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Independent, Interactive, and Species-Specific Responses of Leaf Litter Decomposition to Elevated CO_2 and O_3 in a Northern Hardwood Forest

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Abstract

The future capacity of forest ecosystems to sequester atmospheric carbon is likely to be influenced by CO_2-mediated shifts in nutrient cycling through changes in litter chemistry, and by interactions with pollutants like O_3. We evaluated the independent and interactive effects of elevated CO_2 (560 μl l^{-1}) and O_3 (55 nl l^{-1}) on leaf litter decomposition in trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*) at the Aspen free air CO_2 enrichment (FACE) site (Wisconsin, USA). Fumigation treatments consisted of replicated ambient, $+CO_2$, $+O_3$, and $+CO_2 + O_3$ FACE rings. We followed mass loss and litter chemistry over 23 months, using reciprocally transplanted litterbags to separate substrate quality from environment effects. Aspen decayed more slowly than birch across all treatment conditions, and changes in decomposition dynamics of both species were driven by shifts in substrate quality rather than by fumigation environment. Aspen litter produced under elevated CO_2 decayed more slowly than litter produced under ambient CO_2, and this effect was exacerbated by elevated O_3. Similarly, birch litter produced under elevated CO_2 also decayed more slowly than litter produced under ambient CO_2. In contrast to results for aspen, however, elevated O_3 accelerated birch decay under ambient CO_2, but decelerated decay under enriched CO_2. Changes in decomposition rates (*k*-values) were due to CO_2- and O_3-mediated shifts in litter quality, particularly levels of carbohydrates, nitrogen, and tannins. These results suggest that in early-successional forests of the future, elevated concentrations of CO_2 will likely reduce leaf litter decomposition, although the magnitude of effect will vary among species and in response to interactions with tropospheric O_3.

Key words: *Betula papyrifera*; elevated CO_2; decomposition; elevated O_3; FACE; leaf litter; limit values; litter quality; *Populus tremuloides*.

Introduction

Aspen and birch are important pioneer species, responsible for considerable carbon storage above-

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and belowground, in hardwood forests of the northern conterminous United States and in boreal Canada and Alaska (Burns and Honkala 1990; Hogg and others 2002). Indeed, following stand-replacing disturbance, trembling aspen (*Populus tremuloides* Michaux) and paper birch (*Betula papyrifera* Marsh.) dominate early successional sites in the boreal mixedwoods (Chen and Popadiouk 2002). Leaf litter dynamics of trembling aspen have been the subject of numerous decomposition studies across its enormous geographic range (for example, Van Cleve 1971; Bartos and Debyle 1981; Taylor and others 1989; Bockheim and others 1991; Prescott and others 2004). Numerous studies have also been performed on paper birch decay (for example, Van Cleve 1971; Bockheim and others 1991; Trofymow and others 2002). Few experiments, however, have examined aspen or birch leaf litter production and decomposition under elevated atmospheric CO_2 (Randlett and others 1996; King and others 2001; Parsons and others 2004). More surprisingly, neither production nor decomposition of aspen and birch litter under elevated O_3 concentrations has been investigated thoroughly. Only recently have Liu and others (2005), working at the Aspen free air CO_2 enrichment (FACE) site, characterized the effects of elevated O_3, alone and in combination with CO_2 enrichment, on the chemistry of aspen and birch litterfall.

The Aspen FACE research program was created to explore the independent and interactive effects of elevated CO_2 and O_3 on ecosystem processes, including decomposition, in northern hardwood forests. In a previous study at Aspen FACE (Parsons and others 2004), we followed the decay of paper birch leaves over 12 months, and observed that mass loss (ML) rates were indeed lower for litter generated under elevated vs. ambient CO_2. CO_2 enrichment was the primary driver of direct changes to birch litter quality, as we found that O_3 exposure did not offset or enhance effects of elevated CO_2 on birch litter decay rates, at least in the short-term.

In the current study, our first goal was to compare the decomposition dynamics of trembling aspen with paper birch under the independent and interactive effects of CO_2 enrichment and O_3 exposure. We monitored changes in the mass and chemistry of aspen and birch leaf litter over 23 months. We hypothesized that, consistent with our previous short-term experiments with birch, elevated CO_2 would also reduce the chemical quality of aspen leaves, primarily through reductions in foliar N. Such declines have been observed in a number of species, and are due to dilution effects of accumulated carbohydrates or downregulation of photosynthesis, among other factors (Norby and others 2000). As a consequence of higher C/N and lignin/N, together with higher concentrations of condensed tannins, low-quality aspen and birch litter generated under CO_2 enrichment was predicted, in turn, to decay more slowly than litter produced under ambient CO_2 and O_3. Aspen and birch are shade-intolerant, nutrient-demanding, and fast-growing (Burns and Honkala 1990). Recent evidence suggests, however, that they differ in how carbon is allocated to growth, storage, and defense under ambient and elevated CO_2 (Mattson and others 2005). We sought to determine whether responses of these early successional species also differed in relation to litter decay under ambient and elevated O_3.

Koricheva and others (1998) documented through meta-analysis that O_3 consistently increases phenylpropanoid derivatives (for example, condensed tannins, phenolic glycosides, and lignin), while having little effect on sugar, starch, and N concentrations of woody plant tissues. Yet Findlay and Jones (1990) observed that ozone stress can markedly elevate tissue N beyond that of unexposed leaves. Consequently, the C/N ratio of woody plant foliage can decrease under O_3 exposure, ostensibly increasing its chemical quality at senescence. Nonetheless, O_3 may reduce decay rates of the resulting litterfall (Findlay and Jones 1990). This apparently paradoxical result has been explained by increased polyphenols and lignin, which accumulate in direct response to O_3 stress (Koricheva and others 1998; Peltonen and others 2005) and reduce litter decomposition. Findlay and others (1996) suggested that the latter compounds readily complex with tissue N, thereby reducing its availability to herbivores and decomposers. Therefore, we predicted that O_3 would increase the apparent quality (low C/N) of aspen and birch litter, but simultaneously decrease their respective decay rates because of increased concentrations of secondary metabolites (condensed tannins, lignin). Consequently, elevated CO_2 + O_3 was predicted to depress aspen and birch litter decay rates beyond that predicted for CO_2 alone. This prediction runs counter to other ecosystem-level responses observed at Aspen-FACE, where CO_2 enrichment has been shown to ameliorate short-term O_3 stress (Karnosky and others 2003). More recent studies have demonstrated that chronic O_3 exposure decreases productivity (Karnosky and others 2005), litterfall (*unpublished data*), and soil organic carbon accrual (Loya and others 2003), even in the presence of elevated CO_2.

The second goal of our study was to relate mass loss to litter chemistry. We correlated short-term decomposition rates from the simple negative exponential model (k-values, sensu Olson 1963) with initial substrate quality variables for each species. We performed similar correlation analyses of initial substrate quality with estimates of initial decay rates (k_{init}, Berg and Ekbohm 1991) and asymptotic mass loss (that is, limit values), which were obtained from nonlinear regression. We also used multiple linear regression to explore relationships between mass loss over 23 months and concomitant temporal variation in litter chemical variables.

Methods

Site Description

Our decomposition experiments were conducted at the Aspen FACE (Free-Air Carbon dioxide Enrichment) facility near Rhinelander, Wisconsin, USA. Aspen FACE is the world's largest open-air climate change research facility, and the only FACE site where woody plants are being fumigated with elevated CO_2 and O_3, singly and in combination. Twelve 30-m diameter FACE rings contain aggrading stands of aspen, birch and sugar maple (*Acer saccharum* Marsh.), which have been subjected to ambient CO_2 and O_3 (Controls), elevated CO_2 (''+CO_2''; ambient + 200 μl CO_2 l^{-1}), elevated O_3 (''+O_3''; 1.5 × ambient cumulative exposure), or elevated CO_2 plus O_3 (''+CO_2 + O_3''). Our research was conducted in the southwestern section of each FACE ring, where a single aspen genotype (clone 216) was interplanted with birch (Dickson and others 2000; Karnosky and others 2001).

Litter Bag Deployment and Retrieval

Fiberglass bags (1-mm mesh, 17 cm × 17 cm) were filled with aspen or birch leaf litter (4–6 g oven-dry mass), which had been collected (September and October, 1999) from the aspen-birch communities. Labeled bags (520 per species) were deployed in the FACE rings in early November, 1999, using the same reciprocal transplantation strategy as employed in previous 12-month-long experiments with birch leaf litter (Parsons and others 2004).

Reciprocal transplants were conducted as shown in Table 1, which allowed us to differentiate treatment effects imparted by differences in litter chemical quality (Common Garden) from those imposed by microenvironmental differences among fumigation treatments (Common Substrate). Aspen or birch litter produced in ambient CO_2 and O_3 (that is, Control) plots was placed into the Control, +CO_2, +O_3, and +CO_2 + O_3 plots, forming the Common Substrate experiment. Similarly, aspen or birch litter generated in Control, +CO_2, +O_3, and +CO_2 + O_3 plots was placed into control plots to complete the Common Garden, whereas litter placed back into its plot of origin formed the Native Placement experiment. Ten different treatments resulted from the combinations of plot-of-origin and plot-of-placement (Table 1).

Table 1. Design of Reciprocal Litterbag Transplant Experiments

Experiment	Plot of litter origin	Plot of litter placement
Common Substrate	Control	Control
	Control	+CO_2
	Control	+O_3
	Control	+CO_2 + O_3
Common Garden	Control	Control
	+CO_2	Control
	+O_3	Control
	+CO_2 + O_3	Control
Native Placement	Control	Control
	+CO_2	+CO_2
	+O_3	+O_3
	+CO_2 + O_3	+CO_2 + O_3

Note: Sufficient numbers of litter-filled 2-mm mesh bags were deployed for each set of experiments to permit collection of three litter samples per combination of species (2), fumigation treatment (10), replicate ring (3), and retrieval date (6). A single ''control'' treatment serviced all three experiments, so the total number of placement studies conducted was 10 rather than the 12 shown above.

Litterbags were collected at 30, 187, 281, 369, 523, and 698 days. The litter was flash-frozen in liquid N_2 and freeze-dried within 2 days of retrieval. After cleaning the bags of loose debris, we estimated litter mass in the presence of mineral soil contaminants (difficult to remove from decaying leaves) using the equations of Blair (1988). Lyophilized and cleaned materials were ground to pass a 1-mm mesh screen and converted to an ash-free basis, following loss-on-ignition (LOI, 550°C for 6–12 h), and correction for soil contamination. Similar corrections were made to N, condensed tannin, carbohydrate, fiber, and lignin concentrations (mg g^{-1}), following analytical protocols described by Parsons and others (2004).

Soil contamination consisted of two components: (1) soil material that could not be easily dislodged from the litter and for which we estimated remaining litter mass and its constituent chemical fractions by LOI and (2) debris that loosely adhered to the bags and litter, and that was not included in the aforementioned calculations. This latter fraction typically consisted of casts excreted by large lumbricid worms (unidentified species). We sub-

tracted the mass of this fine mineral soil from total litterbag mass prior to ashing, and used it to calculate ''Wormcast'' (as a percentage of total uncleaned litter mass), a crude index of earthworm presence and activity.

Litter nitrogen was measured by thermoconductometric detection following high temperature combustion (LECO, St. Joseph, MI, USA). Glycine *p*-toluenesulfonic acid served as the standard. Soluble sugars and starch were quantified colorimetrically as glucose equivalents, using a modified dinitrosalicylic acid assay (Lindroth and others 2002). Soluble condensed tannins in aspen and birch were determined colorimetrically by the acid-butanol method (Porter and others 1986), following extraction in 70% acetone (with 1 mM ascorbic acid). Tannins purified from aspen and birch leaves via adsorption chromatography served as standards. Bound condensed tannins were measured by the same method as that used for soluble tannins, except that exhaustively extracted tissues, rather than extracts, were subjected to hot acid hydrolysis. Phenolic glycosides in aspen (salicortin, tremulacin) were quantified by high performance thin-layer chromatography, as described by Lindroth and others (1993). Phenolic glycosides purified from aspen tissues via flash-chromatography were used as standards. Fiber and (klason) lignin were estimated gravimetrically following sequential extraction in hot acid-detergent solution (100°C for 1 h, Ankom 200 Fiber Analyzer, ANKOM Technology, Macedon, NY, USA) and incubation in 72% H_2SO_4 (3 h).

Statistical Analyses

Mass Loss and Litter Chemistry. Mass loss and litter chemistry data from Common Substrate, Common Garden, and Native Placement experiments were analyzed separately for the two species. The ANOVA model was a completely randomized block layout with three-crossed factors, including CO_2 (ambient vs. elevated), O_3 (ambient vs. elevated), and Time (six removal dates), with three replicate rings (blocks) per treatment. Main effects and interactions were tested at $P = 0.10$, followed by 1 degree-of-freedom (*df*) Scheffé contrasts. Consistent with our previous study (Parsons and others 2004) and the recommendations of Filion and others (2000), we adopted an α-value of 0.10, thereby reducing the risk of committing a Type-II error due to the low degree of replication inherent in FACE experiments. Analyses were performed in SAS (Proc Mixed, Restricted Maximum Likelihood, Littell and others 1996), with block and block by treatment interactions considered as random effects.

We further assessed temporal consistency of treatment means for mass loss at each removal, together with litter quality, using Kendall's coefficient of concordance (*W*, Parsons and others 2004). *W* ranges from 0 (no agreement) to 1 (perfect agreement), and measured the consistency with which ranks of treatment means for a given variable were ordered over time.

Litter Decay Rates. Litter decay rates (k) for aspen and birch leaves were estimated using the linear form of Olson's (1963) equation: $\ln M_t = \ln M_0 - kt$, where $\ln M_t$ and $\ln M_0$ are natural logarithms of remaining litter mass (M, %) at times t and 0 (years), respectively, and k is the regression slope coefficient (year^{-1}). We extended the regression model to ANCOVA by including coding for fixed effects of the 10 treatments (Table 1) and a treatment-by-time interaction term. The mass loss data for Common Substrate, Common Garden, and Native Placement experiments were combined in a separate ANCOVA for

Table 2. ANCOVA Summary of Ln Mass-Remaining (%) Over Time for Aspen and Birch Litter

Source	Aspen			Birch	
	df	MS	*P*	MS	*P*
Treatment	9	0.007	0.891	0.009	0.892
Treatment × Time	9	0.070	<0.001	0.050	0.006
Treatment × Wormcast	9	0.052	<0.001	0.033	0.085
Treat × Wormcast × Time	9	0.056	<0.001	0.047	0.011
Time × Wormcast	1	0.028	0.152	0.004	0.648
Time (covariate)	1	13.926	<0.001	26.586	<0.001
Wormcast (covariate)	1	0.141	0.001	0.006	0.591
Error	480	0.014		0.019	

Differences in environment (Common Substrate), substrate quality (Common Garden), and their interaction (Native Placement) are subsumed under Treatment (=10 levels), the effects of which have been adjusted for the covariates Time (years) and Wormcast (%).

each species (Table 2), which was followed by a set of directed contrasts for each experiment. These 1-*df* contrasts tested the main effects of CO_2 and O_3, and the $CO_2 \times O_3$ interaction. A final contrast, that of additivity effects, determined whether the sum of the individual trace gas responses (that is, $+CO_2$ plus $+O_3$) equaled that of the combined gases ($+CO_2 + O_3$) alone.

Comparisons within the Common Substrate experiment tested whether decay coefficients of aspen or birch litter that was produced under Control conditions (ambient CO_2, ambient O_3) were altered by different environments created in the $+CO_2$, $+O_3$, and $+CO_2 + O_3$ plots. Comparisons within the Common Garden experiment determined whether decay coefficients of litter in a common environment were altered by differences in litter chemistry among the four fumigation treatments (Control, $+CO_2$, $+O_3$, $+CO_2 + O_3$). Finally, comparisons within the Native Placement experiment determined whether differences in environments among fumigation treatments obscured or reinforced differences in substrate quality produced in each of those environments. Analyses were conducted in JMP and Statview 5 (SAS Institute, Inc., Cary, NC, USA).

We focused on *k*-values estimated from the single exponential decay model because they offered a familiar framework in which treatments, species, and locations could be compared, and against which predictions could be made, based on substrate quality variables (for example, Taylor and others 1989; Trofymow and others 2002; Valachovic and others 2004). Although these estimates may be useful for assessing litter decay dynamics over the shortterm (or more explicitly, for the first phase of decomposition), decay rates have been observed to decline with time (Howard and Howard 1974; Berg and Ekbohm 1991; Berg and others 1996; Latter and others 1998). Thus, the decomposing litter does not completely disappear; rather, cumulative mass loss (in %) reaches an asymptote, which Berg (2000) described as its limit value (denoted *m*). At this point, litter has been modified into humus and has entered a second phase of decomposition, which is extremely slow (Prescott 2005). We fitted ML against time (*t*, in days) to the nonlinear function: $ML = m^*(1 - e^{-Bt})$, where $B = k_{init}/m$, and k_{init} is the initial decomposition rate (% day^{-1}). The two parameters, *m* and B, were estimated using Prism (version 4, GraphPad, San Diego, CA, USA), under the constraint that *m* is less than 100%.

Predicting Mass Loss from Litter Chemistry. We performed correlation analyses for both species, relating estimates of the decomposition coefficients (*k* and k_{init}) to initial litter chemistry variables. From the estimates of k_{init} and *m*, we estimated limit values for litter N in each fumigation treatment. Limit values were calculated as: $N_{limit} = N_{initial} + (k_{init} * m)$, where $N_{initial}$ was the initial concentration of N in aspen and birch litterfall for each treatment, and k_{init} and *m* were defined as before. Also, multiple regression was performed on mass loss (%), against N, condensed tannins, total nonstructural carbohydrates (TNC = soluble sugars + starch), fiber, lignin, lignin/N, C/N, and Wormcast. Categorical independent variables were added, including leaf color and size. Color classes have been used previously to characterize litter that differs in its chemistry and rates of annual mass loss (Taylor and Parkinson 1988; Cornelissen and others 2000). We constructed color categories (black, green, yellow, multi-hued and mottled, yellow with dark necroses) for aspen that were assigned consecutive ratings (1–5), and that were indicative of damage incurred by frost-kill and premature leaf-drop, and to the prevalence of leaf pathogens such as *Melampsora* (Karnosky and others 2002), all of which could potentially influence decay rates. Five size classes (<2 cm-wide leaf blades; 2–4 cm; 4–8 cm; 8–12 cm; >12 cm), also rated from 1 to 5, reflected transitions from the indeterminate to determinate growth form of aspen leaves, together with potential growth-retarding effects of O_3 and growth-promoting effects of enriched CO_2. We created five birch color classes, also indicating premature leaf drop and O_3 stress.

Data were pooled across reciprocal transplant experiments and FACE treatments, assuming that variation in both the dependent and the independent variables reflected differences among the fumigation treatments, the coding for which was not explicitly incorporated into the models. Stepwise regression model development proceeded with backward elimination of litter quality variables (JMP, SAS Institute, Inc.).

Results

Overall, results from the Common Substrate Study showed few if any effects of fumigation treatments. In contrast, results from the Common Garden Study revealed numerous and significant CO_2 and O_3 effects, which were largely mirrored in the Native Placement Study. In short, our results showed that CO_2- and O_3-mediated changes in decomposition dynamics were dominated by changes in substrate quality rather than changes in fumigation environment. Thus, to streamline and

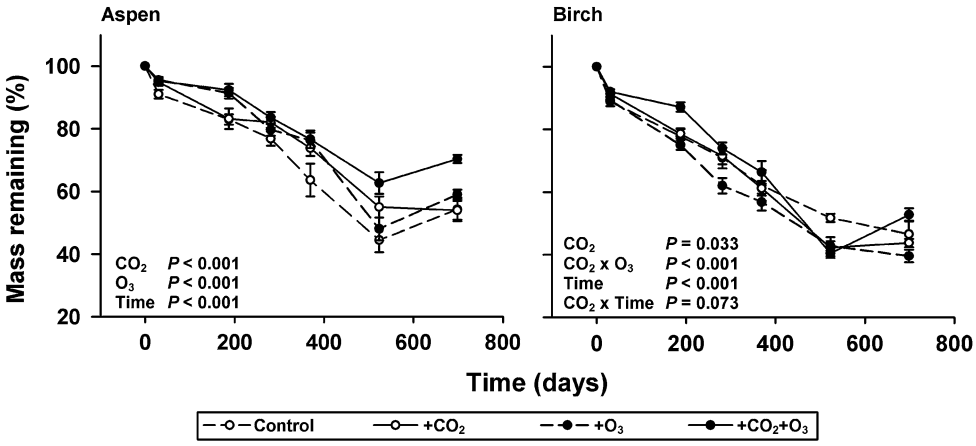

Figure 1. Mass remaining of aspen and birch litter over 698 days in the field for the Native Placement experiment. ANOVA summaries are provided for significant treatment effects on litter mass loss through time.

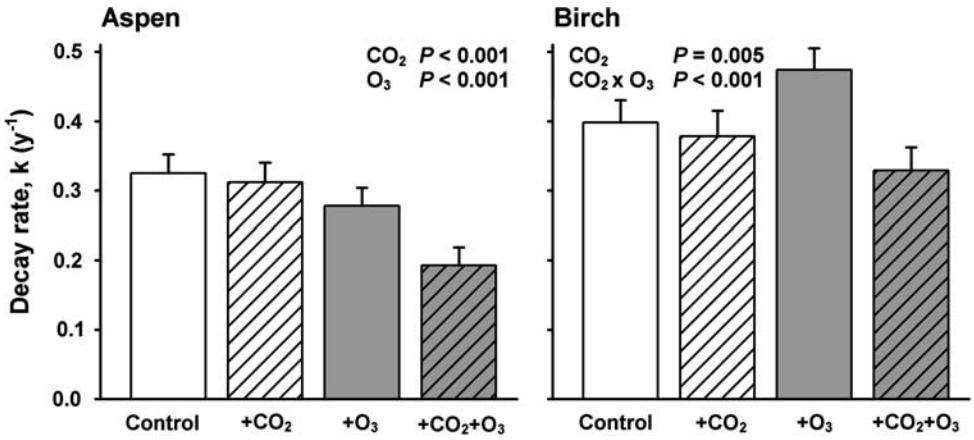

Figure 2. Litter decay coefficients (k, year^{-1}), and their associated standard errors, as estimated for aspen and birch from ANCOVA, including fumigation treatments (main effects), and time (years) and Wormcast (see text) as covariates. Hatched and gray bars denote responses to elevated CO_2 and elevated O_3 treatments, respectively, for the Native Placement experiment. Significance tests of the slope coefficients (k-values) are based on 1-df contrasts for CO_2 and O_3 main effects and the CO_2 by O_3 interaction (see text).

facilitate presentation of major results, we focus here on the Native Placement Study.

Litter mass decreased strongly over the two-year decay period ($P < 0.001$) and more steeply in birch than in aspen (Figure 1). Mass losses responded to CO_2 and O_3 treatments in both tree species. In aspen, CO_2 and O_3 reduced decay rates, such that litter from the Control and $+CO_2 + O_3$ plots lost the most and least mass, respectively. Treatment order (highest to lowest mass loss) was quite consistent over time (Kendall's $W = 0.756$, $P = 0.003$). In birch, CO_2 also decreased decay rates. O_3, however, tended to accelerate mass loss under ambient CO_2, but to reduce mass loss under elevated CO_2 (significant $CO_2 \times O_3$ interaction). The consistency of treatment rankings for birch was weak (Kendall's $W = 0.400$, $P = 0.066$).

Litter Decay Rates

Regression of mass-remaining values (ln-transformed) against time provided significant model fits for both tree species ($R^2 = 0.64$ and 0.74 for aspen and birch, respectively; Table 2). Under ANCOVA, the single exponential decay functions estimated for the ten treatments had a common Y-intercept (Table 2, Treatment, $P > 0.89$) for aspen ($M_0 = 95\%$) and birch ($M_0 = 97\%$). Consequently, mass losses attributable to physical leaching following bag deployment were small (that is, 5% for aspen; 3%, birch), consistent with expectation.

Mass losses that were attributed to earthworm activity were large in comparison to leaching, especially for aspen; the addition of Wormcast improved explained variation in the model by 18% (that is,

Table 3. Initial k-Values (k_{init}) and Limits to Litter Mass Loss Estimated from Nonlinear Regression of Mass Loss (ML) Against Time (Days)

	Aspen					Birch				
Treatment	B (day^{-1})	k_{init} (% day^{-1})	m (%)	$t_{0.95}$ (days)	N_{limit} (mg N g^{-1})	B (day^{-1})	k_{init} (% day^{-1})	m (%)	$t_{0.95}$ (days)	N_{limit} (mg N g^{-1})
Control	0.00185 (0.00071)	0.130	70.5 (16.5)	1620	25.7	0.00219 (0.00058)	0.152	69.4 (10.4)	1368	24.0
$+CO_2$	0.00091 (0.00209)	0.091	100.00 (51.1)	3292	21.2	0.00166 (0.00042)	0.146	88.2 (14.7)	1804	23.4
$+O_3$	0.00118 (0.00065)	0.096	81.6 (32.2)	2538	24.4	0.00246 (0.00036)	0.184	75.1 (5.7)	1218	28.8
$+CO_2 + O_3$	0.00168 (0.00075)	0.081	48.2 (13.5)	1783	15.7	0.00158 (0.00054)	0.128	81.2 (18.3)	1896	21.5

Initial k-values were calculated from estimates of B (=k_{init}/m) and m. The time required to reach 95% of the limit value, that is, maximum asymptotic mass loss (m), is $t_{0.95}$. N_{limit} is the N concentration of the litter at m. Standard errors are shown in parentheses.

R^2 = 0.82) for aspen and 7% (R^2 = 0.81) for birch. Earthworm activity in the aspen litterbags, however, varied strongly with treatment (Treatment × Wormcast; Table 2) and over time (Time × Wormcast, Treatment × Wormcast × Time; Table 2). Differences among slope coefficients estimated for the Wormcast interactions (results not shown) suggested that earthworm activity in aspen litter was highest in Controls and lowest in the $+O_3$ plots, and that their effects were more pronounced in late vs. early decay. Earthworms similarly influenced birch litter decay: annelid activity was highest in the Controls but lowest under $+CO_2 + O_3$ during the latter stages of the experiments (Treatment × Wormcast × Time; Table 2).

Aspen litter decayed more slowly than birch litter across all treatments (Wilcoxon signed-rank test, P = 0.005; Figure 2). The tree species also differed with respect to the effects of CO_2 and O_3 on decay coefficients. Again, we focus on the Native Placement Study, as the effects of fumigation environment on decay were negligible (Common Substrate Study), and the effects of substrate quality were largely similar between the Common Garden and Native Placement experiments.

For aspen litter returned to its site of origin (Native Placement), decay rates (k-values) declined under both $+CO_2$ and $+O_3$ treatments (Figure 2). Litter from the $+CO_2 + O_3$ plots had the lowest mass loss rates among the four source litters deployed (59% of Control). The rate-retarding effects of $+CO_2$ and $+O_3$ were synergistic, as the 41% reduction in aspen decay incurred by $+CO_2 + O_3$ was greater (Additivity contrast, $P < 0.001$) than the cumulative reduction of 18% for $+CO_2$ (96% of Control) and $+O_3$ (86% of Control). Birch litter decay rates also declined under elevated CO_2 treatment (Figure 2). O_3 treatment accelerated birch decay under ambient CO_2, but decelerated decay under enriched CO_2 ($CO_2 \times O_3$ interaction). Thus, the combined effects of $+CO_2$ and $+O_3$ were not additive for birch (Additivity contrast, $P < 0.001$).

Nonlinear regression of mass loss against time-produced estimates of k_{init} (Table 3) that were consistent with the trends in k-values estimated under simple exponential decay. Like k, values of k_{init} were lower for aspen than for birch, and among the treatments, the rate of decomposition was slowest under $+CO_2 + O_3$ (Table 3). Estimates of maximum cumulative mass loss (m), or limit values, ranged from 48% ($+CO_2 + O_3$) to 100% ($+CO_2$) for aspen, compared to 69% (Control) to 88% ($+CO_2$) for birch. Interestingly, litter decomposition was most complete for CO_2-enriched aspen (that is, 100% mass loss), but this $+CO_2$ litter also took the longest

to disappear (3292 days to reach 95% loss) among all treatments for both species (Table 3). Although the percentage of variance explained by regression ranged from 65 to 72% among the four fumigation treatments, it should be noted that several of the standard errors associated with the parameter estimates for aspen were quite large (Table 3). Consequently, the aspen results should be interpreted with caution. Nonlinear regression explained about 75–88% of the variation associated with cumulative birch mass loss. Among the birch treatments, litter that had been enriched with CO_2, either under ambient or elevated O_3, took longer to disappear than Control or $+O_3$ litter (Table 3).

Litter Chemistry

Several general trends characterized the chemistry of decaying aspen and birch leaves. First, consistent with expectation, substrate quality varied markedly over time ($P < 0.001$). Many metabolites strongly decreased (condensed tannins, soluble sugars, phenolic glycosides) or steadily increased (acid-detergent fiber, klason lignin) throughout the period of decomposition. In contrast, other constituents, such as starch and N, attained their highest and lowest concentrations during the course of the experiments, rather than at the beginning or end. Second, even as they varied over time, concentrations of most litter constituents differed significantly between species. Third, with few exceptions (that is, soluble sugars, phenolic glycosides, lignin), temporal changes in aspen and birch litter chemistry were accompanied by significant differences in substrate quality initially imparted by $+CO_2$, $+O_3$, and $+CO_2 + O_3$ fumigation. Lastly, treatment means maintained a consistent rank-order over sampling dates, despite strong interactions between time, CO_2, and O_3 effects. This pattern was especially true for tissue nitrogen and its derivatives, C/N and lignin/N (see below).

Soluble sugars, soluble and bound condensed tannins, and phenolic glycosides (aspen only) exhibited their greatest concentrations, and the strongest differences among treatments, at the outset of leaf decay (Figure 3). In aspen, soluble sugars increased slightly in the elevated O_3 treatments, whereas in birch, sugars increased slightly in the elevated CO_2 treatments (Figure 3). Concentrations of soluble sugars were consistently lower in aspen compared with birch (Wilcoxon tests, $P = 0.005$), until completely lost from decaying litter at 187 days. Soluble and bound fractions of condensed tannins responded positively to CO_2 enrichment in both aspen and birch leaves (Figure 3). Consistent

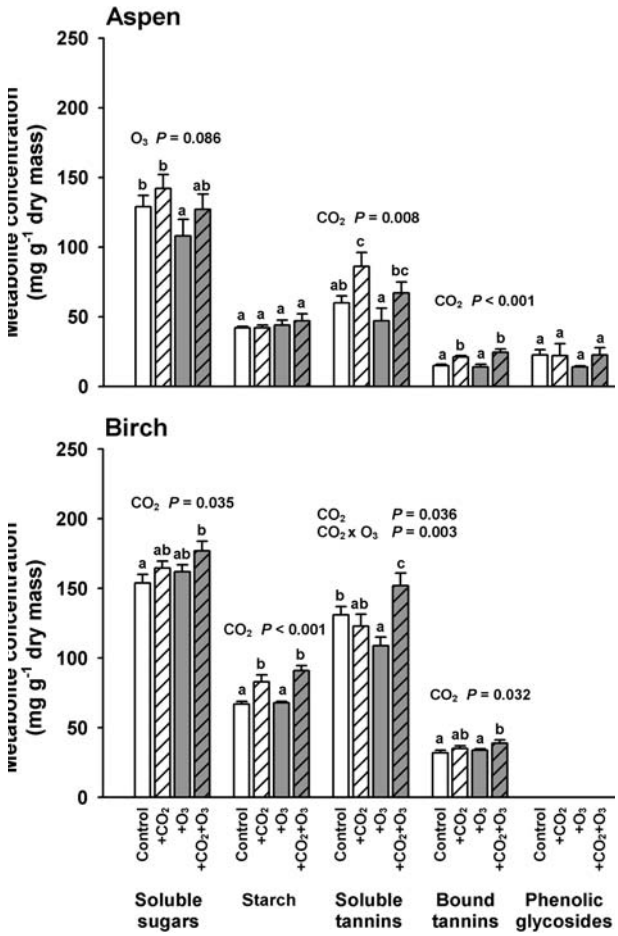

Figure 3. Mean initial chemical composition of aspen and birch litter. Standard errors (±1 SE) are calculated from three replicate rings per treatment. Means with the same letters do not differ significantly at $P = 0.05$. Phenolic glycosides occur in aspen only.

differences among treatments were maintained through time for total tannins (soluble + bound) in birch litter (Kendall's $W = 0.822$, $P = 0.060$; treatment order: $+CO_2 + O_3 \geq +CO_2 > \text{Control} > +O_3$), but not in aspen ($P > 0.45$). With respect to species differences, aspen litter contained about half as much total tannin as did birch, and bound tannins initially constituted about 22% of the total tannin pools in both species. Concentrations of phenolic glycosides did not differ among treatments (Figure 3) and these compounds disappeared within a couple of weeks.

Fiber concentrations (results not shown) were significantly altered by elevated O_3 in both aspen ($P = 0.033$) and birch ($P = 0.009$) leaves. Aspen litter from the $+O_3$ plots initially had the highest concentrations (462 ± 32 mg fiber g^{-1}; 107% of Control) among the treatments, but these differences were not maintained during decay

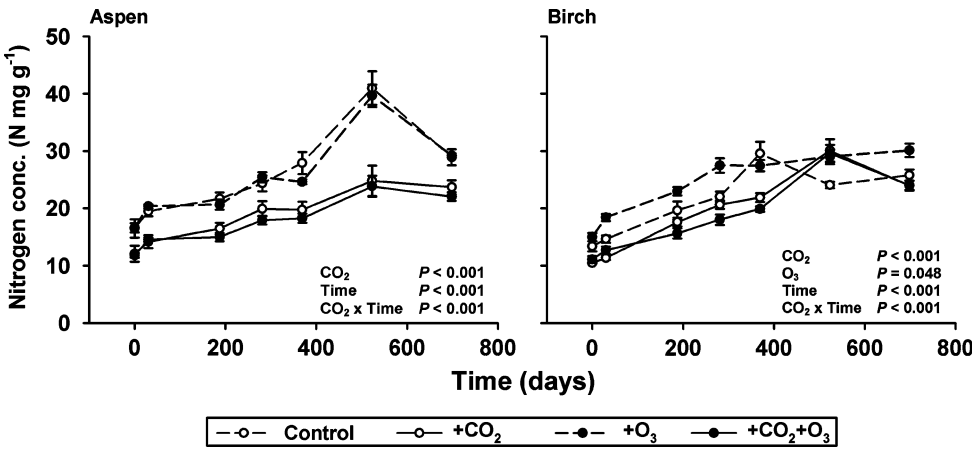

Figure 4. Dynamics of N in aspen and birch litter from the Native Placement experiment. Significant effects from the corresponding ANOVAs are shown.

($P = 0.079$). In contrast, fiber in birch litterfall was lowest in the $+O_3$ plots (308 ± 5 mg g^{-1}; 90% of Control), and this effect was maintained throughout the period of decomposition (Kendall's $W = 0.633$, $P = 0.010$; treatment order: $+CO_2$ > Control = $+CO_2 + O_3 > +O_3$). Differences in fiber concentration between species were maintained throughout litter decay (aspen > birch, Wilcoxon tests, P-values ≤ 0.047). By final bag removal, fiber in aspen and birch litter was 163 ± 3% and 220 ± 3% of initial values, respectively.

Surprisingly, elevated CO_2 and O_3 conditions did not influence lignin concentrations in the source litters ($P > 0.10$), a result underscored by the lack of consistent treatment rank-order over time (Kendall's W, $P > 0.20$). Like fiber, lignin did not vary systematically among treatments when the species were compared through time ($P > 0.15$). Also like fiber, lignin concentrations differed between species (aspen > birch, Wilcoxon tests, P-values ≤ 0.009), and followed a time course such that concentrations almost doubled in aspen (183 ± 4% of initial values) and more than doubled in birch (247 ± 14% of initial values) by 698 days (results not shown).

Concentrations of starch in aspen litter did not respond to $+CO_2$ or $+O_3$, whereas levels in birch litter increased 28% in response to $+CO_2$ (Figure 3). For both species, starch was at its maximum 187 days following litterbag deployment (mean ± SE % of initial levels: aspen, 134 ± 1.5%; birch, 120 ± 5%) and declined thereafter, converging on assay detection limits (6 mg glucose g^{-1}) by 523 days (results not shown). Tissue starch was lower in aspen compared to birch at deployment (Figure 3), a difference that also was maintained during litter decay (Wilcoxon tests, $P < 0.01$).

Patterns of N accumulation and release differed among fumigation treatments, between species, and over time. At deployment, N concentrations were lower under elevated vs. ambient CO_2 for both aspen ($P = 0.016$; 38% initial difference) and birch ($P < 0.001$; 32% initial difference) litter (Figure 4). In addition, N levels in birch litter were slightly increased under elevated O_3 ($P = 0.044$). Tissue N was higher in aspen than in birch litter, initially (Wilcoxon test, $P = 0.004$) and at the first litterbag sampling ($P = 0.005$), but this difference gradually diminished as decomposition progressed.

Differences in tissue N among the four fumigation treatments were strongly maintained during the decay process for aspen (Kendall's $W = 0.856$, $P = 0.001$), but weakly for birch (Kendall's $W = 0.411$, $P = 0.060$; Figure 4). Aspen litter continued to immobilize N for 523 days, and more so in ambient- than in CO_2-enriched treatments (Figure 4.). In birch, immobilization continued through 369 days for control litter, through 523 days for $+CO_2$ and $+CO_2 + O_3$ litter, and through the end of the study for $+O_3$ litter.

Following calculation of the limit values (m) for litter decomposition under the four fumigation treatments (Native Placement experiment), we determined the limits to nitrogen stored in the remaining residues (N_{limit}; Table 3). Nitrogen was initially higher in the foliar litterfall of aspen than of birch, but more N was eventually stored at the limit values for birch leaves (mean ± SE increase among the treatments = 196.7 ± 18.5% of initial N) compared with those of aspen (152.8 ± 17.5% of initial N). Differences in the N_{limit} among the fumigation treatments, however, were greater in aspen than in birch, with the lowest stabilized N concentrations in aspen litter from the $+CO_2 + O_3$ treatment, and the highest in aspen Control litter (Table 3).

Carbon concentrations did not differ among fumigation treatments for either species (results not shown). C/N ratios thus mirrored differences in N, both among source litters and over time. Treatment order was consistent over time for both aspen

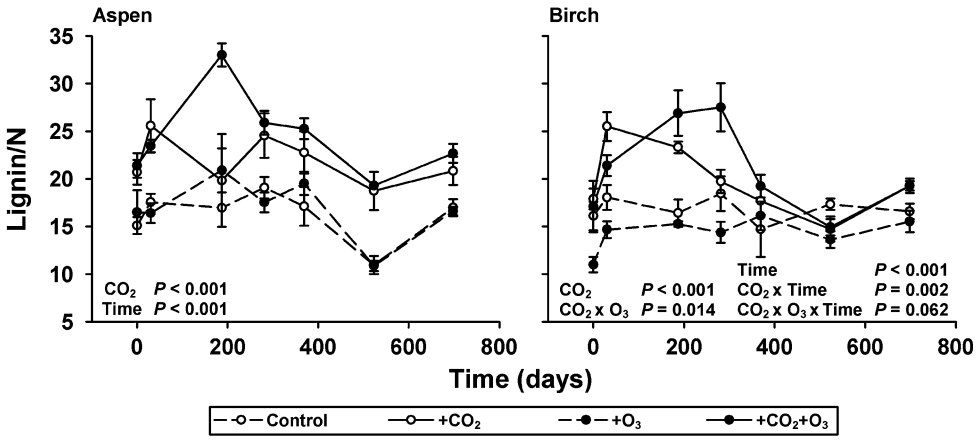

Figure 5. Dynamics of lignin/N ratios in aspen and birch litter from the Native Placement experiment. Significant effects from the corresponding ANOVAs are shown.

(Kendall's $W = 0.866$, $P = 0.001$; $+CO_2 + O_3 > +CO_2 > +O_3 >$ Control) and birch (Kendall's $W = 0.656$, $P = 0.008$; $+CO_2 + O_3 > +CO_2 > >$ Control $> +O_3$). Even as it decreased over time, C/N remained higher in aspen than in birch as the litter decayed (Wilcoxon tests, $P = 0.002$). This was despite the fact that initial C/N ratios were significantly higher among the ten treatments (Table 1) in birch than aspen (Wilcoxon test, $P = 0.005$).

Lignin/N ratios, like C/N ratios, reflected differences in N dynamics among source litters and exhibited complex dynamics over time (Figure 5). In aspen, lignin/N ratios were generally higher in enriched CO_2 litter than in ambient litter (Kendall's $W = 0.574$, $P = 0.016$). In birch, treatment effects on lignin/N ratios were of smaller magnitude (Kendall's $W = 0.700$, $P = 0.006$), but more interactive, than in aspen. Over the first year of decay, O_3 tended to increase lignin/N ratios of ambient-CO_2 litter, but not of elevated CO_2 litter. From 523 days onward, however, no treatment effects were distinguishable. Aspen litter was of poorer quality than birch litter for much of the study (aspen lignin/N > birch lignin/N, Wilcoxon tests, $P < 0.02$).

Predicting Mass Loss from Litter Chemistry

Simple correlation analyses of both the limit values (m) and decomposition coefficients (k and k_{init}) revealed few significant relationships with initial litter chemistry. Estimates of m were not correlated with the chemical properties of either species. Interestingly, the time to reach 95% m ($t_{0.95}$) for birch litter was correlated with initial C/N ($r = 0.689$, $P = 0.028$), initial N ($r = -0.676$, $P = 0.032$), and TNC ($r = 0.650$, $P = 0.042$), but only weakly with lignin via lignin/N ($r = 0.620$, $P = 0.056$). For birch, the decay rates were negatively correlated with initial C/N ($r = -0.770$, $P = 0.004$) and initial lignin/N ($r = -0.760$, $P = 0.005$), whereas k_{init} was also negatively correlated with condensed tannins ($r = -0.726$, $P = 0.015$) and positively correlated with initial N concentrations ($r = 0.778$, $P = 0.006$). For aspen, k- and k_{init}-values were mainly uncorrelated with indices of initial substrate quality (P-values > 0.10), except for a moderate relationship with lignin (k_{init} vs. initial lignin: $r = -0.602$, $P = 0.065$).

Multiple regression (Table 4) explained 80 +% of the variation in mass loss (comparable to ANCOVA, Table 2). Equations for aspen and birch included coefficients with the same signs for N, total condensed tannins, and TNC (soluble sugars + starch). These variables were the only ones required by stepwise, backward elimination to predict mass loss in birch.

The complete prediction equation for aspen included Damage, Size, and Wormcast. As determined from absolute values of the standardized regression coefficients (Table 4, Betas), TNC had the most influence in predicting both aspen (−0.959) and birch (−0.913) mass loss. (Standardized b coefficients essentially measure the correlation between residuals from the regression and the variable of interest, if that variable had been omitted from the analysis.) We calculated TNC to extend the record of soluble sugars and starch over the course of the study, but TNC also showed much stronger ordering of treatment means than did starch alone. Nitrogen and tannin concentrations had moderate impacts on mass loss in both aspen and birch. The remaining variables (Wormcast, Damage, and Size) had weak effects on aspen mass loss.

Discussion

Our experiments suggest that decomposition dynamics in northern ecosystems dominated by

Table 4. Results from Multiple Regressions of Mass Loss (%) vs. Substrate Quality Variables for Aspen and Birch Litter Decaying over 698 Days

Variable	Aspen				Birch			
	b	SE	*P*-value	Beta	*b*	SE	*P*-value	Beta
Intercept	5.912	2.609	0.007	–	23.065	2.605	<0.001	–
N	0.885	0.085	<0.001	0.378	1.127	0.095	<0.001	0.373
Tannins	0.379	0.071	<0.001	0.448	0.155	0.030	<0.001	0.342
TNC	−0.346	0.031	<0.001	−0.959	−0.217	0.016	<0.001	−0.913
Wormcast	0.398	0.087	<0.001	0.135				
Damage	1.452	0.319	<0.001	0.104				
Size	1.208	0.484	0.003	0.057				
Model	R^2_{adj} = 0.808		<0.001	*n* = 412	R^2_{adj} = 0.819		<0.001	*n* = 431

Common Substrate, Common Garden, and Native Placement experiments have been combined for each species. P-values are given for the whole model fit, and for t-tests on individual regression coefficients (b) and their associated standard errors (SE). Standardized coefficients (Betas) are included for each model. Only variables that differ significantly from zero are included in each model.

trembling aspen and paper birch will change under atmospheric conditions predicted for the future. Our results further suggest that the processes of litter accumulation and carbon transfer to the mineral soil beneath will decrease via both direct and indirect effects (*sensu* Dukes and Hungate 2002) of rising atmospheric CO_2 and O_3, and mediated by species-specific mechanisms. These conclusions are supported by the confirmation, particularly in aspen, of our first prediction: that CO_2 enrichment would reduce foliar chemical quality and litter decay. Indeed, high CO_2 produced poorer quality aspen litter (high C/N, lignin/N, and condensed tannins) than did ambient CO_2 (low C/N, lignin/N, and condensed tannins), which resulted in lower decay rates (both k and k_{init}). This pattern was consistent between this current 23-month-long experiment and a previous 12-month-long experiment with aspen at Aspen FACE (*unpublished data*). Moreover, our FACE results lent only weak support to the notion advanced by Berg and coworkers (for example, Berg and others 1996) that low C/N litter (that is, of high initial quality) will have low limit values (leave behind large amounts of residue), and vice versa. Aspen litter had lower initial C/N ratios than birch, but took significantly longer than birch to reach its limit values (Wilcoxon test, $P = 0.013$). Higher apparent initial quality, but slower observed decomposition of aspen litter relative to that of birch was consistent with observations made by Giardina and others (2001) regarding the dynamics of aspen versus pine litter. Decreases in aspen litter quality, which were attributed to increased C/N, were reinforced by high tannin levels in the CO_2-enriched litterfall. CO_2 enrichment of birch leaves also resulted in high C/N and lignin/N ratios, but minimal increases in tannin concentrations, under ambient O_3. CO_2 had a greater effect on these litter quality variables, however, under elevated O_3. Thus, CO_2 more strongly reduced decomposition under elevated, versus ambient, O_3.

This study documents a stronger impact of CO_2 on litter decomposition, especially for aspen, than has been reported in other FACE studies with trees. Enriched CO_2 had only a small inhibitory effect on decomposition of litter from three *Populus* species at POPFACE (Cotrufo and others 2005). Decomposition of litter from loblolly pine and four deciduous species was unaffected by CO_2 treatment at the Duke Forest FACE site (Finzi and others 2001; Finzi and Schlesinger 2002). Differences among studies such as these emphasize the fact that effects of elevated atmospheric CO_2 on litter decay are likely to be species-, and perhaps ecosystem-, specific.

Support for our second prediction, that elevated O_3 would increase quality (low C/N) of litter while reducing its decay rate, differed between the two tree species. In aspen, O_3 did not alter C/N beyond that of the Control treatment (contrary to prediction), but it decreased decay rates nonetheless (consistent with prediction). Reduced decay rates for O_3-fumigated leaves are often attributed to accumulation of phenylpropanoid derivatives (that is, tannins or lignin) in response to chronic O_3 stress (Koricheva and others 1998; Peltonen and others 2005). In our study, however, reduced decomposition occurred in spite of lower tannin concentrations. Our findings are consistent with the initial predictions, but not the results, of work by Liu and others (2005) at the same FACE site. Liu and others posited that phytoxic effects of elevated O_3 would decrease leaf carbohydrate supplies, and in turn lower secondary metabolite concentrations.

They found, however, that O_3-fumigated aspen actually had higher soluble sugar and tannin concentrations. Why our results differ from theirs remains unclear. Evidence from other decomposition experiments suggests that O_3 fumigation causes irreparable biochemical damage to leaves, leading to increased refractory content of the senesced leaf material (Findlay and Jones 1990; Findlay and others 1996). Although lignin was indeed higher in aspen litterfall from $+O_3$ plots (270 ± 34 mg g^{-1}) vs. the controls (246 ± 11 mg g^{-1}), this difference was not significant. However, klason lignin is not a well-defined fraction, and may include various acid-insoluble residues, including cutins and waxes. Thus, the phytotoxic effects of O_3 exposure explain some of the observed changes in aspen litter chemistry, and may offer yet additional reasons as to why $+O_3$ and $+CO_2 + O_3$ litter decayed more slowly than that of the Control, as will be described below.

Results for birch also only partially supported our second prediction, but in a manner opposite to that for aspen. Apparent quality of birch litter increased under elevated O_3 with reduction of its C/N ratio (consistent with prediction). But these reductions resulted in faster rather than slower decay (contrary to prediction). Levels of both condensed tannins and lignin decreased in the $+O_3$ birch litter (contrary to prediction), and likely contributed to its accelerated decay (again, reflected in both k and k_{init} estimates).

With respect to our third prediction, $+CO_2 + O_3$ was expected to reduce decay rates of aspen and birch litter below those anticipated for $+CO_2$ alone. Again, we found support for this prediction from our aspen, but not our birch, data. CO_2 enrichment reduced initial aspen litter quality through reductions in foliar N, regardless of O_3 level. Condensed tannins increased with CO_2 enrichment of aspen litter, but additional increases in concentration were not achieved under fumigation with $+O_3$ or $+CO_2 + O_3$. O_3 fumigation clearly exacerbated the rate-retarding effects of elevated CO_2 on aspen, but we cannot easily provide a mechanism relating mass loss to litter chemistry, by which $+CO_2 + O_3$ dramatically decreased k or k_{init}, relative to those of Control and $+CO_2$ aspen litters.

Differences in aspen decomposition rates among $+CO_2$, $+O_3$, and $+CO_2 + O_3$ treatments may have originated from chemical and physical attributes that we did not measure. Aspen leaves are thicker, tougher, and waxier than birch leaves, which likely accounted for the faster decomposition of the latter species. Aspen leaves were thus less likely to crack or physically fragment, while the thick wax layers coating their epidermal cells were more likely to resist hyphal colonization and penetration by pathogenic or saprophytic fungi. Karnosky and others (1999, 2002) found that O_3 fumigation, alone or in combination with enriched CO_2, increased leaf wettability and, therefore, its susceptibility to fungal attack, possibly by altering the crystalline structure and composition of these surface waxes. At the same time, paradoxically, chronic O_3 stress was found to significantly increase surface wax deposits (Karnosky and others 2002), and therefore, we speculate that the extra ''armoring'' afforded by the waxes could have delayed fungal colonization and effectively retarded the decay of $+O_3$ and $+CO_2 + O_3$ litters.

Ozone exposure of birch leaves raised condensed tannins and lignin to concentrations that would offset effects of low C/N during subsequent litter decomposition, but this response occurred only in the presence of elevated CO_2. Consequently, soluble condensed tannins and sugars (and lignin) were highest in birch litter generated in the $+CO_2 + O_3$ plots, a result that accords with observations made by Liu and others (2005). Increased secondary compounds in $+CO_2 + O_3$ birch litter resulted in slower rates of decay relative to those exhibited under Control or $+CO_2$ conditions.

Birch litter decay was retarded under $+CO_2$, but accelerated under $+O_3$. Thus, effects of the individual trace gases were antagonistic, and we expected that the $+CO_2$ and $+O_3$ treatments would negate one another during decomposition of birch litter that was generated under $+CO_2 + O_3$. This pattern, where the effects of CO_2 and O_3 exposure cancel out, accords with other early ecosystem- and community-level responses (for example, tree growth and productivity, foliar retention, soil respiration) to the two trace gas additions at the Aspen-FACE (Karnosky and others 2003, 2005). We observed this amelioration of response when $+CO_2 + O_3$ birch litter deployed in the Common Garden took 7 years to disappear (that is, to achieve 95% mass loss, $t_{0.95}$ = 7.1 years), compared to $+CO_2$ litter ($t_{0.95}$ = 8.6 years). Yet ambient CO_2 and O_3 concentrations of the common garden environment do not reflect atmospheric conditions projected for the future; $+CO_2 + O_3$ birch litter took 2 years longer to decay when it was placed back into its original plot ($t_{0.95}$ = 9.1 years). Thus, birch litter decay was subject to substrate × environment interactions, a result consistent with our previous, 12-month-long experiments (Parsons and others 2004).

What are the implications of our results for the future of litter decomposition and soil organic

carbon sequestration in northern deciduous forests? First, leaf litter decay rates will be altered by elevated levels of CO_2 and O_3, although the magnitude and direction of changes will differ because of interactions between pollutants and species. Enriched CO_2 decreased decomposition in both tree species, whereas O_3 exposure exacerbated the rate-retarding effects of CO_2 in aspen, but not in birch. Thus, aspen litter is likely to accumulate on the ground surface of future forests, as suggested by our limit value estimates. Birch litter, however, is more likely to be incorporated into soil organic-C pools of the underlying mineral soil, because elevated CO_2 and O_3 canceled one another's effects on this species. Despite these species-related differences, we note that Loya and others (2003) found that soils in the $+CO_2 + O_3$ plots containing aspen and birch gained only about half as much carbon as did soils in similar plots fumigated with CO_2 alone. This differential is likely to change, however, as different species come to dominate forest canopies of the future (Kubiske and others 2007).

Second, this work suggests that substantial CO_2- and O_3-mediated effects on litter decomposition can be effected via changes in litter chemistry. Studies in several herbaceous and woody plant systems have revealed inconsistent effects of CO_2 and O_3 on litter decay, and led to the perception that the pollutants will influence nutrient cycling more through their impacts on litter inputs than on litter quality (Holmes and others 2006). At Aspen FACE, litter production in the aspen-birch community increased by 46% under $+CO_2$, decreased by 10% under $+O_3$, and increased by 23% under $+CO_2 + O_3$, relative to the Control (*unpublished data*). Nonetheless, as demonstrated here, changes in litter decomposition mediated by changes in quality were not trivial. Coupled with changes in litter inputs, they are likely to shift rates of carbon sequestration and nutrient release in forest soils of the future. Indeed, Holmes and others (2006) have found that gross N immobilization was greatest under the $+CO_2 + O_3$ treatment. Thus, the forest floor is likely to continue as a strong sink for nitrogen, although this may not lead to progressive N limitation, given the relatively high fertility of the Aspen FACE site compared to other CO_2-enrichment experiments.

Third, our results highlight the need to evaluate the *combined* effects of greenhouse gases on ecosystem structure and function. The cumulative effects of CO_2 and O_3 on litter decay rates could not be predicted based on simple additive effects alone.

Altogether, these studies suggest that elevated concentrations of atmospheric CO_2 and O_3 have the potential to alter litter quality and decomposition rates in early successional, north-temperate forests. The consequences of such shifts for nutrient cycling rates, forest productivity, and carbon sequestration will be influenced, however, by interactions among the greenhouse gases and tree species, and linked to effects of the pollutants on leaf litter production.

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