Elevated CO_2 reduces leaf damage by insect herbivores in a forest community

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Summary

- By altering foliage quality, exposure to elevated levels of atmospheric CO_2 potentially affects the amount of herbivore damage experienced by plants.
- Here, we quantified foliar carbon (C) and nitrogen (N) content, C : N ratio, phenolic levels, specific leaf area (SLA) and the amount of leaf tissue damaged by chewing insects for 12 hardwood tree species grown in plots exposed to elevated CO_2 (ambient plus 200 μl l^{-1}) using free-air CO_2 enrichment (FACE) over 3 yr.
- The effects of elevated CO_2 varied considerably by year and across species. Elevated CO_2 decreased herbivore damage across 12 species in 1 yr but had no detectable effect in others. Decreased damage may have been related to lower average foliar N concentration and SLA and increased C : N ratio and phenolic content for some species under elevated compared with ambient CO_2. It remains unclear how these changes in leaf properties affect herbivory.
- Damage to the leaves of hardwood trees by herbivorous insects may be reduced in the future as the concentration of CO_2 continues to increase, perhaps altering the trophic structure of forest ecosystems.

Key words: arthropod, carbon content, global change, herbivory, leaf nitrogen, leaf phenolic content, specific leaf area (SLA).

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Introduction

Human activity is increasing the concentration of CO_2 in the atmosphere at the rate of *c.* 0.4% yr^{-1} and it is expected to double from preindustrial levels by the middle of this century (Houghton *et al.*, 2001). Elevated CO_2 can directly affect forest productivity by increasing photosynthesis and nutrient use efficiency (Drake *et al.*, 1997; Curtis & Wang, 1998; Norby *et al.*, 1999; D.M. Moore, unpublished), but it may also indirectly affect productivity by altering the performance of herbivores and pathogens. Insect herbivory typically removes 2–15% of net primary production in temperate deciduous forests (Whittaker, 1970; Ohmart *et al.*, 1983; Cyr & Pace, 1993). By altering the chemical composition of foliage (Bezemer & Jones, 1998), elevated CO_2 may change the impact of herbivores on forest productivity. However, most research has been conducted in controlled environments, and how insect herbivory may be affected by elevated CO_2 in complex natural communities is not fully understood.

The stimulation of photosynthesis and accumulation of nonstructural carbohydrates by elevated CO_2 contributes to dilution of foliar nitrogen (N) and a corresponding increase in C : N ratio (Drake *et al.*, 1997; Hughes & Bazzaz, 1997). This increase in C : N ratio may reduce the palatability and nutritional quality of foliage to insects and is sometimes associated with the production of allelochemicals, such as phenolics, which deter herbivory (Bryant *et al.*, 1983; Agrell *et al.*, 2000; Lindroth *et al.*, 2001). Insect growth and metabolism often are N-limited and can respond strongly to leaf N content (Mattson, 1980; Scriber & Slansky, 1981). Consequently,

reduced N content in foliage under elevated CO_2 can decrease insect performance and increase mortality (Stiling *et al.*, 1999). For plants grown under elevated CO_2, increased per capita feeding by chewing insects (compensatory feeding) generally has been associated with low leaf N content and high C : N ratios (Bezemer & Jones, 1998; Coviella & Trumble, 1999; Hunter, 2001). However, an increase in per capita consumption of foliage under elevated CO_2 in laboratory or greenhouse trials does not necessarily result in increased herbivory in native forest communities (Hamilton *et al.*, 2004).

In nature, plant physiological responses to elevated CO_2 interact with other sources of environmental variation (Arnone *et al.*, 1995; Roth *et al.*, 1997; McDonald *et al.*, 1999) or vary among species (Lindroth *et al.*, 1993; Bezemer & Jones, 1998; Williams *et al.*, 2000) and even among genotypes (Goverde *et al.*, 1999; Mansfield *et al.*, 1999; Lindroth *et al.*, 2001), potentially complicating the interactions between plants and insects. In addition, there is considerable variation in the effect of elevated CO_2 on insect feeding and oviposition preference and demography (Stange *et al.*, 1995; Stiling *et al.*, 1999; Stiling *et al.*, 2002; Kopper & Lindroth, 2003b). Furthermore, larval feeding trials under controlled conditions may not capture the multitrophic interactions that occur in forests ecosystems (Lincoln *et al.*, 1993; Arnone *et al.*, 1995; Coviella & Trumble, 1999; Hunter, 2001).

Hamilton *et al.* (2004) found that most damage to the foliage of four species of hardwood saplings (1–3 m high) growing in the understory of a pine forest was from chewing insects and the amount of damage was substantially lower in plots exposed to elevated atmospheric CO_2 compared with plots under ambient conditions. In that experiment, CO_2 concentration was controlled by free-air CO_2 enrichment (FACE) technology, which distributes CO_2 throughout the forest without enclosures that restrict the movement of insects and other organisms (Hendrey & Kimball, 1994; Lewin *et al.*, 1994; Hendrey *et al.*, 1999).

In the present study we examined the effect of elevated CO_2 on damage by chewing insects on saplings of seven (2002) and 12 (2001, 2003) hardwood species growing in the same forest understory as Hamilton *et al.* (2004). To capture annual variation in the interaction between plant and insect responses to elevated CO_2, measurements were made over three growing seasons. Aspects of leaf chemistry, including N, C and tannin concentration and specific leaf area (SLA), were measured to elucidate potential mechanisms governing the effect of CO_2 on herbivory.

Materials and Methods

Leaf damage by chewing insects was quantified on saplings of 12 species of hardwood trees growing in the understory of a 17-yr-old loblolly pine plantation and exposed to ambient or elevated levels of atmospheric CO_2. Research was conducted at the Forest Atmosphere Carbon Dioxide Transfer and Storage-1 (FACTS-1) research site in the Piedmont region of North Carolina, USA (35°97′ N 79°09′ W). At the FACTS-1 site, the CO_2 concentration in three, 30-m diameter plots was elevated with a free-air CO_2 enrichment (FACE) system (Hendrey & Kimball, 1994; Lewin *et al.*, 1994; Hendrey *et al.*, 1999). Beginning in 1996, the FACE system was used to raise the concentration of CO_2 in the forest canopy by 200 μl l^{-1} above current levels (*c.* 577 μl l^{-1} at 1 m and *c.* 586 μl l^{-1} at 0.25 m height; G.R. Hendrey, pers. comm.). This level was chosen to represent the CO_2 concentration predicted for the year 2050 (Houghton *et al.*, 2001). Three additional fully instrumented control plots received ambient air (CO_2 386 ± 27 μl l^{-1}). The pine canopy provided complete cover (DeLucia *et al.*, 2002) and saplings in the understory were in shade; the average photon flux density in the understory was 121 μmol m^{-2} s^{-1} between 08:00 and 17:00 hours (Singsaas *et al.*, 2000).

The amount of missing tissue and foliar characteristics were measured in three consecutive years from 2001 to 2003 on saplings of hardwood trees species that represent those in regenerating forests in the Piedmont of North Carolina, USA (Mohan, 2002). Because of logistical constraints, not all species were measured every year. Leaf damage was measured on seven of 12 species in all three years: *Acer rubrum* L. (red maple), *Cercis canadensis* L. (redbud), *Liquidambar styraciflua* L. (sweetgum), *Prunus serotina* Ehrh. (black cherry), *Quercus alba* L. (white oak), *Quercus phellos* L. (willow oak), and *Ulmus alata* Michx. (winged elm). Five additional species were measured only in 2001 and 2003: *Acer barbatum* Michx. (sugar maple), *Liriodendron tulipifera* L. (yellow poplar), *Quercus rubra* L. (red oak), *Quercus velutina* Lam. (black oak), and *Robinia pseudo-acacia* L. (black locust). Because these species represent a range of life history traits (Burns & Honkala, 1990), we considered them likely to vary in their responses to elevated CO_2 and herbivory.

Tree saplings were grown from seed and transplanted into the experimental plots. Locally collected, genetically diverse seeds for each species were germinated in a greenhouse, and in October 1998 an equal number of 8-month old saplings of each species were planted at 30-cm intervals in a random order in eight subplots within each of the six FACE plots (2352 seedlings). The eight 1.44 m^2 subplots were evenly distributed within each experimental FACE plot and surrounded by 1-m high wire fencing to reduce vertebrate herbivory (Mohan, 2002). At the time of herbivory measurements, saplings ranged from 10 to 100 cm tall and had been exposed to the elevated CO_2 treatment for at least two full years.

The amount of leaf tissue damaged by arthropod herbivory was quantified using digital photographs taken in mid-June in 2001, late June in 2002, and mid-August in 2003. Damage was defined as tissue area or mass removed by chewing herbivores, which constitutes approximately 66% of all leaf damage in this forest (Hamilton *et al.*, 2004). However, discrete sampling of folivore damage may underestimate total herbivory

losses by not accounting for leaf or hole expansion and changes in photosynthetic activity (Reichle *et al.*, 1973; Lowman, 1984; Sand-Jensen & Jacobsen, 1994; Zangerl *et al.*, 2002), although most leaves were fully expanded by the time they were sampled in June.

Each year, 20 trees of each species were randomly selected in each FACE plot. Every fully expanded leaf on each of the trees was given a number and two leaves were selected randomly and photographed, yielding 120 leaves for each species and treatment (7440 images in total over 3 year). Because saplings were small there was no self-shading within their individual crowns. Leaves were photographed *in situ* against a calibrated background with a high-resolution digital camera (Coolpix 950; Nikon, Melville, NY, USA), and the area of each leaf was determined with image analysis software (Scion Image, Beta Version 4.0.2; Scion Corp., Frederick, MD, USA). Leaf area before damage was estimated by reconstructing the perimeter of the damaged leaf. The damaged area was expressed as the absolute difference between the area of intact and damaged leaves and as a percentage of the area of intact leaves. The mass of tissue removed was estimated from the absolute area damaged divided by the average specific leaf area (SLA, $cm^2\ g^{-1}$) calculated for each subplot.

Leaf tissue was analysed for physical and chemical characteristics thought to affect insect performance. Tissue samples were collected using a cork borer (0.6 cm^2) from randomly selected undamaged leaves within each plot subplot. Primary and second order veins were avoided. From each species and treatment, approximately 45 leaf punches were dried at 70°C to constant mass and weighed to determine SLA. Carbon and N content per unit dry mass were measured on ground subsamples with an Elemental Combustion System (Model 4010; Costech Analytical Technologies, Valencia, CA, USA). Because only limited quantities of leaf tissue were available from these small plants, especially during the drought in 2002, C and N were measured only on black cherry, white oak, willow oak, black oak and winged elm, and SLA was reported for these five species and for red maple and sweetgum in this year.

Protein precipitation capacity was measured for the four oaks species in 2001 and 2003, and six additional species (red maple, redbud, sweetgum, black cherry, winged elm and sugar maple) in 2003. Potential biological tannin activity was quantified, with minor modifications, by a protein precipitation–diffusion assay developed by Hagerman (1987). A crude leaf extract was prepared by grinding dried leaf tissue (5–150 mg) in a 70% acetone solution (150 µl), and the supernatant (40 µl) was loaded onto 0.8-ml glass tubes containing bovine serum albumin (BSA, Fraction V powder, essentially fatty acid free, #A-6003; Sigma, Sigma-Aldrich Co., St Louis, MO, USA) added to a buffered agarose solution (Type I, A-6013, Sigma). During a 9-h incubation at 30°C, tannins diffused into the agarose, creating a visible precipitate. The distance from the top of the tube to the precipitate was measured with a digital dial caliper accurate to 0.01 mm. A linear standard curve was created by loading different concentrations of tannic acid (Baker, Phillipsburg, NJ, USA) onto a subset of tubes and the values for leaf extracts were expressed as tannic acid equivalents, either as the percentage of leaf mass or per unit leaf area.

Leaf damage, foliar C, N and protein precipitation capacity, C : N ratio, and SLA were analysed using a randomized complete block analysis of variance (PROC MIXED; SAS Version 8.1, The SAS Institute, Inc., Cary, NC, USA). Values for plant replicates and subplots were averaged within each FACE plot before statistical analysis. New individuals were sampled from the population each year and were therefore assumed to be statistically independent. The CO_2 treatment, year, species and their interactions were modeled as fixed effects, and block and CO_2 treatment by block were included as random effects within the ANOVA. For herbivory, foliar C and N content, C : N ratio and SLA, separate statistical analyses were performed for the species that were measured in all three years and for the additional species measured only in 2001 and 2003. Separate statistical analyses of protein precipitation capacity were performed for the four oak species that were measured in 2001 and 2003 and the additional six species measured only in 2003. To meet the assumptions of normality, data were log- or square root-transformed as required and herbivory levels were analysed using subplot means. The Kenward–Rogers correction was used to estimate the degrees of freedom because of the nested design. The relationship between the CO_2 treatment effect on insect herbivory and foliar quality (e.g. N and C content, total phenolic content, C : N and SLA) were tested with linear regressions (PROC REG, SAS Institute Inc., Cary, NC, USA). The significance of the treatment effect within individual species was determined by least-squared differences within an analysis of variance. Unless otherwise noted, mean values were considered statistically significant where $P < 0.05$.

At the level of replication in this experiment (three ambient plots and three plots exposed to elevated CO_2) there was a greater than 99% probability of resolving a statistical difference in herbivory between plants grown at ambient and elevated CO_2 at a significance level of $\alpha = 0.05$, if herbivores removed, on average, 1.0 ± 0.2 mg of leaf tissue, and this value decreased to a 62% probability of resolving a statistically significant treatment effect for a lower level of leaf damage (0.5 ± 0.2 mg; PROC POWER).

Results

Across the seven species that were measured in each of the three years, elevated CO_2 caused a reduction in the percentage of leaf area removed by chewing insects (Fig. 1, Table 1, main effect of CO_2, $P = 0.10$). Growth under elevated CO_2 caused a substantial reduction in herbivory in 2001, when across 12 species the average damage per leaf under ambient CO_2 was

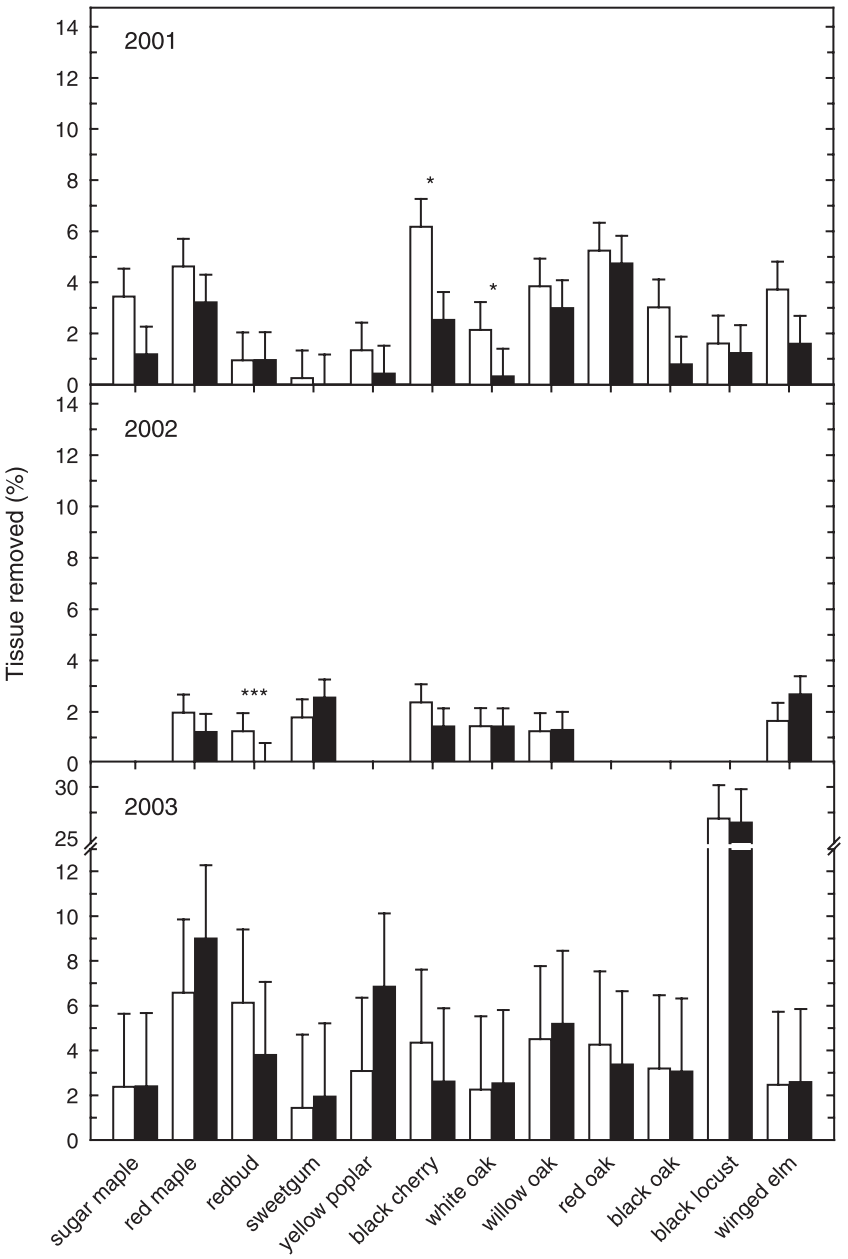

Fig. 1 Percentage of leaf tissue removed by chewing insects for plants grown under ambient (*c.* 386 μl l^{-1}) and elevated (*c.* 586 μl l^{-1}) CO_2 for each of the three years. Columns represent the least-squared means (± 1 SE) of the three ambient and three elevated CO_2 plots (open columns, ambient CO_2; closed columns, elevated CO_2). Within a species and year, statistical significance of pairwise comparisons is indicated above columns (*$P < 0.1$, **$P < 0.05$, ***$P < 0.01$).

3.1% compared with 1.7% for plants under elevated CO_2 (one-way ANOVA, $P < 0.01$). There also was a 46% decrease in the total area (ambient CO_2 plots, 0.69 cm^2; elevated CO_2 plots, 0.37 cm^2; one-way ANOVA, $P = 0.011$) and total mass (ambient CO_2 plots, 2.04 mg; elevated CO_2 plots, 1.20 mg; $P = 0.025$) of leaf tissue damaged by chewing insects in the elevated CO_2 plots. In 2001, elevated CO_2 decreased the proportion of all leaves damaged by insects from 33% to 26% (one-way ANOVA, $P = 0.02$). In contrast to 2001, elevated CO_2 had no effect on overall herbivory of the 12 species in 2003, and, averaged across all species and years, the percentage of leaf tissue damaged by insect herbivores was 3.8% per leaf under ambient CO_2 and 3.3% per leaf under elevated CO_2.

For black cherry in 2001, black locust in 2001 and 2003, and red maple and winged elm in 2002, the reduction in damage expressed as a percentage of total leaf area was greater than when expressed on an absolute basis because leaves were, on average, 4.5% larger for plants grown under elevated CO_2 (data not shown). This was not the case for the other tree species.

Damage by insect herbivores varied significantly from year to year, as indicated by significant year effects in the ANOVA (Table 1, Fig. 1). The average percentage of leaf area damaged per leaf by insect herbivores for the seven species measured in each of the three years and averaged across treatments was 2.4% in 2001, 1.6% in 2002 and 4.0% in 2003. On a mass basis, leaf tissue removed by herbivores followed a similar

Table 1 Analysis of variance for effects of elevated CO_2 on total leaf area damaged, percentage leaf area damaged, and total leaf mass damaged per leaf for seven tree species measured in each of the three successive years and for five additional tree species that were measured in the first and third year of the experiment

Main effects and interactions	Species measured in each of the three years			Species measured in two of three years		
	df	*F*	*P*	df	*F*	*P*
Total area damaged (cm^2)						
CO_2	1	3.04	0.09	1	0.00	0.97
Year	2	23.1	< 0.01	1	28.3	< 0.01
Species	6	21.95	< 0.01	4	2.67	0.05
CO_2 × year	2	0.64	0.53	1	7.4	0.01
CO_2 × species	6	0.43	0.85	4	1.71	0.17
Year × species	12	4.06	< 0.01	4	9.78	< 0.01
CO_2 × year × species	12	0.72	0.72	4	0.74	0.57
Per cent area damaged (%)						
CO_2	1	4.42	0.10	1	0.35	0.62
Year	2	23.67	< 0.01	1	38.29	< 0.01
Species	6	8.15	< 0.01	4	8.3	< 0.01
CO_2 × year	2	2.12	0.13	1	3.66	0.06
CO_2 × species	6	1.02	0.42	4	0.31	0.87
Year × species	12	3.96	< 0.01	4	12.19	< 0.01
CO_2 × year × species	12	0.64	0.80	4	0.58	0.68
Total mass damaged (g)						
CO_2	1	0.19	0.67	1	0.00	0.98
Year	2	16.61	< 0.01	1	24.32	< 0.01
Species	6	18.5	< 0.01	4	3.67	0.01
CO_2 × year	2	0.88	0.42	1	7.2	0.01
CO_2 × species	6	1.23	0.30	4	1.89	0.13
Year × species	11	3.49	< 0.01	4	8.28	< 0.01
CO_2 × year × species	10	0.33	0.97	4	0.66	0.63

Seven tree species measured in each of the three successive years were: red maple, redbud, sweetgum, black cherry, white oak, willow oak and winged elm. The five additional tree species that were measured in the first and third year of the experiment were: sugar maple, yellow poplar, red oak, black oak and black locust (Fig. 1).

Table 2 Analysis of variance for effects of elevated CO_2 on leaf carbon and nitrogen concentration (%, mass basis) and carbon : nitrogen ratio for the five species measured in each of the three successive years and for seven additional tree species that were measured in the first and third year of the experiment

Main effects and interactions	Species measured in each of the three years			Species measured in two of three years		
	df	*F*	*P*	df	*F*	*P*
Carbon (%)						
CO_2	1	0.21	0.67	1	0.68	0.46
Year	2	1.43	0.28	1	6.35	0.01
Species	4	30.41	< 0.01	6	16.32	< 0.01
CO_2 × year	2	0.30	0.74	1	1.46	0.23
CO_2 × species	4	0.83	0.50	6	0.88	0.51
Year × species	8	2.57	0.01	6	0.30	0.94
CO_2 × year × species	8	1.10	0.37	6	0.16	0.99
Nitrogen (%)						
CO_2	1	2.29	0.25	1	4.30	0.14
Year	2	4.25	0.05	1	22.21	< 0.01
Species	4	16.65	< 0.01	6	43.37	< 0.01
CO_2 × year	2	3.52	0.03	1	0.03	0.86
CO_2 × species	4	0.33	0.86	6	0.31	0.93
Year × species	8	4.05	< 0.01	6	0.62	0.71
CO_2 × year × species	8	0.55	0.81	6	1.39	0.22
Carbon : nitrogen ratio						
CO_2	1	3.14	0.20	1	2.34	0.25
Year	2	8.36	< 0.01	1	16.14	< 0.01
Species	4	22.32	< 0.01	6	35.38	< 0.01
CO_2 × year	2	4.50	0.01	1	0.24	0.62
CO_2 × species	4	0.28	0.89	6	0.48	0.83
Year × species	8	4.16	0.00	6	0.49	0.81
CO_2 × year × species	8	0.59	0.79	6	1.49	0.18

The five species measured in each of the three successive years were: black cherry, white oak, willow oak, black oak and winged elm. The seven additional tree species that were measured in the first and third year of the experiment were: sugar maple, red maple, redbud, sweetgum, yellow poplar, red oak, black locust (Figs 2–3).

pattern: 1.0 mg per leaf, 0.9 mg per leaf, and 1.9 mg per leaf in each of the three years, respectively. The average percentage area damaged per leaf for the five additional species measured in 2001 and 2003 was 2.3% and 8.2%, respectively, and the average mass removed per leaf in these years was 2.4 mg and 6.0 mg, respectively.

There was significant variation in the extent of damage among individual species, but the species with greatest susceptibility to damage varied each year (Table 1, Fig. 1). For example, in 2001 damage of red maple, black cherry and red oak was greater than most other species. Red maple also had relatively high levels of damage in 2003, but black locust had substantially greater damage (27% damage per leaf) than all other species this year. Sweetgum typically experienced low levels of damage (0.2–2.2% damage per leaf; Fig. 1).

Although growth under elevated CO_2 decreased N and SLA and increased C : N ratio for individual species (Figs 2–4), it had no detectable main effect on these variables across all three years of the experiment (Tables 2–4). For the four oak species that were measured in 2001 and 2003, there was a marginally significant CO_2 × year interaction on protein precipitation capacity expressed as a percentage of leaf mass ($P = 0.10$, Table 5; Fig. 5) and a significant CO_2 × year interaction when expressed per unit leaf area ($P = 0.03$). The protein precipitation capacity on an area basis increased by 32% under elevated CO_2 compared with ambient CO_2 (ambient CO_2 plots, 1.0 g m^{-2}; elevated CO_2 plots, 1.5 g m^{-2}; one-way ANOVA, $P = 0.07$, Table 5).

In 2001, the only year with a significant effect of CO_2 on herbivory levels, elevated CO_2 altered key aspects of leaf chemistry and structure thought to affect suitability for insects. Averaged across all 12 species in 2001, there was a statistically significant decrease in average foliar N from 2.5% under ambient CO_2 to 2.3% under elevated CO_2 (Fig. 2; one-way ANOVA, $P < 0.01$). In that same year, the C : N ratio was greater (mean for ambient plots, 20.9; mean for elevated CO_2

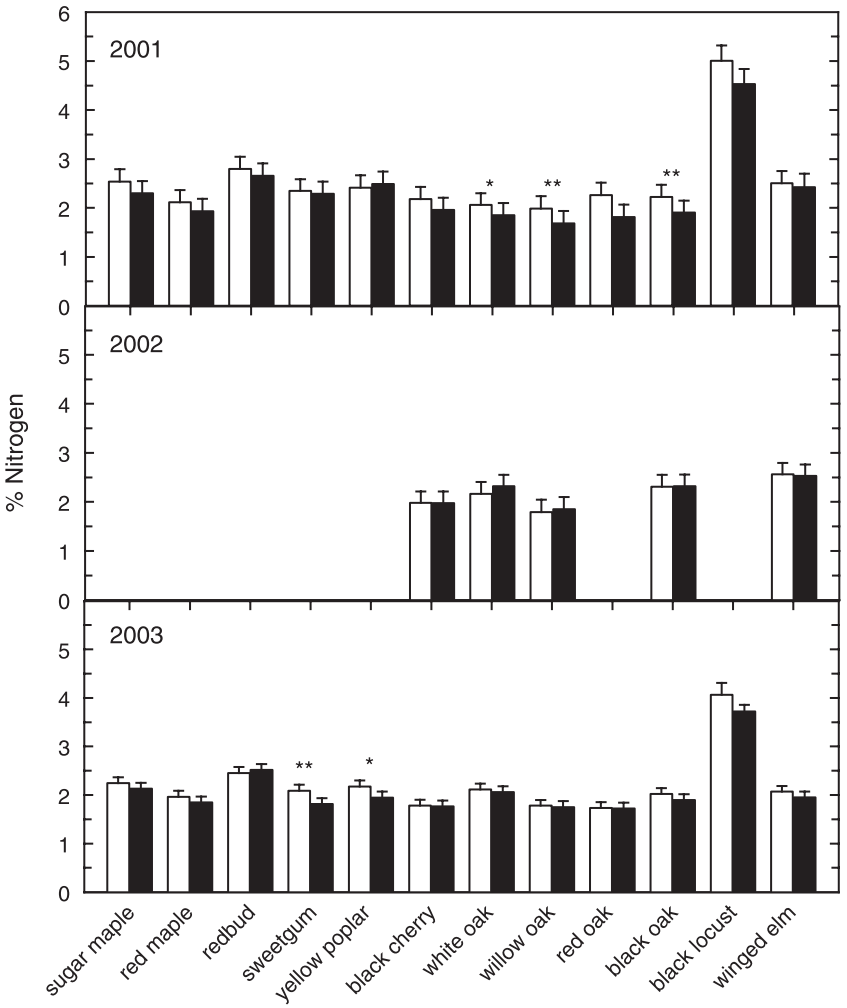

Fig. 2 Effects of elevated CO_2 on leaf nitrogen concentration (% dry mass) for plants grown under ambient (*c.* 386 µl l^{-1}) and elevated (*c.* 586 µl l^{-1}) CO_2 for each of the three years. Columns represent the least-squared means (± 1 SE) of the three ambient and three elevated CO_2 plots (open columns, ambient CO_2; closed columns, elevated CO_2). Within a species and year, statistical significance of pairwise comparisons is indicated above columns (*$P < 0.1$, **$P < 0.05$, ***$P < 0.01$).

plots, 23.0; $P < 0.01$; Fig. 3) and there was a trend towards lower SLA (mean for ambient CO_2 plots, 408 cm^2 g^{-1}; mean for elevated CO_2 plots, 373 cm^2 g^{-1}; $P = 0.09$; Fig. 4) under elevated compared with ambient CO_2. Across the four oak species in 2001, there was a trend toward higher protein precipitation capacity under elevated CO_2 (1.5 g m^{-2}) than ambient CO_2 (1.0 g m^{-2}; $P = 0.10$; data not shown). Elevated CO_2 had no detectable effect on N on an area basis in any year (data not shown).

There was significant between-year variation for most leaf attributes (Tables 2–5). Generally, leaf N concentration was lowest during 2003 and SLA was lowest during the drought year of 2002. For the species for which aspects of leaf chemistry were measured in each of the three years of the experiment (black cherry, white oak, willow oak, black oak and winged elm), average foliar N concentration (Table 2, Fig. 2) was slightly but significantly lower in 2003 (1.9%) than in 2001 (2.0%) and in 2002 (2.1%). Correspondingly, foliar C : N levels were significantly higher in 2003 (25.3) than in 2001 (24.4) and 2002 (22.9) (Table 2, Fig. 3). For the additional species in which aspects of leaf chemistry were measured in only two years (sugar maple, red maple, redbud, sweetgum, yellow poplar, red oak and black locust), average N was higher in 2001 (2.7%) than in 2003 (2.3%; Fig. 2). Carbon content (data not shown) was higher in 2001 (48.9%) than in 2003 (48.3%), and the corresponding C : N ratio (Fig. 3) was lower in 2001 (20.8) than 2003 (22.2). Across the four oak species, protein precipitation capacity was higher in 2001 (3.3%) than 2003 (2.1%, $P < 0.001$; Table 4). There was trend of between-year variation in SLA for the species examined in each of the three years of the experiment (red maple, sweetgum, black cherry, white oak, willow oak, black oak, and winged elm), with lower average SLA in 2002 (283 cm^2 g^{-1}) than in 2001 (328 cm^2 g^{-1}; $P < 0.02$) and 2003 (315 cm^2 g^{-1}; $P < 0.07$; Table 5). The SLA was higher in 2001 than in 2003 ($P < 0.03$) across the species that were measured only in those years (sugar maple, redbud, yellow poplar, red oak and black locust).

Carbon, N and protein precipitation capacity, three potential determinants of foliar quality, expressed on a mass per leaf area basis and on a mass per leaf mass basis varied substantially among species (Tables 2–5). Foliar C concentration was

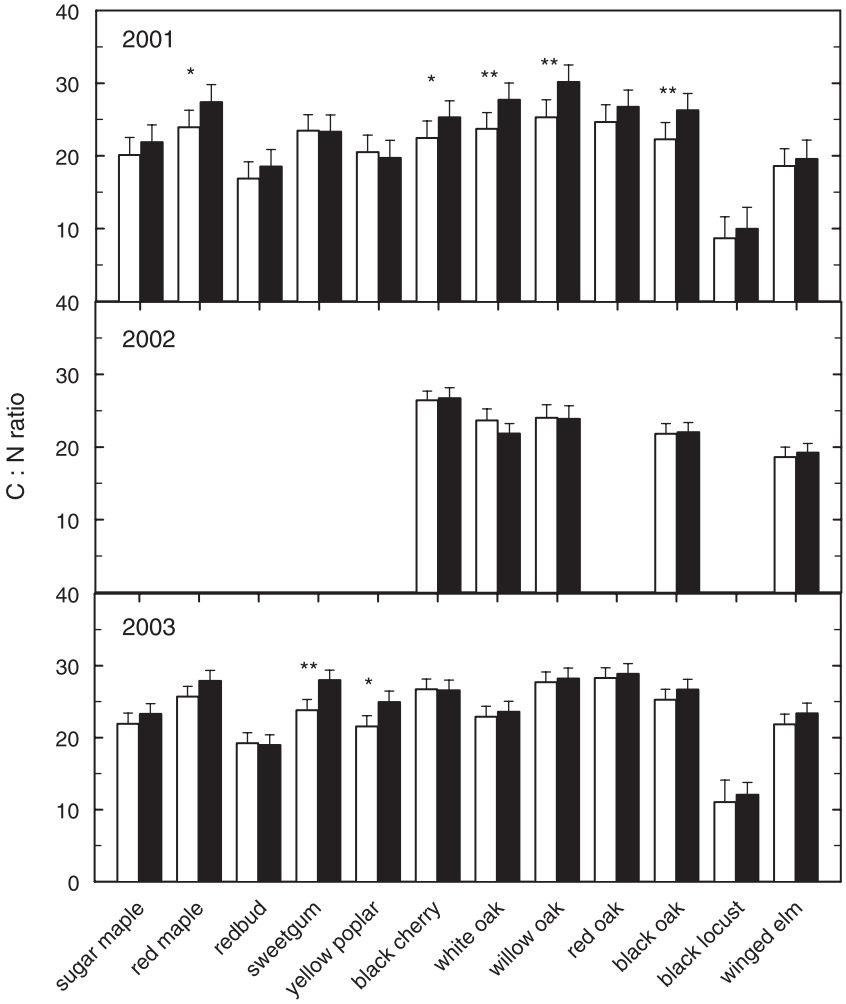

Fig. 3 Effects of elevated CO_2 on carbon : nitrogen (C : N) ratio of leaves for plants grown under ambient (*c.* 386 μl l^{-1}) and elevated (*c.* 586 μl l^{-1}) CO_2 for each of the three years. Columns represent the least-squared means (± 1 SE) of the three ambient and three elevated CO_2 plots (open columns, ambient CO_2; closed columns, elevated CO_2). Within a species and year, statistical significance of pairwise comparisons is indicated above columns (*$P < 0.1$, **$P < 0.05$, ***$P < 0.01$).

higher in red maple than all other species (50.2%) and lowest in winged elm (45.8%). Averaged across years, the highest N concentration (4.3%; Fig. 2) and lowest C : N ratio (10.1%; Fig. 3) were found in black locust, a nitrogen-fixing species (Olesniewicz & Thomas, 1999). Willow oak had the lowest average N concentration (1.8%) and highest C : N ratio (27.0). Yellow poplar had the highest average SLA value, followed by black locust (553 $cm^2\ g^{-1}$ and 513 $cm^2\ g^{-1}$, respectively), while willow oak had lower average SLA than all other species (229 $cm^2\ g^{-1}$; Fig. 4). Willow and red oak had higher protein precipitation capacity (2.9% and 3.5%, respectively) than white and black oak (2.4% and 2.1%, respectively).

When tested within species, no significant correlations were detected between the amount or percentage of leaf tissue removed by herbivores and leaf attributes (e.g. N, C, C : N, SLA and protein precipitation capacity) across all years of the experiment. However, in 2001 the percentage leaf tissue removed by chewing insects significantly decreased as SLA increased across all 12 species ($P = 0.01$, ambient; $P = 0.05$, elevated).

Discussion

Although there was considerable between-year and between-species variation, when differences could be statistically resolved, exposure to elevated CO_2 caused a reduction in the loss of leaf tissue to insect herbivores (Fig. 1). Other field-based herbivory surveys also found that elevated CO_2 decreased the number of leaves damaged by chewing insects (Stiling *et al.*, 2002, 2003) as well as the overall amount of leaf damage (Hamilton *et al.*, 2004). These results are similar to field bioassay experiments that have found a decrease (Kopper & Lindroth, 2003a) or no change (Kopper & Lindroth, 2003a, 2003b) in damage by chewing insects under elevated CO_2. The consistent reduction of herbivory across 12 species in 2001 (Fig. 1) suggests that elevated CO_2 caused a fundamental change in tissue quality beyond potential species-specific changes in secondary chemistry.

Overall herbivory levels were substantially lower during the drought of 2002, when total growing-season precipitation (March–June) was half of the 50-yr average (State Climate

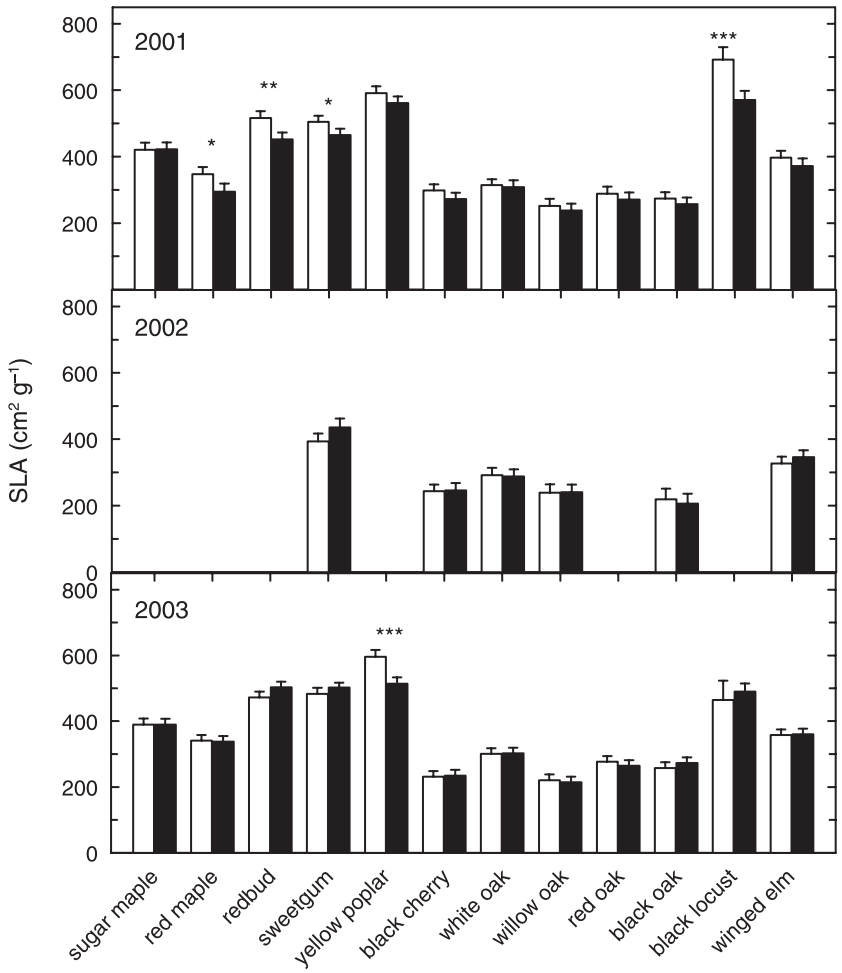

Fig. 4 Effects of elevated CO_2 on specific leaf area (SLA, $cm^2\ g^{-1}$) for plants grown under ambient (*c.* 386 μl l^{-1}) and elevated (*c.* 586 μl l^{-1}) CO_2 for each of the three years. Columns represent the least-squared means (± 1 SE) of the three ambient and three elevated CO_2 plots (open columns, ambient CO_2; closed columns, elevated CO_2). Within a species and year, statistical significance of pairwise comparisons is indicated above columns (*$P < 0.1$, **$P < 0.05$, ***$P < 0.01$).

Office of North Carolina, North Carolina State University, Raleigh, NC, USA). Drought typically reduces foliar quality (Roth *et al.*, 1997) and directly increases insect mortality (Shure *et al.*, 1998). It is unclear why there was no treatment effect on herbivory in 2003, when precipitation was closer to average. Measurements in 2003 were made two months later than the two previous years, and it is possible that herbivory earlier in the growing season may have induced plant defenses, thereby obscuring the effects of CO_2 on herbivore damage in late summer (Wold & Marquis, 1997).

Given the consistent reduction in herbivory under high CO_2 across species in 2001, it appears that some universal feature of chemistry or structure that affected leaf suitability was altered by the treatment. There was a trend of lower SLA and a small but statistically significant decrease in leaf N for plants grown in elevated CO_2 in this year. Thus, reductions in SLA and leaf N, and the corresponding increase in C : N ratio and protein precipitation capacity (at least in oaks), may have contributed to reduce herbivory under elevated CO_2. This inference is supported by the observation that in the two subsequent years, when there was no detectable effect of elevated CO_2 on these key aspects of leaf chemistry and structure, the amount of leaf tissue removed by chewing insects was similar for trees exposed to ambient and elevated CO_2.

A reduction in SLA under elevated CO_2 often is associated with increasing cell wall thickness and an increase in the concentration of total nonstructural carbohydrates (Yin, 2002). Insofar as decreased SLA is related to increased leaf toughness, it may account in part for the decrease in herbivory in 2001; tougher leaves are more difficult for folivores to consume (Bernays, 1986) and leaf toughness is correlated with lower herbivore densities (Peeters, 2002). Alternatively, by eating leaves with lower SLA, folivores consume more mass for a given leaf area, thus potentially explaining reduced area consumption under high CO_2. This was not the case in this study because the mass of leaf tissue removed by herbivores under elevated CO_2 (area removed/SLA) was similar to the leaf mass consumed under ambient CO_2.

Nitrogen content is an important determinant of the nutritional quality of foliage to insect herbivores and often is reduced in plants grown under elevated CO_2 (Lincoln *et al.*, 1993; Bezemer & Jones, 1998; Hunter, 2001). Kerslake *et al.* (1998) found no response of C : N ratio or phenolics to elevated CO_2 when plants were grown under realistic environmental

Table 3 Analysis of variance for effects of elevated CO_2 on leaf carbon and nitrogen content (g m^{-2}, area basis) for the five species measured in each of the three successive years and for the seven additional tree species that were measured in the first and third year of the experiment

Main effects and interactions	Species measured in each of the three years			Species measured in two of three years		
	df	*F*	*P*	df	*F*	*P*
Carbon (g m^{-2})						
CO_2	1	0.04	0.85	1	3.26	0.18
Year	2	43.32	< 0.01	1	0.83	0.36
Species	4	97.39	< 0.01	6	60.99	< 0.01
CO_2 × year	2	2.94	0.05	1	2.80	0.10
CO_2 × species	4	0.86	0.49	6	0.97	0.45
Year × species	8	6.16	< 0.01	6	1.34	0.24
CO_2 × year × species	8	1.27	0.26	6	0.93	0.48
Nitrogen (g m^{-2})						
CO_2	1	0.96	0.42	1	0.01	0.94
Year	2	37.45	< 0.01	1	5.31	0.02
Species	4	20.30	< 0.01	6	35.02	< 0.01
CO_2 × year	2	0.01	0.99	1	2.23	0.14
CO_2 × species	4	0.57	0.69	6	0.39	0.88
Year × species	8	4.45	< 0.01	6	0.77	0.60
CO_2 × year × species	8	1.11	0.36	6	1.10	0.36

The five species measured in each of the three successive years were: black cherry, white oak, willow oak, black oak and winged elm. The seven additional tree species that were measured in the first and third year of the experiment were: sugar maple, red maple, redbud, sweetgum, yellow poplar, red oak, black locust.

Table 4 Analysis of variance for effects of elevated CO_2 on specific leaf area (SLA; cm^2 g^{-1}) for the seven species measured in each of the three successive years and for the five additional tree species that were measured in the first and third year of the experiment

Main effects and interactions	Species measured in each of the three years			Species measured in two of three years		
	df	*F*	*P*	df	*F*	*P*
CO_2	1	0.01	0.93	1	0.98	0.36
Year	2	5.76	0.07	1	8.58	0.03
Species	6	189.48	< 0.01	4	89.36	< 0.01
CO_2 × year	2	7.60	< 0.01	1	2.21	0.14
CO_2 × species	6	0.30	0.94	4	0.56	0.69
Year × species	12	4.05	< 0.01	4	1.94	0.11
CO_2 × year × species	12	0.82	0.63	4	1.52	0.20

The seven tree species measured in each of the three successive years were: red maple, redbud, sweetgum, black cherry, white oak, willow oak and winged elm. The five additional tree species that were measured in the first and third year of the experiment were: sugar maple, yellow poplar, red oak, black oak and black locust (Fig. 4).

Table 5 Analysis of variance for effects of elevated CO_2 on the protein precipitation capacity (tannic acid equivalents, % mass basis, and g m^{-2}, area basis) for four species that were measured in the first and third year of the experiment and six additional species that were measured in the third year of the experiment

	Species measured for two years			Species measured for one year only		
	df	*F*	*P*	df	*F*	*P*
Concentration (%)						
CO_2	1	3.32	0.17	1	0.01	0.94
Year	1	23.05	< 0.01	–	–	–
Species	3	6.46	< 0.01	5	7.07	< 0.01
CO_2 × year	1	2.79	0.10	–	–	–
CO_2 × species	3	0.76	0.52	5	1.17	0.33
Year × species	3	0.38	0.76	–	–	–
CO_2 × year × species	3	1.79	0.15	–	–	–
Content (g m^{-2})						
CO_2	1	3.28	0.07	1	3.70	0.99
Year	1	5.40	0.01	–	–	–
Species	3	11.10	0.01	5	11.10	0.02
CO_2 × year	1	112.00	0.03	–	–	–
CO_2 × species	3	116.00	0.41	5	134.00	0.31
Year × species	3	24.60	0.67	–	–	–
CO_2 × year × species	3	116.00	0.23	–	–	–

The four species that were measured in the first and third year of the experiment were: white oak, willow oak, red oak and black oak. The six additional species that were measured in the third year of the experiment were: sugar maple, red maple, redbud, sweetgum, black cherry and winged elm (Fig. 5).

conditions. In contrast, for the suite of hardwood species examined in this experiment, elevated CO_2 reduced foliar N by 12% and increased C : N by *c.* 11% in 2001 (Figs 2 and 3; Table 2) during the same year when herbivore damage was uniformly reduced under elevated CO_2. Changes in N concentration and C : N ratio under elevated CO_2 in 2001 were similar in magnitude to those observed in previous studies of hardwood tree species (McGuire *et al.*, 1995; Cotrufo *et al.*, 1998; Norby *et al.*, 1999; Yin, 2002).

Phenolic compounds – potentially important defensive compounds – may increase under elevated CO_2. Buse *et al.* (1998) and Dury *et al.* (1998) reported an increase in condensed tannins and total phenolics for leaves of *Q. robur* grown under elevated CO_2. Lindroth *et al.* (1993) and Kinney *et al.* (1997) also found an increase in condensed tannins for *Q. rubra* grown under elevated CO_2, but these studies report conflicting responses of ellagitannin content to elevated CO_2 in red maple. At least for the oak species measured in this study, which tended to have higher concentrations of protein-binding tannins than tree species other than oaks, the concentration of these compounds was greater in leaves on plants grown under elevated than ambient CO_2 in 2001, when there was also a significant reduction of leaf damage under elevated CO_2 (Fig. 5, Table 5). For some species, an increase in leaf phenolics may contribute to lower levels of leaf damage under elevated CO_2.

In field studies, elevated CO_2 decreased (Kopper & Lindroth, 2003a) or had no effect (Kopper & Lindroth, 2003a,b) on per

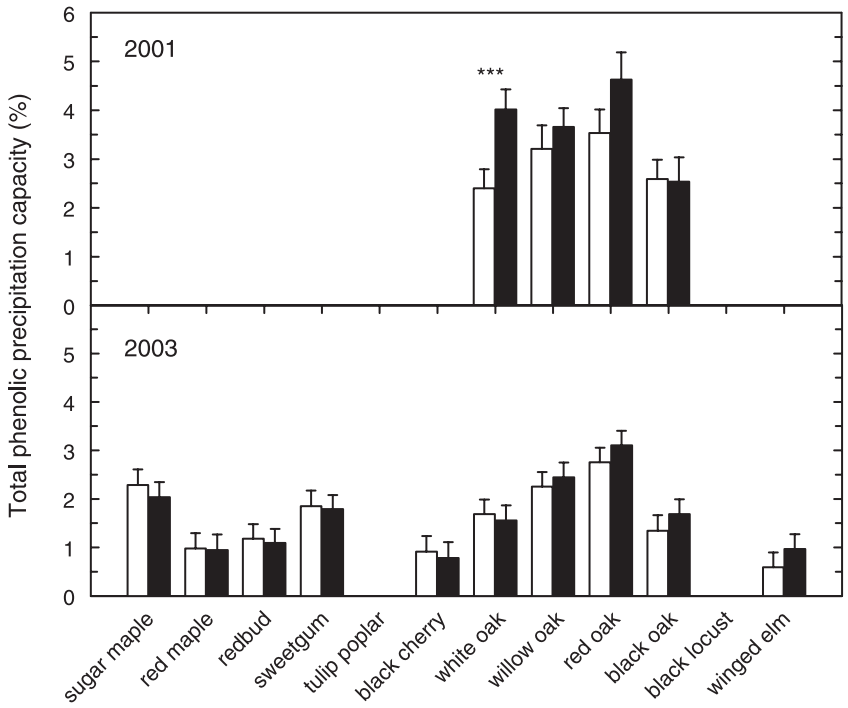

Fig. 5 Effects of elevated CO_2 on the protein precipitation capacity (tannic acid equivalents; % mass) for plants grown under ambient (*c.* 386 μl l^{-1}) and elevated (*c.* 586 μl l^{-1}) CO_2 for each of two years. Columns represent the least-squared means (± 1 SE) of the three ambient and three elevated CO_2 plots (open columns, ambient CO_2; closed columns, elevated CO_2). Within a species and year, statistical significance of pairwise comparisons is indicated above columns (*$P < 0.1$, **$P < 0.05$, ***$P < 0.01$).

capita herbivory by chewing insects. In contrast, numerous laboratory and greenhouse studies reported increased per capita tissue consumption under elevated CO_2 (Bezemer & Jones, 1998; Coviella & Trumble, 1999; Hunter, 2001). Thus, there appear to be fundamental differences in the effect of elevated CO_2 on damage from insects between controlled laboratory/greenhouse settings and field studies despite the fact that elevated CO_2 affects N content, SLA and other leaf constituents similarly in both experimental contexts.

The amount of leaf damage is related to the size of the herbivore population, which is in turn affected by insect mortality and fecundity – processes that potentially are affected by feeding on leaves developed under elevated CO_2. One important difference between greenhouse experiments and those conducted in FACE experiments is that movement of insects through the experimental plots is unfettered in the latter, so that oviposition and feeding preferences also may contribute to different levels of leaf damage. The choice of oviposition sites may be influenced by plant quality, which can be altered by elevated CO_2 (Coviella & Trumble, 1999; Awmack & Leather, 2002). For example, Kopper & Lindroth (2003b) found that leaf miner oviposition was lower on aspen trees growing under elevated CO_2. Insect and mite species representing six orders commonly are observed in the FACTS-1 experiment (Hamilton *et al.*, 2004), but the identity of organisms directly responsible for differences in leaf damage in the ambient and elevated CO_2 is not yet available; neither are there data on the effect of the treatment on oviposition or feeding choice.

Despite increases in per capita consumption (Bezemer & Jones, 1998; Coviella & Trumble, 1999; Hunter, 2001), forest herbivory may decrease under elevated CO_2 because of a decline in the abundance of chewing insects (Stiling *et al.*, 2002, 2003). Slower rates of development under elevated CO_2 prolongs the time that insect herbivores are susceptible to natural enemies, which may be abundant in open-top chambers and FACE experiments but absent from greenhouse experiments (Bezemer & Jones, 1998; Coviella & Trumble, 1999; Stiling *et al.*, 2003). Percy *et al.* (2002) found that natural enemy densities increase under elevated CO_2. Additionally, decreased foliar quality and increased per capita consumption under elevated CO_2 may increase exposure to toxins and insect mortality (Coviella & Trumble, 1999), and CO_2-induced changes in host plant quality directly decrease insect fecundity (Coviella & Trumble, 1999; Awmack & Leather, 2002).

In contrast to the view that herbivore damage will increase under elevated CO_2 as a result of compensatory feeding on lower quality foliage, our results and those of Stiling *et al.* (2002) and Hamilton *et al.* (2004) in open experimental systems suggest that damage to trees may decrease. The mechanism governing this response remains uncertain but may involve universal changes in leaf structure and N content common to many plant species in response to elevated CO_2, which may have indirect effects on feeding success of higher trophic levels, as well as species-specific responses in secondary chemistry.

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