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Decomposition of *Betula papyrifera* leaf litter under the independent and interactive effects of elevated CO_2 and O_3

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Abstract

Litter decay dynamics of paper birch (*Betula papyrifera*) were assessed at the Aspen free-air CO_2 enrichment (FACE) facility in northern Wisconsin, USA. Leaf litter was decomposed for 12 months under factorial combinations of 360 vs. 560 μL CO_2 L^{-1}, crossed with 36 vs. 55 nL O_3 L^{-1}. To differentiate between substrate quality and environment effects, litterbags were placed in their Native Plots of origin or transplanted into the other treatments. CO_2 enrichment, regardless of O_3 concentration, produced poorer quality litter (high C/N, lignin/N and condensed tannins) than did ambient CO_2 (low C/N, lignin/N and condensed tannins). Substrate quality differences were reflected in the mass loss rates (*k*-values), which were high for litter generated under ambient CO_2 (0.887 $year^{-1}$) and low for litter generated under elevated CO_2 (0.674 $year^{-1}$). The rate-retarding effects of CO_2 enrichment were neither alleviated nor exacerbated by O_3 exposure. Decay rates varied, however, depending on whether litter was placed back into its plot of origin or transplanted to Common Gardens. The results of this study are species specific, but they have important implications for understanding the processes regulating storage of fixed C and the release of CO_2 from northern forest ecosystems.

Keywords: *Betula papyrifera*, CO_2, decomposition, FACE, O_3, paper birch

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Introduction

Accumulation of fixed C in the vegetation and soils of northern temperate forests has been proposed as an important global sink for rising atmospheric CO_2 concentrations. The strength and persistence of this sink is likely dictated, in part, by changes in plant C quality and its rate of disappearance. Litter decomposition potentially affects ecosystem source–sink dynamics directly, via release of CO_2, and indirectly, via shifts in nutrient availability and consequent changes in net primary production. Observed shifts in plant nutrient composition, which are engendered by these indirect effects, have motivated the proposal of the 'litter quality hypothesis' (Strain & Bazzaz, 1983). In particular, elevated C/N has been noted to persist beyond abscission of CO_2-enriched foliage (Lambers, 1993; De Angelis *et al*., 2000; Gifford *et al*., 2000).

Small-scale 'pot' experiments, and more recent ecosystem-level manipulations using free-air CO_2 enrichment (FACE) technology, have demonstrated that plant growth and tissue nutrient concentrations can be altered by atmospheric CO_2 additions. Under CO_2 enrichment, phenolic secondary metabolites and non-structural carbohydrates can increase in living plant tissues (e.g., Peñuelas *et al*., 1997; Poorter *et al*., 1997; Lindroth *et al*., 2001), while N decreases (McGuire *et al*., 1995; Cotrufo *et al*., 1998b; Koricheva *et al*., 1998; Gifford *et al*., 2000; Norby *et al*., 2000). By modifying leaf tissue chemistry, CO_2 enrichment has been implicated in reducing subsequent litter quality, slowing its rate of decay and thereby enhancing C sequestration in the soil and forest floor. Yet this pattern has not been universally observed among ecosystems and plant species (O'Neill & Norby, 1996; Hirschel *et al*., 1997; Cotrufo *et al*., 1998a, b; Norby & Cotrufo, 1998; Ceulemans *et al*., 1999; Coûteaux *et al*., 1999; Norby *et al*., 1999, 2001; Finzi *et al*., 2001; Finzi & Schlesinger, 2002).

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The equivocal effects of CO_2 enrichment on lowering litter decomposition rates may stem from several possible sources. First, inconsistencies among study outcomes could be attributed to differences in experimental approach and the scale of observation (O'Neill & Norby, 1996). Second, leaf litter produced under high CO_2 concentrations often is decomposed under ambient-CO_2 conditions (e.g., Boerner & Rebbeck, 1995; Kainulainen *et al*., 2003). Third, the processes regulating C sequestration in forest environments are poorly understood, especially how they will be influenced by other widespread environmental changes. Regarding this last point, changes in atmospheric composition include increases in other air-borne pollutants, particularly tropospheric O_3. O_3 exposure can alter plant tissue composition, but there is a paucity of studies regarding interactions between elevated CO_2 and O_3, which potentially affect litter chemistry in a natural field setting (Scherzer *et al*., 1998; Lindroth *et al*., 2001; Kainulainen *et al*., 2003). Very few experimental field studies exist in forested ecosystems that have examined multiple trace gas additions (i.e., elevated $CO_2 + O_3$), especially regarding their *in situ* effects on litter decomposition after altering plant growth and modifying tissue chemistry (Boerner & Rebbeck, 1995; Scherzer *et al*., 1998).

We endeavoured to redress some of these deficiencies by conducting leaf litter decay experiments at the Aspen FACE facility. The Aspen FACE project was established in 1997 to investigate C sequestration in northern hardwood forests undergoing global change (Dickson *et al*., 2000). The model ecosystems consisted of two early successional tree species, trembling aspen (*Populus tremuloides* Michx.) and paper birch (*Betula papyrifera* Marsh.), and a late-successional species, sugar maple (*Acer saccharum* Marsh.). These aggrading mixed stands were exposed to four combinations of CO_2 and O_3 (ambient $CO_2 + O_3$, elevated CO_2, elevated O_3, elevated $CO_2 + O_3$) for one growing season prior to a 1-year-long study of birch leaf litter decay. We separated substrate quality and environmental effects by putting litter from treatments into the controls and vice versa. Reciprocal transplantation thus allowed us to decompose litter in a Common Garden and the Native Plots, and to deploy a Common Substrate among the treatments.

We hypothesized that chemically distinct signatures would be imparted on paper birch foliage growing under the four treatments and that differences in their respective litter qualities would be retained throughout 1 year of decomposition under field conditions. Based on prior analyses of green and senescing birch leaves (Lindroth *et al*., 2001), we predicted that CO_2 enrichment would decrease decay rates, given that poorer quality leaf litter (i.e., lower N, higher C/N) would be generated under elevated vs. ambient CO_2. We predicted that litter quality and, hence, decomposability would further decrease with elevated condensed tannin levels under CO_2 enrichment, since increased polyphenols, including tannins, have been implicated in retarding litter decay (Hattenschwiler & Vitousek, 2000). We focused mainly on condensed tannins, nonstructural carbohydrates and N because these compounds typically respond to changes in atmospheric CO_2 concentrations, and because they have important ecological roles related to decomposition (Peñuelas & Estiarte, 1998). We also measured lignin, which has been used to predict decay rates, along with lignin/N (Taylor *et al*., 1989; Stump & Binkley, 1993).

O_3 fumigation can exert equivocal effects on litter quality indicators such as N concentrations (Koricheva *et al*., 1998), but simple phenolics and tannins have been demonstrated to accumulate in foliage in response to the oxidative stresses imposed by chronic O_3 exposure (e.g., Jordan *et al*., 1991; Booker *et al*., 1996; Saleem *et al*., 2001). These latter responses may explain the low rates of litter disappearance observed in some studies (Findlay *et al*., 1996; Kim *et al*., 1998), yet O_3 exposure has not always led to reductions in litter decay rates (e.g., Scherzer *et al*., 1998). We predicted that elevated O_3, by itself, would exert little direct influence on leaf chemical quality throughout litterfall and decay, whereas elevated $CO_2 + O_3$ would act like elevated CO_2, by depressing birch leaf decay rates through reduced litter quality (viz., high phenolic, and low N and starch concentrations in the foliage).

Materials and methods

Description of Aspen FACE

The litter decomposition experiments were conducted at the Aspen FACE facility in northern Wisconsin, USA. Twelve 30 m diameter FACE rings were planted with 3–6-month-old saplings (1 m × 1 m spacing) of aspen, birch and maple in the mid-summer of 1997, and assigned to one of four treatments: ambient controls (360 $\mu L\,CO_2\,L^{-1}$, 36 $nL\,O_3\,L^{-1}$), 560 $\mu L\,CO_2\,L^{-1}$, 55 $nL\,O_3\,L^{-1}$ and elevated $CO_2 + O_3$. Each fumigation treatment was replicated in three rings, which were blocked across northern, central and southern regions of the site. Fumigation commenced in May 1998, and has continued during daylight hours (07:00–19:00 hours) of each subsequent growing season, from bud break until bud set (Dickson *et al*., 2000; Karnosky *et al*., 2003).

Litterbag deployment and retrieval

Leaf litter was collected from early September to mid-October 1998, in the southwest quarter of each ring. This quarter was planted with greenwood cuttings of a single aspen genotype (clone 216), alternating with birch saplings that originated from seed. The 12 rings were visited weekly to retrieve freshly fallen leaves beneath individual aspen and birch trees. Weekly collections were air-dried, sorted to species and composited by ring. Subsamples of the pooled litters were periodically withdrawn to calculate air-to-oven-dry (65 °C, for 24–48 h) correction factors.

Birch leaves (4–6 g oven-dry mass) that had been grown and senesced in the four treatments were placed in 17 cm × 17 cm litterbags constructed from 1 mm aperture fibreglass cloth. A 1 mm mesh allowed most decomposer mesofauna to pass into and out of the bags. Each litterbag was sealed with wire attached to a numbered tree tag. Once identified, the bags were placed back into their plots of origin ('Native Placement') or transplanted into other rings within the same block. Because of a lack of litter material, reciprocal transplants were restricted to each block by placing litterbags from the three treatment rings into the control ('Common Garden'), and by placing litterbags from the control ring into the three treatments ('Common Substrate'). Also, as insufficient litterfall had accumulated to create a conspicuous forest floor in each ring, litterbags were pinned to large 1 mm mesh fibreglass mats, thereby reducing soil contamination because of rainfall splash. Three mats were laid in the aspen–birch quadrants, about 10 m from the centre of each ring. From 4–7 November 1998, 450 litterbags were deployed on the mats.

Native Placement, Common Garden and Common Substrate experiments were run concurrently. Reciprocal transplantation (Belyea, 1996; Kim *et al*., 1998; De Angelis *et al*., 2000; Finzi *et al*., 2001) differentiated treatment effects on substrate (leaf litter) quality from treatment effects on microenvironment (microclimate and decomposer activities) over 12 months of litter decay. For the Native Placement study, replication was sufficient for deployment and removal of 180 bags (4 fumigation treatments × 3 blocks × 3 mats × 5 intervals) from the 12 treatment rings from which the litter originated. For the Common Garden study, litterbags from the treatments were assigned with the same degree of replication (180 bags = 4 × 3 × 3 × 5) on the mats in the control rings of each north, centre and south block. For the Common Substrate study, 180 bags of control plot litter also were deployed in the four treatment rings of each block. It should be noted that the Common Garden and Common Substrate studies each shared the same 45 bags of control plot litter, which were placed back into the control rings in the Native Placement study. Litterbags were retrieved after 21, 169, 230, 295 and 343 days in the field. At each removal, litterbags were transported to the laboratory on ice. The bags were quick-frozen on the same day of collection and stored at −20 °C until they could be freeze-dried. Freeze-dried material was ground to pass a 1 mm sieve for chemical analysis.

Litter chemistry

Litter quality was determined at leaf abscission and at each litterbag removal, following protocols described in Lindroth *et al*. (2001). N in the ground leaves (100 mg) was determined by high-temperature combustion, followed by thermoconductometric detection (LECO, St Joseph, MI, USA). Free sugars (sucrose + reducing sugars) and starch were determined as glucose equivalents via a modified dinitrosalicylic acid assay (Lindroth *et al*., 2001). Soluble sugars were extracted with 80% aqueous ethanol (3 × 2 mL, 25 mg tissue); starch in the remaining tissue pellet was converted enzymatically to glucose prior to the colorimetric assay. Condensed tannins were exhaustively extracted by sonicating ground tissues (25–100 mg) in 70% aqueous acetone (4 × 1 mL). Soluble condensed tannins in the extracts were assayed colorimetrically in butanol HCl (Porter *et al*., 1986), against purified birch tannin standards. Bound tannins, which typically comprise 20% of the total tannin pool, were not measured in this study. Fibre and lignin were determined sequentially by digesting 500 mg of tissue in acid-detergent solution, then incubating the residue in 72% H_2SO_4 (Rowland & Roberts, 1994).

The mass of each litter sample, together with concentrations of its chemical constituents, was corrected for soil contamination using Blair's (1988) formula. First, sample ash content was determined gravimetrically following loss on ignition (550 °C for 6–12 h); litter C was calculated from the ash-free dry mass. Second, the fraction of actual remaining litter was estimated by additional ash determinations on the initial litterfall and on surface mineral soil (0–2 cm) from each ring.

Statistical analyses

Litter masses remaining at each time point, and litter chemistry data, were subjected to three-way factorial analysis of variance, which was implemented in SAS (PROC MIXED; Littell *et al*., 1996). The ANOVA design was a completely randomized block layout, with CO_2 (ambient vs. elevated), O_3 (ambient vs. elevated) and

Time (five dates), and their respective two- and three-way interactions, as fixed effects. Random effects were the replicate rings (Block), and Block interactions with CO_2, O_3 and Time. Presentation and interpretation of litter chemistry and mass loss results were simplified by conducting separate analyses on the litterfall data, and on the Native Placement, Common Garden and Common Substrate experiments, respectively. Because of the low number of replicates inherent to FACE experiments, and the corresponding risk of Type II statistical errors, *P*-values ≤ 0.10 are reported as significant (Filion *et al*., 2000). Means and standard errors ($\pm$ SE) of the results were calculated using the LSMEANS procedure.

Coefficients of concordance (Kendall's *W*) were calculated for every litter quality variable, as specified in a previous paper (Lindroth *et al*., 2001), to evaluate the consistency in rank order of the responses to the treatments over time. For each experiment, separate estimates were made for *W*, which ranges from 0 to 1, with 0 indicating no agreement and 1 indicating complete agreement in rank order over time.

Rates of litter decay (*k*) were estimated from the simple negative exponential model (Olson, 1963). This model was expanded to include the fumigation treatments as a fixed effect in an analysis of covariance (ANCOVA), with subsequent pairwise tests of slopes (*k*-values). Data from the three experiments were combined for ANCOVA, since they shared the treatment combination of control litter deployed in the control plots. Hence, the experiments were not truly independent. The fixed effect of the ANCOVA included the 10 different combinations created in the initial design (i.e., control litter placed in control plot, control litter in CO_2 plot, control litter in O_3 plot, control litter in $CO_2 + O_3$ plot, CO_2 litter in control plot, O_3 litter in control plot, $CO_2 + O_3$ in control plot, CO_2 litter in CO_2 plot, O_3 litter in O_3 plot, and $CO_2 + O_3$ in $CO_2 + O_3$ plot).

Maximum residence times (MRTs) of litter were calculated from *k* as the number of years, which were required to reach 95% mass loss. Relationships between initial litter quality and *k*-values, and changes in litter quality between leaf abscission and first litterbag removal, were explored through correlation analyses.

Results

Native Placement generally produced results similar, but not identical to those obtained in the Common Garden. These studies were different, in turn, from the results obtained in the Common Substrate experiment, which yielded few significant main effects or interactions among treatment factors. Except for lignin/N, the other chemical constituents in the Common Substrate only varied significantly with time ($P < 0.001$), and therefore, they also are excluded from the presentation of results. Taken together, the three studies indicate that decomposition was driven primarily by factors associated with substrate quality, not environment. To avoid undue repetition, we emphasize results from Native Placement, while also indicating in greater detail as necessary where those results diverge from the Common Garden study.

Nitrogen

CO_2 enrichment decreased N concentrations in the litterfall by 30% relative to the controls ($P = 0.031$), while O_3 exposure had little effect (Table 1). When placed in the Native Plots, the source litters segregated into two groups, with decaying leaves from ambient CO_2 having consistently higher N than those from the elevated-CO_2 treatments (Fig. 1a). While O_3 had no significant effect on litter N, rank order of the four treatments over time was maintained strongly in the Native Plots (Table 2, $P = 0.007$), and strictly so in the Common Garden ($P < 0.001$).

Environmental differences among FACE rings were not strong enough to (1) mask expression of substrate quality effects when source litters were placed back into their original plots (Table 2, $P = 0.007$) or (2) significantly alter N dynamics of the Common Substrate (Table 2, $P = 0.571$). Other than Time ($P < 0.001$), no other environmental effects significantly influenced patterns of N accumulation patterns in the Common Substrate (results not shown).

Table 1 Birch leaf chemistry in the Aspen free-air CO_2 enrichment (FACE) at litterfall, 1998

Chemical variable	Ambient $CO_2 + O_3$	Elevated CO_2	Elevated O_3	Elevated $CO_2 + O_3$
Nitrogen	11.0^{b} (1.6)	7.6^{a} (0.9)	11.0^{b} (1.4)	7.5^{a} (0.1)
Soluble sugars	136^{a} (9)	156^{a} (15)	146^{a} (7)	154^{a} (8)
Starch	72^{ab} (4)	75^{b} (2)	72^{ab} (4)	67^{a} (3)
Condensed tannins	76^{a} (11)	125^{b} (22)	79^{a} (12)	157^{b} (12)
Acid-detergent fibre	334^{b} (10)	350^{b} (14)	290^{a} (12)	319^{a} (15)
Lignin	199^{a} (4)	209^{a} (18.5)	175^{a} (10)	193^{a} (9)
C/N	45.4^{a} (5.5)	65.3^{b} (8.1)	46.8^{a} (4.7)	64.0^{b} (1.2)
Lignin/N	19.0^{a} (2.4)	29.3^{b} (5.7)	16.8^{a} (1.9)	25.8^{b} (0.4)

Chemical concentrations (mg g^{-1}) are means (SEs) from three replicate rings per treatment combination. Within rows, means followed by the same letter did not differ significantly at $P \leq 0.10$.

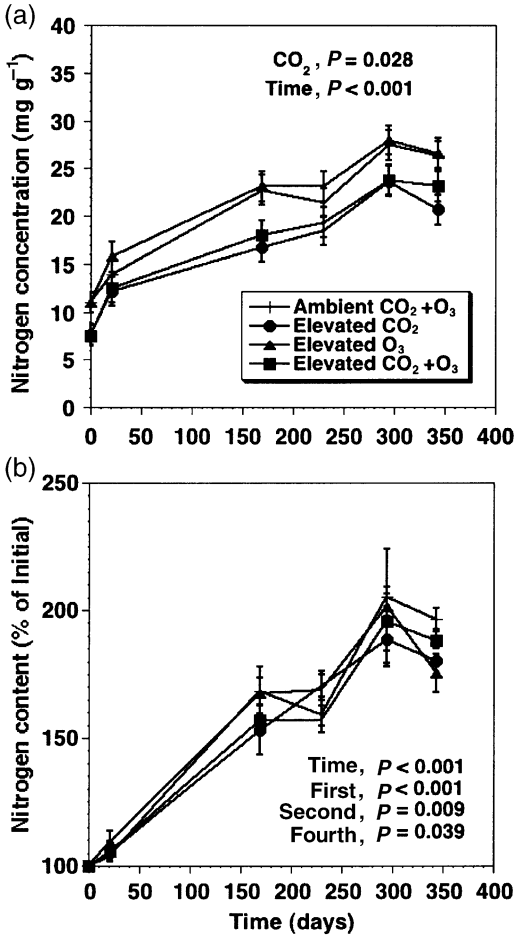

Fig. 1 Dynamics of N over 343 days of birch leaf decomposition. Each value is the mean (± SE) of three replicate rings at five removal dates, for (a) N concentrations (mg g^{-1}) and (b) N contents (% of initial [N]) in litter decaying in the Native Plots. For N content, the Time main effect was decomposed into linear (first-order), quadratic (second-order), cubic (third-order) and quartic (fourth-order) trend components. Fixed effects that were significant at $P \leq 0.10$ have been included in this and all subsequent figures. The fumigation treatments are denoted by the same symbols in all figures.

N was lowest at litterfall, irrespective of treatment, but steadily increased as decay progressed (Fig. 1a). When concentrations were expressed as a percentage of initial litterfall N, a very strong linear N immobilization trend characterized litter decay (Fig. 1b). CO_2 enrichment increased N accumulation in bags returned to their Native Plots, where N content averaged 15% higher in elevated- vs. ambient-CO_2 litter (Fig. 1b). N content also remained 20% higher when elevated-CO_2 litter was deployed in the Common Garden plots (results not shown; CO_2 effect, $P = 0.050$). Neither experiment was characterized by a strong $CO_2 \times$ Time interaction.

Table 2 Kendall's coefficient of concordance (W) for temporal consistency of treatment rankings on tissue quality variables during birch leaf litter decay

Variable	Native Placement	Common Garden	Common Substrate
Nitrogen	0.808**	1.000***	0.136
Sugar	0.300	0.225	0.375
Starch	0.675*	0.450	0.125
Tannins	0.733	0.822	0.378
Fibre	0.152	0.072	0.024
Lignin	0.488	0.664*	0.232
C/N	0.808**	1.000***	0.232
Lignin/N	0.808**	0.936***	0.088

The coefficients were calculated for each Native Placement, Common Garden and Common Substrate experiment.
*$P < 0.05$,
**$P < 0.01$,
***$P < 0.001$.
Significance determined from χ^2 tests with 3 df.

N accumulation peaked around 295 days following litterbag deployment, at twice the initial values (Fig. 1b). Mineralization dominated N dynamics by the fourth and fifth litterbag removals (295 and 343 days, respectively), as suggested by additional curvilinear trends in the N accumulation data. These higher-order time trends were most pronounced for the Common Substrate (results not shown; first-, second- and fourth-order polynomial contrasts, $P < 0.001$), and least pronounced for the Native Plots (Fig. 1b) and Common Garden (results not shown; first-order contrast, $P < 0.001$; second-order contrast, $P = 0.009$; fourth-order contrast, $P = 0.039$).

Carbohydrates

Soluble sugar concentrations in the litterfall were not responsive to elevated-CO_2 or elevated-O_3 exposure (Table 1). Following litterbag placement, sugars decreased sharply from a mean 148 mg glucose g^{-1} (Table 1) to 6 mg glucose g^{-1} (assay detection limits) by the final bag removal (343 days). Throughout decomposition, sugar responses (results not shown) in Native Placement litters were characterized by significant two- and three-way interactions ($CO_2 \times$ Time, $P < 0.001$; $O_3 \times$ Time, $P = 0.002$; $CO_2 \times O_3 \times$ Time, $P = 0.028$). Their mean concentrations thus could not be consistently ranked among the treatments (Table 2, $P = 0.414$). Responses from the Native Placement and Common Garden (results not shown) were comparable, although interactions among factors were stronger in the former compared with the latter experiment ($CO_2 \times$ Time,

$P = 0.021$; $O_3 \times$ Time, $P = 0.043$; $CO_2 \times O_3 \times$ Time, $P = 0.078$).

Litterfall starch concentrations showed marginally negative responses to O_3 exposure, alone (Table 1; O_3 effect, $P = 0.071$) and combined with CO_2 enrichment ($CO_2 \times O_3$, $P = 0.086$). Starch did not disappear immediately following bag deployment, unlike soluble sugars. Starch declined rapidly in all treatments following the winter of 1998–1999, to assay detection limits (6 mg glucose g^{-1}) after 343 days (results not shown). Despite the absence of initial substrate quality differences attributable to CO_2 enrichment, starch tended to be highest and lowest in elevated- and ambient-CO_2 litters, respectively, deployed in the Native Placement study. Treatment rankings were more consistent in the Native Plots than in the Common Garden ($P = 0.045$ vs. $P = 0.160$), which distinguished starch from other litter properties (Table 2). Thus, starch concentrations separated into elevated-CO_2 vs. ambient-CO_2 time courses, which was reinforced by returning source litters to their original plots (results not shown). Starch in the Common Substrate (results not shown) was not altered when it was deployed in the treatment plots (Table 2, $P = 0.689$).

Condensed tannins

Condensed tannins in the litterfall were 81% higher under elevated-CO_2, relative to ambient-CO_2 conditions, but did not respond to O_3 exposure (Table 1). Initial litter groupings based on CO_2 enrichment were significant ($P = 0.001$) and persisted through 230 days in the Native Plots (Fig. 2). By 230 days, tannins were 1–5% of initial concentrations, and at subsequent removals, they were below detection limits (< 1 mg tannin g^{-1}). Across all three experiments, concordance among treatment rankings was rather weak (Table 2, $P > 0.100$) and likely due, in part, to a limited number of sample dates where tannins could be detected in appreciable amounts.

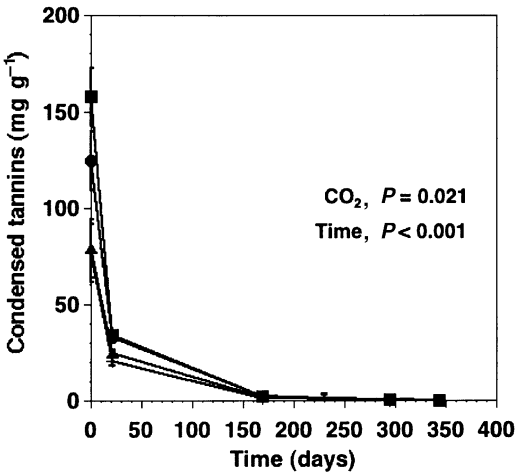

Fig. 2 Dynamics of condensed tannin (mg g^{-1}) over 343 days of birch leaf decomposition. Each value is the mean (± SE) of three replicate rings at each of five removal dates for litter decaying in the Native Plots.

Acid-detergent fibre and lignin

Acid-detergent fibre and lignin concentrations in the litterfall, like those of carbohydrates, were not directly responsive to elevated CO_2, although fibre did respond significantly and negatively to O_3 (Table 1, $P = 0.041$). Neither CO_2 nor O_3 affected subsequent fibre (results not shown) and lignin concentrations (Fig. 3a) in litter decaying in the Common Garden or Native Plots. Fibre

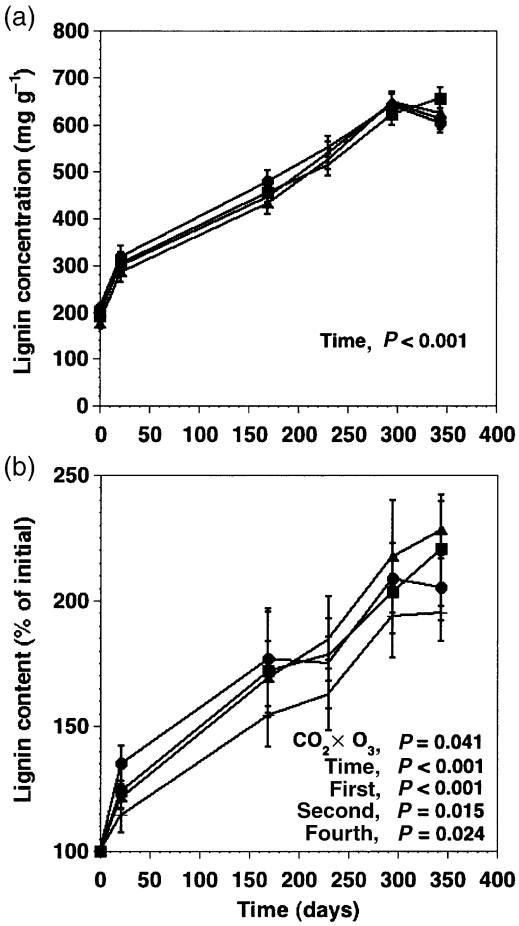

Fig. 3 Dynamics of lignin over 343 days of birch leaf decomposition. Each value is the mean (± SE) of three replicate rings at each of five removal dates, for (a) lignin concentrations (mg g^{-1}) and (b) lignin contents (% of initial concentrations) in litter decaying in the Native Plots. For lignin content, trend components are included for the main effect of Time, as in Fig. 1.

and lignin increased significantly and progressively with litter decay (Time effects, $P<0.001$). Fibre averaged 492 mg g^{-1} at the first removal (21 days), while levels nearly doubled at the final removal (343 days), averaging 839 mg g^{-1} across treatments. Lignin likewise doubled over the same interval (Fig. 3a), but its concentrations maintained a more consistent treatment order than fibre over time (Table 2, Native Placement: $P=0.066$ vs. $P=0.519$). Throughout decomposition, CO_2- and O_3-source litters tended to have highest and lowest lignin, respectively, with Control and CO_2+O_3 litters at intermediate concentrations (Fig. 3a). Lignin in the Common Substrate was influenced by O_3, but to varying degrees over the course of decay ($O_3 \times$ Time, $P=0.049$), and thus, the treatments were not ranked consistently through time (Table 2, $P=0.341$).

A different picture of lignin dynamics emerges when concentrations are expressed as a percentage of initial litterfall values (Fig. 3b). In the Native Plots, percentage lignin contents of the four source litters could be separated on the basis of a significant $CO_2 \times O_3$ interaction ($P=0.010$). Lignin content remained lowest in control litter, and highest in a group formed by elevated-O_3 and elevated-CO_2+O_3 litters (Fig. 3b). Like N, lignin showed a strong accumulation phase (Fig. 3b), as indicated by a significant linear time trend common to the three experiments ($P<0.001$). Mineralization dominated lignin dynamics in control and CO_2-enriched litters by the fourth and fifth litterbag removals, as indicated by significant curvilinear trends in lignin accumulation (Fig. 3b). These additional trends were most pronounced in the Common Substrate (not shown; second-, third- and fourth-order contrasts, $P<0.001$), and least pronounced in Native Placement (Fig. 3b) and Common Garden studies (not shown; second-order contrast, $P=0.015$; fourth-order contrast, $P=0.024$).

C/N and lignin/N

CO_2 enrichment increased litterfall ratios of C/N ($P=0.017$) and lignin/N ($P=0.034$), primarily through its negative effects on N concentrations (Table 1). Moreover, O_3 alone did not alter these ratios, either at litterfall or throughout the course of decomposition. CO_2 enrichment produced litter with C/N that was 42% higher than the controls. Source litters also could be separated into ambient-CO_2 and elevated-CO_2 groups based on their lignin/N ratios. Elevated CO_2 produced the poorest quality litter, with a lignin/N that was 54% higher than the controls (Table 1).

C/N declined throughout litter decomposition, mirroring the progressively increasing tissue concentrations of N. Differentiation in C/N ratios among source litters was attributable neither to initial differences in C (results not shown; $P=0.340$) nor to substantive alterations in C concentrations throughout decomposition. C/N was consistently higher for elevated-CO_2 vs. ambient-CO_2 source litters that were placed in the Common Garden (results not shown), a trend that resulted in the absolute ranking of treatment means over time (Table 2: Common Garden, $P<0.001$). Placement of source litters back into their original plots did not substantially weaken the C/N rankings observed in the Common Garden experiment (Table 2: Native Placement, $P=0.007$). Also, environmental differences among the FACE rings were not strong enough to modify C/N dynamics of the Common Substrate (Table 2: Common Substrate, $P=0.341$).

The four source litters clearly separated into two groups, based on their lignin/N ratios, whether they were decayed in their Native Plots (Fig. 4) or in the Common Garden (results not shown). The ratios gradually increased through time, but lignin/N remained lowest in control and elevated-O_3 litters, and highest in those litters produced under elevated CO_2 and elevated CO_2+O_3 (Fig. 4). These trends reflected CO_2 enrichment of the original source materials. Differences between low lignin/N (high-quality) and high lignin/N (low-quality) litter were maintained from the first removal (20.9 vs. 26.4) to the final removal (24.5 vs. 31.6), despite different curvilinear patterns of increase and decrease within each treatment through time (Table 2, $P=0.007$). While consistent treatment groupings also were strongly maintained throughout the Common Garden study (Table 2, $P<0.001$), the

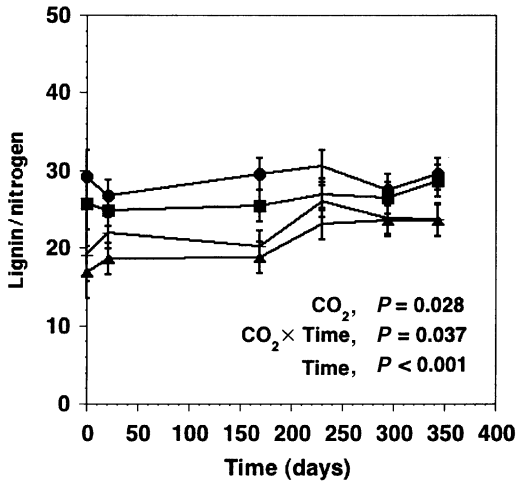

Fig. 4 Dynamics of lignin/N over 343 days of birch leaf decomposition. Each value is the mean (± SE) of three replicate rings at each of five removal dates for litter decaying in the Native Plots.

Common Substrate exhibited no such characteristic ordering (Table 2, $P = 0.637$). These latter results likely were because of strong and variable environmental effects on the lignin/N of the Common Substrate (results not shown; $CO_2 \times O_3 \times$ Time, $P = 0.028$).

Mass loss

Litter mass decreased strongly with time in the Native Placement, Common Garden and Common Substrate studies (Fig. 5). Mass loss dynamics of the four source litters were strongly affected by CO_2 enrichment (Fig. 5a, b). Litter originating from control and O_3-exposed plots lost the most mass, while litter from the CO_2-enriched plots lost the least mass over time. Substrate differences were more pronounced in the Native Placement (Fig. 5a) than in the Common Garden experiment (Fig. 5b). Returning litter to its original plot reinforced the effects of CO_2 enrichment on retarding mass loss, which increasingly varied over time ($CO_2 \times$ Time interaction). Mass loss of the Common Substrate did not respond significantly to elevated-CO_2 or elevated-O_3 exposure (Fig. 5c), suggesting that it was not affected by environmental variation among the treatment rings.

Decomposition rates (k) were estimated from ANCOVA (Table 3). Time, the covariate in the analysis, had the strongest effect ($P < 0.001$), explaining about 78% of variation in mass loss. However, slopes (k-values) differed significantly among regression lines (Table 3; Treatment $\times$ Time, $P < 0.001$), which had a common Y-intercept (98.4% remaining; $P = 0.999$). Among the four source litters, mass loss dynamics were strongly driven by CO_2 enrichment, which decreased decay rates relative to the controls, both in the Native and Common Garden Plots (Table 3). Thus, MRT of the leaf litter was increased by CO_2 enrichment. Under elevated CO_2, 95% of the litter disappeared in 4.4 years, while the same mass of source material that originated from the controls and O_3-exposed plots was lost in about 3.5 years (Table 3). ANCOVA indicated that the mass loss rate for the Common Substrate was highest when it was placed in the O_3-fumigated plots (0.961 year^{-1}), lowest when it was placed into the control plots (0.843 year^{-1}) and that these differences in decay rates were significant (Table 3).

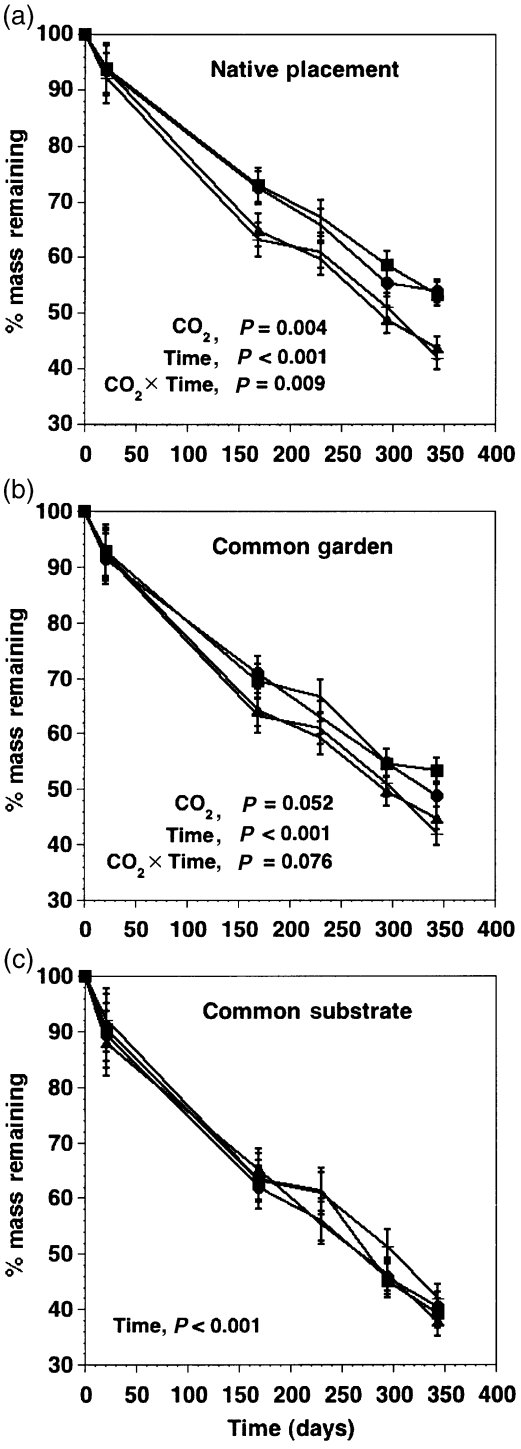

Fig. 5 Dynamics of mass loss (% mass remaining) over 343 days of birch leaf decomposition. Each value is the mean (± SE) of three replicate rings at each of five removal dates for litter decaying in the (a) Native Placement, (b) Common Garden and (c) Common Substrate experiments.

Discussion

Litter quality

Our studies demonstrated that, consistent with expectation, birch litter quality was diminished strongly by CO_2 enrichment. Initially, N was highest in litter that originated from the controls and lowest in litter from the elevated-CO_2 rings, while the converse was true for C/N and condensed tannins. Litter produced under

Table 3 Mass loss rates and maximum residence times (time-to-95%-loss) of decaying birch leaf litter in Native Placement, Common Garden and Common Substrate experiments at Aspen free-air CO_2 enrichment (FACE)

Placement plot	Origin plot	k (year^{-1})	$t_{0.95}$ (years)
Native Placement			
Ambient $CO_2 + O_3$	Ambient $CO_2 + O_3$	0.843^{b}	3.6
Elevated CO_2	Elevated CO_2	0.683^{a}	4.4
Elevated O_3	Elevated O_3	0.879^{bc}	3.4
Elevated $CO_2 + O_3$	Elevated $CO_2 + O_3$	0.669^{a}	4.5
Common Garden			
Ambient $CO_2 + O_3$	Ambient $CO_2 + O_3$	0.843^{b}	3.6
Ambient $CO_2 + O_3$	Elevated CO_2	0.700^{a}	4.3
Ambient $CO_2 + O_3$	Elevated O_3	0.811^{b}	3.7
Ambient $CO_2 + O_3$	Elevated $CO_2 + O_3$	0.643^{a}	4.7
Common Substrate			
Ambient $CO_2 + O_3$	Ambient $CO_2 + O_3$	0.843^{b}	3.6
Elevated CO_2	Ambient $CO_2 + O_3$	0.910^{bc}	3.3
Elevated O_3	Ambient $CO_2 + O_3$	0.961^{c}	3.1
Elevated $CO_2 + O_3$	Ambient $CO_2 + O_3$	0.915^{bc}	3.3

Slope coefficients (k) were estimated from ANCOVA, by regressing $\log_e$ (% mass remaining) against time (years); k-values followed by the same letter did not differ significantly at $P \leq 0.10$.

elevated $CO_2 + O_3$ had the lowest starch levels among the four treatments. In this single instance, interactions between the two trace gases were nonadditive and antagonistic, since starch under elevated $CO_2 + O_3$ did not equal averaged responses to CO_2 and O_3 alone. Starch persisted longer than sugars and tannins, especially during early decay stages of the $CO_2 + O_3$ litter, but O_3 exposure did not alter its concentrations beyond those of the controls.

Like C/N, lignin/N ratios of the initial litterfall were higher under elevated than ambient CO_2. Surprisingly, CO_2 enrichment did not increase lignin concentrations of foliage and litter, a result contrary to the trend identified in the meta-analysis of Coûteaux *et al*. (1999). Also, Cotrufo *et al*. (1994) and Cotrufo & Ineson (1996) found that elevated CO_2 (ambient $+250\,\mu L\,CO_2\,L^{-1}$) raised lignin concentrations in *B. pubescens* leaves, almost doubling the lignin/N of litter deployed in their experiments. Significant increases in lignin/N that we observed under similar levels of CO_2 enrichment, however, were attributable to treatment-related differences in [N]. Indeed, leaf N dynamics likely drove litter quality differences in paper birch more strongly than did C or lignin concentrations, since neither variable differed among fumigation treatments.

Products of the shikimic acid pathway other than lignin typically increase in response to high CO_2 concentrations (Lindroth *et al*., 1993; Poorter *et al*., 1997). Whether this trace gas effect generally carries over from foliage to litter to exert control on decay processes remains questionable, but we found a consistent seasonal ordering of treatment effects on condensed tannins and N from leaf flush to senescence for birch trees growing in the Aspen FACE (Lindroth *et al*., 2001). Treatment-related differences in chemical quality at leaf abscission were strongly maintained through first litterbag removal, especially for condensed tannins (Spearman's rank correlation: $r_s = 0.952$, $P = 0.004$) and lignin/N ($r_s = 0.994$, $P = 0.005$). N was weakly correlated between litterfall and first bag removal ($r_s = 0.556$, $P = 0.096$), while starch was not significantly correlated at all ($P = 0.355$). In a recent study of sugar maple leaf decay under elevated CO_2, King *et al*. (2002) obtained strong positive rank correlations between live foliage and the corresponding litter for condensed tannins and N, and no correlation for starch. These results, together with our own observations, lend credence to the litter quality hypothesis, although King *et al*. (2002) found that differences in leaf quality did not translate into lower decay rates for maple litter produced under CO_2 enrichment.

The Common Garden experiment revealed differences in substrate quality among the source litters in the absence of environmental variation contributed by the treatment plots themselves. CO_2 enrichment affected birch foliar and litter chemistry more strongly than did O_3 exposure for most chemical quality variables, affirming our initial hypothesis. Native Placement incorporated variation in decomposition microenvironments imposed by tree growth and development, which could have confounded substrate quality differences imparted by fumigation. Under elevated CO_2, trees attained greater size, and a greater degree of canopy closure, and contributed more litterfall to the development of a forest floor than did trees in the control rings, while O_3 exposure had opposite effects (Karnosky *et al*., 2003). Redeployment of litter in the original plots did not seriously modify their distinctive signatures, thus suggesting a weak environment by substrate–quality interaction. These results were surprising, since Karnosky *et al*. (2003) reported that seasonal soil moisture was elevated in the treatment rings relative to the controls, leading to environments potentially more favourable to microbial decay processes. Lower soil moisture levels may also explain the lower k-value obtained for the Common Substrate in its Native Plots. Moreover, seasonal temperatures of the surface litter were highest, and diel fluctuations the most extreme, in the elevated-O_3 rings (W. F. J. Parsons, unpublished data), which may explain why the Common Substrate disappeared most rapidly under O_3 exposure.

Mass loss

Variation in decomposition rates of birch leaf litter was strongly influenced by CO_2 enrichment, consistent with our initial predictions. Our results also suggest a species-specific response for paper birch to elevated CO_2, since similar studies have not reported lowered decay rates for other species native to eastern North America (Boerner & Rebbeck, 1995; Scherzer *et al*., 1998; Finzi *et al*., 2001; King *et al*., 2001, 2002; Finzi & Schlesinger, 2002). CO_2 enrichment depressed *k*-values, whereas O_3 neither retarded nor accelerated birch decay. Mass loss was lowest for $CO_2 + O_3$-fumigated birch leaves placed in the Native Plots, but statistically significant differences in *k* were not evident between our elevated-CO_2 and elevated-$CO_2 + O_3$ treatments. Consequently, elevated CO_2 but not O_3 increased litter residence times. For control litter, 5% of mass remained after 3.6 years, while CO_2-enriched litter took $\sim$4.5 years to turn over 95% of its mass. An additional year of decay on the forest floor is certain to affect rates of C transfer to the underlying mineral soil.

For similar litter types in common environments, N, lignin and tannins appear to act as 'a hierarchical series of controls' (Anderson, 1991). In high-quality (i.e., low C/N), readily decomposable leaves, tannins may be rate retardants, whereas in low-quality (high C/N), slowly decomposable leaves, lignin or lignin/N may serve to slow decay (Anderson, 1991 and references therein). Birch leaves produced under elevated CO_2 and $CO_2 + O_3$ did not conform to this pattern; in our study, high C/N litter also had high levels of tannins. Condensed tannins and other litter quality variables showed persistent treatment-related differences that, as shown by correlation analyses, strongly controlled mass loss over time. The *k*-values were inversely correlated with initial litter tannins ($r = -0.869$, $P < 0.001$) and C/N ($r = -0.862$, $P < 0.001$), which were, in turn, uncorrelated ($P = 0.598$). An abrupt downward shift in *k* occurred when it was plotted against increasing C/N or lignin/N, with the break point coinciding with CO_2 enrichment ($r = -0.885$, $P < 0.001$). CO_2 enrichment alone influenced partitioning of photosynthate to structural, storage and defence compounds, thereby imparting distinctive chemical signatures to leaf litter that persisted throughout decomposition. Both the short duration of the current study and young age of the aggrading stands argue, however, that CO_2–O_3 interactions, or the indirect effects of O_3 and CO_2, should not be discounted in further experiments, as suggested by our Common Substrate study.

Schlesinger & Lichter (2001) suggested that incorporation of C into long-term pools and, therefore, the potential size of forest C sinks will be constrained by the turnover times of the short-term C pools, i.e., leaf litter and fine root inputs. Rising atmospheric CO_2 will likely elicit species-specific effects on litter production and decomposition in forested ecosystems, retarding decay rates in some species such as paper birch, while potentially exerting little effect on others. Moreover, rising CO_2 will not exert its influence on litter decay rates in isolation from other factors, although results from this study show that O_3 will have little if any modulating effect on birch decomposition. Our results indicate that atmospheric CO_2 concentrations predicted for this century may markedly alter the chemical composition and decomposition dynamics of at least one early successional, temperate tree species. Such changes are likely to have important implications for our understanding of the processes regulating the storage and release of C from forest ecosystems.

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