Ecophysiological responses of *Quercus gilva*, endangered species and *Q. glauca* to long-term exposure to elevated CO$_2$ concentration and temperature

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**Abstract**

The physiological effects of elevated CO$_2$ concentration and temperature were examined for *Quercus gilva* and *Q. glauca* grown under control (ambient CO$_2$ and temperature) and treatment (elevated CO$_2$ and temperature) conditions for 39 months. The objective of the study was to measure the long-term responses, in physiological parameters, of two oaks species exposed to elevated CO$_2$ and temperature. The photosynthetic rate of *Q. gilva* was found to be decreased, but that of *Q. glauca* was not significantly affected, after long-term exposure to elevated CO$_2$ and temperature. Stomatal conductance of *Q. glauca* was reduced by 21.7% , but that of *Q. gilva* was not significantly affected, by long-term exposure to CO$_2$ and temperature. However, the transpiration rate of the two oak species decreased. Water use efficiency of *Q. gilva* was not significantly affected by elevated CO$_2$ and temperature, while that of *Q. glauca* was increased by 56.6%. The leaves of *Q. gilva* grown under treatment conditions had an increased C:N ratio due to their reduced nitrogen content, while those of *Q. glauca* were not significantly affected by long-term exposure to elevated CO$_2$ and temperature. These results suggest that the long-term responses to elevated CO$_2$ and temperature between *Q. gilva* and *Q. glauca* are different, and that *Q. gilva*, the endangered species, is more sensitive to elevated CO$_2$ and temperature than *Q. glauca*.

**Key words:** endemic plants, evergreen broad-leaved, global climate change, leaf nitrogen, photosynthesis, *Quercus*

**INTRODUCTION**

Increased atmospheric CO$_2$ concentrations associated with increasing temperatures can directly affect the growth and development of plants through the influence of their physiology. Thus, understanding the interactive effects of elevated CO$_2$ and temperature on plant physiological responses is essential in order for accurate predictions of the effects of climate change to be made.

Much research over the past few decades has focused on the effects of rising atmospheric CO$_2$ concentration and temperature on the physiology of woody plants (Zhou et al. 2011). In general, the short-term exposure of plants to high CO$_2$ levels causes a significant increase in photosynthetic rates and decreases in stomatal conductance (Curtis 1996). However, many studies have shown that accelerated net carbon assimilation is not maintained over longer time periods, and a substantial decline in photosynthesis may occur after long-term exposure to elevated CO$_2$ (DeLucia et al. 1985). Also, stomatal conductance may be increased or decreased, when plants are exposed to elevated CO$_2$ concentrations and temperatures over long periods.

Short-term measurements of the effects of CO$_2$ con-
centration on physiological responses of plants are not always good indicators of the effects of long-term exposure (Long et al. 2004). Sour orange grown at +300 ppm CO₂ above control condition showed that the enhancement ratio for net photosynthesis was about 2.8 in 2 year, but had declined to 1.3 by 14 year, indicating some acclimation to the elevated CO₂ (Kimball and Idso 2005), while in a further similar study the photosynthetic rate of loblolly pine was shown to be increased in high CO₂ levels during all four years (Tissue et al. 1997).

Woody plants have lower relative growth rates and are less responsive to elevated CO₂ and temperature than herbaceous species. Also, woody species may be less functionally plastic to elevated CO₂ than non-woody species (Nowak et al. 2004). Thus, it is necessary to conduct long-term experiments on the effects of elevated CO₂ and temperature on the physiological responses of woody plants to obtain more significant data.

In long-term experiments, down-regulation of photosynthesis is often associated with the responses of leaf chemical compositions, such as leaf nitrogen and carbon contents, to elevated CO₂ and temperature (Roumet et al. 2000). In one study, the leaves of seedlings exposed to elevated CO₂ had lower concentrations of nitrogen than seedlings exposed to ambient conditions, and nitrogen levels declined by 25% in Alphitonia petriei and 29% in Flindersia brayleyana, respectively (Kanowski 2001). The C:N ratios of the Phytolacc insularis and P. americana grown at elevated CO₂ and increased temperature were reduced by 24% and 7%, respectively, as compared with the control plants (Kim and You 2010a).

The habitat ranges and growth rates of woody species can often be related to temperature (Walther 2002). In particular, evergreen broad-leaved species are considered to be limited by the length of the growing season and absolute minimum temperature (Walther et al. 2001). Peng et al. (2011) determined that if the air temperature increased by 2°C without changes in precipitation rates, the net primary productivity of evergreen broad-leaved forests would be increased by 5.5%.

The evergreen broad-leaved forest is one of the main vegetation types in warm temperate regions of Korea. It occurs in the region south of 35° N and from 126° to 128° E (Koo et al. 2001). The potential habitats of the evergreen broad-leaved species should expand with increasing temperatures, and hence, it might be suggested that evergreen broad-leaved species could be used as indicators of climate change (Iversen 1944). Thus, for a variety of practical purposes, it is necessary to determine the responses of evergreen broad-leaved plant to elevated CO₂ and high temperature.

In Korea, several studies have made on the influence of elevated CO₂ and/or higher temperatures on the growth and physiological responses of crops and herbs, such as rice (Kim and You 2010b), meadow-grass (Hwangbo and Kwak 2001), cabbage and radish (Choi et al. 2011) as well as native and invasive plants (Kim and You 2010a, Shin et al. 2012), etc. However, within Korea there has been no such study that tried to determine the ecophysiological responses of evergreen woody species to elevated CO₂ and temperature.

Quercus gilva and Q. glauca are evergreen broad-leaved oak species of the warm temperate regions of Korea. Q. glauca is scattered along the southern coasts and islands, but Q. gilva, designated as an endangered plant by the Korean Ministry of Environment, is limited to the lowland mountain regions of Jeju Island in Korea.

We investigated the effects of long-term exposure to elevated CO₂ and increased temperature on these two evergreen broad-leaved oaks species, and measured the physiological responses, including photosynthetic rate, stomatal conductance, transpiration rate, water use efficiency (WUE), N and C content and C:N ratio of treatment and control groups. The objective of this study was to understand the long-term responses of Q. gilva and Q. galuca to increasing ambient CO₂ and temperature.

MATERIALS AND METHODS

CO₂ treatments and experimental plants

This study was conducted in glass greenhouse (12 m length × 7.8 m width × 5 m height). The surface area of control and treatment is 46.8 m² respectively. The control was maintained at ambient CO₂ concentration, which averaged approximately 360 ± 9.2 ppm on a 24-h basis (Fig. 1). Treatment with elevated CO₂ concentration achieved by inputting a small quantity of pure CO₂ through two perforated plastic hoses so as to maintain the concentration at approximately 742.30 ± 16.92 ppm, twice that of the ambient (360.38 ± 9.19 ppm) concentration. CO₂ concentration was monitored by a CO₂ sensor (TEL-7001; Onset Computer, Bourne, MA, USA) at 30-min intervals, and data was stored on data logger (HOBO U12; Onset Computer) to evaluate the stability of the CO₂ concentration in the treatment. The CO₂ treatment lasted from May 2008 to June 2011 (39 months).

The mean temperature in the treatment was about 2.5°C higher than the control (Fig. 1). The air temperature...
Physiological responses of the evergreen oaks to CO₂ and temperature change

Emissive active radiation (PAR) was 400-600 μmol PAR m⁻² s⁻¹. Relative humidity was 70.79 ± 18.56 % in the control greenhouse and 72.04 ± 16.59 % in the treatment greenhouse. There were no significant differences of PAR or relative humidity between control and treatment greenhouses.

The determined physiological parameters included photosynthetic rate (μmol m⁻² s⁻¹), stomatal conductance (mol m⁻² s⁻¹), transpiration rate (mmol m⁻² s⁻¹) and WUE (μmol CO₂/μmol H₂O). WUE was calculated as the ratio between photosynthesis rate and transpiration rate.

Leaf C:N ratio, nitrogen and carbon contents

The samples of leaves were dried for 2 days at 65°C. After the leaves were dried, the samples were pulverized into fine powder with a blender (AKM-369s; Eupa, Seoul, Korea). The nitrogen and carbon contents were determined using an automatic elemental analyzer (Flash EA 1112 series; Thermo Fisher Scientific, Rochester, NY, USA) at the Center for Research Facilities, Chungnam National University. The C:N ratio was calculated as the ratio of carbon content to nitrogen content.

Statistical analysis

The determined effects of elevated CO₂ and temperature on the physiological parameters of two oak species were confirmed via one-way ANOVA, and the statistical

was measured using a thermo recorder (TR-71U; T&D Co., Matsumoto, Japan) at the same height in the control and treatment greenhouses during the study period.

In March 2008, the seeds of two species were sown in pots (diameter 22.5 cm, height 27 cm) filled with equal volumes of sand, and we fertilized 0.5% of the sand weight. We subsequently applied organic fertilizer, which contains an ammonium nitrogen content below 170 mg/L and nitrate nitrogen at a concentration of 150-330 mg/L (Monsanto Korea Inc., Seoul, Korea). The plants were watered twice or three times per week to prevent them from suffering from water stress.

In January 2009, four seedlings of each species was transplanted into larger pots (120 cm × 85 cm × 48 cm) containing sand, in order to remove root growth restrictions.

Gas-exchange measurements

The photosynthetic characteristics of the treatment and control plants of the two species were measured at the vegetative stage, using an LCi Ultra Compact Photosynthesis System (LCi Pro; ADC Bioscientific, Hoddesdon, UK) from 10:00 am to 12:00 pm in June, 2009 and 2011.

Three fully expended leaves from the upper part of the plants, and the current year's growth, were selected from each of the four individuals. All measurements were replicated more than 10 times per leaf section.

The light source utilized natural light, and photosynthetic active radiation (PAR) was 400-600 μmol PAR m⁻² s⁻¹. Relative humidity was 70.79 ± 18.56% in the control greenhouse and 72.04 ± 16.59% in the treatment greenhouse. There were no significant differences of PAR or relative humidity between control and treatment greenhouses.

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Differences between the control and treatment groups were evaluated by Fisher’s least significant difference test as post-hocs, with significance set at $P = 0.05$. Multivariate analysis of variance (MANOVA) was used to analyze the effects of elevated CO$_2$ concentration and temperature, species, term of exposure and their interaction within each species. All statistical analyses were performed at a 0.05 level of confidence with STATISTICA 8 software (Statsoft, Inc., Tulsa, OK, USA).

### Table 1. Overall impacts of elevated CO$_2$ + temperature and term of CO$_2$ exposure on photosynthetic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CO$_2$ + Temperature</th>
<th>Term</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treatment</td>
</tr>
<tr>
<td><strong>2009</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercus gilva</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2.870 ± 0.34</td>
<td>3.412 ± 0.37</td>
</tr>
<tr>
<td>Gs</td>
<td>0.015 ± 0.01</td>
<td>0.012 ± 0.00</td>
</tr>
<tr>
<td>E</td>
<td>0.310 ± 0.05</td>
<td>0.436 ± 0.02</td>
</tr>
<tr>
<td>WUE</td>
<td>8.644 ± 2.04</td>
<td>7.850 ± 1.00</td>
</tr>
<tr>
<td><strong>2011</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercus gilva</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>3.648 ± 0.45</td>
<td>5.454 ± 0.36</td>
</tr>
<tr>
<td>Gs</td>
<td>0.038 ± 0.01</td>
<td>0.027 ± 0.00</td>
</tr>
<tr>
<td>E</td>
<td>0.550 ± 0.06</td>
<td>0.617 ± 0.05</td>
</tr>
<tr>
<td>WUE</td>
<td>6.743 ± 1.46</td>
<td>8.870 ± 0.67</td>
</tr>
<tr>
<td>Quercus glauca</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>3.648 ± 0.45</td>
<td>5.454 ± 0.36</td>
</tr>
<tr>
<td>Gs</td>
<td>0.038 ± 0.01</td>
<td>0.027 ± 0.00</td>
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</tbody>
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A, photosynthetic rate (μmol m$^{-2}$ s$^{-1}$); Gs, stomatal conductance (mol m$^{-2}$ s$^{-1}$); E, transpiration rate (mmol m$^{-2}$ s$^{-1}$); WUE, water use efficiency (μmol CO$_2$/mmol H$_2$O); ns, non-significant ($P > 0.05$).

Effects of elevated CO$_2$ and temperature on photosynthetic responses

Elevated CO$_2$ and temperature stimulated the photosynthetic rate of both oak species in 2009 and 2011, although the difference in photosynthetic rate of Q. gilva in 2009 between control and treatment did not reach a significance level of $P < 0.05$. The percent stimulation of photosynthesis due to elevated CO$_2$ and temperature for Q. glauca seedlings reached 49.5% in 2009 and 38.8% in 2011. The photosynthetic rate of Q. gilva was not significantly affected by elevated CO$_2$ and temperature in 2009, but increased by 27.9% compared with the control in 2011.

Stomatal conductance was significantly reduced in Q. glauca grown under treatment conditions, but not in Q. gilva. The transpiration rate of Q. glauca was significantly reduced by 40.6%, but that of Q. gilva was not significantly affected by elevated CO$_2$ and temperature in 2009. In contrast, the transpiration rate of both species was significantly increased by 31.5% in 2009 and 81.6% in Q. glauca in 2011. Q. gilva's WUE was not significantly affected by elevated CO$_2$ and temperature in 2009 but was increased by 59.9% compared with control in 2011. Elevated CO$_2$ and higher temperatures resulted in a rise of 31.5% in 2009 and 81.6% in 2011 in Q. glauca.

Effects of terms of CO$_2$ exposure on photosynthetic responses

After long-term exposure to elevated CO$_2$ and temperature, the photosynthetic rate of Q. gilva was decreased by 45.8%, but that of Q. glauca was not significantly affected (Tables 1 and 2). Stomatal conductance of Q. glauca was reduced by 21.7%, but that of Q. gilva was not significantly affected by long-term exposure to CO$_2$ and temperature.
The transpiration rate of both oak species was largely decreased after long-term exposure to elevated CO2 and temperature. WUE of *Q. gilva* was not significantly affected by elevated CO2 and temperature, while that of *Q. glauca* was increased by 56.6%.

**Effects of elevated CO2 and temperature on leaf C:N ratio, nitrogen and carbon contents**

Elevated CO2 and temperature resulted in a decline in leaf nitrogen content for two oak species throughout this study (Table 3 and Fig. 2). The nitrogen content for *Q. gilva* under treatment decreased by 34% in 2009 and 36.4% in 2011, when compared to that of the control. For *Q. glauca*, the nitrogen content was found to be increased by 21.6% in 2009, and 27.4% in 2011, when compared to those grown in the control. The carbon content of *Q. gilva* was not found to be significantly affected by elevated CO2 and temperature in either 2009 or 2011. However, within *Q. glauca*, elevated CO2 and temperature caused a significant increase in leaf carbon content in 2011 but not in 2009. C:N ratio increases due to elevated CO2 and temperature for *Q. gilva* and *Q. glauca* reached 52.5% and 57.3% in 2009, and 22.3% and 42.3% in 2011, respectively.

**Effects of term of CO2 exposure on leaf C:N ratio, nitrogen and carbon contents**

The pooled data for *Q. gilva* showed significant term of exposure effects of nitrogen, carbon content and C:N ratio, although the difference in carbon content of seedlings grown at elevated CO2 and temperature between in 2009 and 2011 did not reach a significance level of \( P < 0.05 \) (Table 3 and Fig. 2). After long-term exposure to elevated CO2 and temperature, the nitrogen content of *Q. gilva* declined by 10.1%, with an associated C:N ratio increase of

### Table 2. Effects of elevated CO2 + T and term of CO2 exposure and their interactions on photosynthetic parameters of *Quercus gilva* and *Q. glauca*

<table>
<thead>
<tr>
<th>Factor</th>
<th>A F</th>
<th>p</th>
<th>Gs F</th>
<th>P</th>
<th>E F</th>
<th>p</th>
<th>WUE F</th>
<th>p</th>
</tr>
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<tr>
<td><em>Quercus gilva</em></td>
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</tr>
<tr>
<td>CO2 + T (A)</td>
<td>5.843</td>
<td>&lt;0.020</td>
<td>3.850</td>
<td>ns</td>
<td>5.180</td>
<td>&lt;0.028</td>
<td>1.231</td>
<td>ns</td>
</tr>
<tr>
<td>Term (B)</td>
<td>58.311</td>
<td>&lt;0.000</td>
<td>1.351</td>
<td>ns</td>
<td>146.684</td>
<td>&lt;0.000</td>
<td>0.236</td>
<td>ns</td>
</tr>
<tr>
<td>A × B</td>
<td>0.125</td>
<td>ns</td>
<td>0.012</td>
<td>ns</td>
<td>41.694</td>
<td>&lt;0.000</td>
<td>8.625</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td><em>Quercus glauca</em></td>
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</tr>
<tr>
<td>CO2 + T (A)</td>
<td>23.785</td>
<td>&lt;0.000</td>
<td>13.302</td>
<td>&lt;0.000</td>
<td>0.162</td>
<td>ns</td>
<td>35.283</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>Term (B)</td>
<td>0.490</td>
<td>ns</td>
<td>11.638</td>
<td>&lt;0.001</td>
<td>28.607</td>
<td>&lt;0.000</td>
<td>20.090</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>A × B</td>
<td>0.372</td>
<td>ns</td>
<td>0.281</td>
<td>ns</td>
<td>4.356</td>
<td>&lt;0.043</td>
<td>9.366</td>
<td>&lt;0.004</td>
</tr>
</tbody>
</table>

A, photosynthetic rate (μmol m⁻² s⁻¹); Gs, stomatal conductance (mol m⁻² s⁻¹); E, transpiration rate (mmol m⁻² s⁻¹); WUE, water use efficiency (μmol CO2/mmol H2O); ns, non-significant (\( P > 0.05 \)).
grown at elevated CO2 concentration was 40% lower than for those grown at ambient CO2 concentrations. The CO2-induced enhancement of photosynthesis of *C. aurantium* was shown to have declined after 14 year of exposure to elevated CO2 (Adam et al. 2004) and photosynthetic down-regulation was reported for 1-year-old foliage of *P. taeda* trees exposed to elevated CO2 for 9 years (Crous et al. 2008).

The down-regulation of photosynthesis of plants grown in elevated CO2 and temperature is often associated with starch accumulation (Tissue et al. 1993), reduction in rubisco content and activity (Albert et al. 2011), acclimation of photosynthetic capacity, such as the maximum carboxylation rate and the maximum rate of electron transport (Ainsworth and Rogers 2007), and, in particular, with leaf N content (Chaturvedi et al. 2009).

Many researchers have reported changes in the nitro-

14.4% (Fig. 3).

For *Q. glauca*, however, the term of exposure effects only caused a significant increase in leaf carbon content, but not in nitrogen content and C:N ratio (Table 3 and Fig. 3). The leaf carbon content of *Q. glauca* was about 4% increased, but nitrogen content and C:N ratio was not significantly affected by long-term of exposure.

**DISCUSSION**

Down-regulation of photosynthesis was observed for *Q. gilva* after long-term of exposure to elevated CO2 and temperature (Table 1), similar to the general decreased investment in photosynthesis that has been seen in other species (DeLucia et al. 1985). According to Faria et al. (1996), the net photosynthetic rate of *Q. suber* seedlings grown at elevated CO2 concentration was 40% lower than for those grown at ambient CO2 concentrations. The CO2-induced enhancement of photosynthesis of *C. aurantium* was shown to have declined after 14 year of exposure to elevated CO2 (Adam et al. 2004) and photosynthetic down-regulation was reported for 1-year-old foliage of *P. taeda* trees exposed to elevated CO2 for 9 years (Crous et al. 2008).

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Table 3. Effects of elevated CO2 + T, term of CO2 exposure and their interactions on leaf nitrogen content, carbon content and C:N ratio for *Quercus gilva* and *Q. glauca*

<table>
<thead>
<tr>
<th>Factor</th>
<th>Nitrogen content</th>
<th>Carbon content</th>
<th>C:N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>F</em></td>
<td><em>P</em></td>
<td><em>F</em></td>
</tr>
<tr>
<td><em>Quercus gilva</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO2 + T (A)</td>
<td>443.71</td>
<td>&lt;0.000</td>
<td>0.40</td>
</tr>
<tr>
<td>Term (B)</td>
<td>11.39</td>
<td>&lt;0.010</td>
<td>25.20</td>
</tr>
<tr>
<td>A × B</td>
<td>0.39</td>
<td>ns</td>
<td>0.10</td>
</tr>
<tr>
<td><em>Quercus glauca</em></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>CO2 + T (A)</td>
<td>46.885</td>
<td>&lt;0.000</td>
<td>16.80</td>
</tr>
<tr>
<td>Term (B)</td>
<td>0.762</td>
<td>ns</td>
<td>35.40</td>
</tr>
<tr>
<td>A × B</td>
<td>0.623</td>
<td>ns</td>
<td>9.00</td>
</tr>
</tbody>
</table>

*ns*, non-significant (*P* > 0.05).
Physiological responses of the evergreen oaks to CO₂ and temperature change

...gen contents of plant parts under such conditions (e.g., Cotrufo et al. 1998). The prolonged exposure to elevated CO₂ concentration and temperature a leads to reduction in leaf nitrogen content associated with a down-regulation of photosynthesis (Kuehny et al. 1991). The strong relationship between net CO₂ assimilation and leaf N based on mass is a principal functional relationship frequently utilized for predicting photosynthesis in many current models of plants at high CO₂ levels (Liu et al. 2009) because the light capture, electron transport and the carbon metabolism portion of photosynthesis require large investments of nitrogen in the form proteins (Crous et al. 2010). According to Adam et al. (2004), the photosynthetic acclimation of C. aurantium has resulted in a reduced rubisco protein content relative to leaf N content after 14 years of exposure to elevated CO₂ concentration. We identified significant effects of elevated CO₂ + T and term of exposure on photosynthetic rates and leaf N contents of Q. gilva (Tables 2 and 3). The CO₂ assimilation and leaf nitrogen content of Q. gilva were reduced by 45.8% and 7.9%, respectively, after long-term exposure to elevated CO₂ concentration and temperature (Table 1 and Fig. 3). Thus, the marked down-regulation of photosynthesis in seedlings of Q. gilva may be caused by reduction in leaf N content after long-term exposure to elevated CO₂ and temperature.

The instantaneous photosynthetic rate of Q. gilva under treatment was greater than for those under control, despite the down-regulation of photosynthesis (Table 1). In this study, although the photosynthetic rate of Q. gilva was decreased by 45.8% after long-term exposure to elevated CO₂ and temperature, it was higher in the treatment than in the control in 2009 and 2011 respectively. Unlike Q. gilva, Q. glauca seem to have different photosynthetic response to term of elevated CO₂ and temperature exposure (Table 2). The photosynthetic rate of Q. glauca seedlings grown under treatment was higher than under control in 2009 and 2011, but there was no significant difference between short- and long-term of exposure to elevated CO₂ and temperature (Table 1). According to Herrick and Thomas (2001), the net CO₂ assimilation of Liquidambar styraciflua trees did not show down-regulation for 3 year. During the seventh year of exposure to elevated CO₂ at the Duke free air CO₂ enrichment (FACE) experiment, light-saturated net photosynthetic rates of the understory tree species were increased by elevated CO₂, and showed no evidence of photosynthetic down-regulation for any species (Springer and Thomas 2007).

One of the most consistent effects of growth at elevated CO₂ is a decrease in stomatal conductance (Ainsworth and Rogers 2007), leading to decreased transpiration rate and improved WUE (Leakey et al. 2009).

Significant elevated CO₂ + T and term of exposure effects on stomatal conductance were detected in Q. glauca but not in Q. gilva (Table 2). The stomatal conductance in seedlings of Q. glauca decreased by 27.6% and 26.7% in 2009 and 2011, respectively, for those under treatment compared to the control, respectively. Besides, it determined that the long-term exposure of elevated CO₂ and temperature significantly reduced stomatal conductance (Table 1). Tjoelker et al. (1998) determined that proportional declines in stomatal conductance in plants grown under elevated CO₂ compared to ambient CO₂ were about 25% for Populus tremuloides, Larix laricina, and Picea mariana, and about 10% for Betula papyrifera and Pinus banksiana.

The pooled data of Q. glauca showed significant term of exposure effects on transpiration rate and WUE, as well as stomatal conductance (Table 2). After a long-term of exposure to elevated CO₂ and temperature, the decline in stomatal conductance of Q. glauca caused a 48% reduction in transpiration rate and a 56.6% increase in WUE (Table 1). This improved WUE under elevated CO₂ and temperature is likely due to lower stomatal conductance and transpiration, however, Q. gilva showed no such effects (Tables 1 and 2).

At the beginning of this study, elevated CO₂ and temperature stimulated transpiration rates of both oak species, although, for Q. glauca, the difference in transpiration rate between treatment and control did not reach a significance level of P < 0.05. In the 3rd year, the transpiration rate of Q. gilva and Q. glauca was reduced by 26.4% and 23.6%, respectively (Table 1).

Higher temperatures increase transpiration and stomatal conductance by changing the vapor pressure deficit at the leaf surface. According to Valle et al. (1984), the transpiration of trees grown with high CO₂ was similar that of ambient CO₂ because the increases in leaf resistance caused by elevated CO₂ were partially offset by increases in vapor pressure gradient between leaf surface and air, caused by increased transpiration. Also, stomatal conductance of the Yucca species was found to be increased under ambient CO₂ when high temperature was applied (>45°C) whereas those grown under elevated CO₂ did not increase stomatal conductance when exposed to high temperatures (Huxman et al. 1998), and furthermore, elevated CO₂ largely offset the interactive effects of high temperature on water relations and photosynthesis in seedlings of Larrea tridentata, an evergreen shrub (Smith et al. 1997).
Our results suggest that two species, *Q. gilva* and *Q. glauca*, may respond more strongly to high temperature than to elevated CO₂ concentration for the period of the experiment (1 year). However, after long-term exposure to elevated CO₂ and temperature, elevated CO₂ may largely offset the interactive effects of high temperature on stomatal conductance, transpiration and WUE.

A well known response of plants to elevated CO₂ and temperature is the production of leaves with a higher C:N ratio. Increased C:N ratio is caused by a relative reduction of nitrogen content and/or an increase in carbon content (Curtis 1996). In our study, the leaves of *Q. gilva* had a reduced nitrogen content and high C:N ratio after long-term exposure to elevated CO₂ and temperature. Inauen et al. (2012) found that the C:N ratio of nine perennial plant species was increased by an average of 7% under elevated CO₂ treatment, prescribing the enhanced C:N ratio as being mainly due to an overall 10% lower leaf nitrogen concentration.

Unlike *Q. gilva*, the nitrogen content in leaves of *Q. glauca* was not significantly affected by elevated CO₂ and temperature. Norby et al. (1999) reported varying results, ranging from a decrease of 35% to an increase of 20% for the leaves of trees grown in open-top chambers. This means that the effect of increased CO₂ and temperature on leaf nitrogen content tends to be species-specific. Within our results, there were significant species-specific differences in the leaf nitrogen, as well as carbon content and C:N ratio of leaves.

Plant responses to elevated CO₂ and temperature are generally species-specific. We found that *Q. gilva* and *Q. glauca* responded differently to elevated CO₂ and temperature, and the responses of most physiological parameters were significantly different among each species (Table 4).

After long-term exposure to elevated CO₂ and temperature, no change in the photosynthetic rate of *Q. gilauca* was observed, but the endangered plant, *Q. gilva* showed a photosynthetic down-regulation as well as a reduced leaf N content. The acquisition of carbon and energy through photosynthetic process is an important aspect of plant performance. Thus, if down-regulation of photosynthesis limits the ability to acquire carbon and energy, the growth and development of *Q. gilva* should be negatively impacted under future high-CO₂ levels, whereas it is expected that the impact upon *Q. glauca* would be significantly less.

Unlike *Q. gilva*, the improved water status of *Q. glauca* under elevated CO₂ and temperature was likely due to increased WUE resulting from lower stomatal conduc-

| Factor | A Gs E WUE N C C:N | F | P | F | P | F | P | F | P | F | P | F | P | F | P |
|--------|-------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CO₂ + T (A) | 29.482 | 0.000 | 223.71 | <0.000 | 17.145 | <0.000 | 113.725 | <0.000 | 40.57 | 0.000 | 20.985 | <0.000 | 8.10 | 0.001 | <0.000 |
| Species (B) | 113.725 | <0.000 | 223.71 | <0.000 | 115.691 | <0.000 | 81.611 | <0.000 | 81.857 | <0.000 | 5.46 | <0.000 | 5.96 | <0.000 | 5.96 | <0.000 |
| Term (C) | 20.282 | <0.000 | 12.486 | <0.000 | 12.486 | <0.000 | 83.857 | <0.000 | 83.857 | <0.000 | 1.53 | <0.000 | 1.53 | <0.000 | 1.53 | <0.000 |
| A × B | 20.282 | <0.000 | 12.486 | <0.000 | 12.486 | <0.000 | 83.857 | <0.000 | 83.857 | <0.000 | 1.53 | <0.000 | 1.53 | <0.000 | 1.53 | <0.000 |
| A × C | 8.759 | <0.000 | 4.669 | <0.000 | 4.669 | <0.000 | 8.10 | <0.000 | 8.10 | <0.000 | 2.30 | <0.000 | 2.30 | <0.000 | 2.30 | <0.000 |
| B × C | 0.497 | <0.000 | 0.263 | <0.000 | 0.263 | <0.000 | 0.263 | <0.000 | 0.263 | <0.000 | 0.49 | <0.000 | 0.49 | <0.000 | 0.49 | <0.000 |
| A × B × C | 0.118 | <0.000 | 0.152 | <0.000 | 0.152 | <0.000 | 0.152 | <0.000 | 0.152 | <0.000 | 0.10 | <0.000 | 0.10 | <0.000 | 0.10 | <0.000 |

A photosynthetic rate (μmol m⁻² s⁻¹), Gs, stomatal conductance (mol m⁻² s⁻¹); E, transpiration rate (μmol m⁻² s⁻¹); WUE, water use efficiency (μmol CO₂/mmol H₂O); N, nitrogen content (%); C, carbon content (%); C:N, carbon:nitrogen ratio; ns, non-significant (P > 0.05).
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tance and transpiration. According to Parmesan and Yohe (2003), rare and endangered species with limited climatic ranges or restricted habitat requirements are generally less tolerant to environmental stresses, and have lower phenotypic plasticity in acclimation to a wider range of environmental conditions.

Thus, it is concluded that the *Q. gilva*, might respond more negatively to elevated CO₂ concentrations and temperatures than *Q. glauca*, the non-endangered species. However, more long-term experiments and studies on mature trees under global climate changes is needed, as results obtained from saplings grown in elevated CO₂ and temperature are unlikely to directly translate to mature trees.

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LITERATURE CITED


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