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The Influence of Chemistry, Production and Community Composition on Leaf Litter Decomposition Under Elevated Atmospheric CO_2 and Tropospheric O_3 in a Northern Hardwood Ecosystem

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Abstract

We examined the effects of elevated CO_2 and O_3 and their interaction on leaf litter chemistry and decomposition in pure stands of aspen (*Populus tremuloides*) and mixed stands of birch (*Betula papyrifera*) and aspen at the Aspen Free Air CO_2 Enrichment (FACE) experiment. A 935-day in situ incubation study was performed using litterbags filled with naturally senesced leaf litter. We found that elevated CO_2 had no overall effects on litter decomposition rates, whereas elevated O_3 reduced litter mass loss (−13%) in the first year. The effect of O_3 on mass loss disappeared in the second year. For aspen litter but not mixed birch-aspen litter, decomposition rates were negatively correlated with initial concentrations of condensed tannins and phenolics. Most soluble components (94% of soluble sugars, 99% of condensed tannins, and 91% of soluble phenolics) and any treatment effects on their initial concentrations disappeared rapidly. However, the mean residence time (MRT) of birch-aspen litter (3.1 years) was significantly lower than that of aspen litter (4.8 years). Further, because of variation in total litterfall, total litter mass, C, lignin and N remaining in the ecosystem was highest under elevated CO_2 and lowest under elevated O_3 during the incubation period. Our results indicate that elevated CO_2 and O_3 can alter short-term litter decomposition dynamics, but longer-term effects will depend more on indirect effects mediated through changes in forest community composition. Treatment effects on soluble

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components are likely to influence cyclical microbial processes and carbon pulses in the ecosystem only when coupled with increased (CO_2) or decreased (O_3) litter inputs.

Key words: condensed tannins; FACE; global change; lignin; litter productivity; mean residence time; plant community composition; soluble phenolics; soil carbon storage.

Introduction

Increases in atmospheric concentrations of CO_2 and O_3 can have large but offsetting effects on ecosystem productivity (King and others 2005), yet their independent and interactive effects on other ecosystem process rates remain poorly understood. In temperate deciduous forests, about 41% of aboveground net primary productivity is allocated to leaf production (Litton and others 2007), and litterfall amounts can be altered by both elevated CO_2 and O_3 (Liu and others 2005). Further, these trace gases can impact the timing of leaf senescence (Taylor and others 2008) and tissue concentrations of nutrients and carbon-based constituents including sugars, starch, organic acids, tannins, phenolics, lipids, hemicellulose, cellulose, pectin, and lignin (Norby and others 2001; Scherzer and others 1998; Kainulainen and others 2003; Parsons and others 2004; Booker and others 2005). Because high C:N, high lignin:N or high concentrations of secondary compounds such as phenolics, tannins, and lignin, can reduce litter decomposition and nutrient mineralization rates (Berg and Laskowski 2006), trace gas related alteration of litter chemistry can alter nutrient supply to plants and microbes. For example, elevated CO_2 can lower litter N concentrations (Scherzer and others 1998; Parsons and others 2004; Cotrufo and others 2005) and increase concentrations of lignin, tannins, and phenolics (Parsons and others 2004; Booker and others 2005), although effects are not consistent across studies (Kainulainen and others 2003; Liu and others 2005). Similarly, O_3 has detrimental effects on plant growth and development (EPA 2006), and elevated O_3 can trigger antioxidant defense responses including increased foliar and litter concentrations of phenolic acids (Liu and others 2005), tannins (Booker and others 1996; Liu and others 2005) and terpenes (Kainulainen and others 2003). Despite these important initial findings, our understanding of the effects of elevated CO_2 and O_3 on chemical decomposition dynamics has been constrained by the short-term nature of field and laboratory incubation studies (Parsons and others 2004; Chapman and others 2005).

In addition to changes in chemical composition, elevated CO_2 and O_3 can alter litter production, and these later changes may be as or more important to biogeochemical cycling (Liu and others 2009). A meta-analysis by Curtis and Wang (1998) indicated that leaf production increased by 31% under elevated CO_2, whereas elevated O_3 can significantly reduce forest productivity (King and others 2005; EPA 2006). Further, atmospheric changes in CO_2 and O_3 may alter plant community composition and so the quality and quantity of litter fall. For example, after 7 years of fumigation, elevated O_3 accelerated the conversion of mixed aspen-birch stands to a birch dominated stand in the Aspen FACE experiment, whereas elevated CO_2 delayed conversion (Kubiske and others 2007). Similarly, in a 2-year controlled-environment study, Kozovits and others (2005) found that both elevated CO_2 and elevated O_3 reduced the competitive ability of beech compared with spruce in a mixed spruce-beech assemblage. As foliar chemistry varies across tree species, canopy compositional changes are likely to have consequences for litter decomposition and nutrient cycling (Luo and others 2004; Bradley and Pregitzer 2007). Changes in litter quality and quantity may also influence belowground process rates, with profound effects on substrate availability for microbial metabolism (Zak and others 1993; Giardina and others 2005), forest nutrient availability, carbon storage, and ultimately ecosystem productivity (Strain and Bazzaz 1983).

Elevated CO_2 and O_3 have the potential to alter litter decomposition not only by changing the quality and quantity of litter, but also by modifying forest-floor environmental conditions such as soil moisture and temperature (Pendall and others 2003; Loranger and others 2004; Carney and others 2007). Changes such as these in the forest environment would further affect biogeochemical process rates. However, reciprocal transplant studies with common litter substrate across several FACE experiments indicate that environmental changes under elevated CO_2 (mediated through changes in canopy properties and stand water use) had no significant influence on litter decomposition rates (Finzi and Schlesinger 2002; Parsons and others 2004, 2008; Knops and others 2007). This suggests that any differences in litter decomposition rate observed under elevated CO_2 would be derived

from a change in litter quality and/or quantity, rather than changes in microenvironment or even microbial/faunal community composition.

Parsons and others (2008) conducted a 23-month decomposition study from the 2nd to 4th year of the CO_2 and O_3 fumigation at the Aspen–FACE site, which preceded maximum canopy leaf area. Anticipating that stands are dynamic with time and canopy closure often coincides with important ecosystem level changes in forest productivity (Litton and others 2007), we started a 31-month in situ decomposition study from the 6th to 8th year of the fumigation at the same FACE site, which followed maximum LAI. Our study was designed to investigate the effects of long-term fumigation treatment on litter chemistry and decomposition trends of not only litter mass but also carbon-based constituents (soluble sugars, condensed tannins, soluble phenolics, lipid, hemicellulose, and lignin). In addition, we compared responses of a single species (aspen) with mixed-species litter (birch-aspen), and scaled these results to the ecosystem level by incorporating biochemical and production changes. These analyses will help advance our understanding of how CO_2 and O_3 driven changes in community composition and litter production influence litter decomposition and nutrient cycling.

Method and Materials

Site Description

This study was conducted at the Aspen FACE experiment in Rhinelander, Wisconsin (45°40.5′N, 89°37.5′E), established in 1997. The experiment is a randomized complete block split plot design with three replications. Each block contains four 30-m diameter circular plots: control (ambient CO_2, ambient O_3), elevated CO_2 (elevated CO_2, ambient O_3), elevated O_3 (ambient CO_2, elevated O_3), and elevated CO_2 plus elevated O_3. One half of each plot was planted with five trembling aspen (*Populus tremuloides* Michx.) clones differing in O_3 sensitivity (relatively tolerant: 8L, 216, and 271; relatively sensitive: 42E and 259). The SW quadrant of each plot was planted with aspen clone 216 and paper birch (*Betula papyrifera* Marsh), and the NW quadrant of each plot was planted with aspen clone 216 and sugar maple (*Acer saccharum* Marsh). Saplings were planted in June 1997 at 1 × 1 m spacing and have been exposed to elevated CO_2 and O_3 treatments during daylight hours throughout the growing season (May to October) since 1998. The daily average concentrations for elevated CO_2 and elevated O_3 fumigation in 2003 are 535 ppm and 51 ppb, individually. A complete description of the experimental design and operation of this FACE facility are provided by Dickson and others (2000).

Litter Collection and Field Incubation

Naturally senesced leaf litter was collected every 2 weeks from June to October in 2003 in 43 cm diameter plastic litter traps. Twelve traps in the aspen subplot and six traps in the birch-aspen subplot were evenly placed along an inner concentric circle with a diameter of 15 m in each plot. After removing understory litter and other coarse woody material, leaf litter was aggregated within each plot by community type, air-dried and pooled across collection dates to determine biomass production. A total of 2.5 g of leaf litter was placed in 11 × 7 cm litterbags with 1-mm mesh size. This commonly used mesh size was chosen because it allows for abiotic and biotic interactions of the litter with its surroundings while effectively retaining the sample and so that comparisons can be made with previous decomposition studies (Finzi and Schlesinger 2002; Parsons and others 2004, 2008). For birch-aspen community samples, leaf litter was composited according to the dry biomass ratio of total annual aspen leaf litter to total annual birch leaf litter of the respective subplots in 2003. Litterbags were placed on the soil surface in the respective plot from which the litter was collected and left undisturbed until collection. Decomposition was followed for 935 days from November 2003 to May 2006. Two litterbags were retrieved on the following dates from each treatment section: May 2004 (180 d), July 2004 (270 d), November 2004 (360 d), May 2005 (540 d), August 2005 (660 d), November 2005 (735 d) and May 2006 (935 d). At each removal, the litter samples were sorted to remove foreign material, weighed for mass loss after freeze-drying, then ground in liquid N and stored in a freezer at −20°C for later biochemical analysis. Ash contents of litter samples were determined by combustion of sub-samples overnight in a muffle furnace at 450°C. Values of mass remaining and chemical concentrations were ash corrected.

Litter Chemistry

Litter C and N concentrations were analyzed on a NC 2100 CHN auto-analyzer (CE Instruments Ltd., Hindley Green,Wigan, UK). Soluble sugars were extracted from litter samples (25 mg) with 2 ml of

methanol:chloroform:water (60:25:15) (v/v) (3×) and quantified colorimetrically by the phenol-sulfuric acid method (Poorter and Villar 1997). Absorbance was measured at 490 nm and soluble sugar concentration was expressed as glucose-equivalents using a standard curve (Tissue and Wright 1995). After evaporating off the chloroform from the sugar extracts, lipid content was determined as the weight of the dried residue (Poorter and Villar 1997).

The concentration of soluble phenolics was determined by the Folin-Ciocalteu method (Booker and others 1996). Samples (50 mg) were extracted with 1.5 ml of 70% acetone (3×) and 50-µl aliquots of the extracts were then reacted with 0.475 ml of 0.2 N Folin-Ciocalteu reagent and 0.475 ml of 1 M Na_2CO_3 for 1 h. Absorbance of the solutions was measured at 724 nm and total phenolic concentrations were expressed as catechin-equivalents using a standard curve (Booker and others 1996).

Condensed tannins were measured by the acid-butanol method (Porter and others 1986). Samples of litter residue (100 mg) were extracted with 1 ml ice-cold acetone-ascorbic acid mixture (70% acetone + 10 mM ascorbic acid) (5×). Aliquots (150 µl) of extract were diluted with 350 µl of 70% acetone-ascorbic acid solution, and mixed with 3.0 ml of 1 N butanol (95%) containing 5% HCl (v/v) and 100 µl of iron reagent (0.02 g ml^{-1} $FeNH_4(SO_4)_2 \cdot 12H_2O$ in 2 N HCl). After incubation for 50 min at 100°C, absorbance of the solutions was measured at 550 nm. A standard curve was prepared using purified condensed tannins extracted from senesced aspen and birch leaves as described previously (Booker 2000). The acetone-extracted pellets used in the condensed tannins assay were dried and then extracted with 10% KOH (w/v) at 30°C for 24 h. The extracts were mixed with ice-cold absolute ethanol—4 M acetic acid solution, stored at −20°C for 24 h and then centrifuged. Hemicellulose was determined by the dry weight of the precipitate (Dickson 1979).

Lignin concentration was measured according to the method of Booker and others (1996). Litter samples (50 mg) were first extracted with 50% MeOH (v/v) (3×), methanol:chloroform:water (53:26:21) (v/v) (2×), phenol:acetic acid:water (51:25:24) (v/v) (2×), and then washed with EtOH (5×). The extractive-free cell wall material was oven-dried at 70°C, treated with 5% H_2SO_4 (w/w) at 100°C for 1 h and 72% H_2SO_4 (w/w) at 20°C for 2 h. The digested samples were diluted with water and incubated in boiling water for 2 h. Lignin was determined by the residue weight.

Calculations and Statistical Analysis

Treatment effects on loss of litter mass and carbon-based constituents were analyzed by ANOVA for a randomized completed block design, where CO_2 and O_3 are treated as main effects and community and time are treated as split-plot effects. Detailed descriptions of the ANOVA model are provided by King and others (2001). Measurements of litter samples from the same subplot on a single date were treated as subsamples and averaged. Data were tested for normality and outliers, which are defined as observations that are more than three standard deviations from the mean, were removed before analysis. Relative litter mass loss was calculated as the fraction of initial mass remaining (%) at each collection date.

Litter mass decomposition rates (k) were estimated from a simple negative exponential model as: $m_t = m_o e^{-kt}$, where: m_o is the initial litter mass, m_t is the litter mass at time t and k is the decomposition rate constant (Olson 1963). The mean residence time (MRT) is calculated as $1/k$. Differences in decomposition rates were compared using Fisher's Least Significant Difference (LSD) means test. All statistical analyses were done using SAS (Version 9, SAS Institute Inc., Cary, NC).

Mass loss of decomposing litter in each year was separated into seven fractions: soluble sugars, condensed tannins, soluble phenolics, hemicellulose, lignin and 'other compounds'. Here, 'other compounds' were composed mainly of cellulose, protein, and mineral nutrients. The percent of total mass loss caused by decomposition of each component was calculated as: $[\text{constituent}]_c \times RM_c - [\text{constituent}]_p \times RM_p$, where $[\text{constituent}]_c$ and RMc are the constituent concentration (%) and the remaining litter mass (%) of the last collection in the calculated year, whereas $[\text{constituent}]_p$ and RM_p are the constituent concentration (%) and the remaining litter mass (%) of the last collection in the previous year. Relative mass loss per year was calculated as $RM_c - RM_p$. The effects of elevated CO_2 and O_3 on mass changes during decomposition were analyzed with the ANOVA model described by King and others (2001).

Results

Litter Decomposition

Community Effects

Litter samples from the aspen and birch-aspen communities had similar decomposition rates in the first 270 days (Table 1, Figure 1). Thereafter,

Table 1. ANOVA Summaries of Litter Mass Remaining (%) and Chemistry (mg g^{-1}) Over 935 Days in a Field Incubation Under Experimental Treatments at the Aspen FACE Project

Source	DF	Biomass		Soluble sugars		Soluble tannins		Soluble phenonics		Lipids		Hemicellulose		Lignin	
		MS	*P*	MS	*P*	MS	*P*	MS	*P*	MS	*P*	MS	*P*	MS	*P*
CO_2	1	0.01	*0.98*	0.28	*0.49*	0.13	*0.31*	0.31	*0.33*	1.41	*0.59*	44.45	*0.33*	194.45	*0.20*
O_3	1	244.00	***0.04***	1.24	*0.17*	3.26	***<0.01***	1.91	***0.04***	20.08	*0.07*	12.27	*0.60*	93.90	*0.36*
$CO_2 \times O_3$	1	11.95	*0.59*	0.36	*0.44*	0.00	*0.93*	0.01	*0.83*	2.68	*0.46*	10.49	*0.62*	221.88	*0.18*
Com	1	3392.74	***<0.01***	0.67	*0.34*	0.11	*0.35*	0.01	*0.81*	21.26	***<0.01***	670.01	***<0.01***	33.29	*0.39*
$CO_2 \times$ Com	1	118.03	*0.33*	0.13	*0.67*	0.62	***0.04***	0.01	*0.83*	0.20	*0.71*	220.62	***<0.01***	4.37	*0.75*
$O_3 \times$ Com	1	79.13	*0.42*	0.09	*0.72*	0.00	*0.99*	0.43	*0.18*	0.15	*0.75*	130.40	***0.01***	26.13	*0.45*
$CO_2 \times O_3 \times$ Com	1	4.81	*0.84*	0.20	*0.59*	0.00	*0.97*	0.14	*0.43*	0.01	*0.95*	3.67	*0.58*	15.42	*0.56*
Time	6	8314.40	***<0.01***	15.45	***<0.01***	29.72	***<0.01***	24.22	***<0.01***	35.68	***<0.01***	891.29	***<0.01***	610.97	***<0.01***
$CO_2 \times$ Time	6	60.62	***0.01***	0.82	***0.04***	0.11	*0.32*	0.31	*0.23*	0.25	*0.97*	67.73	***0.01***	17.87	*0.92*
$O_3 \times$ Time	6	11.06	*0.78*	1.92	***<0.01***	2.40	***<0.01***	0.61	***0.02***	0.34	*0.94*	64.66	***0.01***	162.06	***0.01***
$CO_2 \times O_3 \times$ Time	6	69.37	***<0.01***	0.10	*0.93*	0.00	*1.00*	0.01	*1.00*	0.85	*0.69*	18.61	*0.44*	59.33	*0.33*
Com × Time	6	317.41	***<0.01***	0.19	*0.59*	0.08	*0.27*	0.05	*0.87*	0.89	*0.34*	36.91	***<0.01***	14.72	*0.69*
$CO_2 \times$ Com × Time	6	38.22	*0.38*	0.05	*0.98*	0.46	***<0.01***	0.09	*0.70*	1.12	*0.22*	13.35	*0.23*	41.09	*0.09*
$O_3 \times$ Com × Time	6	47.25	*0.25*	0.15	*0.72*	0.00	*1.00*	0.14	*0.40*	0.85	*0.37*	19.92	*0.07*	14.88	*0.68*
$CO_2 \times O_3 \times$ Com × Time	6	75.31	***0.05***	0.09	*0.89*	0.00	*1.00*	0.12	*0.51*	0.65	*0.52*	14.29	*0.20*	35.70	*0.14*

Com, community. P-values in bold italics indicate significance ($P \leq 0.05$). P-values in bold italics indicate significance ($P \leq 0.05$). P-values in italics indicate significance ($P < 0.05$)

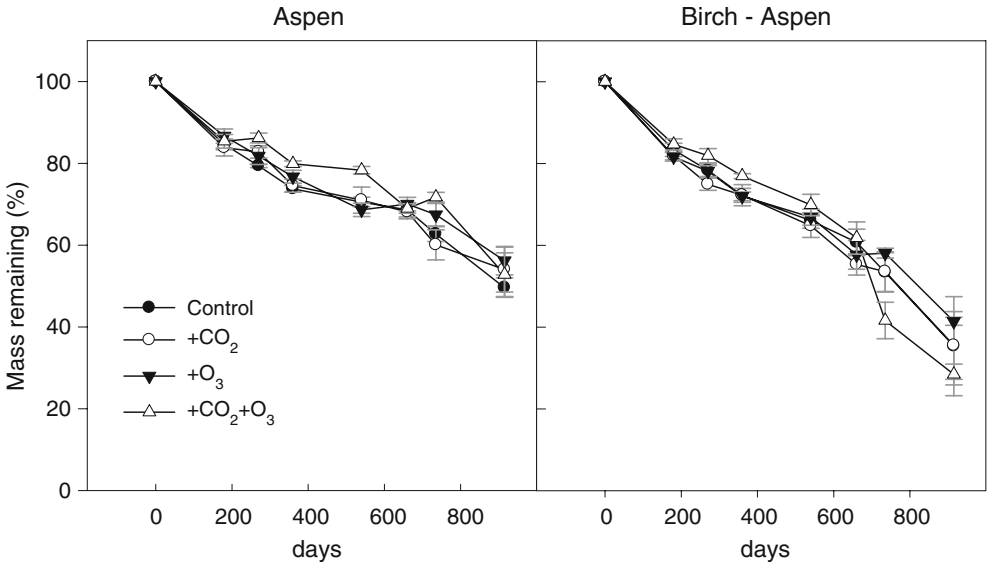

Figure 1. Dynamics of aspen and birch-aspen litter decomposition (% mass remaining in litterbags) over 935 days in a field incubation under experimental treatments at the Aspen FACE project. Litter used in this study was collected from each atmospheric treatment plot during the 2003 growing season and placed in its respective plot for the incubation. Values are means of three replicate plots per treatment combination and bars represent standard error. ANOVA summaries are provided in Table 1.

birch-aspen litter decomposed faster than aspen and differences in mass remaining increased over time, resulting in a significant Community × Time interaction (Table 1, Figure 1). Total litter mass remaining on the ground was higher in the aspen community than in the birch-aspen community ($P < 0.01$) (Table 2). The MRT calculated over the 935-d incubation of aspen (4.8 years) was significantly longer than birch-aspen litter (3.1 years) ($P = 0.01$) (Table 3). Litter decomposition rates of the aspen community were negatively correlated with initial concentrations of condensed tannins

Table 2. Mass Remaining in the Litterbag (g/bag) and Ecosystem (g m^{-2}) for Aspen and Birch-Aspen Litter Samples at the End of Each Incubation Year

Treatment	Year 1 (November 2003–November 2004)		Year 2 (November 2004–November 2005)	
	Aspen	Birch-aspen	Aspen	Birch-aspen
Mass remaining in litterbag (g/bag)				
Control	1.84 ± 0.02^{b}	1.80 ± 0.04^{b}	1.57 ± 0.05^{b}	1.33 ± 0.07^{ab}
+CO_2	1.86 ± 0.04^{b} (101.06%)	1.81 ± 0.06^{b} (100.05%)	1.50 ± 0.12^{b} (95.82%)	1.34 ± 0.03^{ab} (100.31%)
+O_3	1.92 ± 0.04^{ab} (104.00%)	1.80 ± 0.03^{b} (99.68%)	1.69 ± 0.09^{ab} (107.56%)	1.45 ± 0.03^{a} (108.79%)
+CO_2 + O_3	2.00 ± 0.02^{a} (108.28%)	1.92 ± 0.01^{a} (106.53%)	1.79 ± 0.12^{a} (114.37%)	1.04 ± 0.11^{b} (77.94%)
Litter mass remaining on the ground (g m^{-2})				
Control	159.49 ± 1.96^{c}	143.58 ± 8.79^{c}	135.51 ± 3.83^{b}	105.76 ± 11.05^{b}
+CO_2	209.87 ± 8.34^{a} (131.59%)	196.99 ± 13.49^{a} (137.20%)	168.78 ± 11.24^{a} (124.55%)	145.80 ± 14.50^{a} (137.86%)
+O_3	131.37 ± 5.95^{d} (82.37%)	128.49 ± 3.51^{c} (89.49%)	114.95 ± 4.49^{c} (84.83%)	103.65 ± 3.09^{b} (98.01%)
+CO_2 + O_3	184.85 ± 2.98^{b} (115.91%)	175.57 ± 4.37^{b} (122.28%)	165.90 ± 3.33^{a} (122.43%)	95.50 ± 11.71^{b} (90.30%)

Values in parentheses indicate percentage of the control treatment. Initially, 2.5 g of litter from the corresponding treatment was placed in the litterbag. Mass remaining in communities was estimated by: $M_r = M_i \times r/100$, where M_r is mass remaining in communities, M_i is litter production in 2003, r is % mass remaining in litterbags. Values are means ± SE, n = 3. For each community, the values followed by a different letter are significantly different ($P \leq 0.05$).

Table 3. Decomposition Rate Constants (k) and Mean Residence Time (MRT = 1/k) for Aspen and Birch-Aspen Litter Samples at the Aspen FACE Site

Treatment	Aspen		Birch-aspen	
	k (R^2)	MRT (year)	k (R^2)	MRT (year)
Control	0.23 (0.90)	4.43 ± 0.19[a]	0.32 (0.79)	3.19 ± 0.26[ab]
+CO_2	0.23 (0.79)	4.56 ± 0.59[a]	0.32 (0.81)	3.14 ± 0.30[ab]
+O_3	0.20 (0.83)	5.01 ± 0.51[a]	0.28 (0.91)	3.56 ± 0.20[a]
+CO_2 + O_3	0.18 (0.80)	5.57 ± 0.45[a]	0.37 (0.82)	2.69 ± 0.12[b]

Coefficients of determination (R^2) for each exponential rate constant model are shown in parentheses. MRT values are means ± SE, n = 3. For each community, the MRT values followed by a different letter are significantly different ($P \leq 0.05$).

and soluble phenolics, but no such correlation was found in the birch-aspen community (Table 4). Litter decomposition rates were not significantly correlated with the initial concentrations of other constituents.

Elevated CO_2 and O_3 Effects

The main effect of elevated CO_2 on litter mass loss was not significant for either community. Averaged across all treatments, aspen litter mass loss under elevated O_3 was significantly lower than under ambient O_3 (Table 1, Figure 1). Total litter mass remaining on the ground was highest under elevated CO_2 treatment and lowest in the added O_3 treatment (Table 2).

Interactive Effects of the Treatments

A significant $CO_2 \times O_3 \times$ Community × Time interaction occurred because elevated CO_2 had no effect on mass loss early in the incubation, but increased mass loss later on in the birch-aspen litter samples from the combined elevated CO_2 and O_3 treatment (Table 1, Figure 1). Among all treatments, aspen litter from the elevated CO_2 plus O_3 treatment had the longest MRT (5.57 years), whereas birch-aspen litter from the same treatment had the shortest MRT (2.69 years), mainly due to rapid mass loss late in the incubation period (Table 3, Figure 1).

Litter Chemistry During Decomposition

Community Effects

Across atmospheric treatments, litter samples from the birch-aspen community had significantly higher lipid concentrations than those from the aspen community ($P = 0.01$) (Table 1, Figure 3A, D). A significant Community × Time interaction occurred for hemicellulose because aspen litter had higher hemicellulose concentrations early in the incubation period, but lower levels after day 270 compared with birch-aspen litter samples (Table 1, Figure 3B, E). Other constituents showed similar dynamics between the two communities during the incubation period.

Table 4. Spearman's Correlation Coefficients (r_s) between Initial Litter Chemical Concentration (%) and Decomposition Rate Constants (k) at the Aspen FACE Project

Chemical component	Aspen		Birch-aspen	
	r_s	*P*-value	r_s	*P*-value
Sugar	−0.41	*0.17*	0.24	*0.46*
Tannins	−0.50*	***0.05***	0.23	*0.48*
Phenolics	−0.60*	***0.04***	0.27	*0.37*
Lipids	0.27	*0.41*	−0.38	*0.22*
Hemicellulose	−0.05	*0.85*	−0.45	*0.14*
Lignin	0.00	*0.97*	−0.22	*0.44*
N	0.02	*0.94*	−0.05	*0.85*
Lignin/N	−0.01	*0.98*	−0.19	*0.56*

The correlation coefficient values followed by asterisks are significant ($P < 0.05$). n = 12. P-values in bold italics indicate significance ($P \leq 0.05$).

Elevated CO_2 and O_3 Effects

The initial concentrations of condensed tannins and soluble phenolics were higher in extracts of litter samples from the elevated O_3 treatments for both communities, but those differences disappeared by day 180, which resulted in a significant O_3 × Time interaction (Table 1, Figure 2B–F). Lignin concentrations showed a rapid increase during early decomposition stages and were significantly higher under elevated O_3 by day 180, but this difference became statistically non-significant by day 360, resulting in a statistically significant O_3 × Time interaction (Table 1, Figure 3C, F). Elevated CO_2 did not significantly alter decomposition dynamics of any constituent.

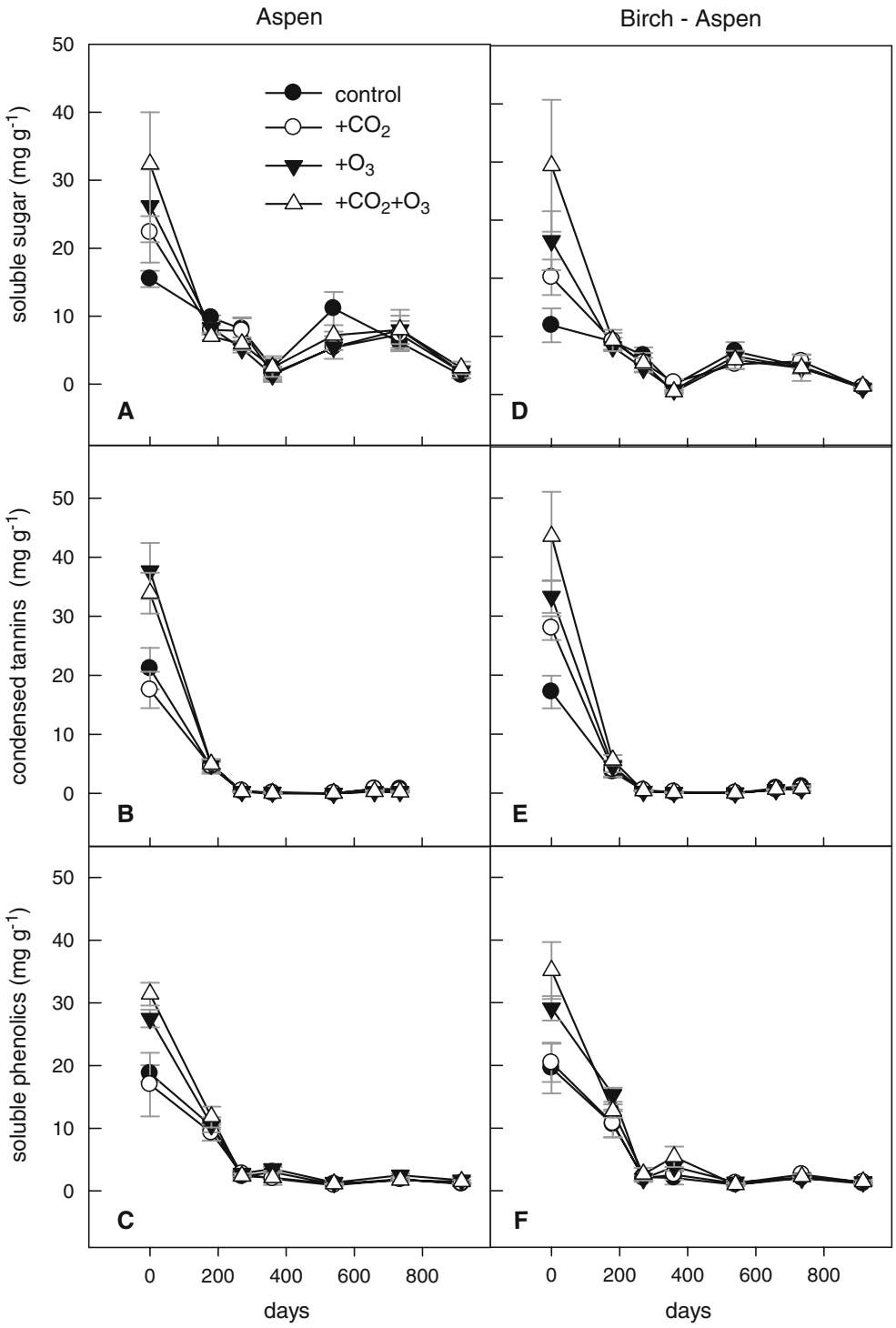

Figure 2. Trends in soluble sugar, condensed tannins and soluble phenolics concentrations in decomposing aspen and birch-aspen leaf litter samples from trees previously grown under all combinations of elevated CO_2 and O_3 at the Aspen FACE site (n = 3). Values are means of three replicate plots per treatment combination and bars represent standard error. ANOVA summaries are provided in Table 1.

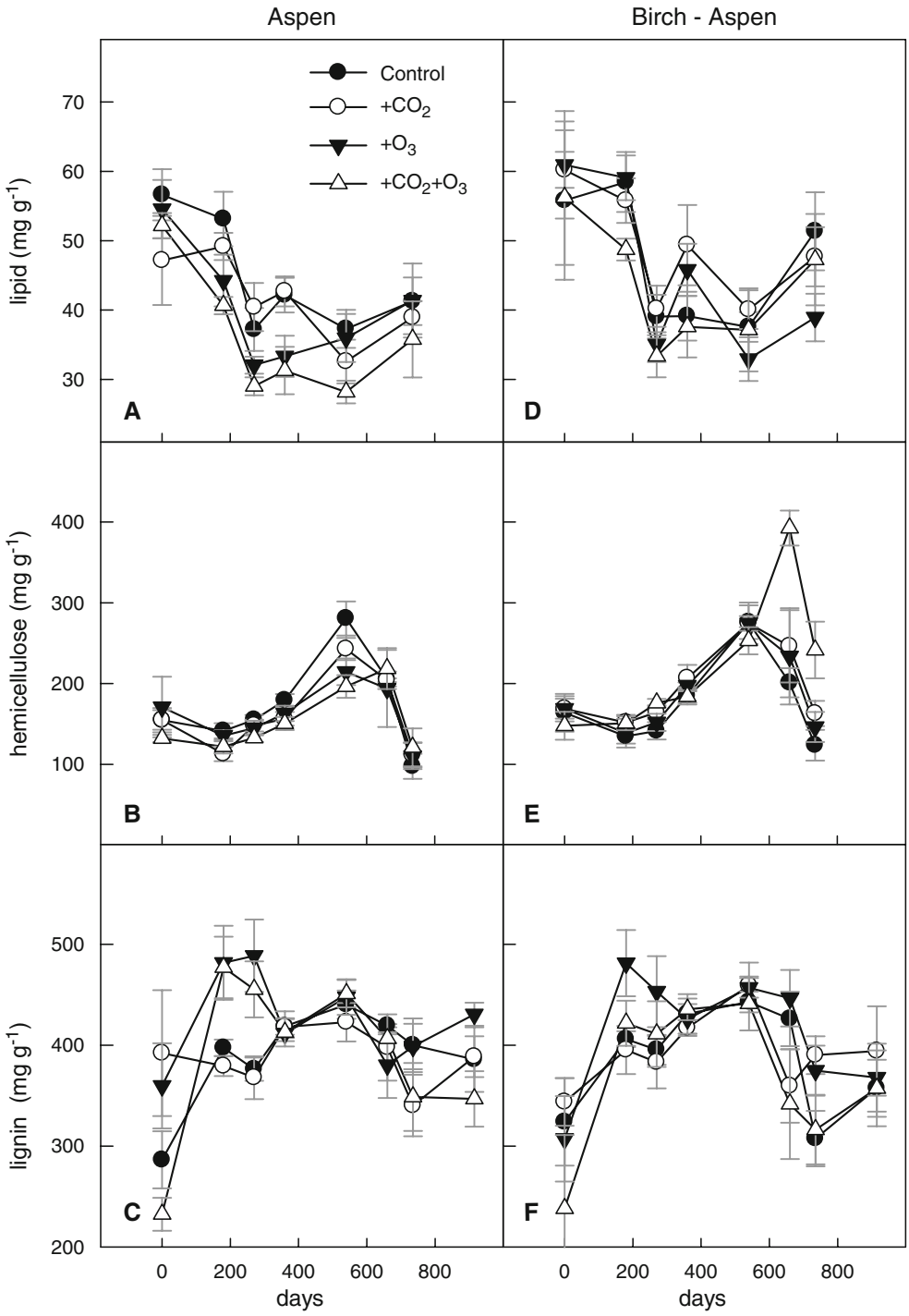

Figure 3. Trends in lipid, hemicellulose, and lignin concentrations in decomposing aspen and birch-aspen leaf litter samples from trees previously grown under all combinations of elevated CO_2 and O_3 at the Aspen FACE site (n = 3). Values are means of three replicate plots per treatment combination and bars represent standard error. ANOVA summaries are provided in Table 1.

Interactive Effects of the Treatments

A significant CO_2 × Community interaction occurred for condensed tannins because concentrations were lower in aspen but higher in birch-aspen litter samples from the elevated CO_2 treatments compared with ambient CO_2 during the first 180 days (Table 1, Figure 2B, E). Averaged across the incubation period, hemicellulose concentrations were higher in aspen litter and lower in birch-aspen litter under both elevated CO_2 and elevated O_3 treatments, which resulted in significant CO_2 × Community and O_3 × Community interactions (Table 1, Figure 3B, E).

Chemical Decomposition Dynamics at Litterbag Level

Averaged across treatments, mass in litter bags decreased 25% during the first year and 16% during the second year (Figure 4). Mass loss in the first year was dominated primarily by losses of 'other compounds', most of which was cellulose (Figure 4A, B). The highest contribution to mass loss during the second year was due to lignin (Figure 4C, D). Most soluble compounds were lost during the first year: 94% of total soluble sugars, 99% of total condensed tannins, and 91% of total soluble phenolics. Primarily structural compounds were lost during the second year including 31% of total lignin and 35% of total hemicellulose. Elevated CO_2 and O_3 had no overall effects on the mass loss of all constituents at litterbag level.

Decomposition Dynamics at the Ecosystem Level

Scaled to the ecosystem level, total C losses after 2 years were 39.5 and 56.5 g C m^{-2} for the aspen and birch-aspen communities, respectively (Figure 5A, B). Similarly, lignin loss in the aspen community (19.6 g lignin m^{-2}) was also lower than in the birch-aspen community (26.7 g lignin m^{-2}) (Figure 5C, D). Total N content slightly increased in the aspen community (0.25 g N m^{-2}), but decreased in birch-aspen (−0.24 g N m^{-2}) (Figure 5E, F). Across the field incubation, elevated CO_2 resulted in more C, lignin and N retained in the forest floor, whereas elevated O_3 reduced them for both communities (Table 5, Figure 5A–F).

Discussion

In this 935-day experiment, elevated CO_2 and O_3 both alter leaf litter chemistry and total leaf litter inputs rates (Liu and others 2005). Elevated CO_2 decreased [N] by 11% while increasing leaf litter production by 34%. In contrast, elevated O_3 increased [tannins] by 77% and [phenolics] by 53%, and decreased leaf litter production by 18% (Liu and others 2005). In this study, we hypothesized

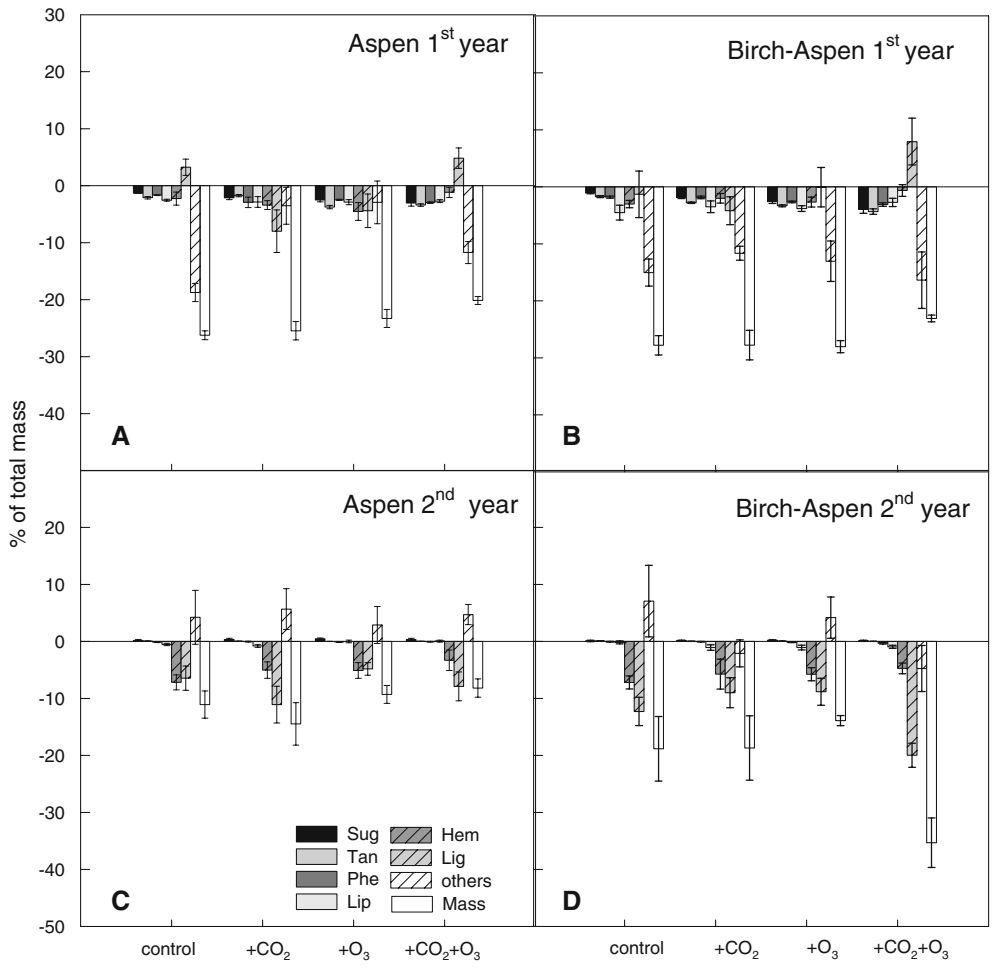

Figure 4. The contribution of C-based constituents and other litter components to leaf litter mass loss of aspen and birch-aspen litter at Aspen FACE site during the 2-year field incubation. Relative mass loss per year is also shown. (*Phe:*. Soluble phenolics; *Sug:*. Soluble sugars; *Tan:* Condensed tannins; *Hemi:* Hemicellulose; *Lig:* Lignin; *other*: other compounds). Values are means of three replicate plots per treatment combination and bars represent standard error.

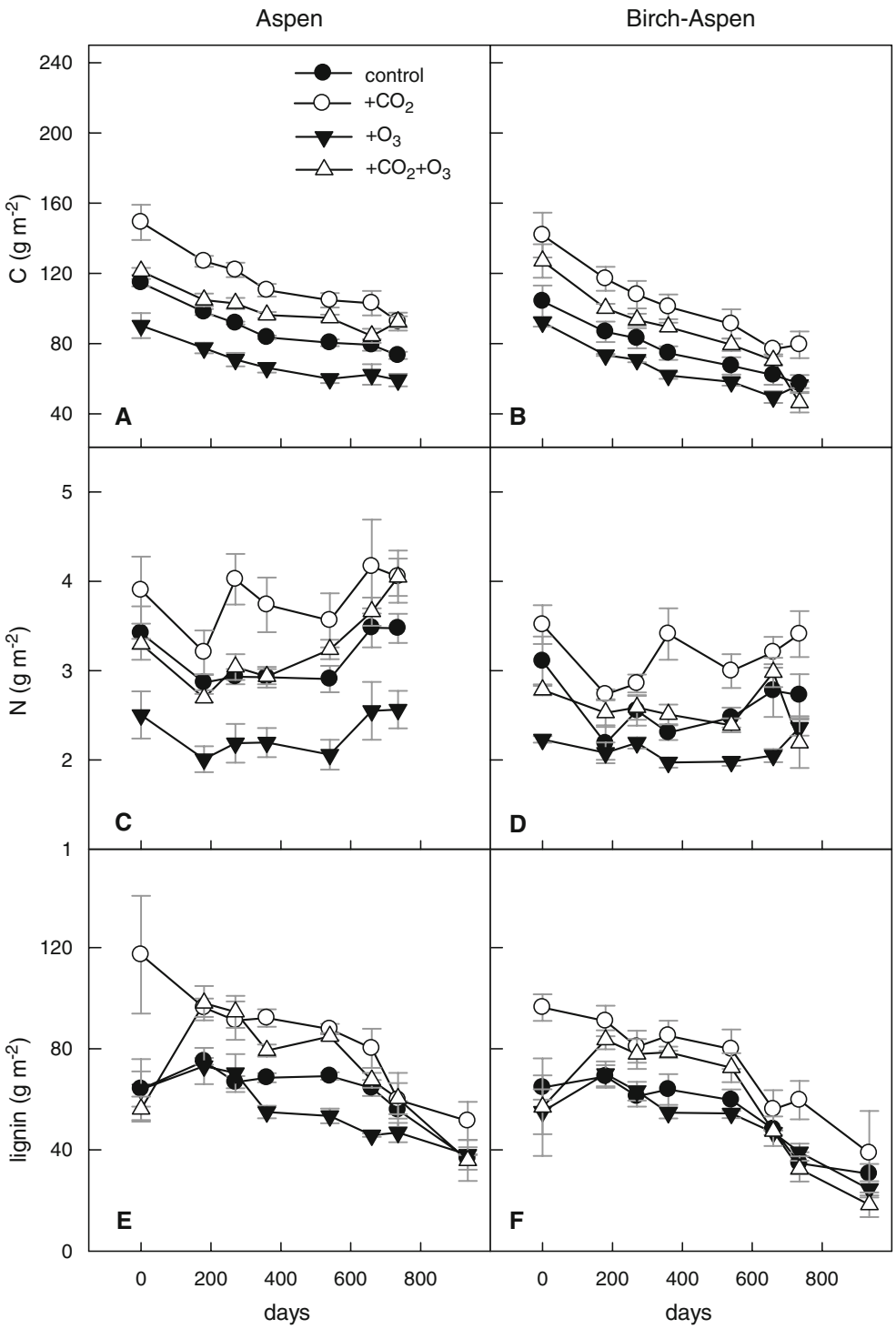

Figure 5. Trends in total C, N, and lignin (g m^{-2}) remaining in aspen and birch-aspen community for litter produced in 2003 under all combinations of elevated CO_2 and O_3 at the Aspen FACE site (n = 3). Values are means of three replicate plots per treatment combination and bars represent standard error. ANOVA summaries are provided in Table 5.

that a reduced N concentration would reduce litter decomposition under elevated CO_2 whereas increased condensed tannin and soluble phenolic concentrations would reduce litter decomposition under elevated O_3. However, we found that changes in leaf chemistry had little influence on litter decomposition rates. The one exception was a weak negative correlation for aspen litter between O_3-induced changes in the concentrations of phenolics and condensed tannins and litter decomposition rates. The lack of a chemistry effect suggests that changes in litter mass inputs caused by the effects of elevated CO_2 and O_3 on tree growth, leaf production and timing of leaf fall will likely have greater impacts on decomposition dynamics.

Table 5. ANOVA Summaries of Ecosystem C, N and Lignin ($g\ m^{-2}$) Content over 935 Days in a Field Incubation Under Experimental Treatments at the Aspen FACE Project

Source	DF	C		N		Lignin	
		MS	*P*	MS	*P*	MS	*P*
CO_2	1	35643.33	***<0.01***	22.99	***0.01***	14373.77	***0.01***
O_3	1	11661.71	***<0.01***	19.31	***0.01***	3974.26	***0.05***
$CO_2 \times O_3$	1	18.37	*0.87*	0.12	*0.77*	837.27	*0.34*
Com	1	5155.96	***0.05***	13.81	***0.02***	3203.86	*0.11*
$CO_2 \times$ Com	1	277.65	*0.61*	1.21	*0.42*	396.68	*0.55*
$O_3 \times$ Com	1	363.61	*0.57*	0.43	*0.62*	0.46	*0.98*
$CO_2 \times O_3 \times$ Com	1	311.13	*0.59*	0.83	*0.50*	291.26	*0.60*
Time	6	8072.27	***<0.01***	1.95	***<0.01***	4556.21	***<0.01***
$CO_2 \times$ Time	6	285.74	***<0.01***	0.10	*0.33*	276.59	*0.26*
$O_3 \times$ Time	6	59.10	*0.15*	0.11	*0.29*	652.74	***0.01***
$CO_2 \times O_3 \times$ Time	6	57.72	*0.16*	0.12	*0.23*	472.65	***0.05***
Com × Time	6	328.54	***<0.01***	0.38	***0.01***	148.74	*0.16*
$CO_2 \times$ Com × Time	6	148.87	***0.02***	0.23	*0.09*	42.03	*0.83*
$O_3 \times$ Com × Time	6	123.13	***0.04***	0.28	***0.04***	72.37	*0.58*
$CO_2 \times O_3 \times$ Com × Time	6	202.65	***<0.01***	0.29	***0.04***	211.79	***0.05***

Com, community. P-values in bold italics indicate significance ($P \leq 0.05$).

Constituent Dynamics

The concentrations of soluble phenolics and condensed tannins in the litter samples dropped to near zero during the first 270 days of incubation in our study. This observation is consistent with previous studies at the Aspen FACE site showing that these constituents are rapidly lost from litter (Parsons and others 2004; Chapman and others 2005) and may be leached, catabolized by microorganisms, immobilized in the soil, or exported in groundwater. In our study, we observed that litter decomposition rates were negatively correlated with initial condensed tannin and soluble phenolics concentrations in the aspen community samples. Although treatment effects on the initial concentrations of the two constituents disappeared rapidly from the litterbag samples, leaching of these compounds into the soil could influence decomposition rates as well as nutrient cycling through inhibitory effects on soil microbial activity and formation of protein-polyphenol complexes resistant to decomposition (Hättenschwiler and Vitousek 2000).

A rapid increase in hemicellulose concentrations was observed from day 360 to day 540. We used 10% KOH to extract hemicellulose from leaf litter (Dickson 1979). Hemicelluloses are closely associated with cellulose or lignin, and so they may not be removed from the cell wall by this procedure (Dickson 1979). Therefore, with the decomposition of cellulose and lignin in year two, bound hemicelluloses may have been released, perhaps explaining the increase in detectable hemicelluoses.

At the Aspen FACE site, elevated CO_2 has increased the activity of cellulose-degrading enzymes, whereas elevated O_3 appears to have eliminated this response (Chung and others 2006). Although cellulose decomposition was not directly measured in this study, we can roughly estimate cellulose mass loss using the 'other component' values (Figure 4). Our results indicate that elevated CO_2 had no significant effect on cellulose decomposition. The lack of an effect could be due to low litter N concentrations, which could be reduced by the activity of cellulose-decomposing microbes (Berg and Laskowski 2006).

Lignin concentrations increased by 43% across community types during the first 200 days of the incubation, with faster mass loss of other constituents likely explaining this effect (Berg and Laskowski 2006). Further, for partly decomposed litter, the gravimetric fraction used to determine lignin concentrations is likely to contain compounds other than native lignin, especially humification products, complexed N compounds and chitin from fungal mycelium (Berg and Laskowski 2006), thus increasing apparent lignin concentrations.

Impacts of Elevated CO_2

Previous studies have found widely ranging responses of litter decomposition rates to elevated CO_2

including decreased rates (Parsons and others 2004, 2008; Cotrufo and others 2005), increased rates (Pendall and others 2003; Carney and others 2007), or no change (Norby and others 2001; Booker and others 2005). We hypothesized that lower initial litter N concentrations under elevated CO_2 at Aspen FACE (Liu and others 2005) would result in lower decomposition rates. However, decomposition rates were on the whole unaffected by the elevated CO_2 treatment. Although consistent with several previous studies (Norby and others 2001; Knops and others 2007), this finding contradicts the negative feedback hypothesis proposed by Strain and Bazzaz (1983). And so any changes to soil C formation are unlikely to relate to altered litter quality and decomposition rates under elevated CO_2, but rather to larger litter production rates (Figure 5). Over long periods of time, these findings indicate that increases in litter production at the Aspen FACE site may be the main factor driving increased carbon storage in response to elevated CO_2 (Loya and others 2003; Liu and others 2009).

Impacts of Elevated O_3

During the first year of our incubation study, litter decomposition rates were suppressed under elevated O_3, which is consistent with the observation of Booker and others (2005) that elevated O_3 significantly decreases leaf residue decomposition of soybean in 20-week field incubations. Similar findings were reported for O_3-treated blackberry (*Rubus cuneifolus* Pursh.) and shoots of broomsedge bluestem (*Andropogon virginicus* L.) (Kim and others 1998). And at Aspen FACE, elevated O_3 treatment resulted in increased tannin and phenolics concentrations (Liu and others 2005), and decreased S, P, Ca, and N concentrations (Liu and others 2007). The slower decomposition rates for this treatment may be due to low litter quality reducing microbial activity, which is supported by the findings of Chung and others (2006) at the Aspen FACE site. Their results showed that elevated O_3 can decrease the activity of cellulose-degrading enzymes and alter fungal community composition. Although the decomposition rate was reduced, lower litter production under elevated O_3 resulted in significantly lower scaled estimates of total remaining C and N content across the incubation time (Figure 5), pointing to reduced C storage and slower N cycle in the forest floor.

Combined Elevated CO_2 and O_3 Treatment

Elevated CO_2 partially ameliorated the inhibitory effects of O_3 on net photosynthesis, growth and litter production at Aspen FACE (King and others 2005). This was attributed to decreased O_3 uptake under elevated CO_2 resulting from greater stomatal closure and lower stomatal density (Volin and others 1998; Noormets and others 2001). In general, litter decomposition rates and chemistry in samples from the combined elevated CO_2 and O_3 treatment were similar to those observed in the elevated O_3 treatment, indicating that even reduced O_3 uptake can stimulate phenylpropanoid metabolism in aspen and so too the concentrations of soluble phenolics and tannins. Results from previous litter decomposition studies have not identified significant $CO_2 \times O_3$ interactions for mass loss, indicating that the effects of CO_2 and O_3 on litter decomposition rates may be independent (Kainulainen and others 2003; Parsons and others 2004; Booker and others 2005).

Decomposition Trends During Stand Development

Litter decomposition contributes to soil C formation, but also sustains ecosystem production through nutrient cycling. However, the long-term responses of litter chemistry and decomposition to elevated CO_2 and O_3 during stand development remain poorly understood. Parsons and others (2008) and the current study together bridge this knowledge gap. Parsons and others (2008) collected litter from aspen and birch seedlings in the 2nd year of the fumigation treatment at Aspen FACE site. They documented that elevated CO_2 reduced [N] by 38% for aspen litter and 32% for birch litter. The dramatic changes in litter chemistry led to significantly slower decomposition rates under elevated CO_2 in their study (Parsons and others 2008). Litter used in our study was collected in the 6th year of the fumigation treatment following maximum stand LAI for these stands, and we found that elevated CO_2 reduced litter [N] by 9.1% and 8.7% for aspen and birch-aspen litter, respectively. These smaller changes in [N] did not alter litter decomposition rates in our study. Parsons and others (2008) and our study together indicate that the influences of atmospheric conditions on litter chemistry and decomposition dynamics may change with stand development, and that extrapolating short-term results may be inappropriate.

Impacts of Species Composition

Substantial experimental evidence indicates that mixed-species litter decomposition patterns cannot

be predicted from those of single-species decomposition studies (Gartner and Cardon 2004). Atmospheric changes have been shown to alter the composition of litter mixtures (Dukes and Hungate 2002; Henry and others 2005; Kubiske and others 2007), but how this variation impacts litter decomposition in forests remains an important question, with the few reports available coming from grassland ecosystems (Dukes and Field 2000; Knops and others 2007).

In the current study, we mixed aspen and birch litter according to the production ratio of the two species in the birch-aspen community in 2003. Compared to pure aspen litter, the birch-aspen mixture had significantly lower initial N and S concentrations, and similar lignin, tannin, and phenolic concentrations (Liu and others 2005). Soil moisture and temperature were similar across the two communities during the incubation period (Pregitzer and others 2006). We expected that the birch-aspen mixture would have a lower decomposition rate due to lower litter quality. Surprisingly, the decomposition rates for birch-aspen litter were significantly faster than that of pure aspen. In a similar decomposition study at Aspen FACE, Parsons and others (2008) found pure birch litter decomposed faster than pure aspen litter although birch litter had lower [N]. They found that changes in litter chemistry associated with atmospheric treatments generally translated into difference in decomposition for aspen litter, but not so for birch litter. Parsons and others (2008) suggested that the slower decomposition of aspen litter may relate to the fact that aspen leaves are thicker and waxier. In addition to chemical and physical attributes of leaves, we suggest that the different responses of aspen and birch litter may originate with decomposer activity. Chung and others (2007) found that plant species richness had a strong positive effect on soil microbial activity, with higher biodiversity resulting in higher total microbial biomass, fungal abundance, and cellulolytic activity. We speculate that the mixed birch-aspen stand may improve decomposition conditions by providing heterogeneous litter substrates, which may change the microenvironment and diversity of the decomposer community (Gartner and Cardon 2004).

Nutrient supply, especially that of N, can modulate plant growth response to elevated CO_2 (Reich and others 2006). Due to faster decomposition rates, birch-aspen litter retained less N, S, Ca, Mg, and B than pure aspen litter at the end of our incubation (Liu and others 2007). Such changes could have indirect effects on forest production under elevated CO_2 by increasing plant nutrient availability. We found that elevated CO_2 showed higher stimulation on total biomass production at the birch-aspen community (+46%) than at the aspen community (+25%) (King and others 2005). Similarly, multispecies plots at the BioCON experiment in Minnesota had faster litter decomposition rates (Knops and others 2007) and higher biomass increases under elevated CO_2 treatment than the monoculture plot (Reich and others 2001).

Conclusion

We found that litter decomposition was not affected by elevated CO_2 although initial litter [N] was significantly reduced by this treatment. Litter decomposition under elevated O_3 was marginally reduced, which may be due to higher initial tannin and phenolic concentrations. Birch-aspen litter mixture decomposed faster than pure aspen litter even with poorer litter quality. Community type exerted a larger effect on litter decomposition rate than expected based on the litter chemistry. Decomposition rates under elevated gas treatments that are obtained from single litter type experiments may be biased for modeling carbon and nutrient dynamics. The changes in estimated forest-floor carbon storage, increased by elevated CO_2 and decreased by elevated O_3, were driven by increases in litter input rates rather than decreases in litter quality. Overall, there is still no consensus in the literature on whether the short-term responses of tree seedlings to elevated CO_2 and O_3 are sustained over time. Previous experiments have shown that ecosystems may become less responsive to elevated CO_2 over time, perhaps as a result of downregulation of photosynthetic capacity (Long and others 2004) or decreased growth rate due to progressive N limitations (Luo and others 2004). In our study, we found that litterfall rates continued to be stimulated by elevated CO_2 well into stand development.

We conclude that small differences in litter chemistry are lost quickly and that differences in litter inputs, along with changes in community composition, will exert a dominant influence on soil C sequestration.

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