Plant Physiol. (1992) 100, 191–198
0032-0889/92/100/0191/08/$01.00/0

Received for publication February 21, 1992
Accepted April 22, 1992

A Comparison of Dark Respiration between C_3 and C_4 Plants[1]

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ABSTRACT

Lower respiratory costs were hypothesized as providing an additional benefit in C_4 plants compared to C_3 plants due to less investment in proteins in C_4 leaves. Therefore, photosynthesis and dark respiration of mature leaves were compared between a number of C_4 and C_3 species. Although photosynthetic rates were generally greater in C_4 when compared to C_3 species, no differences were found in dark respiration rates of individual leaves at either the beginning or after 16 h of the dark period. The effects of nitrogen on photosynthesis and respiration of individual leaves and whole plants were also investigated in two species that occupy similar habitats, *Amaranthus retroflexus* (C_4) and *Chenopodium album* (C_3). For mature leaves of both species, there was no relationship between leaf nitrogen and leaf respiration, with leaves of both species exhibiting a similar rate of decline after 16 h of darkness. In contrast, leaf photosynthesis increased with increasing leaf nitrogen in both species, with the C_4 species displaying a greater photosynthetic response to leaf nitrogen. For whole plants of both species grown at different nitrogen levels, there was a clear linear relationship between net CO_2 uptake and CO_2 efflux in the dark. The dependence of nightly CO_2 efflux on CO_2 uptake was similar for both species, although the response of CO_2 uptake to leaf nitrogen was much steeper in the C_4 species, *Amaranthus retroflexus*. Rates of growth and maintenance respiration by whole plants of both species were similar, with both species displaying higher rates at higher leaf nitrogen. There were no significant differences in leaf or whole plant maintenance respiration between species at any temperature between 18 and 42°C. The data suggest no obvious differences in respiratory costs in C_4 and C_3 plants.

The unique biochemical and structural differences found in leaves of C_4 species allow for more efficient use of N when compared to C_3 species, primarily due to increased catalytic efficiency of Rubisco, the major soluble protein in mesophyll cells of C_3 plants (7, 26). In general, C_4 plants also invest proportionally less N in leaves than C_3 plants (7, 27). Because synthesis of organic N compounds represents a substantial respiratory cost (23), the bioenergetic cost of maintaining protein pools in C_3 leaves may also be greater when compared to C_4 leaves. Carbon lost through respiration can account for up to 50% of the daily carbon gain by photosynthesis (21, 25) and may be critical in habitats dominated by low light or warm temperatures, where respiration can be high relative to daily carbon gain (6). Warm night temperature was the variable most closely correlated with the relative abundance of C_4 grasses in North America (28), indicating that different respiratory costs between C_3 and C_4 plants may have ecological significance. To describe fully the improved performance of C_4 plants as a result of improved N use efficiency, both photosynthetic and respiratory analyses need to be conducted.

R_n[2] in plants has been examined in terms of two conceptual components, growth and maintenance (10, 13, 18, 19, 29). Growth respiration is considered the energy source for the synthesis of new phytomass. Maintenance respiration supplies energy to maintain current phytomass, is independent of substrate concentration, and includes processes such as protein turnover, ion balance, and tissue acclimation to environmental change (1). Because C_4 plants synthesize and maintain less photosynthetic protein in leaves (26), it is possible that both growth and maintenance respiration would be lower than in C_3 species.

Differences in respiration exist among species (18, 25) and among plant parts (10, 15), yet few comparisons have been made between C_3 and C_4 species. In the few respiratory studies including different photosynthetic types, the question of whether C_3 and C_4 differed in their maintenance costs was never specifically addressed. McCree (18) concluded that the maintenance coefficient of grain sorghum (C_4) was lower than for white clover (C_3). Ryle et al. (25) compared $^{14}CO_2$ loss of barley and maize in continuous darkness for several days and found maintenance respiration in the C_4 species, maize, to comprise 24% of the labeled assimilate, compared to 37% for barley. Penning de Vries (24) also implied that differences in rates of maintenance respiration may exist between species based on earlier work (23) where values of maintenance respiration in *Helianthus annuus* (C_3) seemed higher than *Zea mays* (C_4) in all experiments.

To test the hypothesis that greater investment of reduced N in C_3 leaves increases respiratory costs, we compare R_n values of leaves of a number of C_4 species with those of C_3 species of similar form and ecological requirements. In addition, attention is focused on two species, *Chenopodium album* (C_3) and *Amaranthus retroflexus* (C_4), to consider the effects of leaf nitrogen concentration on respiration of individual leaves and whole plants.

MATERIALS AND METHODS

Comparison of Photosynthesis and Respiration in C_3 and C_4 Leaves

Plants listed in Table I were propagated in a greenhouse during late spring from seed or, in the case of *Flaveria*,

[1] Supported by state funds and Hatch funds allocated to the University of Georgia.

[2] Abbreviations: R_n, dark respiration rate; A, CO_2 assimilation rate.

Table I. *A and R_n by Mature Leaves of C_3 and C_4 Species Measured at 30°C, 305 to 370 μL L^{-1} CO_2, 210 mL L^{-1} O_2, and Either 2 mmol Quanta m^{-2} s^{-1} (A) or Complete Darkness (R_n)*

Each value is the mean of three or more replicates.

Plant	*A*	R_n[a]	R_n[b]	A/R_n[a]
		μmol CO_2 m^{-2} s^{-1}		
C_3 species				
Flaveria pringlei	28.4 ± 0.1	−3.1 ± 0.7	−1.1 ± 0.3	9.2
Atriplex patula	26.6 ± 3.0	−2.9 ± 0.4	−0.7 ± 0.2	9.2
Triticum aestivum	27.9 ± 1.0	−2.0 ± 0.1	−0.5 ± 0.1	14.0
Alloteropsis semialata	16.2 ± 1.0	−2.0 ± 0.2	−0.8 ± 0.2	8.1
C. album	27.7 ± 1.4	−2.6 ± 0.3	−1.1 ± 0.2	10.6
Panicum boliviense	18.3 ± 2.1	−2.0 ± 0.4	−0.8 ± 0.3	9.2
Mean	23.5 ± 5.8	−2.4 ± 0.5	−0.8 ± 0.2	10 ± 2
C_4 species				
Flaveria trinervia	47.3 ± 1.1	−2.1 ± 0.6	−1.0 ± 0.2	22.5
Sorghum bicolor	41.1 ± 2.8	−1.8 ± 0.1	−1.0 ± 0.2	22.8
Alloteropsis semialata	28.8 ± 0.2	−3.6 ± 0.3	−1.1 ± 0.3	8.0
Atriplex pentandra	37.4 ± 5.1	−2.3 ± 0.4	−0.8 ± 0.2	16.3
A. retroflexus	36.1 ± 3.6	−2.5 ± 0.4	−1.1 ± 0.2	14.4
Z. mays	35.3 ± 3.0	−2.9 ± 0.5	−0.9 ± 0.2	12.2
Panicum maximum	37.6 ± 4.0	−2.1 ± 0.2	−1.0 ± 0.1	17.9
Mean	37.6 ± 5.6	−2.5 ± 0.6	−1.0 ± 0.1	16 ± 5

[a] Dark respiration at the beginning of the dark period. [b] Dark respiration after 16 h in complete darkness.

vegetatively. Plants were grown at least 4 weeks in 8-L containers in mixtures of equal volumes of peat, sand, and perlite, with mean day/night temperatures of 30/20°C and with natural light. Plants were watered daily and fertilized three times weekly with a full-strength Hoagland solution.

Prior to measurements of respiration, *A* was measured during midday (between 1200 and 1300 h) on young, fully expanded leaves marked for R_n measurements. Both *A* and R_n were measured using a gas exchange system with four leaf cuvettes allowing for the measurement of four leaves (two C_3 and two C_4) during one time period. The IRGA was operated in the differential mode with CO_2 exchange rates based on differences in CO_2 entering and exiting each chamber. Specific details of the gas exchange system have been described in earlier work (8). Leaf temperature during measurements was maintained at 30°C, and PPFD was 2 mmol quanta m^{-2} s^{-1} for *A* and zero for R_n measurements.

The decline in R_n over 16 h for each leaf was sequentially monitored for 15 min of each hour. Measurements were accomplished by four three-way solenoid valves controlled by a data acquisition system that activated only one valve during any 15-min period, allowing gas from one chamber to be monitored. Gas from the other three chambers was released to the atmosphere inside the laboratory when not being monitored. Flow rates through each chamber were maintained at 0.5 L min^{-1}.

Gas Exchange of Leaves Differing in N Content

Seeds of *Chenopodium album* (C_3) and *Amaranthus retroflexus* (C_4) were sown in a greenhouse in June in 4-L pots containing mixtures of equal volumes of sand, perlite, and vermiculite. Greenhouse temperatures ranged from 30 to 35°C during the day and 22 to 26°C during the night. Applications of 0.5 strength Johnson-Hoagland solution (9) modified to contain 12, 3, and 0.75 mM N were administered daily. Each treatment contained identical concentrations of K, P, Ca, Mg, and micronutrients, but in the two N-deficient solutions, SO_4^{2-} and Cl^- were used to replace NO_3^-. Plants were thinned to one per pot with four replicates for each N treatment.

Gas exchange measurements were performed as described above. Measurements of leaf R_n were begun approximately 1 h prior to the end of the natural photoperiod with the initial R_n of each leaf recorded from 5 to 10 min after it had been placed in the cuvette and completely darkened. Reduced leaf N was determined by micro-Kjeldahl procedure using an autoanalyzer following digestion in selenous, sulfuric, and perchloric acid (14).

Gas Exchange of Whole Plants Differing in N Content

Plants of *C. album* and *A. retroflexus* were grown in a growth chamber in 2.2-L (15 cm diameter) pots containing sterilized-washed sand. After germination, seedlings were fertilized daily with 100 mL of 0.5 strength Hoagland solution containing 1.5 mM N until plants reached a height of approximately 5 cm. At this time, 10 pots of each species were harvested and separated into leaves, stems, and roots, and leaf area was determined using a portable leaf area meter. Harvested plants were oven-dried at 75°C and weighed. The remaining pots were fertilized daily with 250 mL of 0.5 strength modified Hoagland solution containing either 6, 3, 1.5, 0.75, or 0.375 mM N in the form of NH_4NO_3.

The plants were grown in a growth chamber from February 27 through May 1, 1991, under the following conditions. Day and night plant canopy temperatures were maintained at 30 ± 2°C and monitored using a Cu-constantan thermocouple

shaded by leaves. A 16-h photoperiod and an 8-h dark period were employed with constant PPFD during the light period of approximately 400 μmol quanta m^{-2} s^{-1} at plant height using fluorescent and incandescent lamps. Dew point of the air during the experiment ranged from 21 to 25°C. The growth chamber was vented with outside air and CO_2 concentrations ranged from 345 to 420 μL L^{-1} during the light and 385 to 450 μL L^{-1} during the dark period.

CO_2 exchange was measured using four cylindrical acrylic chambers (25.08 length; 30 cm diameter), which were placed over each plant and sealed by a shallow pan filled with water to a depth of 8 cm. The pot containing the plant was raised above the water using a small brick. No attempt was made to estimate root and shoot respiration separately. Temperature inside each chamber was measured using a Cu-constantan thermocouple and maintained at 30°C by adjusting the growth chamber temperature. A small blower (4.5 × 6 cm) was attached to the side of the chamber, which allowed for sufficient mixing of air inside the chamber.

Air was supplied to each chamber by an air compressor located outdoors. The air compressor first supplied air to a mini-proportioner at a pressure of 60 p.s.i. The air flow was divided into four tubes using a manifold and four metering valves adjusted to supply air at a rate of 2 to 2.5 L min^{-1} to each chamber as measured by an electronic flow meter, which was monitored continuously by a recorder. The carbon flux of each chamber was monitored for a 15-min period every hour throughout a 16-h light and dark period. Opaque sleeves were fitted over individual chambers to allow measurements of CO_2 efflux to extend through the following light period. Therefore, the normal diurnal cycle of plants not being measured continued uninterrupted.

Measurements of CO_2 exchange on whole plants were accomplished in a similar manner as for individual leaves, except two IRGAs were used in the differential mode. Both were calibrated using 345 and 416 μL L^{-1} CO_2 obtained from gas cylinders. With 345 μL L^{-1} CO_2 flowing through the reference cells of both IRGAs, the CO_2 concentrations of the air entering and exiting each chamber were continuously determined using both analyzers, one determining the CO_2 concentration entering the chamber and one determining the concentration exiting the chamber. Changes in CO_2 levels were recorded using a chart recorder. Water vapor in the air exiting the chambers was removed by passing the air through two condensers maintained at 0°C using a temperature-controlled water bath and then through a column of $Mg(ClO_4)_2$.

Because the gas exchange system could accommodate only four plants, two of each species having approximately the same mass were measured at any given time except near the end of the experiment, when some plants of *A. retroflexus* became too large. At this time, gas exchange of *C. album* plants of similar size was measured. After CO_2 flux measurements, sand was washed from the roots and leaf area determined. Plant parts were then dried at 75°C to a constant weight. Plant dry weight ranged from 1.2 to 4.4 g. Total C and N of leaves were determined by burning finely ground 10-mg samples in a Carlo-Erba analyzer. CO_2 uptake and efflux were integrated over a 16- and 8-h period, respectively, and expressed in mg CO_2 g^{-1} 16 h^{-1} or mg CO_2 g^{-1} 8 h^{-1}, with dry weight converted to CO_2 equivalents (g) as determined by C analysis.

To determine the length of time required for a plant to reach the point where growth respiration ceases, respiration was determined on plants grown at th highest (6.0 mM) and the lowest (0.375 mM) N for different time periods in the dark following the 16-h illumination period. Dark CO_2 efflux was measured for 30 min immediately after lights were off, and at 16, 24, 32, 48, and 64 h of darkness. Because only slight differences existed between measurements at 48 and 64 h for each plant, 48 h was chosen for the dark period required to reach a basal or maintenance rate of respiration. This time period is within the time frame for estimating maintenance as determined by Irving and Silsbury (13). Afterward, CO_2 flux of plants grown at the five N levels was measured during the middle of the photoperiod, immediately following the photoperiod, and after 48 h in the dark at 30°C. Growth respiration was estimated by subtracting maintenance respiration from CO_2 efflux measured in the first hour of darkness.

Temperature Response of R_n of Leaves and Whole Plants Differing in N Content

For plants of *C. album* and *A. retroflexus* grown at 12, 3, and 0.75 mM N, a temperature response of leaf R_n was determined. After measuring R_n for 16 h at 30°C, the temperature was lowered to 18°C and R_n was measured at increasing 4°C increments from 18 to 42°C. Leaf temperature was adjusted to within 0.2°C of the desired temperature using a controlled water bath. A temperature response of R_n of whole plants of *C. album* and *A. retroflexus* was determined on plants grown at 0.375 and 6.0 mM N. After 48 h in darkness, R_n was first measured at 30°C, followed by R_n measurements at 18, 24, 36, and 42°C. The increments of temperature were accomplished by adjusting the growth chamber temperature. In both leaves and whole plants, R_n was expressed in similar units (mg CO_2 g^{-1} dry weight h^{-1}).

RESULTS

Comparison of Photosynthesis and Respiration of C_3 and C_4 Leaves

Maximum A values were higher in C_4 species when compared to C_3 species except *A. semialata*, where the C_4 ecotype did not differ significantly from C_3 species (Table I). In this regard, it should be noted that the species *A. semialata* represents a special case with two putative ecotypes or subspecies, one being C_4 and the other C_3 (5). Differences in leaf R_n were not found between C_3 and C_4 plants either at the beginning of the dark period or after 16 h of complete darkness. The A to initial R_n ratio was greater for most C_4 species, with a mean of 16 compared to 10 for C_3 species.

Respiration of C_3 and C_4 Leaves with Different N Contents

Respiration by leaves of *C. album* and *A. retroflexus* showed a similar rate of decline over a time period of 16 h (Fig. 1), with no significant differences in R_n after 16 h of darkness

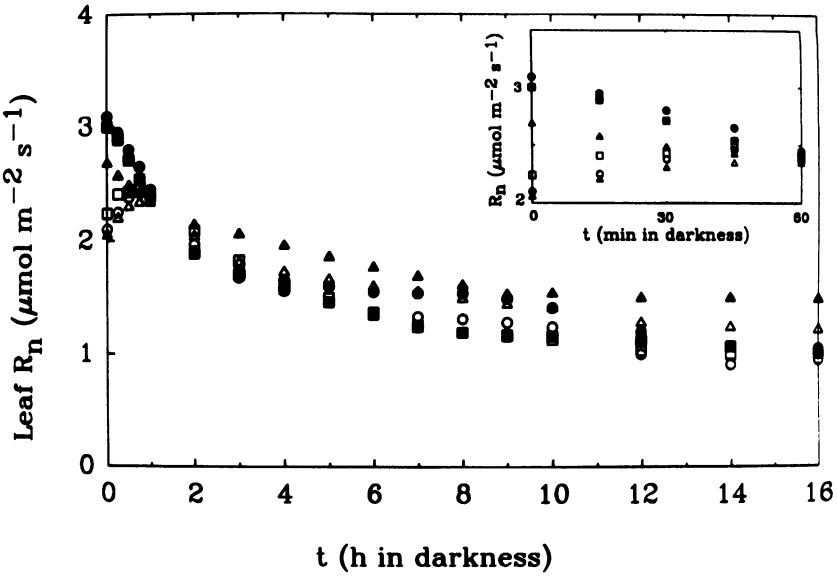

Figure 1. Time course of R_n for leaves of *A. retroflexus* (open symbols) and *C. album* (closed symbols) grown at 12.0 (○, ●), 3.0 (□, ■), and 0.75 (▲, △) mM N. Inset shows the time course for the first hour of darkness. Each point is the mean of three replicates.

between species or among N treatments. As time in darkness progressed, R_n of leaves followed two general patterns. *A. retroflexus* had lower initial rates compared to *C. album* and displayed an increase in R_n for approximately 45 min (Fig. 1, inset) and then declined markedly. The reason for this transient rise was not known, though it may be related to carbohydrate metabolism, as suggested by Heichel (11), or increases in Fru-2,6-P_2 (17). In contrast, R_n of *C. album* leaves declined immediately after darkening. In addition, R_n of some leaves declined rapidly for 3 to 4 h before becoming steady, whereas the decline in other leaves took as much as 12 h to reach a basal rate. The rate of decline in R_n was not related to species or N treatment and contributed to variation in the results.

There was no relationship between leaf N and leaf R_n at 0 or 16 h (Fig. 2A). In contrast, *A* increased with increasing leaf N in both species (Fig. 2B), with the C_4 species, *A. retroflexus*, displaying a greater response to nitrogen compared to the C_3 species, *C. album*. Thus, *A*/N was higher for *A. retroflexus* (347 ± 53 μmol CO_2 mol N^{-1} s^{-1}) than for *C. album* (210 ± 30 μmol CO_2 mol N^{-1} s^{-1}) in the range of leaf N attained. The ratio of *A* to R_n after 16 h of darkness was positively correlated with leaf N (data not shown) in both *A. retroflexus* and *C. album*, with *A. retroflexus* also showing a steeper increase.

Gas Exchange of Whole Plants Differing in N Content

Whole plant gas exchange of *A. retroflexus* and *C. album* differing in N was fitted to a linear equation (Fig. 3) to form a clear relationship between daily net CO_2 uptake and CO_2 efflux ($r = +0.97$). The dependence of nightly CO_2 efflux on CO_2 uptake was similar for both species, even though at higher leaf N, CO_2 uptake and consequently CO_2 efflux were usually higher in *A. retroflexus*. When expressed as a percentage of net daytime CO_2 uptake, daily CO_2 efflux was 20 ± 5% for both species.

The response of CO_2 uptake to leaf N was much steeper for *A. retroflexus* than for the C_3 species, *C. album* (Fig. 4A). A linear increase was found between leaf N and CO_2 efflux by *C. album*, but this relationship was not significant for *A. retroflexus* (Fig. 4B). Analysis of variance indicated CO_2 efflux by *A. retroflexus* increased at higher leaf N, but no significant differences were found between species. Net CO_2 gain per day by both species was calculated by subtracting CO_2 efflux for the normal 8-h night period from net CO_2 uptake over a 16-h photoperiod. A steeper response of net CO_2 gain to leaf N was displayed by *A. retroflexus* when compared to *C. album* and, for both species, the response was significant (Fig. 4C).

Growth and maintenance R_n of plants of *A. retroflexus* and *C. album* grown at different N levels were estimated along with corresponding photosynthetic rates (Table II). No significant differences were found between the two species with respect to growth or maintenance R_n at comparable N levels, with both species displaying higher rates at the higher leaf N. Growth R_n also tended to be higher for both species at the higher N levels. Significant differences were found in CO_2 uptake between the two species, with *A. retroflexus* displaying greater uptake at each N level.

Temperature Response of R_n of Leaves and Whole Plants Differing in N Content

Leaf R_n for the two species displayed a progressive increase to increasing temperature (Fig. 5A), although the temperature effect on *C. album* was less pronounced from 26 to 34°C. There were no significant differences in leaf R_n between species at any measured temperature. When grown at 0.75 mM N, leaves of both species had significantly higher R_n values at 42°C than leaves grown at either 12 or 3 mM N.

After 48 h in darkness, CO_2 efflux was measured at 6°C increments from 18 to 42°C for whole plants of the two species grown at the highest and lowest N levels (Fig. 5B). A significant difference in whole plant CO_2 efflux was found between the two N treatments only at 30°C. No clear differ-

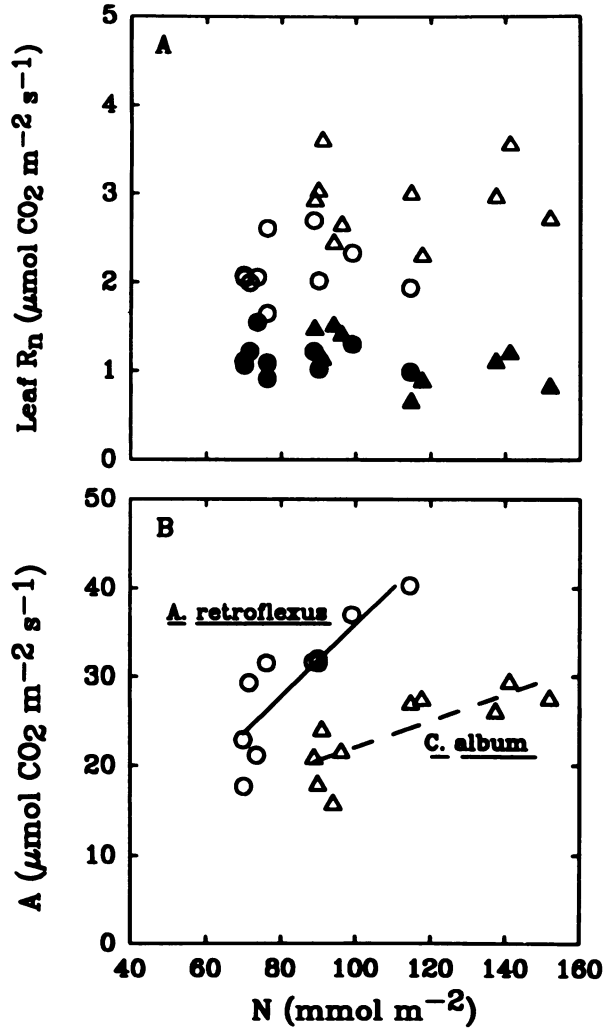

Figure 2. A, The response of leaf R_n at 0 h (open symbols) and at 16 h of darkness (closed symbols) to leaf N in *A. retroflexus* (O, ●) and *C. album* (Δ, ▲). B, The response of A to organic leaf N.

ences in response to temperature were found between species. When expressed on a similar basis, the pattern of response of CO_2 efflux by whole plants to temperature was comparable to that of individual leaves (Fig. 5A).

DISCUSSION

The greater allocation of soluble protein to Rubisco in C_3 species compared to C_4 species (16), and the generally higher N concentration of C_3 species (7, 26, 27), imply a higher maintenance cost for C_3 plants, because the turnover of organic N compounds represents a major expenditure of energy. For example, Penning de Vries (23) calculated that 50 to 60% of the maintenance cost was associated with protein turnover. Barneix et al. (4) calculated a smaller, although a significant proportion (27–36%) of mature leaf respiration in perennial ryegrass (C_3) was due to protein turnover. Based on this, we hypothesized that an additional benefit of the C_4 syndrome is reduced respiratory costs to synthesize and maintain the photosynthetic apparatus. However, there is little evidence from our gas exchange experiments to suggest a difference in R_n between leaves of the two photosynthetic types or for leaves differing in N content. The more efficient use of nitrogen in leaves of the C_4 species, *A. retroflexus* (26), is attributed to greater photosynthesis at any given N concentration rather than to both lower R_n and greater *A*. Greater photosynthetic capacity for most C_4 species in Table I would allow allocation of more carbon for growth if maintenance costs are similar to C_3 species.

Our data do not indicate higher maintenance costs of leaves with higher N concentrations. For both *A. retroflexus* and *C. album* and for other species in Table I, R_n of mature leaves declined over a period of 16 h to reach a basal rate, after which decline was minimal. N concentration of leaves had little relationship to the basal rate of R_n, which contradicts a model described by Amthor (2) in which maintenance is expressed as a linear function of protein. It is possible that leaves at lower N levels had not reached minimum R_n, but at 16 h, the difference between N levels was as great as at any earlier time. It is therefore unlikely that leaves with high N would have substantially higher R_n at some later time. Rather, in mature leaves of similar age, R_n is more likely to be related to the amount of carbohydrate synthesized during the previous light period (3) than to growth and maintenance requirements.

The lack of a relationship between N and leaf R_n has been observed in a number of species. Wilson (30) found that R_n of leaves of *Pavicum maximum* (C_4) was less affected than other growth parameters by increasing N stress and that R_n for leaves of *Lolium perenne* (C_3) was variable over the various levels of N deficiency. Under different N supply, R_n was

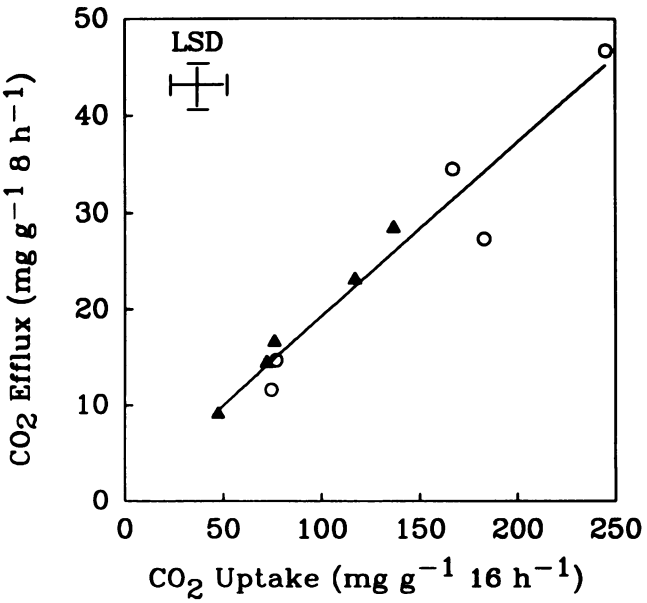

Figure 3. The relationship between nightly CO_2 efflux and CO_2 uptake by whole plants of *A. retroflexus* (O) and *C. album* (▲) grown at five N levels. Each point is the mean of two to five replicates (n = 32). CO_2 efflux = 1.073 + 0.1807 CO_2 uptake (r = + 0.97).

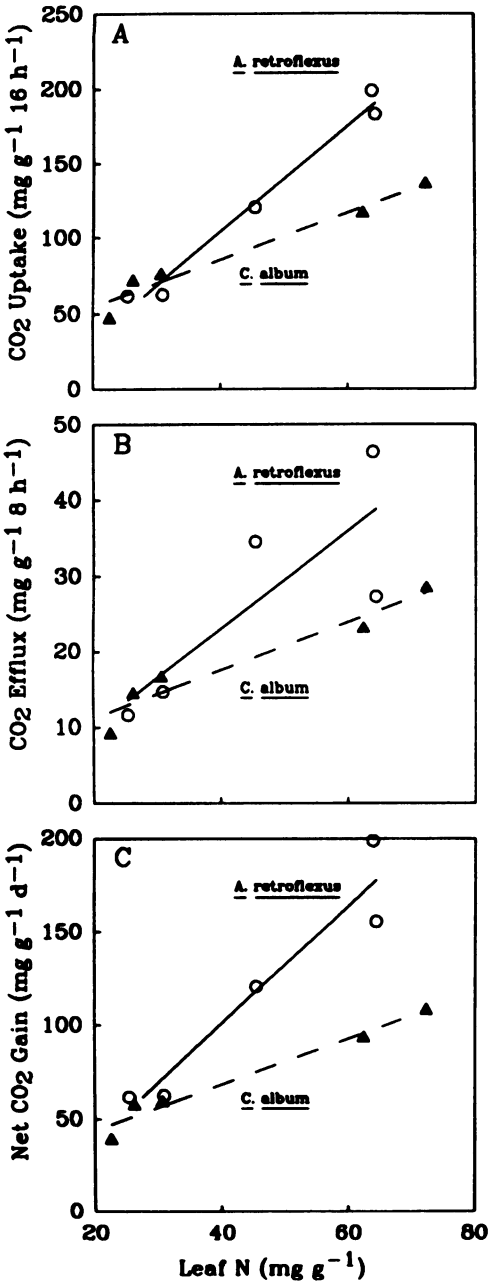

Figure 4. The relationship between whole plant gas exchange and leaf N in *A. retroflexus* (O) and *C. album* (▲). A, Response of CO_2 uptake to leaf N (for *A. retroflexus*, CO_2 uptake = −37.46 + 3.54[leaf N], $r = 0.99$, $P < 0.01$; for *C. album*, CO_2 uptake = 23.46 + 1.55[leaf N], $r = 0.98$, $P < 0.01$). B, Response of CO_2 efflux and leaf N (for *A. retroflexus*, CO_2 efflux = −2.99 + 0.65[leaf N], $r = 0.82$, not significant; for *C. album*, CO_2 efflux = 4.81 + 0.32[leaf N], $r = 0.96$, $P < 0.05$); C, Response of net CO_2 gain to leaf N (for *A. retroflexus*, net CO_2 gain = −25.31 + 3.15[leaf N], $r = 0.96$, $P < 0.05$; for *C. album*, net CO_2 gain = 19.51 + 1.219[leaf N], $r = 0.98$, $P < 0.01$).

unaffected in leaves of barley (20), ryegrass (31), and *Ammophila arenaria* and *Elymus mollis* (22). However, Hirose and Werger (12) found a positive, though weak, correlation between leaf nitrogen and dark respiration in the perennial herb *Solidago altissima*. Therefore, the coupling of N and protein content to maintenance R_n of leaves may be of only minor importance.

In contrast to the lack of a relationship between N and leaf R_n (e.g. Fig. 2A), increased whole plant CO_2 efflux by *C. album* was clearly demonstrated when plotted against leaf N and was also apparent for *A. retroflexus* (Fig. 4B). However, net CO_2 gain also increased with increased leaf N (Fig. 4C). The greater response of net CO_2 gain found in *A. retroflexus* compared to *C. album* reaffirms the ability of this C_4 species to sustain higher growth through greater CO_2 assimilation at similar, high N levels (26). The linear relationship in Figure 3 indicates that both R_n and A are controlled in a similar way by N level, probably because of increases in both the proportion of meristematic tissues with high R_n and the photosynthetic capacity of leaves at high N. The fact that data for both *A. retroflexus* and *C. album* fit the same regression in Figure 3 indicates that the influence of photosynthetic pathway on R_n is through its effect on A. Higher A causes a similar increase in whole plant R_n irrespective of the CO_2 assimilation pathway.

The increase in R_n with higher N was due to increases both in maintenance and growth components (Table II). In fact, the two increased in proportion so that maintenance accounted for 43 ± 4% of total R_n when averaged across N levels and species. This constancy of percentage of maintenance respiration implies that neither N nor photosynthetic pathway has much influence on the proportion of respiration used for maintenance. Because there was no effect of N on leaf R_n, it appears likely that with whole plants, greater R_n was due to increased meristematic activity rather than increased leaf mass.

Table II. *N Effects on CO_2 Flux of Whole Plants of C. album and A. retroflexus*

Growth respiration is considered the difference between CO_2 efflux at the beginning of the dark period and after 48 h. Estimates of maintenance respiration are measurements of CO_2 efflux after 48 h in darkness. CO_2 uptake was measured during the middle of the photoperiod.

Species	Leaf N[a]	Respiration		CO_2 Uptake
		Growth	Maintenance	
	mg g^{-1}	mg g dry wt^{-1} h^{-1}		
C. album	74	6.1	5.4	15.5
	65	5.7	5.4	13.6
	54	6.5	4.6	13.6
	48	5.7	3.8	13.0
	33	4.4	3.8	9.6
A. retroflexus	72	6.7	5.5	19.8
	64	7.4	5.7	20.4
	48	5.9	4.3	22.5
	31	6.0	3.5	14.3
LSD (0.05)	3.1	2.7	0.9	3.1

[a] Plants were grouped according to similar leaf N, with each N concentration representing the mean of two to five measurements.

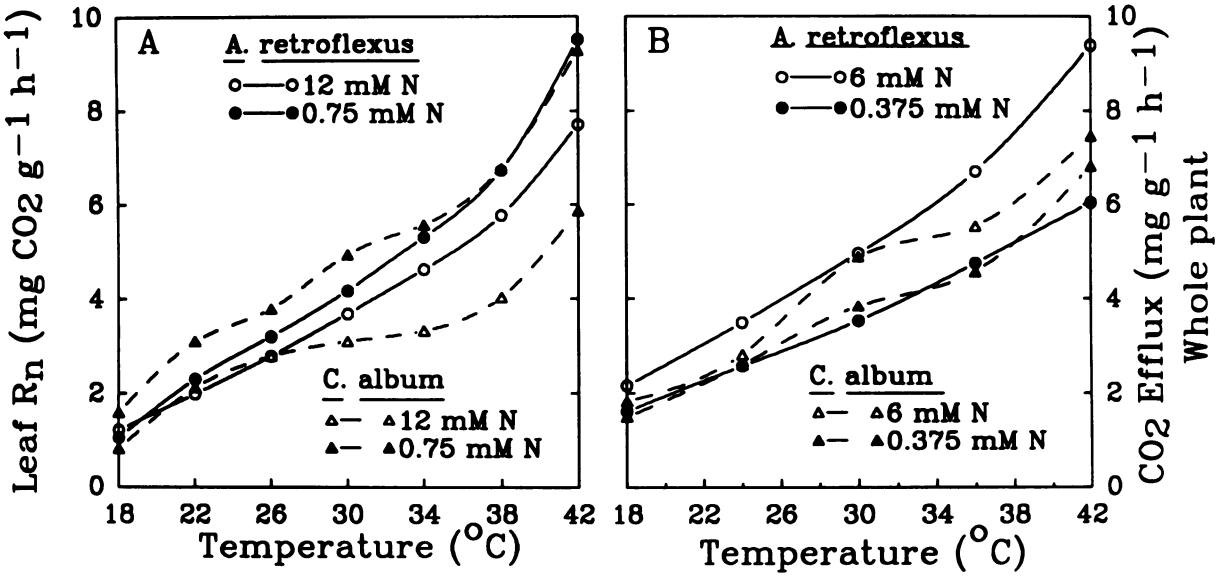

Figure 5. A, Temperature response of leaf R_n after 16 h of darkness in *A. retroflexus* (O, ●) and *C. album* (Δ, ▲) grown at 12 mM N (open symbols) and 0.75 mM N (closed symbols). Specific leaf weights were 33.2 ± 0.4 and 32.6 ± 3.8 g m^{-2} for *A. retroflexus* and 37.8 ± 2.4 and 31.8 ± 2.2 g m^{-2} for *C. album* grown at 12 and 0.75 mM N, respectively; Leaf [N] were 42.7 ± 5.0 and 29.9 ± 2.9 mg g^{-1} for *A. retroflexus* and 53.3 ± 3.7 and 40.8 ± 2.7 mg g^{-1} for *C. album* grown at 12 and 0.75 mM N, respectively. B, Temperature response of CO_2 efflux after 48 h in darkness for whole plants of *A. retroflexus* (O, ●) and *C. album* (Δ, ▲) grown at 6.0 (open symbols) and 0.375 mM N (closed symbols). Leaf [N] were 72 and 31 mg g^{-1} for *A. retroflexus* and 74 and 33 mg g^{-1} for *C. album* grown at 6.0 and 0.375 mM N, respectively (LSD = 3.1).

Because optimum photosynthesis of most C_4 species occurs at temperatures of 30 to 35°C and C_3 species at temperatures of 15 to 25°C, it was interesting to evaluate R_n of the two species at a range of temperatures. The effect of temperature on R_n was similar for leaves (Fig. 5A) and whole plants (Fig. 5B) of *A. retroflexus* and *C. album*. The lack of an effect of N on their temperature dependency of R_n suggests the overriding influence of other processes besides protein turnover. Because maintenance R_n is a strong function of temperature, the similar temperature response provides further evidence that maintenance is similar for the two species.

Any decreases in the maintenance costs of C_4 leaves compared to C_3 leaves could result in enhanced productivity of the whole plant. In the results presented here, it appears that differences do not exist between C_3 and C_4 species in the fraction of respiration used to maintain the plant. It appears that the growth and maintenance of tissue is more dependent on the composition of biomass than on the species or photosynthetic pathway. Even though C_4 leaves have lower levels of N and especially Rubisco, the data suggest no obvious differences in their maintenance requirement.

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