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Consequences of elevated carbon dioxide and ozone for foliar chemical composition and dynamics in trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*)

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"Capsule": *Elevated CO_2 resulted in changes in chemical foliar composition that are likely to impact herbivory and decomposition*

Abstract

Atmospheric chemical composition affects foliar chemical composition, which in turn influences the dynamics of both herbivory and decomposition in ecosystems. We assessed the independent and interactive effects of CO_2 and O_3 fumigation on foliar chemistry of quaking aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*) at a Free-Air CO_2 Enrichment (FACE) facility in northern Wisconsin. Leaf samples were collected at five time periods during a single growing season, and analyzed for nitrogen, starch and condensed tannin concentrations, nitrogen resorption efficiencies (NREs), and C:N ratios. Enriched CO_2 reduced foliar nitrogen concentrations in aspen and birch; O_3 only marginally reduced nitrogen concentrations. NREs were unaffected by pollution treatment in aspen, declined with O_3 exposure in birch, and this decline was ameliorated by enriched CO_2. C:N ratios of abscised leaves increased in response to enriched CO_2 in both tree species. O_3 did not significantly alter C:N ratios in aspen, although values tended to be higher in $+CO_2+O_3$ leaves. For birch, O_3 decreased C:N ratios under ambient CO_2 and increased C:N ratios under elevated CO_2. Thus, under the combined pollutants, the C:N ratios of both aspen and birch leaves were elevated above the averaged responses to the individual and independent trace gas treatments. Starch concentrations were largely unresponsive to CO_2 and O_3 treatments in aspen, but increased in response to elevated CO_2 in birch. Levels of condensed tannins were negligibly affected by CO_2 and O_3 treatments in aspen, but increased in response to enriched CO_2 in birch. Results from this work suggest that changes in foliar chemical composition elicited by enriched CO_2 are likely to impact herbivory and decomposition, whereas the effects of O_3 are likely to be minor, except in cases where they influence plant response to CO_2.

Keywords: *Betula papyrifera*; Carbon dioxide; C:N ratios; Decomposition; FACE; Litter quality; Nitrogen resorption efficiency; Ozone; Phytochemistry; *Populus tremuloides*; Tannins

1. Introduction

Chemical composition is a primary determinant of the fate of plant tissues, and correspondingly, of both energy flow and nutrient cycling dynamics in ecosystems. Qualitative changes in plant composition affect both herbivore and detritivore trophic pathways and these feed back to affect plant and animal community composition (e.g. Pastor and Naiman, 1992). Because plant chemistry is also responsive to changes in environmental conditions (Bryant et al., 1983; Herms and Mattson, 1992; Koricheva et al., 1998), numerous studies

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have begun to address the effects of global environmental changes on plant tissue quality.

The consequences of enriched atmospheric carbon dioxide (CO_2) for plant chemical composition are of particular interest. Not only is CO_2 of singular concern vis à vis climate change, but it has long been known to influence plant chemical composition, e.g. C:N ratios (Strain and Bazzaz, 1983). In general, plants grown under elevated CO_2 exhibit decreased concentrations of nitrogen, and increased concentrations of nonstructural carbohydrates and tannins (Watt et al., 1995; Lindroth, 1996; Peñuelas and Estiarte, 1998; Peñuelas et al., 1997; Koricheva et al., 1998). The magnitude of such effects, however, varies in relation to both plant species and resource (e.g. light, nutrient) availability.

The effects of elevated atmospheric CO_2 on ecosystems will not occur in isolation, but in the context of multiple environmental changes. One such change is increased concentrations of tropospheric ozone (O_3). Ozone is considered one of the most ubiquitous and damaging air pollutants in the USA (Reich, 1987; Broadmeadow, 1998; Heck et al., 1998). Current projections indicate that levels of O_3 will increase well into the twenty-first century (Chameides et al., 1994). Relatively little is known, however, about the effects of O_3 on plant chemical composition, and almost nothing is known about the interactive effects of CO_2 and O_3 on plant chemistry.

Assessment of the individual and combined effects of CO_2 and O_3 on tree chemistry in an outdoor environment is now afforded by the Aspen FACE (Free-air CO_2 Enrichment) project in northern Wisconsin, USA. This FACE site was established to investigate the effects of CO_2 and O_3 on aggrading stands of trembling aspen (*Populus tremuloides* Michx.), paper birch (*Betula papyrifera* Marsh.) and sugar maple (*Acer saccharum* Marsh.). These tree species are important components of northern deciduous forests, and span the range from early successional, fast-growing species (aspen, birch) to a late-successional, slow-growing species (maple). The project employs a full factorial experimental design with four treatments: ambient air (control), $+CO_2$, $+O_3$ and $+CO_2+O_3$.

The primary purpose of the research reported here was to address the independent and interactive effects of CO_2 and O_3 on the foliar chemical composition of aspen and birch over an entire growing season. (At the time of this study, maples were too small to sustain sequential foliar sampling.) We focus on three chemical constituents—nitrogen, starch and condensed tannins—because these are typically the most responsive to changes in atmospheric CO_2 levels, and because they have important ecological roles with respect to herbivory or decomposition (Watt et al., 1995; Lindroth, 1996; Peñuelas and Estiarte, 1998).

Based on a comprehensive review by Koricheva et al. (1998), we predicted that CO_2 enrichment would increase levels of tannins and starch, but decrease levels of nitrogen, in aspen and birch foliage. The consequences of enriched O_3 for foliar chemistry were more difficult to predict. Consistent with the summary of Koricheva et al. (1998), we predicted that O_3 would increase phenolic concentrations but have little effect on starch and nitrogen concentrations. In addition, due to cumulative effects of the pollutants, we predicted that treatment effects on foliar chemistry would increase during the growing season.

A secondary purpose of the research was to assess the effects of CO_2 and O_3 on patterns of nitrogen translocation from senescing leaves to perennial tissues prior to leaf abscission, and whether changes in nitrogen resorption efficiency (NRE) alter leaf litter quality. Nitrogen resorption plays a pivotal role in determining the nitrogen concentration of leaf litter, and, consequently, the dynamics of litter decomposition (McGuire et al., 1995; Norby et al., 2000). As described by Norby et al. (2000), the "litter quality hypothesis" (Strain and Bazzaz, 1983; McGuire et al., 1995) suggests that lower concentrations of nitrogen in foliage of plants grown under enriched CO_2 will decelerate decomposition, decrease nitrogen availability, and ultimately feed back to reduce primary productivity. Thus, if CO_2 and O_3 environments do not significantly alter resorption efficiencies, levels of nitrogen in leaf litter should reflect those in green leaves. For a variety of reasons, however, nitrogen resorption may shift under conditions of enriched CO_2 (see Norby et al., 2000 for detailed discussion). Similarly, O_3, which typically accelerates leaf senescence, may alter nitrogen resorption. If resorption efficiencies decline, then the effects of CO_2 or O_3 on nitrogen levels in green leaves may not carry over to leaf litter; i.e. the "litter quality hypothesis" would not be supported.

Based on the results of preliminary experiments (Parsons, Lindroth and Bockheim, unpublished data), we predicted that CO_2 enrichment would increase the efficiency of nitrogen resorption, such that the litter carbon-to-nitrogen (C:N) ratio would increase relative to the control, and conversely, that O_3 stress would decrease NRE, such that the C:N ratio would decrease relative to the control. When fumigations included the combined trace gases, plant response to elevated CO_2 plus O_3 would equal the average response of the two independent gas additions.

2. Materials and methods

2.1. Experimental design and set-up

The experiment was conducted at the Aspen FACE facility, near Rhinelander, Wisconsin (W 89.7°, N 45.7°). The site contains 12 FACE rings (30 m diameter)

in a 2×2 factorial design, with three rings for each treatment (ambient air, supplemental CO_2, supplemental O_3, and supplemental CO_2 plus O_3). The replicates were blocked to provide adequate coverage of the northern, central and southern regions of the 32-ha site. Additional details of site description, experimental design and operation of the FACE facility are provided by Dickson et al. (2000).

Fumigation of the aggrading forest plots was conducted only during daylight hours of the growing season (0700–1900 h). Carbon dioxide enrichment was set to a target level of 560 μl l^{-1}, a concentration likely to be reached by 2050, as projected from climate change models (Dickson et al., 2000). Earlier testing revealed that CO_2 concentrations were maintained within 10% of the target, 80% of the time (Dickson et al., 2000). Fixed concentrations of O_3 in the elevated-ozone FACE rings were not realistic given the dynamic, photochemical nature of tropospheric O_3 formation. Thus, O_3 concentrations followed a diurnal, stepped-sine function that peaked at 90–100 nl l^{-1} on sunny days, and 50–60 nl l^{-1} on cloudy days. No ozone was administered during cold weather (<15 °C) or when leaf surfaces were wet from fog, dew, or rain events. Earlier testing showed that O_3 exposure was maintained within 10% of daily target concentrations for 66% of the time (Dickson et al., 2000). These target O_3 concentrations were based on regional averages (Pinkerton and Lefohn, 1987), modified to reflect on-site daily O_3 levels (Karnosky et al., 1996). Further details about fumigant exposures are available in Dickson et al. (2000) and Isebrands et al. (2001).

Vegetatively propagated aspen saplings were interplanted with birch seedlings (1 m×1 m spacing; southwest quadrant of each ring) in summer 1997. The aspen derived from a single clone (No. 216), which is responsive to CO_2 enrichment and moderately sensitive to O_3 exposure (Karnosky et al., 1996; Dickson et al., 2000). The birch originated from seed collected in Houghton County, Michigan (Dickson et al., 2000). Sapling establishment was sustained by periodic irrigation during the first growing season (1998). No supplemental fertilizer was applied. Fumigation treatments commenced at budbreak in spring 1998.

2.2. Foliar collections

Foliar samples were collected during the 1999 growing season, when the trees were in their third growing season. Sampling was concentrated on the upper canopy, about breast height (~1.4 m). Foliage was selected from random branches at the same relative position on the tree for all collection dates. Only fully expanded foliage was collected from the middle-to-distal third of a given branch.

Leaf samples were collected at five intervals: June 9, July 6, August 1, August 26, and September 22 for aspen, and June 3, June 29, August 1, August 26, and September 22 for birch. For both species, leaves used for the first two collections had been previously covered with fine mesh material in order to match foliage used in a concurrent study of insect performance. For foliar collections one, two and four, the same three birch and five aspen trees within each ring were used. Additional trees were sampled during collections three and five to increase within-ring sample sizes for the determination of specific leaf areas (SLAs) used in the calculation of nitrogen resorption efficiencies (NREs). Leaves collected for NRE on August 1 were green and fully mature, while those collected September 22 had senesced and were in the process of abscission. Leaching losses and other canopy exchange processes were not measured for any of the leaf collections.

Approximately 2–3 g (fresh mass) of foliage were excised at the petiole and stored under crushed ice until leaves could be returned to the laboratory in Madison (<4 h following field collection). Leaves were then flash-frozen in liquid nitrogen, freeze-dried, ground, and stored at −20 °C. Leaves designated for determination of nitrogen resorption efficiency were passed through a leaf area meter (LI-3100, Licor, Lincoln, Nebraska) prior to freezing. Specific leaf areas (SLAs) were estimated from those area measurements and the corresponding freeze-dried tissue masses.

2.3. Phytochemical analyses

Foliar samples were analyzed for nitrogen, starch, condensed tannins and carbon. Tissue nitrogen concentrations were determined by high-temperature combustion, followed by thermoconductometric detection (LECO FP528 nitrogen analyzer, St. Joseph, Michigan). The nitrogen analyzer was calibrated against glycine *p*-toluenesulfonate (Hach Company, Loveland, Colorado) as a standard. Starch content was determined by enzymatic conversion to glucose (Prado et al., 1998), followed by colorimetric detection of liberated glucose via a modified dinitrosalicylic acid method (Lindroth et al., 2001a). Condensed tannins were measured colorimetrically, using the butanol-HCl method of Porter et al. (1986), which hydrolytically converts proanthocyanidins to anthocyanidins. Condensed tannin standards were purified from aspen and birch leaves by adsorption chromatography (Hagerman and Butler, 1980). Tissue carbon content of pre-abscised leaves (third and fifth collections) was determined by loss-on-ignition (LOI: 550 °C, 4–6 h), assuming that 50% of the resulting ash-free dry mass estimate was carbon. Finally, C:N ratios of leaves at abscission were calculated from the respective carbon and nitrogen tissue concentrations.

2.4. Nitrogen resorption efficiencies

Nitrogen resorption efficiencies (NREs) were calculated using nitrogen concentrations and specific leaf areas of mid-season (collection 3) and senescent (collection 5) leaves. Tissue nitrogen concentrations were converted to a content basis by dividing by specific leaf area (SLA, $cm^2\ g^{-1}$). The difference between the corrected green leaf and senescent leaf nitrogen content (mg N cm^{-2} leaf) was divided by the corrected green leaf content, and multiplied by 100 to yield an estimate of the NRE percentage (Killingbeck et al., 1990; Killingbeck, 1996). NRE estimates were not corrected for leaching losses.

2.5. Statistical analyses

Concentrations (as percentages) of starch, nitrogen and condensed tannin were analyzed in full factorial analysis of variance, with repeated measures (ANOVA; PROC Mixed; Littell et al., 1996). The same model was used for both aspen and birch; however, due to the different collection dates, the effect of species was not added to the model. The statistical model used was:

$$Y_{ijkl} = \mu + B_i + C_j + O_k + \mathrm{CO}_{jk} + e_{ijk} + T_l + \mathrm{CT}_{jl} + \mathrm{OT}_{kl} + \mathrm{COT}_{jkl} + \varepsilon_{ijkl}$$

where Y_{ijkl} was the average response of block i, CO_2 level j, O_3 level k, and time l. Fixed effects were CO_2 level (C_j), O_3 level (O_k), $CO_2 \times O_3$ interaction (CO_{jk}), time (T_l), $CO_2 \times$time (CT_{jl}), $O_3 \times$time (OT_{kl}), and $CO_2 \times O_3 \times$time (COT_{jkl}). Random effects included block (B_i), whole plot error (e_{ijk}), and subplot error (ε_{ijkl}). The whole plot error term e_{ijk} ($= BC_{ij} + BO_{ik} + BCO_{ijk}$) was used to test C_j, O_k, and their interaction, whereas the pooled subplot error term ε_{ijkl} ($= BT_{il} + BCT_{ijl} + BOT_{ikl} + BCOT_{ijkl}$) was used to test T_l and its interactions. Means and standard errors were calculated using the LSMEANS (least-squares means) procedure and are reported for each $CO_2 \times O_3 \times$ time combination. Because of the low number of true replicates ($n = 3$ FACE rings) for the fumigation treatments, and the corresponding risk of Type II statistical errors, we report P-values < 0.10 as significant.

Nitrogen resorption efficiencies and C:N ratios were subjected to a similar analysis, except that time was eliminated from the model. Thus, LSMEANS are reported for the CO_2 and O_3 combinations without a time factor.

We calculated coefficients of concordance (Kendall's W) for the nitrogen, starch and tannin data, to evaluate the consistency of rank order of responses to the fumigation treatments over time. This nonparametric measure gives values from 0 to 1, with 0 indicating no agreement in rank order of treatments over time, and 1 indicating complete agreement in rank order over time (Sprent, 1993). Chi-square tests were conducted to determine the significance of the coefficients of concordance.

Seasonal and treatment-related variation in tissue nitrogen concentrations were also subjected to more comprehensive means comparisons. As a quantitative factor with five levels, the effect of time could be decomposed into linear (first-order), quadratic (second-order), cubic (third-order) and quartic (fourth-order) terms. For each species, four separate sets of trend analyses were performed, one for each treatment. Differences among mean nitrogen concentrations were tested for systematic temporal trends, using contrasts that incorporated orthogonal polynomial coefficients (Woodward et al., 1990). The coefficients were not weighted since the number of days between the five sample periods was about equal. The trend contrasts were applied to the N means to determine whether systematic shifts or plateaus in the data occurred over time, as a prelude to the calculation of nitrogen resorption efficiencies.

3. Results

The chemical composition of aspen and birch leaves changed in response to CO_2 or O_3 treatments. Foliar chemistry also varied over time, and in some cases differently so for the fumigation treatments (i.e. significant fumigant $\times$ time interactions).

3.1. Trembling aspen

Nitrogen concentrations averaged 16% lower in enriched CO_2 treatments relative to unenriched treatments, and 8% lower in high O_3 treatments relative to low O_3 treatments (Fig. 1; Table 1). Also, control trees and trees exposed to either trace gas alone exhibited strong declines in tissue N concentrations in June, with a mid- to late-season plateau in August, followed by a rapid decrease during senescence in mid-September (Fig. 1). In all treatments, tissue N levels declined in a linear fashion over time (first-order polynomial contrasts, $P < 0.001$). Additional, curvilinear trend components were superimposed on this dominant linear trend in the controls (third-order contrast, $P = 0.021$; fourth-order contrast, $P = 0.018$), and aspen exposed to only CO_2 (fourth-order contrast, $P = 0.040$) or O_3 fumigation (third-order contrast, $P = 0.098$). Only the $+CO_2 + O_3$ treatment exhibited no higher order trends. In addition, the magnitude of variation between O_3-fumigated and nonfumigated trees tended to increase late in the growing season, contributing to a significant $O_3 \times$time

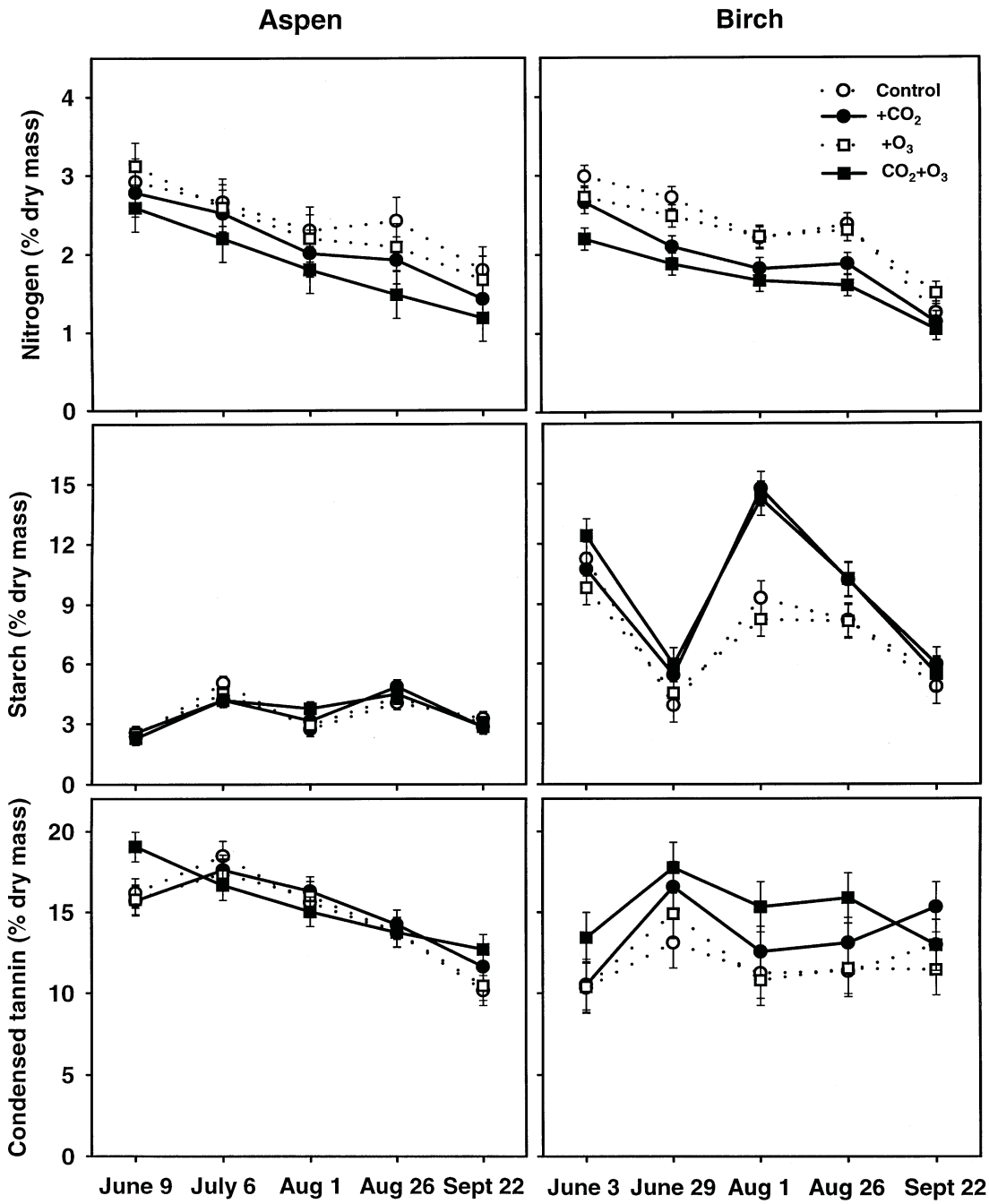

Fig. 1. Effects of CO_2 and O_3 fumigation on chemical composition of trembling aspen and paper birch foliage over a single growing season. Error bars indicate ±1 S.E.

interaction. The rank order of the treatment responses was highly consistent throughout the growing season (Table 2), with highest concentrations in ambient foliage and lowest concentrations in $+CO_2+O_3$ foliage.

Nitrogen resorption efficiencies were not significantly different among aspen in the various treatments, although efficiencies tended to be higher for trees enriched with CO_2 plus O_3 (Fig. 2; Table 1). C:N ratios increased in foliage from high CO_2 treatments (Fig. 2; Table 1), and the general pattern of response was similar to that exhibited by NRE values. In contrast to CO_2, O_3 did not alter C:N ratios in aspen foliage.

Starch concentrations showed little to no response to pollutant treatment (Fig. 1; Table 1). Levels in CO_2-enriched foliage tended to be lower than in unenriched foliage in early July, but higher in early August, resulting in a marginally significant $CO_2 \times$ time interaction. Starch concentrations were dynamic over time, but did

Table 1
Summary of P values for the effects of carbon dioxide and ozone on chemical composition, nitrogen resorption efficiency (NRE) and C:N ratios of aspen leaves

Main effects and interactions			Condensed		
	Nitrogen	Starch	Tannins	NRE	C:N ratio
CO_2	<0.001	0.843	0.464	0.188	0.027
O_3	0.005	0.923	0.886	0.372	0.161
$CO_2 \times O_3$	0.062	0.944	0.724	0.312	0.192
Time	<0.001	<0.001	<0.001	–	–
$CO_2 \times$ time	0.121	0.055	0.061	–	–
$O_3 \times$ time	0.030	0.647	0.112	–	–
$CO_2 \times O_3 \times$ time	0.661	0.631	0.106	–	–

Table 2
Coefficients of concordance (Kendall's W) indicating consistency in ranked mean response of nitrogen, starch and tannin concentrations to four fumigation treatments over a growing season[a]

Species	Nitrogen	Starch	Condensed tannins
Aspen	0.93 (<0.010)	0.04 (>0.800)	0.07 (>0.750)
Birch	0.81 (<0.005)	0.52 (>0.05, <0.100)	0.66 (<0.020)

[a] P values from associated Chi-square tests are shown in parentheses.

not exhibit systematic temporal trends. The rank order of treatment responses was not consistent throughout the year (Table 2).

Condensed tannin concentrations in aspen also exhibited little response to CO_2 and O_3 treatments (Fig. 1; Table 1). Concentrations in foliage exposed to the $+CO_2+O_3$ treatment in June, and to both elevated CO_2 treatments in September, were slightly higher than in unenriched foliage (marginally significant $CO_2 \times$ time interaction). Overall, however, the most substantial change was over time, with an average 36% decline from early to late in the season. The rank order of treatment responses varied during the season (Table 2).

3.2. Paper birch

Nitrogen concentrations averaged 21% lower in enriched CO_2 treatments relative to unenriched treatments (Fig. 1; Table 3). Levels also tended to be lower in O_3-fumigated versus nonfumigated foliage early but not late in the growing season, contributing to a significant $O_3 \times$ time interaction. Nitrogen levels of foliage from all treatments declined during the growing season (linear trend contrasts, $P<0.001$), and tended to converge toward a common value of approximately 1.5% (dry mass) at abscission. Birch exhibited early season declines in tissue N concentrations before leveling-off at mid-season, and significantly declining at senescence. This pattern is similar to that of aspen (Fig. 1), with comparable linear and higher-order trend components to the seasonal pattern. The rank order of treatment responses to the fumigation treatments was consistent over time (Table 2), with highest concentrations in ambient foliage and lowest concentrations in $+CO_2+O_3$ foliage.

Nitrogen resorption efficiencies in birch were not affected by tree exposure to enriched CO_2 alone, but decreased under elevated O_3 (Fig. 2; Table 3). This decrease was ameliorated, however, by co-exposure to high CO_2. As for aspen, C:N ratios of senescent birch leaves increased with CO_2 enrichment. Ozone appeared to affect C:N ratios differently, depending on CO_2 concentration (marginally significant CO_2 x O_3 interaction). Under ambient CO_2, C:N ratio tended to decrease with O_3 exposure, whereas under elevated CO_2, it increased.

Starch concentrations varied in response to CO_2, but not in response to O_3, treatments (Fig. 1; Table 3). The effects of CO_2 were strongly time-dependent, with the largest difference between treatments occurring in early August (significant $CO_2 \times$ time interaction). As for aspen, starch concentrations were dynamic over time (especially for enriched CO_2 treatments), but did not exhibit systematic trends. The rank order of treatment responses tended to be consistent across sampling dates (Table 2).

Condensed tannin concentrations also varied in response to CO_2, but not in response to O_3 (Fig. 1; Table 3). Levels in high-CO_2 trees averaged 21% higher than in ambient CO_2 trees. Tannin concentrations also changed over time; levels increased early in the summer, peaked in July, then declined to stable levels in August. The rank order of treatment responses was generally consistent throughout the growing season, with highest values in the $+CO_2+O_3$ foliage, and lowest values in the control and $+O_3$ foliage.

4. Discussion

Fumigation treatments elicited changes in some, but not all, foliar constituents. The magnitude of responses to CO_2 or O_3 generally varied during the growing season and differed between the two tree species.

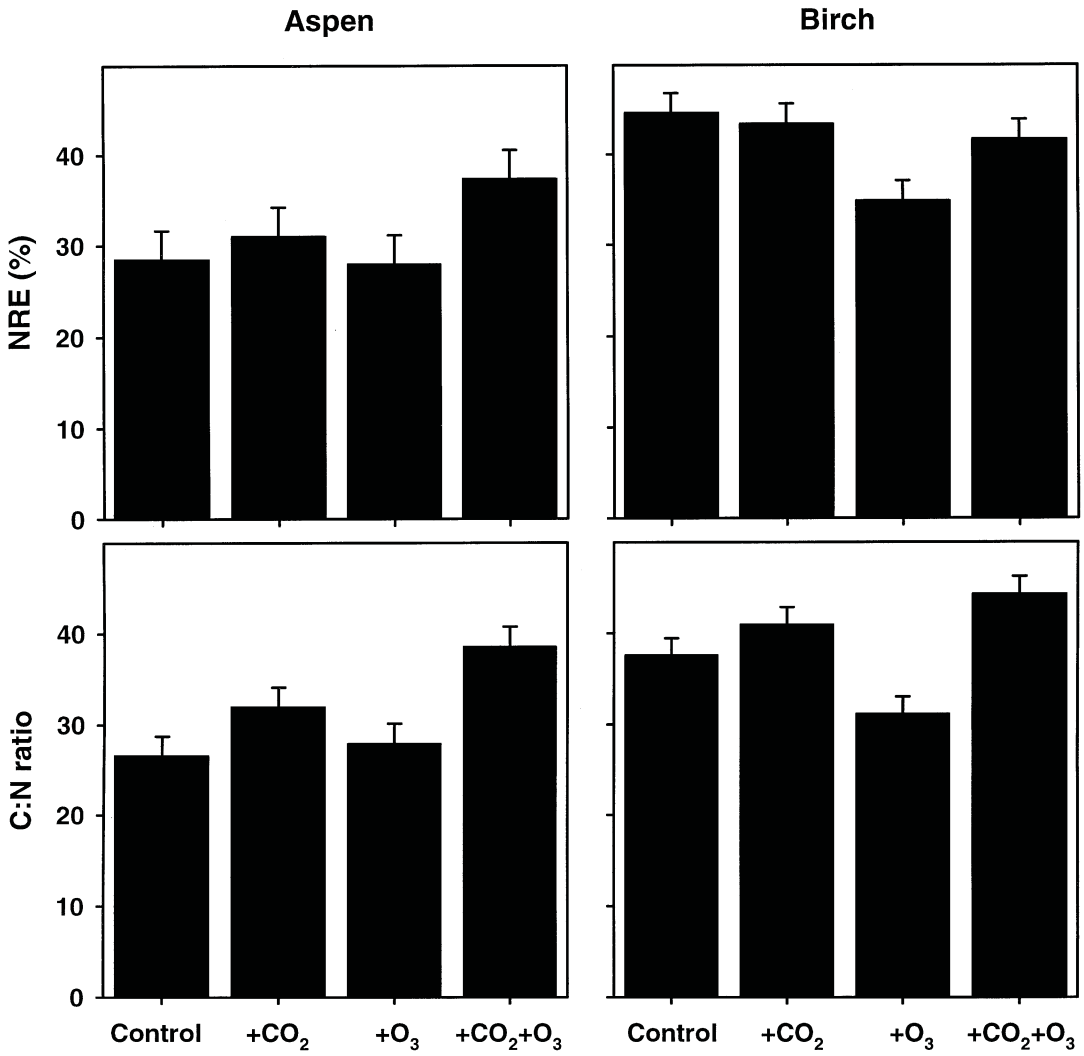

Fig. 2. Effects of CO_2 and O_3 fumigation on nitrogen resorption efficiencies (NRE) and C:N ratios for trembling aspen and paper birch. Error bars indicate +1 S.E.

Table 3
Summary of *P* values for the effects of carbon dioxide and ozone on chemical composition, nitrogen resorption efficiency (NRE) and C:N ratio of birch leaves

Main effects and interactions			Condensed		
	Nitrogen	Starch	Tannins	NRE	C:N ratio
CO_2	<0.001	0.001	0.047	0.443	0.022
O_3	0.148	0.979	0.471	0.036	0.595
$CO_2 \times O_3$	0.349	0.561	0.528	0.071	0.059
Time	<0.001	<0.001	<0.001	–	–
$CO_2 \times$time	0.189	<0.001	0.850	–	–
$O_3 \times$time	0.061	0.823	0.184	–	–
$CO_2 \times O_3 \times$time	0.831	0.438	0.633	–	–

Consistent with our predictions, nitrogen concentrations declined in aspen and birch in response to elevated atmospheric CO_2. The magnitude of decline (16–21%) was similar to that observed in numerous other studies with woody plants (McGuire et al., 1995; Curtis and Wang, 1998; Cotrufo et al., 1998), including other studies of aspen and birch (e.g. Lindroth et al., 1993; Roth and Lindroth, 1995; McDonald et al., 1999). Contrary to our prediction, however, the effects of CO_2 on foliar nitrogen levels did not increase during the

season for either aspen or birch. O_3 affected foliar nitrogen concentrations less than did CO_2, with a marginal effect tending to increase over time for aspen, but to decrease over time for birch. Other studies of O_3 fumigation have reported positive, negative, and no change in foliar nitrogen levels, with the latter result occurring most commonly (Koricheva et al., 1998).

This study revealed little effect of CO_2 enrichment on nitrogen resorption efficiencies in aspen and birch, except where it ameliorated the effects of O_3 exposure in the combination trace gas treatment. The absence of a detectable CO_2 effect is consistent with the results of Norby et al. (2000) and Finzi et al. (2001) for a variety of other tree species. In the absence of CO_2, O_3 exposure did have a strong negative effect on birch N resorption. Thus, our results are mostly consistent with the litter quality hypothesis: pollutant-induced variation in concentrations of nitrogen in green leaves are likely to persist in leaf litter. The signature of the CO_2 enrichment was present not only in senesced leaves, but persisted through the early stages of a subsequent decomposition experiment using the fallen litter (Parsons, Lindroth and Bockheim, unpublished data). Ozone exerted a similar signature that was carried through the period of leaf senescence to litter deposition and beyond. Also, both species exhibited a trend toward higher C:N ratios when exposed to elevated CO_2 plus O_3. These ratios were higher than the averaged response to independent CO_2 and O_3 additions, suggesting a synergistic, and therefore counter-intuitive, plant response to the combined trace gases.

Starch concentrations were low in aspen, and, contrary to our prediction, unresponsive to CO_2 treatment. Roth et al. (1998) reported that enriched CO_2 significantly increased starch concentrations in expanding aspen leaves, but that the difference disappeared upon leaf maturation. If the response of starch concentrations to elevated CO_2 is moderated by leaf phenology, we may have observed little response in this study because leaves were fully mature by early June. Alternatively, that starch concentrations were low and unresponsive to CO_2 enrichment may simply reflect carbohydrate source-sink dynamics [which strongly influence starch accumulation (Stitt, 1991)], and the strong demand for carbohydrates to support rapid growth of these aspen trees. In contrast, starch concentrations were higher, and more responsive to enriched CO_2, in birch. Concentrations were particularly high in early August, following the period of most rapid tree growth but preceding leaf senescence. O_3 fumigation had no effect on starch concentrations in aspen or birch, consistent with results from similar research summarized by Koricheva et al. (1998) and reported more recently by Kainulainen et al. (1998) for Scots pine.

Condensed tannins are carbon-based secondary metabolites derived from the shikimic acid pathway, concentrations of which typically increase in response to enriched atmospheric CO_2 (Lindroth, 1996; Peñuelas and Estiarte, 1998). In contrast to our prediction, however, we found little effect of elevated CO_2 on condensed tannin concentrations in aspen. Numerous other studies have evaluated the consequences of enriched CO_2 for tannins in aspen, and responses have ranged from no effect to substantial increases (e.g. Lindroth et al., 1993, 2001b; Kinney et al., 1997; Mansfield et al., 1999; McDonald et al., 1999; Agrell et al., 2000). Negligible response in this study could be a consequence of several factors. First, the level of CO_2 enrichment used in the study was significantly less than that used in earlier studies (560 vs. 650–700 $\mu l\ l^{-1}$). Second, levels of tannins respond more strongly to enriched CO_2 under conditions of low nutrient availability (Kinney et al., 1997; Mansfield et al., 1999; Lindroth et al., 2001b), which is not the case for soil at the FACE site (Dickson et al., 2000). Third, aspen genotypes differ with respect to accumulation of tannins in response to high CO_2 (Mansfield et al., 1999; Lindroth et al., 2001b); the genotype used in this study may be particularly unresponsive. In contrast, elevated atmospheric CO_2 did elicit increased concentrations of condensed tannins in birch, and these levels were sustained throughout the growing season. Other studies of birch under enriched CO_2 have documented a range of responses, from no change to a doubling of tannin concentrations. As for aspen, the variation in response can be attributed to numerous factors, including CO_2 concentration, soil fertility, light availability, and genetic differences (Lavola and Julkunen-Tiitto, 1994; Lindroth et al., 1995; Roth and Lindroth, 1995; Traw et al., 1996; McDonald et al., 1999; Agrell et al., 2000).

O_3 exposure is known to alter the activity of several enzymes (e.g. phenylalanine ammonia lyase, chalcone synthase) that regulate synthesis of phenolic constituents via the shikimic acid pathway, induction of which is consistent with a generalized stress response. (Kangasjärvi et al., 1994). Although we did not address production of antioxidants such as flavonoids and related simple phenolics, our results showed that O_3 fumigation did not influence levels of condensed tannins in aspen or birch. In a study of European white birch (*B. pendula*), Lavola et al. (1994) also reported no effect of O_3 on concentrations of condensed tannins and numerous simple phenolics. Nearly all of the limited, earlier work documenting increases in tannins and related phenolics in response to O_3 was conducted with conifers (Koricheva et al., 1998). The putative difference in response between early successional deciduous trees and coniferous evergreens could simply be an artifact of small sample size for the former, or may indicate that chemical defense systems of trees with short-lived foliage are less easily induced than those of trees with long-lived foliage.

Of particular interest in this study was the potential for foliar chemical composition to be influenced by the interaction of CO_2 and O_3. Indeed, a principal objective of the Aspen FACE research program is to investigate such responses in the context of aggrading forest communities (Dickson et al., 2000). For the limited, but important, suite of phytochemicals we investigated, no evidence for such interactions was found. Only nitrogen concentrations were influenced by O_3 treatment, and those responses were independent of CO_2 treatment.

Results of this research hold implications for the fate of tree foliage vis à vis herbivory and decomposition. Changes in foliar chemistry, especially nitrogen, due to enriched CO_2 were comparable to those associated with increased feeding but decreased growth efficiency of numerous lepidopteran insects in earlier studies (e.g. Roth and Lindroth, 1995; Lindroth, 1996; McDonald et al., 1999; Agrell et al., 2000). Performance of free-feeding insects at the Aspen FACE site is likely to be similarly affected. In addition, CO_2-elicited changes in chemical composition of green leaves generally carried over to leaf litter. Because litter decomposition is reduced by low nitrogen content, high C:N ratio, and the presence of chemical modifiers such as tannins (Swift, 1979; Taylor et al., 1989, 1991; Anderson, 1991; Peñuelas and Estiarte, 1998), we predict that decomposition rates of high-CO_2 aspen and birch litter may decline. In contrast, the effects of O_3 on herbivory and decomposition, as mediated by changes in foliar nitrogen, starch, tannins, and C:N ratios, are likely to be negligible, with the exception of cases where O_3 influences plant response to CO_2.

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