Hydrobiologia **495:** 203–211, 2003.

Elevated atmospheric CO_2 alters leaf litter quality for stream ecosystems: an *in situ* leaf decomposition study

Decomposition of elevated CO_2-altered leaf litter

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Received 24 July 2001; in revised form 10 February 2003; accepted 1 March 2003

Key words: atmospheric CO_2, leaf litter, leaf chemistry, decomposition, microbes, invertebrates

Abstract

Trembling aspen (*Populus tremuloides*) seedlings were exposed to both elevated (720 ppm; ELEV) and ambient (370 ppm; AMB) concentrations of atmospheric CO_2 for a 6-month growing season after which senesced leaves were collected and analyzed for differences in chemical composition. Elevated levels of atmospheric CO_2 significantly increased total phenolic compounds, lignin levels, and C:N ratios, while decreasing the concentration of foliar nitrogen. ELEV and AMB leaf aggregates were placed into a headwater stream in the autumn of 1999 for 4 months to assess microbial activity, macroinvertebrate colonization, and leaf decomposition rates. Elevated CO_2 significantly reduced 30 day microbial community respiration (-36.8%), and percent leaf mass remaining after 30 and 120 days of stream incubation (-9.4% and -13%, respectively). Low resolution of the experimental design for testing macroinvertebrate responses to altered leaves, including the free movement of macroinvertebrates among leaf aggregates, may explain the lack of treatment effect on invertebrate distribution between AMB and ELEV leaves. Elevated CO_2-induced increases in leaf litter total phenolic compounds, lignins, and C:N appear to have negative effects on leaf decomposition, especially in the early stages of the decay process where microorganisms play a dominant role.

Introduction

Atmospheric CO_2, rising at a rate greater than 1.5 ppm per year, currently measures 380 ppm and is projected to double within the next half-century (Watson et al., 1992). While extensive research concerning the effects of elevated atmospheric CO_2 on vegetation has been conducted in terrestrial ecosystems (e.g. Curtis et al., 1996; Bezemer & Jones, 1998; Norby & Cotrufo, 1998), little is known about the potential effects in aquatic ecosystems. Terrestrially-based CO_2 research has demonstrated that elevated CO_2 can alter green leaf chemistry, and therefore, foliage quality. Because terrestrial leaf litter supports the food webs of many freshwater aquatic ecosystems, providing upwards of 99% of their energetic requirement (Minshall, 1967; Fisher & Likens, 1973; Petersen & Cummins, 1974), the implications of an elevated CO_2 atmosphere could be great for aquatic ecosystems.

Elevated CO_2 directly alters the foliar chemistry of trees and aquatic emergent plants (Strain & Bazzaz, 1983; Wetzel & Grace, 1983; Curtis et al., 1996), reducing nitrogen concentrations (Curtis et al., 1996; Lindroth, 1996a; Cotrufo et al., 1998) and increasing levels of secondary plant defense compounds (Lambers, 1993; Lindroth, 1996b). These alterations typically increase leaf tissue carbon:nitrogen ratios and lower foliage nutritional quality for insect herbivores

(Lindroth, 1996b; Roth et al., 1997; Bezemer & Jones, 1998; Lindroth & Kinney, 1998). Yet the effect on insect larval performance is not always statistically significant (Kopper et al., 2001; Kopper & Lindroth, 2003). CO_2-induced alterations in leaf litter nutritional quality also can negatively affect terrestrial leaf decomposition as shown by Couteaux et al. (1991), Boerner & Rebbeck (1993, 1995), Cotrufo et al. (1994) and Cotrufo & Ineson (1996), however, CO_2 enrichment effects on litter decomposition in general are species-specific (Couteaux et al., 1999).

Foliar nutritional quality is a major determinant of leaf decomposability in aquatic ecosystems (Stout, 1989; Ostrofsky, 1993, 1997). For instance, secondary compounds, such as condensed tannins and lignins, and leaf toughness are known to induce slow decomposition rates (Stout, 1989). Furthermore, considerable evidence demonstrates that reduced leaf nutritional quality, such as high concentrations of lignins and tannins, interferes with the utilization of detritus by detritivores and microbes (Irons et al., 1988; Nicolai, 1988; Stout, 1989; Ostrofsky, 1993, 1997; Canhoto & Graca, 1999). In this study, we explore the possible indirect effects that elevated levels of atmospheric CO_2 could have on leaf litter decomposition in streams. Our specific hypotheses include:

1. Leaf litter of *Populus tremuloides* (trembling aspen), grown under elevated levels of CO_2, will be of lower quality than litter of ambient-CO_2 grown (control) *P. tremuloides*, exhibiting higher levels of secondary C-compounds, lower nitrogen concentrations, and higher C:N ratios.

2. *In situ* community respiration of leaf colonizing aquatic microbes will be lower on elevated CO_2-produced leaf litter compared to control leaves, a consequence of the CO_2-induced alterations in foliar chemistry.

3. Invertebrate colonization of elevated CO_2-grown leaf litter will be lower than that of the control leaves as a result of the CO_2-alteration of leaf litter quality.

4. Decomposition of elevated CO_2-produced leaf litter will be slower than that of control litter, effectively shifting the processing rate of *Populus tremuloides* from a moderately slow processed species to a slower-processed species.

Materials and methods

Growing aspen on elevated atmospheric carbon dioxide

Populus tremuloides was chosen as a leaf source for this study because of its dominant abundance throughout the state of Michigan (Schmidt et al., 1993), and the quantitative importance of its leaf litter for many streams around our study site, the University of Michigan Biological Station (UMBS) (Tuchman et al., 2002). Aspen clones were exposed to both elevated (720 ppm; ELEV treatment) and ambient (360 ppm; AMB treatment) CO_2 conditions in open top chambers from May through late September, 1999 at the UMBS Elevated CO_2 Research Facility (*sensu* Tuchman et al., 2002). Leaves on the saplings were allowed to naturally senesce and abscise, and leaf litter was collected daily, air dried, and stored in airtight containers for both chemical analyses and use in the *in situ* leaf decomposition study.

Leaf litter chemical analyses

Chemical differences between ELEV and AMB leaf litter were determined for green leaves, senesced leaves, and senesced leaves incubated in the East Branch of the Maple River for 30 days. The following parameters were measured on the above leaves: percent carbon and nitrogen measured on a Carlo-Erba Elemental Analyzer, total phenolic compounds using the Folin-Denis procedure (Swain & Goldstein, 1964), and initial lignin concentrations following the thioglycolic acid method by Dean (1997).

Experimental design

Forty 10 g DW leaf aggregates (20 AMB and 20 ELEV) were constructed by placement into commercial pecan bags (6.35 mm mesh diameter) and anchored with 23 cm gutter nails slightly above the rocky substrate within the East Branch of the Maple River, a small third order headwater stream in Cheboygan Co., MI (N 45°, 32.781" W 84°, 45.178") on 26 September 1999. Microbial responses, invertebrate colonization patterns, and breakdown rates of elevated CO_2-altered leaf litter were monitored over a 4-month period. A large mesh size was used to allow free access to the leaves by aquatic invertebrates. At monthly intervals, 10 leaf aggregates (5 AMB and 5 ELEV) were removed, placed into separate containers filled

with stream water, and transported to the laboratory at UMBS. The experimental litter was removed from the pecan bags, gently washed through a sieve (#60) to remove silt and collect invertebrates, and used for microbial respiration analyses and measurements of leaf breakdown rates. It was evident upon examination of leaf litter removed from the bags that leaves were thinner and decomposing, but they were not fragmented. Therefore, mass was not lost via leaf fragmentation.

Microbial and invertebrate responses, and decomposition rates of ELEV and AMB leaf litter

Total microbial community respiration was measured using the dissolved oxygen uptake method (Wetzel & Likens, 2000). On each sampling date, approximately 5 g (or that which remained) of leaf material from each of 10 leaf bags (5 AMB, 5 ELEV) was placed into individual BOD bottles filled with sterile filtered (0.22 μm) stream water. BOD bottles were placed in a darkened environmental chamber at 5 °C, and allowed to incubate for 4 h to ensure a minimum decrease of 1 mg O_2 per liter of H_2O attributable to microbial respiration. Magnetic stir bars were added to the BOD bottles to adequately disperse O_2 throughout the bottles. Under identical temperature, light, water quality and water movement conditions of the environmental chamber, any differences measured in microbial community respiration of ELEV relative to AMB leaves can be attributed to a difference in leaf substrate quality and associated differences in microbial standing stock. After respiration analyses, litter from each leaf aggregate was oven-dried at 60 °C for 48 h and weighed to estimate percentage of leaf litter mass remaining (%R). %R estimates were log-transformed and regressed against the number of stream incubation days (Benfield, 1996) to determine processing rate (k) estimates.

Invertebrates associated with individual leaf aggregates were identified to the lowest taxonomic unit possible and placed into functional feeding groups (Cummins & Klug, 1979; Merritt & Cummins, 1996). After identification, invertebrates were enumerated, oven-dried at 60 °C for 48 h and weighed. Invertebrate densities and biomasses were standardized on a per gram leaf mass basis to determine possible treatment effects on leaf colonization and invertebrate community composition patterns.

Statistical analyses

To determine if elevated levels of atmospheric CO_2 lowered the quality of aspen litter, differences between AMB and ELEV leaf chemistry within green leaves, senesced leaves, and leached/microbially colonized leaf litter were analyzed using a one-way ANOVA tests. Our *a priori* hypothesis stated that ELEV $\geq$ AMB in all chemical parameters measured and for percent leaf mass remaining, while AMB $\geq$ ELEV for total microbial community respiration, invertebrate density and biomass per gram leaf material, and leaf processing rates. Based on the *a priori* hypotheses, these dependent variables were analyzed for AMB and ELEV treatment differences using 1-tailed *t*-tests (Systat 7).

Results

Leaf litter chemistry

The foliar chemistry of *P. tremuloides* was significantly altered by elevated levels of atmospheric CO_2. ELEV green leaves had significantly higher C:N ratios, and total phenolic compound concentrations than their AMB counterparts (1-way ANOVA; $p < 0.05$, Table 1). Leaf senescence further decreased the quality of ELEV leaf litter, as C:N ratios and lignin concentrations were significantly higher in ELEV senesced leaf litter than in ELEV green leaf tissue (1-way ANOVA; $p < 0.05$, Table 1). After 30 days of stream incubation, C:N ratios and total phenolic compound levels did not significantly differ between treatments (1-tailed *t*-test; $p > 0.05$; Table 1).

Microbial response and invertebrate colonization of CO_2-altered leaf litter

After 30 days of incubation in the East Branch of the Maple River, microbial community respiration was 36.8% lower on ELEV leaves than on AMB (ELEV = 0.86 mg O_2 g^{-1}leaf hr^{-1}, AMB = 1.36 mg O_2 g^{-1} leaf hr^{-1}; 1-tailed *t*-test; $p < 0.05$, Fig. 1). This treatment effect trend was consistent through day 90, however, no significant differences in microbial respiration between ELEV and AMB litter could be discerned after day 30 (1-tailed *t*-test; $p > 0.05$, Fig. 1). For both ELEV and AMB litter, microbial community respiration increased with time up to day 60, then decreased slightly thereafter (Fig. 1).

Table 1. C:N ratios,% total phenolic compounds, and % lignin of both ELEV and AMB green leaves, naturally senesced litter, and litter incubated in the East Branch of the Maple River for one month. Values expressed as means ± s.d. Asterisks (*) indicate a statistically significant difference between means ($p < 0.05$)

	AMB	ELEV
Live/Green:		
Lignin (% leaf dry mass)	1.88 ± 0.22	2.19 ± 0.36
Total phenolics (% leaf dry mass)	9.55 ± 0.35	11.15 ± 0.49*
C:N	23.40 ± 0.30	27.50 ± 0.54*
Senesced litter:		
Lignin (% leaf dry mass)	11.70 ± 0.72	15.50 ± 1.21*
Total phenolics (% leaf dry mass)	3.40 ± 0.20	5.64 ± 0.28*
C:N	28.10 ± 1.03	56.10 ± 1.82*
30 Day Incubated litter:		
Lignin (% leaf dry mass)	not measured	not measured
Total phenolics (%leaf dry mass)	n.d.	n.d.
C:N	34.68 ± 1.46	33.16 ± 3.84

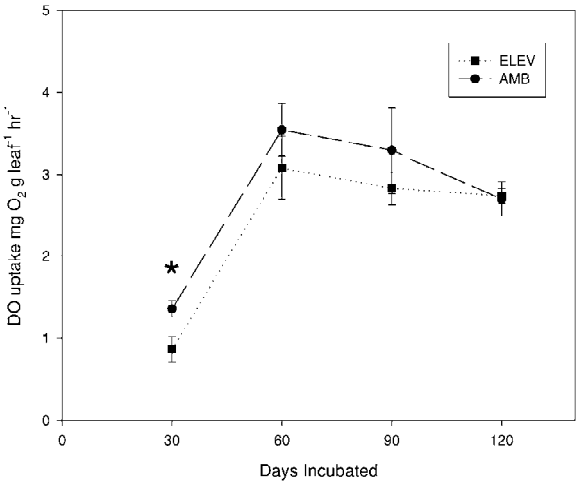

Figure 1. Microbial metabolic activity on ELEV and AMB litter incubated in the East Branch of the Maple River for 30, 60, 90, and 120 days. Values are expressed as means ± S.E. Asterisks (*) indicate a statistically significant difference between means ($p < 0.05$).

Although no statistical differences in invertebrate colonization patterns (densities) on the leaf aggregates were observed between treatments (1-tailed *t*-test; $p > 0.05$, Fig. 2), there was a strong trend; ELEV leaf aggregates contained lower densities of invertebrates on a per gram leaf basis than their AMB counterparts, with the exception of day 120. Throughout the experiment, invertebrate density increased on both ELEV and AMB litter, declining only on AMB litter after day

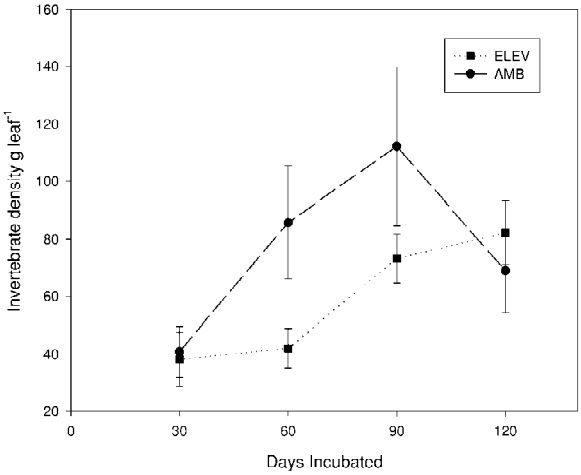

Figure 2. Total invertebrate colonization of ELEV and AMB leaf aggregates incubated in the East Branch of the Maple River for 30, 60, 90, and 120 days. Values are expressed as means ± S.E.

90. Invertebrate abundance was also analyzed based on relative densities and relative biomasses within functional feeding groups to specifically look at the leaf-shredding invertebrate response. While ELEV leaf aggregates supported lower densities of invertebrate shredders, collectors, and scraper/grazers, these trends were not significant (1-tailed *t*-test; all comparisons $p > 0.05$, Fig. 3). Additionally, no trends were apparent for invertebrate biomass, although it is interesting to note that shredder biomass on ELEV leaf aggregates was consistently near zero (1-tailed *t*-test; all comparisons $p > 0.05$, Fig. 3B).

In situ *breakdown of CO_2-altered leaf litter*

Decay rates (k) of ELEV *P. tremuloides* leaf aggregates were significantly slower than for AMB leaf aggregates during the first 30 days of stream incubation (ELEV = -0.018 d^{-1}, AMB = -0.021 d^{-1}; 1-tailed *t*-test; $p < 0.05$, Fig. 4). Additionally, ELEV leaf aggregates maintained significantly more leaf litter mass than AMB leaf aggregates after 30d (ELEV = 58.56%R, AMB = 53.03%R; 1-tailed *t*-test; $p < 0.05$, Fig. 4). As with microbial respiration, treatment effects were masked after 60 days of stream incubation, however, ELEV leaves were more recalcitrant to decay that AMB leaves after 120 days of stream incubation although this trend was not significant (1-tailed *t*-test; $p > 0.05$, Fig. 4). Overall decay rates (k) for 120 days of stream incubation were calculated as 0.0011 d^{-1} for both treatments and did not differ statistically (1-tailed *t*-test; $p > 0.05$, Fig. 4).

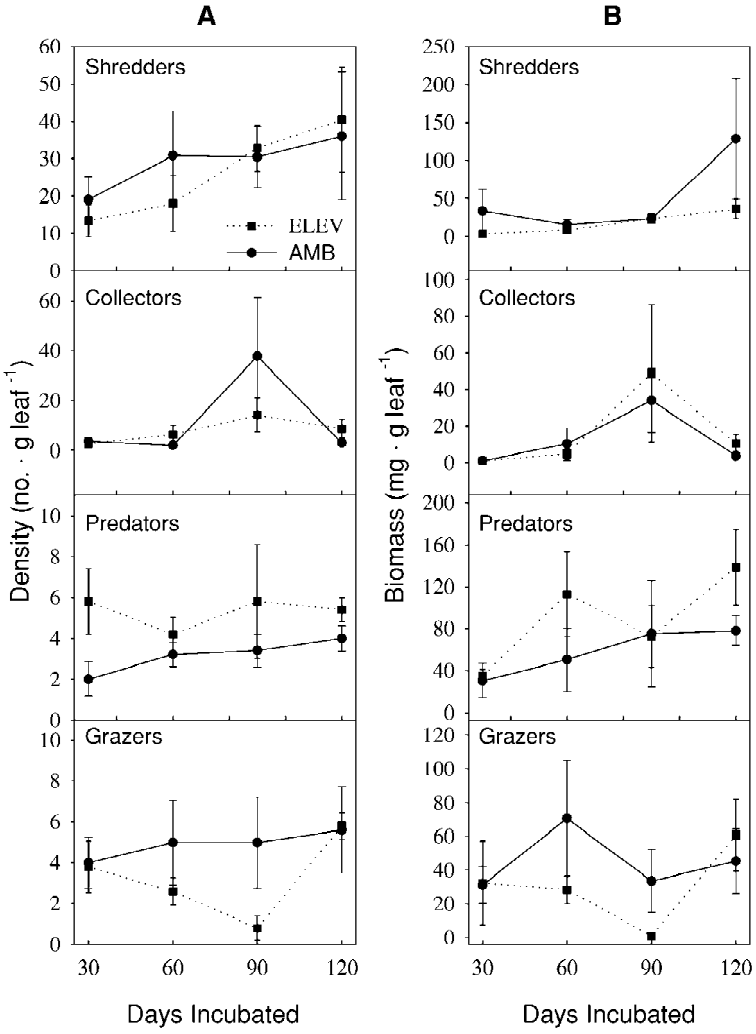

Figure 3. Invertebrate colonization expressed as density (A) and biomass (B) of functional feeding groups of ELEV and AMB leaf aggregates incubated in the East Branch of the Maple River for 30, 60, 90, and 120 days. Values are expressed as means ± S.E.

Discussion

Leaf litter quality

Elevated levels of atmospheric CO_2 significantly altered the chemical quality of *Populus tremuloides* green leaves and leaf litter. Both ELEV green leaves and leaf litter contained higher C:N ratios, percent total phenolic compounds, and lignin concentrations, as well as lower levels of foliar nitrogen than their AMB counterparts. A number of studies have demonstrated similar phytochemical alterations under conditions of enriched CO_2 for green and senescent leaves of *P. tremuloides* and other species. For example, Curtis et al. (1996) reported a 5% decrease in green leaf foliar nitrogen under elevated CO_2 conditions for bigtooth aspen, *Populus grandidentata*. Using *P. tremuloides*, Roth et al. (1997) found a 14% decline in green leaf foliar nitrogen levels as well as large increases in the concentrations of certain secondary carbon-based metabolites. Salicortin and tremulacin, two of these secondary metabolites, increased by 45% and 69%, respectively. Lindroth (1996a) reported a 24% decrease in nitrogen levels and a 75% increase in the concentration of condensed tannins for green leaves of *P. tremuloides* as well. Additionally, Cotrufo et al. (1994) found a significant increase in lignin concentrations and C:N ratios, as well as a decrease in nitrogen concentrations for the senesced litter of ash,

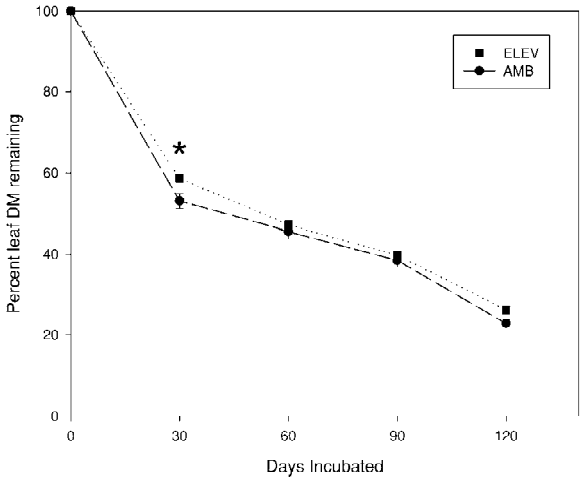

Figure 4. Percent leaf mass remaining (%R) for ELEV and AMB leaf litter decomposing in the East Branch of the Maple River for 30, 60, 90, and 120 days. Values are expressed as means ± S.E. Asterisks (*) indicate a statistically significant difference between means ($p < 0.05$).

birch, sycamore, and spruce. Elevated CO_2-induced changes in green and senesced phytochemistry seem to be consistent among species with similar responses having been documented for several woody, weed, and crop species (see review by Curtis, 1996).

Leaf decomposition rates in both aquatic (Stout, 1989; Ostrofsky, 1993, 1997) and terrestrial (Melillo et al., 1982; Taylor et al., 1989) ecosystems are strongly influenced by leaf chemical quality. Ostrofsky (1997) demonstrated that the best individual predictors of leaf processing rates were percent nitrogen, C:N ratio, condensed tannins, and percent lignin:percent nitrogen ratio, with 50% of the variation in leaf processing rates explained by total phenolics, nitrogen content, and lignin concentration. These factors influence microbial colonization and leaf conditioning as well as macroinvertebrate utilization (Suberkropp et al., 1976; Canhoto & Graca, 1999). In addition, Stout (1989) demonstrated that high condensed tannin and lignin concentrations, and leaf toughness, induced slow decomposition rates by stream organisms. In the present study and our previous studies (Tuchman et al., 2002, 2003; Rier et al., 2002), the quality of *P. tremuloides* leaf litter was altered by CO_2 enrichment, reducing rates of microbial colonization and leaf decomposition (especially in the early stages of leaf decay in the stream) and reducing effective utilization by invertebrate detritivores.

Microbial response and invertebrate colonization patterns of experimental leaf litter

During the first 30 days of stream incubation, the combined effects of lower leaf nitrogen and high C:N ratios and lignin concentrations in ELEV leaves may have contributed to the inhibited colonization and growth of stream microbes. Highly recalcitrant compounds including structural polyphenolics such as lignin, are known to be inhibitors of aquatic microbial colonization and activity (Suberkropp et al., 1976; Gessner & Chauvet, 1994). In a previous study of microbial responses to ELEV and AMB *Populus tremuloides* litter, we found significant reductions in bacterial and fungal biomass and productivity in the East Branch of the Maple River throughout the 35d study (Rier et al., 2002). Microbial reductions in that study were related to higher ELEV leaf C:N and lignin content, while 99% of the total phenolic compounds were removed by leaching within the first 5 d of immersion (Rier et al., 2002).

After 60 days of stream incubation in the present study, microbial respiration on ELEV litter did not statistically differ from AMB. One explanation for this phenomenon is that 60 days of stream incubation may be sufficient for leaching and microbial degradation of the recalcitrant materials, eliminating the original chemical discrepancy between AMB and ELEV leaves. This explanation is supported by the observation that ELEV and AMB leaf litter incubated in the East Branch of the Maple River for more than 30 days did not differ in its concentration of total phenolic compounds or C:N ratios.

Studies of microbial decomposition of CO_2-enriched litter in terrestrial environments have shown mixed responses. Microbial decomposition of straw from plants grown at elevated CO_2 was significantly slower than on ambient CO_2-grown straw after 5 months (Frederiksen et al., 2001). Similarly, Cotrufo et al. (1998) demonstrated significantly slower decomposition of elevated CO_2-produced ash and sycamore after one year. However, a meta-analysis of published and unpublished experiments was conducted by Norby et al. (2001) and suggests that the common changes in leaf chemistry due to CO_2enrichment do not impact the decomposition process in a consistent way.

Colonization of leaf aggregates by invertebrates (densities and/or biomasses) was not significantly affected by the phytochemical alterations found in ELEV leaf litter. Invertebrates associated with leaf aggregates were measured only once every 30 days,

effectively taking a snap-shot of invertebrate distributions, which were likely changing between ELEV and AMB leaf aggregates throughout the study. Most invertebrates typically drift in search of new or better food resources, shelter, or to evade predators (Otto, 1976; Watson et al., 1977). For this reason, whether the invertebrates were abundant or not on ELEV or AMB leaf aggregates at any given time could be attributed solely to chance. Our companion laboratory studies on the effects of elevated CO_2-altered leaf litter as food for macroinvertebrate detritivores have demonstrated that when invertebrates are not given a choice of leaf litter types, ELEV leaf litter significantly decreases *Tipula abdominalis* consumption rates, assimilation, and growth (Tuchman et al., 2002). Likewise, for the 4 species of mosquito larvae tested, all demonstrated significantly reduced development rates and/or higher mortality when reared on ELEV leaf detritus (Tuchman et al., 2003). Cotrufo et al. (1998) demonstrated a similar reduction in isopod performance in field decomposition studies of ash and sycamore leaf litter produced under CO_2 enrichment. A recent study by Adams et al. (2003, in review) demonstrates that when given a choice of ELEV or AMB *P. tremuloides* leaf litter, crayfish show a significant preference for AMB litter. These studies indicate that macroinvertebrate growth, development, and survivorship can be compromised with ELEV leaf litter, and suggest that in natural settings, macroinvertebrates, may choose not to remain on ELEV leaf aggregates unless they are using the leaves as refugia rather than food.

Decomposition of CO_2-altered leaf litter

The decomposition rate of ELEV aspen litter was slower than AMB litter during the first 30 days of stream incubation, a result consistent with the reduced microbial response. After 60 days of stream incubation however, %R for both ELEV and AMB leaves was similar, but started to diverge again after 120 days of stream incubation. We postulate that the highly structural polyphenolic compounds, and the low levels of litter nitrogen, were responsible for the negative effect elevated levels of CO_2 had on the first 30d of aspen litter decomposition; results that are consistent with similar litter decomposition studies (Cotrufo et al., 1998; Frederiksen et al., 2001; Rier et al., 2002). However, as these structural compounds were broken down during the subsequent month of stream incubation, microbial metabolic activity and leaf decomposition rates on ELEV litter became similar to AMB, eliminating the previously observed treatment effect. The terrestrial studies mentioned above by Cotrufo et al. (1998) and Frederiksen et al. (2001) found that CO_2 treatment differences in decomposition rates persist for up to a year. We suggest that in stream ecosystems, the process of chemical leaching and microbial decomposition may be faster than for terrestrial soil systems due to the convection of current increasing diffusion of compounds from the leaves.

Leaf processing rates in streams have been well established for many tree species (Petersen & Cummins, 1974; Ostrofsky, 1997). Depending on certain chemical parameters, such as lignin, phenolic compounds, and nitrogen content, leaves from different tree species can be categorized on a continuum from slow- to fast-decomposing. For example, *Quercus alba* (white oak) is considered a slow decomposing species due to its high lignin content (36.67%) and low nitrogen levels (0.67%), whereas *Alnus rugosa* (speckled alder), containing high nitrogen concentrations (2.30%), is considered a fast decomposing species (Ostrofsky, 1997). *Populus tremuloides* leaves contain moderate levels of polyphenolic compounds and intermediate levels of nitrogen, and fall in the middle of the leaf processing spectrum. However, elevated levels of atmospheric CO_2 cause both significant increases in leaf polyphenolic levels and decreases in nitrogen concentrations, effectively shifting *P. tremuloides* towards the slower end of the processing rate spectrum.

Summary and conclusions

The potential indirect effects of elevated levels of atmospheric CO_2 to aquatic ecosystems may be important. Terrestrial and aquatic plant research has demonstrated impacts on the chemical and nutritional quality of green and senesced plants grown under conditions of elevated CO_2. Previous studies in our lab demonstrate significant negative effects of CO_2-altered leaf litter on aquatic microbial productivity as well as the growth, development, and survival of multiple aquatic detritivore macroinvertebrates. It follows that the process of leaf decomposition in small lakes and headwater streams could be significantly altered. The main goal of this research was to assess, at low resolution, the overall effects of elevated CO_2-altered leaf litter quality on the process of litter breakdown in a headwater stream. We found that while leaf litter quality was significantly reduced, negatively affecting

both microbial respiration and leaf litter decomposition rates during the first month of stream incubation, enhanced leaching and microbial decomposition of leaves in streams appeared to have neutralized the differences in leaf litter chemistry after 30 d incubation. As a result, longer term leaf processing rates and invertebrate colonization patters did not significantly differ between CO_2 treatments of leaf litter.

Acknowledgements

We would like to thank Steven Rier, Michael Grant, David Karowe, Angela Spickard, Christoph Vogel, and Alexander Pavlovich for their contributions to this study. Leaf litter was produced at the University of Michigan Biological Station Elevated CO_2 Research Facility. This research was supported by two grants awarded to NCT, RGW, and JAT from the National Science Foundation (DEB-9903888 and DEB-0108847).

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