Photosynthetic responses of Scots pine to elevated CO₂ and nitrogen supply: results of a branch-in-bag experiment

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Summary Naturally seeded Scots pine (Pinus sylvestris L.) trees, age 25–30 years, were subjected to two soil-nitrogen-supply regimes and to elevated atmospheric CO₂ concentrations by the branch-in-bag method from April 15 to September 15 for two or three years. Gas exchange in detached shoots was measured in a diffuse radiation field. Seven parameters associated with photosynthetic performance and two describing stomatal conductance were determined to assess the effects of treatments on photosynthetic components. An elevated concentration of CO₂ did not lead to a significant downward regulation in maximum carboxylation rate (V_cmax) or maximum electron transport rate (J_max), but it significantly decreased light-saturated stomatal conductance (g_ssat) and increased minimum stomatal conductance (g_smin). Light-saturated rates of CO₂ assimilation were higher (24–31%) in shoots grown and measured at elevated CO₂ concentration than in shoots grown and measured at ambient CO₂ concentration, regardless of treatment time or nitrogen-supply regime. High soil-nitrogen supply significantly increased photosynthetic capacity, corresponding to significant increases in V_cmax and J_max. However, the combined elevated CO₂ + high nitrogen-supply treatment did not enhance the photosynthetic response above that observed in the elevated CO₂ treatment alone.

Keywords: carboxylation rate, electron transport rate, Pinus sylvestris, stomatal conductance.

Introduction Photosynthetic responses of plants to elevated atmospheric concentrations of CO₂ are complex and depend on many physiological and environmental variables. Despite numerous studies on photosynthetic responses to elevated atmospheric CO₂ concentrations, many uncertainties remain (Cure and Acock 1986, Ziska et al. 1990, 1991, Idso et al. 1991, Pettersson et al. 1992, Dufrêne et al. 1993). Depending on growth conditions and experimental methods, responses of leaf-level photosynthesis to elevated CO₂ concentrations may reflect combined changes in biochemical capacity and leaf morphology. Results from gas exchange and metabolic studies (Farquhar and von Caemmerer 1982, Sharkey 1985, von Caemmerer and Edmondson 1986) suggest that changes in the biochemical capacity of photosynthesis can be induced by Rubisco activity, the rate of RuP₂ regeneration or triosephosphate consumption (Sage et al. 1989, Sage 1990, Stitt 1991). In addition, plants grown under inadequate nutritional supplies are often unable to respond to elevated atmospheric CO₂ concentrations (Eamus and Jarvis 1989, Evans 1989, Arp 1991). Morphological changes in response to elevated CO₂ may include accumulation of carbohydrates as a result of inadequate sink demands for photosynthates (Clough et al. 1981, Sage et al. 1989), changes in leaf thickness and changes in mesophyll cell number (Vu et al. 1989). In principle, well-established models can be used to quantify modifications in these key photosynthetic components (Harley et al. 1992, McKee and Woodward 1994, Mitchell et al. 1995).

The response of trees to elevated CO₂ concentrations may be expected to mirror that of other C₃ species; however, forest tree longevity raises questions about long-term effects about which little is known. For example, it is not known whether short-term enhancement of photosynthesis by elevated CO₂ concentration can be sustained over several growing seasons. For several reasons, most CO₂ enrichment experiments have been confined to short-term studies on juvenile trees (Eamus and Jarvis 1989), but it is not known whether these observations can be extrapolated to mature trees (Cregg et al. 1989). The branch-in-bag method makes it possible to examine the effects of elevated CO₂ concentrations on mature trees without the technical difficulties and expense of subjecting entire trees to the CO₂ treatment (Barton et al. 1993, Dufrêne et al. 1993). The validity of branch-in-bag experiments is based on the finding that branches are relatively autonomous with respect to their carbon budget during the growing season. The assumption that branches do not import carbon but only export it to woody tissues and roots is considered valid for large branches after initial shoot elongation has ceased (Sprugel et al. 1991).

We measured gas exchange in shoots of Scots pine (Pinus sylvestris L.) trees grown in two atmospheric CO₂ concentrations and two soil-nitrogen regimes for two to three years. Specifically, we determined: (1) how long-term elevation of CO₂ concentration affects photosynthetic performance; (2) whether a high soil-nitrogen supply enhances the response of photosynthesis to elevated atmospheric CO₂ concentrations; (3) whether different lengths of exposure (two or three years) to elevated CO₂ concentration lead to differences in photosyn-
thetic capacity; and (4) whether there is a coupled relationship between modifications in photosynthetic components and leaf nitrogen concentration. The main physiological parameters concerning photosynthetic processes were determined by fitting the data to a set of photosynthetic models and to an empirical model of stomatal conductance.

Materials and methods

Experimental design

The experiment was established in a naturally seeded stand of Scots pine near the Mekrijärvi Research Station (62°47′ N, 30°58′ E, 145 m elevation), University of Joensuu, Finland. The soil on the site is sandy with a water retention of 7 mm at field capacity and 3 mm at the wilting point for the top 30-cm layer of soil. The mean density of the pure Scots pine stand was about 2500 stems per hectare. The trees were 25–30 years old and had a mean height of 4–5 m.

Three branches of the third whorl from the stem apex on each tree were chosen for the treatments: one branch was placed in a bag and subjected to an elevated CO$_2$ concentration (Bagged + CO$_2$); one branch was placed in a bag and subjected to ambient CO$_2$ concentration (Bagged); and one branch was left as an unbagged control (Unbagged). Starting in early spring of 1993, six sample trees representing six replicates of each treatment were exposed to the CO$_2$ treatments. In early spring of 1994, a similar experiment was set up with 12 trees per CO$_2$ treatment. Within each CO$_2$ treatment, half of the trees were grown under normal soil conditions of the site (Normal-N), whereas the remaining trees received an improved nitrogen supply (High-N); i.e., 0.7 kg of pure nitrogen in the form NH$_4$NO$_3$ was added to a 4 × 4 m area around each tree.

The design of the branch bags was similar to that described by Barton et al. (1993). Briefly, the bags consisted of a cylindrical wire frame covered with a polyethylene sleeve which was made of PVC plastic (thickness 0.4 mm, protected from deterioration by UV radiation) and had a volume of 0.379 m$^3$ (length 1.65 m, diameter 0.6 m). The sleeve material prevented only a small fraction of radiation (approximately 10%), within the spectral range 400–800 nm, from entering the bags, and had only a small impact on the spectral composition of visible radiation.

Air was supplied to each bag through a 10-cm-diameter duct. Air in the duct was driven by a blower fan (maximum air flow = 190 dm$^3$ s$^{-1}$) that was mounted in a weather-proof housing. A butterfly valve was positioned in the duct, close to the housing, to enable manual setting of the air flow rate which was usually 0.02–0.025 m$^3$ s$^{-1}$.

The CO$_2$ concentration inside the bags providing the elevated CO$_2$ treatment was increased to 670–820 µmol mol$^{-1}$ from April 15 to September 15, by injecting pure CO$_2$ into the duct (Figure 1). The CO$_2$ concentration inside the bags was monitored with a CO$_2$ analyzer (Model 7MB1300-0BA00, Siemens, Germany).

Relative humidity and temperature inside and outside the bags were recorded separately with a probe combining a temperature sensor and a Humicap relative humidity sensor (Model HMP131Y, Vaisala, Vantaa, Finland). During most of the day, temperature and relative humidity inside the branch bags were only slightly higher than ambient values (Figures 2a and 2b) except during short periods of intense sunshine, when temperatures inside the bags exceeded ambient by 4–6 °C (about 10% of the time) and relative humidity inside the bags exceeded ambient by 10–15% (about 20% of the time).
Measurement and analysis of gas exchange

Measurements of CO₂ exchange in a diffuse radiation field were conducted in the laboratory with an automatic open-gas-exchange system as described by Wang (1996).

A one-year-old shoot was excised under water and transported to the laboratory. After the shoot had been recut under water, a 10-cm-long section of shoot with only 30 needle fascicles (surplus needles had been thinned evenly one week before conducting the measurements to minimize shoot structure effects) was sealed in the assimilation cuvette. The base of the shoot was inserted in a tube providing a constant water supply throughout the measurements.

Gas-exchange measurements were conducted on two shoots from each branch bag through July and August 1995. Photosynthetic parameters were first estimated on the basis of a single branch bag. Then the mean of the estimated parameters from the six replicated branch bags representing the same treatment was used to analyze the results. During all measurements, air temperature in the assimilation cuvette was maintained at 20 ± 0.5 °C and water vapor pressure deficit remained below 0.6 kPa, except during measurements to estimate stomatal conductance parameters. Gas-exchange parameters were calculated by the method of von Caemmerer and Farquhar (1981). Rate of net assimilation (Aᵣ) was based on projected needle area measured with a leaf-area meter (LI-3100, Li-Cor, Inc., Lincoln, NE).

Immediately after the gas-exchange measurements, all needles were removed from the shoot, after which their projected area, fresh weight, and weight after drying at 75 °C were determined. In addition, their nitrogen concentration was determined by the micro-Kjeldahl method.

Estimation of photosynthetic parameters

Three sets of response curves were obtained for each shoot. First, slopes of Aᵣ versus the calculated intercellular concentration of CO₂ (Aᵣ/Cᵣ curve) at photon flux densities (Qₛ) of 150 and 700 μmol m⁻² s⁻¹ were determined by least-squares linear regression. Respiration rate in light (R_d) and CO₂ compensation point in the absence of nonphotorespiratory respiration (Γ*) were estimated from the intercept of the two slopes, as described by Brooks and Farquhar (1985). Representative examples of Aᵣ/Cᵣ curves, measured at the two Qₛ values used to estimate R_d and Γ*, are shown in Figure 3.

Then Aᵣ/Cᵣ curves were measured at nearly saturating Qₚ (1500 μmol m⁻² s⁻¹). Based on the estimated R_d and Γ*, Equation 1 was fitted to each Aᵣ/Cᵣ curve. The maximum RuP₂-saturated rate of carboxylation (Vᵢ max) was estimated for each branch bag:

\[ Aᵣ = \frac{Vᵢ max(Cᵣ - Γ*)}{Cᵣ + Kᵣ(1 + O_i/K_o)} - R_d, \]

where Aᵣ is RuP₂-saturated rate of CO₂ assimilation, O_i is intercellular concentration of O₂ (198 μmol mol⁻¹), and Kᵣ and K_o are the Michaelis constants for carboxylation and oxygenation, respectively. Values of Kᵣ (358.6) and K_o (255.9) at 20 °C were derived from the measurements as described by Badger and Collatz (1977).

Finally, Aᵣ/Qₚ curves were measured at a CO₂ concentration of 2000 ± 10 μmol mol⁻¹. Three parameters, maximum rate of electron transport (Jₘₐₓ), effectivity factor for use of light (q) and convexity factor of light-response curve (θ) were estimated in Equation 2 by the nonlinear least-squares regression techniques described by Ziegler-Jöns and Selinger (1987):
conditions. First, effects of CO₂ assimilation were analyzed in terms of the parameters of the fitted function. Statistical analysis was applied to test the effects of growth exposure, elevated CO₂ concentration and soil-nitrogen supply on each parameter were analyzed (multifactor ANOVA) using the SPSS/PC software package (SPSS, Chicago, IL) based on shoots grown in bags, assuming that significant responses were attributable to the aforementioned treatments, and not to some unknown bagging effects. Treatment time was analyzed as repeated measures during the two growing seasons. Finally, one-way ANOVA was applied to test for the effect of bagging.

Results

Maximum RuP₂-saturated rate of carboxylation, \( V_{\text{cmax}} \), and maximum rate of electron transport, \( J_{\text{max}} \)

Both \( V_{\text{cmax}} \) and \( J_{\text{max}} \) were significantly affected by the High-N treatment (Tables 1 and 2). Compared to the Normal-N treatment, the High-N treatment increased \( V_{\text{cmax}} \) and \( J_{\text{max}} \) on average by 33 and 11%, respectively. Regardless of duration of exposure, elevated CO₂ alone did not significantly affect \( V_{\text{cmax}} \) or \( J_{\text{max}} \), although \( V_{\text{cmax}} \) was depressed 11% after the third growing season. Effects of elevated CO₂ on \( V_{\text{cmax}} \) and \( J_{\text{max}} \) were not enhanced by High-N (Table 2). Bagging resulted in only a slight decrease in \( J_{\text{max}} \) (Table 1).

Carbon dioxide compensation point in the absence of dark respiration, \( \Gamma^* \), and respiration rates

The CO₂ compensation point (\( \Gamma^* \)), and respiration rates in the light (\( R_\text{d} \)) and the dark (\( R_\text{n} \)) were significantly affected by elevated CO₂ (Tables 1 and 2). Compared to the bagged treatment alone, elevated CO₂ concentration decreased \( \Gamma^* \) on average by 9%, and increased \( R_\text{d} \) and \( R_\text{n} \) by 22 and 18%, respectively. Furthermore, only in the case of \( R_\text{n} \) was this increase significantly enhanced by both High-N and treatment time. The High-N treatment resulted in a slight increase in the CO₂-induced increase in \( R_\text{n} \). There was no effect of bagging on any of the three parameters; however, treatment time led to a significant increase (17%) in \( R_\text{n} \) (Table 1).
PHOTOSYNTHETIC RESPONSES IN SCOTS PINE

Table 1. Parameter estimates as functions of treatments. Data are means ± SE of estimated values from six replicated branch bags per treatment. “Bagged + CO₂” are bagged shoots with CO₂ concentration of 700 µmol mol⁻¹. “Bagged” are bagged shoots with CO₂ concentration of 350 µmol mol⁻¹ and “Unbagged” are unbagged shoots with CO₂ concentration of 350 µmol mol⁻¹.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( V_{\text{cmax}} )</th>
<th>( J_{\text{max}} )</th>
<th>( \Gamma^* )</th>
<th>( R_d )</th>
<th>( R_n )</th>
<th>( q )</th>
<th>( \theta )</th>
<th>( g_{\text{min}} )</th>
<th>( g_1 )</th>
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<tbody>
<tr>
<td>After two growing seasons with High-N</td>
<td></td>
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<tr>
<td>Bagged + CO₂</td>
<td>60.3 ± 2.7</td>
<td>172.6 ± 6.1</td>
<td>32.11 ± 2.4</td>
<td>1.090 ± 0.06</td>
<td>1.678 ± 0.04</td>
<td>0.388 ± 0.03</td>
<td>0.87 ± 0.05</td>
<td>53.7 ± 1.3</td>
<td>3.55 ± 0.20</td>
</tr>
<tr>
<td>Bagged</td>
<td>59.8 ± 1.4</td>
<td>169.7 ± 4.5</td>
<td>35.07 ± 1.8</td>
<td>0.918 ± 0.04</td>
<td>1.311 ± 0.07</td>
<td>0.396 ± 0.04</td>
<td>0.74 ± 0.03</td>
<td>47.9 ± 2.1</td>
<td>3.83 ± 0.30</td>
</tr>
<tr>
<td>Unbagged</td>
<td>61.6 ± 2.3</td>
<td>177.1 ± 4.2</td>
<td>35.22 ± 2.1</td>
<td>0.929 ± 0.07</td>
<td>1.347 ± 0.06</td>
<td>0.401 ± 0.06</td>
<td>0.72 ± 0.07</td>
<td>44.6 ± 1.4</td>
<td>3.91 ± 0.34</td>
</tr>
<tr>
<td>After two growing seasons with Normal-N</td>
<td></td>
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</tr>
<tr>
<td>Bagged + CO₂</td>
<td>43.7 ± 2.1</td>
<td>156.3 ± 6.3</td>
<td>31.86 ± 2.2</td>
<td>0.874 ± 0.07</td>
<td>1.203 ± 0.04</td>
<td>0.349 ± 0.02</td>
<td>0.70 ± 0.04</td>
<td>52.8 ± 2.0</td>
<td>3.08 ± 0.26</td>
</tr>
<tr>
<td>Bagged</td>
<td>47.8 ± 2.7</td>
<td>152.8 ± 7.4</td>
<td>34.79 ± 1.9</td>
<td>0.788 ± 0.05</td>
<td>1.112 ± 0.05</td>
<td>0.347 ± 0.03</td>
<td>0.60 ± 0.03</td>
<td>47.2 ± 1.7</td>
<td>3.57 ± 0.21</td>
</tr>
<tr>
<td>Unbagged</td>
<td>45.5 ± 3.2</td>
<td>158.4 ± 4.1</td>
<td>35.00 ± 1.7</td>
<td>0.780 ± 0.08</td>
<td>1.147 ± 0.04</td>
<td>0.350 ± 0.05</td>
<td>0.59 ± 0.08</td>
<td>42.3 ± 1.8</td>
<td>3.56 ± 0.27</td>
</tr>
<tr>
<td>After three growing seasons with Normal-N</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Bagged + CO₂</td>
<td>39.7 ± 3.7</td>
<td>145.2 ± 9.3</td>
<td>30.84 ± 2.4</td>
<td>0.963 ± 0.04</td>
<td>1.474 ± 0.06</td>
<td>0.320 ± 0.07</td>
<td>0.76 ± 0.06</td>
<td>59.6 ± 2.6</td>
<td>2.90 ± 0.23</td>
</tr>
<tr>
<td>Bagged</td>
<td>44.8 ± 3.1</td>
<td>147.6 ± 8.0</td>
<td>34.01 ± 2.0</td>
<td>0.714 ± 0.05</td>
<td>1.239 ± 0.04</td>
<td>0.346 ± 0.04</td>
<td>0.72 ± 0.02</td>
<td>53.2 ± 2.8</td>
<td>2.22 ± 0.31</td>
</tr>
<tr>
<td>Unbagged</td>
<td>47.3 ± 3.4</td>
<td>156.1 ± 6.1</td>
<td>32.99 ± 2.0</td>
<td>0.744 ± 0.06</td>
<td>1.045 ± 0.05</td>
<td>0.354 ± 0.04</td>
<td>0.54 ± 0.07</td>
<td>37.4 ± 4.0</td>
<td>3.44 ± 0.35</td>
</tr>
</tbody>
</table>

Table 2. Summary of F-values from multifactor ANOVA for the effects of growth CO₂ regime and soil-nitrogen supply on photosynthetic parameters, assuming that significant responses were due to CO₂ and nitrogen, and not to some unknown bagging effect. The environmental treatments are CO₂ concentration (ambient [CO₂] (Bagged) and elevated [CO₂] (Bagged + CO₂)), and soil-nitrogen supply (Normal-N and high soil-nitrogen supply (High-N)). Treatment times were treated as repeated measures during two growth seasons. Bagging effects were analyzed by one-way ANOVA. Least significant differences (LSD) was used for the range test. Legend: * \( P < 0.05; **P < 0.001. \)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>( V_{\text{cmax}} )</th>
<th>( J_{\text{max}} )</th>
<th>( \Gamma^* )</th>
<th>( R_d )</th>
<th>( R_n )</th>
<th>( q )</th>
<th>( \theta )</th>
<th>( g_{\text{min}} )</th>
<th>( g_1 )</th>
<th>( A_{\text{sat}} )</th>
<th>( g_{\text{sat}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂ (Bagged and Bagged + CO₂)</td>
<td>1.02</td>
<td>0.97</td>
<td>4.84*</td>
<td>4.99*</td>
<td>5.06*</td>
<td>0.42</td>
<td>1.99</td>
<td>5.08*</td>
<td>6.40*</td>
<td>7.51*</td>
<td>6.25*</td>
</tr>
<tr>
<td>N (Normal-N and High-N)</td>
<td>8.22**</td>
<td>4.30*</td>
<td>1.01</td>
<td>6.34*</td>
<td>4.76*</td>
<td>4.90*</td>
<td>4.23*</td>
<td>1.14</td>
<td>4.28*</td>
<td>5.04*</td>
<td>3.66</td>
</tr>
<tr>
<td>CO₂ × High-N</td>
<td>1.16</td>
<td>1.67</td>
<td>0.69</td>
<td>0.75</td>
<td>1.10</td>
<td>0.88</td>
<td>4.40*</td>
<td>0.87</td>
<td>0.76</td>
<td>4.73*</td>
<td>2.18</td>
</tr>
<tr>
<td>Season (2 and 3)</td>
<td>1.82</td>
<td>2.03</td>
<td>1.44</td>
<td>0.86</td>
<td>5.00*</td>
<td>1.04</td>
<td>3.78*</td>
<td>4.11*</td>
<td>1.03</td>
<td>2.01</td>
<td>0.62</td>
</tr>
<tr>
<td>Bagging</td>
<td>0.87</td>
<td>0.55</td>
<td>0.08</td>
<td>1.10</td>
<td>1.23</td>
<td>0.04</td>
<td>4.01*</td>
<td>5.22*</td>
<td>0.03</td>
<td>1.44</td>
<td>0.87</td>
</tr>
</tbody>
</table>

“Effectivity factor” for the use of light, q, and convexity factor of light-response curve, \( \theta \)

Values of \( \theta \) were significantly increased 23% by High-N, 13% by elevated CO₂ concentration, 14% by time of treatment, and 13% by the bagged treatment (Tables 1 and 2). In contrast, values of \( q \) were significantly increased (by 13%) only by the High-N treatment. The bagged treatment caused a 2% reduction in values of \( q \).

Minimum stomatal conductance, \( g_{\text{min}} \), and the empirical coefficient, \( g_1 \)

Compared with the bagged treatment alone, elevated CO₂ significantly increased \( g_{\text{min}} \) by 12% on average, but this increase was not enhanced by High-N (Tables 1 and 2). Both time of treatment and the bagged treatment significantly increased \( g_{\text{min}} \) by 13 and 20%, respectively. Treatment effects on \( g_1 \) were opposite of those observed on \( g_{\text{min}} \). Thus, elevated CO₂ significantly decreased \( g_1 \) by 10%, whereas High-N significantly increased \( g_1 \) by 11%, and the bagged treatment and treatment time caused slight decreases in \( g_1 \) (Table 2).

Light-saturated rate of assimilation, \( A_{\text{sat}} \), and stomatal conductance, \( g_{\text{sat}} \)

Figure 5 presents \( A_{\text{sat}} \) and \( g_{\text{sat}} \) as functions of the treatments. Compared to bagged shoots alone, elevated CO₂ concentration increased \( A_{\text{sat}} \) by 31, 29 and 24%, and decreased \( g_{\text{sat}} \) by 17, 19 and 16% separately for shoots grown in High-N, Normal-N after two growth seasons (Norm + 2) and Normal-N after three growth seasons (Norm + 3). However, there were no significant differences in \( A_{\text{sat}} \) or \( g_{\text{sat}} \) between bagged shoots and unbagged shoots.

Nitrogen concentration of needles

Regardless of CO₂ concentration and treatment time, High-N resulted in a significant increase (\( P < 0.01 \)) in area-based needle nitrogen concentration, but not in mass-based needle nitrogen concentration (Figure 6). Elevated CO₂ treatment decreased needle nitrogen concentration, but the decrease was only significant (\( P < 0.05 \)) for mass-based needle nitrogen concentration. Neither the bagged treatment nor treatment...
time led to a significant change in needle nitrogen concentration.

Discussion

We assessed whether long-term treatment with elevated CO\textsubscript{2} concentration and high soil-nitrogen supply modifies the photosynthetic capacity of Scots pine. We applied the branch-in-bag technique (Barton et al. 1993), where single branches in the same whorl are subjected to CO\textsubscript{2} treatments or are used to control the effect of the bag itself. By this means it is possible to reduce the effects of genetic and physiological status of the tree on the results. This technique also facilitates study of the effects of elevated CO\textsubscript{2} concentration on physiological and developmental processes in mature trees. However, despite these advantages, the technique subjects only a small fraction of the tree to elevated CO\textsubscript{2} concentrations, and subsequent source–sink relationships could differ greatly from those of whole trees under natural conditions.

Because of the complex structure of a Scots pine shoot, irradiation of needle surfaces is not uniform (Oker-Blom et al. 1992), and the needle area receiving direct radiation is often difficult to determine (Leverenz and Jarvis 1978, Carter and Smith 1985). We attempted to overcome these technical difficulties by using shoots with relative few needles (i.e., needles were thinned evenly to give 30-needle fascicles per sample shoot) so that no point on the needle surface was shaded in all directions under a uniform diffuse radiation field. Therefore, the photon flux density incident on the needle surface was assumed to be equal to the irradiance incident on any surface in the diffuse radiation field (Oker-Blom et al. 1992). Further, the projected area of needles can be measured directly in the laboratory.

The total radiation in the spectral range 400–800 nm entering the bag was about 90% of that outside the bag, implying that bagged branches did not have to acclimate to a substantially altered radiation regime. Therefore, the photon flux density incident on the needle surface was assumed to be equal to the irradiance incident on any surface in the diffuse radiation field (Oker-Blom et al. 1992). Further, the projected area of needles can be measured directly in the laboratory.

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Although branches were exposed to the elevated-CO\textsubscript{2} treatment from April 15 to September 15, a period longer than the average growing season outside the bags, there was no significant downward regulation in the capacity for CO\textsubscript{2} carboxylation ($V_{\text{cmax}}$) or maximum electron transport ($J_{\text{max}}$) regardless of
soil-nitrogen supply and treatment time (Besford and Hand 1989, Sage et al. 1989, Harley et al. 1992, Nie and Long 1992, Van Oosten et al. 1992) (Tables 1 and 2). When measured at the CO$_2$ concentration in which the shoots were grown, the light-saturated rate of CO$_2$ assimilation was higher (24–31%) in shoots grown at elevated CO$_2$ concentration than in shoots grown at current ambient CO$_2$ concentration (Figure 5).

Reasons for this difference in response could be that: (i) only a small part of each tree was subjected to elevated CO$_2$ concentrations; (ii) the trees were in a stage of rapid growth (a big “sink” in other parts of the trees and a high demand for carbohydrates would have accompanied (i) and (ii)); (iii) compared to trees grown in a pot, relatively unrestricted root growth of field-grown trees may help prevent sink limitation and end-product inhibition; and (iv) the presence of active sinks for recently assimilated metabolites may also be important for maintenance of photosynthetic capacity (Stitt 1991, Thomas and Strain 1991). The finding that Scots pine can sustain increased capacity for carboxylation after long-term exposure to elevated CO$_2$ concentrations suggests that this species will exhibit increased growth and carbon storage with future increases in atmospheric CO$_2$ concentration.

Values of $\Gamma^*$ were similar among treatments, except in the elevated CO$_2$ treatment (Table 1). This absence of treatment effects was expected because the value of $\Gamma^*$ depends mainly on the kinetic characteristics of Rubisco (Laisk 1977, Jordan and Ogren 1981, von Caemmerer et al. 1994), which are unlikely to change rapidly (Long and Drake 1991). The average $\Gamma^*$ value of 33.54 $\mu$mol mol$^{-1}$ is close to the value of 35.5 $\mu$mol mol$^{-1}$ found by Brooks and Farquhar (1985) for Spinacia oleracea L. leaves. The relatively low value of $\Gamma^*$ in the elevated CO$_2$ treatment may have resulted from using the $x$ coordinate (intercellular CO$_2$ partial pressure) at the intercept of the two slopes as the value of $\Gamma^*$ (see Figure 2). This estimate ignores the effect of conductance for CO$_2$ transfer from substomatal cavities to the sites of carboxylation ($g_a$), on the value of $\Gamma^*$ (see Brooks and Farquhar 1985 and von Caemmerer et al. 1994, for detailed discussion on measurements of $\Gamma^*$). If valid, this explanation implies that the decrease in $\Gamma^*$ in response to the elevated CO$_2$ treatment was associated with changes in $g_a$ and $R_e$.

Our results showing a significant increase in $R_d$ with elevated CO$_2$ and high soil-nitrogen supply, are consistent with the results of McKee and Woodward (1994), but differ from Thomas et al. (1993) who found no effect. Studies on the long-term effect of elevated CO$_2$ on dark respiration ($R_d$) have shown increases (Hrubec et al. 1985, Poorter et al. 1988), decreases (Reuveni and Gale 1985, Bunce 1990, Wullschleger et al. 1992) or no effect (Gifford et al. 1985, Hrubec et al. 1985) depending on species, age, plant part, or reference units. Wang et al. (1996) have shown that the direction of change in $R_d$ of Scots pine shoots grown in open-top chambers in elevated CO$_2$ depends on both growing conditions and temperature during measurements.

Many studies (e.g., Sharp et al. 1984, Brooks and Farquhar 1985) have shown that $R_d$ is correlated with $R_n$. We observed that different treatments induced similar changes in $R_d$ and $R_n$ (Table 1) resulting in relatively small variations (0.58–0.72) in the ratio of $R_d$ to $R_n$. The mean ratio value of 0.68 was higher than that of 0.34 estimated by Brooks and Farquhar (1985) for Pseudotsuga menziesii, but still within the range (0.25–1.0) measured by others (Peisker et al. 1981, Azcón-Bieto and Osmond 1983).

The elevated CO$_2$ treatment decreased the slope of stomatal functions ($g_1$ in Equation 3), but increased minimum stomatal conductance ($g_{\text{min}}$: Table 1, Figure 4) (cf. Harley et al. 1992). This finding is consistent with the measured decrease in light-saturated stomatal conductance in shoots grown in bags and elevated CO$_2$ (Figure 5), and implies that elevated CO$_2$ could limit stomata adaptability to changing environmental conditions.

Because leaf nitrogen is a major component of Rubisco (Evans 1989), it is often closely correlated with photosynthetic performance (Brix 1981, Matyssek and Schulze 1987). Although there is not a direct relationship between leaf nitrogen concentration and soil nitrogen content, fertilization usually results in increased foliar nitrogen concentrations in Scots pine, perhaps because pine forests commonly occur on nutrient-poor forest soils (Miller et al. 1979, Sheriff et al. 1986, Näsholm and Ericsson 1990, Mitchell and Hinckley 1993). In response to nitrogen fertilization, the increase in area-based needle nitrogen concentration was closely coupled with the increase in light-saturated assimilation rate (Figures 5 and 6), regardless of growing CO$_2$ concentration. Similar responses have been reported by Mitchell and Hinckley (1993) for Pseudotsuga menziesii (Mirb.) Franco.

Long-term exposure to elevated CO$_2$ often alters photosynthetic capacity as a result of nitrogen reallocation from Rubisco to other photosynthetic components, or to nonphotosynthetic processes (Evans 1989, Newton 1991, Stitt 1991, Conroy 1992, Tissue et al. 1993). Such acclimation is variable because it depends on several factors. Compared to the Normal-N, High-N increased maximum carboxylation rate more than maximum electron transport rate (33 versus 11%), indicating that high soil-nitrogen supply had a greater effect on carboxylation rate than on electron transport capacity (cf. Evans and Terashima 1987, Tissue et al. 1993). However, a comparison of the Bagged shoots and Bagged + CO$_2$ shoots indicated that increasing soil-nitrogen supply did not significantly increase the magnitude of photosynthetic response to elevated CO$_2$ concentration, implying that photosynthetic response to elevated CO$_2$ was not limited by soil-nitrogen content at the site.

An analytical model is only as good as the set of assumptions underlying it. Our parameter estimates were based on simplified model assumptions: Rubisco was assumed to be fully activated; and $C_i$ was assumed to equal the C concentration at the site of fixation, which ignores possible liquid resistances between the intercellular space and carboxylation site. The error resulting from this assumption has been estimated by Farquhar and von Caemmerer (1982) (see also Harley et al. 1985). We believe, however, that this approach represents an advance in our ability to understand CO$_2$ exchange kinetics at the whole-leaf level, and that it enhances our ability to make
ecophysiological comparisons among plants acclimated to different environments.

We conclude that two or three years of exposure to elevated CO$_2$ concentrations did not significantly reduce the photosynthetic capacity of Scots pine. However, elevated CO$_2$ concentrations decreased light-saturated stomatal conductance. A high soil-nitrogen supply significantly increased the photosynthetic capacity in Scots pine as a result of significant increases in maximum carboxylation rate, $V_{\text{max}}$, and maximum electron transport rate, $J_{\text{max}}$. However, high soil-nitrogen supply did not increase photosynthetic response to elevated CO$_2$ concentration.

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References


## Appendix

<table>
<thead>
<tr>
<th>Symbols</th>
<th>Unit</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>$A_n$</td>
<td>$\mu\text{mol m}^{-2}\text{s}^{-1}$</td>
<td>Net assimilation rate of foliage per unit leaf area (projected area)</td>
</tr>
<tr>
<td>$A_{nc}$</td>
<td>$\mu\text{mol m}^{-2}\text{s}^{-1}$</td>
<td>Rubisco-limited rate of net assimilation</td>
</tr>
<tr>
<td>$A_{nj}$</td>
<td>$\mu\text{mol m}^{-2}\text{s}^{-1}$</td>
<td>RuP$_2$-limited rate of net assimilation</td>
</tr>
<tr>
<td>$A_{sat}$</td>
<td>$\mu\text{mol m}^{-2}\text{s}^{-1}$</td>
<td>Light-saturated rate of assimilation</td>
</tr>
<tr>
<td>$C_a$</td>
<td>$\mu\text{mol mol}^{-1}$</td>
<td>Molar fraction of CO$_2$ in the air inside cuvette</td>
</tr>
<tr>
<td>$C_s$</td>
<td>$\mu\text{mol mol}^{-1}$</td>
<td>Molar fraction of CO$_2$ at surface of leaf</td>
</tr>
<tr>
<td>$C_i$</td>
<td>$\mu\text{mol mol}^{-1}$</td>
<td>Intercellular concentration of CO$_2$</td>
</tr>
<tr>
<td>$g_{\text{min}}$</td>
<td>mmol m$^{-2}$ s$^{-1}$</td>
<td>Minimum stomatal conductance to water vapor</td>
</tr>
<tr>
<td>$g_1$</td>
<td>dimensionless</td>
<td>An empirical coefficient</td>
</tr>
<tr>
<td>$g_s$</td>
<td>mmol m$^{-2}$ s$^{-1}$</td>
<td>Stomatal conductance to water vapor</td>
</tr>
<tr>
<td>$g_{\text{sat}}$</td>
<td>mmol m$^{-2}$ s$^{-1}$</td>
<td>Light-saturated stomatal conductance to water vapor</td>
</tr>
<tr>
<td>$Q_p$</td>
<td>$\mu\text{mol m}^{-2}\text{s}^{-1}$</td>
<td>Incident photon flux density</td>
</tr>
<tr>
<td>$J_{\text{max}}$</td>
<td>$\mu\text{mol m}^{-2}\text{s}^{-1}$</td>
<td>Maximum rate of electron transport</td>
</tr>
<tr>
<td>$K_c, K_o$</td>
<td>$\mu\text{mol mol}^{-1}$</td>
<td>Michaelis constants for CO$_2$, O$_2$</td>
</tr>
<tr>
<td>$O_i$</td>
<td>$\mu\text{mol mol}^{-1}$</td>
<td>Intercellular concentration of O$_2$</td>
</tr>
<tr>
<td>$R_d$</td>
<td>$\mu\text{mol m}^{-2}\text{s}^{-1}$</td>
<td>Respiration rate in light</td>
</tr>
<tr>
<td>$R_n$</td>
<td>$\mu\text{mol m}^{-2}\text{s}^{-1}$</td>
<td>Respiration rate in dark</td>
</tr>
<tr>
<td>Rubisco</td>
<td>dimensionless</td>
<td>Ribulose 1,5-bisphosphate carboxylase/oxygenase</td>
</tr>
<tr>
<td>RuP$_2$</td>
<td>dimensionless</td>
<td>Ribulose 1,5-bisphosphate</td>
</tr>
<tr>
<td>$V_{\text{cmax}}$</td>
<td>$\mu\text{mol m}^{-2}\text{s}^{-1}$</td>
<td>Maximum RuP$_2$ saturated rate of carboxylation</td>
</tr>
<tr>
<td>$D_s$</td>
<td>kPa</td>
<td>Leaf-to-air vapor pressure difference at leaf surface</td>
</tr>
<tr>
<td>$\Gamma^*$</td>
<td>$\mu\text{mol mol}^{-1}$</td>
<td>CO$_2$ compensation point in the absence of dark respiration</td>
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<tr>
<td>$\theta$</td>
<td>dimensionless</td>
<td>Convexity factor of light-response curve</td>
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<td>$q$</td>
<td>electron quanta$^{-1}$</td>
<td>Effectivity factor for the use of light</td>
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