 shows net photosynthetic CO_2 exchange at three times (week 13, 23 and 37) during the experiment. The net CO_2 exchange was higher by approximately 30% in leaves growing under elevated [CO_2] compared with those growing under ambient [CO_2] levels (Fig. 4). Net photosynthesis of leaves was not influenced by the chambers. Stomatal conductance was generally lower in branchlets growing under elevated [CO_2], but there was considerable overlap in values between the elevated and ambient [CO_2] treatments (Fig. 4).

Over the growing season the specific leaf area (SLA) of leaves declined by 30–40% (Fig. 5a). The decline was particularly strong in the first 10 weeks of the experiment. There was no difference in SLA between chambered and unchambered branchlets. The SLA of branchlets grown under elevated CO_2 was generally similar to that of those grown under ambient [CO_2], except at week 7 when the SLA of leaves grown under elevated [CO_2] was lower (110 $cm^2\ g^{-1}$ compared with 120 $cm^2\ g^{-1}$). The total nonstructural carbohydrates within the leaves varied over the growing season, but there was no significant effect of elevated [CO_2] or chambers (Fig. 5b). There was a decline in total nonstructural carbohydrate concentrations within leaves at week 10 which preceded the start of the second leaf flush.

The production of flower buds by the branchlets was highly correlated with the leaf area produced in the second flush of leaves (Fig. 6a, r^2 = 0·73), but only weakly correlated with the area produced in the first flush of leaves and the total leaf area (r^2 = 0·11, data not shown). There was no influence of growth in chambers or under elevated CO_2 concentrations on this relationship ($F_{2,7}$ 1·786, P = 0·237). Flower bud production was highly correlated with fruit production (Fig. 6b, r^2 = 0·85). The slope of this line was 0·44 ± 0·06, which is the proportion of flowers successfully fertilized that develop into fruit. The number of fruit in the elevated [CO_2] chambers appeared to be less than in ambient [CO_2] treatments with similar numbers of flower buds initiated, however, this trend was not significant. The weight of individual fruits tended to be lower under elevated CO_2 levels (Table 1, $F_{2,10}$ = 2·63, P = 0·1323). There was no effect of chambers on mean individual fruit weight.

At the end of the experiment, the total nonstructural carbohydrate concentration in woody stem tissue bearing the first flush of leaves (i.e. stems formed the previous year) did not differ among treatments (Fig. 7). In the woody tissue bearing the second flush of leaves (the woody tissue grown under the experimental treatments) wood under elevated [CO_2] had approximately 20% more total nonstructural carbohydrate than the ambient [CO_2]-chambered branchlets and unchambered control branchlets.

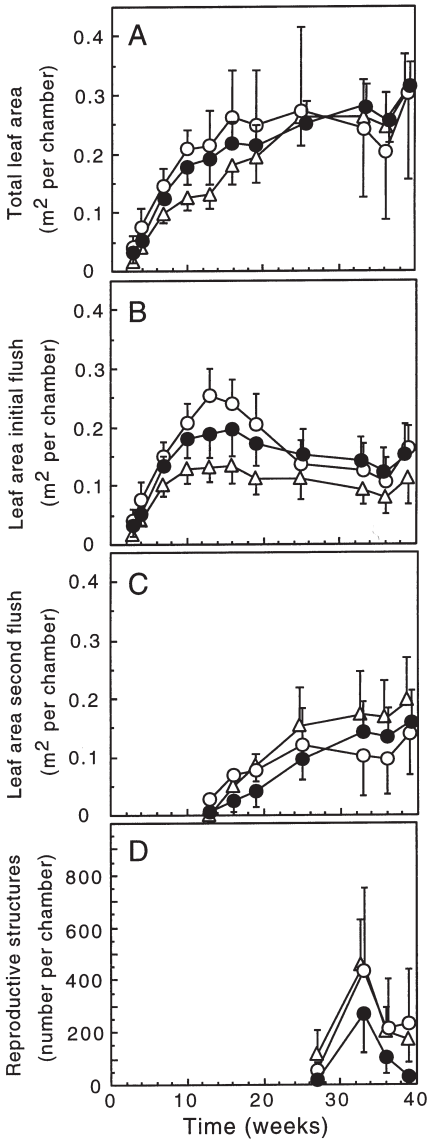

Figure 3. Total leaf area growth (A), leaf area growth of the component first (B) and second flush of leaves (C), and the number of reproductive structures (flower buds, flowers or fruits) (D) produced over time in branchlets of four mature trees of *Luehea seemannii* in 1996. Branchlets were enclosed within chambers (circles) or without chambers (triangles), and were exposed to elevated levels of CO_2 (closed symbols), or ambient levels of CO_2 (open symbols). Values are the means (± standard errors) of four branchlets. For clarity, error bars are shown on only one side of the mean value.

DISCUSSION

Branches as units of study

Using branchlets to infer how whole trees will respond to elevated [CO_2] must be approached with caution because of the difficulties associated with scaling-up from branches to whole trees. The appropriateness of using branches is dependent upon the degree of branch autonomy or the physiological separation of one branch from another (Barton *et al*. 1993). Recently in experiments where part of

Table 1. Biomass and biomass allocation patterns of canopy branchlets of *Luehea seemannii* at final harvest (after 39 weeks of growth). Branchlets were either enclosed in open-top chambers or unchambered controls. Those enclosed in open-top chambers were exposed to ambient or elevated levels of CO_2. Leaf area ratio is the proportion of leaf area to total biomass within the chambers. Specific leaf area is the area per unit biomass of leaves. Values are the means (± standard errors) of four branchlets

Biomass allocation	No chamber	Chamber		*P*-value (Main effect of CO_2)
		Ambient CO_2	Elevated CO_2	
Total branchlet				
Leaf area (m^2)	0·309 ± 0·062	0·304 ± 0·129	0·312 ± 0·045	NS
Leaf dry weight (g)	39·9 ± 8·6	41·3 ± 22·7	40·1 ± 6·4	NS
Wood dry weight (g)	40·0 ± 10·1	35·9 ± 19·2	27·9 ± 5·9	NS
Leaf area ratio ($cm^2\ g^{-1}$)	39·7 ± 1·7	36·5 ± 3·2	46·6 ± 3·1	NS
Specific leaf area ($cm^2\ g^{-1}$)	78·6 ± 3·0	79·6 ± 4·9	78·4 ± 2·2	NS
First flush				
Leaf area (m^2)	0·112 ± 0·045	0·164 ± 0·038	0·153 ± 0·052	NS
Leaf dry weight (g)	13·4 ± 5·4	21·0 ± 6·3	19·1 ± 6·3	NS
Wood dry weight (g)	22·1 ± 6·3	22·3 ± 8·6	18·8 ± 2·8	NS
Leaf area ratio ($cm^2\ g^{-1}$)	29·4 ± 5·3	35·0 ± 4·0	38·1 ± 6·8	NS
Specific leaf area ($cm^2\ g^{-1}$)	83·0 ± 1·9	81·7 ± 5·3	79·0 ± 2·2	NS
Second flush				
Leaf area (m^2)	0·197 ± 0·072	0·105 ± 0·088	0·158 ± 0·055	NS
Leaf dry weight (g)	26·6 ± 9·8	15·2 ± 13·0	21·0 ± 7·7	NS
Wood dry weight (g)	18·0 ± 6·1	13·6 ± 10·9	9·0 ± 4·3	NS
Leaf area ratio ($cm^2\ g^{-1}$)	45·7 ± 3·2	37·7 ± 2·1	55·2 ± 3·2	0·0119
Specific leaf area ($cm^2\ g^{-1}$)	75·6 ± 1·6	72·8 ± 2·0	76·8 ± 2·7	NS
Reproduction				
Mean number of fruit	168 ± 81	173 ± 160	32 ± 12	NS
Mean fruit weight (g)	27·6 ± 12·4	30·5 ± 27·6	4·6 ± 1·7	NS
Mean weight/fruit (g)	0·19 ± 0·03	0·21 ± 0·01	0·13 ± 0·03	0·1323

a canopy was exposed to environmental conditions different to the rest of the canopy, changes in the physiology of the unexposed portions of the canopy were observed (Whitehead *et al*. 1996), indicating a high degree of physiological integration among branches of trees. Despite this problem, many studies have demonstrated a high degree of branch autonomy (Sprugel *et al*. 1991).

In *L. seemannii*, the evidence for some degree of branch autonomy, with respect to carbon allocation, comes from the correlation between leaf area and reproductive effort per branchlet (Fig. 6a). Correlation between the number of leaves and the number of flower buds per branch have also been observed in clonal woody species (Doust & Doust 1988), and in palms (Cunningham 1997). Thus the local resources within branches appear to be more important than resources within trunks to the number of flowers produced on a branch (Doust & Doust 1988; Obeso 1997).

The accumulation of nonstructural carbohydrate accumulation in stem tissue of the current years wood under elevated [CO_2] (Fig. 7) also suggests that branches are relatively autonomous. That is, at least some of the extra CO_2 fixed in photosynthesis under elevated [CO_2] (Fig. 4) was retained in the branchlet and not exported to the rest of the tree. Moreover Newell, Mulkey & Wright (1997) observed that annual variation in the concentrations of total nonstructural carbohydrates in *L. seemannii* was greatest in the small twigs of branchlets compared with the large branches and trunks. In the small branchlets the concentrations of total nonstructural carbohydrates declined rapidly as the leaves flushed early in the wet season and then increased throughout the growing season. They concluded that total nonstructural carbohydrates were stored in the terminal branchlets to fuel leaf growth in the following year. In our experiment leaf carbohydrates were reduced at the onset of the second leaf flush (Fig. 5b), suggesting new leaf growth was a substantial sink for carbohydrates within the branch. Additionally, specific leaf area of leaves decreased dramatically with the onset of the second leaf flush (Fig. 5a). Kitajima, Mulkey & Wright (1997) have shown that leaves produced in the second flush have higher photosynthetic rates than those produced in the first flush of leaves. The correlation between the leaf area of the second leaf flush and the number of flower buds produced suggests that the second flush of leaves is dedicated to the reproductive output of that branch. Thus, evidence for branchlet autonomy, with respect to carbon, in mature individuals of *L. seemannii* appears to be reasonably strong.

Effects of elevated CO_2 concentrations

Net photosynthesis was increased under elevated [CO_2] (Fig. 4), and this effect was not reduced over time.

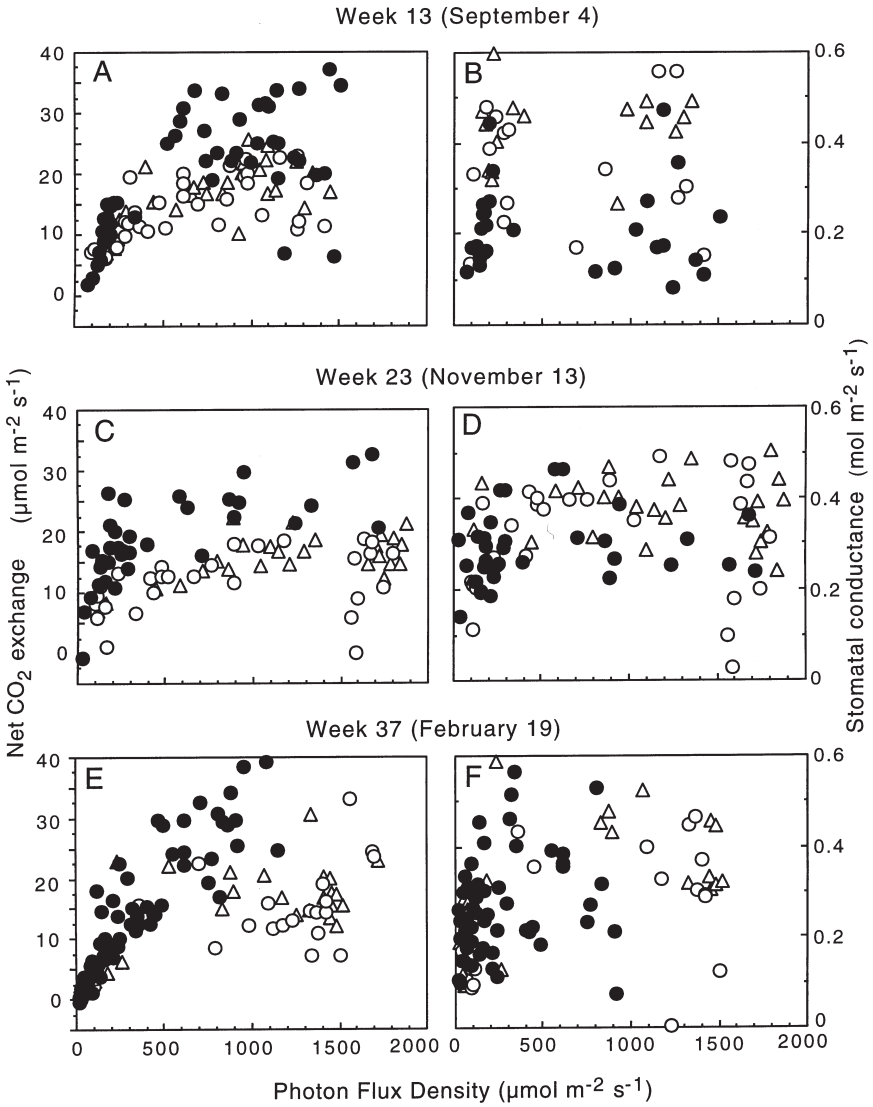

Figure 4. Net photosynthetic CO_2 fixation (A, C and E) and stomatal conductance (B, D and F) of leaves of branchlets of mature trees of *Luehea seemannii* at three times during the growing season (week 13, 23 and 37 after the intiation of leaves). Values at week 13 (A and B) are for the first flush of leaves while at weeks 23 (C and D) and 37 (E and F) observations are for both the first and second flush of leaves. Key to the symbols are the same as Figure 3.

Sustained higher rates of photosynthesis under elevated [CO_2] have been observed in many plant species (reviewed by Drake *et al*. 1997) and in studies using branch chambers (Liu & Teskey 1995; Teskey 1997; but see Marek & Kalina 1996). Despite increases in photosynthetic carbon gain under elevated [CO_2] there were no detectable increases in leaf growth or reproductive effort over the duration of the experiment (Fig. 3, Table 1), although higher levels of carbohydrates were stored in woody tissue under elevated [CO_2] (Fig. 7). In addition, an unknown proportion of the fixed CO_2 would have been respired and transported to the rest of the tree (large supporting branches, trunk and roots). Enhanced storage of carbohydrates in terminal branchlets and elsewhere within the tree may lead to increased growth in the year following the elevated [CO_2] treatment. The small size of the branch chambers did not allow the continuation of the experiment into the following year, and therefore the observed lack of enhanced growth under elevated [CO_2] in branchlets during this study should be viewed as preliminary. However, in an experiment within 2 km of the branch chamber study, where communities of saplings of tropical tree species were exposed to elevated [CO_2] in open-top chambers for 6 months, saplings of *L. seemannii* showed no enhancements in growth rates (Lovelock *et al*. 1998).

The lack of growth enhancement in both branchlets and saplings of *L. seemannii* under elevated [CO_2], may suggest that some other resource, for example the availability

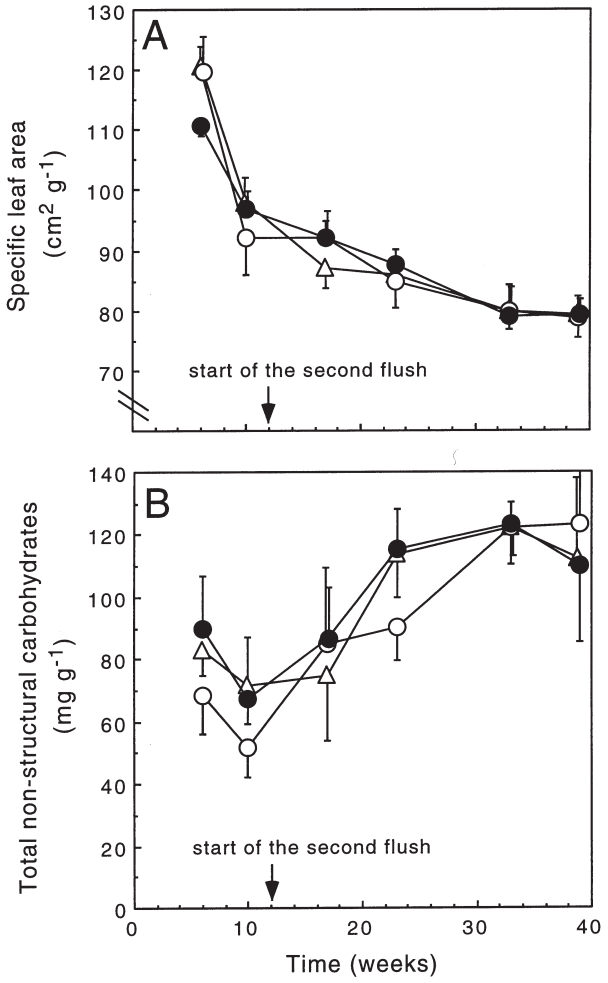

Figure 5. Variation in specific leaf area (cm^2 g^{-1}) (A) and the concentration of total nonstructural carbohydrates within leaves (B) of branchlets of mature trees of *Luehea seemannii* measured throughout the growing season. Values before week 16 are for the first flush of leaves, while those after week 16 are a mixture of first and second flush leaves. Key to the symbols are the same as Figure 3. For clarity, error bars are shown on only one side of the mean value.

of mineral nutrients, rather than the supply of carbon is limiting the growth and reproduction in *L. seemannii*. Enhancements in plant growth under elevated [CO_2] have sometimes been observed to be small or negligible (e.g. Ceulemans & Mousseau 1994; Garbutt & Bazzaz 1984; Schaäpi & Körner 1996), and have been largely attributed to limitation of other resources, mainly low nutrient concentrations, although water availability may also be important (Peñuelas, Biel & Estiarte 1995). In the canopy of tall forest trees, water availability may limit growth due to the high resistance to flow through the trunk and branches (Gower, McMurtrie & Murty 1996). Soil nutrient concentrations, particularly phosphorus, are also low in tropical forest soils (Vitousek & Sanford 1986; Yavitt, Wieder & Wright 1993) and could be responsible for the lack of growth enhancements observed in branchlets (this study), and the study of communities of tropical tree saplings (Lovelock *et al*. 1998).

Although there were no detectable increases in leaf growth under elevated CO_2 concentrations, the ratio of leaf area to total biomass of the second leaf flush was increased when branches were grown under elevated [CO_2] (Table 1). This indicates that proportionally more biomass was allocated to leaf area than supporting stem tissue during the second leaf flush under elevated [CO_2]. In contrast, in a study of the response of communities of tropical tree species to elevated [CO_2], the saplings of *L. seemannii* showed a decrease in the proportion of leaf area to total plant biomass (Lovelock *et al*. 1998). Differences in responses to elevated [CO_2] among saplings and branchlets of the same species could be due to changes in source and sink strength during plant development.

Stomatal conductance was decreased under elevated [CO_2] at photon flux densities (PFDs) saturating for photosynthesis (greater than 600 mmol m^{-2} s^{-1}, Fig. 4). Decreased stomatal conductance was also observed in saplings of *L. seemannii* grown under elevated [CO_2] (Lovelock *et al*. 1998). Reductions in stomatal conductance may lead to decreases in water loss from tropical canopies due to reduced transpiration, providing leaf area within the canopy remains constant (Tyree & Alexander 1993; Cardon, Berry & Woodrow 1995). However, recent work in tropical forest canopies reveals stomatal conductance correlates poorly with canopy water loss, because of the strong effects of the canopy boundary layer on canopy water loss (Meinzer & Goldstein 1996). Therefore, although reductions in stomatal conductance were measured in *L. seemannii* under elevated [CO_2], there may also be changes in canopy leaf area. Whether this will result in reduced water loss from forest canopies in the future remains uncertain.

Changes in the reproductive effort under elevated [CO_2] have been assessed in herbaceous species (Garbutt & Bazzaz 1984; Garbutt, Williams & Bazzaz 1990; Farnsworth & Bazzaz 1995; Peñuelas, Biel & Estiarte 1995) but, to the best of our knowledge, never before examined in woody plant species. In the present study, reproductive effort, in terms of the number of flower buds initiated and the number of fruits produced, was not altered by elevated [CO_2] (Table 1), but there was a tendency ($P = 0{\cdot}132$) for individual fruit weight of *L. seemannii* to be reduced (Table 1). Under elevated [CO_2] flower and fruit production measured in herbaceous species is highly variable. In pepper, the fruit and flower production increased under elevated [CO_2] when there was sufficient water (Peñuelas *et al*. 1995). In natural grassland more seeds per plant were observed in plants growing under elevated [CO_2] (Jackson *et al*. 1994), while in the study of *Abutilon* total seed number decreased under elevated [CO_2] relative to controls (Garbutt & Bazzaz 1984). In a detailed study of nine nonagricultural species elevated [CO_2] enhanced fruit production in two of the nine species, reduced fruit numbers in two of the nine species

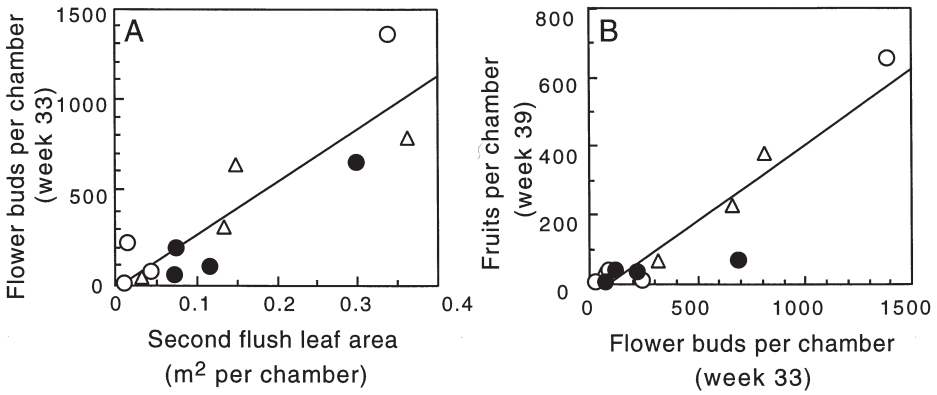

Figure 6. Correlation between the maximum number of flower buds (at week 33) and the leaf area produced per chamber in the second leaf flush (A), and between the number of fruits produced and the number of flower buds produced per chamber (B) of mature trees of *Luehea seemannii*. Key to the symbols are the same as Figure 3.

and showed no change in the remaining five species (Farnsworth & Bazzaz 1995). Changes in reproductive output under elevated [CO_2] varied independently of the response of vegetative growth. Moreover, the weight of seeds produced and germination could be either positively or negatively affected by elevated [CO_2], but these variables did not correlate with each other or with the number of seeds produced. Thus the tendency towards lower fruit weight and increased investment in leaf area in *L. seemannii* under elevated [CO_2] may not indicate that this species will have reduced fitness under these conditions. The range of responses to elevated [CO_2] observed in herbaceous species suggest the response of reproduction to such levels in woody, long-lived species is likely to be complex and warrants further investigation.

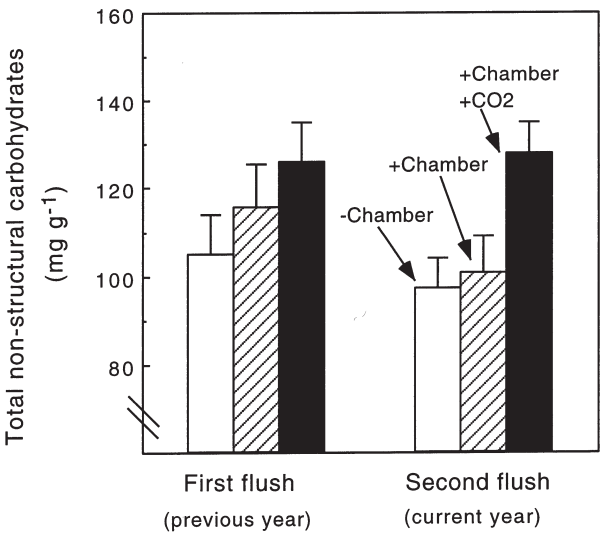

Figure 7. Total nonstructural carbohydrates in woody tissue of stems of branchlets of mature trees of *Luehea seemannii*. Stems of branchlets were either produced during the treatments (current year, bearing the second leaf flush) or the previous year (previous year, bearing the first leaf flush). Branchlets were enclosed within chambers at ambient levels of CO_2 (with chambers, hatched bars) or without chambers at ambient CO_2 (without chambers, open bars), and exposed to elevated levels of CO_2 within chambers (with chamber + CO_2, solid bars). Values are the means (± standard errors) of five replicate samples from four branchlets (n = 20).

The results of this study suggest the implications of elevated [CO_2] for tropical forests may not centre around increases in growth of forest trees, although we cannot exclude the possibility that root growth was enhanced under these conditions, nor that growth could have been enhanced in the year following the elevated [CO_2] treatment due to increases in carbohyrate concentrations in woody tissue. Alterations in carbon allocated to leaf area under elevated [CO_2] may influence seedling recruitment (Bazzaz & Miao 1993) and forest water relations (Tyree & Alexander 1993; Cardon *et al*. 1995). Additionally, changes in the quantity and quality of leaf litter and the chemical composition of woody debris, are likely to be important because they can potentially impact on nutrient availability in forest soils (Comins & McMurtrie 1993; Diaz *et al*. 1993; Zak *et al*. 1993; Berntson & Bazzaz 1996; Cardon 1996; Hungate *et al*. 1997), and on organisms at other trophic levels (Bazzaz 1990).

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