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# Correlation of foliage and litter chemistry of sugar maple, *Acer saccharum*, as affected by elevated $CO_2$ and varying N availability, and effects on decomposition

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Rising atmospheric carbon dioxide has the potential to alter leaf litter chemistry, potentially affecting decomposition and rates of carbon and nitrogen cycling in forest ecosystems. This study was conducted to determine whether growth under elevated atmospheric $CO_2$ altered the quality and microbial decomposition of leaf litter of a widely distributed northern hardwood species at sites of low and high soil nitrogen availability. In addition, we assessed whether the carbon–nutrient balance (CNB) and growth differentiation balance (GDB) hypotheses could be extended to predict changes in litter quality in response to resource availability. Sugar maple (*Acer saccharum*) was grown in the field in open-top chambers at 36 and 55 Pa partial pressure $CO_2$, and initial soil mineralization rates of 45 and 348 μg N $g^{-1}$ $d^{-1}$. Naturally senesced leaf litter was assessed for chemical composition and incubated in the laboratory for 111 d. Microbial respiration and the production of dissolved organic carbon (DOC) were quantified as estimates of decomposition. Elevated $CO_2$ and low soil nitrogen resulted in higher litter concentrations of nonstructural carbohydrates and condensed tannins, higher C/N ratios and lower N concentrations. Soil N availability appears to have had a greater effect on litter quality than did atmospheric $CO_2$, although the treatments were additive, with highest concentrations of nonstructural carbohydrates and condensed tannins occurring under elevated $CO_2$–low soil N. Rates of microbial respiration and the production of DOC were insensitive to differences in litter quality. In general, concentrations of litter constituents, except for starch, were highly correlated to those in live foliage, and the CNB/GDB hypotheses proved useful in predicting changes in litter quality. We conclude the chemical composition of sugar maple litter will change in the future in response to rising atmospheric $CO_2$, and that soil N availability will exert a major control. It appears that microbial metabolism will not be directly affected by changes in litter quality, although conclusions regarding decomposition as a whole must consider the entire soil food web.

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There is little doubt that conditions for plant growth are changing due to human activity. Changes in land use have the greatest direct impact on plant and animal communities (Vitousek 1994), but society is also rendering more subtle, yet continuous changes to global element cycles that may ultimately have a more extensive

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influence on ecosystem function. By increasing the availability of limiting resources for plant growth, human activities may be changing complex feedback relationships with unforeseen results. In particular, concentrations of atmospheric carbon dioxide ($CO_2$) and rates of nitrogen deposition are projected to continue rising well into the next century (Galloway et al. 1995, Houghton et al. 1996), and both are expected to directly affect net primary production and ecosystem C-N cycling. Hypothesized effects range from alteration of competitive relationships and community dynamics (Patterson and Flint 1980, Strain and Bazzaz 1983, Wray and Strain 1987), accelerated rates of insect herbivory (Lincoln et al. 1993, Williams et al. 1994), greater net primary productivity (Eamus and Jarvis 1989, Amthor 1995), and greater C sequestration in soils through reduced rates of decomposition as a result of altered litter quality (Lambers 1993, van de Geijn and van Veen 1993, Cotrufo et al. 1994).

Two main hypotheses have guided our thinking about how changes in resource availability will affect plant secondary metabolism and consequent chemical composition of tissue, as reviewed recently by Koricheva et al. (1998). The carbon–nutrient balance (CNB) hypothesis (Bryant et al. 1983) postulates that concentrations of carbon-based secondary compounds (CBSC) increase under conditions favoring carbohydrate accumulation in excess of that needed for growth. This hypothesis predicts lower concentrations of CBSCs when growth is enhanced more than photosynthesis (e.g. under fertilization), but higher concentrations when photosynthesis is stimulated more than growth (high light, elevated $CO_2$). Very similar predictions are obtained by the growth differentiation balance (GDB) hypothesis (Loomis 1932, Lorio 1986, Herms and Mattson 1992). This model states that growth is generally limited by water and nutrients, whereas differentiation (chemical and morphological changes that occur in maturing cells) depends on available carbohydrates. Therefore, production of CBSCs (a differentiation process) is enhanced when factors other than photosynthate supply are sub-optimal for growth. In general, plant responses are consistent with the two hypotheses when considering pooled CBSCs and carbohydrates, but less so for biosynthetically distinct compounds, such as hydrolyzable tannins and terpenoids (Koricheva et al. 1998). CBSCs are considered a major defense mechanism of plants against herbivores (Feeny 1976, Rhoades and Cates 1976), but little is known about the relationship between altered foliage chemistry and the quality of leaf litter as a substrate for soil microorganisms (Findlay et al. 1996).

Strain and Bazzaz (1983) hypothesized that rates of N and P mineralization might be reduced due to slower decomposition of plant litter produced under elevated atmospheric $CO_2$, caused primarily by increased C/N and C/P ratios, or higher amounts of protective polyphenols. We refer to this as the "negative feedback" hypothesis. Since that time a vigorous debate has ensued in the literature, with researchers reporting results that both support, and fail to support, this hypothesis. In a recent review of the $CO_2$-decomposition literature Norby and O'Neill (1996) found that many of the earlier studies performed with small potted plants, often grown in growth chambers, exhibited reduced litter quality and slower rates of decomposition, while larger-scale experiments (open-top chambers) found little or no effect. Thus, experimental approach must be considered when interpreting results. More recently, scientists convening at an international symposium addressing the effects of elevated $CO_2$ on litter decomposition concluded there is insufficient evidence to support the negative feedback hypothesis (Norby and Cotrufo 1998). As researchers continue to report results that both support (Ball and Drake 1997, Prior et al. 1997; Robinson et al. 1997, Cotrufo et al. 1998, Torbert et al. 1998, Van Ginkel and Gorissen 1998) and fail to support (Coûteaux et al. 1991, Cotrufo et al. 1994, Cotrufo and Ineson 1995, Norby and O'Neill 1996, Randlett et al. 1996, King et al. 2001) the hypothesis, it is clear that a quantitative synthesis (i.e. meta-analysis) of the $CO_2$-decomposition literature, as well as further experimentation at more realistic scales (e.g. free air carbon dioxide enrichment) are warranted. Furthermore, as different stages of the decomposition process may respond differently to elevated $CO_2$ (Hättenschwiler et al. 1999), we must be cognizant of effects specific to both micro- and macro-decomposers in early and late stages of decay.

The current work has two goals. First, to ascertain whether growth under elevated atmospheric $CO_2$ will alter the microbial decomposition of leaf litter of a widely distributed north temperate tree species under conditions of both high and low N availability. Second, by comparing the chemical composition of leaves at the peak of physiological activity and after senescence, we test whether predictions of the CNB and GDB hypotheses can be extended to litter, at least for sugar maple (*Acer saccharum* Marsh.). These hypotheses may form a useful conceptual framework for considering how changes in environmental factors will affect the cycling of C in forest ecosystems as mediated by soil microbial communities. Sugar maple is highly shade-tolerant and has determinate growth (Barnes and Wagner 1981). It is characterized by relatively low rates of photosynthesis and growth, and production of CBSCs is stimulated by elevated atmospheric $CO_2$ (Lindroth 1996). In North America, it is distributed from Nova Scotia, west to Ontario and Minnesota, south to Missouri and east to Tennessee and North Carolina (Godman et al. 1990). Across its range it is an important commercial species, and is used for hardwood lumber and maple syrup.

To examine the interactive effects of elevated atmospheric $CO_2$ and varying soil N availability on sugar maple leaf litter quality and decomposition, we conducted an open-top chamber experiment at the University of Michigan Biological Station. After the second growing season under the experimental treatments, naturally senesced leaf litter was collected from the chambers and incubated in the laboratory, where rates of decomposition were related to chemical quality. In accordance with the CNB/GDB hypotheses, we expected to see higher concentrations of nonstructural carbohydrates in litter produced under elevated $CO_2$ or low N availability. We hypothesized this would be accompanied by higher concentrations of CBSCs in litter and reduced rates of decomposition.

## Methods

### Field site

In the spring of 1997, a randomized complete block design of $CO_2$ and soil N treatments was established in an open-top chamber facility at the University of Michigan Biological Station, near Pellston, MI. The 20 open-top chambers measured 3 m in diameter, and were of a design commonly used in elevated $CO_2$ research (Rogers et al. 1983, Pregitzer et al. 1995, Tissue et al. 1996). The $CO_2$ fumigation system has been described previously (Curtis et al. 2000, King et al. 2001). Briefly, it consisted of a centrally located intake line that monitored ambient atmospheric $CO_2$ concentration and sample lines and $CO_2$ dispensing lines distributed to each elevated $CO_2$ chamber (10 chambers). Ambient and treatment sample lines were switched to a Li-Cor 6262 infra-red gas analyzer by a computer control system that also recorded 2-min averages of $CO_2$ concentration every 22 min over a 24-h period. Carbon dioxide dispensing lines ran from a common manifold connected to a 6-ton liquid $CO_2$ reservoir, through individual volume flow regulators, and out to each elevated $CO_2$ chamber. Carbon dioxide concentrations in each chamber were monitored over the course of the day and flow rates adjusted manually to maintain the treatment differential at + 20 Pa ($\pm$ 10%).

Chambers were fumigated continuously from 17 April 1998 to 23 October 1998. Mean daytime ambient and elevated $CO_2$ concentrations (standard deviation) were 36.85 Pa (2.51) and 55.92 Pa (2.73), respectively. The chambers were installed above open-bottom root boxes (3.5 m $\times$ 3.5 m $\times$ 0.5 m) filled with one of two soil mixes (Pregitzer et al. 1995). The soil mixes resulted in levels of N-availability characteristic of the range found in the northern Great Lakes region (Zak and Pregitzer 1990). The high N-availability soil consisted of a homogenized A-horizon of a Kalkaska series soil (sandy, mixed, frigid, Entic Haplorthod) having a net initial N-mineralization rate of 348 $\mu g\ N\ g^{-1}\ d^{-1}$. The low N-availability soil consisted of a 4:1 mix of Rubicon series C-horizon soil (sandy, mixed, frigid, Entic Haplorthod) with A-horizon of the Kalkaska series soil and had a net initial N-mineralization rate of 45 $\mu g\ N\ g^{-1}\ d^{-1}$. Other physical and chemical properties of the soil mixes can be found in Curtis et al. (2000).

Maple seed was collected from a wild source in Baraga County, Michigan, in autumn 1996, refrigerated moist, and propagated in March 1997. Twelve sugar maple seedlings approximately 10 cm tall were planted in the chambers in early spring 1997 in association with 12 trembling aspen (*Populus tremuloides* Michx.), and the chambers were maintained free of understory vegetation. Because the growth of aspen was much more rapid than that of sugar maple, analysis of litter chemistry and decomposition for that species was performed in the first year of the study (King et al. 2001). Sugar maple was allowed to grow for the entire 1997 and 1998 growing seasons before sampling was conducted for the current study. Live foliage was sampled at the peak of physiological activity (30 August 1998) by randomly selecting a fully expanded leaf from the middle of the canopy of four randomly selected trees. This material was flash frozen with dry ice, lyophilized, ground to pass a 0.5-mm mesh, and stored at $-20°C$. Near the end of the season, all senesced foliage (litter) that had fallen to the floor of the chambers was collected each morning and allowed to air dry in a nearby greenhouse. All litter was composited by chamber and ground in a Wiley mill to pass a 4-mm mesh that created large fragments simulating coarse leaf litter. A subset of this material was ground to pass a 0.5-mm mesh, and chemical determinations for the live foliage and litter were carried out immediately thereafter.

### Laboratory incubations

To assess litter quality effects on decomposition, laboratory incubations were initiated using the coarsely ground, air-dried litter in January 1999. One gram of this material was placed in the top compartment of modified microlysimeters (Falcon Filter Unit # 7102, Becton Dickinson and Co., Cockeysville, MD; Zak et al. 1993). Fiberglass screen with 1 mm mesh separated litter from 40 g of homogenized sand that had been inoculated with sieved A-horizon soil collected from a forest adjacent to the field site. The sieved soil mix was placed on glass-fiber filter paper (1.7 $\mu m$ pore size) that divided the top and bottom portions of the microlysimeters. The four outlets in the microlysimeters (2 top, 2 bottom) were closed with butyl rubber septa and all seams were sealed with silicone

sealant. The incubations were started by initially extracting the litter and soil with 50 ml 0.01 M $CaCl_2$ (Herbert and Bertsch 1995, Randlett et al. 1996), flushing the units with $CO_2$-free air, and placing them in an incubator at 25°C.

The incubation was started with eight complete sets of microlysimeters (25 units/set), which included five replicates of each field treatment ($CO_2$, soil N), and five blanks that contained soil mix but no litter. One set was destructively harvested every two weeks. At each harvest, a sample of headspace gas from each unit was collected through a septum with a syringe and temporarily stored in a sealed 3-ml serum vial. Litter and soil within the units was extracted with 50 mL of 0.01 M CaCl solution (Herbert and Bertsch 1995), which was collected in the bottom half of the microlysimeters by placing them under a vacuum. The extracts were collected with a large syringe, passed through a 0.45-μm filter into sample bottles, acidified to pH 2–3 with HCl and stored at 4°C to inhibit microbial growth. Tissue samples were collected by removing the fiberglass screen to which most of the solid material adhered. These were placed in paper envelopes, frozen, and freeze-dried to constant mass. All remaining units were extracted with 0.01 M $CaCl_2$, fertilized with 25 mL of a dilute nutrient solution containing 0.002 M $CaCl_2$, 0.002 M $MgCl_2$, 0.005 M KCl, and 0.005 M $Ca(H_2PO_4)_2$ to replace nutrients lost during the extractions, flushed with $CO_2$-free air, and returned to the incubator (Randlett et al. 1996).

## Chemical determinations

Carbon dioxide concentrations of the headspace gas were determined by injecting samples into a Tracor Model 540 gas chromatograph (San Jose, CA) equipped with a thermal conductivity detector. Concentrations of total dissolved organic carbon (DOC) in the $CaCl_2$ extracts were determined using a Shimadzu TOC-5000A (Wooddale, IL) total organic carbon analyzer, which oxidizes the sample with a Pt on alumina catalyst (680°C) to quantify total carbon content in samples from which inorganic carbon (i.e. $CO_2$) has been removed. Total carbon (% C) and total nitrogen (% N) in the decomposed litter samples were measured using a Carlo Erba NA1500 Series II elemental analyzer (Beverly, MA) run with National Institute of Standards and Technology pine needle and peach leaf standards.

Total nonstructural carbohydrates (starch and soluble sugars) in the decomposed leaf samples were quantified using the method of Tissue and Wright (1995). Samples were extracted with methanol: chloroform:water to release soluble sugars and the remaining pellet was digested with 35% perchloric acid to hydrolyze starch into soluble sugars. Both soluble and insoluble sugars (i.e. starch) were quantified colorimetrically by comparing sample absorption to that of glucose standards. Condensed tannins were chosen to characterize the response of C-based secondary compounds because they are known to respond to elevated atmospheric $CO_2$ in sugar maple foliage (Lindroth 1996) and play an important role in the decomposition process (Horner et al. 1988, Tiarks et al. 1992, Schimel et al. 1996). Condensed tannins were determined after hydrolysis in acid butanol (Porter et al. 1986, Hagerman and Butler 1989). Samples (75 mg) were extracted with an acetone-ascorbic acid mixture. Extracts (500 μl of sample) were treated with acid butanol and ferric-ammonium sulfate activated with HCl, and heated at 100°C for 50 min. Absorbance of the extracts was read with a spectrophotometer and compared to a standard curve prepared from purified Quebracho tannin. Because the reactivity of condensed tannins varies by species, concentrations expressed here as Quebracho equivalents should only be used as an index of relative treatment responses and not expressions of absolute amounts.

## Statistical analyses

Effects of $CO_2$ and soil N availability on tissue chemistry and decomposition were tested using two-way analyses of variance (ANOVA) for a randomized complete-block design. Effect of physiological status (live foliage vs litter) on chemical parameters was tested by including a "state" variable in the model (live or dead). This split-plot ANOVA converted the analysis to a repeated measures design, with the split-plot effect corresponding to time (Meredith and Stehman 1991). The experimentwise error rate for the multiple statistical tests on the decomposition data was controlled by dividing the overall level of confidence ($\alpha$) by the number of tests on each response variable. Differences in rates of decline of litter constituent concentrations were tested by fitting the semi-log regression model $\ln C = -kt + \ln(C_0)$, where $C_0$ and $C$ are the constituent concentrations at time = 0 and subsequent time periods, respectively, $-k$ is the constant, fractional loss rate and $t$ is time (d). Rearranging and taking antilogarithms of both sides of this equation expresses the fraction remaining as the familiar exponential decay model $C/C_0 = e^{-kt}$ (Olson 1963). Differences in rates of decline were indicated by significant interactions between the experimental treatments ($CO_2$ or N availability) and time. Inspection of residuals and normal probability plots ensured data conformed to the assumptions of ANOVA, and when necessary, data were log or square root transformed to normalize variances across treatments (Sokal and Rohlf 1995). The overall level of confidence was maintained at $\alpha = 0.05$.

## Results

### Foliage and litter quality

Production of foliage as estimated by total litter biomass ranged from 107.6 g to 229.9 g, and increased significantly (Table 1) due to both elevated $CO_2$ (42%) and high soil N availability (45%). Concentrations of soluble sugars ranged from 49.4 to 89.0 mg/g in foliage, and 20.7 to 43.3 mg/g in litter and exhibited a significant $CO_2 \times N \times$ Time interaction. The response to elevated $CO_2$ was modified by level of N availability, and the transition to litter strongly reduced soluble sugar concentrations (Table 1). Under ambient $CO_2$, the reduction in soluble sugar concentration due to high soil N availability was 25% for foliage and 52% in litter, whereas under elevated $CO_2$ the reduction in foliage was 44% while that in litter was 24%. Starch concentrations ranged from 72.4 to 108.0 mg/g in foliage and 68.2 to 81.0 mg/g in litter and, in contrast to soluble sugars, were affected to a similar extent by $CO_2$, soil N availability and time main effects. Averaged over the other factors, elevated $CO_2$ resulted in a 14% increase in starch, while high soil N availability resulted in a 13% decline. Similarly, the transition to litter resulted in an average 16% decline in starch concentrations.

Condensed tannin concentrations were markedly different between foliage (195 to 588 mg/g) and litter (1.31 to 3.31 mg/g) (Table 1). All treatment main effects were highly significant, and significant two-way interactions with time would seem to indicate that responses to the $CO_2$ and soil N availability treatments were different for foliage and litter. However, elevated $CO_2$ caused a 39% increase in condensed tannins in foliage and a 34% increase in litter. Similarly, high soil N availability caused a 49% decrease in foliage and a 46% decrease in litter. We believe this apparent inconsistency between the statistical tests and responses stems from the overwhelmingly large effect of time on condensed tannin concentrations, making interpretation of interactions between time and the other factors in the split-plot analysis problematic. Independent ANOVAs on the foliage and litter confirm the $CO_2$ and soil N availability main effects, with no significant $CO_2 \times N$ interactions (data not shown), and from the data presented here it is obvious the effects of time were highly significant.

Nitrogen concentration ranged from 11.96 to 21.08 mg/g in foliage and 4.96 to 10.12 mg/g in litter, and all main effects were highly significant (Table 1). A significant $CO_2 \times$ Time interaction was due a greater reduction in N concentration in elevated compared to ambient $CO_2$-grown foliage (18%) vs litter (15%). The N main effect resulted from much higher concentrations of N in foliage (43% greater) and litter (75% greater) produced at high soil N availability. C/N ratios ranged from 22.16 to 40.05 in foliage and 43.60 to 86.60 in litter, and all main effects were highly significant (Table 1). The increase in C/N ratio due to elevated $CO_2$ (litter and foliage) ranged from 22 to 26% at both high and low soil N availability, except in litter at low N, which exhibited a 6% decrease, resulting in the significant $CO_2 \times N \times$ Time interaction.

### Litter decomposition

Soluble sugar concentrations declined to less than 10 mg/g after d 28, and approached zero by the end of the incubation (Fig. 1A). Effects of elevated $CO_2$ and soil N availability were not significant (Table 2), the differences in soluble sugar concentrations between treatments being nominal (Fig. 1A). Starch concentrations declined 39% to an average of 47.04 mg/g after 111 d (Fig. 1B). Treatment effects on starch concentrations were not significant at any time during the incubation (Table 2).

Initially, concentrations of condensed tannins in litter were affected by atmospheric $CO_2$ and soil N availability (Table 1). Over the course of the incubation, however, only the N availability treatment remained significant (Table 2). Litter produced at low N had consistently higher concentrations, until the last harvest at 112 d (Fig. 1C). Condensed tannin concentrations declined exponentially, and regression analysis indicated that the rate of decline was significantly greater for litter produced at low compared to high N availability ($P = 0.015$).

Nitrogen concentration of the initial, and decomposing litter was significantly affected by both the N availability and atmospheric $CO_2$ treatments (Fig. 2A, Tables 1, 2). Nitrogen concentration was 60% higher, on average, in litter produced at high compared to low N availability. It was 16% lower, on average, in litter produced at elevated compared to ambient $CO_2$. A $CO_2 \times N$ interaction occurred due to a much greater effect of elevated $CO_2$ at high compared to low N availability (Fig. 2A). Although this appears to have been consistent throughout the incubation, variation in the data was such that it was statistically significant on only one of the eight harvest dates (Table 2). N concentration increased on average 62% over the incubation, and regression analysis indicated the rate of increase did not differ between treatments ($P = 0.439$).

$CO_2$ and N availability main effects were highly significant for C/N ratio in the initial litter, but only N availability remained significant throughout the incubation (Fig. 2B, Table 2). Litter produced at low N had on average 70% higher C/N ratios than that produced at high N throughout the incubation, while the increase due to elevated $CO_2$ averaged only 14%. C/N ratio decreased on average 42% during the incubation, and regression analysis indicated that rates of decline in C/N ratio did not differ between N availability treatments ($P = 0.454$).

Table 1. Means (standard error of the mean; $N = 4$ or 5) and statistical significance ($P$-values) of biomass and chemical constituents of sugar maple foliage and leaf litter produced at two levels of atmospheric $CO_2$ and two levels of soil N availability at the Univ. of Michigan Biological Station. Live foliage was collected on 30 August 1998 and litter was collected after leaf fall.

| Treatment | Total litter biomass[λ] | Soluble sugars | | Starch | | Condensed tannins[§] | | N | | C/N | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | L | F | L | F | L | F | L | F | L | F | L |
| Ambient $CO_2$ Low N | 107.63 | 78.46 | 43.24 | 94.70 | 80.58 | 483.45 | 2.53 | 15.10 | 5.50 | 32.01 | 86.60 |
| | (9.56) | (2.97) | (4.04) | (10.67) | (4.47) | (51.45) | (0.09) | (0.40) | (0.28) | (0.97) | (4.56) |
| Ambient $CO_2$ High N | 171.71 | 59.12 | 20.75 | 72.42 | 68.20 | 195.93 | 1.31 | 21.08 | 10.12 | 22.16 | 43.60 |
| | (33.16) | (3.80) | (0.78) | (6.71) | (0.96) | (11.79) | (0.09) | (0.45) | (0.42) | (0.66) | (2.36) |
| Elevated $CO_2$ Low N | 168.37 | 89.03 | 43.64 | 108.06 | 78.00 | 588 | 3.31 | 11.96 | 4.96 | 40.05 | 80.96 |
| | (46.70) | (0.55) | (4.62) | (2.45) | (5.78) | (49.35) | (0.29) | (0.38) | (0.33) | (1.37) | (8.97) |
| Elevated $CO_2$ High N | 229.94 | 49.46 | 33.32 | 93.64 | 81.00 | 354.57 | 1.83 | 17.54 | 8.26 | 27.09 | 55.09 |
| | (30.08) | (8.26) | (2.77) | (7.66) | (11.27) | (43.89) | (0.08) | (0.30) | (0.13) | (0.50) | (0.62) |
| Source | | | | | | | | | | | |
| $CO_2$ | 0.048 | 0.269 | | 0.045 | | 0.000 | | 0.000 | | 0.005 | |
| N | 0.026 | 0.000 | | 0.040 | | 0.000 | | 0.000 | | 0.000 | |
| $CO_2 \times N$ | 0.953 | 0.411 | | 0.379 | | 0.163 | | 0.206 | | 0.147 | |
| Time[Π] | – | 0.000 | | 0.039 | | 0.000 | | 0.000 | | 0.000 | |
| $CO_2 \times$ Time | – | 0.031 | | 0.263 | | 0.006 | | 0.001 | | 0.083 | |
| N × Time | – | 0.546 | | 0.220 | | 0.000 | | 0.114 | | 0.253 | |
| $CO_2 \times N \times$ Time | – | 0.026 | | 0.856 | | 0.274 | | 0.212 | | 0.035 | |

[λ] Units for biomass are g; soluble sugars, starch, condensed tannins and N concentrations are all mg/g.
[Π] Refers to time of collection, corresponding to column headings "F" and "L", for foliage and litter, respectively.
[§] Because we used Quebracho tannin as a standard, concentrations of condensed tannins should only be used for relative comparisons between treatments and are not reliable estimates of absolute concentrations.

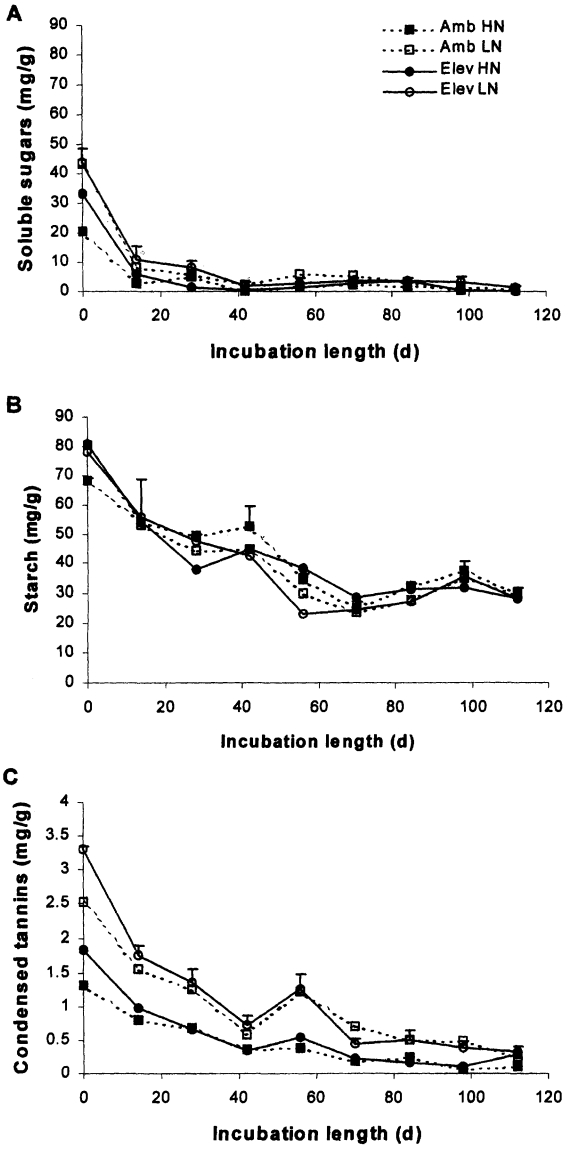


Fig. 1. Mean concentrations of soluble sugars (A), starch (B), and condensed tannins (C) in decomposing sugar maple leaf litter produced at ambient (Amb) and elevated (Elev) atmospheric $CO_2$, and low (LN) and high (HN) soil N availability at the Univ. of Michigan Biological Station. Incubations were carried out in microlysimeters in the dark at 25°C and field capacity soil moisture. Values are means ($N = 4$ to 5) and bars are SEM (given on Elev LN treatment only). Note different scale on $y$-axis of panel C.

### Microbial respiration and soil solution

During the first 14 d of incubation, microbial respiration resulted in the accumulation of an average of 3.26 mg C in the microlysimeters, and there were no significant differences between treatments (Table 2). Microbial respiration declined consistently until 84 d, when it leveled off at approximately 1.49 mg C evolved per 14-d interval, representing a 54% decrease from initial rates (Fig. 3A). Similarly, DOC extracted after 14 d averaged 3.53 mg, and declined exponentially to 0.07 mg per 14-d interval by the end of the incubation. Treatment effects were not significant in influencing the amount of DOC produced (Table 2).

The initial extraction of the litter at the start of the incubation yielded large quantities of dissolved C. The DOC content of the 50-ml extracts averaged 16.81 mg and was not affected by the $CO_2$ or soil N treatments (Table 3). Soluble sugars in the extracts ranged from 3.98 to 6.27 mg and a significant $CO_2$ main effect resulted in an average 38% increase from litter produced at elevated $CO_2$. Condensed tannins in the extracts ranged from 20.43 to 122.6 mg, and were responsive to both atmospheric $CO_2$ and soil N availability (Table 3). Extracts from litter produced at elevated $CO_2$ contained on average 101% more condensed tannins than that from ambient $CO_2$, while litter extracts from the high soil N availability treatment contained 60% less than those from low N. Over the course of the incubation, soluble sugars in the soil extracts declined very rapidly (Fig. 3B) and treatment effects were not significant. Similarly, condensed tannins in the soil extracts after the incubation had begun were essentially zero for the entire experiment.

## Discussion

Overall we found reasonable correspondence between predictions of the CNB/GDB hypotheses and responses of sugar maple litter quality to the availability of C and N. In addition, although we documented large differences in litter quality, rates of decomposition appeared insensitive to the elevated $CO_2$ and soil N availability treatments.

### Foliage production, chemistry, and the C-N balance

Total litter production, which should be very closely correlated to total foliage production, increased approximately 40% due to elevated $CO_2$ and high soil N availability. This indicates that growth was limited at the lower level of availability of both factors. According to the CNB and GDB hypotheses, if growth were reduced more than assimilation we would expect to see higher levels of nonstructural carbohydrates and CBSCs in foliage grown at elevated atmospheric $CO_2$ or low soil N availability (Koricheva et al. 1998). Although statistical inferences based on our data are complicated by the large differences in concentrations of some constituents in foliage vs litter, our data provide compelling support for this model of carbon allocation in plants in response to resource availability.

In general, starch and soluble sugars increased under conditions of elevated $CO_2$ or low soil N availability,

Table 2. *P*-values for biochemical constituents of decomposing sugar maple foliage produced under conditions of ambient and elevated atmospheric $CO_2$, and low and high soil N availability. Column headings: Numbers refer to number of days from beginning of incubation that a set of microlysimeters (25) was harvested. To maintain the overall level of confidence ($\alpha = 0.05$), only *P*-values $\leq 0.002$ are considered significant.

| Constituent | Source | 14 | 28 | 42 | 56 | 70 | 84 | 98 | 112 |
|---|---|---|---|---|---|---|---|---|---|
| Sol. sugar | $CO_2$ | ns | ns | ns | ns | ns | ns | ns | ns |
| | N | ns | ns | ns | ns | ns | ns | ns | ns |
| | $CO_2 \times N$ | ns | ns | ns | ns | ns | ns | ns | ns |
| Starch | $CO_2$ | ns | ns | ns | ns | ns | ns | ns | ns |
| | N | ns | ns | ns | ns | ns | ns | ns | ns |
| | $CO_2 \times N$ | ns | ns | ns | ns | ns | ns | ns | ns |
| Tannin | $CO_2$ | ns | ns | ns | ns | ns | ns | ns | ns |
| | N | 0.001 | 0.002 | ns | 0.000 | 0.001 | ns | 0.000 | ns |
| | $CO_2 \times N$ | ns | ns | ns | ns | ns | ns | ns | ns |
| N | $CO_2$ | ns | 0.001 | ns | ns | ns | ns | 0.000 | ns |
| | N | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 |
| | $CO_2 \times N$ | ns | ns | ns | ns | ns | ns | 0.001 | ns |
| C/N ratio | $CO_2$ | ns | ns | ns | ns | ns | ns | ns | ns |
| | N | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 |
| | $CO_2 \times N$ | ns | ns | ns | ns | 0.002 | ns | ns | ns |
| Microbial respiration | $CO_2$ | ns | ns | ns | ns | ns | ns | ns | ns |
| | N | ns | ns | ns | ns | ns | ns | ns | ns |
| | $CO_2 \times N$ | ns | ns | ns | ns | ns | ns | ns | ns |
| DOC | $CO_2$ | ns | ns | ns | ns | ns | ns | ns | ns |
| | N | ns | ns | ns | ns | ns | ns | ns | ns |
| | $CO_2 \times N$ | ns | ns | ns | ns | ns | ns | ns | ns |

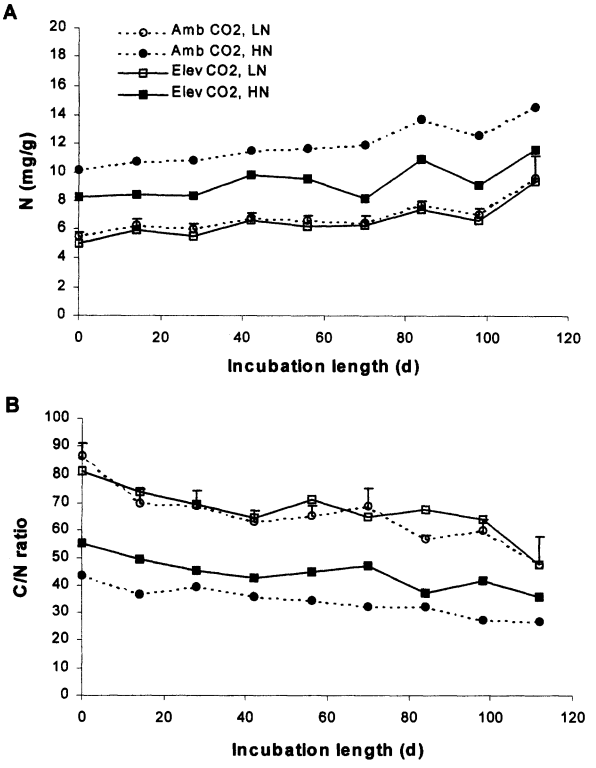


Fig. 2. Mean concentration of nitrogen (A) and C/N ratio (B) of decomposing sugar maple leaf litter produced at ambient (Amb CO2) and elevated (Elev CO2) atmospheric $CO_2$, and low (LN) and high (HN) soil N availability at the Univ. of Michigan Biological Station. Incubations were carried out in microlysimeters in the dark at 25°C and field capacity soil moisture. Values are means ($N = 4$ to 5) and bars are SEM (given on Amb CO2 LN treatment only).

and were highest in the elevated $CO_2$-low soil N treatment. Similarly, condensed tannins in foliage responded in a manner consistent with the CNB/GDB hypotheses: elevated $CO_2$ or low soil N availability caused an increase, and concentrations were highest in foliage produced in the elevated $CO_2$-low soil N availability treatment. Further support for the CNB/GDB hypotheses was the marginally significant positive correlation between foliar concentrations of sugars and tannins.

These results are in contrast to King et al. (2001) who found poor correspondence between predictions of the CNB/GDB hypotheses and the quality of litter of trembling aspen produced under similar treatments of atmospheric $CO_2$ and soil N availability. We attribute this to the contrasting life history patterns of the two species, and consequent responses to changes in resource availability (Borman and Likens 1979, Griffin et al. 1995). Trembling aspen is an early successional species, characterized by relatively high photosynthetic rates and rapid, indeterminate growth; it is extremely shade intolerant. Sugar maple is a late-successional species having lower photosynthetic rates, slower, determinate growth and very high shade tolerance (Barnes and Wagner 1981, Godman et al. 1990, Perala 1990).

As phenylalanine is the rate-limiting precursor for phenylpropanoid synthesis (e.g. lignin, flavonoids, condensed tannins) and also an essential amino acid for protein synthesis, production of secondary defense compounds and growth are in direct competition for C (Margna 1977, McClure 1979, Da Cunha 1987, Margna et al. 1989). Growth-dominated species typically have low constitutive resistance to herbivores (low levels of

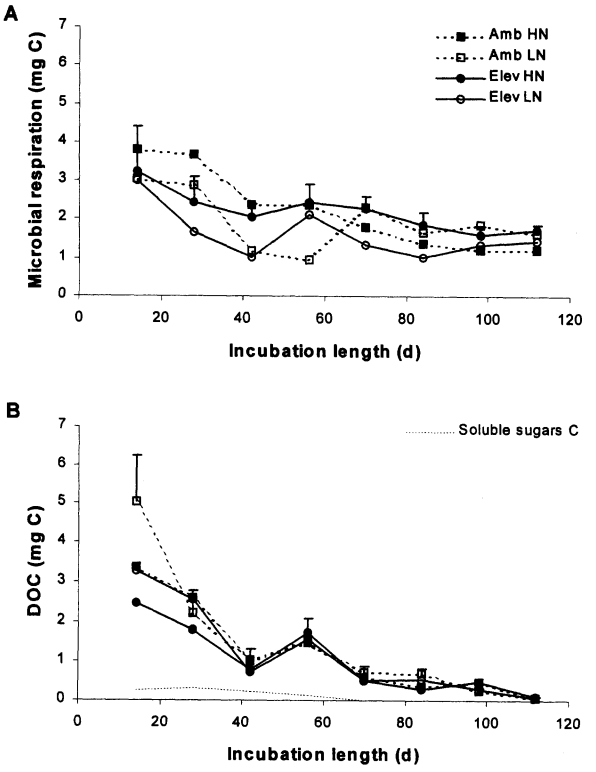


Fig. 3. Decomposition of sugar maple leaf litter produced at two levels of atmospheric $CO_2$ and two levels of soil N availability at the Univ. of Michigan Biological Station. Incubations were carried out in microlysimeters in the dark at 25°C and field capacity soil moisture. Decomposition is expressed as microbially respired carbon (A) and the production of dissolved organic carbon (B). Values are means ($N = 4$ to 5) and bars are SEM (given on Elev HN and Amb LN treatments only).

Table 3. Means (standard error of the mean; $N = 4$) and statistical significance ($P$-values) of DOC, soluble sugars, and condensed tannins content (mg) in initial extracts of litter produced at two levels of atmospheric $CO_2$ and two levels of soil N availability at the Univ. of Michigan Biological Station.

| Treatment | DOC | Soluble sugars | Condensed tannins* |
|---|---|---|---|
| Amb LN | 16.03 (1.22) | 4.87 (0.50) | 68.28 (9.88) |
| Amb HN | 16.24 (1.64) | 3.98 (0.46) | 20.43 (2.83) |
| Elev LN | 16.79 (2.39) | 6.27 (0.58) | 122.64 (11.32) |
| Elev HN | 18.20 (1.90) | 5.91 (0.18) | 56.11 (11.06) |
| Source | | | |
| $CO_2$ | 0.787 | 0.008 | 0.000 |
| N | 0.877 | 0.258 | 0.000 |
| $CO_2 \times N$ | 0.373 | 0.622 | 0.338 |

* Because we used Quebracho tannin as a standard, estimates of condensed tannin contents should only be used for relative comparisons between treatments and are not reliable estimates of absolute carbon content.

secondary metabolites) while differentiation-dominated species invest more heavily in defense (Herms and Mattson 1992, and references therein). We extend this discussion by hypothesizing that accumulation of stable secondary metabolites (little metabolic turnover) in differentiation-dominated species is more responsive to changes in resource availability than that of growth-dominated species, in which growth would be expected to be most responsive. Thus, concentrations of condensed tannins in sugar maple litter varied as a function of soil N availability, while those of trembling aspen were unresponsive (King et al. 2001).

There were large, significant reductions in the concentrations of most constituents due to the transition from fresh foliage to senesced litter, and substantial increases in the C/N ratio. In general, concentrations of all constituents in foliage were highly correlated to those in litter (Spearman rank correlation $r = 0.63$ to 0.92), the exception being that levels of starch in live foliage were not significantly correlated to those in litter (Table 4). This "decoupling" of starch concentrations in foliage and litter could be seen as a limitation to the direct application of the CNB/GDB hypotheses to litter. Nonetheless, the pattern of response of soluble sugars and condensed tannins to the treatments was consistent with that in foliage and the treatment with the highest litter concentrations of soluble sugars and condensed tannins was elevated $CO_2$–low soil N availability, as predicted by the hypotheses. Therefore, our data suggest that the CNB/GDB hypotheses are useful tools for predicting bulk C-N composition of litter in response to changes in resource availability, but may not be useful for predicting some specific chemical constituents such as starch.

The cardinal property of condensed tannins, the ability to strongly bind to protein and carbohydrates, and their wide distribution across plant taxa (Hagerman et al. 1997) makes this class of compounds especially likely to affect the decomposition of plant litter. In the present study, concentrations of condensed tannins contained in litter were highly correlated with those of the live foliage ($r = 0.73$), but were less than 25% of the original content. Although this might at first appear to decrease the role of tannins as a direct control on litter decomposition, effects on the activity and/or composition of soil microbial communities could be significant after tannins have leached from the litter. The initial extraction of the litter resulted in leaching of from 10 to 21% of the condensed tannins contained in the live foliage. This would explain, in part, the tea-brown color of the initial extracts, and is analogous to the tannin stained waters of acidic forest streams and lakes that occur throughout North America. It is reasonable to expect the soluble fraction of condensed tannins to have less of an influence on the decomposition of the litter than the recalcitrant fraction, which is probably bound to proteins in the tissue. The remainder of the condensed tannins that were "lost" probably leached from the senescing foliage while it was still attached to

Table 4. Spearman rank correlation coefficients of different biochemical constituents of sugar maple foliage and litter produced at two levels of atmospheric $CO_2$ and two levels of soil N availability at the Univ. of Michigan Biological Station. Values are the correlation coefficient ($r$), $P$-value, and number of paired observations ($N$). "F" and "L" refer to foliage and litter constituents, respectively. To maintain the overall level of confidence ($\alpha = 0.05$), only $P$-values $\leq 0.005$ are considered significant.

| | Fsugar | Lsugar | Fstarch | Lstarch | Ftannin | Ltannin | F[N] | L[N] | F(C/N) | L(C/N) |
|---|---|---|---|---|---|---|---|---|---|---|
| Fsugar | 1.000 | 0.632 | 0.275 | 0.196 | 0.616 | 0.680 | −0.739 | −0.751 | 0.762 | 0.523 |
| | 0.000 | 0.006 | 0.268 | 0.451 | 0.006 | 0.002 | 0.000 | 0.000 | 0.000 | 0.025 |
| | 18 | 17 | 18 | 17 | 18 | 18 | 18 | 18 | 18 | 18 |
| Lsugar | | 1.000 | 0.394 | −0.138 | 0.508 | 0.849 | −0.818 | −0.729 | 0.825 | 0.721 |
| | | 0.000 | 0.117 | 0.571 | 0.026 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | | 19 | 17 | 19 | 19 | 19 | 17 | 19 | 17 | 19 |
| Fstarch | | | 1.000 | 0.034 | 0.558 | 0.496 | −0.492 | −0.396 | 0.463 | 0.263 |
| | | | 0.000 | 0.896 | 0.016 | 0.036 | 0.037 | 0.103 | 0.053 | 0.291 |
| | | | 18 | 17 | 18 | 18 | 18 | 18 | 18 | 18 |
| Lstarch | | | | 1.000 | 0.282 | 0.017 | −0.185 | −0.211 | 0.208 | 0.181 |
| | | | | 0.000 | 0.241 | 0.943 | 0.476 | 0.386 | 0.422 | 0.459 |
| | | | | 19 | 19 | 19 | 17 | 19 | 17 | 19 |
| Ftannin | | | | | 1.000 | 0.735 | −0.738 | −0.678 | 0.729 | 0.654 |
| | | | | | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.002 |
| | | | | | 20 | 20 | 18 | 20 | 18 | 20 |
| Ltannin | | | | | | 1.000 | −0.928 | −0.849 | 0.927 | 0.714 |
| | | | | | | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | | | | | | 20 | 18 | 20 | 18 | 20 |
| F[N] | | | | | | | 1.000 | 0.916 | −0.996 | −0.724 |
| | | | | | | | 0.000 | 0.000 | 0.000 | 0.000 |
| | | | | | | | 18 | 18 | 18 | 18 |
| L[N] | | | | | | | | 1.000 | −0.915 | −0.817 |
| | | | | | | | | 0.000 | 0.000 | 0.000 |
| | | | | | | | | 20 | 18 | 20 |
| F(C/N) | | | | | | | | | 1.000 | 0.723 |
| | | | | | | | | | 0.000 | 0.000 |
| | | | | | | | | | 18 | 18 |
| L(C/N) | | | | | | | | | | 1.000 |
| | | | | | | | | | | 0.000 |
| | | | | | | | | | | 20 |

the tree. In addition, condensed tannins become less extractable upon drying of the tissue (during senescence) and more difficult to detect due to the formation of oxidation products that inhibit formation of the chromophore (anthocyanidin) in the acid-butanol assay (P. E. Laks pers. comm.).

The concentration of N (or C/N ratio) also has been implicated as a strong control on decomposition (Melillo et al. 1982, Enríquez et al. 1993). Concentrations of N in foliage were very highly correlated with those in litter, and inversely correlated with concentrations of carbohydrates and condensed tannins in both foliage and litter, providing further support for the CNB/GDB hypotheses. Soil N availability exerted a much greater effect on foliage and litter N concentrations than did the $CO_2$ treatment, causing a 75% increase in litter at high N, compared to 15% higher concentrations at ambient $CO_2$. The treatment with the highest N concentration and lowest C/N ratio was ambient $CO_2$-high soil N, again, as predicted by the CNB/GDB hypotheses. We found no evidence for differences in N retranslocation due to elevated $CO_2$, with 55–56% of N being retained in the litter of both $CO_2$ treatments, consistent with Norby et al. (2000). Litter produced at low soil N availability retained only 38% of the N, while that produced at high soil N availability retained 47%, although the lack of a statistically significant N × Time interaction would suggest this difference was not important. If we consider the C/N ratio an index of "decomposability" (sensu Enríquez et al. 1993) we would expect litter produced at ambient $CO_2$–low soil N (C/N = 86.6) to decompose much more slowly than that from ambient $CO_2$–high soil N (C/N = 43.6), but as discussed below, this was not the case.

## Atmospheric $CO_2$, soil N availability, and decomposition

Our measures of decomposition were selected to capture both short-term (microbially respired $CO_2$) and longer-term (DOC) responses of the microbial community to litter produced under differing levels of resource availability. It has been demonstrated that increases in soil respiration under elevated $CO_2$ can be due to more rapid cycling of labile carbon substrates by the soil microbial community (Hungate et al. 1997). In contrast, formation of DOC is thought to result from the degradation of more recalcitrant forms of carbon (tannins, lignin, humic substances), but may contain significant quantities of labile constituents such as low molecular weight organic acids and carbohydrates

(Herbert and Bertsch 1995). In agreement with similar microlysimeter studies using *Populus* litter (Randlett et al. 1996, King et al. 2001), we found few consistent treatment effects on either measure of decomposition. This is surprising, given the two-fold differences in C/N ratios of litter produced in our experiment. In a recent study, Hättenschwiler et al. (1999) report that beech leaf litter produced under elevated $CO_2$ decomposed more slowly than that produced under ambient $CO_2$, but the effect was small to non-existent during the first 100 d of incubation, and there was no effect on spruce branchlets. They found increased litter consumption and feces production by isopod detritovores, and proposed this could offset the reduced quality of litter produced at elevated $CO_2$, resulting in no net effect on decomposition (see also Lussenhop et al. 1998). These results illustrate the importance of understanding the effects of altered litter quality on both micro- and macro-decomposers in early and late stages of the decomposition process. Our data indicate that even large differences in litter quality are unlikely to directly affect the dynamics of soil microbial communities during the early stages of leaf litter decay.

## Changes in litter quality through time

To gain insight into the dynamics of microbial processing of litter as affected by resource availability, it is helpful to examine changes in litter quality through time. Concentrations of soluble sugars dropped rapidly to very low levels, while starch concentrations declined only 39% by the end of the incubation. This is in contrast to King et al. (2001), who reported that starch concentrations decreased to near zero in trembling aspen litter after just 55 d of incubation, and may be related to the much higher N concentrations (average 22 mg/g) of the litter in that study. Initial treatment effects on litter soluble sugars and starch disappeared during the incubation, suggesting changes in these constituents in response to elevated atmospheric $CO_2$ or soil N availability will be rapidly neutralized by soil microorganisms and/or physical effects (leaching). Concentrations of condensed tannins in decomposing litter declined to near zero by the end of the incubation, and interestingly, the strong reduction in concentration due to high soil N availability persisted almost until the end of the incubation, at which point all treatments converged. The rate of decline in condensed tannin concentrations was significantly faster in litter produced at low soil N availability, indicating greater microbial utilization until an apparent threshold was reached after 112 d of incubation. As a recalcitrant material, we would expect the concentration of condensed tannins to increase with time (Waring and Schlesinger 1985), but clearly our data show the opposite. This suggests that condensed tannins may be utilized by microbial decomposers as an energy source to a greater extent than has previously been suspected.

Concentrations of N in the decomposing litter of this study were similar to the range of concentrations reported for decomposing sugar maple litter during the period in which approximately 50% of the original mass had been lost (Aber and Melillo 1980). This reassures us that the quality of the litter produced in this study is representative of that produced naturally. In contrast, King et al. (2001) found that leaf litter of trembling aspen produced in open-top chambers had much higher N concentrations than natural litter, indicating that some species may be more appropriate as model systems in growth chambers than are others. Apparently, the rapidly growing, "exploitive" aspen (sensu Griffin et al. 1995) responded to the experimental treatments by assimilating more N relative to wild plants, whereas the slower growing "conservative" sugar maple was less likely to alter nutrient uptake and biochemistry. Regression analysis established that rates of increase in N concentration (and decrease in C/N ratios) as the litter decomposed did not differ by treatment, supporting the conclusion that our treatments had little effect on rates of decomposition.

## Dynamics of carbon loss

By comparing microbial respiration and DOC over time we can get an idea of the relative magnitudes of these contrasting pathways of C loss from the decomposing litter. Microbial respiration was greatest early in the incubation, then declined to a steady rate at approximately 40 to 60 d of incubation. This closely approximates the pattern of starch degradation, indicating utilization of labile substrates at the very early stages of decomposition. Total C lost as microbially respired $CO_2$ was 15.97 mg, averaged across the treatments. In contrast, the initial extraction of the litter leached an average of 16.81 mg C as DOC. Although the coarse grinding of the litter (necessary to ensure uniform substrate) may have artificially increased fragment surface area, we feel these data illustrate the potentially large C fluxes that occur in forest ecosystems during the period of leaf senescence. Autumn is typically a wet time of year at our field site with precipitation occurring over several months; a longer period of leaching that should to some extent compensate for grinding of the litter. As demonstrated, much of the initial DOC was composed of highly labile soluble sugars (31%) and condensed tannins (actual quantity unknown). In nature, much of this material is trapped in the soil profile where it can be consumed by microorganisms (Herbert and Bertsch 1995); however, cooling soil temperatures at this time of year may reduce microbial activity allowing this material to be leached from the system. DOC in the extracts gradually

declined during the incubation, approaching zero by 112 d. Chemical analyses revealed that this material contained very little soluble sugars or condensed tannins, and probably resulted from microbial degradation of more recalcitrant litter constituents. By the end of the incubation the amount of C lost from the decomposing litter as DOC totalled 26.57 mg, representing a 66% greater flux than that of microbial respiration. These results differ from MacDonald et al. (1999), who found that microbial respiration dominated C fluxes ( $>50$ mg C) compared to DOC losses ( $\sim 2.7$ mg C) during a 32-wk incubation of soil from the same site as ours. The carbon in our study was fresh litter containing large quantities of labile C, while MacDonald et al. (1999) used intact soil monoliths that had been collected in April of 1994, from which most of the easily leached labile C had probably been lost during the previous fall and winter. The DOC in their experiment must have originated from the degradation of recalcitrant forms of C, whose low rate of flux resembled that found in the later stages of decomposition in this study.

## Conclusions

By varying the availability of atmospheric $CO_2$ or soil N, we found that the biochemical response of sugar maple foliage is in agreement with predictions of the CNB/GDB hypotheses. Conditions favoring accumulation of nonstructural carbohydrates relative to growth enhanced production of condensed tannins, a major carbon-based secondary compound (CBSC). Further, high correlation between biochemical constituents in foliage and litter indicate that the CNB/GDB hypotheses provide a useful tool for predicting litter quality in response to changing environmental conditions, for some but not all chemical constituents. Our data indicate that elevated $CO_2$, and especially soil N availability, can substantially alter litter C/N ratios and levels of condensed tannins that persist through time, but these changes have little direct effect on rates of decomposition by soil microorganisms. Finally, large quantities of C may be lost from forest ecosystems in autumn as labile constituents are leached from senescing litter and exported as DOC.

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