Functional Ecology 2001
15, 497–505

Leaf dark respiration as a function of canopy position in *Nothofagus fusca* trees grown at ambient and elevated CO_2 partial pressures for 5 years

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Summary

1. Mass-based and area-based rates of respiration, leaf nitrogen content, leaf total protein content, non-structural carbohydrates and leaf mass per unit area (LMA) all decreased with depth in the canopy of *Nothofagus fusca* (Hook. F.) Oerst. (Red beech) trees grown for 5 years at ambient (36 Pa) or elevated (66 Pa) CO_2 partial pressures.

2. Elevated CO_2 partial pressure had a strong effect on dark respiration, decreasing both mass-based and area-based rates at all canopy positions, but had little or no effect on leaf physical and biochemical properties.

3. Leaf sugars, starch, protein, N and LMA were all correlated with respiration rate, and are therefore strong predictors of area-based dark respiration rates. The *y* axis intercept of regressions of respiration rate on mean leaf N, protein, starch and LMA was lower for plants grown at elevated compared to ambient CO_2 partial pressures because of the differential effect of growth at elevated CO_2 partial pressure on leaf gas-exchange, chemical and physical characteristics.

4. The lower respiration rates for leaves from trees grown at elevated CO_2 partial pressure resulted in a significant increase in the ratio of light-saturated net photosynthesis to respiration, increasing the potential carbon-use efficiency of these leaves.

Key-words: Carbohydrates, photosynthesis, protein, Red beech

Functional Ecology (2001) **15**, 497–505

Introduction

The distribution of photosynthetic capacity (A_{max}, light-saturated photosynthesis) within plant canopies has been characterized in many studies, and some (Field 1983; Field 1991; Hirose & Werger 1987; Hollinger 1989), but not all (Anten, Schieving & Werger 1995; Evans 1993; Hollinger 1996), find that this distribution is optimized in relation to light. Furthermore, due to the robust relationship between A_{max} and leaf N (g N m^{-2}, Field 1983; Field & Mooney 1986; Mooney & Gulmon 1979), these studies also show that leaf N is optimally distributed (Ellsworth & Reich 1993; Evans 1989; Field 1983; Hirose & Werger 1987; Hollinger 1989; Leuning, Cromer & Rance 1991), and a strong predictor of the distribution of A_{max} within the canopy. As a result, the A_{max}/N relationship is often used in canopy and ecosystem models (Aber, Reich & Goulden 1996; Leuning *et al*. 1995; Running & Hunt 1993; Sellers *et al*. 1992) and appears to be robust across a wide range of plant types and environmental conditions. Still, the productivity of plant canopies is not determined solely by the rate of photosynthetic carbon gain, but is modulated by the rate of carbon loss. The distribution of respiration rates (R) within plant canopies has not been characterized well, but a general relationship between leaf N and R has been reported (Reich, Oleksyn & Tjoelker 1996; Reich *et al*. 1998a; Reich *et al*. 1998b; Ryan 1991; Ryan 1995; Ryan *et al*. 1996), suggesting that a relationship may exist similar to that between A_{max} and canopy position.

More generally, a relationship among leaf N, A_{max} and R exists across terrestrial ecosystems and, to a lesser extent, across functional groups (Reich *et al*. 1998a). Furthermore, physiological mechanisms may explain the close relationships between R and leaf structure

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and/or R and N across functional groups and biomes (Reich *et al*. 1998a). At the scale of the individual tree canopy, much less work has been done to characterize the distribution of respiration among leaf populations, or how this distribution may be affected by resource availability. Here we suggest that understanding the within-tree distribution of physiological activity is important for interpreting whole-tree carbon gain, a prerequisite for scaling up to canopy and regional level carbon gain. Given anthropogenic changes in the CO_2 partial pressure of the Earth's atmosphere (Keeling *et al*. 1995), a mechanistic understanding is required of how the distribution of physiological activity will respond to increased CO_2 partial pressure.

Within the global carbon cycle, forest trees are a major biological carbon sink (Houghton 1993; Vitousek 1991; Waring & Schlesinger 1985), emphasizing the need to understand canopy and regional level carbon gain. Furthermore, both short- and long-term photosynthetic and respiratory responses to elevated CO_2 partial pressure have been demonstrated (Curtis & Wang 1998; Drake, Gonzàlez-Meler & Long 1997; Gonzàlez-Meler & Siedow 1999; Griffin & Seemann 1996; Norby *et al*. 1999). Our objective is to quantify the distribution of respiration within *Nothofagus fusca* (Hook. F.) Oerst. (Red beech) canopies, and the effect of CO_2 enrichment on this distribution. We hypothesize that respiration rates decrease with depth in the canopy as a result of lower metabolic activity due to decreasing irradiance. We further predict that growth in elevated CO_2 will lead to reduced respiration rates. Red beech is native to New Zealand, where it dominates large areas of forests on rich soils from sea level to 1000 m elevation (Wardle 1984), and thus we consider our results in relation to forest responses to elevated CO_2.

Materials and methods

TREE GROWTH

Our experimental site was established in 1994 on the east coast of the South Island of New Zealand at Bromley, Christchurch (43°32′ S, 172°42′ E, 9 m a.s.l.). Sixteen circular open-top chambers (3·6 m tall, 4·6 m diameter; Heagle *et al*. 1989) were established on recently stabilized free-draining Kairaki dune sand. Native beech forest soil (50 mm) was placed on top of the sand to allow for inoculation of the beech roots with native fungi. The design and performance of the chambers are described elsewhere (Whitehead *et al*. 1995). The CO_2 partial pressure supplied to the chambers was obtained by separation from biogas using a three-stage filtration process at a nearby wastewater treatment facility (Rogers & Whitehead 1998). Experimental CO_2 partial pressure treatments were automatically monitored and set 24 h a day. Within half of each chamber, 20 *Nothofagus* trees were planted (the other half chamber contained three *Pinus radiata* seedlings).

Physiological, biochemical and morphological measurements were made during the early growing season (November 1998) when the trees were 5 years old and approximately 4 m high. A range of biochemical, physiological and morphological measurements was made at nine canopy heights of single trees from three different chambers for the ambient and elevated treatments.

GAS-EXCHANGE MEASUREMENTS

At each canopy height for each tree, two leaves were removed and four 10 mm diameter leaf disks were taken (two per leaf). These leaf disks were kept in the dark, floating in 20 mM MES buffer (pH 6·0) saturated with air for 20 min prior to initiation of respiration measurements. Oxygen exchange was then assayed in the dark at 25 °C using a liquid-phase O_2 electrode (Clark-type, Rank Brothers, Cambridge, UK) containing 20 mM MES buffer (pH 6·0) that had been equilibrated in ambient air. Intact leaf punches were placed in the electrode cuvette and the depletion of oxygen was recorded. All measurements were terminated before the oxygen was depleted by 50% to avoid O_2 limitation. Disks were removed from the electrode and dried to a constant mass in a 60 °C oven for determination of leaf mass per unit area (LMA).

Net photosynthetic rate versus internal CO_2 partial pressure (A/C_i) response curves were measured using four infrared gas analysis systems equipped with CO_2 control modules and LED light sources (Model Li-6400, LiCor Inc., Lincoln, NE, USA). To accomplish this, external CO_2 partial pressures (C_a) were decreased in 13 steps from 150 to 0 Pa. Measurements were made on clear, warm days between 09:30 and 16:00 h. Leaf temperatures were maintained near 20 °C using thermoelectric coolers, and the water vapour pressure deficit generally was held between 1·0 and 1·5 kPa. All measurements were made at saturating light intensities (a constant photon flux density of 2000 μmol m^{-2} s^{-1} of photosynthetically active radiation). Gas-exchange measurements were recorded automatically once a stable rate was observed.

Photosynthetic response curves (A/C_i) were analysed using the model of Farquhar, von Caemmerer & Berry (1980) and von Caemmerer & Farquhar (1981). The model was parameterized and run as described by Lewis *et al*. (1994); net photosynthesis (A, the light-saturated net assimilation rate at the growth CO_2 concentration) was then calculated from the A/C_i response curves.

BIOCHEMICAL MEASUREMENTS

The soluble sugar and starch content of adjacent leaves were determined colorimetrically using a phenol–sulphuric acid technique (Tissue & Wright 1995). Total non-structural carbohydrate content, expressed as a percentage of leaf dry mass, was calculated as the sum of soluble sugars and starch. Leaf N content was determined on dried and ground material using a CNS

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autoanalyser (Carlo Erba Na 1500, Milan, Italy). Total leaf protein was extracted in a 100 mM Tris Buffer (pH 7·5) and assayed in a Bradford Reagent (Sigma B-6916, Sigma Chemical Co, St Louis, MO, USA).

STATISTICAL ANALYSES

Analysis of variance (ANOVA) was used to test for the effects of CO_2 treatment and canopy position on all calculated parameters, and mean treatment effects were separated with least-squares estimates using linear models (Data Desk, Data Description Inc., Ithaca, NY, USA). Multiple linear regression analysis was used to examine relationships between continuous variables. The tolerance of each variable was examined to determine if colinearity existed between variables, and was considered to be reasonable in all cases (indicating that colinearity was not significant). An analysis of covariance (ANCOVA) was used to compare regression results after determining the homogeneity of the slopes of the regression. Treatment and canopy height effects were considered significant if $P = 0{\cdot}05$. All data were normally distributed; no problems were detected with heteroscedasticity among the residuals and thus a log transformation was not necessary.

Results

The nine canopy positions sampled were roughly evenly spaced every 45 cm from the top of the canopy (Table 1). The trees grown in the ambient CO_2 partial pressure chambers were slightly taller than those in the elevated CO_2-grown chambers, so sampling heights were an average of 30 cm higher in the canopies of trees grown in the ambient CO_2 partial pressure chambers. Nevertheless, the proportional sampling heights were similar, and were equally distributed from top to bottom of the living canopy.

Leaf respiration rate decreased with depth in the canopy (Fig. 1). On a leaf-area basis, respiration decreased by 51%, from 0·51 μmol m^{-2} s^{-1} in the upper canopy to 0·25 μmol m^{-2} s^{-1} in the lower canopy for trees in the ambient treatment and by 61%, from 0·47 to 0·18 μmol m^{-2} s^{-1} for trees in the elevated treatment. The effects of both elevated CO_2 partial pressure and canopy position were significant statistically but not interactively. Growth at elevated CO_2 partial pressure reduced the mean area-based respiration rate by 18%, from 0·39 ± 0·03 to 0·32 ± 0·03 μmol m^{-2} s^{-1} (averaged across all canopy positions). No significant differences were detected in the slopes of the linear regressions describing the relationship between respiration and canopy position for ambient versus elevated CO_2-grown trees, but the intercept of the elevated CO_2 regression was significantly lower than that for the ambient treatment regression line (Fig. 1, top).

A similar, but less pronounced, response was found when leaf respiration was expressed on a mass basis. Mass-based leaf respiration decreased 32%, from 7·1 μmol kg^{-1} s^{-1} in the upper canopy to 4·8 μmol kg^{-1} s^{-1} in the lower canopy of ambient CO_2 partial pressure-grown trees, and by 38%, from 5·3 μmol kg^{-1} s^{-1} in the upper canopy to 3·3 μmol kg^{-1} s^{-1} in the lower canopy of trees from the elevated treatment. Again, the effects of elevated CO_2 and canopy position were both significant statistically, but not interactively.

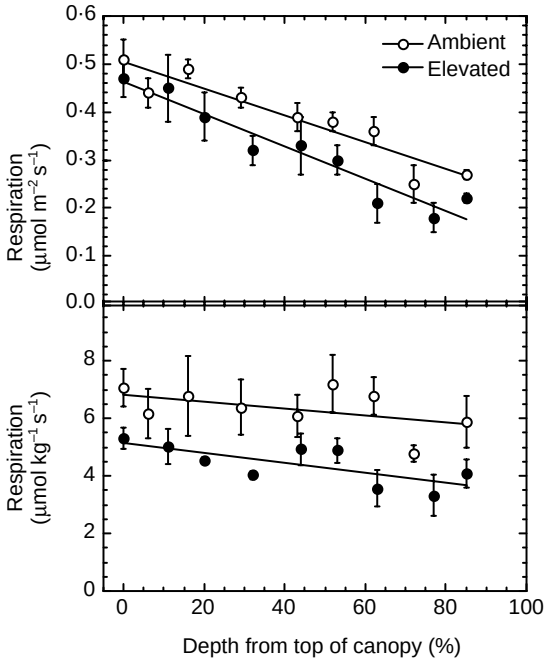


Fig. 1. Leaf respiration as a function of canopy position in *Nothofagus fusca* trees grown at ambient (36 Pa, open symbols) and elevated (66 Pa, closed symbols) CO_2 partial pressures. Each point represents the mean of three to four leaves from each canopy position of three trees, and is subtended by the SEM. Area-based respiration (top) decreased with depth in the canopy in a linear fashion: Ambient CO_2 respiration = $-0{\cdot}0028 \times$ canopy depth + 0·505, $R^2 = 0{\cdot}88$; elevated CO_2 respiration = $-0{\cdot}0034 \times$ canopy depth + 0·464, $R^2 = 0{\cdot}92$. Mass-based respiration (bottom) is more weakly predicted by canopy position: Ambient CO_2 respiration = $-0{\cdot}012 \times$ canopy depth + 6·84, $R^2 = 0{\cdot}25$; elevated CO_2 respiration = $-0{\cdot}017 \times$ canopy depth + 0·514, $R^2 = 0{\cdot}53$.

Table 1. Relationship between canopy position, canopy height and proportional canopy height for the nine sampling positions of *Nothofagus fusca* trees grown at ambient (36 Pa) and elevated (66 Pa) CO_2 partial pressures. Canopy height is expressed as the mean of three trees from the ambient or elevated CO_2 treatment ± the standard error of the mean

Canopy position (1 = top)	Canopy height (m)		Proportional height (%)	
	36 Pa CO_2	66 Pa CO_2	36 Pa CO_2	66 Pa CO_2
1	4·3 ± 0·15	4·0 ± 0·12	100	100
2	4·1 ± 0·06	3·5 ± 0·13	94	89
3	3·6 ± 0·04	3·2 ± 0·09	84	80
4	3·1 ± 0·05	2·7 ± 0·08	71	68
5	2·5 ± 0·06	2·2 ± 0·14	57	56
6	2·1 ± 0·13	1·9 ± 0·12	48	47
7	1·6 ± 0·19	1·5 ± 0·11	38	37
8	1·2 ± 0·10	0·9 ± 0·14	28	23
9	0·7 ± 0·22	0·6 ± 0·09	15	15

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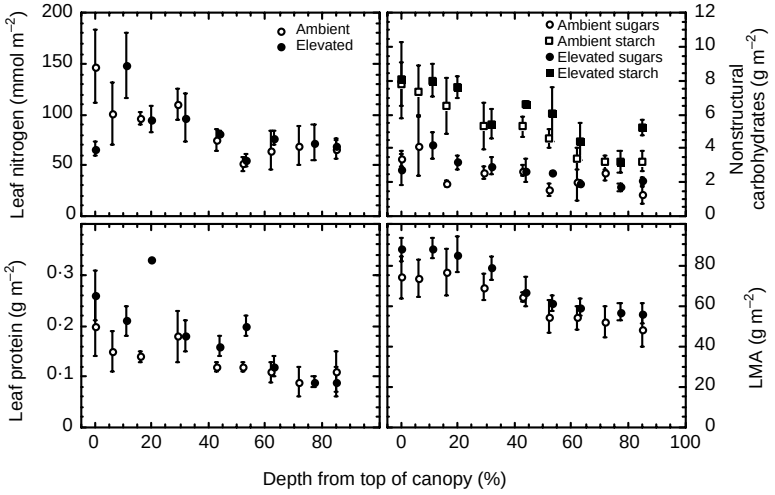


Fig. 2. Leaf structural and biochemical characteristics as a function of canopy depth in *Nothofagus fusca* trees grown at ambient (36 Pa, open symbols) and elevated (66 Pa, closed symbols) CO_2 partial pressures. Each point represents the mean of three to four leaves from each canopy position of three trees, and is subtended by the SEM. Upper left, leaf N; upper right, leaf starch and sugar; lower left, leaf protein; lower right, leaf mass per unit area.

Growth in elevated CO_2 partial pressure did not affect the rate at which respiration decreased with canopy depth. Growth at elevated CO_2 partial pressure reduced the mean mass-based respiration rate by 31%, from $6{\cdot}3 \pm 0{\cdot}25$ to $4{\cdot}4 \pm 0{\cdot}23$ μmol kg^{-1} s^{-1} (averaged across all canopy positions). Similar to the area-based estimates, no significant differences were detected in the slopes of the linear regressions describing the relationship between mass-based respiration and canopy position for trees from the ambient versus elevated treatments, but the intercept of the regression for the elevated treatment was significantly lower than that for the ambient treatment (Fig. 1, bottom).

Leaf structural and biochemical properties also varied with canopy position. Leaf N content decreased with depth in the canopy, but was not significantly affected by growth at elevated CO_2 (with the exception of the upper canopy, position 1, Fig. 2 top left). For trees grown at ambient CO_2 partial pressure, there was a decrease of 65% from the highest N leaves (position 1) to the lowest N leaves (position 6). For trees grown at elevated CO_2 partial pressure, the reduction in leaf N was 63% (canopy position 2 to position 6). Across all canopy positions and CO_2 treatments, the mean leaf N content was $85{\cdot}7 \pm 6{\cdot}6$ mmol m^{-2}. Total leaf protein similarly decreased with depth in the canopy, and was increased by growth in elevated CO_2 partial pressure from $0{\cdot}14 \pm 0{\cdot}012$ to $0{\cdot}18 \pm 0{\cdot}026$ g m^{-2} (Fig. 2, bottom left). Leaf N and protein were not well correlated with each other, with the variation in leaf N accounting for only 15% of the variation in leaf protein ($P = 0{\cdot}11$, data not shown).

Soluble sugars and starch concentrations decreased with depth in the canopy (by an average of 59%), but neither was affected by the growth CO_2 partial pressure (Fig. 2, top left). The regression of soluble sugars against canopy position was significantly influenced by canopy position 2 (ambient treatment = $4{\cdot}2 \pm 1{\cdot}80$; elevated treatment = $4{\cdot}2 \pm 0{\cdot}75$ g m^{-2}), and if these points were excluded from the regression, canopy position was no longer significant statistically.

The LMA (g m^{-2}) was significantly affected by both canopy position and growth CO_2 partial pressure, but the two factors were not interactive (Fig. 2, bottom right). The LMA decreased with depth in the canopy, and this decrease was most significant between canopy positions 3 and 6 regardless of CO_2 concentration. Overall, the reduction in LMA from top to bottom of the canopy was 37% in canopies of ambient CO_2-grown trees, and 36% in canopies of elevated CO_2-grown trees. Growth at elevated CO_2 partial pressure increased LMA by 13%, from $63{\cdot}0 \pm 3{\cdot}62$ to $71{\cdot}2 \pm 4{\cdot}59$ g m^{-2}.

Leaf respiration and respiratory carbon substrates (sugars and starch) were positively and significantly correlated (Fig. 3, top two panels). Mean leaf-soluble sugar content was more tightly correlated to leaf respiration in the elevated than in the ambient treatment ($R^2 = 0{\cdot}69$ elevated, $0{\cdot}23$ ambient; Fig. 3, top left). Mean leaf starch content explained 82% (ambient CO_2-grown) and 92% (elevated CO_2-grown) of the variation in mean leaf respiration (Fig. 3, second from top). Growth at elevated CO_2 partial pressure did not affect the slope of the respiration versus starch regression, but did result in a significantly reduced intercept. Leaf respiration increased with mean leaf protein content, and the rate of increase was nearly twice as high for ambient compared to elevated CO_2-grown trees (Fig. 3, middle). Mean leaf protein was better correlated to leaf respiration than was mean leaf N content (Fig. 3, second from bottom). Respiration was strongly correlated to mean LMA, accounting for 84% of the variation in respiration in the ambient CO_2-grown plants and 87% in the elevated CO_2-grown plants (Fig. 3, bottom). Growth at elevated CO_2 partial pressures did not affect the slope of this regression, but did reduce the intercept.

Light-saturated net photosynthetic rates (A), measured at the growth CO_2 partial pressure, did not decline consistently through the canopy (Fig. 4, top). On average, growth at elevated CO_2 partial pressure increased photosynthesis from $9{\cdot}7 \pm 0{\cdot}53$ μmol m^{-2} s^{-1} for trees from the elevated treatment to $6{\cdot}0 \pm 0{\cdot}50$ μmol m^{-2} s^{-1} for trees from the ambient treatment. Carbon efficiency (the ratio of net photosynthetic rate to respiration rate, $A : R$) increased significantly with depth in the canopy for the elevated CO_2-grown trees, varying from 24 in canopy position 1 to 52 in canopy position 7 of the elevated CO_2-grown trees (Fig. 4, bottom). The average $A : R$ of the trees grown in ambient CO_2 partial pressure was half that of the trees grown in elevated CO_2 partial pressure ($15 \pm 1{\cdot}1$ versus $33 \pm 3{\cdot}4$). Light-saturated rate of net photosynthesis was not a good predictor of the rate of respiration, particularly at elevated CO_2 partial pressure (Table 2). Mass-based estimates of both photosynthesis and respiration were even more poorly correlated (data not shown).

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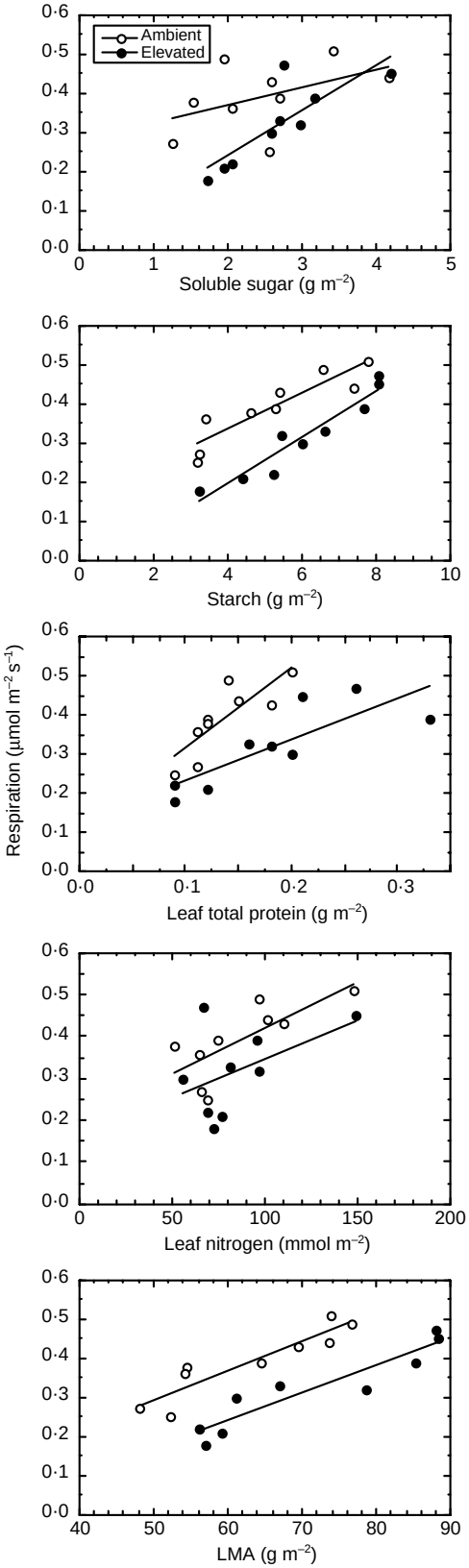


Fig. 3. Regression among area-based leaf respiration rate (R, μmol O_2 m^{-2} s^{-1}) and soluble sugar content (top); starch content (second from top); total protein content (middle); leaf nitrogen content (second from bottom); and leaf mass per unit area (LMA) (bottom) in *Nothofagus fusca* trees grown at ambient (36 Pa, open symbols) or elevated (66 Pa, closed symbols) CO_2 partial pressures. Each point represents the mean of three to four leaves from each canopy position of three trees. Regressions are as follows: ambient CO_2 $R = 0{\cdot}047 \times \text{sugar} + 0{\cdot}274$, $R^2 = 0{\cdot}23$; elevated CO_2 $R = 0{\cdot}113 \times \text{sugar} + 0{\cdot}017$, $R^2 = 0{\cdot}69$; ambient CO_2 $R = 0{\cdot}046 \times \text{starch} + 0{\cdot}153$, $R^2 = 0{\cdot}82$; elevated CO_2 $R = 0{\cdot}060 \times \text{starch} - 0{\cdot}043$, $R^2 = 0{\cdot}92$; ambient CO_2 $R = 1{\cdot}93 \times$ total protein $+ 0{\cdot}128$, $R^2 = 0{\cdot}64$; elevated CO_2 $R = 1{\cdot}04 \times$ total protein $+ 0{\cdot}128$, $R^2 = 0{\cdot}67$; ambient CO_2 $R = 0{\cdot}0022 \times$ leaf N $+ 0{\cdot}198$, $R^2 = 0{\cdot}56$; elevated CO_2 $R = 0{\cdot}0018 \times$ leaf N $+ 0{\cdot}165$, $R^2 = 0{\cdot}23$; ambient CO_2 $R = 0{\cdot}007 \times \text{LMA} - 0{\cdot}082$, $R^2 = 0{\cdot}84$; elevated CO_2 $R = 0{\cdot}007 \times \text{LMA} - 0{\cdot}182$, $R^2 = 0{\cdot}87$.

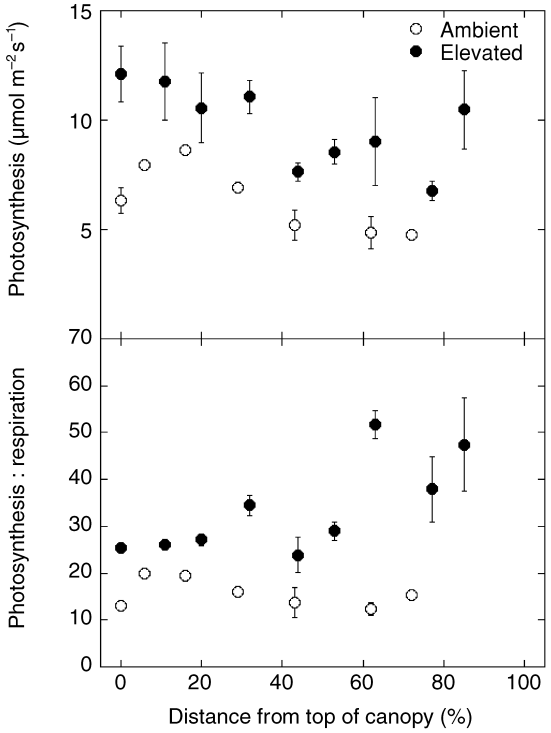


Fig. 4. Light-saturated net photosynthesis rate as a function of canopy position in *Nothofagus fusca* trees grown and measured at ambient (36 Pa, open symbols) and elevated (66 Pa, closed symbols) CO_2 partial pressures (top). Each point represents the mean of three trees and is subtended by the SEM. The ratio between light-saturated net photosynthesis (above) and leaf respiration (from Fig. 1) as a function of canopy position.

Table 2. Summary of regression relationships between the light-saturated net photosynthetic rate and respiration (both on an area basis) in *Nothofagus fusca* trees grown at ambient (36 Pa) and elevated (66 Pa) CO_2 partial pressures

Growth CO_2 (Pa)	y intercept	Slope	P	R^2
36 (ambient)	0·18	0·023	0·04	0·46
66 (elevated)	0·02	0·029	0·13	0·29
Combined	0·17	0·019	0·07	0·19

Discussion

A strong gradient of respiration rate was observed within the canopy of Red beech trees grown in open-top chambers, such that R declined with depth in the canopy. The reduction in respiration was nearly linear with depth in the canopy, and was observed for both area- and mass-based rates of respiration from both ambient

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and elevated treatments. We suggest that this is a complex response to the ambient light environment. The upper canopy leaves received the most direct sunlight and therefore had the largest daily total carbon gain compared with leaves in the lower canopy. As a result these upper canopy leaves contained more soluble sugars and starch, which in turn provided the substrate to support the increased respiration rates (Azcón-Bieto & Osmond 1983). Energy demand (ATP and reducing power) ultimately increases respiration rates (Farrar 1985), and therefore the more active upper canopy leaves would have greater energy demands. Decreased leaf N and protein content with depth in the canopy support the hypothesis that upper canopy leaves were metabolically more active and had greater demands for the products of growth and maintenance respiration (cf. De Visser, Spitters & Bouma 1992; McCree 1974; Penning de Vries 1975; Thornley 1982) than the lower canopy leaves. Few other studies have examined the distribution of respiration through a canopy, but our results are consistent with the few individual plant (Bolstad, Mitchell & Vose 1999; Carswell *et al*. 2000) multibiome (Reich *et al*. 1998a) studies that exist.

Leaf mass per unit area also decreased with depth in the canopy, suggesting that leaves get thinner or less dense lower in the canopy, a result that is consistent with previous studies of this species (Hollinger 1996) and others (Field 1983; Field 1991; Hirose & Werger 1987; Hollinger 1989). Nonetheless, while the observed decrease in LMA diminished the effect of canopy position on mass-based respiration, it was not sufficient to mask it completely. Because we suggest that light absorption and the subsequent process of carbon fixation drive the observed effects on respiration, emphasis was placed on the area-based estimates. Similar to the findings of Reich *et al*. (1996); Reich *et al*. (1998a); Reich *et al*. (1998b), who found that across species and functional types, thin leaves tended to have large N, *A* and *R*, we found these relationships to hold for leaves within a canopy. Furthermore, we also conclude that predicting *R* from a combination of leaf traits (in our case sugars, starch, N or protein, and LMA) is better than using N alone (data not shown).

Previous work focusing on photosynthetic characteristics in Red beech showed that physiological and functional properties of leaves vary with depth in the canopy (Hollinger 1996). Our findings are similar to this previous study, finding decreased dark respiration with canopy depth (Figure 4; Hollinger 1996). While the results of Hollinger (1996) are qualitatively similar to ours, the rate of dark respiration reported by Hollinger was estimated from A/C_i curves (R_d from Farquhar *et al*. 1980) and was not a direct measurement of CO_2 or O_2 exchange in the dark. We also estimated R_d from A/C_i curves (data not shown), but found no significant relationship between these estimates and the direct measurement of respiration, particularly for plants grown at elevated CO_2 partial pressure. Furthermore, Hollinger's estimated R_d is significantly higher than our direct measurements (as are our estimates of R_d compared to R), resulting in potentially lower leaf level carbon efficiency (Hollinger's $A : R$ results range from 10 to 17, while ours range from 19 to 30 at ambient CO_2 partial pressure). Still, both our estimates and those of Hollinger (1996) significantly exceed the 10 : 1 ratio commonly reported (Reich *et al*. 1998b). Both studies suggest that, hypothetically, leaves become more efficient lower in the canopy but at different absolute rates. It is interesting to consider the impact this distribution would have on the conclusions of Hollinger (1996) that the N distribution within Red beech canopies results in higher canopy carbon gain than does a completely random distribution, but that it is not optimal. Hollinger's (1996) model results suggest that the canopy could gain 10% more carbon if more N were allocated to the best illuminated microsites. Our results suggest smaller returns from such a re-allocation of N, as the efficiency of these leaves would be less.

In the fifth year of growth at elevated CO_2 partial pressure, the rate of respiration from trees maintained at 66 Pa CO_2 were consistently less than in the ambient treatment, on both a mass and an area basis. Literature reviews consistently find the average response to growth in elevated CO_2 is reduced mass-based respiration, but increased or unresponsive area-based respiration (Amthor 1994; Curtis & Wang 1998; Poorter *et al*. 1992). While the exact mechanism of this response is unknown, it has been suggested that growth at elevated CO_2 partial pressure may lead to smaller mass-based maintenance cost resulting from a decrease in leaf protein and *N* content, while the starch content increases. The latter accounts for increases in leaf mass, but has a very low maintenance cost if stored or exported (Thomas & Griffin 1994). In this study, growth at elevated CO_2 partial pressure did not significantly affect leaf protein or N content, nor did the level of starch or soluble sugar respond to the CO_2 treatment, suggesting that reduced maintenance respiration was not contributing to the reduced mass-based respiration rates. Our findings are consistent with the hypothesis that respiration can be reduced in plants exposed to elevated CO_2 partial pressures, due to an inhibition of respiratory enzymes such as succinate dehydrogenase and cytochrome *c* oxidase (Azcón-Bieto *et al*. 1994; Gonzàlez-Meler *et al*. 1996). If the final step of the mitochondrial electron transfer chain is inhibited by growth at elevated CO_2 partial pressure, it is interesting to note that the effect is uniform with canopy depth. In addition, it is apparently not influenced by leaf-level energy demand or ambient irradiance, both of which will decrease with depth in the canopy.

Growth at elevated CO_2 partial pressure did affect the relationship between various leaf traits and respiration, reducing the intercept of the regressions between leaf starch, total protein, leaf N, LMA and respiration. Peterson *et al*. (1999) reported that the intercept of the relationship between photosynthesis and leaf N was increased by growth in elevated CO_2

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partial pressure, yet similarly to our findings, the slope of the relationship was unchanged. Our results are consistent with a direct inhibition of respiration by elevated CO_2 partial pressure such that, at an equivalent level of starch or protein for example, plants grown at ambient CO_2 partial pressure would have higher respiration rates.

Previous work from the first 2 years of this experiment reported that Red beech trees grown and measured at elevated CO_2 partial pressure have higher rates of photosynthesis (Hogan *et al*. 1996, Hogan *et al*. 1997). Less excess light energy was released for trees growing at elevated CO_2 partial pressure, even though photoinhibition was not detected in either ambient or elevated CO_2-grown trees (Hogan *et al*. 1997). To our knowledge, no previous direct measurements of the effect of growth at elevated CO_2 on leaf respiration exist for this species. As a result, our estimates of $A : R$ and our finding of increasing leaf efficiency with depth in the canopy and growth at elevated CO_2 are unique.

We found growth in elevated CO_2 partial pressure increased light-saturated photosynthetic rates, and then examined the ratio of light-saturated photosynthesis to respiration as an index of the potential carbon-gaining capacity of the leaves. This index increased in trees grown at elevated CO_2 partial pressure compared with trees grown in the ambient treatment, suggesting there was an increase in the overall potential carbon efficiency of leaves; this response was particularly strong in the lower half of the canopy. As a result, we did not find that R varied strictly as a proportion of A as has been suggested previously (Reich *et al*. 1998a; Waring, Landsberg & Williams 1998). Increased $A : R$ ratio might have direct effects on the total canopy leaf area index and tree level carbon gain, by increasing the number of leaves maintaining a positive carbon balance and thus being retained. We agree with Reich *et al*. (1998a) that the adaptive strategy of plants to differing levels of environmental resources is likely to include regulation of R to enhance positive carbon balance and/or survival. It is important, however, to note that the $A : R$ ratio presented here is merely an indicator of potential rather than a direct measurement of carbon gain versus loss. The degree to which the lower canopy leaves are light-limited is unknown, as are the potential effects of canopy position and/or atmospheric CO_2 partial pressure on physiological parameters such as the light compensation point or quantum efficiency.

Eddy-correlation measurements above a Red beech canopy (Hollinger *et al*. 1994), and many other forest types (e.g. Valentini *et al*. 2000), stress the importance of night-time respiratory flux in determining the canopy- and regional-scale carbon balance of forested ecosystems on daily, seasonal and annual cycles. Yet, despite the importance of respiration in determining the productivity of forested ecosystems, the process is poorly represented in whole-plant or ecosystem models (Cannell & Thornley 2000; Thornley & Cannell 2000). We concur with Cannell & Thornley (2000) that mechanistic models need to be developed that do not constrain the ratio between whole-plant photosynthesis and respiration, and with Thornley & Cannell (2000) that measurements of respiratory fluxes need to be accompanied by measurements of other leaf traits such as sugars, starch, protein, N and carbon assimilation. We further stress the importance of understanding the variation in leaf respiration through the canopy of individual trees, and the effect this has on ecosystem carbon balance.

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Acknowledgements

We thank G.D. Rogers, T.M. McSeveny, Dr J.E. Hunt, J.N. Byers, N. Lauren and Dr A. Peterson. This research was supported in part by National Science Foundation, Division of International Programs Grant INT-9515449 to D.T.T. and K.L.G., and by National Science Foundation grant IBN 96 03940 to K.L.G. Additional funding came from the Columbia University Center for Environmental Research and Conservation. Majority funding for this work was provided by the New Zealand Foundation for Research, Science and Technology. The research is part of a larger Project 1105 of the Core Research Programme for the GTCE (Global Change and Terrestrial Ecosystems) component of the IGBP (International Geosphere–Biosphere Programme). We acknowledge the generous loan of the open-top chambers from USDA Forest Service, North Carolina, USA and assistance provided by Christchurch City Council. This is Lamont–Doherty Earth Observatory contribution number 6177.

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Received 26 March 2001; accepted 5 April 2001

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