



## Nitrogen resorption in senescing tree leaves in a warmer, CO<sub>2</sub>-enriched atmosphere

Richard J. Norby<sup>1,\*</sup>, Tammy M. Long<sup>2,3</sup>, Jennifer S. Hartz-Rubin<sup>4,5</sup> and Elizabeth G. O'Neill<sup>1</sup>

<sup>1</sup>Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, 37831-6422, USA;

<sup>2</sup>Department of Biology, University of Michigan, Ann Arbor, Michigan, 48109-1048, USA. <sup>3</sup>Current address:

Department of Biology, University of Toledo, Toledo, Ohio 43606, USA. <sup>4</sup>St. Olaf College, Northfield, Minnesota,

55057, USA. <sup>5</sup>Current address: Department of Plant Biology, University of Illinois, Urbana, Illinois 61801, USA.

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### Abstract

The prediction that litter quality, and hence litter decomposition rates, would be reduced when plants are grown in a CO<sub>2</sub>-enriched atmosphere has been based on the observation that foliar N concentrations usually are lower in elevated [CO<sub>2</sub>]. The implicit assumption is that the N concentration in leaf litter reflects the N concentration in green leaves. Here we evaluate that assumption by exploring whether the process of seasonal nutrient resorption is different in CO<sub>2</sub>-enriched plants. Nitrogen resorption was studied in two species of maple trees (*Acer rubrum* L. and *A. saccharum* Marsh.), which were planted in unfertilized soil and grown in open-top chambers with ambient or elevated [CO<sub>2</sub>] in combination with ambient or elevated temperature. In the second growing season, prior to autumn senescence, individual leaves were collected and analyzed for N and dry matter content. Other leaves at the same and an adjacent node were collected for analysis as they senesced and abscised. This data set was augmented with litter samples from the first growing season and with green leaves and leaf litter collected from white oak (*Quercus alba* L.) saplings grown in ambient and elevated [CO<sub>2</sub>] in open-top chambers. In chambers maintained at ambient temperature, CO<sub>2</sub> enrichment reduced green leaf N concentrations by 25% in *A. rubrum* and 19% in *A. saccharum*. CO<sub>2</sub> enrichment did not significantly reduce resorption efficiency so the N concentration also was reduced in litter. There were, however, few effects of [CO<sub>2</sub>] on N dynamics in these leaves; differences in N concentration usually were the result of increased dry matter content of leaves. The effects of elevated [CO<sub>2</sub>] on litter N are inherently more difficult to detect than differences in green leaves because factors that affect senescence and resorption increase variability. This is especially so when other environmental factors cause a disruption in the normal progress of resorption, such as in the first year when warming delayed senescence until leaves were killed by an early frost. The results of this experiment support the approach used in ecosystem models in which resorption efficiency is constant in ambient and elevated [CO<sub>2</sub>], but the results also indicate that other factors can alter resorption efficiency.

### Introduction

The responses of natural ecosystems to rising concentrations of atmospheric CO<sub>2</sub> begin with the well characterized effect of [CO<sub>2</sub>] on the rate of photosyn-

thesis, but this primary response may be modified by secondary responses, such as changes in plant C and N allocation. Feedback loops have been proposed that might alter the availability of other resources in the environment and the subsequent capacity for continued stimulation of photosynthesis and plant growth (Norby et al., 1995; Zak et al., 1993). Feedbacks through the

\* FAX No: 1 423 576 9939. E-mail: rjn@ornl.gov

N cycle may be particularly important given that N often is a limiting resource in many ecosystems, and there are multiple points of interaction between C and N cycles (Norby, 1998).

Strain and Bazzaz (1983) proposed one possible feedback between elevated  $[\text{CO}_2]$  and N availability that has been the focus of experimental research, ecosystem modeling and general predictions of the long-term responses of ecosystems to global change (Norby and Cotrufo, 1998). They proposed that since leaves of plants grown in high  $[\text{CO}_2]$  usually have lower concentrations of N, the leaf litter from  $\text{CO}_2$ -enriched plants would be of poorer quality and decompose more slowly. This would slow the release of N from litter and the conversion of organic N to inorganic N available for plant uptake (Strain and Bazzaz, 1983). This putative effect of high  $[\text{CO}_2]$  on N availability would result in a negative feedback on net primary productivity (NPP). The importance of this scenario on ecosystem response to  $[\text{CO}_2]$  was highlighted in the model of Rastetter et al. (1992) and has been a major rationale for speculation that the positive effect of  $[\text{CO}_2]$  on NPP would not be sustained (Field et al., 1992).

The 'litter quality' hypothesis of Strain and Bazzaz (1983), and its restatement by others (McGuire et al., 1995), rests on an unstated assumption that the lower N concentration observed in green leaves during the growing season would be reflected in the N concentration of dead leaves that fall to the ground (i.e. leaf litter). The relationship between N in green leaves and N in litter is dependent on the process of resorption (also called retranslocation). Resorption is a proximate controller of leaf litter N concentration, and any consideration of  $\text{CO}_2$  effects on litter quality should explicitly consider  $\text{CO}_2$  effects on resorption. Resorption is a particularly important consideration in deciduous forests, as it represents an important nutrient conservation mechanism (Melillo and Gosz, 1983).

The possibility of a  $\text{CO}_2$  effect on resorption is implied by the apparent disparity between the response of green leaf N to elevated  $[\text{CO}_2]$  and that of leaf litter N. Several compilations of the literature are consistent in showing lower N concentration (mass basis) in  $\text{CO}_2$ -enriched tree species, with the average reduction in trees given as 21% (McGuire et al., 1995) or 16% (Cotrufo et al., 1998; Curtis and Wang, 1998). These compilations may be skewed by the inclusion of experiments with pot-bound plants with many artifacts on N cycling. Focusing solely on the responses

of trees grown in the field with an unconstrained rooting environment, Norby et al. (1999) reported a somewhat smaller 11–14% decline in foliar N concentration which decreased with duration of exposure to the elevated  $[\text{CO}_2]$ . There have been many fewer measurements made of litter N concentration (Cotrufo et al., 1998). Although significant declines in litter N concentration in elevated  $[\text{CO}_2]$  have been reported (e.g. Cotrufo and Ineson, 1996), few significant effects have been reported in field-grown plants (O'Neill and Norby, 1996), and the current consensus is that there is no compelling evidence to support the premise that litter N concentration will be lower in response to  $\text{CO}_2$  enrichment (Mooney et al., 1999; Norby and Cotrufo, 1998). This being the case, there is reason to surmise that the proportion of the N in green leaves that is resorbed during senescence and prior to abscission is smaller in  $\text{CO}_2$ -enriched plants, thereby minimizing the difference in N concentration as green leaves become litter.

Prior to formulating hypotheses about how  $\text{CO}_2$  enrichment might alter the resorption process, it is necessary to consider what regulates resorption. Resorption entails the hydrolysis of proteins and other nitrogenous constituents. Amino acids resulting from protein hydrolysis can account for as much as 91% of the total N resorbed (Chapin and Kedrowski, 1983). Amino acids (particularly amino acids with a low C–N ratio such as glutamine) are loaded into phloem and transported into perennial tissues (e.g. the stems of woody plants). Hydrolysis and phloem-loading are active processes, so resorption proceeds only in active, living leaves. Once a leaf is killed by freezing temperatures or an abscission layer is formed in the petiole, resorption stops. Even in the absence of these events, not all of the N in a leaf can be resorbed. Phloem transport requires maintenance of an osmotic difference between source (senescing leaf) and sink (perennial tissue), and amino acids are presumed to produce a large proportion of the osmotically active substances in a senescing leaf (Chapin and Kedrowski, 1983). Hence, leaves are shed with high levels of amino acids, which can stimulate rapid colonization by decomposing fungi (Chapin and Kedrowski, 1983). The final concentration of N in a leaf as it becomes litter will depend both on the amount of N remaining as well as the amount of dry matter per leaf. Carbon compounds also are resorbed during senescence – if all of the N is translocated as N-rich glutamine, then 2.1 g of C (5.3 g of dry matter) will accompany every gram of N. This amount is but a small percentage of the total C content

of a leaf, and the decline in dry matter content during senescence is usually only about 10% (Chapin et al., 1990).

The extent of N withdrawal can be expressed either as resorption efficiency (the proportion of green leaf N that is resorbed) or resorption proficiency (the terminal concentration in the litter) (Killingbeck, 1996). Plants that are adapted to different nutrient conditions will tend to resorb N until a certain minimum concentration is reached. Year-to-year variation in litter N concentration occurs because the resorption process is sometimes interrupted and the plants do not realize their potential resorption. It has been stated as a paradigm of plant ecology that species from nutrient-poor environments have a higher resorption efficiency than species from nutrient-rich environments, but there is actually little evidence for this (Aerts, 1966). Chapin and Kedrowski (1983) reported lower resorption efficiency in plants with low N status and concluded that high efficiency is not an important adaptation to nutrient stress. Litter with low N concentration is due more to lower initial N or to dilution by structural components than to effective resorption (Chapin and Kedrowski, 1983). Killingbeck (1996) and Aerts (1996) conclude that resorption efficiency is not significantly related to plant nutrient status or site fertility.

There are several ways in which CO<sub>2</sub> enrichment could alter resorption efficiency or proficiency. CO<sub>2</sub> enrichment could influence efficiency indirectly through its effect on green leaf N concentration if leaves with lower initial N concentration have lower (or higher) resorption efficiency (Field et al., 1992), the discussion above notwithstanding. The partitioning of N compounds between soluble and structural compounds is an important regulator of resorption (Pugnaire and Chapin, 1993) that could be sensitive to elevated [CO<sub>2</sub>] (Arp et al., 1997). Elevated [CO<sub>2</sub>] can interact with water supply, sink strength, or phloem-loading (Saxe et al., 1998), all of which are likely to influence the resorption process (Aerts, 1996). Any secondary effect of CO<sub>2</sub> enrichment that alters the timing or completeness of leaf senescence could influence resorption efficiency. Resorption proficiency is influenced not only by initial N content and resorption efficiency, but also by C dynamics. If [CO<sub>2</sub>] alters the dry matter content of litter, terminal N concentration also is affected.

There have been no explicit experimental analyses of CO<sub>2</sub> effects on resorption, despite the apparent importance of this process in controlling litter quality. Indirect evidence has come primarily from post

hoc comparisons of the mean values of [N] in green leaves and litter (Curtis et al., 1989) and inferred from published data on green leaf and litter [N], although in many studies the measurements of the two tissues were made in different ways and the data are not directly comparable. Reliance on mean values of green leaf and litter N concentration preclude any statistical analyses of CO<sub>2</sub> effects. Arp et al. (1997) calculated the resorption efficiencies in four CO<sub>2</sub> enrichment experiments comprising eight plant comparisons. Resorption efficiency averaged 40% in ambient [CO<sub>2</sub>] compared to 26% in elevated [CO<sub>2</sub>], leading them to conclude that differences in green-leaf N concentration would not be reflected in litter. Their analysis was hampered, however, by the few experiments with appropriate data available at that time and by apparent problems in some of the data they did include. Only one of the experiments (with three of the observations) was a field experiment (Curtis et al., 1989). Some of the other observations clearly suffered from experimental artifacts. For example, *Quercus alba* seedlings were grown in growth chambers in which the leaves abscised because of severe N deficiency and not in response to simulated autumn conditions of declining photoperiod and temperature. The litter quality in that experiment was markedly different from that of *Q. alba* litter from a forest (Norby et al., 1986). The data set also included *Bromus mollis* plants (Larigauderie et al., 1988) in which the abscised leaves had very high N concentrations (19–27 mg g<sup>-1</sup>), and resorption efficiency was calculated to be -21%, presumably because of a loss of nonstructural carbohydrates that had accumulated in the pot-bound plants.

As part of a four-year study of the responses of maple trees to elevated [CO<sub>2</sub>] and temperature (TACIT: Temperature and CO<sub>2</sub> Interactions in Trees), we measured N resorption as the change in N content in senescing leaves of *Acer saccharum* and *A. rubrum*. Our expectation was that an explicit examination of resorption would provide a more rigorous test of this critical link in the 'litter quality' hypothesis than post hoc analysis of the difference in mean concentrations in green leaves and litter. Our alternate hypothesis was that resorption efficiency would be lower in CO<sub>2</sub>-enriched plants, thereby minimizing any effects of [CO<sub>2</sub>] on litter N. Data from the first year of the experiment and from a previous field experiment with *Quercus alba* (Norby et al., 1995) will be used to illustrate additional considerations about N resorption. Taken together, the results should be useful for

providing guidance to models of ecosystem response to rising  $[\text{CO}_2]$  and climatic change.

## Methods

### *Research site and experimental design*

The research was conducted in open-top chambers at the Oak Ridge National Laboratory's Global Change Field Research Facility on the National Environmental Research Park, Oak Ridge, Tennessee, USA. Twelve open-top chambers (Rogers et al., 1983) were constructed at the facility on soils classified as Captina silt loam (fine-silty, siliceous, mesic Typic Fragiudult) with moderate-to-medium granular structure and medium internal drainage. The chambers were 3.0 m in diameter and 2.4 m high. An additional 1.2-m panel was installed at the beginning of the third growing season (1996) to accommodate the height growth of the seedlings. The chambers were modified to operate at either ambient temperature or 4 °C above ambient, in combination with ambient or elevated ( $+300 \mu\text{mol mol}^{-1}$ ) atmospheric  $\text{CO}_2$  concentration (Norby et al., 1997). A randomized complete block design was used with four treatments (the factorial combinations of two  $\text{CO}_2$  concentrations with two temperature regimes) in each of three blocks. Over the 1994 growing season the daytime (0600–1800 h)  $\text{CO}_2$  enrichment in elevated  $[\text{CO}_2]$  chambers ( $\pm\text{SD}$ ) was  $301 \pm 72 \mu\text{mol mol}^{-1}$  in ambient temperature chambers and  $302 \pm 78 \mu\text{mol mol}^{-1}$  in elevated temperature chambers. The comparable enrichment values over all 24 h were about  $20 \mu\text{mol mol}^{-1}$  higher. From May to December, the average temperature outside the chamber was 18.5 °C. Average temperature was 18.9 °C in the ambient-temperature chambers and 22.4 °C in the elevated-temperature chambers. Atmospheric humidity was similar between chambers, so vapor pressure deficit was higher in elevated-temperature chambers (Norby et al., 1997).

One-year-old red maple (*Acer rubrum* L.) and sugar maple (*A. saccharum* Marsh.) seedlings from a commercial nursery were planted directly into the soil within the chambers in spring, 1994. Additional seedlings (which were not used in the current study) were planted in spring, 1995, for a total of 10 plants of each species per chamber. The sides and tops of the OTCs were covered with 73% shade cloth to avoid unnatural levels of side light to these shade-tolerant maple seedlings. At the end of the second growing

season (1995) when the resorption measurements were made, the trees averaged 1.3 m tall, and were larger in elevated  $[\text{CO}_2]$  and smaller in elevated temperature (Table 1).

### *Leaf senescence and nitrogen resorption*

On 6 October, prior to any visible color change or chlorophyll loss, one mid-canopy leaf was collected from each of three trees per species per chamber. The opposite leaf at the same node and the two leaves at the next node were marked with flagging tape to identify them for subsequent collections on 25 October and 13 November. The last collection was made as leaves abscised, and the date of abscission was recorded. Interveinal tissue of the collected leaves was sampled with a cork borer, removing 15 6.3-mm disks (total area 444 mm<sup>2</sup>). The disks were dried (70 °C), weighed and ground in a dental amalgamator. The leaf tissue was analyzed for N and C content in a C–N analyzer (Carlo Erba, Milano), with atropine as a standard and NIST apple leaf (SRM1515; N concentration of 22.5 mg g<sup>-1</sup>) as an internal quality check (National Institute of Standards and Technology, Gaithersburg, MD).

Nitrogen concentration data were converted to N content using the leaf mass per unit area (LMA) of the disks. Nitrogen resorption was calculated as the difference in N content of leaves from the initial collection ('green leaves') and N content of the abscised leaves ('litter'), and resorption efficiency was calculated as resorption divided by the initial N content. Resorption efficiency also was calculated as the percentage change in N concentration to facilitate comparisons with literature values, but in this calculation, changes in N content are confounded with simultaneous changes in LMA. Losses in N due to leaching during the sampling period were not accounted for, and therefore are included as part of resorption.

Subsamples of the first collection were analyzed for the relative proportions of soluble and insoluble N. A portion of the dried leaf powder was extracted in 70% acetone followed by 0.05% sodium dodecyl sulfate in 0.1 M NaOH. After centrifugation the pellet was washed, dried, weighed and analyzed for N content with the C–N analyzer. The N content of the insoluble residue was expressed as a proportion of the N content of the leaf disks prior to extraction, and the soluble fraction was assumed to account for the remainder of the N content.

Table 1. Size of *Acer rubrum* and *Acer saccharum* trees at the end of the second growing season (1995) in open-top chambers with ambient or elevated [CO<sub>2</sub>] combined with ambient or elevated temperature. Data are means (±SE) of three replicate chambers per treatment (n=3); five plants of each species were measured in each chamber

Treatment	<i>Acer rubrum</i>				<i>Acer saccharum</i>			
	Height (m)	Stem diameter (mm)	Estimated dry mass (g/plant)	Relative dry mass	Height (m)	Stem diameter (mm)	Estimated dry mass (g/plant)	Relative dry mass
Ambient temp.								
Ambient [CO <sub>2</sub> ]	1.2±0.2	14.0±1.6	49.7±15.9	1.00	1.5±0.1	12.3±0.5	45.4±4.2	1.00
Elevated [CO <sub>2</sub> ]	1.6±0.2	17.2±1.3	95.7±5.2	1.92	1.3±0.1	13.6±0.6	58.2±2.2	1.29
Elevated temp.								
Ambient [CO <sub>2</sub> ]	1.0±0.2	10.7±0.1	27.6±4.0	0.55	1.0±0.1	9.2±0.5	22.4±3.4	0.52
Elevated [CO <sub>2</sub> ]	1.6±0.1	16.4±0.9	76.0±13.9	1.52	1.2±0.1	12.5±1.2	44.8±11.4	1.07

The effects of [CO<sub>2</sub>] and temperature treatments were analyzed for the two species separately using analysis of variance for the randomized complete block design. Treatment×block interaction was used as the error term, thereby using the chamber as the experimental unit. The treatment sum of squares was partitioned into an orthogonal set of single-degree-of-freedom contrasts, which compared ambient temperature vs. elevated temperature, ambient vs. elevated [CO<sub>2</sub>] within ambient temperature, and ambient vs. elevated [CO<sub>2</sub>] within elevated temperature.

#### Chlorophyll determination

Chlorophyll concentration of intact leaves was measured in 1994 and 1995 as an index of the time course of leaf senescence. Total chlorophyll of leaves from throughout the canopy was estimated nondestructively (Fanizza et al., 1991) using a SPAD-502 meter, which measures the ratio of leaf absorbance at 650 and 940 nm (Minolta Camera Co., Osaka). To convert the SPAD meter units to chlorophyll content, a calibration set comprising 64 leaf samples from both *Acer* species over the full range of senescence was established. After measuring the calibration leaves with the SPAD meter, chlorophyll was extracted in ethanol and measured spectrophotometrically (Lichtenthaler and Wellburn, 1983). A quadratic regression provided the best fit over the full range of chlorophyll content:

$$\begin{aligned} \text{chl}_{a+b} (\text{g m}^{-2}) &= 0.0106 + 0.0024 * \text{SPAD} \\ &+ 0.0001775 * (\text{SPAD})^2 \\ R^2 &= 0.91 \end{aligned}$$

#### Abscission and litter chemistry in 1994

The timing of leaf abscission was determined by collecting leaves from each plant as they fell. Dry mass of the abscised leaves was converted to leaf area based on the LMA of a subset. The data were expressed as the percentage of total plant leaf area that had abscised by certain dates. Nitrogen concentration of a homogenized subsample of leaf litter was determined as previously described.

#### Nitrogen in *Quercus alba* – 1992

Nitrogen concentration was measured in green and senescent leaves of *Quercus alba* saplings during the last growing season of a 4-year experiment. The experiment is described by Norby et al., (1995). The trees were rooted in unconstrained and unfertilized soil within open-top chambers from May, 1989, until November, 1992. There were two replicate chambers for each of three concentrations of atmospheric CO<sub>2</sub>: 350, 500 and 650 μmol mol<sup>-1</sup>. On 19 August 1992, eight first-flush leaves were collected from throughout the canopy of each of the five plants per chamber. A second sample of first-flush leaves was collected on 3 November when the trees were harvested. The leaves had senesced and most had formed an abscission layer. Nitrogen concentration was determined with the C–N analyzer as previously described. LMA determinations of the November samples included the petioles, whereas the LMA of the August samples were for leaf blades only. Therefore, comparable N content data

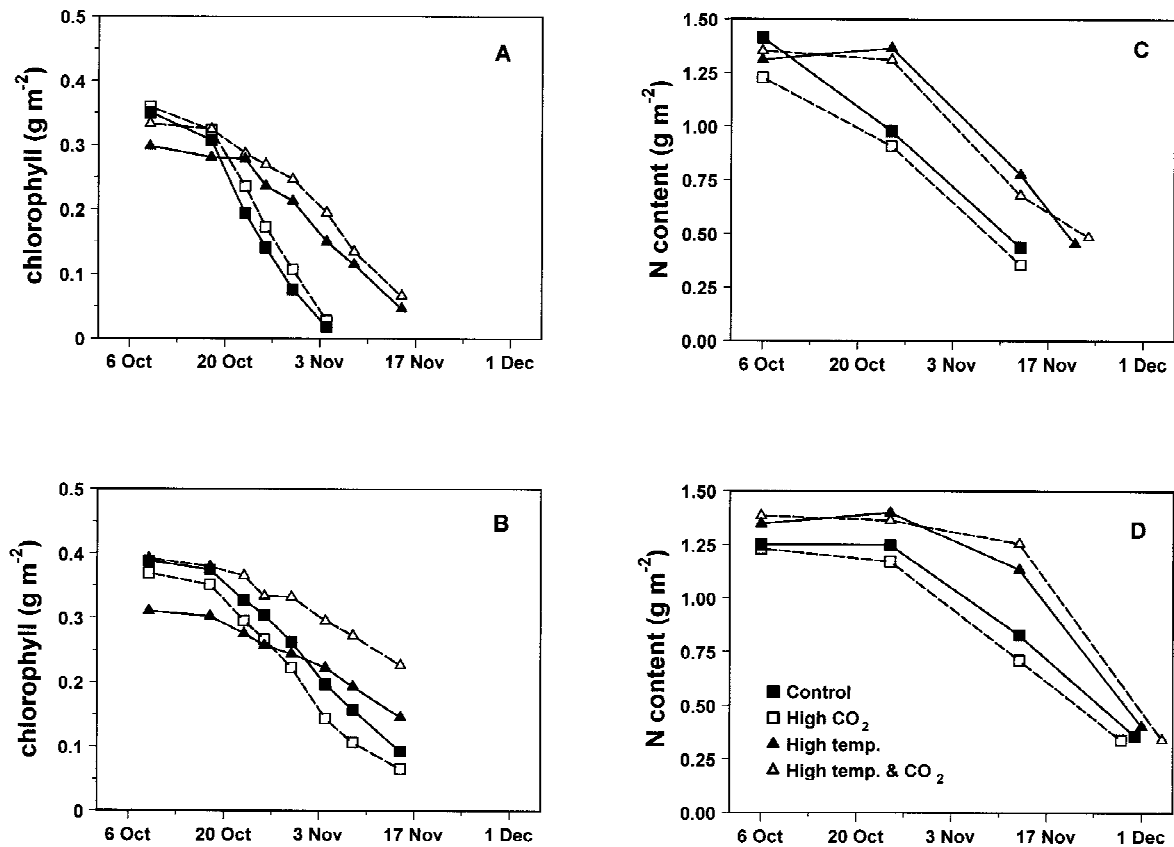


Figure 1. Decline in chlorophyll and nitrogen content during senescence in autumn, 1995. (A) chlorophyll content of *Acer rubrum* and (B) *Acer saccharum*. Chlorophyll was measured on all of the leaves of a plant; data are the means of three chambers per treatment, with five plants measured in each chamber. (C) N content of *Acer rubrum* and (D) *Acer saccharum*. N content was measured on one mid-canopy leaf from three plants per chamber. A different leaf from two adjacent nodes was used at each sample date. The last point in each series is plotted at the average date of abscission.

could not be determined, and resorption efficiency could be calculated only on a mass basis.

## Results

Nitrogen content declined fairly steadily from early October until the leaves abscised in mid-November or early December (Figure 1c,d). N resorption and leaf abscission occurred earlier in *A. rubrum* than in *A. saccharum*, and in both species resorption and abscission were delayed in elevated temperature. However, there was little effect of CO<sub>2</sub> concentration on these dynamics. The decline in N concentration during senescence paralleled the loss of chlorophyll (Figure 1a,b). When leaf sampling for N content started on 6 October, chlorophyll concentrations were at a peak, supporting the assumption that resorption had not yet started.

In ambient temperature prior to senescence, N concentration (mass basis) of *A. rubrum* leaves was 25% lower in elevated [CO<sub>2</sub>] than in ambient [CO<sub>2</sub>] (Table 2), and this difference carried over into litter in which N concentration was 22% lower in elevated [CO<sub>2</sub>]. Elevated temperature reduced N concentration in green leaves in ambient [CO<sub>2</sub>], but increased N concentration in litter. The response of *A. saccharum* leaves to CO<sub>2</sub> enrichment (Table 3) was generally similar to that of *A. rubrum*. There was a 19% decline in green leaf N concentration in ambient temperature and a 17% decline in litter. Most of the effects of [CO<sub>2</sub>] on N concentration of green leaves and litter in both species can be explained by the difference in leaf dry matter content; there was little or no significant difference in N content. Coefficients of variation (CV) were always higher for litter than for green leaves.

Table 2. Nitrogen resorption in *Acer rubrum* trees in open-top chambers based on N concentration and content of green leaves (6 October) and abscised leaves (mid-November). Data are means±SE of three replicate chambers per treatment ( $n=3$ ); three plants (one leaf per plant) were measured in each chamber. Statistical analysis was based on the chamber as the experimental unit; ns, not significant ( $P > 0.10$ )

Treatment	N concentration (mg g <sup>-1</sup> )		N content (g m <sup>-2</sup> )		Resorption efficiency (%)		Soluble fraction in green leaf	Leaf mass per area (g m <sup>-2</sup> )		Dry matter loss (%)
	Green leaf	Litter	Green leaf	Litter	Concn. basis	Content basis		Green leaf	Litter	
Ambient temperature										
Ambient [CO <sub>2</sub> ]	25.6±1.8	7.6±0.4	1.41±0.09	0.38±0.03	70.3±0.7	72.9±2.1	0.87±0.03	55.6±1.9	50.3±4.5	9.1±4.9
Elevated [CO <sub>2</sub> ]	19.2±0.5	5.9±0.6	1.23±0.04	0.33±0.03	68.8±3.2	72.4±3.0	0.88±0.02	64.1±2.4	56.8±4.3	11.2±6.2
Elevated temperature										
Ambient [CO <sub>2</sub> ]	20.7±0.7	8.3±0.1	1.31±0.10	0.49±0.02	59.8±0.7	62.0±3.6	0.79±0.04	64.8±3.7	59.6±2.8	6.3±5.9
Elevated [CO <sub>2</sub> ]	20.0±0.3	7.1±0.8	1.35±0.23	0.49±0.06	64.4±4.7	64.2±4.9	0.86±0.01	67.6±0.9	67.8±1.0	-0.4±2.7
Statistical summary										
CV (%)	7.9	11.1	9.6	17.8	7.3	8.8	4.4	5.5	9.6	137.1
$P_{CO_2}$ in amb. temp.	0.001	0.02	0.09	ns	ns	ns	ns	0.007	ns	ns
$P_{CO_2}$ in elev. temp.	ns	0.09	ns	ns	ns	ns	0.05	ns	0.09	ns
$P_{temperature}$	0.05	0.05	ns	0.005	0.02	0.01	0.04	0.004	0.005	ns

Table 3. Nitrogen resorption in *Acer saccharum* trees in open-top chambers based on N concentration and content of green leaves (6 October) and abscised leaves (about 1 December). Data are means±SE of three replicate chambers per treatment ( $n=3$ ); three plants (one leaf per plant) were measured in each chamber. Statistical analysis was based on the chamber as the experimental unit; ns, not significant ( $P > 0.10$ )

Treatment	N concentration (mg g <sup>-1</sup> )		N content (g m <sup>-2</sup> )		Resorption efficiency (%)		Soluble fraction in green leaf	Leaf mass per area (g m <sup>-2</sup> )		Dry matter loss (%)
	Green leaf	Litter	Green leaf	Litter	Concn. basis	Content basis		Green leaf	Litter	
Ambient temperature										
Ambient [CO <sub>2</sub> ]	22.5±0.8	6.7±0.6	1.25±0.07	0.36±0.04	70.2±1.9	71.2±1.7	0.83±0.03	55.6±2.6	53.3±1.9	3.7±1.2
Elevated [CO <sub>2</sub> ]	18.2±0.9	5.5±0.3	1.23±0.11	0.34±0.03	69.5±2.0	72.0±0.6	0.86±0.04	67.3±3.1	63.3±7.5	6.8±5.3
Elevated temperature										
Ambient [CO <sub>2</sub> ]	21.2±0.3	6.7±0.5	1.35±0.05	0.40±0.03	68.5±2.0	70.0±1.8	0.85±0.04	63.1±1.6	60.1±1.1	4.5±0.4
Elevated [CO <sub>2</sub> ]	20.3±0.8	5.4±0.1	1.38±0.03	0.34±0.01	73.6±1.2	75.3±1.0	0.82±0.02	68.6±1.8	63.8±2.4	6.7±3.0
Statistical summary										
CV (%)	6.2	12.7	9.4	16.5	4.7	3.6	6.4	7.3	12.3	104.0
$P_{CO_2}$ in amb. temp.	0.001	0.08	ns	ns	ns	ns	ns	0.005	ns	ns
$P_{CO_2}$ in elev. temp.	ns	0.05	ns	ns	0.08	0.03	ns	ns	ns	ns
$P_{temperature}$	ns	ns	0.09	ns	ns	ns	ns	ns	ns	ns

Resorption efficiency is best calculated as the percentage change in N content so changes in N are not confounded with changes in leaf dry mass during senescence (Chapin and Kedrowski, 1983). Approximately 70% of the N in green leaves was resorbed during October and November (Tables 2 and 3). This is a higher resorption efficiency than the mean (50–52%) of many plants (Aerts, 1996; Chapin and Kedrowski, 1983) but is consistent with previous measurements of resorption in *Acer rubrum* (Grizzard et al., 1976). The fraction of N resorbed was somewhat less than the soluble fraction of N, which averaged 85% and did not differ significantly between treatments. Resorption efficiency was lower in elevated temperature in *A. rubrum* and higher in elevated [CO<sub>2</sub>] in *A. saccharum* when combined with elevated temperature, but otherwise there were no significant differences. In particular, CO<sub>2</sub> enrichment had no effect on resorption efficiency in ambient temperature – the comparison that is most relevant for the ‘litter quality’ hypothesis – and never was found to significantly reduce resorption efficiency. The dry matter content of leaves declined 0–11% during senescence, but no differences between treatments were significant. Hence, resorption efficiency calculated on a concentration basis, as it is often reported in the literature, did not differ substantially from the content-based resorption and showed similar trends with treatment. The C concentration of dry matter remained constant during senescence (47% in *A. rubrum*, 45% in *A. saccharum*) and was not affected by either [CO<sub>2</sub>] or temperature (data not shown).

The relationships between the initial N concentration in green leaves and either resorption proficiency (the N concentration in litter) or resorption efficiency were determined for ambient temperature only. There was a weak ( $R^2=0.41$ ), but statistically significant ( $P < 0.001$ ) relationship between N concentration in litter and the N concentration in paired leaves prior to senescence (Figure 2a). Leaves from ambient and elevated [CO<sub>2</sub>] were described by the same regression, the slope of which (0.235) corresponds approximately to 1- (resorption efficiency/100). There was no significant correlation ( $R^2=0.02$ ) between resorption efficiency and green leaf N concentration (Figure 2b).

#### Litter nitrogen in 1994

Although N resorption was not specifically studied in the first year (1994) of this experiment, the course of autumn leaf senescence, abscission, and litter forma-

tion provides some insights into one of the important modifiers of the resorption process. In 1994 (and in subsequent years as well) leaf senescence and abscission were delayed in plants grown in elevated temperature (Figure 3). When sub-freezing temperatures occurred on 23 and 24 November, at least 60% of the total plant leaf area in ambient temperature had already abscised, and most of the remaining leaves were senescent. In the elevated temperature chambers, however, three-fourths of the leaf area was still attached and much of it was still green. These attached, green leaves were killed by the low temperature, precluding any further senescence and resorption, and these leaves eventually abscised while still green. This disruption of the resorption process by the phenological response to warming was reflected in the higher N concentrations in litter (Table 4). In ambient [CO<sub>2</sub>], warming increased litter N concentration by 35% in *A. rubrum* and 46% in *A. saccharum*. N contents of litter also were significantly higher in the warmer chambers compared to ambient temperature chambers (62% and 73% increase in the two species), suggesting that resorption was incomplete. Resorption efficiency cannot be calculated for this data set because of the lack of comparable data from green leaves. There were no effects of elevated [CO<sub>2</sub>] on N concentration or content in either temperature regime.

#### Nitrogen resorption in *Quercus alba*

The foliar N concentration in *Quercus alba* (Table 5) was lower than that of the *Acer* species, attributable to the greater LMA in *Q. alba* since N content was similar. Resorption efficiency also was much less than that of the *Acer* species, and the N concentration in litter was similar to or somewhat higher than that in *Acer* species. Elevated [CO<sub>2</sub>] reduced N concentration in green leaves of *Q. alba* primarily through its effect on LMA as there was no significant effect on green leaf N content, litter N concentration, or resorption efficiency.

#### Discussion

The leaf litter of the trees grown in elevated concentrations of CO<sub>2</sub> in these experiments had lower concentrations of N than the litter from trees grown in ambient levels of [CO<sub>2</sub>]. CO<sub>2</sub> enrichment did not reduce resorption efficiency (the proportion of N in green leaves that was resorbed during autumn senescence), and there was no effect on the content or



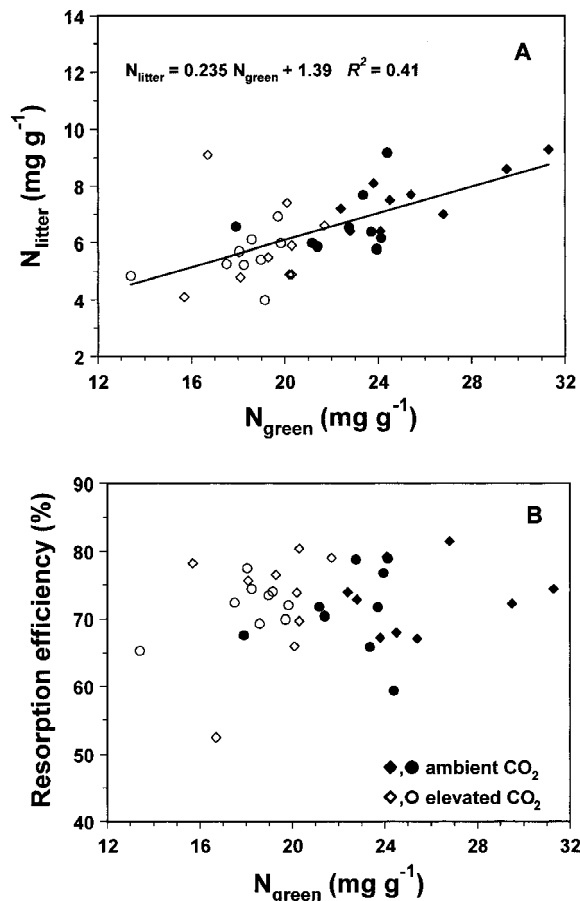


Figure 2. (A) The relationship between green leaf N concentration and litter N concentration. Each point represents an individual plant in ambient  $[\text{CO}_2]$  (solid symbols) or elevated  $[\text{CO}_2]$  (open symbols);  $\diamond$  *Acer rubrum*;  $\circ$  *Acer saccharum*. The linear regression is significant at  $P < 0.001$ . (B) The relationship between green leaf N concentration and resorption efficiency. Resorption was calculated as the relative change in N content (area basis).

dynamics of N. Rather, the  $\text{CO}_2$  effect on N concentration occurred through changes in the dry matter content of leaves. Since resorption efficiency was not reduced,  $\text{CO}_2$  effects on green leaf N concentration were reflected in the litter, and a critical assumption in the ‘litter quality’ hypothesis is supported.

The observations of resorption in these experiments were consistent with the general understanding of the resorption process in many plants. Killingbeck (1996) discussed potential resorption proficiency (the terminal concentration in litter) as the expression that best describes differences in the adaptations of various species to their nutrient environment. As long as resorption is complete such that realized proficiency is equal to potential proficiency, then we might expect litter N concentration of our experimental plants to be

the same across different  $\text{CO}_2$  and temperature manipulations, regardless of initial N concentration in green leaves. This was indeed the case with respect to the  $\text{CO}_2$  regime when N was expressed per unit leaf area. N content of leaves is the better term for describing N dynamics of a system – the amount of N returned to the soil and the amount of N resorbed and available to support a new flush of plant growth. The concept of litter quality and its link to decomposition dynamics, however, is based on the C:N ratio of substrates, which is directly comparable to the inverse of N concentration (since the C concentration of dry matter is almost constant in leaves and fresh litter). In terms of N concentration, the concept of resorption proficiency does not explain the effect of elevated  $[\text{CO}_2]$  in these experiments. The disparity is entirely due to differences in

Table 4. Nitrogen concentration and content of abscised leaves (litter) of *Acer rubrum* and *Acer saccharum* trees after their first year (1994) of exposure to elevated [CO<sub>2</sub>] and elevated temperature in open-top chambers. Data are means±SE of three replicate chambers per treatment ( $n=3$ ); subsamples of all the leaves from each of five plants were measured in each chamber. Statistical analysis was based on the chamber as the experimental unit; ns, not significant ( $P > 0.10$ )

Treatment	<i>Acer rubrum</i>		<i>Acer saccharum</i>	
	N (mg g <sup>-1</sup> )	N (g m <sup>-2</sup> )	N (mg g <sup>-1</sup> )	N (g m <sup>-2</sup> )
Ambient temp.				
Ambient [CO <sub>2</sub> ]	7.1±0.2	0.39±0.09	7.2±0.3	0.45±0.02
Elevated [CO <sub>2</sub> ]	6.1±0.8	0.33±0.10	7.5±0.3	0.52±0.05
Elevated temp.				
Ambient [CO <sub>2</sub> ]	9.6±0.5	0.63±0.14	10.6±0.6	0.79±0.09
Elevated [CO <sub>2</sub> ]	9.6±1.0	0.62±0.13	9.7±1.1	0.70±0.14
Statistical summary				
CV (%)	17.3	34.5	18.9	27.7
P <sub>CO2</sub> in amb. temp.	ns	ns	ns	ns
P <sub>CO2</sub> in elev. temp.	ns	ns	ns	ns
P <sub>temperature</sub>	0.001	0.001	0.001	0.001

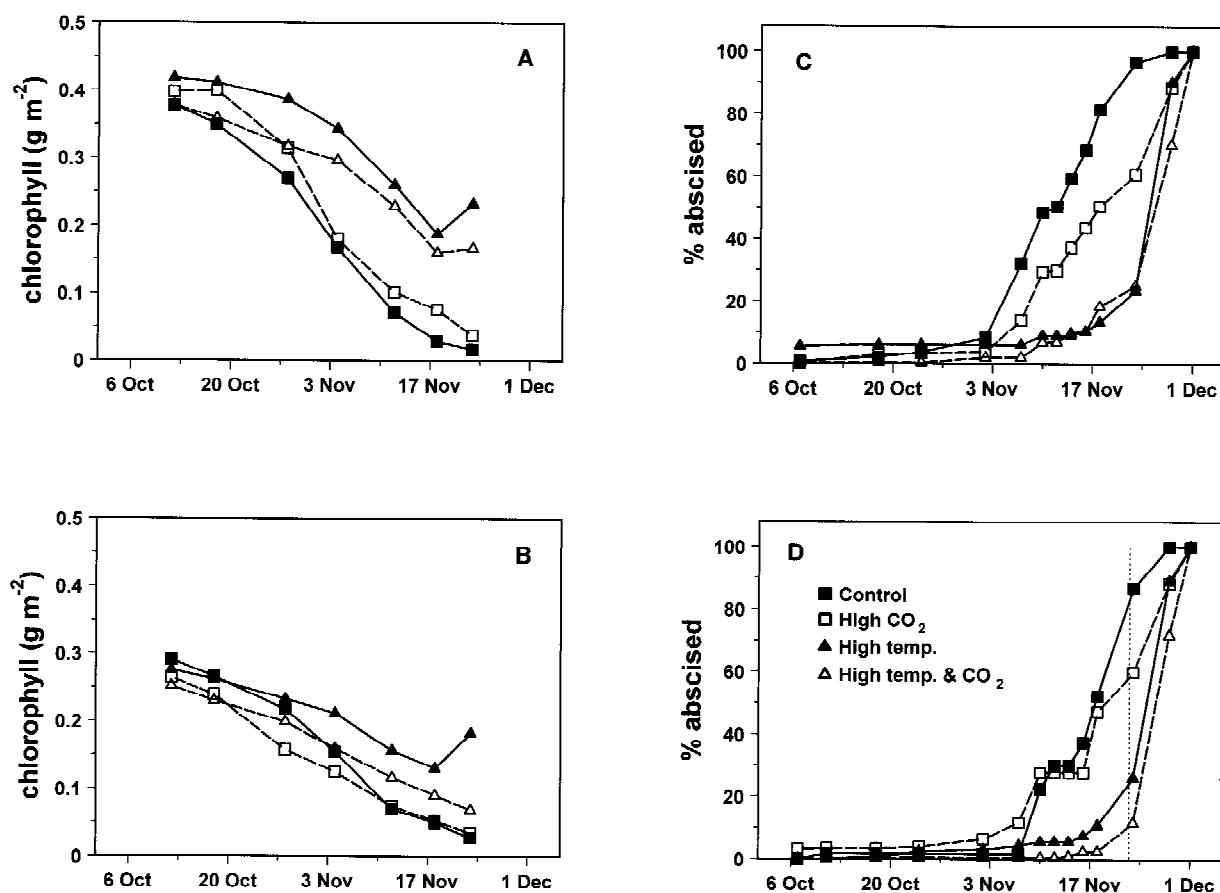


Figure 3. Leaf senescence in autumn, 1994. (A) Decline in chlorophyll content of *Acer rubrum* and (B) *Acer saccharum*. (C) Percentage of total plant leaf area that had abscised by certain dates in *Acer rubrum* and (D) *Acer saccharum*. Dotted line indicates date of first killing frost.

Table 5. Nitrogen concentration, content, and resorption in *Quercus alba* trees in their fourth year of exposure to different CO<sub>2</sub> concentrations in open-top chambers. Data are means±SE of two replicate chambers per treatment (n=2); five plants were measured in each chamber. Statistical analysis was based on the chamber as the experimental unit; ns, not significant ( $P > 0.10$ )

CO <sub>2</sub> concentration ( $\mu\text{mol mol}^{-1}$ )	N concentration ( $\text{mg g}^{-1}$ )		N content ( $\text{g m}^{-2}$ )	Resorption efficiency (%)	Leaf mass per area ( $\text{g m}^{-2}$ ) green leaf
	Green leaf	Litter	Green leaf	Concentration basis	
360	16.6±0.1	9.1±0.1	1.21±0.01	45.1±0.1	73.0±1.2
500	14.1±0.1	7.7±0.1	1.26±0.06	45.0±0.7	89.4±3.7
660	14.8±0.1	8.9±1.0	1.30±0.05	39.7±6.7	87.5±2.0
$P_{\text{CO}_2}$	0.008	ns	ns	ns	0.09

the dry matter content of leaves from different CO<sub>2</sub> regimes and is unrelated to any fundamental change in N resorption. Our results support the view that resorption efficiency is not related to plant nutrient status (Aerts, 1996; Chapin and Moilanen, 1991). Rather, the concentration of N in litter was primarily a function of the initial N concentration, again with the recognition that the differences in initial N resulted from CO<sub>2</sub> effects on the dry matter content of leaves, not the N content.

The increase in dry matter content of leaves (LMA) grown in elevated [CO<sub>2</sub>] is a commonly observed response to elevated [CO<sub>2</sub>] (Saxe et al., 1998). Although increases in LMA are sometimes ascribed to an accumulation of starch and other non-structural carbohydrates, in field grown plants with unconstrained root systems, structural differences – thicker or denser leaves – are probably a larger contributor than carbohydrate accumulation to increased LMA. The distinction is important to the issue of resorption because a dilution of N with nonstructural carbohydrates would be expected to be a temporary phenomenon as the carbohydrates are respired, leached or resorbed from senescing leaves (Mooney et al., 1998). Since C concentration did not change during senescence, we can assume that there was not a large shift from carbohydrates (44% C) to lignin (ca. 64% C). The relative change in LMA during senescence was generally less than 10%. The magnitude of this decline in dry matter content (5.3 g m<sup>-2</sup> in *A. rubrum* in ambient [CO<sub>2</sub>] and temperature) corresponds closely to the expected change if all of the N that was resorbed (1.03 g) was translocated as glutamine. The relative difference in LMA of green leaves and litter was highly variable, contributing to the increased variability in litter N concentration.

Growth of the *Acer* trees in elevated temperature introduced additional variability to the analysis of resorption and litter N. In *Acer rubrum* green leaf N concentration (but not N content) was lower in elevated temperatures, but CO<sub>2</sub> enrichment compensated for the effect of temperature. The summer of 1995 was unusually hot and dry, and the trees grown in elevated temperature showed signs of stress – lower mid-season chlorophyll concentrations and decreased growth rates (Norby et al., 1998). CO<sub>2</sub> enrichment ameliorated or compensated for some of this stress, and the partial reversal of the decline in N concentration may be another indication of a compensation. Elevated temperature decreased resorption efficiency in *A. rubrum*, and N concentrations in litter were higher in the warmer chambers. In contrast, in *A. saccharum* there was no effect of temperature on green leaf N concentration, and elevated temperature (in combination with elevated [CO<sub>2</sub>]) increased resorption efficiency. The difference between the two species is difficult to explain, but may be related to their different timing of senescence (Figure 1). The interactions between the effects of CO<sub>2</sub> enrichment and other environmental variables on the resorption process make it more difficult to generalize about the response of litter N to elevated [CO<sub>2</sub>].

The observations of resorption in *Acer* spp. in 1995 do not support our alternate hypothesis or the prediction of Arp et al. (1997) that resorption efficiency should be lower in CO<sub>2</sub>-enriched plants. They assumed that the fraction of N in soluble compounds should be reduced, related to suggestions that the content of rubisco is lower in CO<sub>2</sub>-enriched leaves. Pugnare and Chapin (1993) demonstrated that the soluble-to-insoluble ratio was an important determinant of resorption efficiency, although in their study

with evergreen mediterranean species, the soluble fraction was considerably less than that observed in our *Acer* species. The soluble fraction was not altered by CO<sub>2</sub> enrichment in the leaves of *Acer* species, however, and the relatively small variation in soluble fraction did not explain any of the variance in resorption efficiency. An apparent CO<sub>2</sub> effect on resorption efficiency was noted in some of the growth chamber experiments included in the analysis of Arp et al. (1997), but much less so in the one field experiment included in the analysis (Curtis et al., 1989). There are several reasons why the results from the growth chamber experiments may not apply to field-grown plants and, therefore, not be relevant to the ecological issue of litter quality and N cycling. Senescence in these experiments did not occur in response to a changing photoperiod and temperature, and the terminal N concentrations in the dead plant tissue was much higher than typical for field-grown plants, indicating that resorption did not progress to completion during the experimental period. The general observation that resorption efficiency is not related to initial N status does not apply to plants grown in laboratory studies where plants are grown at very high nutrient availability and have accumulated to luxury levels (Chapin and Moilanen, 1991). Analyses of N resorption and litter N concentration from N-fertilized, chamber-grown plants probably are not useful for predicting ecosystem responses to elevated [CO<sub>2</sub>].

Although there have been many prior reports of CO<sub>2</sub> enrichment reducing leaf litter N concentration (lowering litter quality), there are few observations of such effects in field-grown plants with unconstrained root systems (O'Neill and Norby, 1996). The current observations, then, seem to be at odds with the view that changes in litter quality with rising [CO<sub>2</sub>] are unlikely to be important (Norby and Cotrufo, 1998). This study suggests several reasons why CO<sub>2</sub> effects on litter N concentration usually are difficult to detect. The resorption process introduced increased variability in litter N compared to green leaf N through variability in dry matter resorption as well as variability in N resorption. Several plants had considerably lower resorption efficiency than the average (Figure 2b), resulting in litter with much higher than predicted N concentrations. Much of the year-to-year variability in litter N concentration of an individual species may be related to various events that prevent complete resorption (Killingbeck, 1996). Many environmental factors can contribute to this, including water stress, premature leaf loss from storm damage or herbivores and

premature disruption of senescence because of early frost. The increased variability in litter N concentration compared to green leaves will inevitably make it more difficult to detect significant effects of elevated [CO<sub>2</sub>]. The total litter produced by a tree will usually include leaves of a variety of physiological and morphological states and leaves that abscised before resorption was complete. In the current study with *Acer* species, variability in litter N was minimized by analyzing only preselected leaves of similar age and canopy position. Note that the analysis of litter N in 1994 (Table 4) was based on a bulk collection; the coefficients of variation were considerably higher than for the 1995 analysis of carefully selected individual leaves (Tables 2 and 3), and there were no significant effects of [CO<sub>2</sub>].

There are large differences among species in resorption efficiency. Resorption efficiency in *Quercus alba* was much less than that of *Acer* sp. This may have been related to high levels of phenolic compounds in *Quercus* sp. Phenolic compounds can bind proteins and interfere with their hydrolysis into smaller, mobile compounds (Pugnaire and Chapin, 1993). If CO<sub>2</sub> enrichment affects the phenolic content of leaves (e.g. Lindroth et al., 1993), there could be secondary effects on resorption efficiency, but there were no effects of [CO<sub>2</sub>] on phenolic content of *Quercus alba* leaves in a parallel experiment at our site (Williams et al., 1998), and there were no effects on resorption efficiency.

The effect of warming on senescence and litter N in 1994 provides an example of how disruption of the resorption process can have a large effect on litter chemistry. Senescence was delayed in the elevated temperature chambers, and attached leaves that still retained chlorophyll (and presumably N) when the first frost of the autumn occurred eventually fell without completing the resorption process. Hence, the N concentration (and N:lignin ratio) was considerably higher than that of litter that formed under ambient temperature. Elevated temperature delayed senescence in 1995 as well, but the leaves in the warmer chambers nevertheless completed the normal course of senescence and resorption before the first killing frost (Figure 1). Unlike elevated temperature, elevated [CO<sub>2</sub>] does not have large or consistent effects on leaf phenology (Norby et al., 1999), but this dramatic effect of temperature on litter chemistry in 1994 illustrates how unpredictable events (e.g. early frost) can alter the relationship of green leaf N to litter N.

The importance of unpredictable events to the process of resorption creates large difficulties in predict-

ing litter N concentration from green leaf N. Ecosystems models, however, must use simple relationships. Whether resorption is specified explicitly or is invoked implicitly from the relationship between green leaf and litter N, it is an important component of models that include N cycling, and many of these models are being used to evaluate the potential effects of elevated CO<sub>2</sub> on ecosystem productivity (Mooney et al., 1999). Litter N concentration (or C:N ratio) is usually set as a constant fraction (typically 50%) of green leaf N, which varies in relation to N availability; hence resorption efficiency is a constant. As foliar N changes with changes in N supply from the soil, litter N changes commensurately (Comins and McMurtrie, 1993). This is a reasonable assumption, supported by the linear, albeit weak, relationship observed in ambient CO<sub>2</sub> in this study. When elevated [CO<sub>2</sub>] effects are included in ecosystem models, green leaf N usually is lower as a result of the CO<sub>2</sub> stimulation of photosynthesis and litter N, therefore, is lower as well in elevated CO<sub>2</sub>. Rastetter et al. (1992) concluded that the increase in litter C:N ratio (lower N concentration) was a key driver of N immobilization in soil and increased N limitation to plant productivity, although actually the increased litter C:N was an inevitable consequence of the model parameterization. None of the models in an intercomparison of CO<sub>2</sub> effects (CMEAL) originally included any CO<sub>2</sub> effects on resorption efficiency, and, therefore, CO<sub>2</sub> effects on green leaf N were reflected in litter, with subsequent effects on decomposition and N availability (Mooney et al., 1999).

The results of our experimental observations suggest that the modeling assumption of a constant resorption efficiency is reasonable. This approach, however, will miss the complexity imposed by occasional disruption of the resorption process. Also, the use of a single resorption efficiency of 50% will not capture the wide variation among species in this trait, as was apparent in the difference between *Quercus alba* and the *Acer* species. Regardless, assumptions about resorption efficiency and litter N concentration may matter little. McMurtrie et al. (1999) have explored the sensitivity of the forest model G'DAY to different assumptions about resorption. With the C:N ratio in litter forced to be the same in ambient and elevated CO<sub>2</sub> (implying a lower resorption efficiency in elevated CO<sub>2</sub>), there were only small effects on NPP and C storage compared to model runs with resorption efficiency set at 60% in both [CO<sub>2</sub>] levels (McMurtrie et al., 1999). Other models in the CMEAL model intercomparison also suggested that reducing resorp-

tion efficiency had little influence on the predicted response of NPP to [CO<sub>2</sub>] (Mooney et al., 1999). Other factors that influence decomposition dynamics at the ecosystem scale, such as species composition, total litter production, and soil moisture, are likely to have a far greater impact on N cycling and ecosystem productivity (Norby and Cotrufo, 1998).

## Conclusions

Nitrogen resorption is recognized to be a primary influence on litter N concentration and therefore must be an important consideration in any analysis of the effects of elevated [CO<sub>2</sub>] on litter quality. Our analysis of resorption in *Acer saccharum*, *Acer rubrum* and *Quercus alba* indicates that CO<sub>2</sub> enrichment does not significantly reduce resorption efficiency and, therefore, the N concentration in litter largely reflects the concentration in green leaves. Since the N concentration in green leaves was lower in elevated [CO<sub>2</sub>], as is most commonly the case, the N concentration was lower in litter as well. There were, however, few effects of CO<sub>2</sub> on N dynamics in these leaves – differences in N concentration were instead a result of increased dry matter content of leaves. The effects of elevated [CO<sub>2</sub>] on litter N are inherently more difficult to detect than differences in green leaves because factors that affect the processes of senescence and resorption increase variability. This is especially so when other environmental factors cause a disruption in the normal progress of resorption, such as when warming delayed senescence. The results of this experiment support the approach used in ecosystem models in which resorption efficiency is constant in ambient and elevated [CO<sub>2</sub>], but they also highlight aspects of resorption that will not be captured by simple relationships.

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