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Effects of nitrogen on *Pinus palustris* foliar respiratory responses to elevated atmospheric CO_2 concentration

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Abstract

Indirect effects of atmospheric CO_2 concentration [CO_2], on longleaf pine (*Pinus palustris* Mill.) foliage respiration were studied by growing trees in a factorial arrangement of low and high [CO_2] (369 and 729 μmol CO_2 mol^{-1}) and low and high N (40 and 400 kg ha^{-1} yr^{-1}). Direct effects of [CO_2] on leaf respiration were tested by measuring respiration rates of foliage from all treatments at two CO_2 levels (360 and 720 μmol CO_2 mol^{-1}) at the time of measurement. Elevated CO_2 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 [CO_2] (indirect response to elevated [CO_2]). The lack of a [CO_2] effect on respiration, when analysed on an area or mass basis, may have resulted from combined effects of [CO_2] 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, [CO_2] affected factors that influence respiration, but in opposing ways.

Key words: *Pinus palustris*, elevated CO_2, nitrogen, foliar respiration.

Introduction

Due to burning of fossil fuels and changes in land use world-wide, atmospheric CO_2 concentration [CO_2], 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). [CO_2] affects a wide range of plant functions, both directly and indirectly, and so increasing atmospheric [CO_2] 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 [CO_2] (Mooney, 1991). Most studies of plant responses to elevated [CO_2] 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 [CO_2], even though respiration may consume more than 50% of the carbon fixed at the whole plant level (Amthor, 1989), and despite evidence suggesting that CO_2 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 [CO_2] is not known, however, extant 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 measurements 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 (Hicklenton and Jolliffe, 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 $[CO_2]$ during a measurement may affect respiration rate. For example, Amthor *et al.* (1992) found that apparent respiration rate was inhibited 20–30% by a 300 μmol mol^{-1} increase in $[CO_2]$ during a respiration measurement. This effect was readily reversible, i.e. a decrease in $[CO_2]$ during a measurement increased respiration rate. Although this response to short-term changes in $[CO_2]$ at the time of measurement has been commonly observed (Begg and Jarvis, 1968; Gale, 1982; Reuveni and Gale, 1985; Bunce, 1990, 1992; El Kohen *et al.*, 1991; Mousseau, 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 $[CO_2]$ on respiration *per se* or reflect the influence of $[CO_2]$ on other metabolic reactions such as dark fixation of HCO_3^- by PEPcase (Amthor, 1995).

Difficulty in comparing studies of respiratory responses to elevated $[CO_2]$ 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 $[CO_2]$ can alter relationships between plant mass, leaf area, and protein content. For example, elevated $[CO_2]$ often increases plant C:N ratio (Norby *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 $[CO_2]$ on photosynthesis and productivity may be mediated by nutrients (Larigauderie *et al.*, 1988; Eamus and Jarvis, 1989; Bazzaz, 1990; Fajer *et al.*, 1992; Griffin *et al.*, 1993), the extent to which elevated $[CO_2]$ increases N use efficiency, thus ameliorating N deficiencies, is controversial (see Norby *et al.*, 1986; Coleman *et al.*, 1991; Hilbert *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 $[CO_2]$.

This study examined the respiration response of longleaf pine (*Pinus palustris* Mill.) foliage from trees grown in ambient and elevated atmospheric $[CO_2]$ and at two levels of soil N. All respiration measurements were conducted at night in the dark. Two $[CO_2]$ (360 and 720 μmol CO_2 mol^{-1}) were used during measurements to assess any direct effects of $[CO_2]$ on respiration rate. Also, effects of N on respiration and on respiratory responses to $[CO_2]$ were quantified. Results are reported per unit dry mass, leaf surface area and foliar N content.

Materials and methods

Plant growth and exposure system

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 (root collar diameter mean = 13 mm; standard deviation = 2 mm; range = 9 to 21 mm), and planted (3 per pot) into each of 192, 45 l 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 $[CO_2]$ of 365 μmol CO_2 mol^{-1} (ambient) or 720 μmol CO_2 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 aluminium frame covered by a clear PVC plastic film (0.2 mm thickness). Carbon dioxide is supplied from a 12.7 Mg liquid CO_2 receiver through a high volume dispensing manifold and added to the chambers by injection into plenum boxes (Rogers *et al.*, 1983). Air is dispensed into each chamber through the bottom half of each chamber cover which is double-walled; the inside wall is perforated with 2.5 cm diameter holes to serve as ducts that distribute air uniformly into the chamber. Carbon dioxide concentrations in each chamber are monitored 24 h d^{-1} using a time-shared sampling manifold; a solenoid bank directed samples to an infra-red gas analyser which recorded an instantaneous value at the end of that sampling period (Rogers *et al.*, 1983). Carbon dioxide exposures were initiated on 30 March 1993, and $[CO_2]$ values were continuously recorded every 15–30 min for each chamber, depending upon whether or not an additional CO_2 study was on line. Average ambient $[CO_2]$ ($\pm$standard deviation) through the duration of the experiment was 368.6 μmol mol^{-1} ($\pm$17.1) during the day (7 a.m. to 7 p.m.) and 403.1 ($\pm$34.4) during the night. Average $[CO_2]$ in the elevated chambers was 728.9 μmol mol^{-1} ($\pm$52.1) during the day and 792.9 ($\pm$53.5) during the night. In order to maintain temperatures within 3 °C of ambient, three chamber volumes were exchanged every minute.

Nitrogen treatments were similar to those described by Bazzaz and Miao (1993). They consisted of applying either 0.20 or 0.02 mg N g^{-1} soil per year (as sulphur-coated urea; 38–0–0 [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. Fertility of other nutrients was maintained at non-limiting levels in all pots by application of sulphur-coated potassium (0–0–47 [N–P–K]; 0.04 mg K g^{-1} soil yr^{-1}) and MicroMax™ Plus (0–4–0 [N–P–K]; P = 0.14, Ca = 0.57, Mg = 0.28, and S = 0.05 mg g^{-1} soil yr^{-1}, plus a complete complement of micronutrients) which was mixed into the sand at the time the pots were filled. Iron chelate (0.007 mg Fe g^{-1} soil) was applied once in April 1993.

Treatments were arranged in a 2 × 2 factorial design with six 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.

Respiration measurements

Respiration was measured four times over a 6-week period. Average daily temperature (range = 27–30 °C) and solar radiation (range = 5652–6521 W m^{-2}) on the day of respiration measurement varied only slightly throughout this 6-week period. On each seedling, a cohort of actively growing needles (that expanded under previously described CO_2 treatments) was delimited with pipe cleaners. Starting 25 June 1993, and continuing every 2 weeks for a 6-week period, one fascicle from the delimited cohort of fascicles from each seedling was excised. All fascicles within a treatment were immediately enclosed in a 1 l cuvette and CO_2 efflux was measured, over a 30–90 s period, with a Li-Cor 6200 portable photosynthesis system (Li-Cor, Inc., Lincoln, NE). Measurements were started at 9 p.m. and continued until approximately 3 a.m. and were conducted in the dark. All measurements were conducted at two CO_2 partial pressures (360 and 720 μmol mol^{-1}, the order of which was randomly assigned) within the cuvettes. Respiration rate was determined by the amount of time required to change [CO_2] by 6 μmol mol^{-1}. After all respiration rate measurements during a single night were made, the average time per measurement was calculated and, in an empty (no fascicles) cuvette, the change in [CO_2] during the average measurement time ($\approx$45 s) was determined at both 360 and 720 μmol mol^{-1} CO_2. These values were used to correct the respiration rate measurements for any leaks in the system. Changes in cuvette [CO_2] due to leaks were always low compared to changes in [CO_2] with needles present; in most cases, leak rates were at least 10-fold less than respiration measurements.

After respiration rate determinations, needle surface area was measured. Needles were then dried at 55 °C for 4 d (to constant mass) and fascicle dry mass was recorded. Foliar N and C content per fascicle was then determined with a LECO CHN-600 analyser (LECO Corp. St Joseph, MI). Total non-structural carbohydrates (starch plus sugar) were analysed according to the methods described by Thomas *et al.* (1993). Construction costs (g glucose required to construct 1 g dry weight of needle tissue) were determined using a bomb calorimeter and adjusted for ash content and N concentration, as described by Williams *et al.* (1987), Griffin *et al.* (1993), and Amthor *et al.* (1994).

Data analysis

Treatment main effects and interactions were tested using a repeated measures multivariate analysis of variance. The main effects of [CO_2] and N were determined using a split-plot factorial analysis with CO_2 treatments as the whole plot and N treatments as the subplot. Trends through time and interactions of main effects with measurement dates were tested using the Wilk's lambda test.

Results and discussion

Increasing [CO_2] did not alter apparent respiration rate of *Pinus palustris* foliage either directly (cuvette [CO_2]) or indirectly (open top chamber [CO_2]) when expressed on a leaf area or dry mass basis (Table 1). Needle respiration rates averaged 1.04 μmol CO_2 m^{-2} s^{-1} and 10.8 nmol CO_2 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 54% 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 [CO_2], N treatment, and their interaction (Fig. 2). Long-term elevated atmospheric [CO_2] resulted in greater respiration rates per unit foliar N;

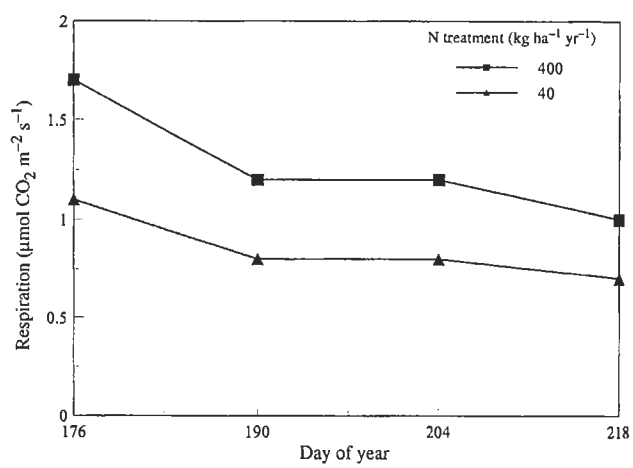

Fig. 1. Respiration of longleaf pine foliage per unit leaf area for the low (40 kg ha^{-1} yr^{-1}) and high (400 kg ha^{-1} yr^{-1}) nitrogen treatments across the four measurement dates ($Pr > F = 0.0001$ for the nitrogen treatment and $Pr > F = 0.0003$ for the date by N interaction).

Table 1. *Effects of long-term (open top chamber) [CO_2] (indirect), nitrogen, and short-term (cuvette) [CO_2] (direct) treatments on CO_2 efflux from longleaf pine needles expressed per unit: needle area, needle dry mass, and needle N*

Respiration measurement	Main effect treatment variable								
	Long-term [CO_2] (μmol CO_2 mol^{-1})			N (kg ha^{-1} yr^{-1})			Cuvette [CO_2] (μmol CO_2 mol^{-1})		
	360	720	$Pr > F$[a]	40	400	$Pr > F$	360	720	$Pr > F$
μmol CO_2 m^{-2} s^{-1}	1.04	1.05	0.937	0.85	1.24	<0.001	1.05	1.04	0.761
nmol CO_2 g^{-1} s^{-1}	10.90	10.66	0.584	8.50	13.06	<0.001	10.82	10.74	0.797
nmol CO_2 mg^{-1} N s^{-1}	1.04	1.22	0.007	1.21	1.06	<0.001	1.14	1.13	0.876

[a] Probability of a greater F value by chance for the difference between the CO_2 or N treatments, and for the CO_2 by N interaction, where the interaction was significant.

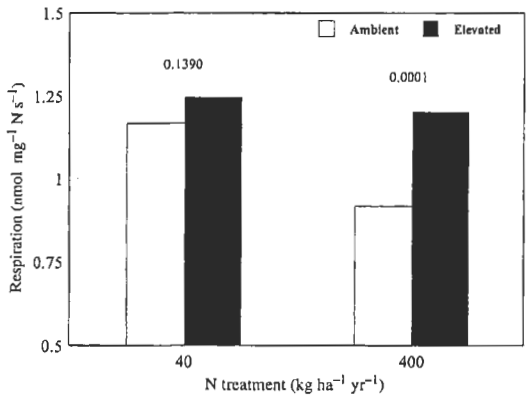

Fig. 2. Respiration of longleaf pine foliage per unit of foliar nitrogen for the ambient (360 μmol mol^{-1}) and elevated (720 μmol mol^{-1}) CO_2 treatments at the low (40 kg ha^{-1} yr^{-1}) and high (400 kg ha^{-1} yr^{-1}) nitrogen treatments. Values above each set of bars are probabilities of a greater F from contrasts run under General Linear Models. The CO_2 by N interaction was significant ($Pr > F = 0.0087$).

however, this effect was much greater in the high N treatment than in the low N treatment, even though increasing N availability reduced respiration rate per unit foliar N. Similarly, N had a greater influence on dark respiration rate of loblolly 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 μmol 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, 1990; 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; Hrubec *et al.*, 1985; Kendall *et al.*, 1985; Williams *et al.*, 1992), decrease (Gifford *et al.*, 1985; Kendall *et al.*, 1985; Spencer and Bowes, 1986; Azcón-Bieto *et al.*, 1994), and remain constant (Gifford *et al.*, 1985; Hrubec *et al.*, 1985; Baker *et al.*, 1992; Azcón-Bieto *et al.*, 1994 [C_4 species]; Downton and Grant, 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; Wullschleger *et al.*, 1994). Yet several reports indicate that respiration rate per unit N (or protein) is depressed following long-term growth in elevated [CO_2] (Ziska and Bunce, 1993; Ceulemans 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

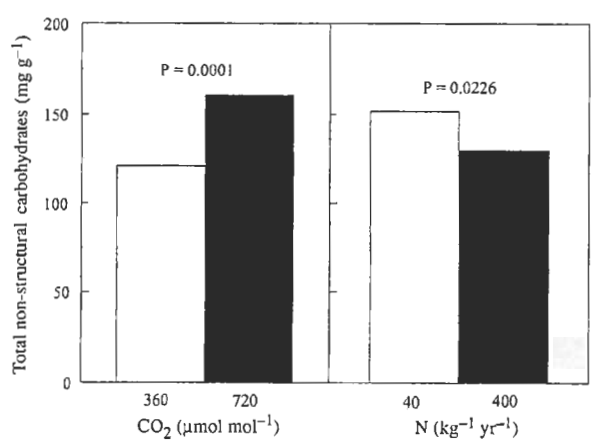

Fig. 3. Total non-structural carbohydrate content of longleaf pine foliage for the ambient (360 μmol mol^{-1}) and elevated (720 μmol mol^{-1}) CO_2 treatments and for the low (40 kg ha^{-1} yr^{-1}) and high (400 kg ha^{-1} yr^{-1}) nitrogen treatments. Values above each set of bars are probabilities of a greater F from contrasts run under General Linear Models. The CO_2 by N interaction was not significant ($Pr > F = 0.7456$).

increased availability of non-structural carbohydrates may stimulate respiration rates (Azcón-Bieto and Osmond, 1983; Hrubec *et al.*, 1985; Poorter *et al.*, 1992; Williams *et al.*, 1992). Azcón-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 photosynthate produced 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.

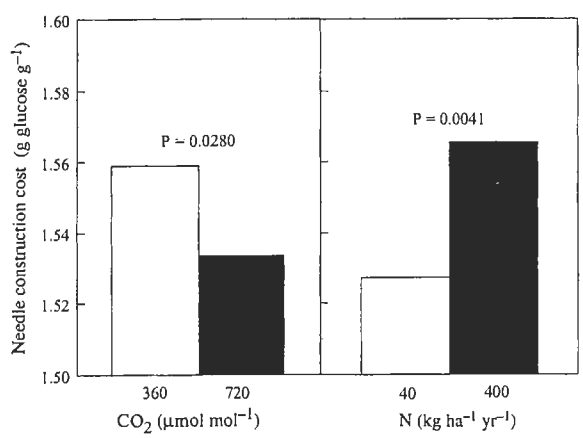

Fig. 4. Costs of constructing longleaf pine foliage for the ambient (360 μmol mol^{-1}) and elevated (720 μmol mol^{-1}) CO_2 treatments at the low (40 kg ha^{-1} yr^{-1}) and high (400 kg ha^{-1} yr^{-1}) nitrogen treatments. Values above each set of bars are probabilities of a greater F from contrasts run under General Linear Models. The CO_2 by N interaction was not significant ($Pr > F = 0.1757$).

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 growth 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 longleaf pine needles*

Nitrogen (kg ha^{-1} yr^{-1})	Long-term [CO_2] (μmol CO_2 mol^{-1})	n	% N[a]	% Ash	Δ Heat of combustion (kJ g^{-1})	Leaf weight:area ratio (g m^{-2})
40	360	6	0.77 c	3.25 a	20.13 b	116.31 a
40	720	6	0.60 d	2.97 ab	19.97 b	122.31 a
400	360	6	1.28 a	3.32 a	20.74 a	121.99 a
400	720	6	0.97 b	2.61 b	20.13 b	113.08 a

[a] Letters which differ within a column indicate a significant difference ($\alpha = 0.05$) according to contrasts conducted under General Linear Models.

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