

# Elevated CO<sub>2</sub> reduces leaf damage by insect herbivores in a forest community

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## Summary

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- By altering foliage quality, exposure to elevated levels of atmospheric CO<sub>2</sub> 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<sub>2</sub> (ambient plus 200 µl l<sup>-1</sup>) using free-air CO<sub>2</sub> enrichment (FACE) over 3 yr.
- The effects of elevated CO<sub>2</sub> varied considerably by year and across species. Elevated CO<sub>2</sub> 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<sub>2</sub>. 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<sub>2</sub> 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<sub>2</sub> in the atmosphere at the rate of *c.* 0.4% yr<sup>-1</sup> and it is expected to double from preindustrial levels by the middle of this century (Houghton *et al.*, 2001). Elevated CO<sub>2</sub> 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and nutrient use efficiency (Drake *et al.*, 1997; Curtis & Wang, 1998; Norby *et al.*, 1999; D.M. Moore, unpublished), but it may also indirectly affect productivity by altering the performance of herbivores and pathogens.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<sub>2</sub> 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<sub>2</sub> in complex natural communities is not fully understood.

The stimulation of photosynthesis and accumulation of nonstructural carbohydrates by elevated CO<sub>2</sub> 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<sub>2</sub> can decrease insect performance and increase mortality (Stiling *et al.*, 1999). For plants grown under elevated CO<sub>2</sub>, 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<sub>2</sub> 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<sub>2</sub> interact with other sources of environmental variation (Arnore *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<sub>2</sub> 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; Arnore *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<sub>2</sub> compared with plots under ambient conditions. In that experiment, CO<sub>2</sub> concentration was controlled by free-air CO<sub>2</sub> enrichment (FACE) technology, which distributes CO<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub>, 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<sub>2</sub> 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<sub>2</sub>. 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<sub>2</sub> concentration in three, 30-m diameter plots was elevated with a free-air CO<sub>2</sub> 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<sub>2</sub> in the forest canopy by 200 µl l<sup>-1</sup> above current levels (*c.* 577 µl l<sup>-1</sup> at 1 m and *c.* 586 µl l<sup>-1</sup> at 0.25 m height; G.R. Hendrey, pers. comm.). This level was chosen to represent the CO<sub>2</sub> concentration predicted for the year 2050 (Houghton *et al.*, 2001). Three additional fully instrumented control plots received ambient air (CO<sub>2</sub> 386 ± 27 µl l<sup>-1</sup>). 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<sup>-2</sup> s<sup>-1</sup> 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<sub>2</sub> 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<sup>2</sup> 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<sub>2</sub> 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,  $\text{cm}^2 \text{g}^{-1}$ ) 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 ( $0.6 \text{ cm}^2$ ) 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  $70^\circ\text{C}$  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  $\mu\text{l}$ ), and the supernatant (40  $\mu\text{l}$ ) 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  $30^\circ\text{C}$ , tannins diffused into the agarose, creating a visible precipitate. The distance from the top of the tube to the precipitate was measured with a

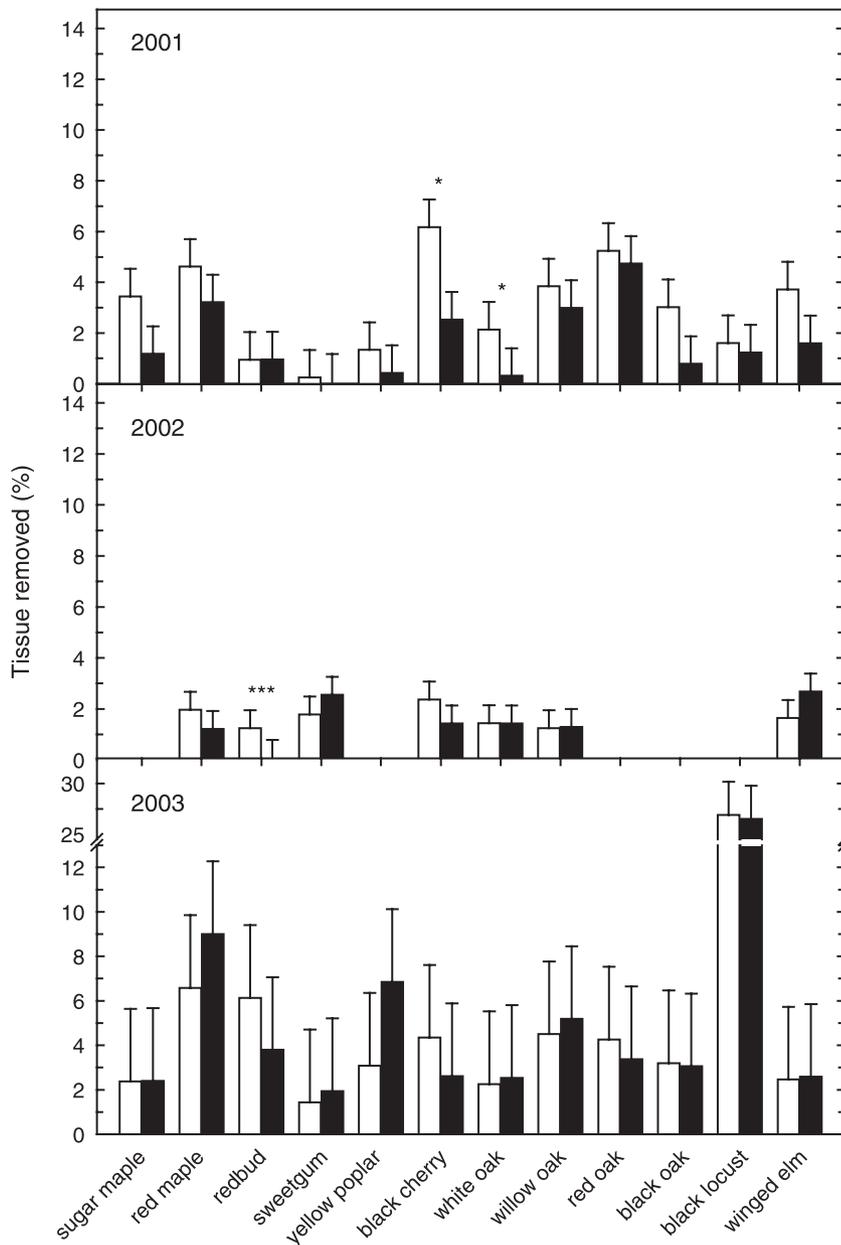
digital dial caliper accurate to 0.01 mm. A linear standard curve was created by loading different concentrations of tannic acid (Baker, Phillipsburg, NJ, USA) onto a subset of tubes and the values for leaf extracts were expressed as tannic acid equivalents, either as the percentage of leaf mass or per 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  $\text{CO}_2$  treatment, year, species and their interactions were modeled as fixed effects, and block and  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$ ) there was a greater than 99% probability of resolving a statistical difference in herbivory between plants grown at ambient and elevated  $\text{CO}_2$  at a significance level of  $\alpha = 0.05$ , if herbivores removed, on average,  $1.0 \pm 0.2 \text{ mg}$  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 ( $0.5 \pm 0.2 \text{ mg}$ ; PROC POWER).

## Results

Across the seven species that were measured in each of the three years, elevated  $\text{CO}_2$  caused a reduction in the percentage of leaf area removed by chewing insects (Fig. 1, Table 1, main effect of  $\text{CO}_2$ ,  $P = 0.10$ ). Growth under elevated  $\text{CO}_2$  caused a substantial reduction in herbivory in 2001, when across 12 species the average damage per leaf under ambient  $\text{CO}_2$  was



**Fig. 1** Percentage of leaf tissue removed by chewing insects for plants grown under ambient (c.  $386 \mu\text{l l}^{-1}$ ) and elevated (c.  $586 \mu\text{l l}^{-1}$ )  $\text{CO}_2$  for each of the three years. Columns represent the least-squared means ( $\pm 1$  SE) of the three ambient and three elevated  $\text{CO}_2$  plots (open columns, ambient  $\text{CO}_2$ ; closed columns, elevated  $\text{CO}_2$ ). 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  $\text{CO}_2$  (one-way ANOVA,  $P < 0.01$ ). There also was a 46% decrease in the total area (ambient  $\text{CO}_2$  plots,  $0.69 \text{ cm}^2$ ; elevated  $\text{CO}_2$  plots,  $0.37 \text{ cm}^2$ ; one-way ANOVA,  $P = 0.011$ ) and total mass (ambient  $\text{CO}_2$  plots,  $2.04 \text{ mg}$ ; elevated  $\text{CO}_2$  plots,  $1.20 \text{ mg}$ ;  $P = 0.025$ ) of leaf tissue damaged by chewing insects in the elevated  $\text{CO}_2$  plots. In 2001, elevated  $\text{CO}_2$  decreased the proportion of all leaves damaged by insects from 33% to 26% (one-way ANOVA,  $P = 0.02$ ). In contrast to 2001, elevated  $\text{CO}_2$  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  $\text{CO}_2$  and 3.3% per leaf under elevated  $\text{CO}_2$ .

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  $\text{CO}_2$  (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<sub>2</sub> 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
<b>Total area damaged (cm<sup>2</sup>)</b>						
CO <sub>2</sub>	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 <sub>2</sub> × year	2	0.64	0.53	1	7.4	0.01
CO <sub>2</sub> × species	6	0.43	0.85	4	1.71	0.17
Year × species	12	4.06	< 0.01	4	9.78	< 0.01
CO <sub>2</sub> × year × species	12	0.72	0.72	4	0.74	0.57
<b>Per cent area damaged (%)</b>						
CO <sub>2</sub>	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 <sub>2</sub> × year	2	2.12	0.13	1	3.66	0.06
CO <sub>2</sub> × species	6	1.02	0.42	4	0.31	0.87
Year × species	12	3.96	< 0.01	4	12.19	< 0.01
CO <sub>2</sub> × year × species	12	0.64	0.80	4	0.58	0.68
<b>Total mass damaged (g)</b>						
CO <sub>2</sub>	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 <sub>2</sub> × year	2	0.88	0.42	1	7.2	0.01
CO <sub>2</sub> × species	6	1.23	0.30	4	1.89	0.13
Year × species	11	3.49	< 0.01	4	8.28	< 0.01
CO <sub>2</sub> × 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).

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<sub>2</sub> 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

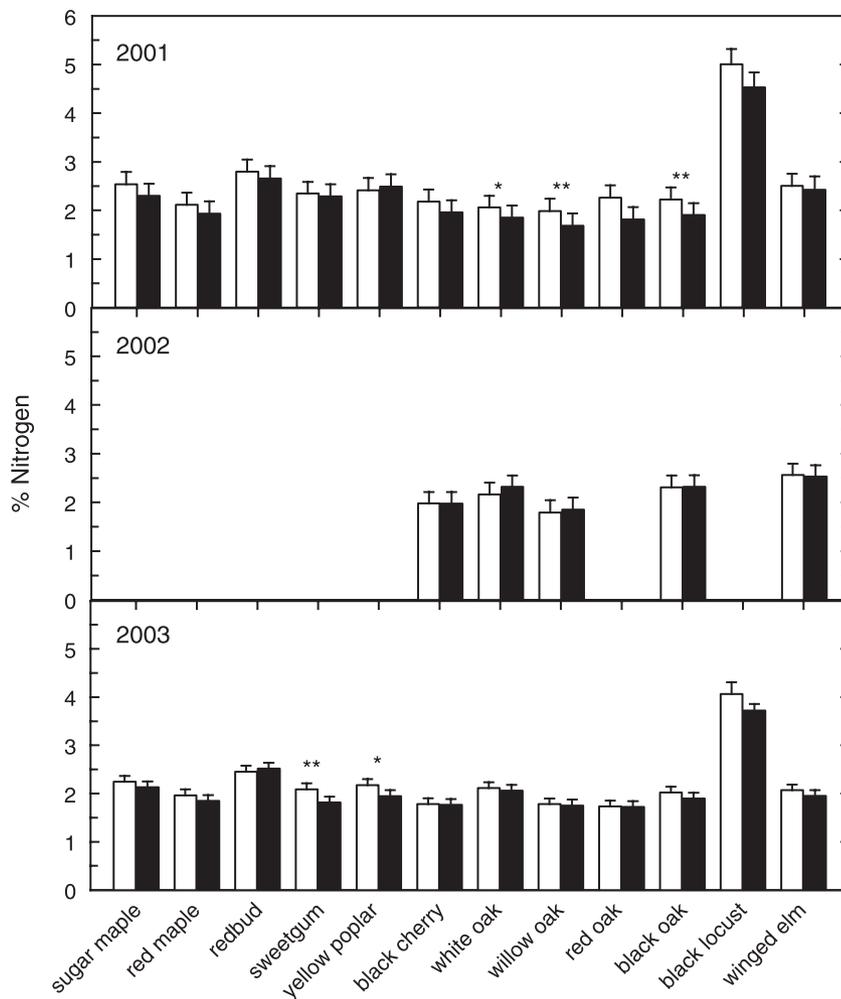
**Table 2** Analysis of variance for effects of elevated CO<sub>2</sub> on leaf carbon and nitrogen concentration (% mass basis) and carbon : nitrogen ratio for the five species measured in each of the three successive years and for seven additional tree species that were measured in the first and third 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
<b>Carbon (%)</b>						
CO <sub>2</sub>	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 <sub>2</sub> × year	2	0.30	0.74	1	1.46	0.23
CO <sub>2</sub> × species	4	0.83	0.50	6	0.88	0.51
Year × species	8	2.57	0.01	6	0.30	0.94
CO <sub>2</sub> × year × species	8	1.10	0.37	6	0.16	0.99
<b>Nitrogen (%)</b>						
CO <sub>2</sub>	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 <sub>2</sub> × year	2	3.52	0.03	1	0.03	0.86
CO <sub>2</sub> × species	4	0.33	0.86	6	0.31	0.93
Year × species	8	4.05	< 0.01	6	0.62	0.71
CO <sub>2</sub> × year × species	8	0.55	0.81	6	1.39	0.22
<b>Carbon : nitrogen ratio</b>						
CO <sub>2</sub>	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 <sub>2</sub> × year	2	4.50	0.01	1	0.24	0.62
CO <sub>2</sub> × species	4	0.28	0.89	6	0.48	0.83
Year × species	8	4.16	0.00	6	0.49	0.81
CO <sub>2</sub> × 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).

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<sub>2</sub> × year interaction on protein precipitation capacity expressed as a percentage of leaf mass ( $P = 0.10$ , Table 5; Fig. 5) and a significant CO<sub>2</sub> × 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<sub>2</sub> compared with ambient CO<sub>2</sub> (ambient CO<sub>2</sub> plots, 1.0 g m<sup>-2</sup>; elevated CO<sub>2</sub> plots, 1.5 g m<sup>-2</sup>; one-way ANOVA,  $P = 0.07$ , Table 5).

In 2001, the only year with a significant effect of CO<sub>2</sub> on herbivory levels, elevated CO<sub>2</sub> 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<sub>2</sub> to 2.3% under elevated CO<sub>2</sub> (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<sub>2</sub>



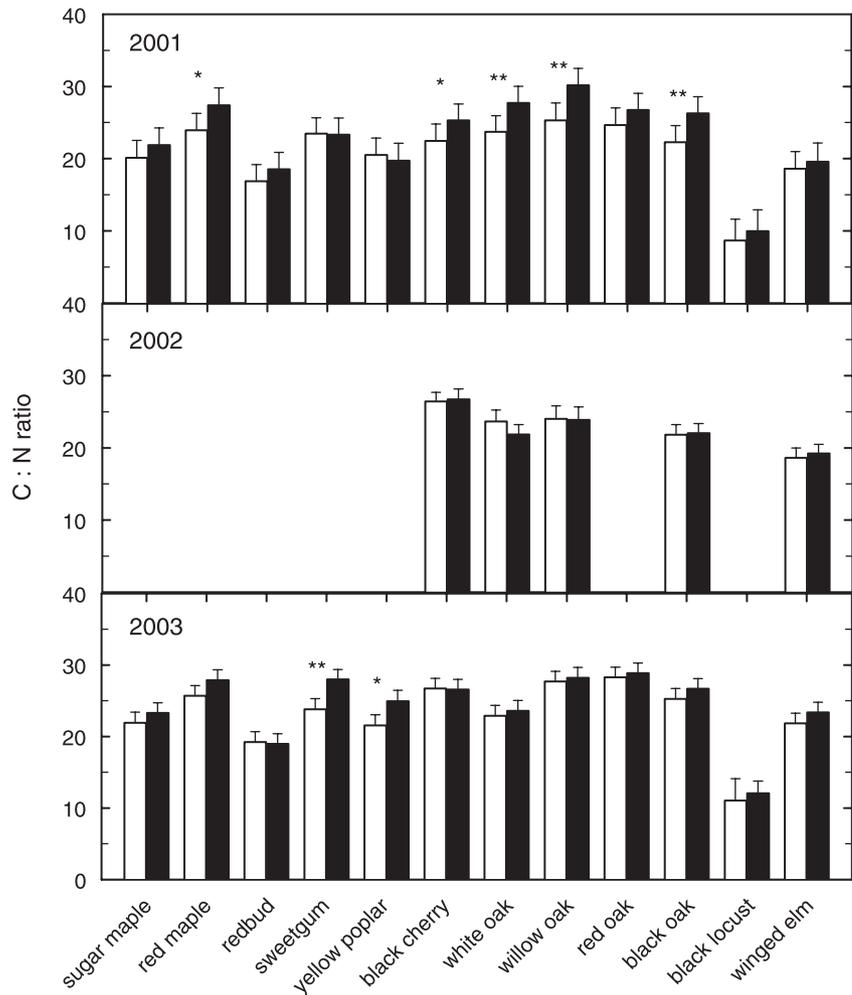
**Fig. 2** Effects of elevated CO<sub>2</sub> on leaf nitrogen concentration (% dry mass) for plants grown under ambient (c. 386  $\mu\text{l l}^{-1}$ ) and elevated (c. 586  $\mu\text{l l}^{-1}$ ) CO<sub>2</sub> for each of the three years. Columns represent the least-squared means ( $\pm 1$  SE) of the three ambient and three elevated CO<sub>2</sub> plots (open columns, ambient CO<sub>2</sub>; closed columns, elevated CO<sub>2</sub>). 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<sub>2</sub> plots, 408  $\text{cm}^2 \text{g}^{-1}$ ; mean for elevated CO<sub>2</sub> plots, 373  $\text{cm}^2 \text{g}^{-1}$ ;  $P = 0.09$ ; Fig. 4) under elevated compared with ambient CO<sub>2</sub>. Across the four oak species in 2001, there was a trend toward higher protein precipitation capacity under elevated CO<sub>2</sub> (1.5  $\text{g m}^{-2}$ ) than ambient CO<sub>2</sub> (1.0  $\text{g m}^{-2}$ ;  $P = 0.10$ ; data not shown). Elevated CO<sub>2</sub> 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  $\text{cm}^2 \text{g}^{-1}$ ) than in 2001 (328  $\text{cm}^2 \text{g}^{-1}$ ;  $P < 0.02$ ) and 2003 (315  $\text{cm}^2 \text{g}^{-1}$ ;  $P < 0.07$ ; 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<sub>2</sub> on carbon : nitrogen (C : N) ratio of leaves for plants grown under ambient (c. 386 μl l<sup>-1</sup>) and elevated (c. 586 μl l<sup>-1</sup>) CO<sub>2</sub> for each of the three years. Columns represent the least-squared means (± 1 SE) of the three ambient and three elevated CO<sub>2</sub> plots (open columns, ambient CO<sub>2</sub>; closed columns, elevated CO<sub>2</sub>). Within a species and year, statistical significance of pairwise comparisons is indicated above columns (\**P* < 0.1, \*\**P* < 0.05, \*\*\**P* < 0.01).

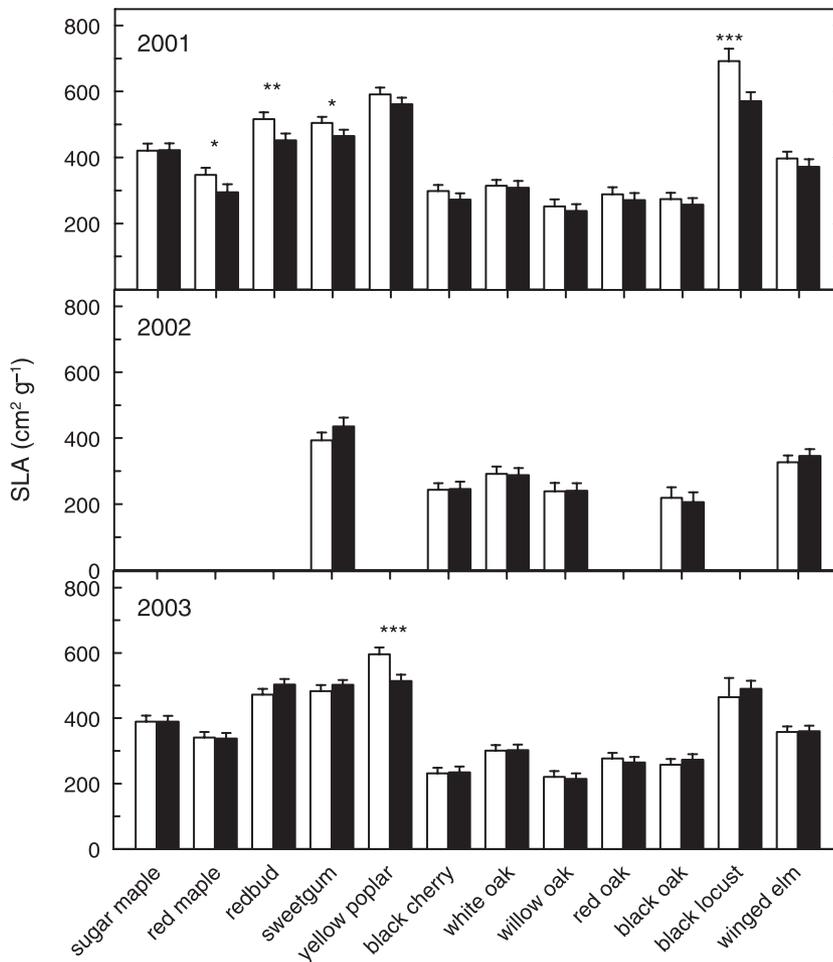
higher in red maple than all other species (50.2%) and lowest in winged elm (45.8%). Averaged across years, the highest N concentration (4.3%; Fig. 2) and lowest C : N ratio (10.1%; Fig. 3) were found in black locust, a nitrogen-fixing species (Olesniewicz & Thomas, 1999). Willow oak had the lowest average 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<sup>2</sup> g<sup>-1</sup> and 513 cm<sup>2</sup> g<sup>-1</sup>, respectively), while willow oak had lower average SLA than all other species (229 cm<sup>2</sup> g<sup>-1</sup>; 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<sub>2</sub> caused a reduction in the loss of leaf tissue to insect herbivores (Fig. 1). Other field-based herbivory surveys also found that elevated CO<sub>2</sub> 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<sub>2</sub>. The consistent reduction of herbivory across 12 species in 2001 (Fig. 1) suggests that elevated CO<sub>2</sub> 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<sub>2</sub> on specific leaf area (SLA, cm<sup>2</sup> g<sup>-1</sup>) for plants grown under ambient (c. 386 μl l<sup>-1</sup>) and elevated (c. 586 μl l<sup>-1</sup>) CO<sub>2</sub> for each of the three years. Columns represent the least-squared means (± 1 SE) of the three ambient and three elevated CO<sub>2</sub> plots (open columns, ambient CO<sub>2</sub>; closed columns, elevated CO<sub>2</sub>). 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<sub>2</sub> on herbivore damage in late summer (Wold & Marquis, 1997).

Given the consistent reduction in herbivory under high CO<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub>. This inference is supported by the observation that in the two subsequent years, when there was no detectable effect of elevated CO<sub>2</sub> 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<sub>2</sub>.

A reduction in SLA under elevated CO<sub>2</sub> 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<sub>2</sub>. This was not the case in this study because the mass of leaf tissue removed by herbivores under elevated CO<sub>2</sub> (area removed/SLA) was similar to the leaf mass consumed under ambient CO<sub>2</sub>.

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<sub>2</sub> (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<sub>2</sub> when plants were grown under realistic environmental

**Table 3** Analysis of variance for effects of elevated CO<sub>2</sub> on leaf carbon and nitrogen content (g m<sup>-2</sup>, 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
<b>Carbon (g m<sup>-2</sup>)</b>						
CO <sub>2</sub>	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 <sub>2</sub> × year	2	2.94	0.05	1	2.80	0.10
CO <sub>2</sub> × species	4	0.86	0.49	6	0.97	0.45
Year × species	8	6.16	< 0.01	6	1.34	0.24
CO <sub>2</sub> × year × species	8	1.27	0.26	6	0.93	0.48
<b>Nitrogen (g m<sup>-2</sup>)</b>						
CO <sub>2</sub>	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 <sub>2</sub> × year	2	0.01	0.99	1	2.23	0.14
CO <sub>2</sub> × species	4	0.57	0.69	6	0.39	0.88
Year × species	8	4.45	< 0.01	6	0.77	0.60
CO <sub>2</sub> × 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.

conditions. In contrast, for the suite of hardwood species examined in this experiment, elevated CO<sub>2</sub> 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<sub>2</sub>. Changes in N concentration and C : N ratio under elevated CO<sub>2</sub> 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<sub>2</sub>. 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<sub>2</sub>. Lindroth *et al.* (1993) and Kinney *et al.* (1997) also found an increase in condensed tannins for *Q. rubra* grown under elevated CO<sub>2</sub>, but these studies report conflicting responses of ellagitannin content to elevated CO<sub>2</sub> 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<sub>2</sub> in 2001, when there was also a significant reduction of leaf damage under elevated CO<sub>2</sub> (Fig. 5, Table 5). For some species, an

**Table 4** Analysis of variance for effects of elevated CO<sub>2</sub> on specific leaf area (SLA; cm<sup>2</sup> g<sup>-1</sup>) 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 <sub>2</sub>	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 <sub>2</sub> × year	2	7.60	< 0.01	1	2.21	0.14
CO <sub>2</sub> × species	6	0.30	0.94	4	0.56	0.69
Year × species	12	4.05	< 0.01	4	1.94	0.11
CO <sub>2</sub> × 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).

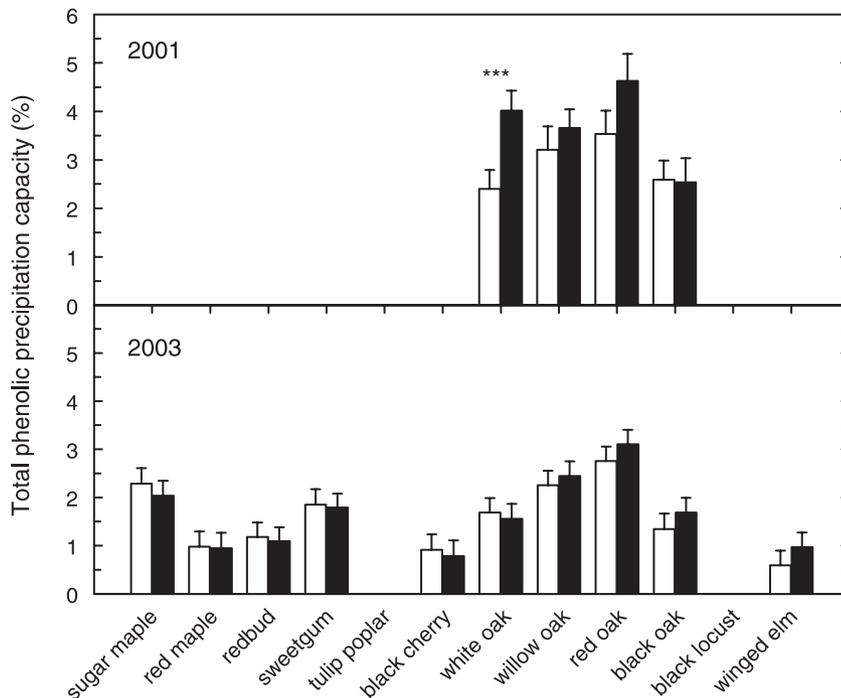
**Table 5** Analysis of variance for effects of elevated CO<sub>2</sub> on the protein precipitation capacity (tannic acid equivalents, % mass basis, and g m<sup>-2</sup>, area basis) for four species that were measured in the first and third year of the experiment and six additional species that were 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
<b>Concentration (%)</b>						
CO <sub>2</sub>	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 <sub>2</sub> × year	1	2.79	0.10	–	–	–
CO <sub>2</sub> × species	3	0.76	0.52	5	1.17	0.33
Year × species	3	0.38	0.76	–	–	–
CO <sub>2</sub> × year × species	3	1.79	0.15	–	–	–
<b>Content (g m<sup>-2</sup>)</b>						
CO <sub>2</sub>	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 <sub>2</sub> × year	1	112.00	0.03	–	–	–
CO <sub>2</sub> × species	3	116.00	0.41	5	134.00	0.31
Year × species	3	24.60	0.67	–	–	–
CO <sub>2</sub> × 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).

increase in leaf phenolics may contribute to lower levels of leaf damage under elevated CO<sub>2</sub>.

In field studies, elevated CO<sub>2</sub> decreased (Kopper & Lindroth, 2003a) or had no effect (Kopper & Lindroth, 2003a,b) on per



**Fig. 5** Effects of elevated CO<sub>2</sub> on the protein precipitation capacity (tannic acid equivalents; % mass) for plants grown under ambient (c. 386  $\mu\text{l l}^{-1}$ ) and elevated (c. 586  $\mu\text{l l}^{-1}$ ) CO<sub>2</sub> for each of two years. Columns represent the least-squared means ( $\pm 1$  SE) of the three ambient and three elevated CO<sub>2</sub> plots (open columns, ambient CO<sub>2</sub>; closed columns, elevated CO<sub>2</sub>). 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<sub>2</sub> (Bezemer & Jones, 1998; Coviella & Trumble, 1999; Hunter, 2001). Thus, there appear to be fundamental differences in the effect of elevated CO<sub>2</sub> on damage from insects between controlled laboratory/greenhouse settings and field studies despite the fact that elevated CO<sub>2</sub> 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<sub>2</sub>. 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<sub>2</sub> (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<sub>2</sub>. 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<sub>2</sub> 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<sub>2</sub> because of a decline in the abundance of chewing insects (Stiling *et al.*, 2002, 2003). Slower rates of development under elevated CO<sub>2</sub> 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<sub>2</sub>. Additionally, decreased foliar quality and increased per capita consumption under elevated CO<sub>2</sub> may increase exposure to toxins and insect mortality (Coviella & Trumble, 1999), and CO<sub>2</sub>-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<sub>2</sub> 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<sub>2</sub>, 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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## References

- Agrell J, McDonald EP, Lindroth RL. 2000. Effects of CO<sub>2</sub> and light on tree phytochemistry and insect performance. *Oikos* 88: 259–272.
- Arnore JAI, Zaller JG, Ziegler C, Zandt H, Korner C. 1995. Leaf quality and insect herbivory in model tropical plant communities after long-term exposure to elevated atmospheric CO<sub>2</sub>. *Oecologia* 104: 72–78.
- Awmack CS, Leather SR. 2002. Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology* 47: 817–844.
- Bernays EA. 1986. Diet-induced head allometry among foliage-chewing insects and its importance for graminivores. *Science* 231: 495–497.
- Bezemer TM, Jones TH. 1998. Plant–insect herbivore interactions in elevated atmospheric CO<sub>2</sub>: quantitative analyses and guild effects. *Oikos* 82: 212–222.
- Bryant JP, Chapin FSI, Klein DR. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* 40: 357–368.
- Burns RM, Honkala BH. 1990. *Silvics of North America*. Washington, DC, USA: Forest Service.
- Buse A, Good JEG, Dury S, Perrins CM. 1998. Effects of elevated temperature and carbon dioxide on the nutritional quality of leaves of oak (*Quercus robur* L.) as food for the Winter Moth (*Operophtera brumata* L.). *Functional Ecology* 12: 742–749.
- Cotrufo MF, Ineson P, Scott A. 1998. Elevated CO<sub>2</sub> reduces the nitrogen concentration of plant tissues. *Global Change Biology* 4: 43–54.
- Coviella CE, Trumble JT. 1999. Effects of elevated atmospheric carbon dioxide on insect–plant interactions. *Conservation Biology* 13: 700–712.
- Curtis PS, Wang X. 1998. A meta-analysis of elevated CO<sub>2</sub> effects on woody plant mass, form, and physiology. *Oecologia* 113: 299–313.
- Cyr H, Pace ML. 1993. Magnitude and patterns of herbivory in aquatic and terrestrial ecosystems. *Nature* 361: 148–150.
- DeLucia EH, George K, Hamilton JG. 2002. Radiation-use efficiency of a forest exposed to elevated concentrations of atmospheric carbon dioxide. *Tree Physiology* 22: 1003–1010.
- Drake BG, Gonzalez-Meler MA, Long SP. 1997. More efficient plants: a consequence of rising atmospheric CO<sub>2</sub>? *Annual Review of Plant Physiology and Plant Molecular Biology* 48: 609–639.
- Dury SJ, Good JEG, Perrins CM, Buse A, Kaye T. 1998. The effects of increasing CO<sub>2</sub> and temperature on oak leaf palatability and the implications for herbivorous insects. *Global Change Biology* 4: 55–61.
- Goverde M, Bazin A, Shykoff JA, Erhardt A. 1999. Influence of leaf chemistry of *Lotus corniculatus* (Fabaceae) on larval development of *Polyommatus icarus* (Lepidoptera, Lycaenidae): effects of elevated CO<sub>2</sub> and plant genotype. *Functional Ecology* 13: 801–810.
- Hagerman AE. 1987. Radial diffusion method for determining tannins in plant extracts. *Journal of Chemical Ecology* 13: 437–449.
- Hamilton JG, Zangerl AR, Berenbaum MR, Phippen JS, Aldea M, DeLucia EH. 2004. Insect herbivory in an intact forest understory under experimental CO<sub>2</sub> enrichment. *Oecologia* 138: 566–573.
- Hendrey GR, Kimball BA. 1994. The FACE program. *Agricultural and Forest Meteorology* 70: 3–14.
- Hendrey GR, Ellsworth DS, Lewin KF, Nagy J. 1999. A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO<sub>2</sub>. *Global Change Biology* 5: 293–309.
- Houghton JT, Ding J, Griggs DJ, Nougier M, van der Linden JJ, Dai X, Maskell K, Johnson CA. 2001. *Climate change 2001: the scientific basis. Contribution of working group 1 to the third assessment report of the intergovernmental panel on climate change*. Cambridge, UK and New York, NY, USA: Cambridge University Press.
- Hughes L, Bazzaz FA. 1997. Effect of elevated CO<sub>2</sub> on interactions between the western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae) and the common milkweed, *Asclepias syriaca*. *Oecologia* 109: 286–290.
- Hunter MD. 2001. Effects of elevated atmospheric carbon dioxide on insect–plant interactions. *Agricultural and Forest Entomology* 3: 153–159.
- Kerslake JE, Woodin SJ, Hartley SE. 1998. Effects of carbon dioxide and nitrogen enrichment on a plant–insect interaction: the quality of *Calluna vulgaris* as a host for *Operophtera brumata*. *New Phytologist* 140: 43–53.
- Kinney KK, Lindroth RL, Jung SM, Nordheim EV. 1997. Effects of CO<sub>2</sub> and NO<sub>3</sub><sup>-</sup> availability on deciduous trees: phytochemistry and insect performance. *Ecology* 78: 215–230.
- Kopper BJ, Lindroth RL. 2003a. Effects of elevated carbon dioxide and ozone on the phytochemistry of aspen and performance of an herbivore. *Oecologia* 134: 95–103.
- Kopper BJ, Lindroth RL. 2003b. Responses of trembling aspen (*Populus tremuloides*) phytochemistry and aspen blotch leafminer (*Phyllonorycter tremuloidiella*) performance to elevated levels of atmospheric CO<sub>2</sub> and O<sub>3</sub>. *Agricultural and Forest Entomology* 5: 17–26.
- Lewin KF, Hendrey GR, Nagy J, LaMorte RL. 1994. Design and application of a free-air carbon dioxide enrichment facility. *Agricultural and Forest Meteorology* 70: 15–29.
- Lincoln DE, Fajer ED, Johnson RH. 1993. Plant–insect herbivore interactions in elevated CO<sub>2</sub> environments. *Tree* 8: 64–68.
- Lindroth RL, Kinney KK, Platz CL. 1993. Responses of deciduous trees to elevated atmospheric CO<sub>2</sub>: productivity, phytochemistry, and insect performance. *Ecology* 74: 763–777.
- Lindroth RL, Roth SK, Nordheim EV. 2001. Genotypic variation in response of quaking aspen (*Populus tremuloides*) to atmospheric CO<sub>2</sub> enrichment. *Oecologia* 126: 371–379.
- Lowman MD. 1984. An assessment of techniques for measuring herbivory: is rainforest defoliation more intense than we thought? *Biotropica* 16: 264–268.
- Mansfield JL, Curtis PS, Zak DR, Pregitzer KS. 1999. Genotypic variation for condensed tannin production in trembling aspen (*Populus tremuloides*, Salicaceae) under elevated CO<sub>2</sub> and in high- and low-fertility soil. *American Journal of Botany* 86: 1154–1159.
- Mattson WJJ. 1980. Herbivory in relation to plant nitrogen content. *Annual Review of Ecology System* 11: 119–161.
- McDonald EP, Agrell J, Lindroth RL. 1999. CO<sub>2</sub> and light effects on deciduous trees: growth, foliar chemistry, and insect performance. *Oecologia* 119: 189–199.
- McGuire AD, Melillo JM, Joyce LA. 1995. The role of nitrogen in the response of forest net primary production to elevated atmospheric carbon-dioxide. *Annual Review of Ecology and Systematics* 26: 473–503.
- Mohan JE. 2002. Atmospheric carbon dioxide effects on temperate forests: implications for plant evolution, forest succession, and nutrient turnover. Doctoral Dissertation. Durham, NC, USA: Duke University.
- Norby RJ, Wullschlegel SD, Gunderson CA, Johnson DW, Ceulemans R. 1999. Tree responses to rising CO<sub>2</sub> in field experiments: Implications for the future forest. *Plant, Cell & Environment* 22: 683–714.
- Ohmart CP, Stewart LG, Thomas JR. 1983. Leaf consumption by insects in 3 eucalyptus forest types in southeastern Australia and their role in short-term nutrient cycling. *Oecologia* 59: 322–330.
- Olesniewicz KS, Thomas RB. 1999. Effects of mycorrhizal colonization on biomass production and nitrogen fixation of black locust (*Robinia*

- pseudoacacia*) seedlings grown under elevated atmospheric carbon dioxide. *New Phytologist* 142: 133–140.
- Peeters PJ. 2002. Correlations between leaf structural traits and the densities of herbivorous insect guilds. *Biological Journal of the Linnean Society* 77: 43–65.
- Percy KE, Awmack CS, Lindroth RL, Kubiske ME, Kopper BJ, Isebrands JG, Pregitzer KS, Oksanen E, Sober J, Harrington R, Karnosky DF. 2002. Altered performance of forest pest under atmospheres enriched by CO<sub>2</sub> and O<sub>3</sub>. *Nature* 420: 403–407.
- Reichle DE, Goldstein RA, van Hook RI, Dodson GJ. 1973. Analysis of insect consumption in a forest canopy. *Ecology* 54: 1076–1084.
- Roth SK, McDonald EP, Lindroth RL. 1997. Atmospheric CO<sub>2</sub> and soil water availability: consequences for tree–insect interactions. *Canadian Journal of Forest Research* 27: 1281–1290.
- Sand-Jensen K, Jacobsen D. 1994. Herbivory and resulting plant damage. *Oikos* 69: 545–549.
- Scriber JM, Slansky FJ. 1981. The nutritional ecology of immature insects. *Annual Review of Entomology* 26: 183–211.
- Shure DJ, Mooreside PD, Ogle SM. 1998. Rainfall effects on plant–herbivore processes in an upland oak forest. *Ecology* 79: 604–617.
- Singsaas EL, Ort DR, DeLucia EH. 2000. Diurnal regulation of photosynthesis in understory saplings. *New Phytologist* 145: 39–49.
- Stange G, Monro J, Stowe S. 1995. The CO<sub>2</sub> sense of the moth *Cactoblastis cactorum* and its probable role in the biological control of the cam plant *Opuntia stricta*. *Oecologia* 102: 341–352.
- Stiling P, Rossi AM, Hungate B. 1999. Decreased leaf-miner abundance in elevated CO<sub>2</sub>: reduced leaf quality and increased parasitoid attack. *Ecological Applications* 9: 240–244.
- Stiling P, Cattell M, Moon DC, Rossu A, Hungate BA, Hymuss G, Drakes B. 2002. Elevated atmospheric CO<sub>2</sub> lowers herbivore abundance, but increases leaf abscission rates. *Global Change Biology* 8: 658–667.
- Stiling P, Moon DC, Hunter MD, Colson J, Rossi AM, Hymus GJ, Drake BG. 2003. Elevated CO<sub>2</sub> lowers relative and absolute herbivore density across all species of a scrub-oak forest. *Oecologia* 134: 82–87.
- Whittaker RH. 1970. *Communities and Ecosystems*. New York, NY, USA: MacMillan.
- Williams RS, Norby RJ, Lincoln DE. 2000. Effects of elevated CO<sub>2</sub> and temperature-grown red and sugar maple on gypsy moth performance. *Global Change Biology* 6: 685–695.
- Wold EN, Marquis RJ. 1997. Induced defense in white oak: Effects on herbivores and consequences for the plant. *Ecology* 78: 1356–1369.
- Yin X. 2002. Responses of leaf nitrogen concentration and specific leaf area to atmospheric CO<sub>2</sub> enrichment: a retrospective synthesis across 62 species. *Global Change Biology* 8: 631–642.
- Zangerl AR, Hamilton JG, Miller JT, Crofts AR, Oxborough K, Berenbaum MR, DeLucia EH. 2002. Impact of folivory on photosynthesis is greater than the sum of its holes. *PNAS* 99: 1088–1091.



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