

# The impact of climate change on the growth of tropical agroforestry tree seedlings

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**Abstract** Several studies have been conducted on the response of crops to greater concentrations of atmospheric CO<sub>2</sub> (CO<sub>2</sub> fertilization) as a result of climate change, but only few studies have evaluated this effect on multipurpose agroforestry tree species in tropical environments. The objectives of this study were to quantify differences in growth parameters and in leaf carbon (C) and nitrogen (N) concentrations of *Cedrela odorata* L. and *Gliricidia sepium* (Jacq.) Walp. seedlings under current ambient temperature (32°C daytime, 22°C night time) and CO<sub>2</sub> (360 ppm) (AMB); CO<sub>2</sub> fertilization (800 ppm, 32°C daytime, 22°C night time) (fCO<sub>2</sub>); elevated ambient temperature (360 ppm, 34°C daytime, 25°C night time) (TEMP); and a combination of elevated temperature (32°C daytime, 22°C night time) and CO<sub>2</sub> fertilization (800 ppm) (TEMPxfCO<sub>2</sub>). Results showed significant differences ( $P < 0.05$ ) in seedling growth parameters (seedling height, number of stem leaves, leaf area ratio, shoot and root biomass, and shoot/root ratio) between treatments for both tree species. The greatest increases in growth parameters occurred in the TEMP and TEMPxfCO<sub>2</sub> treatments compared to the AMB treatment for both tree species. However, growth

parameters were significantly lower ( $P < 0.05$ ) in the fCO<sub>2</sub> treatment compared to that of the AMB treatment. Leaf N concentration was 1.1 to 2.1 times lower ( $P < 0.05$ ) in all treatments when compared to current ambient conditions (AMB) in both tree species, but no significant changes in leaf C concentrations were observed. Results from our study suggested that fCO<sub>2</sub> had the greatest negative impact on tree growth parameters, and leaf N concentrations were affected negatively in all treatments compared to current ambient conditions. It is expected that such changes in growth parameters and plant N content may impact the long-term cycling of nutrients in agroforestry systems.

**Keywords** *Cedrela odorata* · CO<sub>2</sub> fertilization · Controlled environmental growth chambers · *Gliricidia sepium* · Global warming · Leaf nutrient concentrations · Seedling growth parameters

## Introduction

The effects of climate change as a result of rising ambient temperatures and atmospheric CO<sub>2</sub> concentrations are currently observed in a range of ecosystems and species on a global level (Warren et al. 2011), including tropical central America (Walther et al. 2002) where temperatures are predicted to increase between 1.5 and 5.6°C compared to current conditions (IPCC 2007). The majority (95%) of

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plants including agricultural crops, fruit and nut bearing trees, timber trees and service trees such as those used in agroforestry practices are characterized by the C<sub>3</sub> photosynthesis pathway (Ainsworth and Long 2005). However, the ecological competitive advantage of C<sub>3</sub> plant species may be negatively affected under greater than current ambient temperatures and atmospheric CO<sub>2</sub> concentrations (Lee 2011). C<sub>3</sub> species may be negatively affected under elevated temperatures but may respond positively under elevated CO<sub>2</sub>. For example, Lee (2011) found that higher temperatures may inhibited specific plant growth stages, including flowering and fertilization, and lead to greater tree mortality (Lee 2011).

In tropical environments, remarkably few studies have been conducted on the response of C<sub>3</sub> agricultural crops and tropical forest trees to elevated levels of atmospheric CO<sub>2</sub> concentrations, commonly referred to as CO<sub>2</sub> fertilization or enrichment (Lovelock et al. 1999; Keller and Lerdau 1999; Chambers and Silver 2004; Lloyd and Farquhar 2008). In Panama, *Luehea seemanii* Planch. and Triana branchlets had a greater rate of photosynthesis as a result of CO<sub>2</sub> fertilization (Lovelock et al. 1999). Lovelock et al. (1999) suggested that enhanced rates of photosynthesis may lead to a greater tree growth rate. However, Chambers and Silver (2004) suggested that a greater rate of photosynthesis among other physiological responses due to CO<sub>2</sub> fertilization in C<sub>3</sub> trees may be dependent on changes in ambient temperature, and could also be species specific. For example, Clark (2004) and Cramer et al. (2004) found that CO<sub>2</sub> fertilization reduced tree growth rates and their potential to sequester carbon (C) in two tropical forest tree species [*Beilschmiedia pendula* (Sw.) Hemsl., *Tachigalia versicolor* Standl. and L.O. Wms.]. Cox et al. (2000) noted that it is important to understand the combined effects of higher temperatures and CO<sub>2</sub> fertilization on plant productivity because of their collective occurrence as a result of global warming. As such, changes in ambient temperatures and atmospheric CO<sub>2</sub> concentrations may result in a lower tree productivity and C sequestration potential, and altered tree growth patterns in some species (Bradley and Pregitzer 2007). In agroforestry systems, this may also negatively affect timber production and wood quality (Zieche and Overdieck 2004), impact fruit and nut yield and quality, and influence productivity and quality of livestock fodder

and pruning biomass commonly used as green manure or mulch to maintain soil fertility.

To date, only a few studies have evaluated the response of multipurpose agroforestry trees to CO<sub>2</sub> fertilization in temperate (Gebauer et al. 1996; Tupker et al. 2003; Scarascia-Mungozza et al. 2005) and tropical (Tissue et al. 1997; Carswell et al. 2000) biomes. Uncertainty still remains on how an interactive effect of increased temperature and CO<sub>2</sub> fertilization will impact tree ecophysiology in tropical environments (Chambers and Silver 2004; Clark 2004); and to date only a few studies have investigated this concept in tropical forest ecosystems (Lloyd and Farquhar 2008; Wertin et al. 2009; Lukac et al. 2010; Lee 2011). However, no information is currently available on the response of tropical multipurpose agroforestry trees to a combined effect of enhanced ambient temperature and CO<sub>2</sub> fertilization.

This study quantified the effect of CO<sub>2</sub> fertilization, enhanced ambient temperature, and their combined effect on two commonly used multipurpose agroforestry tree species in Costa Rica. It was hypothesized that CO<sub>2</sub> fertilization, and greater ambient temperature or the combined effect would negatively affect tree seedling growth parameters compared current ambient conditions. The objectives of this study were to quantify differences in growth parameters and changes in leaf C and nitrogen (N) concentrations of *Cedrela odorata* L. and *Gliricidia sepium* (Jacq.) Walp. tree seedlings under current ambient temperature and CO<sub>2</sub> conditions, under CO<sub>2</sub> fertilization, elevated ambient temperatures, and a combination of elevated temperature and CO<sub>2</sub> fertilization.

## Materials and methods

### Experimental design

Seeds of *C. odorata* and *G. sepium* were obtained from the tree seed bank at the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), located in Turrialba, Costa Rica in August 2008. The experiment was conducted at the University of Waterloo, Canada and the experimental design was a complete randomized design (CRD) with four treatments per tree species and each treatment was

replicated eight times. The treatments used were (1) experimental control using current ambient conditions (AMB) at a CO<sub>2</sub> concentration of 360 ppm, a mean day time temperature of 32°C and a mean night time temperature of 22°C; (2) CO<sub>2</sub> fertilization (fCO<sub>2</sub>) using a CO<sub>2</sub> concentration of 800 ppm with mean day and night time temperatures of 32 and 22°C; (3) elevated temperature (TEMP) at mean day and night time temperatures of 34 and 25°C, and at a CO<sub>2</sub> concentration of 360 ppm; and (4) a combination of elevated temperature and CO<sub>2</sub> (TEMPx<sub>f</sub>CO<sub>2</sub>) with mean day and night time temperatures of 34 and 25°C and a CO<sub>2</sub> concentration of 800 ppm. Relative humidity was 80% during the day and 90% at night for all treatments throughout the experiment. The different mean day and night time temperatures and CO<sub>2</sub> concentrations were based on the predictions of the UK Hadley Centre Global Climate Model (A2 scenario) predicted for the year 2100 in central America, including Costa Rica (IPCC 2007). Climate data from Puntarenas, Costa Rica was used to establish ambient day and night time temperatures, and provided a baseline for establishing predicted increases in temperature under the A2 scenario for the TEMP and TEMPx<sub>f</sub>CO<sub>2</sub> treatments (IPCC 2007).

#### Tree seedling germination and growth

Prior to initiating the experiment, and after transportation to Canada, the tree seedlings were germinated under controlled environmental conditions using the same germination procedure for all treatments. Seeds were submerged in water at room temperature (21°C) and kept in darkness for 12 (*C. odorata*) and 24 h (*G. sepium*), and subsequently covered in moist sand and kept in darkness.

The germinated seeds were planted in large pots (20 cm diameter, 14 cm height) using a general purpose soil (Premier Horticulture, Pro-mix Bx Mycorise Pro, Canada) with mycorrhizal inoculum (*Glomus intraradices*). The soil was derived from Canadian sphagnum peat moss (75–85%/volume), horticultural grade perlite and vermiculite and dolomitic and calcitic limestone. The pots with the germinated seeds were placed into controlled environmental growth chambers (Conviron PGR-15, Controlled Environments Inc., Winnipeg, Manitoba) to determine their response to the different treatments (AMB, fCO<sub>2</sub>, TEMP, and TEMPx<sub>f</sub>CO<sub>2</sub>) over a

35-day period. Light intensity, temperature, humidity and CO<sub>2</sub> were monitored hourly, and environmental conditions were assumed to be identical between treatments and chambers except for differences in atmospheric CO<sub>2</sub> concentrations and temperature for the actual treatments (fCO<sub>2</sub>, TEMP and TEMPx<sub>f</sub>CO<sub>2</sub>).

The seedlings were watered every second day, with each tree receiving 120 ml of ultra-pure water. On a weekly basis, the tree order within and between chambers was rotated randomly to avoid systematic bias due to slight differences between the chambers (Thomas and Strain 2000). As a nutrient supplement to the potting soil, a general NPK (10-15-10) fertilizer (Schultz Liquid Plant Food, Spectrum Brands, Madison, WI) was added to the 120 ml of pure water on day 21 using a dilution of four drops for every 1,000 ml of ultra-pure water.

Quantification of tree seedling growth, and Carbon and Nitrogen concentrations

Observations on tree height (cm) and leaf number were quantified every second day over the entire 35 days of growth. Leaf surface area for each tree was measured on day 34, one day prior to destructive sampling (Beerling and Fry 1990). Leaf surface area was quantified by tracing each leaf onto grid paper, and subsequently cutting out the shapes of the individual leaves and analyzing these with the LI-COR (LI-3000, Lincoln, Nebraska).

Trees were removed from their potting medium after 35 days of growth and separated into above- and below-ground components. Soil was carefully removed from seedling roots (Oelbermann et al. 2005), and both shoots and roots were weighed (wet weight). Subsequently, seedling shoot and root components were placed in an oven for 48 h at 72°C and weighed to determine their dry weight and to quantify the shoot/root ratio (S/R). The leaf area ratio [(LAR (cm<sup>2</sup>/g)] was determined with a LI-COR LI-3000. The root mass fraction (RMF) was quantified by dividing the root biomass into the total seedling biomass (Raizada et al. 2009). The dried shoots from each tree species within each treatment were ground using a Kinematica Polymix plant grinder (Px-MFC 90D, Lucerne, Switzerland) using a 2 mm sieve and subsequently ground in a Retsch Ball Mill (MM-200, Haan, Germany) and prepared for C and N analysis

on a Costec Elemental Analyzer (ECS-1410, Cernusco, Italy).

### Statistical analysis

All data were examined for homogeneity of variance using Levene's Test and normal distribution was determined with the Kolmogorov–Smirnov (K–S) Test and the Shapiro-Wilks Test. If the data did not meet Levene's Test it was natural log transformed (Steel et al. 1997). For all statistical analyses, the threshold probability level for determining significant differences was  $P < 0.05$ . Data for the growth parameters and leaf nutrient content was evaluated using the general linear model (ANOVA) in SPSS (SPSS Science Inc. 2009). Significant differences were evaluated using Tukey's least significant difference multiple comparison test (Steel et al. 1997). Prior to statistical analyses, data for tree height was corrected by subtracting the first day of measurement from all of the subsequent days of measurement in order to quantify the change in growth over the 5 week period.

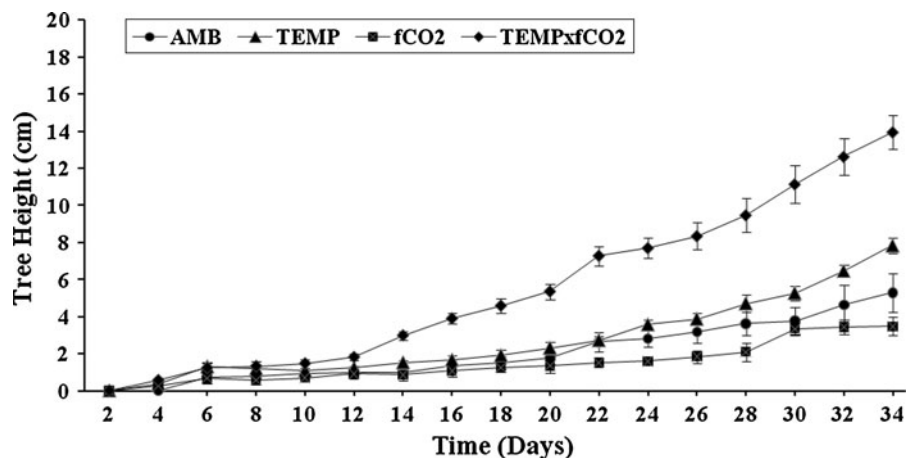
## Results

### Tree seedling growth

Both tree species showed a steady increase in seedling growth over the 35-day growing period in

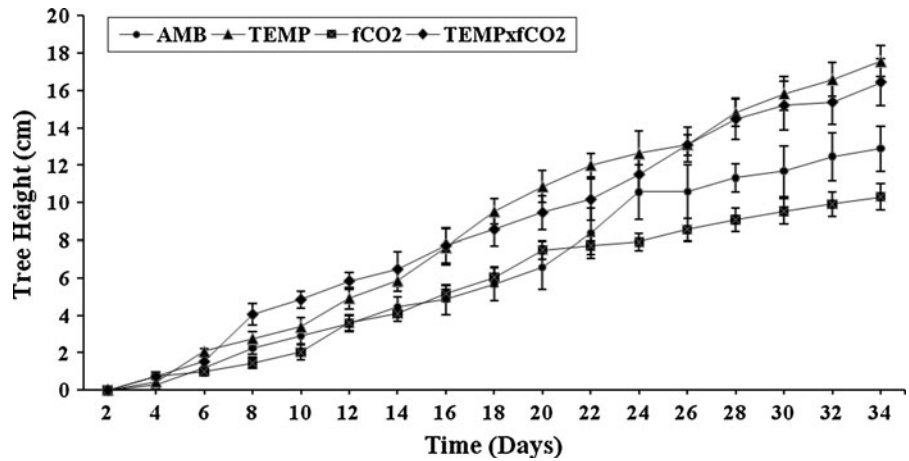
all treatments. The final tree seedling heights were significantly different between treatments for *C. odorata* (Fig. 1), and followed a sequence of  $TEMP_{x_fCO_2} > TEMP > AMB > fCO_2$ . The  $TEMP_{x_fCO_2}$  and TEMP treatments had a 58 and 26% greater height compared to the AMB treatment, whereas in the  $fCO_2$  treatment, *C. odorata* tree height was 43% lower compared to that of the AMB treatment. Differences in tree height for *G. sepium* seedlings were also significant and followed a sequence of  $TEMP \approx TEMP_{x_fCO_2} > AMB > fCO_2$  (Fig. 2). Differences in seedling height at the end of the 35 days were less pronounced compared to that of *C. odorata*. For example,  $TEMP_{x_fCO_2}$  and TEMP treatments showed a 21 and 22% greater seedling height compared to the AMB treatment whereas the  $fCO_2$  treatment had a reduction in height by 21% compared to the AMB treatment.

Other growth parameters, including number of leaves, shoot and root biomass, S/R and RMF were significantly different between treatments for *C. odorata* (Table 1). Shoot biomass was 58 and 83% greater for the TEMP and  $TEMP_{x_fCO_2}$  treatments respectively, whereas shoot biomass in the  $fCO_2$  treatment was 40% lower than that of the AMB treatment. Root biomass was 55% greater in the  $TEMP_{x_fCO_2}$  treatment compared to the AMB treatment whereas the TEMP and  $fCO_2$  treatments were 9 and 80% lower than the AMB treatment. The number of *C. odorata* leaves was lower by 54 to 24% in the treatments compared to the AMB control, however



**Fig. 1** Mean *C. odorata* height (cm) under AMB,  $fCO_2$ , TEMP, and  $TEMP_{x_fCO_2}$  over a 5 week growing period under controlled environmental conditions ( $n = 8$ )

**Fig. 2** Mean *G. sepium* height (cm) under AMB,  $f\text{CO}_2$ , TEMP, and  $\text{TEMP} \times f\text{CO}_2$  over a 5 week growing period under controlled environmental conditions ( $n = 8$ )



**Table 1** Growth parameters under current ambient (AMB),  $\text{CO}_2$  fertilization ( $f\text{CO}_2$ ), elevated temperature (TEMP), and combined elevated temperature and  $\text{CO}_2$  fertilization ( $\text{TEMP} \times f\text{CO}_2$ ) for *C. odorata*

	Number of leaves	LAR ( $\text{cm}^2/\text{g}$ )	Shoot biomass (g)	Root biomass (g)	S/R ratio	RMF	Water content (%)
AMB	15.14 (1.42) <sup>a</sup>	153.27 (12.98) <sup>a</sup>	0.15 (0.07) <sup>a</sup>	0.35 (0.08) <sup>a</sup>	0.43 (0.04) <sup>a</sup>	0.33 (0.02) <sup>a</sup>	86.6 (0.7) <sup>a</sup>
$f\text{CO}_2$	7.00 (1.88) <sup>b</sup>	121.05 (17.17) <sup>a</sup>	0.09 (0.01) <sup>b</sup>	0.07 (0.01) <sup>b</sup>	1.29 (0.20) <sup>b</sup>	0.49 (0.01) <sup>b</sup>	86.4 (0.7) <sup>a</sup>
TEMP	11.50 (1.33) <sup>a</sup>	143.64 (12.14) <sup>a</sup>	0.35 (0.73) <sup>c</sup>	0.32 (0.08) <sup>a</sup>	0.97 (0.07) <sup>c</sup>	0.54 (0.03) <sup>c</sup>	85.0 (0.9) <sup>a</sup>
$\text{TEMP} \times f\text{CO}_2$	14.86 (1.42) <sup>a</sup>	112.01 (12.98) <sup>a</sup>	0.87 (0.08) <sup>d</sup>	0.78 (0.09) <sup>c</sup>	1.12 (0.09) <sup>b</sup>	0.53 (0.02) <sup>c</sup>	85.1 (0.7) <sup>a</sup>

Standard errors are given in parentheses ( $n = 8$ )

Values followed by the same lowercase letter, comparing between treatments, for each *C. odorata* growth parameter are not significantly different at  $P > 0.05$

S/R was the greatest in the TEMP and  $f\text{CO}_2$  treatments compared to the control (AMB). The RMF was significantly different between treatments with the greatest fraction of the root mass occurring in the  $\text{TEMP} \times f\text{CO}_2$  and  $f\text{CO}_2$  treatments. However, the LAR was 6% (TEMP), 21% ( $f\text{CO}_2$ ), and 27% ( $\text{TEMP} \times f\text{CO}_2$ ) lower compared to the AMB treatment.

For *G. sepium*, tree seedling growth parameters were significantly different between treatments and showed a similar pattern between the number of leaves and leaf area ratio (Table 2). For example, the greatest number of leaves and the highest LAR occurred in the AMB treatment followed by the TEMP,  $f\text{CO}_2$  and  $\text{TEMP} \times f\text{CO}_2$  treatments (Table 2). Shoot biomass accumulation was 55 and 36% greater in the TEMP and  $\text{TEMP} \times f\text{CO}_2$  treatments compared to the AMB treatment, whereas the  $f\text{CO}_2$  had a 43% lower biomass accumulation compared to that of the AMB treatment. Comparatively, root biomass allocation for *G. sepium* tree seedlings was 30% greater

in the TEMP treatment compared to that of AMB, but was 29 and 74% lower in the  $\text{TEMP} \times f\text{CO}_2$  and  $f\text{CO}_2$  treatments than that of AMB. The S/R and RMF were significantly greater in the  $\text{TEMP} \times f\text{CO}_2$  and  $f\text{CO}_2$  treatments followed by the TEMP and AMB treatments.

Leaf nutrient concentrations

Leaf C concentrations for both tree species were not significantly different between treatments (Table 3). However, leaf N concentrations were significantly different between treatments showing a 9% greater leaf N concentration in the TEMP treatment compared to that of the AMB treatment for *C. odorata*. However, the  $\text{TEMP} \times f\text{CO}_2$  and  $f\text{CO}_2$  treatments had a 1 and 62% lower leaf N concentration compared to that of the AMB treatment. For *G. sepium* tree seedlings, leaf N concentrations were significantly lower compared to that of the AMB treatment. For example, leaf N concentration was 12, 48 and 52%

**Table 2** Growth parameters current ambient (AMB), CO<sub>2</sub> fertilization (fCO<sub>2</sub>), elevated temperature (TEMP), and combined elevated temperature and CO<sub>2</sub> fertilization (TEMPx<sub>f</sub>CO<sub>2</sub>) for *G. sepium*

	Number of leaves	LAR (cm <sup>2</sup> /g)	Shoot biomass (g)	Root biomass (g)	S/R ratio	RMF	Water content (%)
AMB	16.33 (2.73) <sup>a</sup>	95.02 (16.29) <sup>a</sup>	1.91 (0.33) <sup>a</sup>	2.84 (0.42) <sup>a</sup>	0.67 (0.12) <sup>a</sup>	0.40 (0.02) <sup>a</sup>	86.8 (1.1) <sup>a</sup>
fCO <sub>2</sub>	10.38 (2.20) <sup>b</sup>	59.09 (10.73) <sup>b</sup>	1.08 (0.29) <sup>b</sup>	0.74 (0.14) <sup>b</sup>	1.46 (0.11) <sup>b</sup>	0.51 (0.02) <sup>b</sup>	83.4 (1.7) <sup>a</sup>
TEMP	13.00 (0.58) <sup>b</sup>	80.33 (16.01) <sup>a</sup>	4.21 (0.31) <sup>c</sup>	4.04 (0.60) <sup>c</sup>	1.04 (1.06) <sup>c</sup>	0.58 (0.02) <sup>c</sup>	83.7 (1.4) <sup>a</sup>
TEMPx <sub>f</sub> CO <sub>2</sub>	10.00 (0.93) <sup>b</sup>	56.60 (6.18) <sup>b</sup>	2.98 (0.29) <sup>d</sup>	2.02 (0.99) <sup>a</sup>	1.48 (0.11) <sup>b</sup>	0.61 (0.02) <sup>c</sup>	82.4 (1.5) <sup>b</sup>

Standard errors are given in parentheses ( $n = 8$ )

Values followed by the same lowercase letter, comparing between treatments, for each *G. sepium* growth parameter are not significantly different at  $P > 0.05$

**Table 3** C and N concentrations and C/N ratio of *C. odorata* and *G. sepium* leaves under current ambient (AMB), CO<sub>2</sub> fertilization (fCO<sub>2</sub>), elevated temperature (TEMP), and combined elevated temperature and CO<sub>2</sub> fertilization (TEMPx<sub>f</sub>CO<sub>2</sub>)

	Treatment	C (g/kg)	N (g/kg)	C/N ratio
<i>C. odorata</i>	AMB	437.8 (4.96) <sup>a</sup>	32.0 (0.41) <sup>a</sup>	13.67 (0.06) <sup>a</sup>
	fCO <sub>2</sub>	436.6 (2.17) <sup>a</sup>	12.1 (0.33) <sup>b</sup>	36.23 (0.83) <sup>b</sup>
	TEMP	450.6 (6.12) <sup>a</sup>	35.3 (1.24) <sup>a</sup>	12.78 (0.43) <sup>a</sup>
	TEMPx <sub>f</sub> CO <sub>2</sub>	445.7 (0.87) <sup>a</sup>	31.6 (2.54) <sup>a</sup>	14.27 (1.12) <sup>a</sup>
<i>G. sepium</i>	AMB	437.8 (4.96) <sup>a</sup>	23.9 (2.57) <sup>a</sup>	18.75 (2.13) <sup>a</sup>
	fCO <sub>2</sub>	436.6 (2.18) <sup>a</sup>	11.5 (0.27) <sup>b</sup>	37.90 (0.99) <sup>b</sup>
	TEMP	437.3 (2.34) <sup>a</sup>	21.1 (0.46) <sup>a</sup>	20.71 (0.35) <sup>a</sup>
	TEMPx <sub>f</sub> CO <sub>2</sub>	423.9 (3.89) <sup>a</sup>	12.5 (0.88) <sup>b</sup>	34.10 (1.96) <sup>b</sup>

Standard errors are given in parentheses ( $n = 8$ )

Values followed by the same lowercase letter, comparing between treatments, for each *G. sepium* or *C. odorata* for C, N or C/N ratio are not significantly different at  $P > 0.05$

lower in the TEMP, TEMPx<sub>f</sub>CO<sub>2</sub> and fCO<sub>2</sub> treatments respectively (Table 3).

## Discussion

### Tree seedling growth

Tree height is necessary for the survival and successful establishment of agroforestry trees, and rapid seedling growth is valued by farmers because it is an indicator of successful agroforest establishment and survival (Nair 1993). In our study, tree seedlings from both species had the greatest response to the TEMP and TEMPx<sub>f</sub>CO<sub>2</sub> treatments compared to AMB conditions with respect to height. Similarly, Overdieck et al. (2007) also found a greater tree height of European beech (*Fagus sylvatica* L.) seedlings when grown under warmer temperatures

(+4°C from current ambient conditions). Allen and Vu (2008) showed an increase in citrus tree (*Citrus reticulata* B., *Citrus limon* (L.) Burm.) height in Florida when grown under a combination of enhanced temperature and CO<sub>2</sub> fertilization.

Root and shoot biomass accumulation responded positively under greater than ambient temperature (TEMP) or a combination effect (TEMPx<sub>f</sub>CO<sub>2</sub>), and was similar to the findings of Lee (2011). This may be due to a higher than normal soil temperature (Lewis et al. 2009), suggesting that the necessary resources required for seedling growth were available over the short-term (Khurana and Singh 2001) due to greater rates of soil nutrient mineralization (Lewis et al. 2009), and changes in soil microbial activity and community structure (Lukac et al. 2010). However, the various tree seedling growth parameters in our study responded negatively to fCO<sub>2</sub> compared to the AMB treatment. Previous studies from tropical



and temperate environments reported a positive effect on tree seedling growth parameters under CO<sub>2</sub> fertilization relative to current ambient conditions (Wurth et al. 1998; Raizada et al. 2009; Xiao et al. 2005; Lindroth 2010). For example, Sigurdsson et al. (2001) found a 45% increase in *Populus trichocarpa* Torr. and Gray ex Hook. shoot and a 58% increase in root biomass under CO<sub>2</sub> fertilization. Similarly, Carswell et al. (2000) found that when *C. odorata* was exposed to a CO<sub>2</sub> concentration of 700 ppm compared to the current ambient concentration, shoot and root biomass accumulation increased by 12% when a high level of fertilizer was added, but observed a much lower growth response under low soil fertility conditions relative to current ambient conditions. Raizada et al. (2009) suggested that seedling growth may vary among species and found a decrease or increase in biomass accumulation under CO<sub>2</sub> fertilization relative to current ambient conditions. Sigurdsson et al. (2001) proposed that such differences may be due to nutrient availability. They noted that biomass accumulation of *P. trichocarpa* under CO<sub>2</sub> fertilization only occurred when N availability was very high, but showed no effect or a decrease in biomass, compared to current ambient conditions, when N availability was low (Sigurdsson et al. 2001). Results from these studies and our study illustrated a discrepancy in plant growth with respect to CO<sub>2</sub> fertilization and suggested there is likely no 1:1 effect (CO<sub>2</sub> fertilization:plant growth) (Körner 2009).

A significantly lower number of leaves in all treatments compared to AMB suggested that smaller amounts of organic matter from litterfall or tree prunings may be returned to the soil in an agroforestry system. This could negatively affect the long-term cycling of organic matter, C and nutrients. For example, Oelbermann et al. (2004) found that 28% of the C returned to the soil via pruning biomass was derived from *G. sepium* leaves. Similarly, Gielen et al. (2001) and Na et al. (2011) also found a lower number of stem leaves on tree seedlings exposed to greater than ambient temperatures and CO<sub>2</sub> concentrations.

An increase in the S/R ratio for both tree species in the TEMP, fCO<sub>2</sub> and TEMP<sub>x</sub>fCO<sub>2</sub> treatments compared to AMB conditions was also reported in other studies (Wilson 1988; Shenglei and Ferris 2006; Xiao et al. 2005; Overdieck et al. 2007; Allen and Vu

2008). Markesteijn and Poorter (2009) suggested that an increase in S/R may occur to maximize growth under stress conditions. A significant increase in RMF in the TEMP<sub>x</sub>fCO<sub>2</sub> and TEMP treatments compared to ambient conditions was also reported by Xiao et al. (2005) and Raizada et al. (2009). For example, Raizada et al. (2009) found a greater RMF when *Caragana intermedia* Kuang and H.C. Fu was exposed to 700 ppm, and suggested that this may indicate insufficient nutrient availability.

A decrease in LAR in both *G. sepium* and *C. odorata*, was also reported by others (Hui et al. 2002; Xiao et al. 2005; Raizada et al. 2009; Körner 2009), and may be due to an increased productivity on a smaller photosynthetic area (Xiao et al. 2005). As such, fCO<sub>2</sub> and TEMP<sub>x</sub>fCO<sub>2</sub> may be more efficient photosynthetically compared to seedlings grown under AMB conditions. A decrease in LAR may also be due to increased levels of non-structural carbohydrates, or starch content in leaves, or changes in leaf anatomy (Raizada et al. 2009). Körner (2004) suggested that such changes in levels of non-structural carbohydrates or leaf starch content may also lead to shorter-lived trees, and a higher tree mortality rate.

#### Leaf nutrient concentrations

Our study showed no change in leaf C concentration for either tree species under any of the different treatments, and was consistent with results observed by Liu et al. (2009). A decrease in leaf N concentration however, was also reported in other studies (Hui et al. 2002; Hoorens et al. 2003; Liu et al. 2009; Norby et al. 2010). Carswell et al. (2000) found a decrease in leaf N and phosphorus (P) concentrations when *C. odorata* was exposed to 700 ppm compared to AMB conditions. Cotrufo et al. (2005) suggested that CO<sub>2</sub> fertilization may alter litter quality leading to a reduction in the long-term availability of nutrients. Lukac et al. (2010) showed that changes in tree litter quality may be far-reaching and may depend on the differential responses of nutrient demand under increased concentrations of atmospheric CO<sub>2</sub>. Any change in nutrient availability, utilization or cycling in plant biomass, soil and soil water may affect the nutrient status of other ecosystem components causing a potentially cascading

effect that may be most prevalent for non-leguminous species (Bradley and Pregitzer 2007).

The greater decrease in leaf N concentration in the leguminous tree seedling *G. sepium* compared to the non-leguminous *C. odorata* was also observed in other studies. For example, Rogers et al. (2006) found leaf N content in soybean (*Glycine max* L.) was significantly lower at elevated concentrations of CO<sub>2</sub> in the first 50 days after emergence, which they equated to the initial growth phase of the soybean crop. However, as soybean growth and maturation proceeded, leaf N concentration was similar to that of other non-leguminous plants exposed to ambient levels of CO<sub>2</sub> (Rogers et al. 2006). In our study, tree seedling leaf N concentration was evaluated after 35 days of growth, which likely corresponded to the initial growth phase described by Rogers et al. (2006). This suggested that leguminous species commonly used as multipurpose agroforestry trees may not exhibit reduced leaf N content under CO<sub>2</sub> fertilization, or a combination effect, after their initial growth phase. Thomas and Strain (2000) suggested that legumes may respond to increased N demands induced by elevated CO<sub>2</sub> through increased N<sub>2</sub>-fixation, even if soil N levels are low or if conditions of drought occur. They found that under CO<sub>2</sub> fertilization (700 ppm), the net uptake of N by *G. sepium* seedlings stimulated N<sub>2</sub>-fixation (Thomas and Strain 2000).

## Conclusions

This study demonstrated the effect of elevated ambient temperature, CO<sub>2</sub> fertilization or the combined effect on two different tree species commonly used in Costa Rican agroforestry practices. Both tree species responded similarly to the TEMP and TEMP<sub>x</sub>CO<sub>2</sub> treatments with respect to growth parameters (height, number of leaves, LAI, shoot and root biomass, and S/R ratio) and leaf C and N concentrations. Results from our study suggested that CO<sub>2</sub> fertilization may negatively impact tree growth parameters, but a combined effect likely results in a positive growth effect. However, leaf N concentration was affected negatively in all treatments in both tree species compared to ambient conditions. This may impact the long-term cycling of nutrients in agroforestry systems, especially those with non-leguminous

tree species, which may alter litter quality and ultimately lead to changes in nutrient availability in agroforestry systems. As such, further studies using common leguminous and non-leguminous multipurpose agroforestry tree species are required. Additionally, changes in soil dynamics as a result of a combined effect of CO<sub>2</sub> fertilization and enhanced temperature are required over the long-term to advance our understanding of the response of these plants to changing atmospheric and environmental conditions. It is especially important that pot trials in growth chambers are paralleled with field trials in order to evaluate plant response to climate change under conditions that resemble as close as possible the plant's natural environment. Additionally, knowledge of these ecophysiological responses to a combined effect of CO<sub>2</sub> fertilization and temperature under different conditions of moisture may also play a key role in understanding the functioning of the whole agroforest.

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