Effects of nitrogen on Pinus palustris foliar respiratory responses to elevated atmospheric CO₂ concentration

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Abstract

Indirect effects of atmospheric CO₂ concentration [CO₂], on longleaf pine (Pinus palustris Mill.) foliage respiration were studied by growing trees in a factorial arrangement of low and high [CO₂] (369 and 729 µmol CO₂ mol⁻¹) and low and high N (40 and 400 kg ha⁻¹ yr⁻¹). Direct effects of [CO₂] on leaf respiration were tested by measuring respiration rates of foliage from all treatments at two CO₂ levels (360 and 720 µmol CO₂ mol⁻¹) at the time of measurement. Elevated CO₂ did not directly or indirectly affect leaf respiration when expressed on a leaf area or mass basis, but a significant increase in respiration per unit leaf N was observed in trees grown in elevated [CO₂] (indirect response to elevated [CO₂]). The lack of a [CO₂] effect on respiration, when analysed on an area or mass basis, may have resulted from combined effects of [CO₂] on factors that increase respiration (e.g. greater availability of non-structural carbohydrates stimulating growth and carbon export from leaves) and on factors that decrease respiration (e.g. lower N concentration leading to lower construction costs and maintenance requirements). Thus, [CO₂] affected factors that influence respiration, but in opposing ways.

Key words: Pinus palustris, elevated CO₂, nitrogen, foliar respiration.

Introduction

Due to burning of fossil fuels and changes in land use world-wide, atmospheric CO₂ concentration [CO₂], has increased during the past 100 years at a rate that may be unprecedented in the historical records for the past 160,000 years (Raynaud et al., 1993; Sundquist, 1993). [CO₂] affects a wide range of plant functions, both directly and indirectly, and so increasing atmospheric [CO₂] may alter plant function and community structure (Field et al., 1992). However, predicting the direction, much less the magnitude, of changes in plant function is difficult since there is a lack of understanding of the mechanisms that control overall plant response to [CO₂] (Mooney, 1991). Most studies of plant responses to elevated [CO₂] have focused on photosynthesis and growth of crops and trees. (Strain, 1987; Bowes, 1993; Rogers et al., 1994). Much less effort has been directed to understanding respiratory responses to elevated [CO₂], even though respiration may consume more than 50% of the carbon fixed at the whole plant level (Amthor, 1989), and despite evidence suggesting that CO₂ may directly and indirectly influence respiration (see Amthor, 1991, 1995; Ryan, 1991; Wullschleger et al., 1994, for recent reviews). The extent to which respiration is increased or decreased by elevated [CO₂] is not known, however, extent results conflict, in part, because of non-conformity in methods used to quantify respiration and the manner in which respiration rate has been expressed (Ryan, 1991; Wullschleger et al., 1994). Also, respiration is often measured only at one point in time, although variation in respiration has been observed due to plant ontogeny (Poorter et al., 1992; Mousseau, 1993). Farrar and Williams (1991) suggest that solitary monitoring of leaf or whole plant respiration are of little use in understanding respiratory responses and their relationship to growth; rather, time-course

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studies are needed. Furthermore, the procedure by which respiration is measured may complicate comparisons of different studies. Several investigators have determined respiration rates by shading the plant or leaf during the day (Ziska et al., 1990) or by extrapolating respiratory rates from light response curves (Hickleton and Jolliet, 1978; Nijs et al., 1988). These estimates of respiratory rates may differ substantially from steady-state night-time respiration (Wullschleger et al., 1994), possibly as a result of alteration in diurnal sink-source relationships. Additional complications can arise because the [CO2] during a measurement may affect respiration rate. For example, Amthor et al. (1992) found that apparent respiration rate was inhibited 20–30% by a 200 μmol mol−1 increase in [CO2] during a respiration measurement. This effect was readily reversible, i.e. a decrease in [CO2] during a measurement increased respiration rate. Although this response to short-term changes in [CO2] at the time of measurement has been commonly observed (Begg and Jarvis, 1968; Gale, 1982; Revenu and Gale, 1985; Bunce, 1980, 1992; El Kohen et al., 1991; Moussau, 1993; Ziska and Bunce, 1993, 1994; Downton and Grant, 1994; Villar et al., 1994), it is unknown whether changes in apparent respiration represent a direct effect of [CO2] on respiration per se or reflect the influence of [CO2] on other metabolic reactions such as dark fixation of HCO3 by PEPease (Amthor, 1995).

Difficulty in comparing studies of respiratory responses to elevated [CO2] is exacerbated by variability in the manner in which respiration rates are expressed. That is, respiration rates have been expressed in numerous ways (e.g. per unit dry mass, leaf area, and protein content) and long-term elevated [CO2] can alter relationships between plant mass, leaf area, and protein content. For example, elevated [CO2] often increases plant C:N ratio (Norbey et al., 1986, 1992; Field et al., 1992) and, thus, may alter relationships between respiration per unit dry mass and respiration per unit N or protein.

Although recent work has indicated that effects of [CO2] on photosynthesis and productivity may be mediated by nutrients (Larigaudeire et al., 1988; Eamus and Jarvis, 1989; Bazza, 1990; Fager et al., 1992; Griffin et al., 1993), the extent to which elevated [CO2] increases N use efficiency, thus ameliorating N deficiencies, is controversial (see Norbey et al., 1986; Coleman et al., 1991; Hillert et al., 1991, for contrasting views). Moreover, even though the availability of N has been shown to elicit strong respiratory responses (Ryan, 1991), little effort has been given to determining how soil fertility may influence respiratory responses to elevated [CO2].

This study examined the respiratory response of long-leaf pine (Pinus palustris Mill.) foliage from trees grown in ambient and elevated atmospheric [CO2] and at two levels of soil N. All respiration measurements were conducted at night in the dark. Two [CO2] (360 and 720 μmol CO2 mol−1) were used during measurements to assess any direct effects of [CO2] on respiration rate. Also, effects of N on respiration and respiratory responses to [CO2] were quantified. Results are reported per unit dry mass, leaf surface area and foliar N content.

Materials and methods

Longleaf pine (Pinus palustris Mill.) seedlings, from a wild seed source, were lifted from a Florida nursery in February 1993. Seedlings were stored (5°C) for less than 1 week, graded (from collar diameter mean = 13 mm; standard deviation = 2 mm; range = 9 to 21 mm), and planted (3 per pot) into each of 192, 45.7 black plastic pots containing construction grade sand (pH 5.1) which was low in mineral elements. Seedlings were irrigated with deionized water as needed.

Seedlings were exposed to target [CO2] of 365 μmol CO2 mol−1 (ambient) or 720 μmol CO2 mol−1 (elevated) within an open top chamber system similar to that described by Rogers et al. (1983). The chambers, which are 3 m in diameter and 2.4 m in height, are constructed of a structural aluminum frame covered by a clear PVC plastic film (0.2 mm thickness). Carbon dioxide is supplied from a 12.7 Mq liquid CO2 vower through a high volume dispensing manifold and is vented to the chambers by efflux into plexus boxes (Rogers et al., 1983). Air is dispersed into each chamber through the bottom half of each chamber over which is double-walled, the inside wall is performed with 2.5 cm diameter holes to serve as ducts that distribute air uniformly into the chamber. Carbon dioxide concentration in each chamber are monitored 24 h−1 using a time-averaged sampling system. Time-averaged samples were collected to an infra-red gas analyzer which recorded an instantaneous value at the end of that sampling period (Rogers et al., 1983). Carbon dioxide exposure was initiated on 30 March 1993, and [CO2] values were continuously recorded every 15–30 min for each chamber, depending upon whether or not additional CO2 study was on line. Average ambient [CO2] (standard deviation) through the duration of the experiment was 368.8 μmol mol−1 (±17.1) during the day (7 a.m. to 7 p.m.) and 403.1 (±34.4) during the night, Average [CO2] in the elevated chambers was 728.9 μmol mol−1 (±21.2) during the day and 792.9 (±53.5) during the night. In order to maintain temperature and relative humidity of ambient, three chamber volumes were exchanged every minute.

Nitrigen treatments were similar to those described by Bazza and Miao (1993). They consisted of applying either 0.20 or 0.02 mg N g−1 soil per yea (slightly-coated urea; 28–0–0 [N-P-K], which correspond to high (400 kg N ha−1 yr−1) and low (40 kg N ha−1 yr−1) N treatments. Nitrigen treatments were maintained at non-limiting levels in all pots by application of sulphur-coated potassium, N-P-K, 0.04 kg K2SO4, 0.02 kg S, and 0.007 kg Fe2O3. Iron chloride (0.007 mg Fe2O3 per pot) was applied once in April 1995.

Treatments were arranged in a 2 x 2 factorial design with eight replications. Carbon dioxide treatments were randomly assigned to chambers while N treatments were randomly assigned to a total of 16 pots within each chamber. To avoid location effects within a chamber, pot locations were re-randomized monthly.
Respiratory responses to CO₂

Results and discussion

Increasing [CO₂] did not alter apparent respiratory rate of Pinus palustris foliage either directly (cuvette [CO₂]) or indirectly (open top chamber [CO₂]) when expressed on a leaf area or dry mass basis (Table 1). Needle respiration rates averaged 1.04 μmol CO₂ m⁻² s⁻¹ and 10.8 nmoI CO₂ g⁻¹ s⁻¹, respectively. Nitrogen supply strongly influenced respiration rate per unit needle mass, per unit leaf area, and per unit foliar N content (Table 1).

Longleaf pine foliage from trees grown in the high N treatment showed a 45% increase in respiration rate on a leaf area basis and a 56% increase on a dry mass basis when compared to trees grown at the low N treatment. Although respiration (expressed either per unit leaf area or per unit leaf dry mass) demonstrated a significant N by measurement date interaction (data not shown), this interaction was one of magnitude rather than rank (Fig. 1). Respiration rates per unit foliar N were significantly influenced by [CO₂], N treatment, and their interaction (Fig. 2). Long-term elevated atmospheric [CO₂] resulted in greater respiration rates per unit foliar N.

Table 1. Effects of long-term (open top chamber) [CO₂] (indirect), nitrogen, and short-term (cuvette) [CO₂] (direct) treatments on CO₂ efflux from longleaf pine needles expressed per unit: needle area, needle dry mass, and needle N

<table>
<thead>
<tr>
<th>Respiration measurement</th>
<th>Main effect treatment variable</th>
<th>N (kg ha⁻¹ yr⁻¹)</th>
<th>Cuvette [CO₂] (μmol CO₂ mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long-term [CO₂] (μmol CO₂ mol⁻¹)</td>
<td>360</td>
<td>720</td>
<td>Pr &gt; F*</td>
</tr>
<tr>
<td>μmol CO₂ m⁻² s⁻¹</td>
<td>1.04</td>
<td>1.05</td>
<td>0.937</td>
</tr>
<tr>
<td>mmol CO₂ g⁻¹ s⁻¹</td>
<td>10.90</td>
<td>10.66</td>
<td>0.584</td>
</tr>
<tr>
<td>mmol CO₂ mg⁻¹</td>
<td>1.04</td>
<td>1.22</td>
<td>0.007</td>
</tr>
</tbody>
</table>

*Probability of a greater F value by chance for the difference between the CO₂ or N treatments, and for the CO₂ by N interaction, where the interaction was significant.
however, this effect was much greater in the high N treatment than in the low N treatment, even though increasing N availability reduced respiration per unit foliar N. Similarly, N had a greater influence on dark respiration rate of lobolly pine (Pinus taeda L.) shoots than did [CO\(_2\)] under similar [CO\(_2\)] and N supply and with a similar effect of N supply on tissue N concentration (Griffin et al., 1993).

It has been suggested that increased atmospheric [CO\(_2\)] can influence plant respiration directly (i.e. a response to [CO\(_2\)] within minutes that is reversible) and indirectly (Amthor, 1991). The indirect response might be mediated by altered N or carbohydrate content which, in turn, regulate growth and respiration rates. A common response to short-term step changes in [CO\(_2\)] has been a reduction in respiration rate (Amthor, 1995). Direct effects of [CO\(_2\)] on respiration, however, may be affected by plant ontogeny. For example, El Kohen et al. (1991) observed an immediate and readily reversible decrease in springtime dark respiration rate of leaves and whole shoots of 2-year-old sweet chestnut (Castanea sativa Mill.) trees grown in 350 or 700 mmol mol\(^{-1}\) CO\(_2\), but the effect of [CO\(_2\)] on respiration rate decreased through summer and was negligible in autumn. In addition, several reports suggest that plants grown in elevated [CO\(_2\)] for long time periods are less responsive to short-term changes in [CO\(_2\)] compared with plants grown in ambient [CO\(_2\)] (Bunce, 1998; El Kohen et al., 1991; Thomas and Griffin, 1994). Amthor (1995) suggests that the direct inhibition of apparent respiration from short-term night-time increases in [CO\(_2\)] may be due to dark CO\(_2\) fixation by PEP carboxylase activity, rather than a respiratory response per se. Further research is required to resolve this issue.

Indirect (long-term) effects of atmospheric [CO\(_2\)] on plant respiration per unit dry mass have been variably reported to increase (Gifford et al., 1985; Yozobii et al., 1985; Kendall et al., 1985; Williams et al., 1992), decrease (Gifford et al., 1985; Kendall et al., 1985; Spencer and Bowes, 1986; Azkón-Bieto et al., 1994), and remain constant (Gifford et al., 1985; Hrubec et al., 1985; Baker et al., 1992; Azkón-Bieto et al., 1994 [C3 species]; Downsson and Granat, 1994) in elevated [CO\(_2\)] (reviewed in Amthor, 1995). In many cases maintenance respiration rates have been shown to be correlated with tissue N content (Ryan, 1991), and elevated [CO\(_2\)] often decreases N content of tissue (Field et al., 1992). This decrease in N content of plants grown in elevated [CO\(_2\)] has been suggested to be an important factor in decreasing respiration per unit dry mass in plants grown in elevated CO\(_2\) (Amthor, 1991, 1995; Walschger et al., 1994). Yet several reports indicate that respiration rate per unit N (on protein) is depressed following long-term growth in elevated CO\(_2\) (Ziska and Bunce, 1993; Creutzen and Mousseau, 1994). The data presented here, and those of Griffin et al. (1993), are contrary to this trend. Foliar respiration of longleaf pine was strongly related to N concentration; however, greater respiration rates per unit N were observed when trees were grown in elevated [CO\(_2\)], at least for the high N treatment. Griffin et al. (1993) also found a decrease in N concentration and an increase in apparent respiration rate due to elevated [CO\(_2\)] with a high N treatment; thus, apparent respiration per unit N increased.

Atmospheric [CO\(_2\)] may also influence apparent respiration rates by altering carbohydrate availability. Non-structural carbohydrate concentration of foliage in this study was increased by elevated [CO\(_2\)] and decreased by increased N availability (Fig. 3); the interaction among main treatment variables was not significant. Several reports of increased respiration rates due to long-term elevated atmospheric [CO\(_2\)] exposure have indicated that...
increased availability of non-structural carbohydrates may stimulate respiration rates (Azcon-Bieto and Osmond, 1983; Hrubec et al., 1985; Peart et al., 1992; Williams et al., 1992). Azcon-Bieto et al. (1983) suggested that respiration is limited by the supply of glucose and fructose to mitochondria, although Amthor (1994) suggested that, with the exception of actively growing cells, respiration is regulated mainly by the rate at which respiratory products (ATP, NAD(P)H, and carbon skeletons) are used to meet the metabolic demands of growth, maintenance, transport, and ion uptake. Perhaps the increased photosynthetic production in these trees grown in elevated CO$_2$ data (not shown) was—in addition to accumulating in leaves—being exported out of the leaves at greater rates and being used for additional leaf growth, both of which would result in increased respiration. The decrease in leaf N content, however, may have partially offset increased respiration as a result of reduced costs of growth and maintenance so that no net effect of CO$_2$ on respiration rate was observed. In this study, the magnitude of the decrease in growth cost due to CO$_2$ enrichment was small (1.6%) while increasing N supply increased construction costs by a similar magnitude (2.5%). The interaction among main treatment variables was not significant (Fig. 4). The effects of CO$_2$ on growth cost that were estimated in this study are about the same as those calculated by Griffin et al. (1993) in both magnitude and direction. Thus, effects of CO$_2$ on pine leaf growth respiration may be due mainly to increased growth rate rather than changes in costs of construction.

We emphasize that, in addition to CO$_2$, N influenced apparent leaf construction cost (Fig. 4) and we reiterate the suggestion of Griffin et al. (1993) that with elevated CO$_2$ and low N, pine growth may be sink limited, resulting in an increased non-structural carbohydrate concentration, and that with elevated CO$_2$ and high N, pine growth may be source limited. Trends in leaf weight area ratio, although not statistically significant (Table 2) are consistent with this view and indicate a greater accumulation of non-structural carbohydrates at low compared to high N. Carbohydrate data also follow this pattern (Fig. 3) which was also reported by Thomas and Strain (1991).

In summary, we did not observe a significant effect of CO$_2$ on leaf respiration rate per unit leaf area or mass, but respiration per unit leaf N was greater in elevated CO$_2$. The lack of a respiratory response per unit leaf mass or area is likely due to indirect effects that influenced respiration in opposing ways. Increased carbohydrate content resulted in increased transport and/or increased growth, thus increasing respiration rate, while lower N content decreased maintenance respiration rate. The increased respiration per unit foliar N may reflect more growth per unit N or greater carbohydrate transport at a given level of foliar N. Although elevated CO$_2$ increased growth rate, the effect of increased growth rates on respiration were partially offset by decreased costs of constructing and maintaining a unit of leaf tissue. Nitrogen supply had a greater effect on both maintenance respiration and cost of constructing needle tissue than did atmospheric CO$_2$.

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Table 2. Effects of long-term open top chamber CO$_2$ and nitrogen treatments on construction cost variables for loblolly pine needles

<table>
<thead>
<tr>
<th>Nitrogen (kg ha$^{-1}$ year$^{-1}$)</th>
<th>Long-term CO$_2$ (atmos CO$_2$ mol$^{-1}$)</th>
<th>n</th>
<th>% N*</th>
<th>% Ash</th>
<th>Heat of combustion (4kJ g$^{-1}$)</th>
<th>Leaf weight area ratio (g m$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>360</td>
<td>6</td>
<td>0.01</td>
<td>0.77</td>
<td>3.25 a</td>
<td>20.13 b</td>
</tr>
<tr>
<td>40</td>
<td>720</td>
<td>6</td>
<td>0.60</td>
<td>1.28</td>
<td>2.97 ab</td>
<td>19.97 b</td>
</tr>
<tr>
<td>400</td>
<td>360</td>
<td>6</td>
<td>1.28</td>
<td>0.75</td>
<td>3.25 a</td>
<td>20.13 b</td>
</tr>
<tr>
<td>400</td>
<td>720</td>
<td>6</td>
<td>0.97</td>
<td>1.28</td>
<td>2.97 ab</td>
<td>19.97 b</td>
</tr>
</tbody>
</table>

* Letters which differ within a column indicate a significant difference (α=0.05) according to contrasts conducted under General Linear Models.
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