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Chemical and anatomical changes in *Liquidambar styraciflua* L. xylem after long term exposure to elevated CO_2

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ABSTRACT

The anatomical and chemical characteristics of sweetgum were studied after 11 years of elevated CO_2 (544 ppm, ambient at 391 ppm) exposure. Anatomically, branch xylem cells were larger for elevated CO_2 trees, and the cell wall thickness was thinner. Chemically, elevated CO_2 exposure did not impact the structural components of the stem wood, but non-structural components were significantly affected. Principal component analysis (PCA) was employed to detect differences between the CO_2 treatments by considering numerous structural and chemical variables, as well as tree size, and data from previously published sources (i.e., root biomass, production and turnover). The PCA results indicated a clear separation between trees exposed to ambient and elevated CO_2 conditions. Correlation loadings plots of the PCA revealed that stem structural components, ash, Ca, Mg, total phenolics, root biomass, production and turnover were the major responses that contribute to the separation between the elevated and ambient CO_2 treated trees.

1. Introduction

Globally, the mean atmospheric carbon dioxide level has risen steadily since pre-industrial times, which is largely attributable to human activities such as increased emissions from fossil fuel burning and clearing of forests. Implications of elevated CO_2 on ecosystems have been largely limited to mechanistic models or to studies on seedling and small trees in growth chambers until the development of new technology extended experiments to intact ecosystems. Free Air CO_2 Enrichment (FACE) studies were designed to address long-term responses of ecosystems to elevated level of atmospheric CO_2 through targeted release of air enriched with CO_2 into the plant canopy.

Little is known about the potential impacts of elevated CO_2 on xylem structural properties, which complement stomatal dynamics, to provide additional controls on water flux through plants. A change in xylem anatomy such as lumen diameter (Tyree et al., 1994) or scalariform perforation plate bar thickness (Schulte, 1999) would affect resistance to water flux and thereby hydraulic conductivity. In birch and oak seedlings, elevated CO_2 was found to reduce water flux and leaf specific conductivity (Eguchi et al., 2008) which directly suggests reduced stem hydraulic capacity as was exhibited in beech trees (Overdieck et al., 2007). Elevated CO_2 has been shown to affect xylem cell size, increasing cell size in *Larix decidua* (Handa et al., 2006) and dogwood (Domec et al., 2010), but reducing cell size in *Picea abies* (Kostiainen et al., 2004) and *Fagus sylvatica* (Overdieck et al., 2007), illustrating the species specificity of response. Thicker cell walls have been seen in *Pinus sylvestris* exposed to elevated CO_2 (Kilpelainen et al., 2007), but thinner cells developed when elevated temperature and CO_2 treatments were applied together, illustrating the complexity of response.

Along with the atmospheric CO_2 concentration, soil nutrient status has also been shown to be a crucial factor to the plant growth (Norby et al., 2010; Oren et al., 2001). At the Oak Ridge National Laboratory's (ORNL) FACE site and other forest FACE sites, elevated CO_2 initially enhanced stem wood growth by ~25% (Norby et al., 2005). However, with limited soil N availability on this site, net primary productivity (NPP) was reported to gradually decrease over the course of the study (Norby et al., 2010). Nutrient balance is also important for differentiation of photosynthate, yet several studies showed no significant differences in the amount of total extractives with elevated CO_2 treatment, although the concentration of proteins and mineral nutrients decreased and the lipid

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compositions were altered (DaMatta et al., 2010; Kilpelainen et al., 2005, 2003). Phenolic compounds of plants grown under elevated CO_2 condition have been also studied (Ghasemzadeh et al., 2010; Johnson and Pregitzer, 2007; Penuelas et al., 1996). Non-structural sugars and starch content of CO_2 enriched xylem from several forest FACE sites increased by 30–40 % compared to the control samples (Ainsworth and Long, 2005). Kaakinen et al. (2004) also reported that soluble sugars and starch concentration increased with 3 years of CO_2 treatment in the Aspen FACE site.

In general, the primary effects of elevated CO_2 concentration on trees include higher rates of photosynthesis, enhanced water use efficiency, enhanced productivity, and alteration of secondary metabolites. Such physiological changes may lead to changes in chemical composition of the different parts of trees including leaves, xylem (wood), phloem (bark), and roots. However, as it was pointed out earlier, most of the previous studies were conducted on samples from controlled environments (potted plants or open-top chamber) and some of the previous FACE research was conducted on juvenile wood. The findings could differ with the age of the trees since the anatomical, physical, and chemical properties of juvenile wood are significantly different from that of mature wood (Haygreen and Bower, 1996), suggesting changes in wood chemistry due to elevated CO_2 could also be different between juvenile and mature wood. A recent study by Kostiainen et al. indicated that short-term impact studies, conducted with young seedlings, may not give a realistic view of long-term tree responses (Kostiainen et al., 2014). We expanded on the initial study of wood properties after 11 years of treatment to look at long term responses following the progressive reduction in NPP exhibited in later years at the site. The present study on sweetgum grown under long term exposure of elevated CO_2 at the ORNL FACE site could provide a better understanding of tree responses to higher atmospheric CO_2 in a natural environment.

The goal of this research was to investigate the effects of long-term (11-years) application of elevated CO_2 in the atmosphere on the anatomical and chemical changes of the xylem part of sweetgum (*Liquidambar styraciflua* L.) from the ORNL FACE site. The first objective was to compare anatomical differences of branch xylem growing under elevated and ambient CO_2. Analyses included xylem anatomical measurements such as hydraulic mean diameter, double cell wall thickness, and area of largest cell – characteristics important for tree vigor under drought stress. The second objective was to compare chemical changes of the xylem growing under elevated and ambient CO_2. Analyses included comparison of structural and non-structural chemical composition – characteristics important for wood quality and recalcitrance. The amount of cellulose, hemicellulose, and lignin, which are the main structural components of xylem tissues, was quantified. The non-structural components analyzed included ash, extractives, and macronutrients. Lastly, the sensitivity of the physical and chemical tree responses under elevated CO_2 was investigated using multivariate principal component analysis (PCA). Based on the results from short-term CO_2 experiments, or FACE experiments on young trees, we hypothesized that the older sweetgum trees exposed to FACE treatments would show little change in wood composition. The contribution of this study may improve the understanding of a tree's anatomical and chemical responses under long term exposure of elevated CO_2.

2. Materials and methods

2.1. Materials

Sweetgum (*L. styraciflua* L.) branch and stem material was harvested from the Oak Ridge FACE site located in the Oak Ridge National Environmental Research Park in eastern Tennessee, USA (35°54′N; 84°20′W). The Oak Ridge FACE site consisted of five 25 m-diameter plots (rings), with vertical PVC pipes releasing CO_2-enriched or ambient air. Among those five rings, two were elevated CO_2 rings (targeting 550 ppm and measured 544 ppm) and three ambient CO_2 rings (measured at 391 ppm, two surrounded by the FACE structure and a third ambient CO_2 plot without structure). One-year old sweetgum trees were planted on the site in 1988 and the CO_2 treatment was initiated 10 years later (in 1998). The CO_2 treatment was applied during daytime between April and November from 1998 to 2009. In July 2009, the CO_2 treatment stopped and trees were harvested for this study and for allometric analysis.

For anatomical experiments, fully sun-exposed two-year old upper branches were collected from six trees in each ring in early October, 2007. The apical ends of branches used earlier for xylem vulnerability to embolism curves (Warren et al., 2011) were radially sectioned (40 μm) using a microtome. For each branch, six sections were bleached (10:1 dilution of household bleach), stained with toluidine-blue and mounted on a single slide using a gelatin-glycerin medium.

Stem log samples from the harvested trees at 0.6–1.1 m height from the ground were collected for the chemical analyses and stored at −20 °C until processing. Eight trees from each treatment (elevated or control CO_2 rings) were selected for the experiments. A 25 mm thick disc was cut from the frozen logs, and the annual rings were examined and marked to only collect wood that was produced during the CO_2 treatment. The bark was removed and the CO_2 treated xylem sections (1998–2009) were freeze-dried and chipped with a chisel into small pieces. The chips were then ground with a Wiley Mill 4 (Thomas Scientific, Swedesboro, NJ, USA) equipped with a 40-mesh sieve.

2.2. Methods

For anatomical analyses, one high-level resolution (0.72 pixel μm^{-1}) image of the entire radial branch section was taken at low magnification (15X) for analysis of xylem sapwood area using a Leica M165 stereomicroscope and digital camera. Stem diameter, one- and two-year-old sapwood area, and pith area were measured using Image J software (Rasband, 2012). Six to eleven images per branch that included one or two-year-old xylem tissue were taken at various radial directions at higher magnification (1.96 pixels μm^{-1}; 200X) using a Leica M1000 light microscope system. Each image was fully analyzed for vessel area, vessel diameter, and vessel number using automated software (WinCELL 2001a; Régent Instruments Inc). Cell wall diameter was measured using Image J software. Branch hydraulic conductivity depends on the sum of the weighted mean cell diameters, or mean hydraulic diameter, which was calculated based on Sperry et al. (Sperry et al., 1994). Cells that were smaller than 100 μm^2 were not included in the analysis of xylem vessels. In total, 29,236 xylem vessel cells were analyzed in 202 different images. Results from each image were averaged by branch, and statistical analysis was performed at the branch level ($n = 10$–11 per treatment).

For chemical analyses, approximately 5 g of the finely ground sweetgum samples were sequentially extracted with water and ethanol using an automated extraction system (ASE 350, Dionex Corp.) following the National Renewable Energy Laboratory protocol "Determination of extractives in biomass (NREL/TP 510-42619)". Each extracted wood sample was dried in a low temperature oven (35 °C) until it reached a constant weight, and the extractives content was calculated on dry basis. The total phenolics content in the water and ethanol fractions was determined by the Folin-Ciocalteau method (Singleton and Rossi, 1965).

The dried and extracted sweetgum samples were used for structural chemical analyses following the protocol "Determination of structural carbohydrates and lignin in biomass (NREL/TP-510-42618)". This protocol was used to measure cellulose, hemicellulose, acid soluble and acid insoluble lignin content by a two-stage acid hydrolysis. During the hydrolysis, the polymeric carbohydrates were hydrolyzed into monomeric sugars (glucose, xylose, mannose, arabinose, and galactose) and quantified using a High Pressure Liquid Chromatography (HPLC) (Perkin Elmer, Shelton, CT) equipped with a refractive index detector and an Aminex HPX-87P column (300 × 7.8 mm ID, 9 μm particle sizes) attached to a deashing guard column (Biorad, Hercules, CA). The HPCL's oven temperature was set at 85 °C and the injection volume was 20 μL. Quantified glucose was calculated as % cellulose in the biomass and the other monomeric sugars were calculated as % hemicellulose. Acid insoluble lignin was measured gravimetrically, and acid soluble lignin was determined by UV/VIS spectrophotometer (Lambda 650, Perkin Elmer).

The gravimetric ash content was measured by combusting 0.7 g of biomass at 575 °C for 24 h. 0.5 g of raw biomass was microwave digested then analyzed for content of inorganic elements by inductively coupled plasma-optical emission spectroscopy using an Optima 7300 DV spectrometer (ICP-OES, Perkin Elmer). All of the experiments were performed in triplicate except total phenolics analysis which was conducted in duplicate for each sample.

2.3. Data analysis

To compare responses in structural and non-structural chemical components of sweetgum to CO_2 treatments, two types of statistical methods were employed. First, analysis of variance (ANOVA) methods were used to determine if differences existed between the means of sample groups from elevated and ambient CO_2 sites at a significance level of $\alpha < 0.05$. For the mean separation, 2-sided t-test was used for anatomical analysis and Fisher's Least Significant

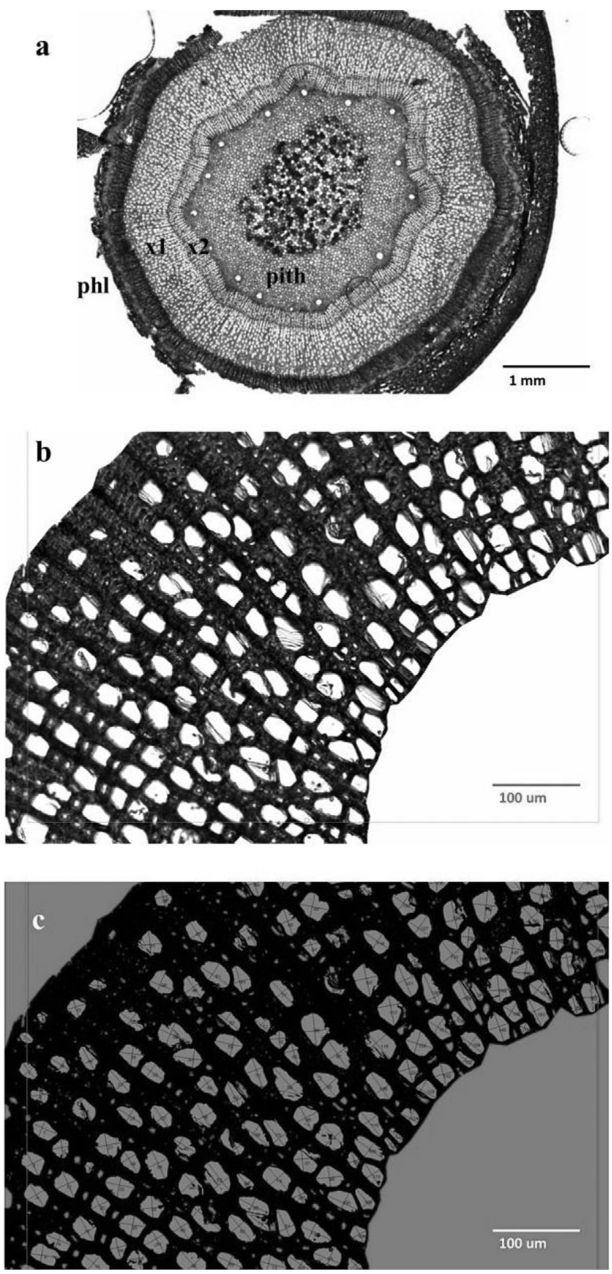

Fig. 1. Typical light microscopy image of a two-year old sweetgum branch collected in 2007 at the ORNL FACE research site. (a) indicates that the pith, one-year old xylem (x1), two-year old xylem (x2) and outer phloem and bark (ph1) tissues; (b) two-year old xylem tissue at 200x magnification; (c) automated image analysis of (b).

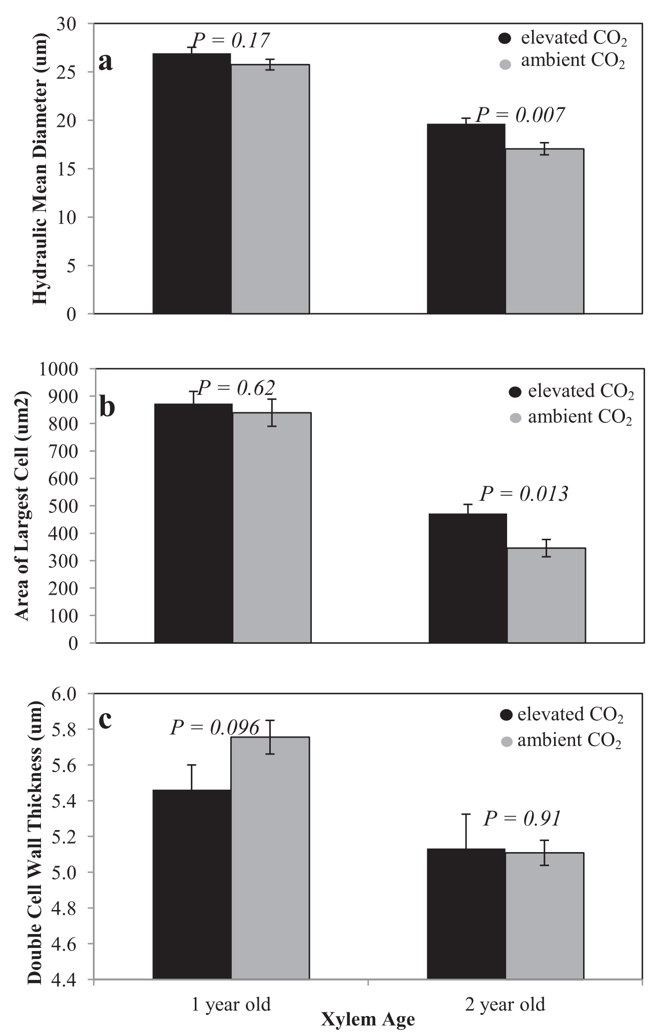

Fig. 2. The hydraulic mean diameter (a), the area of largest cell (b), and double cell wall thickness of elevated and ambient CO_2 treated branch tissue of sweetgum. Significance level ($p < 0.05$) among control and CO_2 enriched samples are shown based on 2 sided t-test.

Differences (LSD) was employed for chemical analysis. The high cost of the FACE infrastructure limits true replication in these types of studies, and thus the analyses are often based on pseudoreplication (e.g., by tree instead of by ring), which increases the chance of type 1 errors (Hurlbert, 1984). Thus results should be considered in this context.

To detect differences of physical and chemical tree responses to the CO_2 treatments and to determine the relative strength of CO_2 effects on physical and chemical tree responses under elevated CO_2, principal component analysis (PCA) was employed. The basic assumption for the use of multivariate analysis is that all the data (tree responses in this research) carry information regarding the effects of elevated CO_2. PCA was also used to visualize correlation structures among the tree responses. The scores plot of PCA shows the cluster of the sample categories and helps visualize any trends in the data sets in the new system of axes of principal components (PCs). Correlation loadings are useful in interpreting the correlation structure between the variables and the PCs by presenting significant levels when loadings plot cannot reveal the actual correlation structure. This statistical method offers a reliable separation between the sample categories (CO_2 treatments in this study) by using the responses of tree under the treatment (Esbensen, 2001). In the PCA, variables included cellulose, hemicellulose, lignin, Ca, Mg, K, P, S, total phenolics, extractives, and ash (tree responses to the CO_2 treatments in this study). Other variables from previously published results such as total root production (gm^{-2}), root turnover (mortality) (gm^{-2}), root peak standing crop (biomass) (gm^{-2}), height and circumference of the sweetgum at harvest time were also included to address the relative importance of CO_2 impacts on tree responses on both above-ground and below-ground changes (Iversen, 2010; Ledford et al., 2008). The data were imported and standardized prior to PCA in the Unscrambler software (v.9.0, CAMO, Woodbridge, NJ.).

3. Results

3.1. Anatomical changes

Fig. 1 is a typical light microscopic image of a two-year old sweetgum branch collected in 2007 from the upper canopy at the ORNL FACE research site. Ladder-like scalariform perforation plates are visible in several cells in Fig. 1, b. The automated image analysis (c) indicates cell length and width.

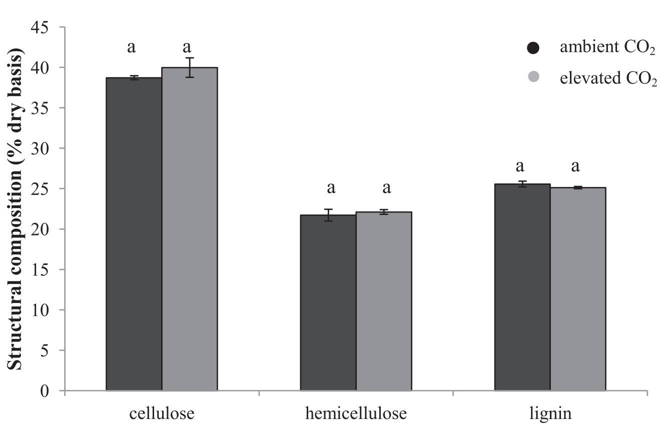

Fig. 3. The structural composition of elevated and ambient CO_2 exposed sweetgum stem wood. Estimated mean values from three replicate measurements of each tree sample with standard error on the bar. Same letters indicate no significant difference ($\alpha < 0.05$) among control and CO_2 enriched samples based on Least Significant Difference (LSD).

Hydraulic mean diameter was significantly larger for elevated CO_2 branch tissue grown in 2006, but not for the 2007 cells (Fig. 2, a). There was also a larger maximum cell size for elevated CO_2 xylem in 2006, but not in 2007 (Fig. 2, b). The double cell wall thickness, as measured from the lumen of one xylem cell to the lumen of the next cell, was thinner for elevated CO_2 branches in 2007, but not during the previous year (Fig. 2, c).

The anatomical results reveal differences in xylem development under elevated CO_2, as well as an interaction with other environmental conditions. In 2006, the site experienced moderate weather conditions with 1184 mm of annual rainfall, and without significant drought or heat waves. In contrast, 2007 was a year of extremes, with a severe early-season frost, low annual rainfall (905 mm), extended 50-year summer drought, and record-breaking summer temperatures (Warren et al., 2011). During non-drought years, net C uptake and availability, and water use efficiency were greater for elevated CO_2 trees. Such conditions are conducive to greater rates of growth and cell expansion, consistent with the larger cell sizes found in elevated CO_2 branches in 2006. Larger cell size is also consistent with increased vulnerability to xylem embolism (Tyree et al., 1994), and *L. styraciflua* trees exposed to elevated CO_2 at this site (Warren et al., 2011) and at the Duke FACE site (Domec et al., 2009) do have increased vulnerability to embolism, which could lead to reduced competitive ability within future forest ecosystems if other species are not similarly affected. In 2007, the severe drought reduced stomatal conductance in elevated CO_2 branches to such an extent that C availability was substantially reduced as compared with ambient branches (Warren et al., 2011). Xylem cell size was not affected by the reduced C availability in elevated CO_2 branches, yet cell wall thickness was slightly reduced ($P = 0.096$). The differences in CO_2 treatments on tree growth and xylem development reflect both the CO_2 treatment, as well as the interaction between CO_2 and other limiting conditions, such as drought (Warren et al., 2011) or lack of nutrients (Norby et al., 2010). While the mild summer of 2006 allowed CO_2 treatments to enhance xylem cell size, the extreme conditions in 2007 limited any positive effect of elevated CO_2 on branch development. Hydraulic function depends on the characteristics of xylem cells developed across multiple years, thus subtle elevated CO_2 impacts to xylem structure can be amplified through time with implications to the whole plant.

3.2. Chemical changes

3.2.1. Structural composition

The structural composition of sweetgum exposed to elevated and ambient CO_2 for 11 years is shown in Fig. 3. Sweetgum exposed to the ambient level of CO_2 had 38.73% cellulose, 21.71% hemicellulose, and 25.57% lignin while sweetgum treated with elevated CO_2 had 39.98% cellulose, 22.11% hemicellulose, and 25.13% lignin. The amount of cellulose and hemicellulose appeared slightly higher for the CO_2 enriched samples, and lignin tended to be lower with

Table 1
Total extractives and total phenolics in sweetgum stem wood.[a]

		Extractives (% dry basis)	Total phenolics[b] (mg GAE g^{-1} extractives)
Sweetgum	Ambient CO_2	3.86 (0.25) a	91.24 (4.36) b
	Elevated CO_2	3.93 (0.10) a	108.46 (4.04) a

[a] Mean values of extractives and total phenolics content from ambient and elevated CO_2 treated sweetgum with standard error values in parentheses. “a” and “b” represent statistical significant difference (LSD, $\alpha < 0.05$).
[b] GAE = gallic acid equivalent.

CO_2 enrichment. However, the results from LSD indicated that there were no significant differences in cellulose, hemicellulose, and lignin content between ambient and elevated CO_2 conditions for sweetgum ($\alpha < 0.05$).

These findings are in agreement with previous research (Atwell et al., 2003; Entry et al., 1998; Kilpelainen et al., 2003; Olszyk et al., 2005; Tingey et al., 2003), which also indicated that there were no significant changes in structural components of wood due to elevated CO_2 treatments. It has been acknowledged that the increased CO_2 in the atmosphere would increase photosynthesis of plant, but the allocation of photosynthate depends on the sink demand of tissues within the plant. In other words, increased photosynthate by elevated CO_2 is most likely transported to the tissues that can obtain the most limiting resources (Entry et al., 1998), in the case of soil nutrients, this would be the root system. Indeed, many studies have pointed out that upon increased CO_2 treatment, fine root growth also increased (Chapman et al., 2005; Iversen, 2010; King et al., 2001; Norby et al., 2004; Nosberger et al., 2006). This result suggests that much of the available photosynthate could be transferred belowground (Nosberger et al., 2006) to enhance the growth of roots rather than partitioned into stem xylem. According to Norby and co-workers, above-ground wood production was 35% greater in CO_2 enriched rings during the first year of exposure, 15% higher in the second year (Norby et al., 2002) and continued to decline through the course of the experiment (Norby et al., 2010). While the enhancement of above-ground wood production gradually declined with time, below-ground production increased continuously (Norby et al., 2005). The cause for the enhanced root growth rather than wood after CO_2 exposure can be explained with soil nutrition status. At the ORNL FACE site, N deficiency occurred after just a few years of elevated CO_2 treatment (Norby et al., 2010). It appears that effects of CO_2 in trees may be mostly observed in highly demanding tissues (Nosberger et al., 2006). Enhanced root growth may be a strategy to acquire limiting nutrients from the soil to offset N sequestration in larger CO_2 stimulated tree biomass. Indeed across CO_2 enrichment studies, root proliferation at depth is a common response (Iversen, 2010) presumably to mine for resources to sustain the greater biomass and supplement decreased N availability in the soil after prolonged elevated CO_2 treatment (Johnson et al., 2004).

3.2.2. Non-structural composition

The estimated mean values of total extractives and total phenolics content are presented in Table 1 (LSD, $\alpha < 0.05$). Total extractives from sweetgum stem wood were 3.86% and 3.93% for ambient or elevated CO_2 trees, respectively. While no significant difference was observed in the total extractives content, the total phenolics concentration for enriched CO_2 sweetgum was 16% higher than that of the control. The amount of total phenolics was 91.24 mg GAE/g of extracts in the ambient sweetgum and 108.46 mg GAE/g of extracts in the elevated CO_2 sweetgum, a difference that was statistically significant.

Increased total phenolic levels under elevated CO_2 have been observed in many tree species, including aspen, birch, oak, maple, eucalyptus, spruce, and pine (Lindroth, 2010; Tuchman et al., 2003). Phenolics can potentially be influenced by changes in carbon inputs (Johnson and Pregitzer, 2007), and elevated CO_2 may influence the chemical pathways that regulate gene expression and synthesis of secondary compounds (Lindroth, 2010). The shikimic acid pathway, known to produce phenolic compounds in trees, was found to be the most influenced pathway by CO_2 treatment (Lindroth, 2010). The total phenolics concentration in sweetgum confirms previous findings that trees exposed to higher CO_2 have higher level of phenolics. Another possible explanation for these increased total phenolics could be the growth-differentiation balance hypothesis of a tree. Growth refers to the production of new cells while differentiation refers to carbon flow into compounds that enhance the structure or function of existing cells (Stamp, 2003). Secondary metabolism is one of the examples of differentiation related processes (Herms and Mattson, 1992). The growth-differentiation balance hypothesis states that any environmental factor that slows growth of the tree can result in C allocation to differentiation-related products. When there is a limiting factor (N availability in the soil at ORNL FACE site) which slows down the tree growth rate, C can accumulate in the differentiation-related products (secondary metabolites and total phenolics in this study) with low cost to plant fitness (Stamp, 2003). Differentiation leads to changes in

Table 2
Elemental composition of sweetgum stem wood.[a,b,c]

Elemental composition	Sweetgum	
	Ambient CO_2	Elevated CO_2
Ash (%)	0.44 (0.01) b	0.53 (0.01) a
Ca (mg/kg)	713.77 (14.72) b	787.58 (21.07) a
K (mg/kg)	690.90 (16.51) a	669.64 (23.55) a
Mg (mg/kg)	239.76 (12.74) b	283.50 (13.16) a
P (mg/kg)	77.52 (6.90) a	74.74 (4.17) a
S (mg/kg)	79.62 (0.92) a	80.74 (1.03) a

[a] Mean values of ash and elemental content of ambient and elevated CO_2 treated sweetgum with standard error values in parentheses.
[b] "a" and "b" represent statistical significant difference.
[c] Concentrations were calculated on the basis of the dry weight of samples (mg/kg).

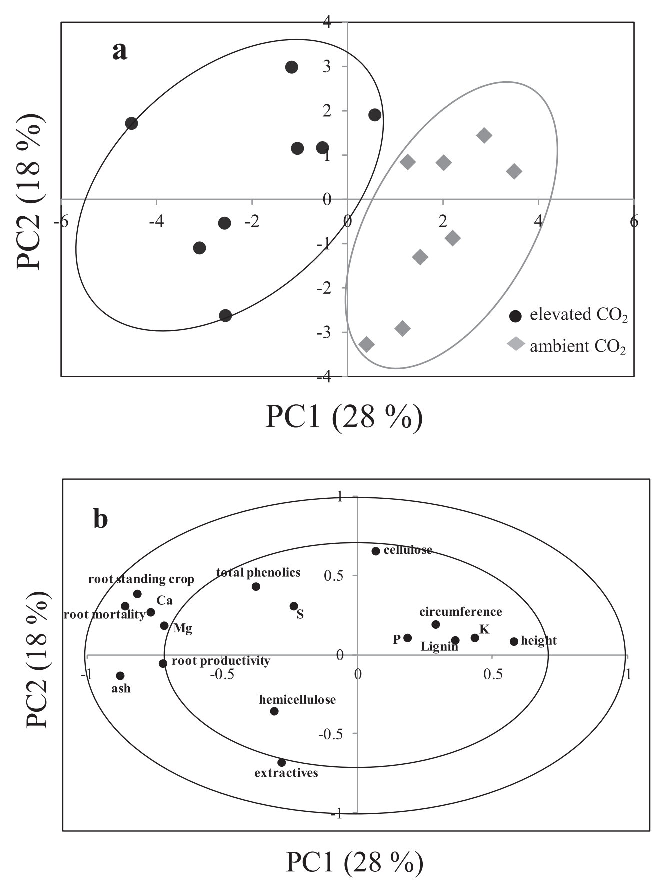

Fig. 4. Scores (a) and correlation loadings (b) plots show the relationships between samples and variables of sweetgum, respectively. Outer and inner circles in correlation loadings (b) plot represent 100% and 50% additive explained variance, respectively.

chemical quality, which has implications for herbivory and decomposition.

3.2.3. Inorganic composition

The total ash content (%) and macronutrients in the xylem part of the sweetgum after CO_2 treatment are shown in Table 2. Statistical analysis (LSD, $\alpha < 0.05$) revealed that total ash content of sweetgum stem wood grown under elevated CO_2 was significantly greater (+19.0%) than trees grown under ambient CO_2. The higher ash content in elevated CO_2 exposed sweetgum led to in-depth study of the inorganic nutrients in the biomass that contribute to the greater ash content.

General elements found in wood are Ca, K, Mg, P, Mn, Fe, Zn, S, Al, and Si. Among those major elements, the inorganic macronutrients such as Ca, K, Mg, and P and the micronutrient S in the biomass are shown in Table 2. The amount of Ca and Mg in the biomass significantly increased with CO_2 treatment (LSD, $\alpha < 0.05$). Sweetgum grown under elevated CO_2 contained 10.0% greater Ca and 18.0% greater Mg than the trees grown under ambient CO_2. No differences were found for K, P, and S.

The increased total ash content and some of the inorganic elements in the elevated CO_2 stem wood samples may be linked to the increase in fine root growth and subsequent increased access to large soil exchangeable pools of P, K, Ca, and Mg. We hypothesized that with the enlarged fine root biomass, uptake of cations was not necessarily related to demand. The tree may take up excess amounts of Ca, Mg, K, and P while 'mining' the soil for additional limiting elements, especially N. Similar published results on inorganic nutrients composition support our hypothesis (Hagedorn et al., 2002; Johnson et al., 2004; Luo et al., 2005). The inorganic content of tree biomass largely depends on tree species and soil type, which suggest that further study is needed to determine why only Ca and Mg increased among the other inorganic elements in sweetgum after 11 years of elevated CO_2 treatment.

3.3. Effect of elevated CO_2 on multiple tree responses

As described earlier, the structural components (cellulose, hemicelluloses and lignin) remained unchanged, while total phenolics content and inorganic components increased upon CO_2 enrichment. Altered tree responses under elevated CO_2 could be due to several environmental factors. For instance, physical tree growth largely depends on variables such as photosynthetic rate, soil N availability, water, inorganic nutrient availability, seasonal variations and duration of treatment (Kaakinen et al., 2004; Kilpelainen et al., 2007, 2005; Kostiainen et al., 2009; Luo and Polle, 2009; Norby et al., 2010). The previously used statistical method (LSD), also known as a univariate method, dealt with one variable at a time and provided a direct comparison between the two levels of CO_2 treatments. Although univariate methods carry important information, they are insufficient for more complex data analyses (Esbensen, 2001). Multivariate analysis uses more than one independent variable and involves mathematical treatment of the data to detect hidden differences or similarities (Esbensen, 2001). Therefore, principal component analysis (PCA), a type of multivariate approach, was selected to simultaneously study multiple tree responses to the elevated CO_2. In order to compare several tree responses at the same time, published data from the ORNL FACE study (Ledford et al., 2008) was also used, which included data regarding fine roots, final year of tree height and circumference at 1.3 m from the ground. The results from PCA illustrate clear separation of responses to the treatments (Fig. 4).

The scores plot is a map of samples and shows a separation between the ambient and elevated CO_2 exposed sweetgum by PC1 and PC2. PC1 accounts for 28% of the variance and PC2 for 18%. The correlation loadings plot provides the relative importance of tree responses (variables) and the visualization of explained variance. Tree responses closer to the outer circle show a higher explained variance level than those in the inner circle. In this study, ash, Ca, Mg, root mortality, root standing crop, and root productivity, located on the negative PC1 close to the outer circle, show higher explained variance level to the elevated CO_2 treated sweetgum.

Most of the variables that show higher degrees of CO_2 effects after long term CO_2 exposure match those from the LSD results. Indeed, Ca, Mg, and ash content were higher in the elevated CO_2 treated sweetgum (Tables 1 and 2). Elevated CO_2 treated sweetgum had higher amounts of root production and shorter height in comparison to the control sweetgum.

4. Conclusion

This study investigated the effects of free-air CO_2 enrichment on the wood chemistry of 22-year old sweetgum (*L. styraciflua*) after 11 years of elevated CO_2 treatment. In conclusion, elevated CO_2 concentration did not have large effects on structural components after long term CO_2 exposure, while non-structural components, xylem cell sizes, and cell wall thickness were significantly impacted by higher CO_2 concentration. The xylem cell sizes from branches growing under elevated CO_2 were larger and cell wall thickness was thinner. Ash, Ca, Mg, and total phenolics content increased in the elevated CO_2 exposed sweetgum. PCA showed clusters by CO_2 treatment, and correlation loadings plot revealed the degree of CO_2 impact on tree responses in the model. According to the correlation loadings plot, ash, Ca, Mg, root mortality, root standing crop, and root productivity were higher in elevated CO_2 exposed sweetgum. In other words, ash, Ca, Mg, root mortality, root standing crop and root productivity were more impacted by higher level of atmospheric CO_2 than the other tree responses such as content of cellulose, hemicellulose, lignin, total extractives, S, P, K, circumference, and height. Long term (11 years) application of elevated CO_2 was also found to affected branch anatomy. Upper branches of sweetgum trees are known to be sensitive to drought (Toole and Broadfoot, 1959), and the elevated CO_2 changes in xylem hydraulic characteristics suggest enhanced potential vulnerability to hydraulic failure under drought. This could increase tree/branch dieback, especially by more intensive and frequent drought expected due to global climate change. The changes in chemical properties, especially ash, Ca, Mg could impact chemical and thermal utilization of xylem (wood).

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