Global Change Biology (2000) **6**, 727–733

Leaf senescence of *Quercus myrtifolia* as affected by long-term CO_2 enrichment in its native environment

J.-H. LI,* P. DIJKSTRA,§ G. J. HYMUS,§ R. M. WHEELER,† W. C. PIASTUCH,‡ C. R. HINKLE‡ and B. G. DRAKE§
**National Research Council, Mail Code JJ-G, †NASA Biomedical Office, Mail Code JJ-G, ‡Dynamac Corporation, Mail Code DYN-3 and 1, Kennedy Space Center, FL 32899, §Smithsonian Environmental Research Center, PO Box 28, Edgewater, MD 21037, USA.*

Abstract

The long-term effects of elevated (ambient plus 350 μmol mol^{-1}) atmospheric CO_2 concentration (C_a) on the leaf senescence of *Quercus myrtifolia* Willd was studied in a scrub-oak community during the transition from autumn (December 1997) to spring (April 1998). Plants were grown in large open-top chambers at the Smithsonian CO_2 Research Site, Merritt Island Wildlife Refuge, Cape Canaveral, Florida. Chlorophyll (a + b) concentration, Rubisco activity and N concentration decreased by 75%, 82%, and 52%, respectively, from December (1997) to April (1998) in the leaves grown at ambient C_a. In contrast, the leaves of plants grown at elevated C_a showed no significant decrease in chlorophyll (a + b) concentration or Rubisco activity, and only a 25% reduction in nitrogen. These results indicate that leaf senescence was delayed during this period at elevated C_a. Delayed leaf senescence in elevated C_a had important consequences for leaf photosynthesis. In elevated C_a the net photosynthetic rate of leaves that flushed in Spring 1997 (last year's leaves) and were 13 months old was not different from fully-expanded leaves that flushed in 1998, and were approximately 1 month old (current year's leaves). In ambient C_a the net photosynthetic rate of last year's leaves was 54% lower than for current year's leaves. When leaves were fully senesced, nitrogen concentration decreased to about 40% of the concentration in non-senesced leaves, in both CO_2 treatments. In April, net photosynthesis was 97% greater in leaves grown in elevated C_a than in those grown at ambient. During the period when elevated C_a delayed leaf senescence, more leaves operating at higher photosynthetic rate would allow the ecosystem dominated by *Q. myrtifolia* to gain more carbon at elevated C_a than at ambient C_a.

Keywords: carbon dioxide, chlorophyll, leaf senescence, nitrogen, Rubisco

Received 6 May 1999; resubmitted 23 October and accepted 6 January 2000

Introduction

Leaf senescence is a highly organized process in the acclimation of higher plants to changing environmental conditions (Thomas & Stoddart 1980; Feller & Fischer 1994; Peñarrubia & Moreno 1995). It is influenced by both exogenous (irradiance, temperature, nutrient availability, water relationships, symbiotic interactions, and pathogens) and endogenous (hormonal balance, source/sink relationships for nutrients and assimilates) factors (Noodén 1980; Kelly & Davies 1988; Grabau 1995). Elevated atmospheric CO_2 concentration (C_a) is known to increase photosynthesis and assimilate availability (Bowes 1993), and improve water- and nitrogen-use efficiency (Drake *et al.* 1997). All of these effects of elevated C_a may also affect leaf senescence.

Previous studies of the effects of elevated C_a on leaf or whole plant senescence have produced conflicting results. Elevated C_a accelerated rates of senescence for leaves of tobacco (*Nicotiana tabacum* L.) (Miller *et al.* 1997), winter wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) (Sicher & Bunce 1997), montane grass (*Festuca vivpara* L.; Baxter *et al.* 1994), and two varieties of

Correspondence: Bert G. Drake, fax +1/301-2617954, e-mail drake@serc.si.edu

ponderosa pine (*Pinus ponderosa* Dougl. Vari. scopulorum and ponderosa; Houpis *et al.* 1988). In contrast, elevated C_a delayed the senescence of *Scirpus olneyi* Grey, a wetland sedge grown in its native environment (Curtis *et al.* 1989a). The rates of leaf-fall in white oak (*Quercus alba*) and yellow poplar (*Liriodendron tulipifera*) were not altered (Gunderson *et al.* 1993). Leaf-fall in five temperate deciduous tree species grown at different nutrient levels in a controlled environment was accelerated, delayed, or unaffected by elevated C_a (Mcconnaughay *et al.* 1996). Thus, it appears that elevated C_a may accelerate, delay or have no effect on leaf senescence depending on plant species and environmental conditions.

Where elevated C_a changes rates of leaf senescence, it may impact ecosystem carbon fixation (Mcconnaughay *et al.* 1996), litter quality, decomposition and nutrient cycling. Clearly, understanding the potential effects on leaf senescence should be an important aspect of studies of the effects of elevated atmospheric C_a on ecosystem-level processes. The aim of this study was to determine whether elevated C_a increased, decreased or had no effect on the pattern of leaf-fall and leaf senescence of *Quercus myrtifolia* Willd., the dominant species in the Florida scrub-oak ecosystem. As a measure of senescence, we monitored the decline in chlorophyll, nitrogen concentration, and Rubisco activity (Crafts-Brandner & Egli 1987; Ford & Shibles 1988; Ono *et al.* 1996; Benbella & Paulsen 1998). For plants grown at elevated and ambient C_a in open top chambers in their native environment, we monitored changes in these important parameters of leaves as they aged from 9 to 13 months.

Materials and methods

Experimental site

The study site was located at Merritt Island Wildlife Refuge on the east coast of central Florida (28°38′N, 80°42′W) USA. The climate is warm, humid and subtropical. Annual precipitation averages 131 cm; a dry period typically occurs from April to June and the rest of the year is wet. The mean daily maximum and mimimum temperatures (respectively) are 22.3 and 9.6 °C for January and 33.3 and 21.9 °C for July. Thunderstorms occur often in summer with frequent lightning strikes, which can cause wildfires.

The soil consists primarily of sand and sandy coquina deposited since the Pleistocene, and has an organic layer about 20 cm deep. The composition of the above-ground biomass at the study sites enclosed in open-top chambers was 76% *Q. myrtifolia*, 15% *Q. geminata* Small, 7% *Q. chapmanii* Sarg. The remaining species were mainly *Myrica serifera* L. (wax myrtle), and *Lyonia ferruginea* (Walt.) Nutt. (rusty lyonia).

Before the study, above-ground biomass was measured and the site was burned (January 1996). After burning, plots were assigned to blocks of three plots with similar biomass characteristics. Sixteen open-top chambers (eight at ambient and eight at elevated C_a) were erected over burned plots. The chambers were octagons, 3.6 m in diameter and 2.1 m in height. Pure CO_2 was added to the air-stream blown into the elevated treatment chambers. Shoots of plants that had begun to grow after the site had been burned were removed before beginning treatment with elevated C_a, on 14 May 1996. The treatments were ambient C_a and ambient plus 350 μmol mol^{-1} CO_2 day and night.

Leaf sampling

Quercus myrtifolia is a perennial oak species. From our observations, plants usually stay green and show no sign of senescence until winter (about January). Therefore, we started our leaf sampling for monitoring the changes of chlorophyll, Rubisco activity, leaf N and C in December 1997. Five plants of *Q. myrtifolia* of similar size were chosen randomly from six ambient C_a chambers and six elevated C_a chambers on December 16, 1997. The first sampling took place on 17 December 1997. The second to fifth samplings were carried out on 3, 10, 24 March and 3 April 1998. Each plant was used for sampling only once. On 3 April 1998, six fully senesced leaves (brown) were collected from each chamber. These fully senesced leaves were still attached to plants, and dropped when they were touched. On 17 April 1998, five nonshaded current year's fully expanded leaves from the new shoots were also collected. Sample collection was carried out from 10.00 to 12.30 hours on sunny days. For each sampling, three leaves from the third to fifth leaves from the top of a shoot were taken to determine specific leaf area and N concentration, with the sixth leaf used for chlorophyll and the seventh leaf used for Rubisco activity. All leaf samples were frozen immediately after collection, and stored at −81 °C until further processing occurred. From this point on, leaves that flushed in Spring 1997 and were sampled between December 1997 and April 1988 are referred to as last year's leaves. Leaves that flushed in Spring 1998 are referred to as current year's leaves.

Leaf chlorophyll and Rubisco activity assays

Frozen leaf tissue (about 100 mg fresh weight) was ground to fine power in liquid nitrogen, mixed with 10 mL of 96% ethanol and left overnight at room temperature in the dark. After centrifugation, the absorption of the supernatant was determined at 647, 654 and 665 nm, and the chlorophyll a and b concentrations were calculated according to Wintermans & Mots (1965).

Frozen leaf tissue (about 100 mg fresh weight) was ground in liquid N and homogenized in 4 mL of 100 mm KH_2PO_4/K_2HPO_4 (pH 7.8), 1 mm EDTA, 5 mm DDT, 1 mm PMSF, 2% triton X-100, 10 mm $NaHCO_3$, 2% PVPP. The extract was centrifuged at 13 000 rpm for 3 min in a microcentrifuge. Five µL of the supernatant was incubated for 5 min in 985 µL of 50 mm Tris (pH 8.0), 10 mm NaCl, 1 mm EDTA, 15 mm $MgCl_2$, 5 mm DTT, 10 mm $NaHCO_3$, 5 mm ATP, 5 mm phosphocreatine, 4 unit/mL creatine phosphokinase, 7 unit/mL glyceraldehyde-3-phosphate dehydrogenase/3-phosphoglyceric phosphokinase, and 0.2 mm NADH. The reaction was started by adding 10 µL 50 mm RuBP. Rubisco activity was determined by monitoring the oxidation of NADH spectrophotometrically (Beckman, DU 640, CA), as described by Lilley & Walker (1974). The extraction and assay buffers were prepared daily and bubbled with N gas for 10 min before use.

Leaf nitrogen and carbon assays

The leaves for measuring N and C concentration were dried in a ventilated oven at 70 °C for 72 h. Analysis was carried out using a CHNS/O Analyser 2400 (Perkin-Elmer Co., CT).

Leaf photosynthesis

The rate of net photosynthesis of the last year's old leaves, from the top of canopy, were measured using a LI-6400 Portable Photosynthesis System (LI-COR, Lincoln, Nebraska) in the field from 10.00 to 13.00 hours on a sunny day (10 April 1998) at a constant light intensity of 1000 $\mu mol\ m^{-2}\ s^{-1}$ PPFD. Photosynthesis was not measured throughout the entire period of leaf senescence because of the lack of required equipment. The net photosynthetic rate of the current year's fully expanded leaves from the top of canopy was measured at the same time of day and light levels on a sunny day (17 April 1998).

Statistics

For measurements made on last year's leaves in December 1997 and April 1998, and on current year's leaves, the effects of C_a and leaf age on leaf chlorophyll content, Rubisco activity, nitrogen concentration and carbon/nitrogen ratio, were tested using two-way analysis of variance (ANOVA). Two-way ANOVA was also used to test for an effect of growth CO_2 and senescence on leaf nitrogen concentration and carbon/nitrogen ratio of non-senesced and fully senesced leaves. Where a statistically significant interaction between C_a and leaf age of senescence was found, differences between individual means were analyzed using a *post hoc* Tukey test, the results of which appear within the text. Differences in rates of net photosynthesis between last year's leaves and current year's leaves were tested for both CO_2 treatments individually using a students *t* test. All statistical analysis was performed using a statistical software package (SYSTAT 7.0.1, SPSS Inc, Chicago, IL, U.S.A.).

Results

Leaf chlorophyll concentration and Rubisco activity

The chlorophyll (a + b) concentration of last year's leaves grown in ambient C_a decreased by 75% with leaf age from December 1997 to April 1998 ($P < 0.001$). For leaves grown in elevated C_a, the 27% decline in leaf chlorophyll (a + b) concentration over the same time period was not statistically significant (Fig. 1a, Table 1). By April 1998 chlorophyll (a + b) was 57% lower in ambient C_a than elevated ($P = 0.023$). Chlorophyll (a + b) concentration of last year's leaves in December did not differ from current year's fully-expanded leaves in April 1998 at either ambient C_a, or elevated C_a.

Rubisco activity in the last year's leaves grown at ambient C_a decreased 82% from December 1997 to April 1998 ($P < 0.001$) (Fig 1b, Table 1). However, leaf aging had no effect on Rubisco activity in the leaves grown at elevated C_a during the same period. Rubisco activity was 44% lower at elevated C_a than at ambient C_a in December ($P = 0.033$). By April 1998 there was no C_a effect on Rubisco activity of last year's leaves. Rubisco activity of last year's leaves in December 1997 did not differ from current year's fully-expanded leaves in April 1998 at either ambient C_a, or elevated C_a.

Leaf N and C/N ratio

Between December 1997 and April 1998 leaf nitrogen concentration of last year's leaves decreased by 52% ($P < 0.001$) at ambient C_a, but by only 25% at elevated C_a ($P = 0.004$) (Fig. 1c, Table 1). Last year's leaves in December 1997 had the same nitrogen concentration as current year's leaves in April 1998 at both ambient C_a and elevated C_a. When leaves were fully senesced, nitrogen concentration decreased to about 40% of the concentration in non-senesced leaves (last year's leaves in December 1997), in both CO_2 treatments ($P < 0.001$) (Fig 2, Table 2). For leaves that were fully-senesced, elevated C_a had no effect on nitrogen concentration. This contrasted with non-senesced leaves where elevated C_a reduced leaf nitrogen concentrations by 15% ($P = 0.012$).

Elevated C_a did not affect leaf carbon concentration (data not shown) and as a consequence, the leaf carbon/nitrogen ratio showed the opposite changes of leaf nitrogen (Fig. 1d, Fig. 2, Table 1, Table 2).

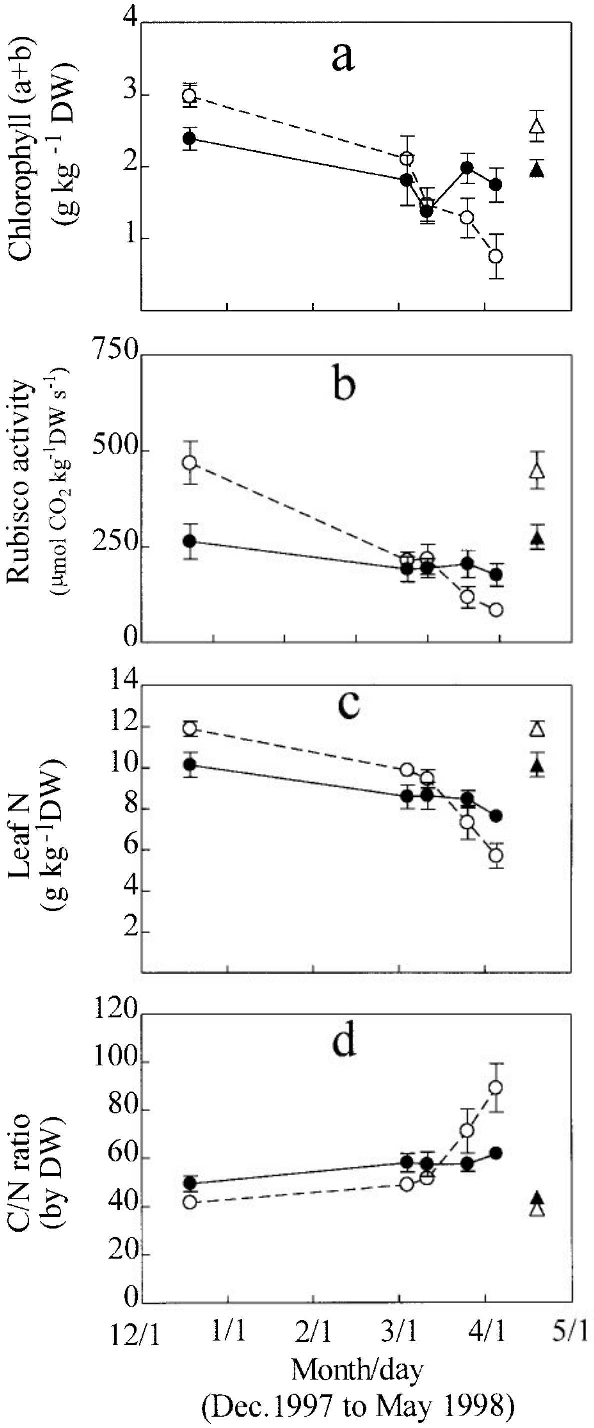

Fig. 1 Chlorophyll concentration (a), Rubisco activity (b), leaf N concentration (c) and C/N ratio (d) in the previous year's leaves (—○— at ambient C_a, —●— at elevated C_a) and current year's fully expanded leaves (Δ at ambient C_a, ▲ at elevated C_a) of *Q. myrtifolia*. Data shown are means ± SE ($n = 6$ plants).

The relationship between leaf N and chlorophyll concentration and Rubisco activity

Data for nitrogen and chlorophyll concentrations and Rubisco activity of *Q. myrtifolia* from last year's leaves and current year's fully expanded leaves at ambient and elevated C_a were pooled in Fig. 3. Elevated C_a did not affect the relationship between either chlorophyll and N concentration (Fig. 3a) or between Rubisco activity and leaf N concentration (Fig. 3b). Both chlorophyll (a + b) concentration and Rubisco activity were correlated with leaf nitrogen (for chlorophyll (a+b), $y = 0.282x - 0.736$, where y–chlorophyll (a + b) content (g kg^{-1} DW), $R = 0.879$; for Rubisco activity, $Z = 35.85x - 120.5$, where z– Rubisco activity (μmol CO_2 kg^{-1} DW s^{-1}), $\times$ – leaf nitrogen (g kg^{-1} DW), $R = 0.729$, $n = 12$).

Leaf photosynthesis

Compared with the current year's fully-expanded leaves net photosynthetic rate of last year's leaves was reduced by 54% ($t_{10} = 3.70$; $P = 0.004$) at ambient C_a, but the 20% reduction at elevated C_a was not statistically significant.

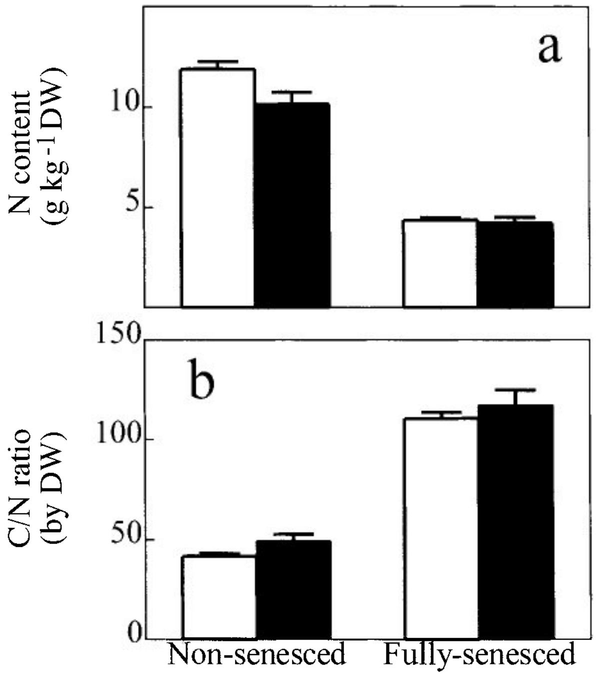

Fig. 2 Nitrogen concentration (a) and C/N ratio (b) of non-senesced and fully-senesced leaves of *Q. myrtifolia* grown under ambient (□) and elevated (■) C_a. Values are means ± SE ($n = 6$ plants).

Due to these effects elevated C_a stimulated net photosynthesis by 97% in last year's leaves but only by 33% in the current year's leaves

Discussion

Collectively, the results presented here indicate that elevated C_a delayed leaf senescence, measured as chlorophyll and N concentrations and Rubisco activity of *Q. myrtifolia*, in its natural environment. Chlorophyll (a + b) concentration, Rubisco activity and N concentration, have been used commonly as indices for leaf senescence (Crafts-Brandner & Egli 1987; Ford & Shibles 1988; Ono *et al.* 1996; Benbella & Paulsen 1998). In this study, chlorophyll (a+b) concentration, Rubisco activity and nitrogen concentration were not different between last year's old leaves in December 1997 and the current year's new fully-expanded leaves in April 1998 (Fig. 1), providing strong evidence that the senescence process had not started when measurements began. Between December 1997 and April 1998, chlorophyll (a+b) concentration, Rubisco activity and nitrogen concentration decreased 75%, 82% and 52% respectively, for leaves grown at ambient C_a (Fig. 1). However, over this same time-period the leaves of elevated C_a grown plants showed no significant decrease in chlorophyll (a + b) concentration and Rubisco activity (Fig. 1) and only a 25% reduction in N concentration.

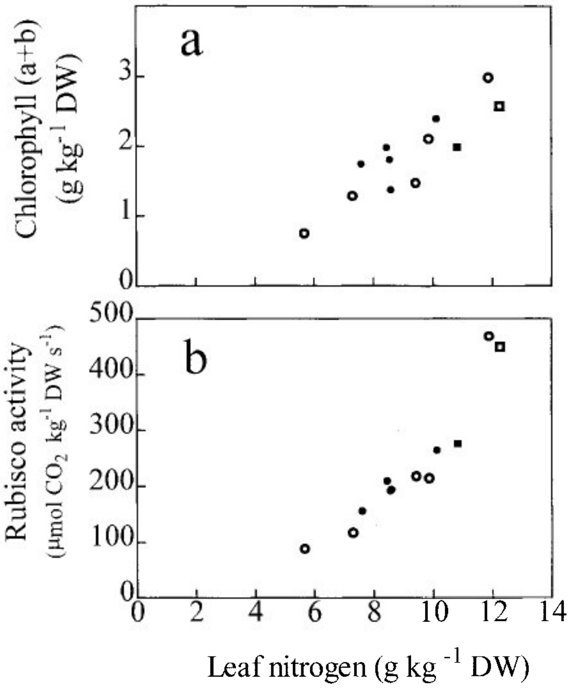

Fig. 3 Correlation of N concentration with leaf chlorophyll concentration (a) and Rubisco activity (b) of *Q. myrtifolia* grown under ambient (○ for last year's leaves, □ for current year's fully-expanded leaves) and elevated (● for last year's leaves, ■ for current year's fully-expanded leaves) CO_2 concentrations. Data are means ($n = 6$ plants).

Decreasing leaf nitrogen may be the first step of leaf senescence. Thomas & Stoddart (1980) explained conflicting results of effects of removal of plant organs on leaf senescence by competition between leaves and other plant structures (fruits, seeds) for nutrients and growth regulators. Ono *et al.* (1996) reported that the demand for nitrogen in the growing organ affected leaf senescence. The close correlation between leaf N and leaf chlorophyll concentration or Rubisco activity in this study (Fig. 3) indicates that the metabolism of nitrogen was probably one of the main factors controlling leaf senescence of *Q. myrtifolia*. In this study competition for nitrogen may have triggered the leaf senescence of *Q. myrtifolia* which was indicated by mobilization of nitrogen from old leaves (Fig. 1, Fig. 2). The leaf nitrogen concentration in the current year's leaves was 15% lower at elevated C_a than at ambient C_a (Fig. 2a). This suggested that the demand for nitrogen was lower at elevated C_a as reported for other C3 species (Wong 1979; Conroy 1992; Drake *et al.* 1997): the plant may require nitrogen from fewer old leaves in order to satisfy the nitrogen demand of new leaves in elevated C_a than at ambient C_a.

Senescence is an orderly loss of functions and structures, comprising an array of biochemical and physiological processes whose ultimate goal is the removal of nutrients from decaying tissues (Peñarrubia & Moreno 1995). Nitrogen and some other nutrients are exported from senescing leaves and transported to sinks (e.g. vegetative storage organs, developing leaves or fruits) (Pate 1980; Simpson *et al.* 1982). Compared with last year's leaves in December (1997) or current year's fully expanded leaves in April (1998), the N concentra-

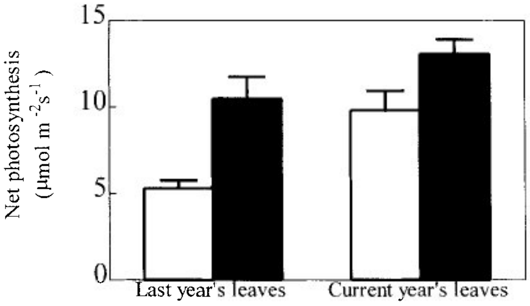

Fig. 4 Leaf net photosynthetic rates in last year's leaves and current year's fully-expanded leaves of *Q. myrtifolia* grown under ambient (□) and elevated (■) CO_2 concentrations and measured at their growing CO_2 concentrations and 1000 μmol m^{-2} s^{-1} PPFD in April (1998) Data shown are means ± SE ($n = 6$ plants).

Table 1 Analysis of the effect of elevated C_a and leaf age on chlorophyll (a + b), Rubisco activity, nitrogen concentration ([N]), and carbon/nitrogen ratio (C/N). Leaves were 9 and 13 months old from the first flush of 1997 and approximately 1 month old from the first flush of 1998.

	Factor	F ratio	P
Chlorophyll (a + b)	CO_2	0.135	0.716
	Leaf age	26.5	0.001
	CO_2 *leaf age	10.0	0.001
Rubisco activity	CO_2	6.30	0.018
	Leaf age	15.7	0.001
	CO_2 *leaf age	5.75	0.008
[N]	CO_2	1.35	0.254
	Leaf age	73.0	0.001
	CO_2 *leaf age	10.4	0.001
C/N	CO_2	1.81	0.189
	Leaf age	34.8	0.001
	CO_2 *leaf age	9.60	0.001

tion of old leaves decreased by about 60% ($P < 0.001$) at ambient or elevated C_a (Fig. 2), i.e. more than 60% of N of fully senesced leaves of *Q. myrtifolia* was exported from the old leaves. Because the scrub-oak ecosystem is considered to be nutrient-limited (Schmalzer & Hinkle 1992), this redistribution of nitrogen is a very important mechanism for optimizing nutrient use in this ecosystem.

Elevated C_a decreased N concentration and increased the C/N ratio in the non senesced leaves (Fig. 2). This agreed with general findings of other studies (Rouhier *et al.* 1994; Robinson *et al.* 1997). Similarly, the absence of any C_a treatment effect on N concentration and the C/N ratio in the fully senesced leaves (Fig. 2) has been reported for salt marsh plants (*Scirpus olneyi*) (Curtis *et al.* 1989b) and alpine plants (*Carex curvula* & *Leontodon helveticus*) (Schäppi & Körner 1997). It has been proposed that the decomposition rate of leaves at elevated C_a might be reduced because the leaf N concentration is lower and C/N ratio is higher (Conroy 1992; Rogers & Runion 1994; Cotrufo *et al.* 1998). The results from this study suggest that decomposition of leaf litter in this scrub-oak ecosystem may not be affected by elevated C_a and unpublished data confirms this (R. Gifford, pers. comm.).

Leaf photosynthetic rate is also an important indicator of leaf senescence. For leaves growing at ambient C_a net photosynthesis was 54% lower in senescing leaves than in young fully-expanded leaves. However, in elevated C_a the small difference between the rates of net photosynthesis in the senescing and the young fully-expanded leaves was not statistically significant. This was consistent with the main finding of this study that leaf senescence was delayed in elevated C_a. For *Q. myrtifolia*, delayed leaf senescence in elevated C_a resulted in a greater stimulation of photosynthesis by elevated C_a during senescence than was observed for young leaves in the spring. In this ecosystem, dominated by *Q. myrtifolia*, this may be another mechanism by which ecosystem carbon uptake may be stimulated in elevated C_a.

Table 2. Analysis of the effect of elevated C_a and senescence on nitrogen concentration ([N]), and carbon/nitrogen (C/N) of non-senesced and fully-senesced leaves.

	Factor	F ratio	P
[N]	CO_2	7.34	0.014
	Senescence	614	0.001
	CO_2 *senescence	4.82	0.040
C/N	CO_2	1.91	0.182
	Senescence	303	0.001
	CO_2 *senescence	0.053	0.821

Acknowledgements

Support for this work came from the Smithsonian Institution, the US Department of Energy and the National Research Council-NASA Kennedy Space Center, USA. We wish to thank Dr John Sager, Dr William Knott, Mr Dave Johnson, Ms. Donna Wayshner, and Ms. Debra Colavito for their help and enthusiastic technical support. Appreciation is also extended to Dr Peter Stiling for constructive reviews of an early version of this paper. We acknowledge the support and encouragement of the NASA Kennedy Space Center, Biomedical Office and the U.S. Fish and Wildlife Service, Merritt Island National Wildlife Refuge.

References

Baxter R, Ashenden TW, Sparks TH, Farar JF (1994) Effect of elevated carbon dioxide on three montane grass species: I. Growth and dry matter partitioning. *Journal of Experimental Botany*, **45**, 305–315.

Benbella M, Paulsen GM (1998) Efficacy of treatments for delaying senescence of wheat leaves: II. Senescence and grain yield under field conditions. *Agronomy Journal*, **90**, 332–338.

Bowes G (1993) Facing the inevitable: plants and increasing atmospheric CO_2. *Annual Review of Plant Physiology and Plant Molecular Biology*, **44**, 309–332.

Conroy JP (1992) Influence of elevated atmospheric CO_2 concentrations on plant nutrition. *Australian Journal of Botany*, **40**, 445–456.

Cotrufo MF, Ineson P, Scott A (1998) Elevated CO_2 reduces the nitrogen concentration of plants tissues. *Global Change Biology*, **4**, 43–54.

Crafts-Brandner SJ, Egli DB (1987) Sink removal and leaf senescence in soybean. *Plant Physiology*, **85**, 662–666.

Curtis PS, Drake BG, Leadley PW, Arp WJ, Whigham DF (1989a) Growth and senescence in plant communities exposed to elevated CO_2 concentration on an estuarine marsh. *Oecologia*, **78**, 20–26.

Curtis PS, Drake BG, Whigham DF (1989b) Nitrogen and carbon dynamics in C_3 and C_4 estuarine marsh plants grown under elevated CO_2 in situ. *Oecologia*, **78,** 297–301.

Drake BG, Gonzalez-Meler MA, Long PS (1997) More efficient plants: a consequence of rising atmospheric CO_2. *Annual Review of Plant Physiology and Plant Molecular Biology*, **48,** 607–637.

Feller U, Fischer A (1994) Nitrogen metabolism in senescing leaves. *Critical Reviews in Plant Science*, **13,** 241–273.

Ford DM, Shibles R (1988) Photosynthesis and other traits in relation to chloroplast number during soybean leaf senescence. *Plant Physiology*, **86,** 108–111.

Grabau LJ (1995) Physiological mechanisms of plant senescence. In: *Handbook of Plant and Crop Physiology* (ed. Pessarakli M), pp. 483–496. Marcel Dekker, New York.

Gunderson CA, Norby RJ, Wullschleger SD (1993) Foliar gas exchange responses of two deciduous hardwoods during 3 years of growth in elevated CO_2: no loss of photosynthetic enhancement. *Plant, Cell and Environment*, **16,** 797–807.

Houpis JLJ, Surano KA, Cowles S, Shinn JH (1988) Chlorophyll and caroteniod concentrations in two varieties of pine-ponderorosa seedlings subjected to long-term elevated carbon dioxide. *Tree Physiology*, **4,** 187–193.

Kelly MO, Davies PJ (1988) The control of whole plant senescence. *Critical Reviews in Plant Sciences*, **7,** 139–173.

Lilley RMcC, Walker DA (1974) An improved spectrophotometric assay for ribulosebiphosphate carboxylase. *Biochemica et Biophysica Acta*, **358,** 226–229.

Mcconnaughay KDM, Bassow SL, Berntson GM, Bazzaz FA (1996) Leaf senescence and decline of end-of-season gas exchange in five temperate deciduous tree species grown in elevated CO_2 concentrations. *Global Change Biology*, **2,** 25–33.

Miller A, Tsai C-H, Hemphill D, Endres M, Rodermel S, Spalding M (1997) Elevated CO_2 effects during leaf ontogeny: a new perspective on acclimation. *Plant Physiology*, **115,** 1195–1200.

Noodén LD (1980) Senescence in whole plants. In: *Senescence in Plants* (ed. Thimann KV), pp. 219–258. CRC Press, Boca Raton, FL.

Ono K, Terashima I, Watanabe A (1996) Interaction between nitrogen deficit of a plant and nitrogen concentration in the old leaves. *Plant Cell Physiology*, **37,** 1083–1089.

Pate JS (1980) Transport and partitioning of nitrogen solutes. *Annual Review of Plant Physiology and Plant Molecular Biology*, **31,** 313–340.

Peñarrubia L, Moreno J (1995) Senescence in plants and crops. In: *Handbook of Plant and Crop Physiology* (ed. Pessarakli M), pp. 461–481. Marcel Dekker, New York.

Robinson CH, Michelson A, Lee JA, Whitehead SJ, Callaghan TV, Press MC, Jonasson S (1997) Elevated atmospheric CO_2 affects decomposition of *Festuca vivipara* (L.) Sm. litter and roots in experiments simulating environmental change in two arctic ecosystem. *Global Change Biology*, **3,** 37–49.

Rogers HH, Runion GB (1994) Plant responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. *Environment Pollution*, **83,** 155–189.

Rouhier H, Billès G, El Kohen A, Mousseau M, Bottner P (1994) Effect of elevated CO_2 on carbon and nitrogen distribution within a tree (*Castanea sativa* Mill) -soil system. *Plant and Soil*, **162,** 281–292.

Schäppi B, Körner CH (1997) *In situ* effects of elevated CO_2 on the carbon and nitrogen status of alpine plants. *Functional Ecology*, **11,** 290–299.

Schmalzer PA, Hinkle CR (1992) Recovery of oak-oaw palmetto scrub after fire. *Castanea*, **57,** 158–173.

Sicher RC, Bunce JA (1997) Relationship of photosynthetic acclimation to changes of Rubisco activity in field-grown winter wheat and barley during growth in elevated carbon dioxide. *Photosynthesis Research*, **52,** 27–38.

Simpson RJ, Lambers H, Dalling MJ (1982) Translocation of nitrogen in a vegetative wheat plant (*Triticum aestivum*). *Physiologia Plantarum*, **56,** 11–17.

Thomas H, Stoddart JL (1980) Leaf senescence. *Annual Review of Plant Physiology*, **31,** 83–111.

Wintermans JFGM, De Mots A, (1965) Spectrophotometric characteristics of chlorophyll *a* and *b* and their pheophytins in ethanol. *Biochemica et Biophysica Acta*, **109,** 448–453.

Wong SC (1979) Evaluated atmospheric partial pressure of CO_2 and plant growth. I. Interactions of nitrogen nutrition and photosynthetic capacity in C3 and C4 plants. *Oecologia*, **44,** 68–74.