

## Elevated CO<sub>2</sub> alters leaf-litter-derived dissolved organic carbon: effects on stream periphyton and crayfish feeding preference

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**Abstract.** Elevated atmospheric CO<sub>2</sub> increases plant C fixation, and much of the soluble C content of deciduous leaf litter entering streams is leached as dissolved organic C (DOC). The effects of DOC from trembling aspen (*Populus tremuloides* Michaux) leaf litter grown under elevated (ELEV = 720 ppm) and ambient (AMB = 360 ppm) CO<sub>2</sub> on stream periphyton were measured during a 35-d experiment in outdoor artificial stream chambers. Crayfish feeding preferences for periphyton grown in AMB and ELEV treatments were evaluated in short-term foraging trials using a Y-maze. Periphyton was sampled through time for ash-free dry mass (AFDM), chlorophyll *a*, total C:N, algal biovolume and species composition, and bacterial productivity and biomass. Leaf litter from plants grown under ELEV CO<sub>2</sub> produced higher concentrations of refractory DOC than did leaf litter from plants grown under AMB CO<sub>2</sub>, and chlorophyll *a* concentrations were lower in periphyton enriched with ELEV DOC than in periphyton enriched with AMB DOC. ELEV DOC did not significantly affect bacterial productivity and biomass or total periphyton C:N, but cyanobacterial biovolume was higher in ELEV algal assemblages than in AMB algal assemblages after 35 d. AMB algal assemblages were dominated by the diatom *Epithemia adnata* var. *proboscidea*, which contains N-fixing endosymbionts. *Orconectes virilis* crayfish preferred AMB periphyton stimulus when offered the choice of AMB and ELEV stimuli or AMB and control stimuli. Our results suggest that DOC from trembling aspen leaf litter produced under ELEV CO<sub>2</sub> alters algal accrual and species assemblages of stream periphyton, and this shift in basal resource quantity and quality could affect feeding preferences of crayfish.

**Key words:** elevated CO<sub>2</sub>, DOC, periphyton assemblage, *Populus tremuloides* leaf litter, streams, *Orconectes virilis* crayfish.

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Rising atmospheric CO<sub>2</sub> levels, which are expected to double by the middle of this century (Houghton et al. 2001), probably will affect both terrestrial and aquatic ecosystems through increased plant C fixation (Strain and Bazaaz 1983). Elevated atmospheric CO<sub>2</sub> results in higher foliar C:N (Bazaaz 1990, Lindroth et al. 1993,

Curtis et al. 1996) and increased structural (e.g., lignin, cellulose, hemicellulose) and polyphenolic compounds (Bazaaz 1990, Lindroth et al. 1993, Curtis et al. 1996) in leaf tissue. These changes in leaf chemistry negatively affect herbivores (Lindroth et al. 1993, Lindroth and Kinney 1998) and litter breakdown (Cotrufo and Ineson 1996, De Angelis et al. 2000) in terrestrial ecosystems, and negatively affect aquatic ecosystems through low-quality leaf-litter inputs (Tuchman et al. 2002). Leaf litter from plants grown under elevated CO<sub>2</sub> has higher C:N, phenolic compounds, and lignins; supports lower bacterial and fungal biomass in aquatic ecosystems (Rier et al. 2002, Tuchman et al. 2002, 2003a, b, Wetzel and Tuchman 2005); and can inhibit growth and development of aquatic arthropods (Tuchman et al. 2002, 2003a, b, Adams et al. 2005).

Terrestrially derived leaf litter is the dominant component of organic matter in many headwater stream ecosystems (Fisher and Likens 1973, Webster and Meyer 1997), and a large proportion of dissolved organic C (DOC) in forest streams is derived from terrestrial leaf litter (Meyer et al. 1998). Aquatic bacteria (Meyer 1994, Hall and Meyer 1998, Bernhardt and Likens 2002) and algae (Tuchman et al. 2006, Frost et al. 2007) rely on DOC as a resource for growth and metabolism. Labile DOC in streams can stimulate growth of epilithic bacteria (Sobczak 1996), and DOC quantity and quality have been linked positively with epilithic algal growth (Vinebrook and Leavitt 1998, Frost et al. 2007). However, leaf litter from plants grown under elevated CO<sub>2</sub> leaches higher concentrations of refractory DOC than does leaf litter of plants grown under ambient CO<sub>2</sub>, and this chemically resistant leachate might support lower bacterial productivity than does labile leachate (Wetzel and Tuchman 2005). Therefore, a change in DOC quantity and quality as a result of elevated atmospheric CO<sub>2</sub> might alter periphyton (bacteria and algae) quantity and quality, which might affect this resource for subsequent consumers.

In streams, many foraging consumers can detect chemicals leaching from food sources. For example, crayfish use chemosensory organs to detect chemicals diffusing from food sources (Dunham et al. 1997, Kreider and Watts 1998, Giri and Dunham 1999) and can discriminate between trembling aspen (*Populus tremuloides* Michaux) leaf litter grown under elevated vs ambient CO<sub>2</sub> levels (Adams et al. 2003, 2005). Leaf-litter detritus has been assumed to be the major resource of crayfish diets (Lorman and Magnuson 1978), but crayfish actively graze stream periphyton (Charlebois and Lamberti 1996). The ability of crayfish to discriminate among periphyton sources amended with different concentrations of DOC has not been tested, and this information

could increase our ability to predict bottom-up effects of rising atmospheric CO<sub>2</sub> on stream food webs.

We tested the effects of elevated CO<sub>2</sub> on leaf-litter-derived DOC concentration and subsequent effects on stream periphyton and crayfish feeding preferences. The objectives of our study were to: 1) examine quantitative differences in DOC concentration from *P. tremuloides* leaf litter grown under ambient (AMB) and elevated (ELEV) CO<sub>2</sub>, 2) measure how stream periphyton is modified by enrichment with leachate from AMB vs ELEV leaf litter, and 3) measure crayfish foraging responses to periphyton grown with leachate from AMB vs ELEV leaf litter. We predicted that leachate from ELEV leaf litter would have higher concentrations of refractory DOC than leachate from AMB leaf litter. We also predicted that these differences in DOC would affect accrual, stoichiometry, and algal assemblages of stream periphyton and that these effects on periphyton would subsequently affect crayfish feeding preferences.

## Methods

### *Elevated CO<sub>2</sub> and trembling aspen*

Clones of trembling aspen (*Populus tremuloides*) trees were grown at the University of Michigan Biological Station Elevated CO<sub>2</sub> Facility (45°33'N, 84°42'W) in Cheboygan County, Michigan, USA. *Populus tremuloides* is the dominant tree species in Michigan (Schmidt et al. 1997) and makes up ~22% of leaf litter that enters the East Branch of the Maple River, Pellston, Michigan (Tuchman et al. 2002). Aspen trees (2 treatments × 5 replicates; *n* = 10) were grown in large root boxes with homogenized sand (80%) and soil (20%) mixtures and fumigated in open-top chambers with ELEV (720 ppm) and AMB (320 ppm) CO<sub>2</sub> throughout the growing season (May–early November) for a total of 5 successive years. During abscission, leaves were collected daily, air-dried, and stored in brown paper bags until chemical analyses and experimental use. Leaf litter from *P. tremuloides* exposed to ELEV CO<sub>2</sub> has consistently higher concentrations of tannins and phenolics and higher C:N than leaf litter from trees exposed to AMB CO<sub>2</sub> (Rier et al. 2002, 2005, Tuchman et al. 2002, 2003a, b).

### *DOC preparation*

Concentrated DOC stock solutions were prepared by placing 23.8 g of *P. tremuloides* leaf litter from multiple trees exposed to AMB or ELEV CO<sub>2</sub> into separate 3-L carboys of nutrient-poor well water for 48 h at 5°C. Solutions were strained using a 2-mm-mesh sieve to remove leaf litter and coarse particulate matter. For

tractability, only 1 DOC stock solution was prepared per treatment. Aliquots for daily amendment of DOC (2 treatments  $\times$  5 replicates  $\times$  35 d,  $n = 350$ ) were made from concentrated stock solutions that were diluted with well water (7:1) to approximate DOC levels in the East Branch of the Maple River, Emmet County, Michigan. Aliquots were stored at  $-20^{\circ}\text{C}$  until needed. Concentrations of DOC for both AMB and ELEV treatments were analyzed using a Shimadzu TOC 5000 analyzer (Shimadzu Scientific Instruments, Columbia, Maryland), and concentrations were compared to ambient DOC from the East Branch of the Maple River.

#### *Periphyton experimental methods*

To test the effect of the AMB and ELEV DOC on periphyton communities,  $4.8\text{-cm}^2$  unglazed clay tiles were placed in artificial stream channels at the University of Michigan Biological Station Stream Facility ( $45^{\circ}32'\text{N}$ ,  $84^{\circ}45'\text{W}$ ) in Emmet County, Michigan. Natural stream water was pumped from the East Branch of the Maple River into a head tank, which distributed water at a flow rate of  $10 \pm 2\text{ cm/s}$  to 8 artificial stream channels ( $2.4\text{ m} \times 9.0\text{ cm} \times 1.5\text{ cm}$ ) covered with standard shade cloth that reduced light levels by 50% to mimic natural benthic light levels in our study stream. Tiles (20 tiles  $\times$  8 stream channels,  $n = 160$ ) were incubated in stream channels for 3 wk to allow colonization by benthic periphyton (epilithic algae and bacteria), after which 6 tiles were randomly selected from across stream channels and transferred to each of 10 shaded, temperature-controlled Plexiglas recirculating stream chambers (20.0-cm diameter  $\times$  5.0-cm height; 2 treatments  $\times$  5 replicates,  $n = 10$ ). Each chamber was randomly assigned a DOC treatment (AMB or ELEV). Treatment solutions were changed daily to maintain DOC concentrations and water levels ( $\sim 3.5\text{-cm}$  depth) within each chamber. Colonized tiles ( $n = 6$ ) remained in recirculating chambers from 8 July to 9 August 2002, and 5 replicate tiles (1 per chamber) from both AMB and ELEV treatment chambers were randomly collected on days 3, 7, 12, 25, and 35. The 6<sup>th</sup> tile from each chamber was sampled on day 35 for use in crayfish preference trials (see *Crayfish experimental methods* below).

Periphyton was removed from tiles using stiff brushes and deionized water, and samples were homogenized with a handheld blender to disperse algal and bacterial cells. Subsamples of homogenized periphyton were used to determine ash-free dry mass (AFDM), chlorophyll *a*, algal biovolume, total C:N, bacterial productivity and biomass, and algal species assemblages. AFDM was measured as the differential mass between pre- and postcombustion ( $550^{\circ}\text{C}$  for 1 h) of oven-dried ( $60^{\circ}\text{C}$  for 24 h) periphyton. To estimate

chlorophyll *a* concentrations, known volumes of periphyton were collected on  $250\text{-}\mu\text{m}$  cellulose nitrate filters (Whatman, Florham Park, New Jersey) and frozen. Chlorophyll *a* was determined fluorometrically after extraction with 90% buffered acetone (APHA 1992). Total periphyton C:N from days 12, 25, and 35 was measured using a PerkinElmer 2400 elemental analyzer (PerkinElmer, Waltham, Massachusetts).

Bacterial productivity ( $\mu\text{g C mm}^{-2}\text{ h}^{-1}$ ) was determined as the incorporation of  $^3\text{H}$ -leucine into protein (modified from Wetzel and Likens 2000). Periphyton samples were incubated for 30 min at  $4^{\circ}\text{C}$  with an addition of  $10\text{ }\mu\text{L}$  of  $^3\text{H}$ -leucine (99.9 Ci/mmol). Bacterial productivity was estimated after subtracting the activity of a killed control (Kirchman 1993). Bacterial biomass was measured from periphyton samples that were preserved in a solution of 3.7% formaldehyde and 0.1 mol/L tetrasodium pyrophosphate (Velji and Albright 1986). Bacterial cells were removed from periphyton by sonication for 30 s on ice. Cells were stained with 2 mL of  $10\text{ }\mu\text{g/mL}$  4',6-diamidino-2-phenylindole (DAPI) solution and counted from 10 random fields per slide using epifluorescent microscopy (Porter and Feig 1980). Bacterial cell biovolumes were estimated using geometric shapes (Bratbak 1985, Psenner 1993, Wetzel and Likens 2000), and total C was estimated by multiplying biovolumes by  $5.6 \times 10^{-13}\text{ g C}/\mu\text{m}^3$  (Bratbak 1985).

Algal subsamples used for species identification were preserved in 2% glutaraldehyde and mounted on glass slides (Stevenson 1984). At least 200 cells were counted from each slide with an Olympus BH-2 microscope (1000 $\times$ ) using phase-contrast optics (Olympus America, Center Valley, Pennsylvania). Algae were identified to species level when possible (Hustedt 1930, Prescott 1962, Patrick and Reimer 1966, 1975, Krammer and Lange-Bertalot 1988). Mean relative biovolumes for diatoms, cyanobacteria, and chlorophytes were estimated using geometric shapes (Hillebrand et al. 1999).

#### *Crayfish experimental methods*

Periphyton from tiles exposed to DOC treatments (ELEV and AMB) for 35 d was homogenized and placed in gelatin, which served as the resource medium for crayfish because of its ability to diffuse in water and its subsequent delivery of chemical stimulus (Keller et al. 2001). Gelatin containing periphyton was made by adding 250 mL of homogenized periphyton and 750 mL of well water to 4 packets of plain Spartan<sup>®</sup> gelatin (Spartan Chemical Company, Wyoming, Michigan). Crayfish do not respond to chemicals leaching from plain gelatin (Moore and Grills 1999); thus, gelatin served as our

control (CON) stimulus. Gelatin was cut into 1 × 2 × 2-cm blocks to standardize volume.

*Orconectes virilis* crayfish were used because of their ability to detect chemical differences in *P. tremuloides* leaf litter from plants grown under AMB and ELEV CO<sub>2</sub> (Adams et al. 2003). Male and female *O. virilis* were collected between 2300 and 0030 hours from Burt and Douglas lakes near Pellston, Michigan. These lakes are hydrologically connected to the East Branch of the Maple River. Each crayfish had a complete set of chemosensory appendages (1 pair of antennae, 1 pair of antennules, and 2 chelae). Animals were housed in 120-L aquaria for ≤3 d, and all animals were starved for ≥24 h prior to experimental trials.

Crayfish preferences for periphyton exposed to AMB and ELEV DOC were tested using a flow-through Y-maze (tank: 77.5 × 42 × 18 cm, arm: 56 × 21 × 18 cm, 56.4 L) (Adams et al. 2005). Prior to each experimental trial, fresh Douglas Lake water was added to the Y-maze to a depth of 15.0 cm. Two reservoir tanks (24 × 13 × 14 cm, 4.2 L) above the maze also were filled with Douglas Lake water, and water flowed from the reservoir tanks to the upstream end of each arm of the Y-maze through 1.0-cm (ID) tubing. Gelatin blocks containing different periphyton stimuli were placed inside each reservoir tank, and discharge from reservoir tanks into the Y-maze was maintained at 4.2 cm<sup>3</sup>/s using inline flow meters (Manostat Riteflow #3; Manostat, Pequannock, New Jersey). Crayfish were acclimated to the Y-maze in a mesh enclosure at the downstream end (the confluence of both arms of the Y-maze [confluence area]) for 20 min prior to trials (sensu Adams et al. 2005), after which time trials began, and flow from the reservoir tanks delivered periphyton chemical stimulus through each arm of the Y-maze and out of the Y-maze at the confluence area.

Forty-five trials were conducted using pairwise combinations of periphyton stimuli (15 AMB vs CON, 15 ELEV vs CON, and 15 AMB vs ELEV). Crayfish preference was measured as differential time spent in each side of the Y-maze during 10-min videotaped trials (Sony Hi-8 Handycam, model #CCD-TR700; Sony, New York, New York). Trials where crayfish did not move from the confluence area of the Y-maze were not used in analyses. Periphyton stimulus (AMB, ELEV, CON) assigned to each Y-maze arm was alternated between trials to control for arm preference by crayfish, and crayfish were used only once during experimental trials.

#### Statistical analyses

Treatment DOC concentrations were compared using a *t*-test. Two-way analyses of variance (AN-

OVAs) were used to test effects of DOC treatment and time on periphyton AFDM, chlorophyll *a*, algal biovolume, total periphyton C:N, and bacterial productivity and biomass. Tukey's Honestly Significant Difference (HSD) post hoc comparisons were conducted for significant interactions. Effects of DOC treatment and time on algal biovolume within each algal division were analyzed with 2-way multiple analysis of variance (MANOVA) (DOC treatment × time) and Tukey's HSD. The effects of DOC treatment on the relative contribution of each algal division to total algal biovolume on day 35 were analyzed with MANOVA and Tukey's HSD, and separate *t*-tests were used to identify treatment effects on dominant algal taxa.

Videotapes were analyzed to determine the amount of time crayfish spent in each arm of the Y-maze as a measure of resource preference. Crayfish preferences were tested using pairwise comparisons of AMB, ELEV, and CON stimuli, and the time crayfish spent in either arm of the Y-maze was analyzed with separate *t*-tests. All statistical analyses were conducted using SAS version 9.1 (SAS Institute, Cary, North Carolina). A Type I error rate of  $\alpha = 0.05$  was selected for all analyses. Chlorophyll *a* and AFDM data were ln(*x*)-transformed and total periphyton C:N data were arcsine  $\sqrt{(x)}$ -transformed to meet assumptions of homoscedasticity.

## Results

### DOC concentrations

Ambient [DOC] in the East Branch of the Maple River was 21.9 ± 3.2 mg C/L. Mean [DOC] was ~36% greater for the ELEV (34.1 ± 1.2 mg C/L) than for the AMB treatment (25.0 ± 0.40 mg C/L) ( $t_{4,0.05} = 2.77$ ,  $p < 0.01$ ).

### DOC effects on periphyton biomass and C:N

Periphyton biomass (AFDM) did not differ between AMB and ELEV treatments throughout the experiment (Fig. 1A). However, chlorophyll *a* was nearly 50% lower in the ELEV than in the AMB treatment ( $F_{1,40} = 5.37$ ,  $p = 0.02$ ; Fig. 1B), particularly after day 12. Algal biovolume increased throughout the experiment (Fig. 1C), but neither DOC treatment nor time significantly affected algal biovolume. Total periphyton C:N did not differ between ELEV and AMB treatments on any sampling date (Fig. 1D).

### DOC effects on algal species assemblages

Diatoms, cyanobacteria, and chlorophytes were the main constituents of algal biovolume, and accounted for >90% of total algal biovolume throughout the experiment. Diatoms dominated algal biovolume throughout the experiment in both DOC treatments

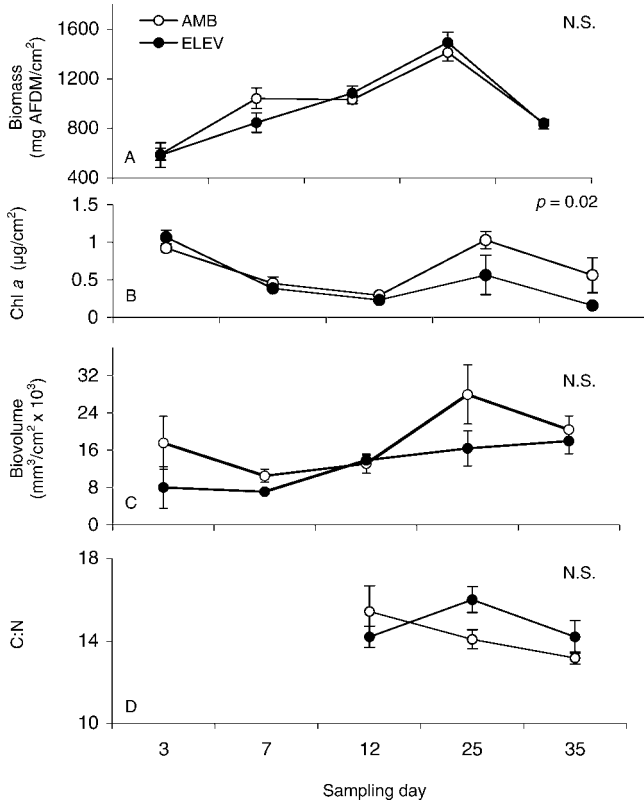


FIG. 1. Mean ( $\pm 1$  SE) periphyton biomass (A), chlorophyll *a* (chl *a*) (B), algal biovolume (C), and total periphyton C:N (D) of periphyton colonizing tiles in artificial streams enriched for 35 d with dissolved organic C (DOC) from leaf litter from trembling aspen (*Populus tremuloides*) grown under ambient (AMB=360 ppm) and elevated (ELEV=720 ppm) CO<sub>2</sub>. AFDM=ash-free dry mass, N.S.=nonsignificant treatment difference.

(Fig. 2A). Diatom biovolume was affected by DOC treatment and time ( $F_{1,49} = 5.59$ ,  $p = 0.02$ ,  $F_{4,49} = 5.95$ ,  $p = 0.0007$ , respectively). Algal assemblages had higher diatom biovolumes in the AMB than in the ELEV treatment ( $p < 0.05$ ), and diatom biovolumes were greater toward the end of the experiment than at the beginning in both DOC treatments ( $p < 0.05$ ). Cyanobacterial biovolume was not significantly affected by DOC treatment or time ( $F_{4,40} = 2.01$ ,  $p = 0.11$ ; Fig. 2B). Contribution of cyanobacteria to total algal biovolume was initially higher in the AMB than in the ELEV treatment; however, by the end of the experiment, assemblages in the ELEV treatment had higher cyanobacterial biovolume than in the AMB treatment (Table 1). Chlorophyta biovolume was not significantly affected by DOC treatment or time ( $F_{1,40} = 0.34$ ,  $p = 0.56$ ,  $F_{4,40} = 0.78$ ,  $p = 0.55$ , respectively; Fig. 2C). Chlorophyta biovolume remained consistently low in both DOC treatments throughout the experiment, and DOC treatment did not affect the proportion of total algal biovolume from dominant Chlorophyta taxa (Table 1).

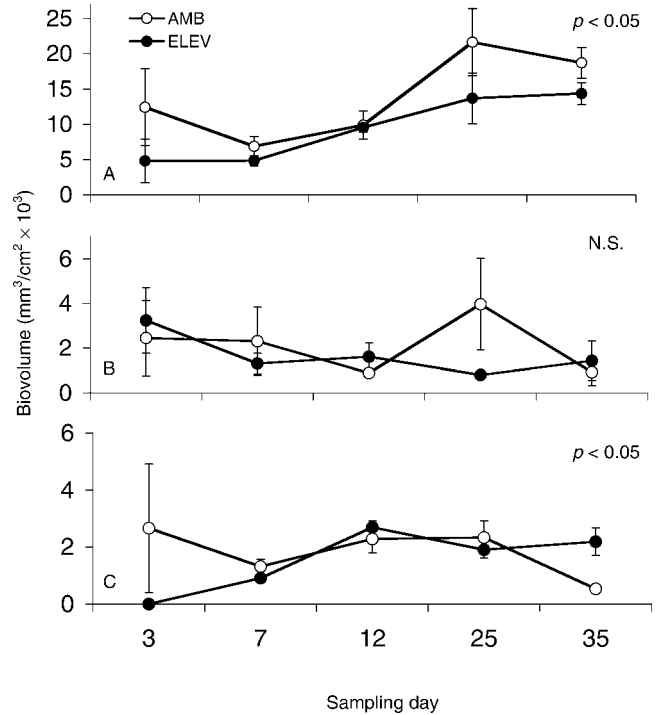


FIG. 2. Mean ( $\pm 1$  SE) biovolume of Bacillariophyta (diatoms) (A), Cyanophyta (cyanobacteria) (B), and Chlorophyta (green algae) (C) in periphyton colonizing tiles in artificial streams enriched for 35 d with dissolved organic C (DOC) from leaf litter from trembling aspen (*Populus tremuloides*) grown under ambient (AMB = 360 ppm) and elevated (ELEV = 720 ppm) CO<sub>2</sub>. N.S. = nonsignificant treatment difference.

DOC treatment had a significant effect on relative algal biovolumes within algal assemblages in samples collected on day 35. The proportion of cyanobacteria was higher in the ELEV than in the AMB treatment ( $F_{1,8} = 23.5$ ,  $p = 0.001$ ), and the proportion of diatoms was higher in the AMB than in the ELEV treatment ( $F_{1,8} = 7.86$ ,  $p = 0.023$ ) (Table 1). Assemblages in both DOC treatments had similar common (>1% biovolume) algal taxa, such as the diatoms *Achnanthes minutissimum* and *Nitzschia palea* (Table 1). However, the proportion of biovolume from *Epithemia adnata* var. *proboscidea* was high only in assemblages in the AMB treatment ( $t_{8,0.05} = 2.29$ ,  $p = 0.051$ ), whereas the proportion of biovolume from cyanobacteria, such as *Anacystis* sp. ( $t_{8,0.05} = -5.35$ ,  $p = 0.0007$ ), *Chroococcus* sp. ( $t_{8,0.05} = -2.73$ ,  $p = 0.026$ ), and *Microcystis* sp. ( $t_{8,0.05} = -1.37$ ,  $p = 0.21$ ), was higher in assemblages in the ELEV than in the AMB treatment (Table 1). The diatom *Synedra ulna* was rare, but because of its large cell size, it contributed >10% of total algal biovolume in assemblages in the ELEV treatment. *Synedra ulna* was rarely found in assemblages in the AMB treatment (Table 1).

TABLE 1. Mean (SE) % of total algal biovolume contributed by dominant (>1% by biovolume) algal taxa from periphyton after 35 d of amendment with dissolved organic C from leaf litter from trembling aspen (*Populus tremuloides*) grown under ambient (360 ppm) and elevated (720 ppm) CO<sub>2</sub>. Bacillariophyta = diatoms, Cyanophyta = cyanobacteria, Chlorophyta = green algae. \* denotes significant differences at  $\alpha=0.05$  (*t*-test).

Algal species	Ambient	Elevated
<b>Bacillariophyta</b>		
<i>Achnanthyidium minutissimum</i>	29.3 (6.0)	24.4 (1.3)
<i>Cocconeis placentula</i>	2.2 (1.5)	6.6 (0.9)
<i>Cymbella affinis</i>	0.8 (0.5)	1.4 (0.9)
<i>Epithemia adnata</i> var. <i>proboscidea</i> *	17.7 (7.8)	0.0
<i>Eunotia pectinalis</i>	4.7 (1.6)	1.6 (0.8)
<i>Fragilaria construens</i> var. <i>venter</i>	6.4 (2.1)	3.1 (0.4)
<i>Melosira varians</i>	1.3 (1.3)	3.7 (4.1)
<i>Nitzschia archibaldii</i>	7.6 (2.1)	7.5 (0.8)
<i>Nitzschia dissipata</i>	0.4 (0.3)	2.6 (0.8)
<i>Nitzschia palea</i>	13.8 (6.4)	19.1 (2.3)
<i>Synedra ulna</i>	0.0	10.7 (4.2)
<i>Tabellaria fenestrata</i>	4.9 (4.9)	0.0
Total	89.1	80.7
<b>Cyanophyta</b>		
<i>Anacystis</i> sp.*	0.4 (0.2)	3.9 (0.6)
<i>Chroococcus</i> sp.*	1.8 (0.1)	5.6 (1.5)
<i>Microcystis</i> sp.	0.4 (0.2)	1.8 (1.0)
Total	2.6	11.3
<b>Chlorophyta</b>		
<i>Coleochaete</i> sp.	0.0	2.9 (3.2)
<i>Cladophora</i> sp.	1.8 (1.8)	0.0
<i>Schizothrix</i> sp.	1.6 (0.2)	2.9 (1.0)
Total	3.4	5.8

#### DOC effects on bacterial productivity and biomass

DOC treatment did not significantly affect bacterial biomass (Fig. 3A) or productivity (Fig. 3B). Bacterial productivity in both treatments was highest on day 12, but bacterial biomass in both treatments was lowest on that date, indicating that the highest rates of bacterial turnover occurred during the middle of the experiment.

#### Crayfish feeding preferences

Crayfish spent more time in the arm of the Y-maze containing AMB stimulus than in the arm containing CON stimulus ( $t_{24,0.05}=1.07, p < 0.05$ ; Fig. 4A); however, time spent in the arm containing ELEV stimulus did not differ from time spent in the arm containing CON stimulus (Fig. 4B). When exposed to both AMB and ELEV stimuli, crayfish spent more time in the arm containing AMB stimulus than in the arm containing ELEV stimulus ( $t_{20,0.05}=6.37, p < 0.05$ ; Fig. 4C).

### Discussion

Concentrations of DOC from trembling aspen leaf litter were higher in ELEV than in AMB treatments,

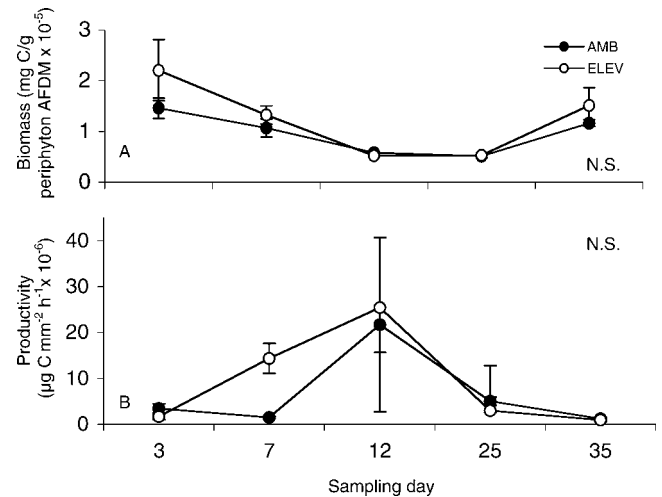


FIG. 3. Mean ( $\pm 1$  SE) bacterial biomass (A) and bacterial productivity (B) in periphyton colonizing tiles in artificial streams enriched for 35 d with dissolved organic C (DOC) from leaf litter from trembling aspen (*Populus tremuloides*) grown under ambient (AMB = 360 ppm) and elevated (ELEV = 720 ppm) CO<sub>2</sub>. AFDM = ash-free dry mass, N.S. = nonsignificant treatment difference.

and the difference affected the algal component of periphyton and crayfish preferences. By the end of the experiment, chlorophyll *a* content was lower and the proportion of total biovolume contributed by cyanobacteria was higher in periphyton amended with ELEV vs AMB DOC. Crayfish preferred AMB periphyton stimulus when offered a choice between AMB or ELEV stimuli and between AMB or CON stimuli. Crayfish did not prefer ELEV stimulus over CON stimulus, suggesting that neither stimulus was perceived as a food source. Our results suggest that rising atmospheric CO<sub>2</sub> may have bottom-up effects on stream food webs through inputs of leaf litter with high concentrations of nonlabile DOC, which could alter organic matter processing and periphyton species composition in streams.

#### Effects of DOC treatment on periphyton

Our findings expand on previous work examining effects of DOC concentrations on aquatic ecosystems. Wetzel and Tuchman (2005) measured significantly higher concentrations of refractory DOC from ELEV than from AMB leaf litter of the same *P. tremuloides* trees used in our experiment. Refractory DOC from ELEV leaf litter decreased benthic bacterial productivity in the absence of ultraviolet (UV) radiation exposure, but when DOC was exposed to natural sunlight, humic compounds were converted to labile fatty acids via UV photolysis and bacterial productiv-

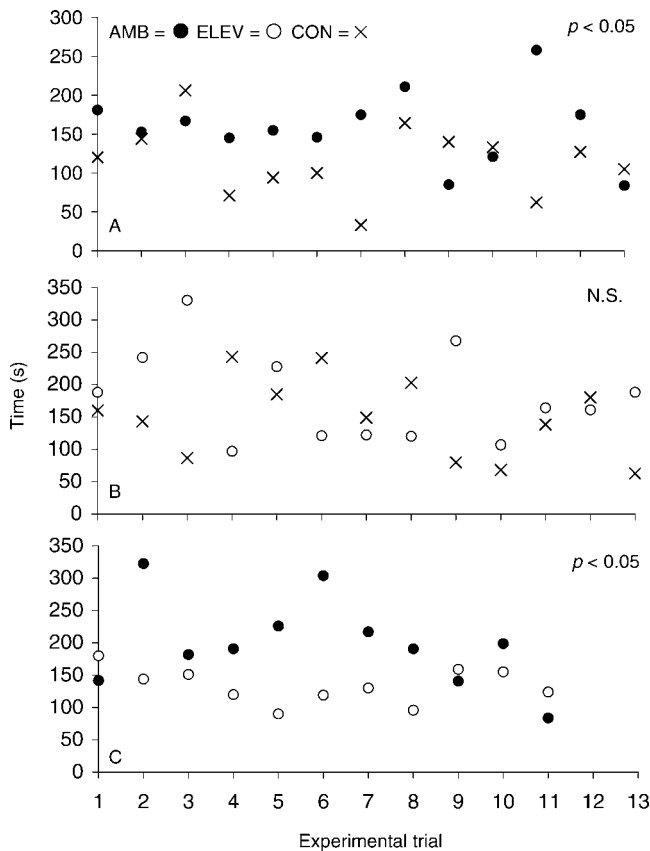


FIG. 4. Time spent by individual crayfish in each arm of the Y-maze during experimental trials in which crayfish were offered chemical stimulus from periphyton enriched with dissolved organic C (DOC) from leaf litter from trembling aspen (*Populus tremuloides*) grown under ambient (AMB = 360 ppm) and elevated (ELEV = 720 ppm) CO<sub>2</sub> or stimuli with no periphyton (CON). A.—AMB vs CON (*n* = 13). B.—ELEV vs CON (*n* = 13). C.—AMB vs ELEV (*n* = 11). Values represent raw data for each experimental trial. N.S. = nonsignificant treatment difference.

ity remained higher in ELEV than AMB DOC (Wetzel and Tuchman 2005). In our study, bacterial productivity and biomass did not differ between ELEV and AMB treatments, despite differences in DOC concentrations between treatments. Shade cloth was used to reduce ambient light levels in our experimental streams and incubation chambers by 50%. However, the DOC was still exposed to UV radiation, which might have reduced the amount of refractory DOC in the ELEV treatment and yielded more labile DOC for both bacteria and algae.

Relative concentrations of dissolved nutrients can regulate species composition and growth rates of algal communities (Wetzel 2001). Frost et al. (2007) reported a significant effect of dissolved organic matter (DOM)

concentration on periphyton accrual and algal species composition in artificial streams. Periphyton receiving higher DOM concentrations had increased AFDM and chlorophyll *a*, and algal communities exposed to higher DOM concentrations had significantly higher chlorophyte biomass (Frost et al. 2007). Higher DOC concentrations (e.g., ELEV DOC) increased cyanobacterial rather than chlorophyte biovolume in our study. The higher proportion of cyanobacterial biovolume in ELEV assemblages might have reflected a heterotrophic response (Tuchman et al. 2006) to higher DOC concentration in the ELEV treatment. The dominance of the diatom *Epithemia adnata* var. *proboscidea* (known to house N-fixing endosymbionts; Lowe et al. 1984) in AMB assemblages at the end of the experiment suggests that algae might have become N-limited in the AMB treatment, which we anticipated but did not find in the ELEV treatment.

*Effects of DOC treatment on crayfish feeding preferences*

The taxonomic composition of algae may affect its palatability as a food source to consumers. Periphyton stoichiometry did not differ between ELEV and AMB treatments despite differences in chlorophyll *a* and algal species composition between treatments. Nevertheless, we detected differences in feeding preferences of crayfish for AMB and ELEV periphyton stimuli. Crayfish preference for AMB stimulus over ELEV stimulus might have been caused by differences in cyanobacterial biovolume between DOC treatments. ELEV algal assemblages had higher cyanobacterial biovolume than AMB assemblages, and many cyanobacteria produce secondary compounds that inhibit consumption and assimilation by macroinvertebrates (Porter and Orcutt 1980, Gregory 1983). Crayfish probably were able to detect these chemical differences and chose AMB over ELEV periphyton stimuli.

Crayfish can detect chemicals leaching from plant material and alter their behavior in response to different chemical stimuli (Tierney and Atema 1988). For example, crayfish avoid certain food sources because they contain specific chemical compounds such as lignins, alkaloids, tannins, and phenolics (Lodge 1991, Bolser et al. 1998, Kubanek et al. 2000, 2001). Concentrations of these secondary defense compounds increase in leaf tissue produced under elevated CO<sub>2</sub> conditions (Lindroth et al. 1993, Curtis et al. 1996) and are released from leaf litter as DOC upon entering streams (Rier et al. 2002, 2005). Crayfish can detect these phytochemical differences between AMB and ELEV detritus and prefer AMB over ELEV leaf litter (Adams et al. 2003, 2005). Our results indicate that crayfish also can detect chemical differences between AMB and

ELEV DOC-enriched periphyton and prefer AMB DOC-enriched periphyton. Thus, crayfish seem to perceive AMB DOC-enriched periphyton as a higher-quality resource and spend more time foraging near it.

Crayfish are considered keystone species in many aquatic ecosystems (Lodge et al. 1994) and exert top-down regulation on primary producers and consumers. If, as our results suggest, crayfish prefer not to consume periphyton grown under ELEV CO<sub>2</sub>, it is possible that they will consume other food types to compensate for the lower nutritional quality of ELEV periphyton. For example, crayfish are active consumers of aquatic macroinvertebrates (Olsen et al. 1991, Momot 1995, Charlebois and Lamberti 1996), and they can alter the relative biomass of macroinvertebrates in aquatic environments (Hanson et al. 1990, Lodge et al. 1994, Momot 1995, Guan and Wiles 1998). Recent experiments using detritus found that crayfish feeding decisions were determined by the nutritional quality of the present food source and not by past experience with a food source (Adams et al. 2005). Therefore, a shift away from consumption of detritus (Adams et al. 2003, 2005) or periphyton (our study) under future elevated CO<sub>2</sub> conditions potentially could increase predation pressure by crayfish on macroinvertebrates, increase benthic organic matter standing crops, and decrease organic matter processing in aquatic ecosystems.

Our results add to the expanding list of potential effects of rising atmospheric CO<sub>2</sub> on stream ecosystems. Specifically, our results show that elevated CO<sub>2</sub>-induced changes in DOC concentration could affect periphyton and subsequently alter stream food webs and nutrient cycling. Direct effects of elevated atmospheric CO<sub>2</sub> on the structure and function of aquatic biofilms through C fixation are understudied and could have significant bottom-up effects on stream food webs. We were able to measure indirect effects of elevated CO<sub>2</sub> on chlorophyll *a*, algal assemblages, and crayfish feeding preferences, but our findings suggest that indirect effects of elevated CO<sub>2</sub>-altered leaf-litter chemistry on stream periphyton might be attenuated (e.g., no effect on periphyton C:N, biovolume, bacterial productivity, or biomass) relative to direct effects of elevated CO<sub>2</sub>-altered leaf litter on detritivores (Adams et al. 2003, 2005, Tuchman et al. 2003a). As atmospheric CO<sub>2</sub> continues to increase, further research will be needed to identify additional effects of elevated atmospheric CO<sub>2</sub> on aquatic ecosystem structure and function.

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