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Elevated atmospheric CO_2 lowers leaf litter nutritional quality for stream ecosystem food webs

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Abstract

Up to 99% of the carbon fuelling the food webs of temperate woodland streams is derived from inputs of terrestrial leaf litter. Aquatic bacteria, fungi, and detritivore invertebrates directly utilize these inputs, transferring this energy to other components of the food web. Increases in atmospheric CO_2 could indirectly impact woodland stream food webs by chemically altering leaf litter. This study evaluated CO_2-induced chemical changes in aspen (*Populus tremuloides*) leaf litter, and the corresponding effects on stream bacteria, fungi and leaf-shredding cranefly larvae (*Tipula abdominalis*: Diptera). Leaf litter from plants grown under elevated CO_2 had decreased nutritional value to aquatic decomposers and detritivores because of higher levels of structural compounds and lower nitrogen content. Consequently, elevated CO_2-grown leaf litter supported 59% lower bacterial production in a stream than litter grown at ambient CO_2 levels, while not affecting fungal biomass. Larval craneflies fed elevated CO_2-grown microbially colonized leaves consumed less, assimilated less, and grew 12 times slower than their ambient fed counterparts.

Keywords: atmospheric CO_2, bacteria, C : N, leaf litter, phenolic compounds, *Tipula abdominalis*

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Introduction

Rising levels of atmospheric CO_2 will likely have countless effects on terrestrial and aquatic ecosystems ranging from immediate physiological changes in plants to long-term effects on ecosystem-level processes such as nutrient cycling, herbivory and detrital decomposition. Some studies assessing the effects of CO_2 enrichment have demonstrated that the chemical composition of leaf tissues of C3 plants can be modified greatly as a result of higher rates of carbon fixation and production (e.g. Strain & Bazaaz 1983; Wetzel & Grace 1983; Curtis *et al*. 1996). Increases in carbon coupled with decreases in nitrogen compounds, result in significantly higher C : N ratios of elevated CO_2-produced leaf tissues. Considerable evidence also indicates that elevated CO_2-induced increases in carbon-rich structural compounds (e.g. lignin) and phenolic-based defence compounds (e.g. tannin) can inhibit herbivory on fresh plant tissues (Levin 1971; Cates & Rhoades 1977; Swain 1979) and interfere with utilization of detritus by detritivores and microbes (Nicolai 1988; Stout 1989).

Although the direct effects of elevated atmospheric CO_2 on terrestrial plants (e.g. Jacob *et al*. 1995) and the indirect effects on herbivory (e.g. Lindroth 1996a; Lindroth 1996b; Lindroth & Kinney 1998; Coviella & Trumble 1999) and soil microbes (e.g. O'Neill & Norby 1996; Randlett *et al*. 1996) have been investigated, effects on detrital-based aquatic ecosystems remain unknown. In most forested streams the autumnal input of leaf litter exceeds in-stream primary production as a source of carbon fuelling the food web. In many streams, terrestrial detritus accounts for up to 99% of the food base for the ecosystem (Minshall 1967; Fisher & Likens 1973). Hence, a significant decrease in leaf litter nutritional quality induced by elevated atmospheric CO_2 conditions will have impacts on the stream organisms utilizing leaf litter as their primary source of energy.

The objectives of this study were to determine the effects of elevated CO_2 on the chemistry of leaf litter entering streams and potential indirect effects on the

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utilization of the litter by microbes and detritivores. Three specific hypotheses were tested: (i) leaf litter of *Populus tremuloides* (trembling aspen) grown under elevated CO_2 conditions will exhibit higher C : N, greater concentrations of phenolic compounds, and higher lignin concentrations; (ii) biomass and productivity of aquatic microbes will be lower on elevated CO_2-produced leaf litter as compared to ambient CO_2-produced leaves; and (iii) consumption, assimilation, and growth of the aquatic detritivore, *Tipula abdominalis*, will be reduced when given a diet of elevated CO_2-produced litter as a result of a general decrease in food quality.

Methods

Growing aspen on elevated atmospheric carbon dioxide

In order to determine the effect of elevated concentrations of atmospheric CO_2 on leaf litter phytochemistry, trembling aspen trees (*Populus tremuloides*) were grown under both elevated (720 ppm; ELEV treatment) and ambient (360 ppm; AMB treatment) atmospheric CO_2 conditions from leaf-out through natural senescence at the University of Michigan Biological Station (UMBS) in northern lower Michigan. *Populus tremuloides* was chosen because it is the most abundant tree species in Michigan (Schmidt *et al*. 1993), and because a previous survey conducted in November 1996 on the Maple River, a 3rd-order forested stream at UMBS, estimated that this species accounts for a significant contribution (22%) of the litter entering the stream. Clear plastic open-top chambers ($1\,m \times 1\,m \times 2\,m$; see Curtis & Teeri 1992 for design) were placed over 16 cloned 4-year-old saplings of *P. tremuloides*, which were all planted in open-bottom root boxes containing a premixed soil homogenate of 80% native rubicon sand amended with 20% topsoil to provide soil nutrients. In addition to promoting aspen growth, the addition of topsoil provided moderate nitrogen levels to produce experimental soil conditions that have a wider geographical application than the extremely nutrient poor granitic sands of northern lower Michigan. CO_2 partial pressure was elevated in 8 of the 16 experimental chambers via manually adjusted flowmeters that dispensed 100% CO_2 into chamber input fans allowing for equal diffusion of CO_2 throughout the chamber (Curtis & Teeri 1992). Chamber gas concentrations were monitored continuously in eight ELEV chambers and one AMB chamber by an infrared gas analyser (LiCor model LI-6252) that logged data into a personal computer.

The aspens were treated with CO_2 enrichment from May through leaf senescence in November of 1999. During the treatment period, all aspens were watered twice weekly or more as needed. Throughout autumnal leaf abscission, leaves were collected on a daily basis, air dried, and stored in airtight containers for both chemical analyses and use in microbial and detritivore experiments.

Leaf litter chemical analyses

Phytochemical differences between AMB and ELEV treatment leaves were determined for green leaf tissue, senesced abscissed leaves, and senesced leaves that were incubated in the Maple River for 14 days, which induced chemical changes resulting from leaching and microbial colonization. Analyses of live vs. senesced leaf chemistries allowed parallel comparisons to be made between detritivore responses and herbivore responses to ELEV and AMB green foliage as reported in the literature. Comparisons of senesced leaf chemistry with microbially colonized leaves offered information on changes in ELEV and AMB leaf quality resulting from initial leaching and incorporation of bacterial and fungal biomass into the leaf matrix. The following parameters were measured on all leaves: percent carbon and nitrogen measured on a Carlo-Erba Elemental Analyser, lignin concentrations using the thioglycolic acid method (Dean 1997), and total phenolic compounds following the Folin–Denis assay (Swain & Goldstein 1964).

Leaf toughness was measured on wet leaves that had been incubated in the stream for 40 days. A simple penetrometer was used to measure the mass required to force a stainless steel rod (3 mm diameter) completely through each leaf. Five replicate punches were made on each leaf with care being taken to avoid the veins. Four replicate leaves from each treatment were examined.

Microbial responses to ELEV and AMB leaf litter

Forty-eight leaves (24 AMB and 24 ELEV) were individually placed into 1 mm mesh packets and suspended in the current of the East Branch of the Maple River (N 45°, 32.781′ W 84°, 45.178′) for 14 days in order to measure microbial responses to CO_2-induced leaf chemical changes, and to provide a food quality estimate for colonized leaves used in the tipulid feeding studies. Prior to submersing leaves in the stream, the bottom right quarter of each leaf was removed for initial analyses of C : N and total phenolic compounds. After the 14-d incubation, leaves were removed and assayed for microbial colonization parameters as well as final C : N and total phenolics. Total microbial community respiration was measured on colonized leaves using the dissolved oxygen uptake method (modified from Wetzel & Likens 2000). Five leaves from each treatment were selected randomly and placed into 10 individual biological oxygen demand (BOD) bottles filled with sterile filtered (0.22 μm pore size) stream water which was continuously stirred using magnetic stir bars. BOD bottles were placed in a darkened

environmental chamber at stream temperature (17 °C) and allowed to incubate for 4 h to ensure a minimum decrease of 1 mg O_2 per litre of water. Fungal biomass was estimated by the extraction and quantification of ergosterol content per mg ash free dry mass of leaf detritus by HPLC analyses (Newell & Fallon 1991; Suberkropp & Weyers 1996). The growth of bacteria is much faster than that of fungi, so while a measure of fungal biomass served to indicate the fungal response to ELEV CO_2 by day 14, bacterial biomass would not sufficiently indicate the turnover rate of bacteria at a single time interval. Therefore, we measured bacterial biomass-specific productivity which was estimated by using productivity per unit biomass, where biomass was a measure of cell biovolume times the density of cells of a given geometric shape. Bacterial density was estimated by sonicating samples for 30 s to remove cells from 1.4-mm dia leaf disks in sterile filtered (0.2 μm) tetrasodium pyrophosphate (Velji & Albright 1986), then staining with DAPI (4′6-diamidino-2-phenylindole), and enumerating cells using epifluorescent microscopy. Biovolumes were estimated using geometric shapes (Psenner 1993; Wetzel & Likens 2000) and total bacterial carbon was estimated by multiplying biovolumes by 5.6×10^{-13} gC μm^{-3} (Bratbak 1985). Bacterial productivity was estimated by sonicating a 1.4-mm diameter leaf disk in 10 mL sterile filtered (0.2 μm pore size) stream water for 30 s to remove cells from the leaf (Thomaz & Wetzel 1995). While productivity of the intact attached bacterial assemblage on leaf disks would be preferrable to disturbing their natural orientation by sonication, we chose to remove bacterial cells from the leaf disks prior to incubation in ^{3}H-leucine in order to separate bacterial uptake from the fungi whose hyphae were growing within the leaf matrix, and therefore could not be dislodged effectively with 30 s sonication. It also proved to be advantageous to remove the bacterial cells from the leaf disk because the leaf disk was seated in the bottom of the scintillation vial so that the majority of the radiation emitted by bacteria on the underside of the leaf was absorbed by the leaf disk, significantly underestimating bacterial productivity. Dislodged bacterial cells were incubated in sterile filtered stream water with 10 μL of 99.9 Ci $mmol^{-1}$ ^{3}H-leucine for 30 min at stream temperature (17 °C), and productivity was estimated as the rate of ^{3}H-leucine uptake and incorporation into intracellular protein (Kirchman 1993). Biomass-specific bacterial productivity was calculated as scintillation counts per minute (cpm)/μg bacterial carbon.

Detritivore responses to ELEV and AMB leaf litter

In order to determine the indirect effects of elevated atmospheric CO_2 on detritivore growth and assimilation, we conducted laboratory feeding experiments using larvae of the leaf-shredding cranefly, *Tipula abdominalis* (Say). *Tipula abdominalis* was used because it is a dominant leaf-shredding invertebrate in Michigan headwater streams (Sharma *et al*. 1984) and its life history is well documented (Alexander 1965; Vannote & Sweeney 1985). Tipulids were collected from Stoney Creek, Isabella Co., MI, in early January 2000. Larvae were wet-weighed in the laboratory and placed into 100-mg size classes. Twenty-eight individuals from the 600–700 mg size-class were placed in small glass chambers (12 cm diameter) containing 200 mL filtered (0.45 μm pore size) stream water. Experimental leaf litter was leached and colonized microbially in natural waters for 2 weeks; half of the tipulids were fed *ad libitum* colonized ELEV litter, and half were given colonized AMB litter. Feeding chambers containing larvae and leaf litter were placed within an environmental chamber with a 9 : 15 light : dark photoperiod at 5 °C, the typical photoperiod and temperature of Stoney Creek in early January.

Tipulids were grown on ELEV or AMB leaf litter for 7 days (a length of time used effectively for estimations of assimilation efficiency and growth in *T. abdominalis* by Lawson *et al*. 1984) to estimate relative consumption rates (RCR), efficiencies of conversion of ingested food into biomass (ECI), and relative growth rates (RGR) according to Waldbauer (1968). Consumption rates were measured as the difference between initial and final leaf mass, and standardized by tipulid mass as follows:

$$\text{RCR (mg)g}^{-1}\text{d}^{-1} = \frac{\text{leaf dry mass ingested}}{\text{(mean animal dry mass) (days)}}$$

The efficiency of conversion of ingested food into biomass was estimated as a proportion of tipulid mass increase per leaf mass ingested:

$$\text{ECI (\%)} = \frac{\text{(animal dry mass gained)}}{\text{(leaf dry mass ingested)}} \times 100$$

Relative growth rates were estimated as a change in tipulid mass over time relative to its size:

$$\text{RGR (mg) g}^{-1}\text{d}^{-1} = \frac{\text{animal dry mass gained}}{\text{(mean animal dry mass) (days)}}$$

On day 7, larvae were blotted dry systematically to remove external water and wet-weighed. Initial and final wet weight measurements were converted to dry weight using a conversion factor for *T. abdominalis* (wet weight × 0.0797 = dry weight) reported by Vannote & Sweeney (1985). Leaf material was oven-dried at 100 °C for 48 h and weighed for estimates of ingestion (total leaf weight loss).

Statistical analyses

The differences between AMB and ELEV leaf chemistry within green, senesced, and microbially colonized leaves were analysed using one-tailed *t*-tests (null hypothesis; ELEV ≤ AMB). One-tailed t-tests were also used to measure the differences between the ELEV and AMB treatments for the following dependent variables: fungal biomass, bacterial biomass-specific productivity, microbial community respiration, and tipulid RCR, ECI, and RGR (null hypothesis; ELEV ≥ AMB).

Results

Leaf litter chemical analyses

Nutritional quality of *P. tremuloides* leaves for decomposers and detritivores was significantly altered by elevated levels of atmospheric CO_2, and some of the important differences were conserved after leaf senescence and 14 days of leaching and microbial colonization in the stream. Carbon:nitrogen ratios, total phenolic compounds, and lignin concentrations were all significantly higher in ELEV senesced leaves than in AMB litter (*t*-tests; $P < 0.05$, Fig. 1). A comparison of ELEVgreen leaf tissues with ELEV senesced leaves revealed a significant decline in leaf nutritional quality with increases in C : N and lignin following leaf senescence (*t*-tests; $P < 0.05$; Fig. 1). Two weeks of leaching and microbial colonization of leaf litter in a stream removed nearly all of the soluble phenolic compounds, lowered lignin content, and decreased C : N in both AMB and ELEV leaf litter. However, significant qualitative differences in C : N between treatments were conserved (*t*-test; $P < 0.01$; Fig. 1); C : N was twice as high in ELEV as in AMB leached leaf litter.

Leaf toughness, after 40 days incubation was approximately twice as high in the ELEV leaves (*t*-test; $P = 0.041$). Leaf toughness units were 113 ± 21 g and 59 ± 12 g (mean $\pm$ 1 se.) for ELEV and AMB, respectively.

Microbial responses to ELEV and AMB leaf litter

Productivity of aquatic bacteria colonizing leaf litter was negatively affected by plant growth under CO_2 enrichment. Biomass-specific bacterial productivity on ELEV leaf litter was two times lower than on AMB litter after 14 days of incubation in the stream (*t*-test; $P < 0.01$; Fig. 2). Fungal biomass on AMB and ELEV litter did not differ significantly after 14 days of incubation ($P > 0.05$; Fig. 2). The trends seen in the bacterial responses to ELEV leaf litter held for the composite total microbial community respiration, although these differences were not significant (*t*-test; $P > 0.05$; Fig. 2).

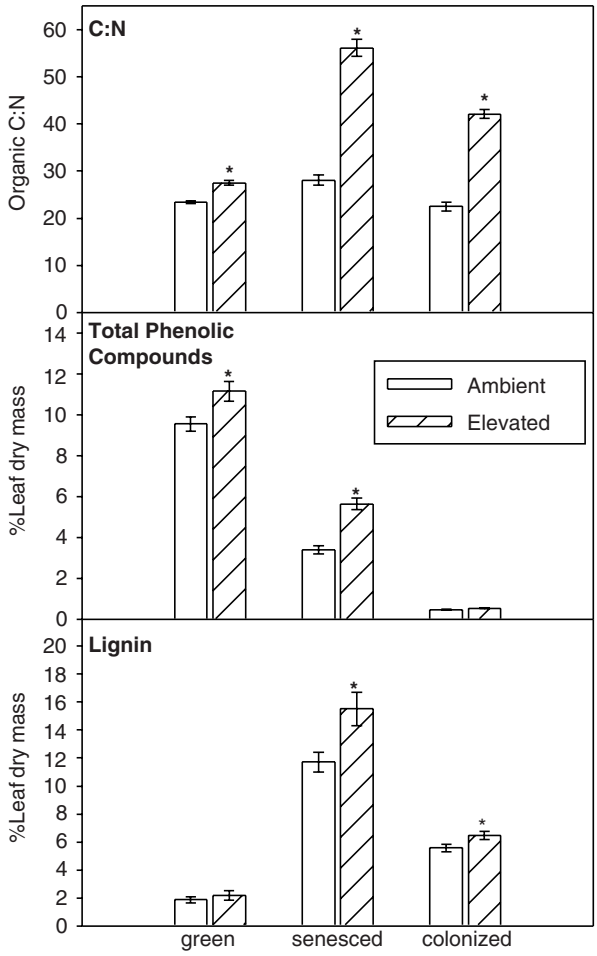

Fig. 1 C : N ratios, % total phenolic compounds and % lignin of live green, naturally senesced, and microbially colonized senesced *Populus tremuloides* leaves which were produced under AMB and ELEV atmospheric CO_2 conditions. Values are expressed as means $\pm$ 1 SE. Asterisks denote significant differences ($P < 0.05$).

Detritivore responses to ELEV and AMB leaf litter

Consumption rate, efficiency of conversion of ingested food (assimilation), and relative growth rates of *T. abdominalis* larvae were negatively impacted by the chemical changes in aspen litter grown under CO_2 enrichment. Cranefly larvae fed ELEV litter consumed significantly less (< 50%) leaf litter than those insects that were given AMB litter (*t*-test; $P < 0.0001$; Fig. 3). In addition, the efficiency of conversion of ingested food (ECI) of *T. abdominalis* fed ELEV litter was significantly lower than that of AMB larvae; ECI for tipulids fed ELEV litter was negative compared to the modest, positive conversion efficiency of the AMB treatment insects (*t*-test; $P < 0.01$; Fig. 3). Relative growth rate for these insects was significantly higher in the AMB than in the ELEV treatment (*t*-test; $P < 0.0001$;

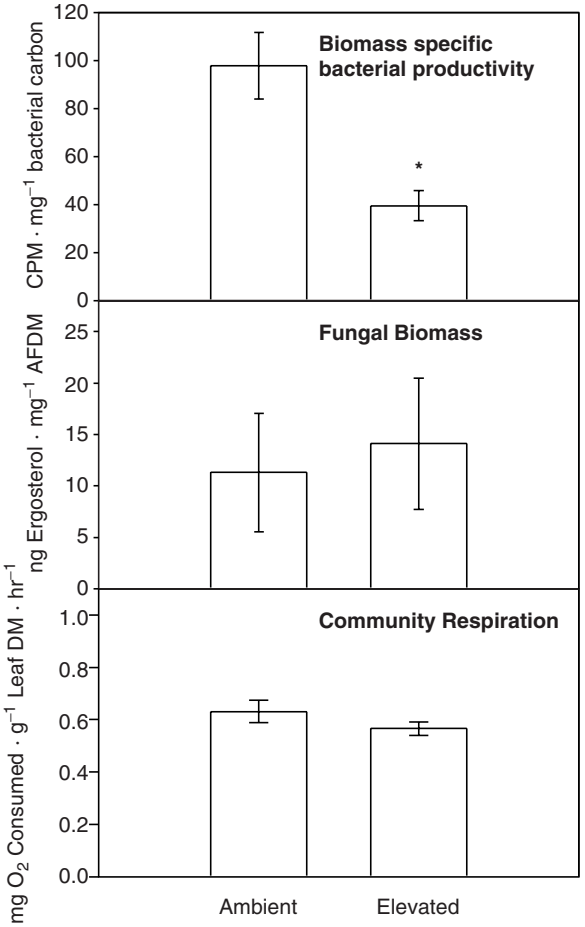

Fig. 2 Biomass-specific bacterial productivity, fungal biomass, and microbial community respiration on AMB and ELEV leaf litter colonized in the East Branch of the Maple River for 14 days. Values are expressed as means ± 1 SE. Asterisks denote significant differences ($P < 0.05$).

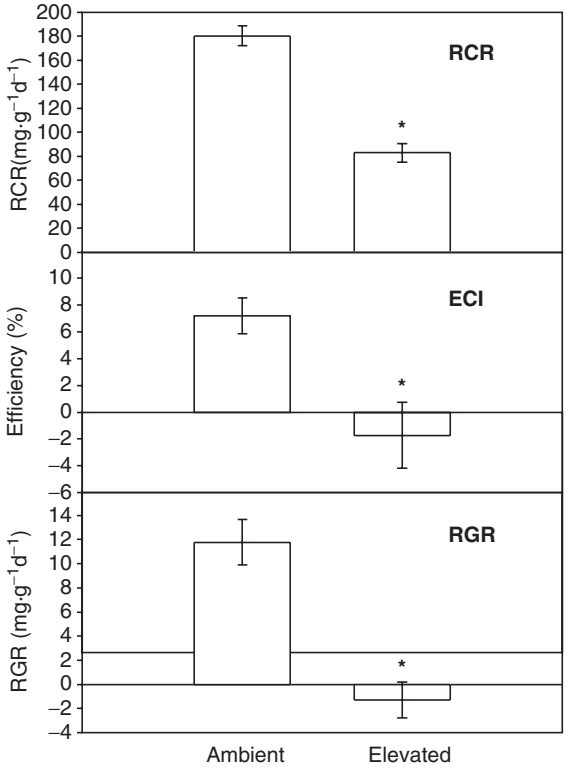

Fig. 3 Relative consumption rate (RCR), efficiency of conversion of ingested food (ECI) and relative growth rate (RGR) of *Tipula abdominalis* on ELEV and AMB leaf litter that was microbially colonized in the East Branch of the Maple River for 14 days. Values are expressed as means ± 1 SE. Asterisks denote significant differences ($P < 0.01$).

Fig. 3). Mortality of *T. abdominalis* individuals during the experiment was not significantly greater than zero for either treatment (*t*-test; $P > 0.05$).

Discussion

Elevated CO_2 levels decreased the quality of *Populus tremuloides* leaf litter for aquatic decomposers and shredders by producing higher C : N,% total phenolic compounds, and lignin content. Similar phytochemical changes have been induced by CO_2 enrichment in *P. tremuloides* in other studies. For example, Lindroth (1996a) reported a 24% decrease in nitrogen, 205% more starch, a 52% increase in phenolic glycosides, and 75% more condensed tannins in elevated CO_2-grown *P. tremuloides* when compared to control leaves. Zak *et al*. (1993) also demonstrated similar phytochemical changes in CO_2-enriched *P. tremuloides* grown in sandy soils that were nutrient amended with rich organic top soil. Several studies have demonstrated similar trends among numerous weed, crop, and woody plant species (e.g. Lincoln *et al*. 1984, 1986; Williams *et al*. 1986; Osbrink *et al*. 1987; Fajer *et al*. 1989; Norby *et al*. 1992), suggesting a potentially broad-based effect of rising global atmospheric CO_2 levels on plant foliage chemistry. However, the magnitude of the effect of elevated CO_2 on foliar C : N may depend on soil N content, suggesting that not all geographical regions will be affected as strongly as the sandy regions of the Mid-West USA.

Elevated CO_2-induced increases in carbon fixation often result in the production of high levels of carbon-based secondary (plant defence) compounds (Lambers 1993; Lindroth 1996a). For example, under high carbon fixation, overflow carbon can be shunted to the production of phenolic compounds via the phenylalanine ammonia lyase catalysed pathway (Lincoln 1993). Production of secondary phenolic compounds can be stimulated by elevated CO_2 under conditions of both low and high soil nitrate concentrations (Kinney *et al*. 1997). Phenols inhibit herbivory on fresh plant tissues (e.g. Levin 1971; Cates & Rhoades 1977; Haukioja *et al*. 1985) and can also interfere

with utilization of detritus by detritivores and microbes (Nicolai 1988). Lignin, a cell wall constituent of vascular and many herbaceous plants, is a complex polymer of phenolic compounds that is difficult for heterotrophs to utilize (Swain 1979; Wetzel *et al*. 1995) and is an example of a secondary metabolite whose concentration generally increases with CO_2 enrichment. Nitrogen is both diluted in leaves by increases in carbon based compounds, and its absolute concentration is lower because of decreased production of the CO_2-binding protein ribulose-1, 5-bisphosphate carboxylase in high CO_2 environments (Rowland-Bamford *et al*. 1991).

Natural differences among tree species in their leaf litter nutritional quality for aquatic decomposers and shredders have been well documented (e.g. Webster & Benfield 1986; Stout 1989; Ostrofsky 1997). Typical 'low-quality' species such as oak and conifers are relatively high in polyphenolic compounds including lignin and tannins, while species that exhibit lower concentrations of secondary compounds and relatively higher nitrogen contents (such as alder) tend to be more rapidly utilized by decomposers and detritivores (see review by Stout 1989). Within the spectrum of high to low nutritional quality leaves, *Populus tremuloides* is in the middle, displaying moderate rates of decomposition (Ostrofsky 1997). However, *P. tremuloides* leaf litter produced under elevated CO_2 conditions has significantly higher lignin and total phenolic compounds and lower nitrogen, conferring a lower quality litter compared to its ambient CO_2 counterpart.

Leaves that were grown under CO_2 enrichment and incubated in the East Branch of the Maple River for 2 weeks demonstrated significantly lower bacterial biomass-specific productivity than control leaves. Increased lignin coupled with decreased leaf nitrogen likely made the leaves less susceptible to microbial degradation. The effects of lower leaf nitrogen were likely augmented by low levels of dissolved nitrogen in the East Branch of the Maple River (mean annual $NH_4 = 23.4\,\mu g\,L^{-1}$; $NO_3 = 10.6\,\mu g\,L^{-1}$; J.A. Teeri, unpubl. UMBS database). In moderately nutrient-rich streams, bacteria can utilize N from the water column so that CO_2 induced changes to leaf nitrogen will likely have less of an effect on rates of decomposition (Ostrofsky 1997; Gessner *et al*. 1998; Tank & Webster 1998).

The combined effects of lower chemical nutritional value, and lower biomass and productivity of microbes colonizing leaf litter produce a negative impact on aquatic macroinvertebrate detritivores (Anderson & Sedell 1979; Cummins & Klug 1979). Leaching and microbial colonization likely contributed to the decreased C : N, total phenolic compounds, and lignin in both AMB and ELEV leaves, yet the differences between ELEV and AMB C : N and leaf toughness were conserved. From the detritivore perspective, food quality of leaves colonized for 14 days in the East Branch of the Maple River was improved both by chemical leaching of soluble secondary compounds and by incorporation of bacterial and fungal biomass. However, performance of tipulid larvae reared on elevated CO_2-produced leaf litter was reduced greatly. Although most terrestrial insect herbivores respond to CO_2-enriched leaves with compensatory consumption in order to counterbalance the low nitrogen (Lindroth *et al*. 1993; Roth & Lindroth 1995; Lindroth & Kinney 1998), tipulid consumption rates were greatly diminished when fed elevated CO_2-produced leaves. Similar reduced consumption rates have been observed for amphipods (*Gammarus pseudolimnaeus*) and isopods (*Asellus communis*) on ELEV leaves (Tuchman, unpubl. data). Lower consumption rates in these detritivores compared with herbivorous insects likely result from alteration of leaf chemistry and subsequent palatability during the process of senescence. In general, woody deciduous and perennial herbaceous plants resorb up to 50% of their leaf nitrogen and to a much lesser extent, carbon, prior to leaf abscission (Chapin *et al*. 1990), which ultimately decreases the nutritional quality of senesced leaves compared to their green leaf counterparts. In addition, because trees retranslocate much of the soluble nitrogen just prior to leaf senescence, the secondary carbon compounds tend to become greatly concentrated in senesced leaves. For example, in *P. tremuloides*, C : N ratios increased twofold and percentage lignin increased eightfold when comparing ELEV live green leaf to ELEV senesced tissues in the present study. This difference in nutritional quality in addition to greater leaf toughness could render leaf litter less palatable than green leaves and account, in part, for the opposing responses elicited for leaf consumption in insect herbivores vs. detritivores.

Efficiency of conversion of ingested food into tipulid biomass was minimal to negative on ELEV leaf litter. ECI is directly related to leaf nitrogen content (Coviella & Trumble 1999), and may also be affected negatively by accumulation of secondary carbon compounds. The observed lack of growth in tipulids eating ELEV leaf litter indicates food quality and/or the quantity ingested were too low to maintain basal metabolic requirements. In contrast, lack of weight gain and weight loss are uncommon responses to elevated CO_2 in terrestrial herbivorous insects, probably because they compensate for low food quality by increasing consumption, as described above. Terrestrial insects reared on elevated CO_2-produced leaves gain weight, but at a significantly reduced rate compared with their control counterparts (Lincoln *et al*. 1986; Fajer *et al*. 1989). Thus, high secondary compounds, low nitrogen, and leaf toughness interact to provide formidable barriers to heterotrophic utilization of leaf tissues (Herms & Mattson 1992) and elevated atmospheric CO_2 appears to amplify the efficacy of these barriers.

Elevated atmospheric CO_2 levels decreased the quality of *P. tremuloides* leaf litter for aquatic decomposers and shredders by producing significantly higher C : N, % total phenolic compounds and lignin content. Lower nutritional quality in this leaf litter produced poor growth responses in aquatic bacteria and an invertebrate shredder. An important global function of most small shallow lakes, streams, wetlands, and flood plains is the collection, storage, and metabolism of organic carbon imported from terrestrial and land–water interface regions. Because the nutritional quality of many species of plant detritus may be lowered by global increases in atmospheric CO_2 as was shown in the present study, we suggest that a major impact upon aquatic ecosystems is through an indirect pathway where CO_2-induced alterations of terrestrial leaf litter negatively affects aquatic microbial and detritivore production. These effects could result in a change in species composition where microbes capable of utilizing more recalcitrant organic compounds may replace others. Similarly, strict detritivores may be replaced by opportunistic detritivores who may suppliment their diets with alternative food sources. Overall, changes in decomposer and detritivore community structure and production could result in lower production at higher trophic levels such as predators (Wallace *et al*. 1997).

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