Atmospheric CO 2 Enrichment Alters Leaf Detritus: Impacts on Foraging Decisions of Crayfish (Orconectes virilis)

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Atmospheric CO_2 enrichment alters leaf detritus: impacts on foraging decisions of crayfish (*Orconectes virilis*)

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Abstract. Many tree species demonstrate altered foliar chemical composition when grown under elevated CO_2 conditions, decreasing the nutritional quality of leaves for herbivores and detritivores. Leaf litter comprises a substantial portion of the organic input into some headwater stream ecosystems, so changes in the chemistry of leaf detritus may affect the food-selection behavior of organisms, such as crayfish, that feed on it. Detritus from trembling aspen (*Populus tremuloides*) was produced under either the current CO_2 concentration of 360 ppm (ambient, AMB) or twice the current concentration, 720 ppm (elevated, ELEV). A Y-maze was used to determine crayfish preference for AMB or ELEV detritus. Experimental conditions consisted of: 1) fresh detritus, 2) detritus that had been leached in water for 24 h, and 3) leachate from detritus (dissolved organic matter, DOM) made into a slow-releasing gelatin block. Pairwise combinations of stimuli (AMB, ELEV, and a no-stimulus control, CON) were compared within each of the experimental conditions. Chemical analyses (%C and N, C:N, and % total phenolics) were done for each stimulus. Behavioral parameters measured from videotapes included initial arm choice (χ^2 test), proportion of time spent in each arm, and proportion of time spent at each stimulus source (arcsine transformed, paired *t*-tests). Percent C, C:N, and % total phenolics were significantly higher and %N significantly lower in both fresh detritus and leachate produced from leaves grown at elevated CO_2. ELEV-leached detritus showed a significantly higher % total phenolics than the AMB-leached detritus. Crayfish preferred AMB over ELEV or CON when offered either fresh detritus or DOM gelatin. There were no differences in preference for ELEV vs CON for all 3 experimental conditions. Crayfish showed no preference in any treatment when offered leached detritus. These results demonstrate that crayfish can discriminate chemically between AMB and ELEV detritus, that AMB detritus is preferred, and that crayfish are attracted by chemicals diffusing from the detritus.

Key words: crayfish, *Orconectes virilis*, elevated CO_2, preference, climate change, detritus.

The concentration of atmospheric CO_2 is steadily rising because of anthropogenic activities such as the burning of fossil fuels and deforestation (Keeling et al. 1995). Atmospheric CO_2 concentrations are expected to at least double within the next 50 y, from the current concentration of ~360 ppm to 720 ppm or more (White 1990, Watson et al. 1992, Tans and Bakwin 1995). CO_2 is a greenhouse gas that traps irradiated heat from the earth's surface in the atmosphere. An increase in atmospheric CO_2 can directly affect terrestrial plant C-fixation rates, plant growth processes, and foliar chemistry, in addition to causing global increases in temperature and the incidence and intensity of catastrophic storms (Bengtsson et al. 1996, Knutson et al. 1998, Meehl et al. 2000).

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CO_2 enrichment in C_3 plants has shown a concomitant increase in C fixation leading to higher growth rates and C:N ratios in leaf tissues. Enhanced C fixation results in relatively higher amounts of structural and nonstructural carbohydrates and plant defense compounds, including lignin and phenolics (Strain and Bazzaz 1983, see Coviella and Trumble 1999 for review). Overall, lower leaf N and higher concentrations of defense compounds result in a decrease in nutritional quality of leaf material for herbivores (Körner and Arnone 1992, Lavola and Julkunen-Tiitto 1994). Herbivores feeding on the foliage of plants grown under elevated CO_2 conditions tend to increase biomass consumption to compensate for lower nutritional value (Johnson and Lincoln 1990, Lindroth et al. 1993), but still show retarded growth (Fajer et al. 1989, Lincoln et al. 1993). In ecosystems where terrestrial plants are the base of the food web, organisms of higher trophic levels may be affected similarly by changes in the nutritional quality of plants because of elevated atmospheric CO_2 (Osbrink et al. 1987, Roth and Lindroth 1995, Awmack et al. 1997).

Terrestrial plant detritus, mainly deciduous leaf litter, forms the foundation of the food web in headwater streams, contributing up to 99% of the organic C input for the ecosystem (Minshall 1967, Fisher and Likens 1973). Leaf litter is the energy base of these ecosystems, so leaf nutritional quality altered by CO_2 concentration can negatively affect aquatic decomposers and invertebrate detritivores. Bacterial and fungal growth in headwater streams is suppressed by >50% on leaf litter produced at elevated CO_2 levels (Rier et al. 2002). In addition, cranefly larvae fed detritus grown under elevated CO_2 conditions consumed less leaf material, assimilated less leaf biomass, and grew slower than those reared on detritus produced at ambient CO_2 (Tuchman et al. 2003). Similarly, 4 species of mosquito larvae exhibited delayed development and higher mortality when reared on detritus produced at elevated CO_2 (Tuchman et al. 2002). Changes at the microbial and detritivore levels of the food web reduce fish growth and fitness (NCT, unpublished data), resulting in alterations of energy and C fluxes through the food web through trophic interactions.

Crayfish are omnivores that can serve as important detritivores in litter-based food webs (Momot et al. 1978, Lodge et al. 1994, Momot 1995). Crayfish consume and process much of the leaf litter in streams (~20–70% in different ecosystems), so they often regulate community structure in these ecosystems (Lodge and Hill 1994, Momot 1995, Usio 2000) and may function as keystone species (Hill and Lodge 1995, Charlebois and Lamberti 1996, Usio 2000).

The influence of crayfish in a stream is a direct result of their behavior and choice of food sources. Many behaviors of crayfish, including orientation to food sources, are mediated through chemical senses (Tierney and Atema 1988, Willman et al. 1994, Moore and Grills 1999). Chemical compounds or mixtures of compounds diffuse from potential food sources into the environment where they are detected by crayfish chemosensory organs (Dunham et al. 1997, Kreider and Watts 1998, Giri and Dunham 1999). Alterations in the composition of chemicals diffusing from detritus produced at elevated CO_2 levels may translate into changes in the perception of the detritus by crayfish.

The objective of our experiment was to determine whether crayfish could perceive a difference in, and demonstrate a preference or aversion to, detrital material altered by atmospheric CO_2 enrichment. We tested 2 hypotheses: 1) crayfish prefer detritus derived from leaves exposed to ambient CO_2 levels (higher nutritional quality) to detritus derived from leaves exposed to elevated CO_2 levels (lower nutritional quality), and 2) the attraction to detritus is mediated by chemicals leaching from it. Our study determined whether the perception of detritus by crayfish and their subsequent foraging decisions, which can affect other levels of the food web, are influenced by the changes in foliar chemistry that accompany elevated-CO_2 detritus.

Methods

Growing aspen on elevated atmospheric CO_2

Trembling aspen (*Populus tremuloides* Michaux) trees were grown under both the ambient CO_2 concentration of 1999 (360 ppm; AMB) and twice the ambient concentration (720 ppm; ELEV). Leaves were gathered after natural senescence and abscission from the tree (late November) and were dried at room temperature. All leaves used for treatments were grown and collected at the Elevated CO_2 Research Facility of The University of Michigan Biological Station (UMBS) in Pellston, Michigan, during the fall of 1999. Trembling aspen is the most common tree species in Michigan (Schmidt et al. 1993) and accounts for ~22% of leaf litter entering the East Branch of the Maple River near UMBS (Tuchman et al. 2002). For detailed methods of growing the aspen trees, see Tuchman et al. (2002) and Rier et al. (2002).

Experimental animals

Male and female crayfish (*Orconectes virilis* Hagen) were collected between 2230 and 2400 h from June through August 2001 from Maple Bay in Burt Lake, Pellston, Michigan. All experiments were performed between 0800 and 1800 h from July through August 2001. Crayfish were housed outdoors in a flow-through metal trough located at the UMBS Stream Research Facility under ambient summer light and temperature conditions in northern Michigan. Densities of crayfish in the tank never exceeded 35 animals/m^2. Adequate shelter was provided to limit the number of aggressive interactions between crayfish. Crayfish were allowed to feed on periphyton in the tank throughout their brief housing period (2 wk maximum). Nylon stocking filters were fitted over the inflow pipes to decrease the amount of detritus crayfish were exposed to during holding and to prevent the accumulation of organic matter in the population tank, and were changed once per week or when full. Filters were checked daily for the buildup of organic matter. Both male (form I, reproductive) and female crayfish were used for trials. Crayfish used in experiments had a carapace length of 3.89 ± 0.5 cm and had a full set of chemosensory appendages (1 pair of antennae, 2 pairs of antennules, 2 chelae). Each crayfish was only used once in an experiment.

Y-maze design

A flow-through Y-maze was used to test crayfish response to different odors (working section = 190 × 114 × 20 cm, arms = 152 × 57 × 20 cm; Fig. 1). The test arena was constructed at the UMBS Stream Research Facility using standard concrete cinder blocks (38 × 19 × 19 cm) as a frame, lined with 4 mm plastic sheeting secured over the cinder blocks. The bottom of the Y-maze was lined with cobble (4.5 ± 0.2 cm diameter, $n = 24$). A collimator constructed of plastic egg crating (1.69 cm^2 aperture) with fiberglass sheeting (1 mm^2 aperture) was placed 25 cm downstream of the flow input to facilitate laminar flow in the test arena. To maintain a constant depth, a 0.35 m baffle was placed perpendicular to the flow at the downstream end of the Y-maze channels, with 2.54 cm diameter holes evenly spaced throughout, allowing water passage. The water depth was maintained at 20 ± 0.5 cm. The Y-maze was covered with an opaque tarp to reduce ambient light conditions to levels similar to those in Maple Bay.

Water from the East Branch of the Maple River was pumped into a constant head tank to regulate the amount of water entering the Y-maze. Pipes leading from the head tank to the Y-maze were covered with nylon mesh to limit the amount of organic matter and macroinvertebrate fauna entering from the Maple River, which would contaminate the treatments. Water flowed over a step where it spilled into the Y-maze to ensure even flow across the entire width of the tank and into the arms. Flow speed was measured at 17 points in the tank (height of 0.5 cm from bottom) with a Marsh–McBirney flow meter (Model 2000 Portable Flowmeter) to ensure equal flow at all points in the Y-maze (see Fig. 1 for spacing of flow measurement points). Flow was 3.0 ± 0.5 cm/s at all points. Dye trials were conducted to visualize odor distribution in the Y-maze.

Stimuli and preparation methods

Crayfish were tested in 3 experimental conditions, where they were offered either 1) fresh detrital material, 2) detritus leached in water for 24 h, or 3) leachate (dissolved organic matter, DOM) collected for 24 h from detritus that was made into slow-diffusing gelatin blocks. Stimuli consisted of AMB, ELEV, and a control (CON) consisting of no chemical stimulus. Pair-wise combinations of stimuli were presented within each experimental condition to crayfish for a total of 3 treatments: AMB vs CON, ELEV vs CON, and AMB vs ELEV. Stimuli were placed in mesh bags (1 mm^2 aperture, 10.0 × 8.5 cm) marked with reflective tape and attached to a weight. The reflective tape made the source visible for later computer analysis. The weight ensured that the stimulus would remain on the bottom of the test arena. Mesh bags containing

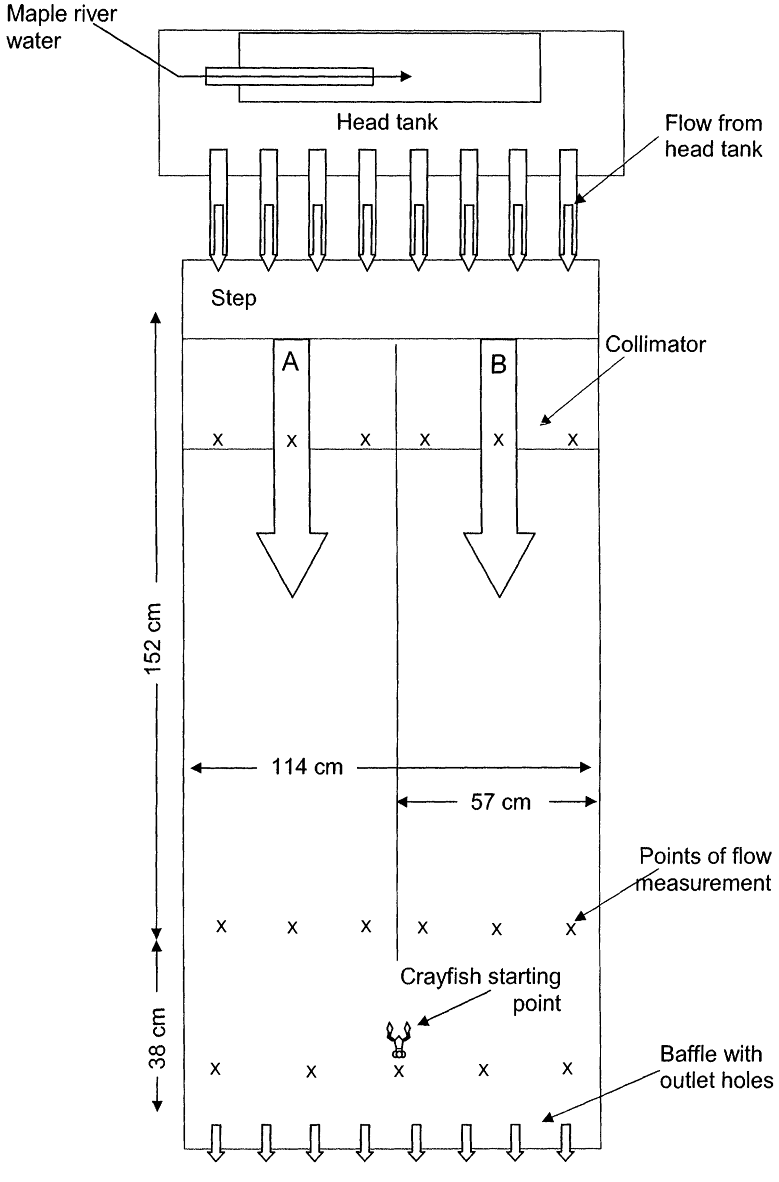

FIG. 1. Y-maze design. Flow is from top to bottom in the diagram. Crayfish begin the experiment in the downstream portion of the Y-maze. X = points where flow rate was measured. A and B = arms.

stimuli were placed at the upstream end of each arm, immediately downstream of the collimator and equidistant from the side walls (Fig. 1).

Fresh detritus.—For all experiments using fresh detritus as a stimulus, 0.070 ± 0.006 g of either AMB or ELEV detritus was placed in a mesh bag. The CON stimulus consisted of an empty mesh bag.

Leached detritus.—Detritus that had lost >90% of its DOM (leached leaves) as well as the DOM leachate were presented to crayfish to determine whether they were attracted to leaf litter by its visual presence or by the chemicals leaching downstream from the litter. Five g of fresh leaf detritus were soaked for 24 h in 1 L of sterile spring water to remove the DOM (Rier et al. 2002). The detritus was agitated on a magnetic stir plate to facilitate the diffusion of DOM from leaves. Detrital material was removed and stored at 4°C in a Ziploc® bag until experimentation. All experiments were conducted within 10 h of leaching.

Approximately 0.240 ± 0.004 g towel-dried leached ELEV or AMB detritus (corresponding to 0.07 g dry mass) were placed in a mesh bag for crayfish preference studies. The CON stimulus for this experiment consisted of an empty mesh bag.

DOM.—DOM was made by placing 21.5 ± 0.1 g of dried AMB or ELEV detritus into a plastic container with 2 L of sterile spring water, and agitating it on a magnetic stir plate for 24 h. Rier et al. (2002) demonstrated that most (>98%) of the soluble phenolic compounds leached out of this detritus within 24 h. Detrital material was filtered from the leachate using a sieve (60-μm mesh). The leachate was stored in 2 L plastic containers in the refrigerator at 4°C for 48 h until use.

The DOM was suspended in gelatin to make a solid matrix from which the chemicals could diffuse into the flowing water of the Y-maze. Four packets of Knox® unflavored gelatin (37.3 g) were placed into a beaker to which 355 mL of boiling spring water were added. An additional 355 mL of cold DOM leachate were added for the AMB and ELEV treatments. An additional 355 mL of cold spring water were added instead of leachate for the CON stimulus. The gelatin was allowed to solidify, and then was cut into 1.0 × 1.0 × 1.5 cm blocks, such that 1 block of gelatin contained the same amount of DOM that would come from 0.07 g of fresh detritus (calculated by multiplying the volume of leachate used to make the gelatin and the g leaves/L water in the original leachate, divided by the number of blocks of gelatin made). The blocks were individually wrapped in cellophane and refrigerated until experimentation. All experiments were conducted within 2 wk of making the gelatin. Gelatin blocks were placed in a mesh bag for experiments.

Experimental protocol

Each stimulus was randomly assigned to an arm by flipping a coin. A crayfish was acclimated to the test arena with no stimuli for 20 min prior to each trial. Crayfish were stimulated to move to the back of the arena by disrupting the water in front of them without physical contact with the animals. Crayfish began each trial at the starting point (Fig. 1) and were then allowed to explore the Y-maze for 10 min while being videotaped from above (Sony Hi-8 Handycam Model # CCD-TR700). Trials in which the experimental animal did not move, escaped from the maze, or appeared visibly disturbed by the researchers were removed from analysis, including 3 of 48 trials (<6.5%) for fresh detritus, 4 of 49 trials (<8.5%) for leached detritus, and 11 of 56 trials (<20%) for DOM. A sample size of $n = 15$ was used for all treatments in each experimental condition.

Leaf litter and DOM chemical analysis

Phytochemical differences between AMB and ELEV stimuli for all experimental conditions were determined by measuring total phenolic compounds (expressed as % dry mass), %C and N, and C:N. Leaf litter was dried at 60°C for 48 h and ground in a mill for analyses. DOM was concentrated by lyophilization prior to analysis. Percent phenolics was measured using the Folin–Denis assay (Swain and Goldstein 1964), and %C and N (and C:N) were measured on a Carlo–Erba Elemental Analyzer.

Data and statistical analysis

Measures of chemical parameters in detritus and DOM were analyzed with a 1-way MANOVA and LSD post-hoc tests for differences between treatments (AMB vs ELEV) within each experimental condition.

Videotapes were analyzed using Peak Motus Motion Analysis® software to digitize the x and y coordinates of the crayfish. The x and y coordinates of the crayfish rostrum were digitized once every second for the total length of the trial. Any possible observer bias was removed because all behavioral measures were analyzed and calculated using computers.

Behavioral parameters obtained from the computer analysis included initial arm choice, time spent in each arm, and time spent at the source. Initial arm choice was defined as the first arm the crayfish entered. A χ^2 test ($n = 15$ for all treatments) was used to determine whether initial arm choices were different from random (the random expectation is selecting a certain arm in 50% of trials for a particular treatment) within each treatment for all stimuli. The total amount of time spent in each arm was the sum of all the individual times when a crayfish stayed in a particular arm (including time spent at the source). Time at the source was defined as the total amount of time a crayfish spent touching the source with at least one chela. These raw times were converted to the proportion of time spent in a particular arm or source relative to total time spent in both of the arms. Trials lasted for 10 min and not all time was spent in the arms (crayfish could spend time in the downstream portion of the test arena), so the proportions were calculated to minimize the influences of a few crayfish that had entered the arms but spent most of their time in the back of the test arena. Proportions were transformed using arcsine square root. Two-tailed paired t-tests were used to detect significant differences in the transformed data for both proportion of time spent within each stimulus and at each source.

Results

Leaf litter and DOM chemical analysis

AMB and ELEV detritus demonstrated differences in leaf chemical quality. ELEV leaf litter and ELEV DOM had significantly higher % total phenolics, %C, and C:N, and significantly lower %N (MANOVA, Rao's $R_{4,15,0.05} = 14.91, p < 0.001$; Fig. 2A, C). Over 90% of the total phenolics was removed from leached leaves of both treatments, which lowered C:N values, yet the treatment differences in C:N were conserved (LSD post-hoc test, $p < 0.001$; Fig. 2B).

Preference tests

Fresh detritus.—Crayfish initially chose the AMB arm more frequently than the ELEV arm in the AMB vs ELEV treatment when offered fresh detritus ($\chi^2_{1,0.05} = 8.07, p < 0.005$; Fig. 3A). They also selected the AMB arm first more often than the CON arm in the AMB vs CON treatment ($\chi^2_{1,0.05} = 11.27, p < 0.001$). Initial arm choice was not significantly different from random in the ELEV vs CON treatment ($\chi^2_{1,0.05} = 0.07, p > 0.05$).

The proportion of time spent in the AMB arm was significantly higher in the AMB vs ELEV treatment (t-test, $t_{14,0.05} = 4.95, p < 0.001$; Fig. 3B). Crayfish spent 98% of time in the AMB arm and 2% in the CON arm in the AMB vs CON treatment (t-test, $t_{14,0.05} = 18.74, p < 0.001$). The proportion of time spent in the ELEV arm did not differ from that spent in the CON arm in the ELEV vs CON treatment (t-test, $t_{14,0.05} = 0.07, p > 0.05$).

Crayfish spent more time in contact with the AMB leaf detritus source than the ELEV source in the AMB vs ELEV treatment (t-test, $t_{14,0.05} = 4.25, p < 0.001$; Fig. 3C). Crayfish also spent significantly more time with the AMB source than the CON source in the AMB vs CON treatment (t-test, $t_{14,0.05} = 3.83, p < 0.002$). There was no difference in time spent at the ELEV vs CON sources (t-test, $t_{14,0.05} = 0.457, p > 0.05$).

Leached detritus.—Crayfish demonstrated no significant initial arm choice in any of the treatments when leached detritus was presented (Fig. 4A). There were also no significant differences in the proportion of time spent in each arm or at each detritus source for any treatment (Fig. 4B, C).

DOM.—In experiments with DOM gelatin as a stimulus, crayfish demonstrated a significant initial arm choice for the AMB arm in both the AMB vs ELEV ($\chi^2_{1,0.05} = 8.07, p < 0.005$) and the AMB vs CON ($\chi^2_{1,0.05} = 5.40, p < 0.02$) treatments (Fig. 5A). Initial arm choice was not significantly different in the ELEV vs CON treatment ($\chi^2_{1,0.05} = 0.07, p > 0.05$).

The proportion of time spent in the AMB arm was significantly higher in the AMB vs ELEV (t-test, $t_{14,0.05} = 4.00, p < 0.001$) and the AMB vs CON treatments (t-test, $t_{14,0.05} = 4.95, p < 0.001$; Fig. 5B). The proportion of time spent in the ELEV arm did not differ from the CON arm in the ELEV vs CON treatment (t-test, $t_{14,0.05} = 0.73, p > 0.05$).

Crayfish spent more time at the AMB source in both the AMB vs ELEV (t-test, $t_{14,0.05} = 3.96, p < 0.001$) and the AMB vs CON ($t$-test, $t_{14,0.05} = 4.00, p < 0.001$; Fig. 5C) treatments. There was no difference in time spent at the ELEV vs CON sources (t-test, $t_{14,0.01} = 0.62, p > 0.05$).

Discussion

Overall, these experiments demonstrated that elevated atmospheric CO_2 alters leaf-litter chemical composition, which in turn may affect crayfish detritus foraging decisions. Crayfish distinguished between AMB and ELEV detritus when presented with either fresh detritus or DOM leachate, and were more attracted to AMB when offered either fresh detritus or DOM leachate.

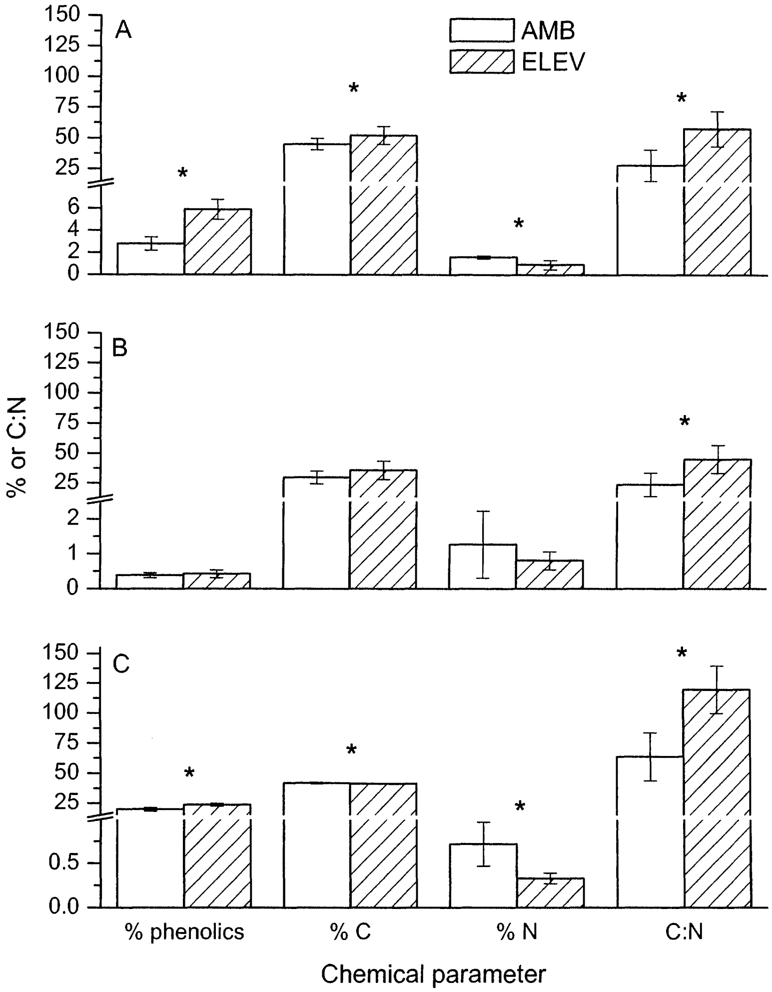

FIG. 2. Partial chemical composition of ambient (AMB) or elevated (ELEV) *Populus tremuloides* detritus offered to crayfish in each experimental condition. A.—Fresh detritus. B.—Detritus leached for 24 h. C.—Dissolved organic matter (DOM). Values are expressed as means (± SEM), $n = 8$ for leaf litter, $n = 12$ for DOM. Asterisks indicate a significant difference between ELEV and AMB within an experimental condition ($p < 0.05$).

However, crayfish could not discriminate between AMB or ELEV leaf material after leaching had occurred, indicating that crayfish detect detritus through compounds leaching from the leaves and that those compounds are more attractive from AMB detritus than ELEV detritus.

The role of foliar chemistry in crayfish preference

Crayfish may select AMB detritus over ELEV detritus because they perceive the former as more nutritious. Optimal foraging theory predicts that animals will select a food item that maximizes energy intake per unit time and minimizes energy used in capturing and processing food (Pulliam 1974, Charnov 1976, Sih and Christensen 2001). Some crustaceans may store leaf detritus for various lengths of time before consumption to reduce C:N ratio and tannin concentration through microbial degradative processes and leaching, a strategy known as "leaf-ageing" (Giddens et al. 1986, Neilson et al. 1986), which may require the detection of C:N concentration. Crayfish may have detected the higher C:N ratio of ELEV detritus in our experiment and made a decision not to eat those leaves, which may cause a shift in dietary focus to other food items.

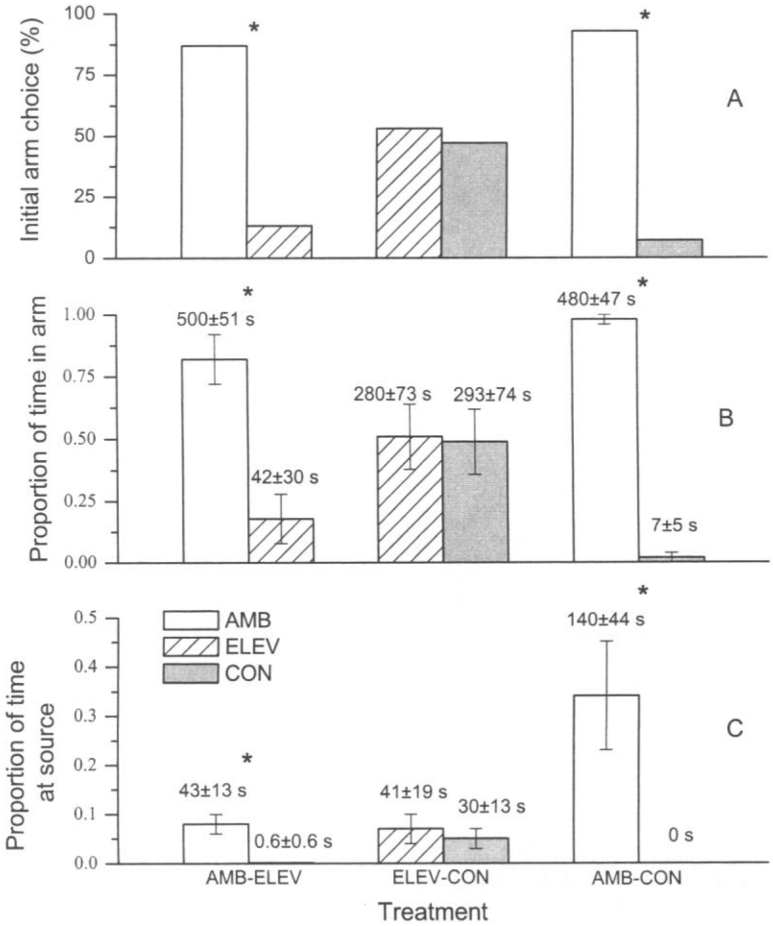

FIG. 3. Behavioral parameters measured in response to fresh detritus. AMB = ambient, ELEV = elevated, CON = control. A.—Initial arm choice (χ^2 test). B.—Mean proportion of time spent in each arm (± SEM; arcsine transformed, paired *t*-tests). C.—Mean proportion of time spent at each source (± SEM; arcsine transformed, paired *t*-tests). The mean (± SEM) raw time (s) spent in each arm and at each source is listed above the bars in panels B and C. * = significant difference at $p < 0.05$.

The DOM released from detritus mediated the foraging decisions of crayfish in our experiment. Crayfish may use these leached chemicals to pinpoint a favorable location in which to search for detritus food sources or to select particular food types. Many sources of DOM are present in stream ecosysytems (Fisher and Likens 1973), so certain compounds leaching from leaf detritus such as sugars (demonstrated as excitatory stimuli for crayfish; Tierney and Atema 1988) and secondary defensive chemicals (demonstrated as an aversive stimulus for crayfish; Lodge 1991, Kubanek et al. 2000, 2001) may enable crayfish to accurately identify detrital material. Although actual consumption of detrital material may be a better indicator of the food crayfish would ultimately select, attraction to the location of the food is a necessary prerequisite for that decision to occur. Thus, the selection of the AMB detritus in the DOM stimulus preparation may indicate that, in nature, crayfish are attracted to that site to locally search for food, or use the chemical cues from the leaves to approach and select a detritus type. The selection of detritus by chemoreception does not depend on long-term exposure to, and feeding on, the detritus grown at different CO_2 concentrations, demonstrating that selection is determined by the chemicals present in the DOM and not some aspect of experience with the food

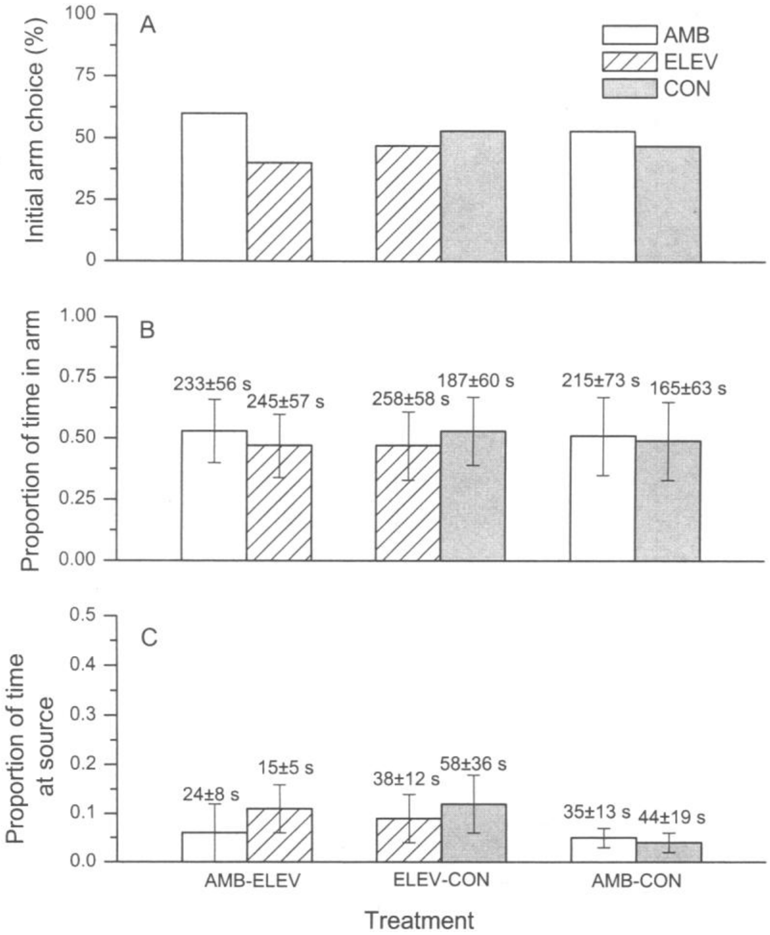

FIG. 4. Behavioral parameters measured in response to leached detritus. AMB = ambient, ELEV = elevated, CON = control. A.—Initial arm choice (χ^2 test). B.—Mean proportion of time spent in each arm (± SEM; arcsine transformed, paired *t*-tests). C.—Mean proportion of time spent at each source (± SEM; arcsine transformed, paired *t*-tests). The mean (± SEM) raw time (s) spent in each arm and at each source is listed above the bars in panels B and C. * = significant difference at $p < 0.05$.

type (JAA, NCT, and PAM, unpublished data). Instead, the chemical components of the food signal are the basis for detritus selection.

Crayfish may be deterred from feeding on plant material by phenolic and other defense compounds (Bolser et al. 1998, Kubanek et al. 2000, 2001). Lodge (1991) demonstrated a negative correlation between crayfish preference for macrophytes and presence of plant defense compounds. In our experiment, the higher phenolic content of ELEV detritus, coupled with a higher C:N ratio, may have mediated the food preference of crayfish for AMB vs ELEV detritus.

Tree species differences

Most terrestrial vegetation will be affected by elevated CO_2, but individual species responses may differ. Changes observed in C:N ratio and defensive compound concentration is variable in different tree species (Peñuelas and Estiarte 1998, Coley et al. 2002). Some trees have higher natural levels of phenolics and condensed tannins (Norby et al. 1986), whereas others produce low amounts of defensive chemicals but dramatically increase production of these compounds in response to elevated CO_2 (Roth et al. 1994, Hemming and Lindroth 1995, Kinney et

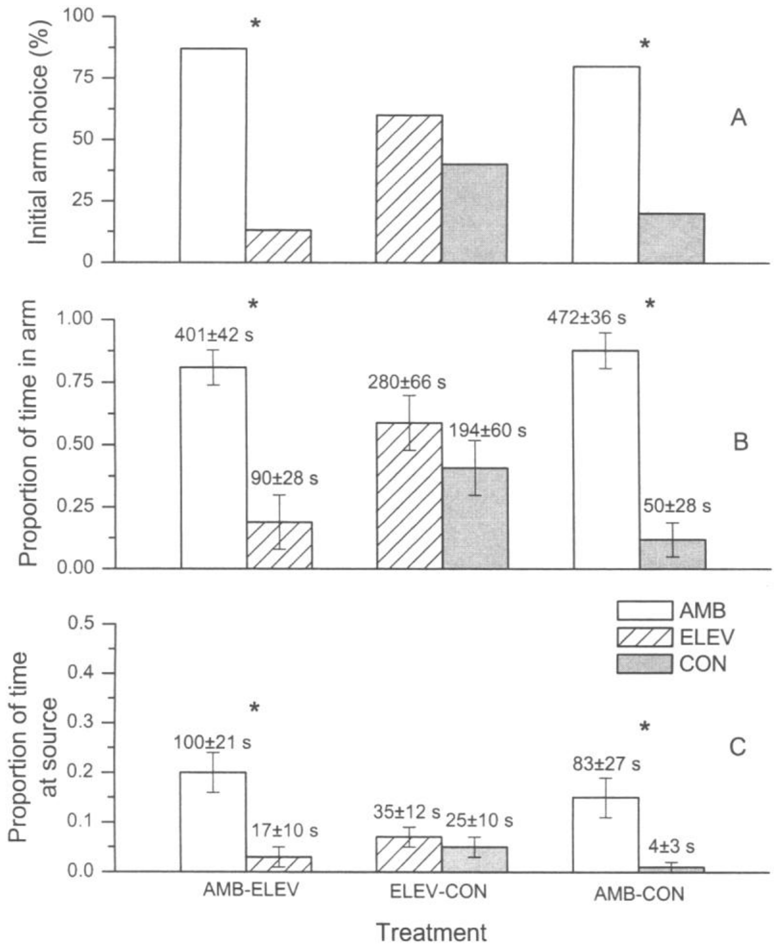

FIG. 5. Behavioral parameters measured in response to dissolved organic matter (DOM) leachate. AMB = ambient, ELEV = elevated, CON = control. A.—Initial arm choice (χ^2 test). B.—Mean proportion of time spent in each arm (± SEM; arcsine transformed, paired *t*-tests). C.—Mean proportion of time spent at each source (± SEM; arcsine transformed, paired *t*-tests). The mean (± SEM) raw time (s) spent in each arm and at each source is listed above the bars in panels B and C. * = significant differences at $p < 0.05$.

al. 1997). This natural variability between species may affect crayfish foraging decisions, resulting in consumption of detritus from a leaf species that is a low producer of defensive compounds. Also, the overall background concentration of defensive compounds in the stream from multiple leaf litter species may increase, interfere with chemoreception, and influence crayfish foraging behavior.

Ecological implications

Alterations in foliar chemistry in response to elevated CO_2 are expected to occur gradually over the next 50 to 100 y, which may allow crayfish time to physically and behaviorally adapt to the lower nutritional quality of detritus and increased concentration of secondary defensive compounds in detritus. However, previous experience with detritus does not alter food preferences of crayfish; chemical composition alone seems to mediate choice between detrital food sources (JAA, NCT, and PAM, unpublished data). Therefore, because crayfish are omnivorous and have multiple food types at their disposal, it is more likely that they will eat detritus species with lower concentrations of secondary defensive compounds, or will avoid detritus as a food source. In either case, this will change the way that C flows in stream ecosystems.

Because crayfish are keystone species in stream food webs, altering their behavioral decisions and feeding activities can have large-scale effects. These potential effects include changes in mechanical damage to vegetation and periphyton (Lodge and Lorman 1987, Lodge et al. 1994), changes in the interaction with predators (Hill and Lodge 1995) and competitors (Lodge et al. 1994), and altering the selection of food types (Momot 1995, Perry et al. 1997, Usio 2000) and prey size classes (Olsen et al. 1991, Lodge et al. 1994). Particularly relevant for our study, crayfish consume and shred a substantial portion of leaf litter in streams, with estimates ranging from 20% to 70% in different systems (Griffith et al. 1994, Momot 1995, Usio 2000). A potential shift in crayfish diet from leaf detritus to macrophytes, periphytic algae, or invertebrates as a result of elevated CO_2 could affect foodweb structure by increasing competition with grazers and predators and releasing competitive pressure with detritivores. More comprehensive field experiments with other species of invertebrates and vertebrates are needed to determine exactly how changes in foliar chemistry and consequent changes in crayfish behavior can affect foodweb dynamics in aquatic ecosystems.

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