Global Change Biology (2001) **7,** 65–74

Chemistry and decomposition of litter from *Populus tremuloides* Michaux grown at elevated atmospheric CO_2 and varying N availability

JOHN S. KING,* KURT S. PREGITZER,§* DONALD R. ZAK,† MARK E. KUBISKE,‡ JENNIFER A. ASHBY* and WILLIAM E. HOLMES†

**School of Forestry and Wood Products, Michigan Technological University, Houghton, MI, 49931 USA, †School of Natural Resources and Environment, The University of Michigan, Ann Arbor, MI 48109 USA, ‡Department of Forestry, Box 9681, Mississippi State University, MS 39762, §USDA Forest Service, North Central Research Station, Houghton, MI, 49931 USA*

Abstract

It has been hypothesized that greater production of total nonstructural carbohydrates (TNC) in foliage grown under elevated atmospheric carbon dioxide (CO_2) will result in higher concentrations of defensive compounds in tree leaf litter, possibly leading to reduced rates of decomposition and nutrient cycling in forest ecosystems of the future. To evaluate the effects of elevated atmospheric CO_2 on litter chemistry and decomposition, we performed a 111 day laboratory incubation with leaf litter of trembling aspen (*Populus tremuloides* Michaux) produced at 36 Pa and 56 Pa CO_2 and two levels of soil nitrogen (N) availability. Decomposition was quantified as microbially respired CO_2 and dissolved organic carbon (DOC) in soil solution, and concentrations of nonstructural carbohydrates, N, carbon (C), and condensed tannins were monitored throughout the incubation. Growth under elevated atmospheric CO_2 did not significantly affect initial litter concentrations of TNC, N, or condensed tannins. Rates of decomposition, measured as both microbially respired CO_2 and DOC did not differ between litter produced under ambient and elevated CO_2. Total C lost from the samples was 38 mg g^{-1} litter as respired CO_2 and 138 mg g^{-1} litter as DOC, suggesting short-term pulses of dissolved C in soil solution are important components of the terrestrial C cycle. We conclude that litter chemistry and decomposition in trembling aspen are minimally affected by growth under higher concentrations of CO_2.

Keywords: C/N ratio, carbohydrates, global change, microlysimeter, soil carbon, tannin

Received 5 April 2000; revised version received 7 June and accepted 15 June 2000

Introduction

The inverted latitudinal gradients of global net primary production and soil organic matter indicate that decomposition is the major controller of carbon (C) storage in terrestrial ecosystems (Olson 1963). Rising levels of atmospheric carbon dioxide (CO_2) are expected to alter many ecosystem processes (Strain & Bazzaz 1983; Eamus & Jarvis 1989; Melillo *et al*. 1996), but its effects on decomposition through changes in the quantity and quality of plant litter are especially important (Torbert *et al*. 1998). The carbon:nutrient balance hypothesis (CNBH) (Bryant *et al*. 1983; Bazzaz *et al*. 1987) predicts that under conditions favouring C uptake (e.g. elevated atmospheric CO_2) C not needed for growth will be stored as nonstructural carbohydrates and subsequently shunted into production of C-based secondary compounds, possibly deterring herbivory or reducing rates of decomposition (Horner *et al*. 1988). Therefore, rising atmospheric CO_2 has the potential to influence decomposition by altering the chemical constituents of plant tissues. It is widely recognized that growth under elevated atmospheric CO_2 generally increases total plant biomass production (Ceulemans & Mousseau 1994; Rogers *et al*. 1994; Amthor 1995; Curtis & Wang 1998), and recent evidence suggests that it can alter plant tissue chemistry (Lindroth 1996; Poorter *et al*. 1997; Gebauer *et al*. 1998; Martijn Bezemer & Hefin Jones 1998). The most commonly observed change in leaf tissue chemistry

Correspondence: Dr John S. King, tel +1/906 487-2915, fax +1/906 487-2915, e-mail jsking@mtu.edu

under elevated CO_2 is an increase in total nonstructural carbohydrates (TNC) (Poorter *et al*. 1997). Reductions in tissue N concentration have been linked to increases in nonstructural carbohydrates (Kuehny *et al*. 1991), and can also result from greater nutrient-use efficiency (NUE) (Norby *et al*. 1986; King *et al*. 1997a). Both mechanisms of reducing tissue N concentration contribute to the reported increase in C/N ratio under elevated CO_2 (Pregitzer *et al*. 1995; Randlett *et al*. 1996). In addition, elevated atmospheric CO_2 has been found to result in increases in tannins and phenolics (Lindroth 1996; Gebauer *et al*. 1998), but effects on lignin were minimal in a wide range of species (Poorter *et al*. 1997). Currently, research indicates that elevated CO_2 has inconsistent effects on litter chemistry and rates of decomposition. In a recent review of the literature, O'Neill & Norby (1996) concluded that litter produced in pot studies frequently exhibited increased C/N ratio in response to elevated CO_2, and decreased rates of decomposition. Conversely, they found that litter produced in open-top chambers was not affected by growth under elevated CO_2, and that rates of decomposition did not differ from those of control litter. Other evidence from a variety of controlled environment facilities (growth chambers, open-top chambers, and free-air carbon dioxide enrichment), suggests that litter of some herbaceous plants, crops and trees produced under elevated CO_2 exhibits altered chemical composition and decomposes more slowly than ambient-grown litter (Ball & Drake 1997; Prior *et al*. 1997; Robinson *et al*. 1997; Cotrufo *et al*. 1998; Torbert *et al*. 1998; van Ginkel & Gorissen 1998). Recently, scientists convening at an international symposium on litter chemistry and decomposition under elevated CO_2 concluded that there is insufficient evidence to support the hypothesis that rates of decomposition will decrease as a result of reduced quality litter (Norby & Cotrufo 1998).

In order to evaluate the effects of elevated atmospheric CO_2 on litter chemistry and decomposition in a widespread northern tree species, we collected leaf litter of trembling aspen (*Populus tremuloides* Michaux) produced in an open-top chamber experiment and performed a laboratory incubation for 111 days. Trembling aspen has the largest distribution of any tree species in North America, spanning 111° of longitude and 48° of latitude (Perala 1990), and on productive sites in the Lake States regions can sequester 2.9 MgC $ha^{-1} y^{-1}$ (Alban & Perala 1992). Decomposition was measured as microbial respiration and the production of dissolved organic C (DOC). Total nonstructural carbohydrates, N-concentration, and condensed tannins were monitored for the duration of the experiment to link dynamics of decomposition with changing litter chemistry. We hypothesized that leaf litter developed under elevated atmospheric CO_2 would have higher levels of TNC resulting in higher C/N ratios, but that this effect would be reduced in foliage grown at high soil-N availability. Additionally, we hypothesized that under conditions of elevated CO_2, higher levels of C-based secondary compounds (tannins) would be produced with this effect also expected to be smaller for foliage grown at high soil-N availability. For both of these reasons, we expected foliage produced under elevated CO_2 to decompose more slowly than ambient-grown foliage, especially when grown at low soil-N availability.

Materials and methods

Field site

A randomized complete block design of ambient and elevated CO_2, and high and low soil-N availability treatments replicated five times was established in an open-top chamber facility at the University of Michigan Biological Station, near Pellston, MI, in the spring of 1997. The 20 open-top chambers measured 3 m in diameter (Rogers *et al*. 1983). The chambers were installed atop open-bottom root boxes (3.5 m × 3.5 m × 0.5 m) filled with one of two soil mixes (Pregitzer *et al*. 1995). The soil resulted in levels of N-availability characteristic of the range found across the northern Great Lakes region (Zak & Pregitzer 1990). High N-availability soil consisted of a homogenized A-horizon of a Kalkaska series soil (sandy, mixed, frigid, Entic Haplorthod) with a net initial N-mineralization rate of 348 $\mu gN\, g^{-1}\, d^{-1}$. The low N-availability soil was a 4:1 mix of Rubicon series C-horizon soil (sandy, mixed, frigid, Entic Haplorthod) with A-horizon of the Kalkaska series soil; it had a net initial N-mineralization rate of 45 $\mu gN\, g^{-1}\, d^{-1}$ (Randlett *et al*. 1996). The CO_2 fumigation system has been previously described (Curtis *et al*. 2000), but briefly, consisted of a centrally located intake line to monitor ambient atmospheric CO_2 concentration, a sample line, and CO_2 dispensing lines going to each of the 10 elevated CO_2 chambers. Ambient and treatment sample lines were sequentially switched to a Li-Cor 6262 infra-red gas analyser by a computer control system that recorded 2-minute 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 flow meters, and out to each elevated CO_2 chamber. Carbon dioxide concentrations in each elevated CO_2 chamber were monitored and flow rates adjusted manually to maintain the treatment differential at ± 20 Pa (± 10%). Chambers were fumigated 24 h per day, beginning on 9 June 1997 and ending on 29 October 1997. Mean daytime ambient and elevated CO_2 concentrations (SD) were 35.8 Pa (0.7) and 56.0 Pa (1.3), respectively.

During the last week of CO_2 fumigation, naturally senescing litter from all clones in each chamber was collected in approximately equal proportions. Most of the litter consisted of dry brown leaves that had naturally fallen, but some were still attached to the tree and were easily dislodged by gently brushing the branches. The litter was dried in an oven to constant mass at 60 °C to arrest any changes that might have occurred between the time of collection and the beginning of the decomposition experiment. We are aware that oven drying may affect concentrations of carbohydrates and some secondary compounds in foliage (Lindroth & Koss 1996). It has been shown that even air-drying litter may greatly reduce rates of decomposition (Taylor 1998), and it was considered herein that artefacts introduced by oven drying were minimal in this instance because the litter was already largely dry. Observations of relative constituent concentrations in foliage and litter, and similar decomposition dynamics of material that had been freeze-dried vs. oven-dried support this contention (data not shown).

Laboratory incubations

In late January 1998, the aggregated litter from each chamber was coarsely fragmented in a large Wiley mill (Arhur H. Thomas Co., Philadelphia, PA) equipped with a 4-mm mesh screen and mixed thoroughly to provide a uniform starting medium for the incubations. Forty grammes of homogenized A-horizon soil collected from a forest adjacent to the field site (Typic Udipsamment) was placed in the top half of modified microlysimeters (Falcon Filter Unit #7102, Becton Dickinson and Co., Cockeysville, MD) (Zak *et al*. 1993). A common inoculum was used rather than soil from the chambers because a previous experiment (Randlett *et al*. 1996) found that soil-N availability dominated the microbial response. In this experiment we were interested in characterizing the direct effects of substrate quality on microbial metabolism, rather than gross differences in decomposition as a result of changes in microbial community composition or soils of greatly differing nutrient availability. Visible organic matter was first removed from the forest soil with a fine mesh soil sieve and the soil placed on 1.7 µm filter paper that separated the top and bottom portions of the microlysimeters. A 1-mm mesh fibreglass screen was placed on top of the soil, followed by 1 g of the homogenized leaf litter. The four outlets in the microlysimeters (two top, two bottom) were closed with appropriately sized butyl rubber septa and all seams were sealed with silicone sealant.

The incubation was started by adding 10 mL of deionized water to each microlysimeter, 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 (20 units per set), with one microlysimeter corresponding to each of the open-top chambers, duplicating the statistical design of the field experiment. Ten 'blank' microlysimeters were also constructed, using soil inoculum only (i.e. no added litter) to correct estimates of microbial respiration and DOC production for that arising from native soil carbon. There was a total of 170 units and one set (20) was destructively harvested every two weeks. At each harvest, a sample of headspace gas from each unit was collected through the septa with a syringe and temporarily stored in a sealed 3-mL serum vial. Litter within the units was then extracted with 50 mL of 0.01 m CaCl solution, which was collected in the bottom half 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. Litter samples were collected by removing the fibreglass screen to which most of the solid material adhered. These were placed in paper envelopes and oven dried to constant mass at 35 °C. 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 following Randlett *et al*. (1996), flushed with CO_2-free air to prevent end-product inhibition of further decomposition, and returned to the incubator. Samples in the microlysimeters remained moist throughout the experiment.

Chemical determinations

Carbon dioxide concentrations of the headspace gas were determined by injecting samples into a Tracor Model 540 gas chromatograph equipped with a thermal conductivity detector. Concentrations of total dissolved organic carbon (DOC) in the $CaCl_2$ extracts were determined using a Shimadzu TOC-5000 A total organic carbon analyser, 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 leaf samples were measured using a Carlo Erba NA1500 Series II elemental analyser run with National Institute of Standards and Technology pine needle and peach leaf standards as a quality control. Total nonstructural carbohydrates (starch and soluble sugars) in the decomposed leaf samples were quantified using the method of Tissue & Wright (1995).

Twenty-five mg subsamples were extracted with methanol:chloroform:water to release soluble sugars and the remaining pellet was digested with 35%

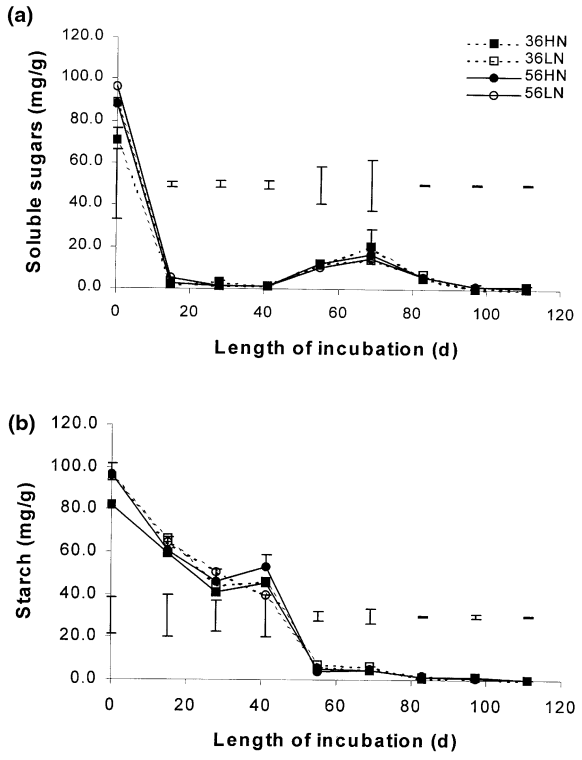

Fig. 1 Mean ($N = 4$ to 5) soluble sugar (a) and starch (b) concentrations in aspen leaf litter grown at ambient and elevated atmospheric CO_2 and two levels of soil N availability, incubated for 111 d at 25° C. Bars are SE. I-beams are Fisher's least significant difference (LSD) for each *a posteriori* comparison of means. Figure legend: factorial combinations of atmospheric CO_2 partial pressure (36, 56 Pa), and high and low soil N availability (HN, LN), respectively.

perchloric acid to hydrolyse starch into soluble sugars. Both soluble and insoluble sugars (i.e. starch) were quantified colourimetrically by comparing sample absorption (490 nm λ) to that of glucose standards. Condensed tannins were determined by the acid butanol method (R.L. Lindroth, pers. comm.; Porter *et al*. 1986; Hagerman & Butler 1989). Seventy-five mg samples were extracted with an acetone–ascorbic acid mixture. Extracts (500 µl) were extracted with acid butanol and an iron reagent and incubated at 100 °C for 50 min. Absorbance (550 nm λ) of the extracts was read with a spectrophotometer and compared to a standard curve prepared from purified Quebracho tannin.

Statistical analyses

Effects of CO_2 and soil-N availability on litter chemistry and decomposition were tested using two-way analyses of variance (anova) for a randomized complete-block design (Proc GLM, Statistical Analysis Systems software version 6.12, SAS Institute, Inc., Cary, NC). Inspection of residuals and normal probability plots indicated that log transformation was required to normalize the variance across treatments, meeting the assumptions of anova (Sokal & Rohlf 1995). Data are presented as back-transformed means and standard errors. The α level was set at 0.05 and to assess the potential of making Type II statistical errors (accepting the null hypothesis when it is false) Fisher's least significant difference (LSD) was calculated *a posteriori* for each statistical test and plotted as I-beams for each date samples were collected.

Results

Total nonstructural carbohydrates

Soluble sugars in the preincubation, aspen litter averaged 86 mg g^{-1} leaf dry weight (Fig. 1a), and did not vary significantly by treatment (Table 1). After two weeks of incubation, soluble sugar concentration fell to almost zero and remained there for the duration of the incubation, except at days 55 and 69, at which times it rose to approximately 11–16 mg g^{-1} leaf dry weight, respectively. Treatment effects remained insignificant for the entire incubation (Table 1). Initial concentrations of starch in the leaf litter averaged 96 mg g^{-1} dry weight (Fig. 1b) and, as with soluble sugars, there were no statistically significant treatment effects. Starch concentration approached zero over the course of the incubation, and treatment effects remained insignificant.

C/N ratio and N

Changes in litter C/N ratio (Fig. 2a), while statistically significant (Table 1), were minor for litter produced at elevated CO_2 (C/N = 22) compared to ambient CO_2 (C/N = 21). Over the course of the incubation, the C/N ratios declined to 15 for the elevated CO_2-low N availability treatment vs. an average of 13 for all other treatments, which were similar to one another (Fig. 2a). The elevated atmospheric CO_2, low N availability treatment (56LN) consistently had a higher C/N ratio than the other treatments and was responsible for the statistically significant CO_2 effect at most harvests (Fig. 2a, Table 1). Initial N concentration averaged 23 mg g^{-1} for ambient grown foliage while that grown under elevated CO_2 averaged 21 mg g^{-1} (Fig. 2b), and this difference was again statistically significant (Table 1). Nitrigen concentrations decreased to approximately 10 mg g^{-1} at Day 28, but then increased to 17 mg g^{-1} by Day 69 before declining to 7 mg g^{-1} through the remaining 40 days. Litter N concentrations were rarely affected by growth CO_2, and never by soil-N availability, or their interaction (Table 1).

Table 1 *P*-values (≤ 0.05) for biochemical constituents of decomposing aspen foliage produced under conditions of ambient and elevated atmospheric CO_2, and low and high soil N availability. Column headings: 'Initial' refers to pre-incubation litter, and numbers refer to number of days from beginning of incubation that a set of units (20) was harvested.

			Day							
Constituent	Source	Initial	15	28	41	55	69	83	97	111
Sol. Sugar	CO_2	NS	NS	NS	NS	NS	NS	NS	NS	NS
	N	NS	NS	NS	NS	NS	NS	NS	NS	NS
	$CO_2 \times N$	NS	NS	NS	NS	NS	NS	NS	NS	NS
Starch	CO_2	NS	NS	NS	NS	NS	NS	NS	NS	NS
	N	NS	NS	NS	NS	NS	NS	NS	NS	NS
	$CO_2 \times N$	NS	NS	NS	NS	NS	NS	NS	NS	NS
C/N ratio	CO_2	0.012	NS	NS	NS	0.016	0.007	NS	0.005	0.000
	N	NS	NS	NS	NS	NS	NS	NS	NS	0.002
	$CO_2 \times N$	NS	NS	NS	NS	NS	NS	NS	0.052	NS
N CO_2	0.005	0.028	NS	NS	NS	NS	0.054	NS	NS	
	N	NS	NS	NS	NS	NS	NS	NS	NS	NS
	$CO_2 \times N$	NS	NS	NS	NS	NS	NS	NS	NS	NS
Tannin	CO_2	NS	NS	NS	NS	NS	NS	NS	NS	NS
	N	NS	NS	NS	NS	0.043	NS	NS	NS	NS
	$CO_2 \times N$	NS	NS	NS	NS	NS	NS	NS	NS	NS
Microbial respiration	CO_2	–	NS	0.013	NS	NS	NS	NS	NS	NS
	N	–	NS	NS	NS	NS	0.009	NS	NS	NS
	$CO_2 \times N$	–	NS	NS	NS	NS	NS	NS	NS	NS
DOC-C	CO_2	NS	NS	NS	NS	NS	NS	NS	NS	NS
	N	NS	NS	NS	NS	NS	NS	NS	NS	NS
	$CO_2 \times N$	NS	NS	NS	NS	NS	NS	NS	NS	NS

Condensed tannins

Condensed tannin concentrations in the senesced leaf material were 51, 57, 64, and 74 mg g^{-1} for the ambient CO_2, low and high N availability, and elevated CO_2, low and high N availability treatments (36LN, 36HN, 56LN, and 56HN), respectively, but were not significantly different from one another (Table 1). After two weeks of decomposition, condensed tannin concentrations dropped more than 90% to 5 mg g^{-1} and continued decreasing to less than 2 mg g^{-1} over the course of the 111-d incubation (Fig. 3). Treatment effects on condensed tannin concentrations remained insignificant for the duration of the experiment, except for a single N-effect on the harvest at Day 55 (Table 1).

Microbial respiration and DOC

Carbon dioxide evolution from microbial respiration of the decomposing leaf substrate peaked 41 days after beginning the incubation. The amount of C respired during the 2-week interval preceding this harvest was 6 mgCO_2-C g^{-1} leaf averaged over all treatments (Fig. 4a). For the two-week period immediately prior to the harvest at Day 111, the amount of C respired decreased by 50% to an average of 3 mgCO_2-C g^{-1} leaf. Microbial respiration was significantly affected by the concentration of atmospheric CO_2 at which the leaf litter was produced only on the Day 28 harvest ($P = 0.013$, Table 1). Over this interval, less C was respired from substrate that had been produced under elevated atmospheric CO_2 (Fig. 4a). Microbial respiration was significantly affected by the level of soil-N availability at which the leaf litter had been produced only on the harvest on Day 69 ($P = 0.009$, Table 1). In this case, less C was respired from litter that had been produced under higher N availability. The mean total amount of C respired over the 111-d incubation was 39, 40, 37 and 37 mgCO_2-C g^{-1} litter for the 36HN, 36LN, 56HN and 56LN treatments, respectively.

Initial quantities of DOC-C leached from the aspen leaf litter were very high, averaging 133, 124, 143, and

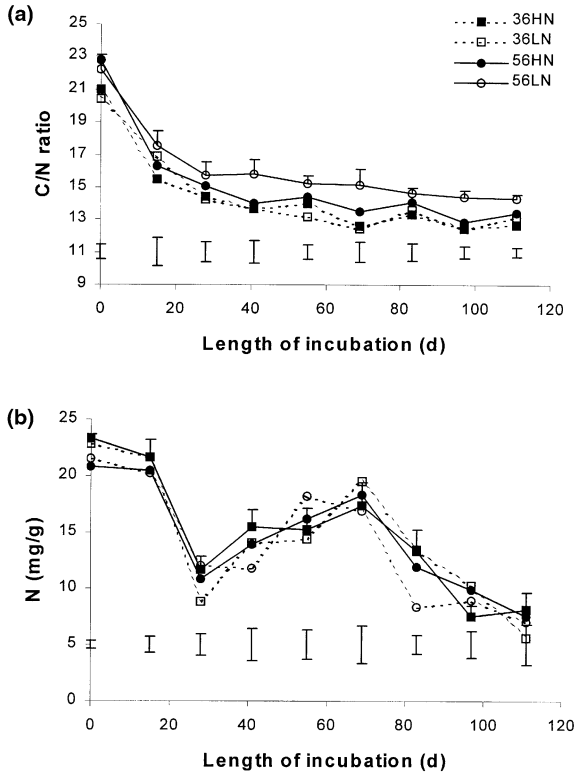

Fig. 2 Mean ($N = 4$ to 5) C/N ratio (a) and N concentration (b) of trembling aspen leaf litter grown at ambient and elevated atmospheric CO_2 and two levels of soil N availability, incubated for 111 d at 25° C. Bars are SE. I-beams are Fisher's least significant difference (LSD) for each *a posteriori* comparison of means. Figure legend: factorial combinations of atmospheric CO_2 partial pressure (36, 56 Pa), and high and low soil N availability (HN, LN), respectively.

146 mgC g^{-1} leaf for the 35HN, 35LN, 55HN and 55LN treatments, respectively, and were not significantly different from one another (Table 1). Fourteen days after the incubations had begun, DOC concentrations in the extracts had dropped precipitously to an average of only 1.5 mgC g^{-1} leaf (Fig. 4b). Thereafter, DOC concentrations in the extracts approached zero, and growth conditions (CO_2, N) continued to have no significant effect (Table 1).

Discussion

We hypothesized that growth under elevated atmospheric CO_2 would cause greater accumulation of TNC in leaf litter of trembling aspen, resulting in higher C/N ratios and higher concentrations of condensed tannins resulting in slower rates of decomposition. We found very minor changes in litter chemistry under elevated atmospheric CO_2, and rates of decomposition were unaltered.

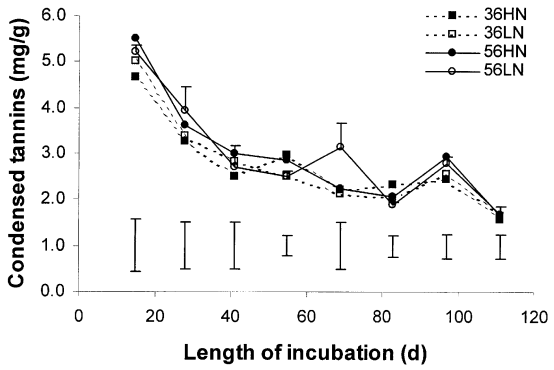

Fig. 3 Mean ($N = 4$ to 5) concentrations of condensed tannins as determined with Quebracho standard in aspen leaf litter grown at ambient and elevated atmospheric CO_2 and two levels of soil N availability and incubated for 111 d at 25° C. Bars are SE, and I-beams are Fisher's least significant difference (LSD) for each *a posteriori* comparison of means. Figure legend: factorial combinations of atmospheric CO_2 partial pressure (35, 55 Pa), and high and low soil N availability (HN, LN), respectively. Initial values are given in Results and were not plotted because trends over time would have been obscured.

Effects of elevated CO_2 and soil-N on litter chemistry

Many investigators have found increases in plant tissue TNC, C/N ratio, and production of secondary compounds in response to elevated CO_2 (Lindroth 1996; Poorter *et al*. 1997; Entry *et al*. 1998; Gebauer *et al*. 1998), in apparent support of the Carbon Nutrient Balance Hypothesis. Increases in the production of secondary compounds under elevated atmospheric CO_2 are, however, often small or are not statistically significant (Peñuelas *et al*. 1996; Heyworth *et al*. 1998; Kainulainen *et al*. 1998). As noted in an earlier study (Randlett *et al*. 1996), differences in foliage chemistry caused by growth under elevated atmospheric CO_2 may not translate into differences in leaf litter chemistry. The most common changes in foliage tissue chemistry in a wide variety of species grown under elevated CO_2 are increases in TNC, and reductions in N concentration (Poorter *et al*. 1997; Martijn Bezemer & Hefin Jones 1998). In a survey of clonal variation in foliage tissue chemistry, Lindroth & Hwang (1996) observed that wild aspen of the same provenance as those used in our study exhibited starch concentrations ranging from less than 10 mg g^{-1} to approximately 50 mg g^{-1} during their late-June sampling.

Concentrations of starch and soluble sugars in naturally senescing aspen litter from this study averaged 96.2 mg g^{-1} and 86.1 mg g^{-1}, respectively. This suggests there was greater allocation of C to TNC late in the season, possibly in response to the growth conditions of our experiment, but also a result of growth sink limitation (Dickson & Nelson 1982; Nguyen *et al*. 1990). We found that concentrations of TNC in this material

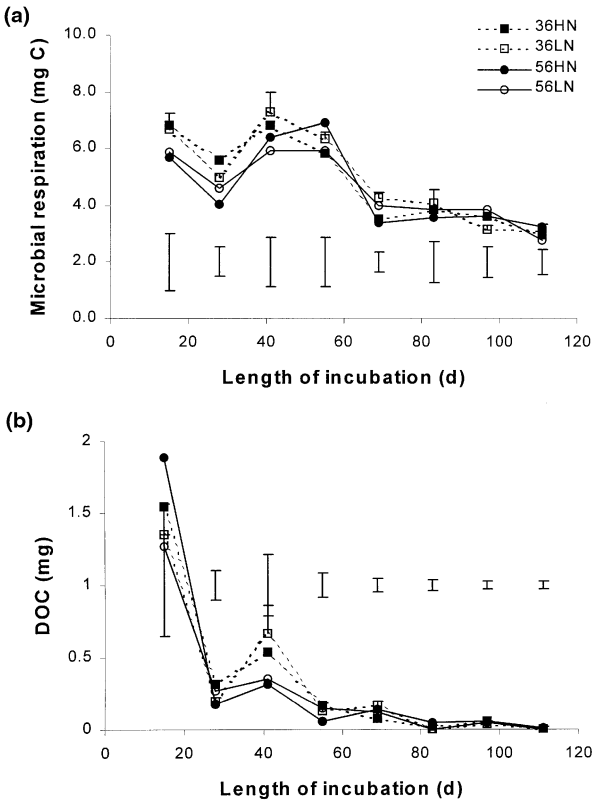

Fig. 4 Carbon lost during decomposition as respired CO_2 (a) or dissolved organic C (b) in aspen leaf litter grown at ambient and elevated atmospheric CO_2 and two levels of soil N availability and incubated for 111 d at 25° C. Values are means ($N = 4$ to 5) and bars are standard errors. I-beams are Fisher's least significant difference (LSD) for each *a posteriori* comparison of means. Figure legend: factorial combinations of atmospheric CO_2 partial pressure (36, 56 Pa), and high and low soil N availability (HN, LN), respectively. Since microbial respiration was quantified as CO_2 accumulation during the 14 days preceding each harvest, no 'initial' values are available.

were not affected by growth CO_2 or soil-N availability. These findings are partially consistent with Randlett *et al*. (1996), who found no change in starch concentrations in litter of *Populus* × *euramericana* in response to elevated CO_2, and contrasting effects on soluble sugars depending on soil-N availability. Wild aspen litter has been reported to have C/N ratios of 78 (Taylor 1998) and 54 (Bockheim *et al*. 1991; assuming C concentration of 47.5%), while the litter used in this study had C/N ratios averaging 20.66 and 22.46 for foliage grown at ambient and elevated CO_2, respectively. The much lower C/N ratios found in our study resulted from the high concentrations of N retained in the senesced foliage, which has been observed in other studies using open-top chambers (Randlett *et al*. 1996). As nutrient concentrations in litter are generally proportional to live tissue concentrations (Nambiar & Fife 1991), discrepancies in C/N ratio could be caused by differences in site fertility, or as shown by our data, effects of the growth chambers. In agreement with Randlett *et al*. (1996), we found very minor changes in C/N ratio of litter produced under elevated atmospheric CO_2. The extremely high N content of our leaf-litter substrate probably minimized N-limitation to microbial growth, as C/N ratios were less than the generally accepted limit of 30, below which N mineralization occurs (Waring & Schlesinger 1985). Concentrations of condensed tannins in wild trembling aspen from the area of the study site were found to range from approximately 130–270 mg g^{-1} depending on genotype (Lindroth & Hwang 1996).

In an open-top chamber study similar to ours, Mansfield *et al*. (1999) quantified genotypic variation in condensed tannin production in trembling aspen in response to elevated CO_2 and found a range from 47 to 167 mg g^{-1} with higher concentrations generally occurring late in the growing season. Although condensed tannins ranged from 50 to 70 mg g^{-1} in our litter, using a different method of tannin determination, effects of elevated atmospheric CO_2 on condensed tannin production should be comparable. Mansfield *et al*. (1999) found greater production of condensed tannins in some aspen clones grown under elevated atmospheric CO_2, while others were unresponsive. We found higher concentrations of condensed tannins in litter produced under elevated CO_2, but differences were small and not statistically significant. This suggests that tannin production in the clones we selected was unresponsive to elevated CO_2, or differences did not persist after senescence. Overall, we found little effect of elevated atmospheric CO_2 on concentrations of TNC or condensed tannins in aspen leaf litter, suggesting the Carbon Nutrient Balance Hypothesis may not be directly applicable to trembling aspen foliage after senescence.

Effects of litter chemistry on decomposition

We investigated the effects of growth CO_2 and N availability on decomposition by quantifying two measures of C loss from decomposing aspen leaf litter: microbially respired CO_2 and dissolved organic C (DOC). Carbon lost as respired CO_2 reflects the rapid cycling of labile substrates (Hungate *et al*. 1997) comprising a short-term response to elevated atmospheric CO_2. Loss of DOC results from the breakdown of more recalcitrant forms of organic compounds largely composed of humic substances, but DOC may also contain significant quantities of labile constituents such as low molecular weight organic acids and carbohydrates (Herbert & Bertsch 1995). Increases in the production of DOC under elevated CO_2 would be expected if litter contained significantly higher quantities of recalcitrant

materials, constituting a longer-term response. For the length of this experiment, however, both measures of decomposition were little affected by growth CO_2 or soil-N availability, supporting the contention that changes to leaf litter decomposition in a future, high-CO_2 world will be minimal (Norby & Cotrufo 1998). In addition, the fact that we did not observe a significant effect resulting from growth N availability while Randlett *et al*. (1996) saw very strong effects resulting from N availability of the soil in the microlysimeters, illustrates the importance of exogenous sources of N to decomposition. Recently, Zak *et al*. (2000) observed that microbial biomass was five times greater and microbial community composition changed (Gram+ bacteria increased, Gram- decreased) under our high compared to our low soil-N availability treatment, but was unaffected by elevated CO_2. This very likely played a role in the large response of microbial respiration to N reported by Randlett *et al*. 1996 and the lack of one in the current study.

Our results agree with previous microlysimeter studies on *Populus* leaf litter decomposition (Randlett *et al*. 1996) but disagree with many recent reports showing reduced rates of decomposition under elevated CO_2 (Ball & Drake 1997; Prior *et al*. 1997; Robinson *et al*. 1997; Cotrufo *et al*. 1998; Torbert *et al*. 1998; van Ginkel & Gorissen 1998). The challenge now is to determine why there is so much disagreement among investigations, a task possibly suited to meta-analysis (Curtis 1996; Curtis & Wang 1998) of the CO_2-decomposition literature.

The dynamics of C loss

It is interesting to contrast the loss of C from decomposing leaf litter as microbial respiration and DOC, while observing the dynamics of starch and soluble sugars, N, and the C/N ratio. The single greatest loss of C occurred during the initial extraction of leaf litter in which an average of 128 and 144 mgC g^{-1} tissue were lost as DOC in ambient and elevated CO_2 ($P = 0.059$), respectively. As soluble sugars comprised 86.1 mg g^{-1} of initial litter, the large loss of C as DOC was largely due to the leaching of this highly labile material. Interestingly, large amounts of 'recalcitrant' condensed tannins may also have been lost at this time, as concentrations dropped from an average 60 mg g^{-1} to less than 10 mg g^{-1} by the time of the first harvest. Leaching of soluble polyphenolics (tannins) in nature has been documented (Suberkropp *et al*. 1976; Schofield *et al*. 1998), and this might partially explain the tea-brown colour we observed in the initial extracts. Additionally, condensed tannins may complex strongly with carbohydrates and have varying solubility in alkaline aqueous solutions, depending on pH (Porter 1992). It is also possible that some of the reduction in condensed tannins was a consequence of drying the decomposed samples at 35 °C after each harvest (Lindroth & Koss 1996). However, the progressive decline in condensed tannins during the incubation does indicate that at least some fraction was subject to microbial degradation. Similar patterns of loss have been observed in decomposing pine litter (Tiarks *et al*. 1992). The marginal significance of the growth CO_2 effect on initial DOC concentrations suggests that the effect of elevated CO_2 on decomposition may occur during the short-lived, but large pulse of labile C in the soil profile leached from freshly senesced foliage (Meyer & Tate 1983). The amount of microbially respired C remained relatively high up to Day 60, but then declined as most of the starch and soluble sugars in the leaf litter had been consumed. Even though microbial respiration accounted for almost all C lost after Day 40, by the end of the experiment the total amount of C lost as DOC was still 3.6 times greater than the total respired as CO_2 (138 mg vs. 38 mg, respectively).

A common response in a series of similar experiments at UMBS is consistently higher C/N ratios in foliage grown at elevated CO_2 and low N availability (Curtis *et al*. 1995 (calculated); Pregitzer *et al*. 1995; Mansfield *et al*. 1999). This pattern continued in the current study (albeit in reduced form) and, remarkably, persisted through the entire course of the incubation. We believe this is a direct result of strong microbial control over C and N dynamics in the decomposing tissue, N and C being lost from the samples in more or less constant proportions. Higher C/N ratio was the most consistently significant response of tissue chemistry to elevated CO_2 and suggests there are long-term changes to tissue chemistry induced by growth under elevated CO_2, but these may not necessarily alter rates of decomposition. Nitrogen concentrations declined a total of 69% and exhibited the three-stage pattern of initial leaching loss, then accumulation, followed by a final release phase (Berg & Staaf 1981). This pattern of N loss was accelerated compared to many field studies (Bockheim *et al*. 1991; King *et al*. 1997b; Taylor 1998) and was probably a result of the lack of fungal hyphae importing N from a surrounding soil volume. In addition, the high N content of the initial substrate may have minimized microbial demand for exogenous N.

Conclusions

Conclusions arrived at in this study must be tempered by the realization that the litter was produced under highly controlled conditions, and the laboratory incubations were far removed from litter decomposing on the forest floor. However, we found little evidence to suggest that the rate of microbial metabolism in decomposing leaf litter will be reduced in a future, high-CO_2 world. If

discrepancies in the literature are indicative of species-specific responses, the agreement between our results and those of previous studies suggests that the decomposition of *Populus* leaf litter will not be affected. The major change in *Populus* litter chemistry under elevated CO_2 was a minor, but significant, increase in C/N ratio when plants were grown under conditions of low N availability. Elevated CO_2 did result in significantly more labile C being leached from litter samples, but effects on the dynamics of more recalcitrant C and N were minimal under the experimental conditions, even though small differences in C/N persisted throughout the course of decomposition. Finally, concentrations of condensed tannins in aspen litter did not appear to be affected by elevated atmospheric CO_2, suggesting that predictions of the Carbon Nutrient Balance Hypothesis may not be directly extended to litter; a hypothesis we feel deserves further investigation.

Acknowledgements

We would like to acknowledge the continued support for our global change research by the University of Michigan Biological Station. Jamie Johnson, Angela Bartnik, Evan Kane, Mike Duscheine and Rebecca Parker provided much needed help with the harvests of the microlysimeter units. This research was funded by the U.S. Department of Energy PER and U.S. Forest Service Global Change Research programs.

References

Alban DH, Perala DA (1992) Carbon storage in Lake States aspen ecosystems. *Canadian Journal of Forest Research*, **22,** 1107–1110.

Amthor JS (1995) Terrestrial higher-plant response to increasing atmospheric [CO_2] in relation to the global carbon cycle. *Global Change Biology*, **1,** 243–274.

Ball AS, Drake BG (1997) Short-term decomposition of litter produced by plants grown in ambient and elevated atmospheric CO_2 concentrations. *Global Change Biology*, **3,** 29–35.

Bazzaz FA, Chiariello NR, Coley PD, Pitelka LF (1987) Allocating resources to reproduction and defense. *Bioscience*, **37,** 58–67.

Berg B, Staaf H (1981) Leaching, accumulation and release of nitrogen in decomposing forest litter. In: *Terrestrial Nitrogen Cycles* (eds Clark FE, Rosswall T). *Ecological Bulletins*, **33**, 163–178.

Bockheim JG, Jepsen EA, Heisey DM (1991) Nutrient dynamics in decomposing leaf litter of four tree species on a sandy soil in northwestern Wisconsin. *Canadian Journal of Forest Research*, **21,** 803–812.

Bryant JP, Chapin FS, IIIKlein DR (1983) Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos*, **40,** 357–368.

Ceulemans R, Mousseau M (1994) Tansley Review no. 71: Effects of elevated atmospheric CO_2 on woody plants. *New Phytologist*, **127,** 425–446.

Cotrufo MF, Briones MJI, Ineson P (1998) Elevated CO_2 affects field decomposition rate and palatability of tree leaf litter: importance of changes in substrate quality. *Soil Biology and Biochemistry*, **30,** 1565–1571.

Curtis PS, Vogel CS, Pregitzer KS, Zak DR, Teeri JT (1995) Interacting effects of soil fertility and atmospheric CO_2 on leaf area growth and carbon gain physiology in *Populus* ×. *euramericana. New Phytologist*, **129,** 253–263.

Curtis PS, Vogel CS, Wang X, Pregitzer KS, Zak DR (2000) Gas exchange, leaf nitrogen, and growth efficiency of *Populus tremuloides* in a CO_2 enriched atmosphere. *Ecological Applications*, **10,** 3–17.

Curtis PS (1996) A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. *Plant, Cell and Environment*, **19,** 127–137.

Curtis PS, Wang X (1998) A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. *Oecologia*, **113,** 299–313.

Dickson RE, Nelson EA (1982) Fixation and distribution of ^{14}C in *Populus deltoides* during dormancy induction. *Physiologia Plantarum*, **54,** 393–401.

Eamus D, Jarvis PG (1989) The direct effects of increase in the global atmospheric CO_2 concentration on natural and commercial temperate trees and forests. *Advances in Ecological Research*, **19,** 1–55.

Entry JA, Runion GB, Prior SA, Mitchell RJ, Rogers HH (1998) Influence of CO_2 enrichment and nitrogen fertilization on tissue chemistry and carbon allocation in longleaf pine seedlings. *Plant and Soil*, **200,** 3–11.

Gebauer RLE, Strain BR, Reynolds JF (1998) The effect of elevated CO_2 and N availability on tissue concentrations and whole plant pools of carbon-based secondary compounds in loblolly pine (*Pinus taeda*). *Oecologia*, **113,** 29–36.

Hagerman AE, Butler LG (1989) Choosing appropriate methods and standard for assaying tannin. *Journal of Chemical Ecology*, **15,** 1795–1810.

Herbert BE, Bertsch PM (1995) Characterization of dissolved and colloidal organic matter in soil solution: A review. In: *Carbon Forms and Functions in Forest Soils* (eds McFee WW, Kelly JM), pp. 63–88. Soil Science Society of America, Madison, WI.

Heyworth CJ, Iason GR, Temperton V, Jarvis PG, Duncan AJ (1998) The effect of elevated CO_2 concentration and nutrient supply on carbon-based plant secondary metabolites in *Pinus sylvestris* L. *Oecologia*, **115,** 344–350.

Horner JD, Gosz JR, Cates RG (1988) The role of carbon-based plant secondary metabolites in decomposition in terrestrial ecosystems. *American Naturalist*, **132,** 869–883.

Hungate BA, Holland EA, Jackson RB, Chapin FS, III, Mooney HA, Field CB (1997) The fate of carbon in grasslands under carbon dioxide enrichment. *Nature*, **388,** 576–579.

Kainulainen P, Holopainen JK, Holopainen T (1998) The influence of elevated CO_2 and O_3 concentrations on Scots pine needles: changes in secondary metabolites over three years exposure. *Oecologia*, **114,** 455–460.

King JS, Thomas RB, Strain BR (1997a) Morphology and tissue quality of seedling root systems of *Pinus taeda* and *Pinus ponderosa* as affected by varying CO_2, temperature, and nitrogen. *Plant and Soil*, **195,** 107–119.

King JS, Allen HL, Dougherty P, Strain BR (1997b) Decomposition of roots in loblolly pine: Effects of nutrient and water availability and root size class on mass loss and nutrient dynamics. *Plant and Soil*, **195,** 171–184.

Kuehny JS, Peet MM, Nelson PV, Willits DH (1991) Nutrient

dilution by starch in CO_2-enriched *Chrysanthemum*. *Journal of Experimental Botany*, **42,** 711–716.

Lindroth RL (1996) CO_2-mediated changes in tree chemistry and tree–Lepidoptera interactions. In: *Carbon Dioxide and Terrestrial Ecosystems* (eds Koch GW, Mooney HA), pp. 105–120. Academic Press, San Diego, CA.

Lindroth RL, Hwang S-Y (1996) Clonal variation in foliar chemistry of quaking apsen (*Populus tremuloides* Michx.). *Biochemical Systematics and Ecology*, **24,** 357–364.

Lindroth RL, Koss PA (1996) Preservation of Salicaceae leaves for phytochemical analyses: further assessment. *Journal of Chemical Ecology*, **22,** 765–771.

Mansfield JL, Curtis PS, Zak DR, Pregitzer KS (1999) Genotypic variation for condensed tannin production in trembling aspen (*Populus tremuloides* Salicaceae) under elevated CO_2 and in high- and low-fertility soil. *American Journal of Botany*, **86,** 1154–1159.

Martijn Bezemer T, Hefin Jones T (1998) Plant–insect herbivore interactions in elevated atmospheric CO_2: Quantitative analyses and guild effects. *Oikos*, **82,** 212–222.

Melillo JM, Prentice IC, Farquhar GD, Schulze E-D, Sala OE (1996) Terrestrial biotic responses to environmental change and feedback to climate. In: *Climate Change 1995: the Science of Climate Change* (eds Houghton JT *et al.*), pp. 447–481, Cambridge University Press, Cambridge.

Meyer JL, Tate CM (1983) The effects of watershed disturbance on dissolved organic carbon dynamics of a stream. *Ecology*, **64,** 33–44.

Nambiar EKS, Fife DN (1991) Nutrient translocation in temperate conifers. *Tree Physiology*, **9,** 5–207.

Nguyen PV, Dickmann DI, Pregitzer KS, Hendrick R (1990) Late-season changes in allocation of starch and sugar to shoots, coarse roots, and fine roots in two hybrid poplar clones. *Tree Physiology*, **7,** 95–105.

Norby RJ, Cotrufo MF (1998) A question of litter quality. *Nature*, **396,** 17–18.

Norby RJ, O'Neill EG, Luxmooore RJ (1986) Effects of atmospheric CO_2 enrichment on the growth and mineral nutrition of *Quercus alba* in nutrient-poor soil. *Plant Physiology*, **82,** 83–89.

Olson JS (1963) Energy storage and the balance of producers and decomposers in ecological systems. *Ecology*, **44,** 322–331.

O'Neill EG, Norby RJ (1996) Litter quality and decomposition rates of foliar litter produced under CO_2 enrichment. In: *Carbon Dioxide and Terrestrial Ecosystems* (eds Koch GW, Mooney HA), pp. 87–103. Academic Press, San Diego, CA.

Peñuelas J, Estiarte M, Kimball BA *et al.* (1996) Variety of responses of plant phenolic concentration to CO_2 enrichment. *Journal of Experimental Botany*, **47,** 1463–1467.

Perala DA (1990) *Populus tremuloides* Michx., trembling aspen. In: *Silvics of North America*, Vol. 2, *Hardwoods.* (eds Burns RMB, Honkala BH), USDA Forest Service Handbook Number 654, pp. 555–569.USDA, Washington, D.C.

Poorter H, Van Berkel Y, Baxter R *et al.* (1997) The effect of elevated CO_2 on the chemical composition and construction costs of leaves of 27 C_3 species. *Plant, Cell and Environment*, **20,** 472–482.

Porter LJ, Hrstich LN, Chan BG (1986) The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry*, **25,** 223–230.

Porter LJ (1992) Structure and chemical properties of the condensed tannins. In: *Plant Polyphenols* (eds Hemingway RW, Laks PE), pp. 245–258. Plenum Press, New York.

Pregitzer KS, Zak DR, Curtis PS, Kubiske ME, Teeri JE, Vogel CS (1995) Atmospheric CO_2, soil nitrogen, and fine root turnover. *New Phytologist*, **129,** 579–585.

Prior SA, Torbert HA, Runion GB *et al.* (1997) Free-air carbon dioxide enrichment of wheat: Soil carbon and nitrogen dynamics. *Journal of Environmental Quality*, **26,** 1161–1166.

Randlett DL, Zak DR, Pregitzer KS, Curtis PS (1996) Elevated atmospheric carbon dioxide and leaf litter chemistry: Influences on microbial respiration and net nitrogen mineralization. *Soil Science Society of America Journal*, **60,** 1571–1577.

Robinson CH, Michelsen A, Lee JA, Whitehead SJ, Callaghan TV, Press MC, Jonasson S (1997) Elevated atmospheric CO_2 affects decomposition of *Festuca vivipara* (L.) Sm. litter and roots in experiments simulating environmental change in two contrasting arctic ecosystems. *Global Change Biology*, **3,** 37–49.

Rogers HH, Heck WW, Heagle AS (1983) A field technique for the study of plant responses to elevated carbon dioxide concentrations. *Air Pollution Control Association Journal*, **33,** 42–44.

Rogers HH, Runion GB, Krupa SV (1994) Plant responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution*, **83,** 155–189.

Schofield JA, Hagerman AE, Harold A (1998) Loss of tannins and other phenolics from willow leaf litter. *Journal of Chemical Ecology*, **24,** 1409–1420.

Sokal RR, Rohlf FJ (1995). *Biometry: the Principles and Practice of Statistics in Biological Research*, 3rd edn. W.H. Freeman, New York.

Strain BR, Bazzaz FA (1983) Terrestrial plant communities. In: *CO_2 and Plants* (ed. Lemon ER), pp. 177–222. Westview Press, Boulder, CO.

Suberkropp K, Godshalk GL, Klug MJ (1976) Changes in the chemical composition of leaves during processing in a woodland stream. *Ecology*, **57,** 720–727.

Taylor BR (1998) Air-drying depresses rates of leaf litter decomposition. *Soil Biology and Biochemistry*, **30,** 403–412.

Tiarks AE, Meier CE, Flagler RB, Steynberg EC (1992) Sequential extraction of condensed tannins from pine litter at different stages of decomposition. In: *Plant Polyphenols* (eds Hemingway RW, Laks PE), pp. 597–608. Plenum Press, New York.

Tissue DT, Wright SJ (1995) Effect of water availability on phenology and the annual shoot carbohydrate cycle of tropical forest shrubs. *Functional Ecology*, **9,** 519–527.

Torbert HA, Prior SA, Rogers HH, Runion GB (1998) Crop residue decomposition as affected by growth under elevated atmospheric CO_2. *Soil Science*, **163,** 412–419.

van Ginkel JH, Gorissen A (1998) In situ decomposition of grass roots as affected by elevated atmospheric carbon dioxide. *Soil Science Society of America Journal*, **62,** 951–958.

Waring RH, Schlesinger WH (1985) *Forest Ecosystems: Concepts and Management*. Academic Press, San Diego, CA.

Zak DR, Pregitzer KS (1990) Spatial and temporal variability of nitrogen cycling in northern Lower Michigan. *Forest Science*, 36, 367–380.

Zak DR, Grigal DF, Ohmann LF (1993) Kinetics of microbial respiration and nitrogen mineralization in Great Lakes forests. *Soil Science Society of America Journal*, 57, 100–106.

Zak DR, Pregitzer KS, Curtis PS, Holmes WE (2000) Atmospheric CO_2 and the composition and function of soil microbial communities. *Ecological Applications*, **10**, 47–59.