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Influence of elevated As on leaf breakdown in an Appalachian headwater stream

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Abstract. A headwater stream adjacent to an abandoned As mine was investigated to determine the influence of As on stream biota and organic matter processing using an upstream (reference reach) and downstream (mine-influenced reach) comparative approach. Field assessments of stream chemistry, macroinvertebrate abundance and composition, and leaf breakdown were coupled with laboratory experiments addressing As influences on leaf biofilm respiration. Streamwater As concentrations varied from below detection limit (2.5μg/L) in the upstream reach to >12,000 μg/L. Concentrations were low in the reference reach, increased immediately adjacent to tailing piles, and climbed significantly with distance along the mine-influenced reach. Compared to the reference reach, macroinvertebrate density (7869 vs 154 individuals/m^2), shredder abundance (3340 vs 22 individuals/m^2), and species richness (11.9 vs 0.8 species/sample) were significantly lower in the mine-influenced reach. For both white oak and red maple leaf packs, breakdown rates in the reference reach (k = 0.0048 and 0.009/d, respectively) were significantly greater than in the mine-influenced reach immediately downstream of waste piles (k = 0.0019 and 0.003/d) and further downstream (k = 0.0014 and 0.005/d). In one experiment, laboratory assays showed that short-term exposure to elevated As concentrations did not alter leaf biofilm respiration rates. In a 2nd experiment addressing chronic exposure, respiration rates for extant leaf biofilms in the reference reach (0.37 ± 0.01 μg O_2 mg ash-free-dry-mass [AFDM]$^{-1}$ h^{-1}) were significantly greater than in the mine-influenced reach (0.29 ± 0.01 μg O_2 mg $AFDM^{-1}$ h^{-1}), but rates in both reaches were typical of forested headwater streams not exposed to elevated As concentrations. Together, these data suggest that elevated As concentrations in the stream have led to altered organic matter processing not by reducing microbial activity but primarily by decreasing invertebrate densities, limiting shredder abundance, and decreasing litter breakdown rates.

Key words: leaf breakdown, arsenic, shredders, leaf biofilm respiration, benthic macroinvertebrates, headwater stream.

Arsenic is an element found in trace amounts in the earth's soils, waters, and organisms (Smedley and Kinniburgh 2002). It is a known carcinogen to humans (NRC 1999), and presumably also has negative effects on the vitality of many other organisms. This concern is substantial because anthropogenic inputs of As to the environment during the past century have been estimated to be as high as 28,000 t/y, nearly four times the 7800 t produced globally each year from natural sources (Nriagu and Pacyna 1988). Classified as a semimetal or metalloid, As forms complexes with other metals and readily forms covalent bonds with C, H, and O_2 (Gorby 1994). In natural waters, As commonly exists in 2 oxidation states: As^{+3} (as arsenite, AsO_2^{-2}), the most toxic form to humans, and As^{+5} (as arsenate, AsO_4^{-3}), the form most toxic to algae (Knauer et al. 1999).

Arsenic has been used for various purposes throughout history, including in medicine, poison, wood preservative, pesticide, and insecticide, and it occurs as a by-product in other industrial activities (Welch et al. 2000). Although As concentrations unsafe for drinking water may exist in many natural geologic settings (Schreiber et al. 2000, Welch et al. 2000, McArthur et al. 2001, Nordstrom 2002), runoff

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from applied uses has contaminated surface water and groundwater in many parts of the world (Azcue and Nriagu 1994, Bhumbla and Keefer 1994, Smedley and Kinniburgh 2002).

Previous studies have documented increased mortality among aquatic invertebrates living in aquatic systems with elevated As concentrations (Eisler 1994, Jeyasingham and Ling 2000, Canivet et al. 2001). Mortality among zoobenthic species in streams may have substantial influences on foodweb dynamics and ecosystem processes, particularly in headwater systems. For example, Wallace et al. (1986) showed that macroinvertebrate shredders (i.e., invertebrates that shred coarse particulate organic matter [CPOM]; sensu Cummins 1973) play a critical role in leaf breakdown. In eastern North America, detritus inputs from surrounding forests greatly exceed primary production in headwater streams (e.g., Webster et al. 1995) and leaf litter fuels allochthonous-based food webs (Fisher and Likens 1973, Vannote et al. 1980). Besides acting as a resource for macroconsumers (Wallace et al. 1986), leaf litter can also serve as a resource for microbial assemblages (Findlay et al. 2001) and influence nutrient transport and retention (Mulholland et al. 1985). Recently, Gessner and Chauvet (2002) provided a cogent argument for the use of litter breakdown as a measure of stream functional integrity in the context of anthropogenic stresses. Leaf breakdown rates influence trophic dynamics and biogeochemical fluxes in streams, so it is important to know how this process is affected by anthropogenic influences such as elevated concentrations of toxic compounds.

There is a long history of work addressing how land use alters rates of leaf breakdown (e.g., Webster and Waide 1982, Sponseller and Benfield 2001, Huryn et al. 2002) and studies have also addressed the influence of some toxic metals (e.g., Gray and Ward 1983, Leland and Carter 1985, Schultheis et al. 1997, Schultheis and Hendricks 1999, Niyogi et al. 2001). Research in streams has demonstrated that elevated concentrations of some metals decrease leaf breakdown. Schultheis et al. (1997) identified Cu as the cause of altered zoobenthic community structure and reduced leaf breakdown in a 2nd-order mountain stream. Niyogi et al. (2001) found that shredder biomass declined with increasing concentrations of Zn in Rocky Mountain streams reflecting the influence of mine drainage. Further, their study tied decreased rates of leaf breakdown to declining shredder abundance. Leland and Carter (1985) used leaf microbial respiration as an index of leaf decomposition in the presence of experimentally added Cu and found that the rate of microbial processing, and hence leaf breakdown, was reduced at all test concentrations. Other researchers have found that respiration and growth of aquatic fungi responsible for breakdown are not readily depressed by elevated metal concentrations (Abel and Bärlocher 1984, Miersch et al. 1997). Thus, elevated metal concentrations may alter leaf breakdown by influencing invertebrate detritivores, microbial processing, or both.

The purpose of our project was to investigate how elevated levels of As, resulting from an abandoned arsenopyrite mine, have altered the biota and organic matter breakdown rates in a 2nd-order headwater mountain stream. A combination of field monitoring and experimental approaches was used to assess how As influenced both microbial and macroinvertebrate effects on leaf litter breakdown. Assessing both microbial and metazoan responses allowed us to elucidate mechanisms by which As may alter organic matter processes.

Methods

Study site

The study was conducted on a perennial headwater stream located in Floyd County, Virginia, USA, at the site of the former Brinton mine (Fig. 1). The mine was in operation from 1903 to 1919 during which time arsenopyrite (FeAsS) was converted to ''white arsenic'' (As_2O_3) before being shipped to pesticide manufacturers (Dietrich 1959, Dove and Rimstidt 1985). Besides scattered foundations, all that remains of the mine are large waste piles located next to the stream. The watershed is located in the Blue Ridge Province where parent lithology consists of metamorphic rocks including sericite schist (Hunt 1974). The catchment is now heavily forested with deciduous trees (Rocovich and West 1975), including white oak (*Quercus alba*), chestnut oak (*Quercus prinus*), and red maple (*Acer rubrum*), as well as eastern white pine (*Pinus strobus*). The stream discharges into Purgatory Creek ~1 km downstream, which eventu-

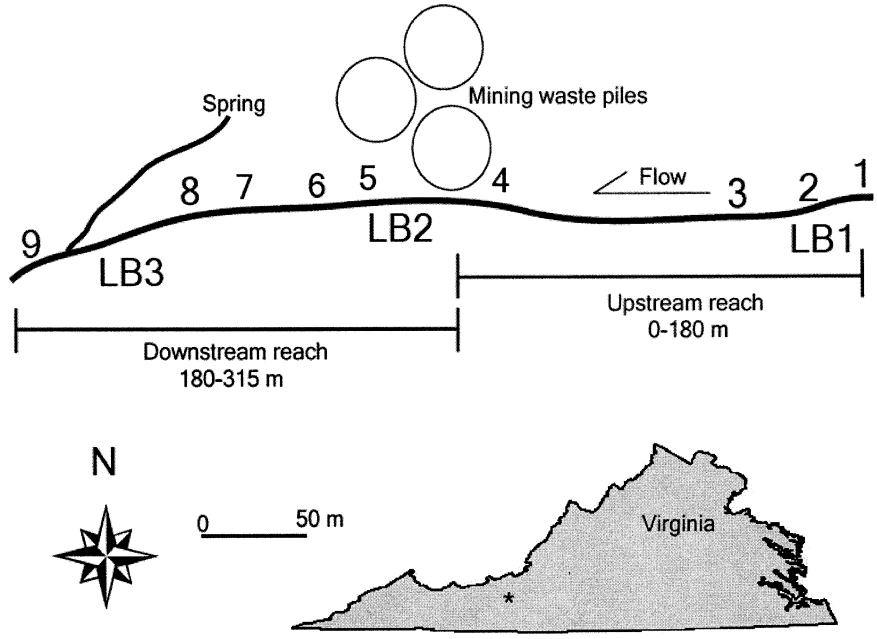

FIGURE 1. Diagram of the Brinton Mine Creek, Floyd County, Virginia, study site. Water sampling sites (numbers) were distributed between upstream and downstream reaches. Site 4 is located near the upstream extent of the mining activity and ~15 m upstream from extant waste piles (circles). LB1 through 3 are leaf breakdown sites.

ally empties into the South Fork of the Roanoke River.

The study site (Fig. 1) included 315 m of stream near the mine, with 180 m (i.e., the reference reach) occurring upstream where no mine activities are evident. The mine-influenced reach extended an additional 135 m adjacent to and downstream from the mine and was characterized by streamside waste piles, increased fine sediments in the stream that included metal oxides originating from the waste piles, and elevated instream As concentrations. Arsenic concentrations increased in the mine-influenced reach, but concentrations of other metals were not comparably elevated (i.e., 32, 20, 10, <5, <5, <1, and <25 μg/L for Cu, Zn, Mn, Ni, Md, Cd, and Pb, respectively). The upstream reference reach was heavily shaded but riparian vegetation in the mine-influenced reach was less dense and the stream channel was more open. The mine-influenced reach supported abundant benthic algae during summer. Baseflow stream velocity (10 cm/s), width (0.57 m), and depth (1.4 cm) were similar between reaches (N. Lottig, Virginia Polytechnic Institute and State University, unpublished data). Distinct riffles and pools were not evident in either reach.

Chemical characterization

Water samples were collected monthly from October 2002 through March 2003 at 9 sites along the stream (Fig. 1). Sites 1 to 3 were 100 m upstream of the tailing piles and represented unimpaired (reference) conditions. Sites 4 to 9 were adjacent to and downstream of the mine. On each occasion, water samples were collected in triplicate, filtered in the field (Whatman GF/F, pore size 0.7 μm), and preserved with HNO_3. Samples were analyzed for total As using a Perkin Elmer Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS) following EPA methods (USEPA 1991) with a detection limit of 2.5 μg/L. One additional filtered water sample from each of the 9 sites was preserved with EDTA, elutriated through an anion exchange cartridge (Fisherbrand, SPE-SAX, 3 mL), and analyzed within 24 h for reduced (AsO_2^{-2}) and oxidized (AsO_4^{-3}) As species (Garbarino et al. 2003).

Additional water samples (n = 3/site) were collected over the same time period, filtered, and frozen for later analysis. Dissolved organic C (DOC) was analyzed by wet persulphate digestion (Menzel and Vaccaro 1964) using an Oceanography International 700 Total Carbon Analyzer (College Station, Texas, USA). PO_4^{-3} was below detection limit (<10 μg/L) using ion chromatography on samples collected in early phases of site monitoring and was not analyzed for the remainder of the study. SO_4^{-2} and NO_3-N were analyzed by ion chromatography with a Dionex DX-500 (Sunnyvale, California, USA) using an IonPac AS14 anion column. NH_4-N was analyzed on a Technicon Autoanalyzer (Saskatoon, Saskatchewan, Canada) using a modified phenol hypochlorite method (USEPA 1997). Dissolved oxygen (DO) and conductivity (specific conductance) were measured monthly at each site using YSI DO and conductivity probes (Model 55 DO probe and Model 30 conductivity probe, YSI Inc., Yellow Springs, Ohio, USA).

Benthic macroinvertebrates

Invertebrate samples were collected in March, April, and October 2002, and January and March 2003. On each sampling date, mean values for benthic abundance and composition were derived from 5 samples collected within the reference and mine-influenced reaches (i.e., n = 5/reach). Over the course of the experiment, samples were taken from sites broadly distributed within each reach. Location of the first sample within each reach was randomly determined for each sampling date and 4 additional samples were then collected at ~5-m intervals downstream. Initial sample locations were never identical among sampling dates. Invertebrates were collected using a stovepipe method, where a sampling pipe (diameter = 14.5 cm) was pressed into the substrate and all CPOM hand collected. Water in the pipe was collected with a hand pump and/or large turkey baster and filtered through a 250-μm sieve. All material in the sieve was preserved in ethanol and transported to the laboratory. Invertebrates were later separated from the sample, sorted, and identified to genus, except for Diptera, which were identified to family, and oligochaetes, which were identified to class. We calculated %EPT ((Ephemeroptera + Plecoptera + Trichoptera/total abundance) * 100) as a bioassessment index (Resh and Jackson 1993) for samples from each reach and collection date. All specimens were assigned to functional feeding groups (sensu Cummins 1973) using keys of Merritt and Cummins (1996). For each sampling date, average values for dependent variables were determined from the 5 samples collected within a given reach. Species richness, invertebrate density, shredder density, and %EPT were compared between reaches with a Mann–Whitney Rank Sum Test using the individual means derived from the 5 sampling dates (i.e., n = 5/reach).

Leaf breakdown

Three sites (LB1–LB3, Fig. 1) with differing As concentrations were chosen for leaf litter breakdown assays. To provide broad assessment of the physical–chemical characteristics of the 3 reaches, water-monitoring sites were grouped for LB1 (sites 1–3), LB2 (sites 4–5), and LB3 (sites 6–8) based on patterns of groundwater input (MES, unpublished data) and the distribution of leaf packs along the stream. Arsenic concentrations were compared among breakdown sites using a 1-way analysis of variance (ANOVA) with breakdown site as the main effect. Differences among sites were assessed with the Student–Newman–Keuls (SNK) Multiple Comparison Test (MCT) following Day and Quinn (1989).

Leaf breakdown assays began on 30 October 2002 using red maple and white oak leaves. Twenty-four (12/species) 10-g leaf packs (1-cm mesh) were placed at LB1, LB2, and LB3. Leaf packs were secured with a 15-cm gutter nail hammered into the substrate. After deployment, 3 red maple and 3 white oak leaf packs were returned to the lab and processed to assess handling loss. Leaf packs (n = 3/species) were collected every 3 (red maple) or 5 (white oak) wk over the course of 102 and 144 d, respectively.

Sediments and invertebrates were rinsed from collected leaf packs. Invertebrates were later identified following techniques similar to those used for benthic samples with specific focus on quantifying shredder abundance. Sediments and remaining leaf material were separated and placed into a drying oven at 50°C for 48 h and then weighed to obtain dry mass. Leaf material

was ashed (550°C for 1 h) to quantify organic matter as ash-free dry mass (AFDM).

Mean shredder density was compared among breakdown sites with a 1-way ANOVA, (site = main effect) using mean shredder abundance from each site over the 4 sample dates (i.e., n = 12/site). Differences among sites were assessed with the SNK MCT.

The natural log of %AFDM regressed against time was used to obtain a breakdown rate (k) calculated using an exponential decay model (Petersen and Cummins 1974, Benfield 1996). Data were analyzed using the SAS (version 7, SAS Institute, Cary, North Carolina, USA) General Linear Model procedure. Because burial of leaf packs may reduce leaf breakdown rate, breakdown rates were compared using sediment mass as a covariate to account for its influence and address how loss of AFDM varied among sites. Data were analyzed using a 2-way ANOVA with site (3 levels) and time (4 levels) as main factors and sediment mass as a covariate. Slopes (AFDM vs time) were then compared to determine differences in breakdown rates among sites.

Microbial respiration and As concentration: influence of acute exposure

A toxicity experiment was done to measure the influence of short-term (i.e., acute) exposure to elevated As concentration on microbial respiration of unexposed (i.e., upstream reference reach) leaf biofilms. Red maple and white oak leaf packs were placed at Site 1 on 25 February 2003 where they were exposed to instream microbial processing for 3 to 5 wk. Using upstream water as a diluent and control concentration, $NaH_3AsO_4^{-3}$ was used to create 4 concentrations (0.01, 0.1, 1, and 10 mg As/L) representative of the As gradient observed along the length of the study reach. Five treatments were established (control and 4 levels of As addition), each with 4 replicates. Leaf packs were collected after 21 (red maple) and 35 (white oak) d to allow for biofilm accumulation and to address comparable stages of breakdown (Webster and Benfield 1986). Two interveinal leaf disks (diameter = 1.6 cm) were cut from randomly selected leaves and immediately placed into 30 mL Erlenmeyer flasks fitted with gastight caps. Four flasks with leaf disks were randomly assigned to each treatment level, filled with the appropriate solution, and carefully capped to avoid introducing bubbles. Leaf biofilm respiration was then measured as O_2 uptake over 9-h periods during which flasks were stored in the dark at stream temperature. DO concentrations were determined using Winkler titrations following Hauer and Hill (1996). Initial concentration was determined as the mean (n = 4) of upstream diluent water samples. Following the assays, leaf disks were dried and ashed to determine AFDM, and respiration rates were reported as $\mu g\ O_2\ mg\ AFDM^{-1}\ h^{-1}$. Arsenic concentration and species identity were used as main effects in a 2-way ANOVA to assess how leaf respiration responded to short-term exposure to As. Tukey's Honest Significant Difference Test (Day and Quinn 1989) was used to identify significant differences among factor levels.

Microbial respiration and As concentration: influence of chronic exposure

In April 2004, chestnut oak leaves were collected separately from numerous locations throughout the upstream and downstream reaches to assess the influence of chronic exposure to elevated As concentrations. Simultaneously, 4 L of filtered (Whatman GF/F) water were collected from sites 1 (upstream reference reach) and 8 (downstream mine-influenced reach) for use in the respiration assays. Leaves were returned to the laboratory and cleaned of sediment by light agitation in stream water. Disks (1.6-cm diameter) cut either from upstream or downstream leaves were used to create 20 flasks containing 2 leaf disks each, and flasks were separated into 2 groups based upon leaf disk origin (i.e., 10 upstream flasks and 10 downstream flasks). Five of the flasks from each group were filled with water from the upstream site, whereas the remaining 5 received water collected from the downstream site. Flasks were carefully capped and kept in the dark at stream temperature for ~9 h. Initial DO concentrations were determined separately for upstream and downstream water sources as the mean of 4 samples. Respiration rates were reported as $\mu g\ O_2\ mg\ AFDM^{-1}\ h^{-1}$ as determined by Winkler titration. Results from this chronic exposure assay were analyzed with a 2-way ANOVA using leaf source (upstream, downstream) and water source (upstream, downstream) as main effects.

TABLE 1. Chemical characterization of the Brinton Mine Creek. Sites 1 to 3 are reference sites; sites 4 to 9 are mine-influenced sites. Dissolved oxygen (DO) and conductivity (Cond) are the mean (± SE) of 6 samples (1/mo), whereas all other chemical variables are the mean (± SE) of 18 samples (3/mo). DOC = dissolved organic C.

Site	As (μg/L)	DOC (mg/L)	NH_4^+-N (μg/L)	NO_3^--N (μg/L)	SO_4^{-2} (mg/L)	DO (% sat.)	Cond (μS/cm)
1	3 ± 1	4.7 ± 0.5	5 ± 0.6	884 ± 132	4.6 ± 0.5	51.7 ± 8.2	53.1 ± 9.3
2	7 ± 2	5.1 ± 0.5	5 ± 0.9	858 ± 124	4.9 ± 0.5	51.4 ± 8.1	56.7 ± 4.9
3	8 ± 1	4.9 ± 0.4	5 ± 0.5	775 ± 152	4.5 ± 0.6	52.3 ± 8.2	50.8 ± 3.5
4	26 ± 5	4.9 ± 0.3	5 ± 0.8	409 ± 87	4.8 ± 0.6	59.9 ± 8.6	42.9 ± 4.2
5	158 ± 41	4.3 ± 0.5	4 ± 0.4	506 ± 99	10.3 ± 1.1	54.6 ± 7.7	58.4 ± 7.7
6	558 ± 95	3.2 ± 0.3	6 ± 0.9	478 ± 84	16.2 ± 1.9	56.6 ± 7.4	69.2 ± 9.3
7	391 ± 59	3.9 ± 0.5	5 ± 0.6	454 ± 81	15.5 ± 1.9	55.5 ± 8.3	67.0 ± 10.0
8	536 ± 89	2.8 ± 0.3	6 ± 1.0	415 ± 74	14.4 ± 1.9	57.1 ± 7.9	67.0 ± 9.9
9	1335 ± 205	2.8 ± 0.3	9 ± 1.8	430 ± 66	17.5 ± 1.9	56.7 ± 7.8	71.3 ± 9.3

Differences among factor levels were assessed following ANOVA using Fisher's Least Significant Difference Test (LSD, Sokal and Rohlf 1981).

Results

Chemical characterization

Mean NO_3-N concentrations generally decreased with distance downstream, from an average of 884 μg/L at Site 1 to 430 μg/L at Site 9 (Table 1). Concentrations of NH_4-N concentrations were much lower (~5 μg/L) and did not vary spatially. DOC was higher in the reference reach (4.7–5.1 mg/L) compared to the mine-influenced reach where concentrations declined with distance from 4.9 to 2.8 mg/L. Sulfate concentrations downstream were as much as 4 times greater than those observed upstream (17.5 vs 4.5 mg/L) and concentrations increased with distance downstream adjacent to the mine. Conductivity also increased with distance in the mine-influenced reach, whereas there was no evident longitudinal gradient within the reference reach. DO concentrations ranged from 6 to 10 mg/L and averaged ~50% saturation.

Average As concentrations increased from 3 to 1335 μg/L along the length of the study site (Table 1). Concentrations of As and SO_4^{-2} across all study dates were highly correlated along the study reach (Pearson Product–Moment Correlation, $r = 0.83$, $p = 0.03$). Arsenic occurring as AsO_2^{-2} was <5% of total As measured for 95% of the collected samples (data not shown). Arsenite was below detection limit (<2.5 μg/L) in ≥½ of those measurements.

Benthic macroinvertebrates

Very low densities of benthic macroinvertebrates characterized the mine-influenced study reach (Fig. 2). Out of a total of 25 downstream samples, only 2 had >10 total invertebrates and 13 of 25 samples had no organisms. Average invertebrate density in the downstream reach was more than an order of magnitude lower than the upstream reach where mean density approached 8000 individuals/m^2. The benthic community of the reference reach contained several functional groups including shredders, collector-gatherers, collector-filterers, and predators (data not shown). Individuals collected in the reference reach were mostly (42.4%) shredders (e.g., *Amphinemura*, *Leuctra*, *Lepidostoma*), with the remainder nearly equally split between collector-gatherers (e.g., *Leptophlebia*) and predators (e.g., *Cordulegaster*). The few individuals collected from the downstream reach were similar genera, either shredders or predators, with shredders accounting for just 14% of total invertebrate numbers. Species richness and %EPT abundance in the reference reach were ≥10 times greater compared to the mine-influenced reach, and the difference was significant (Fig. 2; $p < 0.001$).

Arsenic concentration at leaf breakdown sites

Arsenic concentrations differed significantly (1-way ANOVA, SNK, $p < 0.001$) among the 3

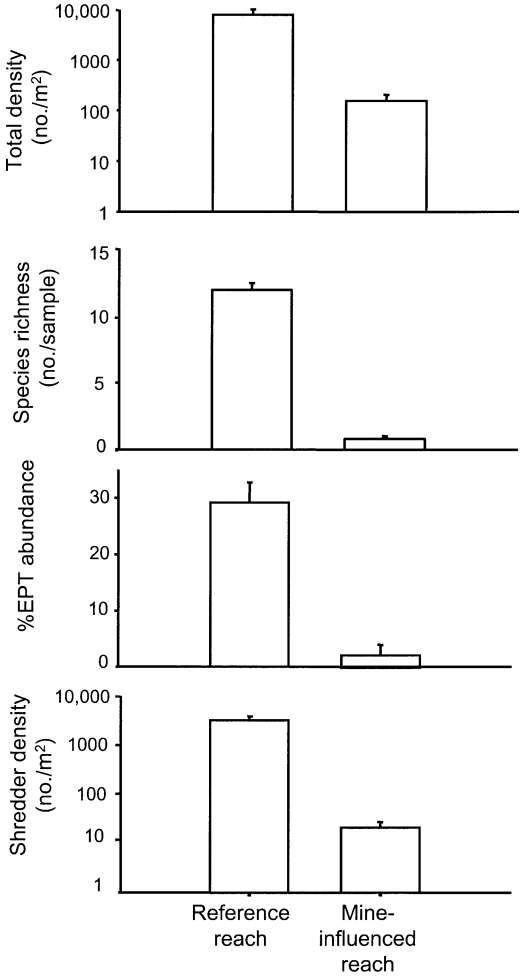

FIGURE 2. Invertebrate communities in reference (upstream) and mine-influenced reaches. Bars are means (+ SE) of 25 samples. % EPT = % Ephemeroptera, Plecoptera, and Trichoptera. All differences between reference and mine-influenced reaches were statistically significant ($p \leq 0.05$).

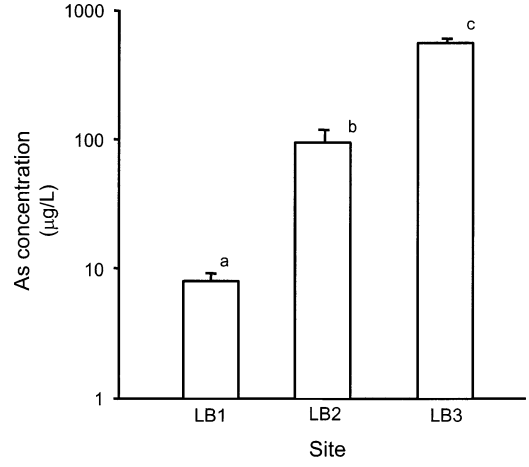

FIGURE 3. Mean (+ SE) total As concentrations at leaf breakdown (LB) sites (LB sites as in Fig. 1). Bars with similar letters were not statistically different ($p \leq 0.05$).

subreaches used for leaf breakdown assays (Fig. 3). Mean As concentration at LB1 (8 ± 1 μg/L) was 10-fold lower than at LB2 (96 ± 21 μg/L) and As concentration climbed to 561 ± 37 μg/L at LB 3.

Leaf breakdown rates

Breakdown rates differed among sites (2-way ANOVA, site main effect, $p < 0.001$; Table 2). Interaction between site and sediment mass was not a good predictor of breakdown rate for red maple ($p = 0.642$) or white oak ($p = 0.374$). Breakdown rate for both species in the reference reach (i.e., LB1) was 2 to 3 times greater ($p < 0.05$) than rates at the other sites (Fig. 4). Rates were statistically similar within the mine-influenced reach ($p > 0.05$).

Sediment had accumulated in leaf packs collected from all sites by the end of the breakdown assay but the timing and extent of accumulation varied among sites (Fig. 5). Dry mass

TABLE 2. Influence of site, time, and sedimentation on leaf pack breakdown rates (*k*s) for white oak and red maple leaf packs. Results from 2-way analysis of variance with site and time as random main effects and sedimentation assessed as a covariate.

Source	DF	MS	*F*	*p*
Red maple				
Site	2	0.541	31.17	<0.0001
Time	1	1.675	98.69	<0.0001
Time*site	3	0.681	39.21	<0.0001
Sediment*site	3	0.009	0.56	0.6421
White oak				
Site	2	0.121	24.18	<0.0001
Time	1	0.827	151.36	<0.0001
Time*site	3	0.364	72.80	<0.0001
Sediment*site	3	0.011	1.07	0.3735

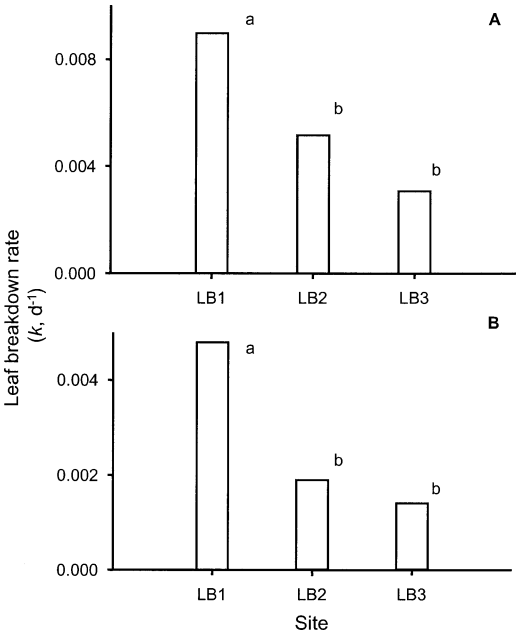

FIGURE 4. Leaf breakdown rates (k) for red maple (A) and white oak (B) leaf packs. Leaf breakdown (LB) sites as in Fig. 1. Within a panel, bars with similar letters were not statistically different ($p \leq 0.05$).

of sediment in leaf packs collected at LB1 and LB2 averaged 8.5 ± 4.3 and 9.3 ± 7.1 g/pack, respectively, and did not differ significantly (1-way ANOVA, SNK, $p > 0.05$). In contrast, average sediment mass at LB3 (201.55 ± 43.6 g/pack) was significantly ($p < 0.0001$) greater than at either of the upstream sites. In addition, sediment was present in all leaf packs collected from LB3, whereas leaf packs at LB1 and LB2 were free of sediments for the first 47 to 70 d (Fig. 5).

Sediment accumulation was not tightly related to leaf breakdown (Fig. 6). The 2 lowest breakdown rates were recorded for white oak at LB2 and LB3 ($k = 0.0019$ and 0.0014, respectively) despite the fact that sediment accumulation at LB2 (14.0 ± 13.9 g/pack) was much lower than at LB3 (246.8 ± 71 g/pack, Fig. 5). Furthermore, breakdown rate for white oak at LB1 ($k = 0.0045$) was more than twice that at LB2 despite more sediment per leaf pack at LB1 (16.3 ± 8.3 g/pack). Average sediment mass was not related to breakdown rate (Pearson Product–Moment Correlation, $p > 0.05$) for either red maple or white oak, but statistical power was low given only 3 observations per species.

Shredder species found in leaf packs were taxonomically similar to those found in benthic samples (e.g., *Tipula*, *Lepidostoma*, *and Leuctra*). White oak and red maple leaf packs at LB1 were inhabited by 20 times the shredder densities found at LB2 and LB3; at LB1 shredder densities ranged from 1.5 to 24.2 individuals/leaf pack, whereas downstream densities were <1 individual/leaf pack (Fig. 7). Numbers of shredders were statistically related to breakdown rates for red maple ($r = 0.98$, $p < 0.05$) and white oak ($r = 0.99$, $p < 0.05$), although correlation coefficients were generated from very small sample sizes (i.e., $n = 3$).

Leaf biofilm respiration: responses to As exposure

Acute.—Biofilm respiration on red maple and white oak leaves did not vary significantly with As concentration (2-way ANOVA, As concentration main effect, $p > 0.05$; Fig. 8). Respiratory response to As exposure did not differ between species (species × As factor interaction, $p = 0.96$). There was no indication of suppressed microbial activity with increasing As concentrations for white oak (Fig. 8). Instead, white oak respiration in the control (0.15 ± 0.04 μg O_2 mg $AFDM^{-1}$ h^{-1}, mean ± SE) was identical to that in the 0.01 mg/L treatment and rates in the 0.1, 1.0, and 10 mg/L treatments were 1.5 to 2 times greater (Fig. 8). Average red maple respiration was virtually unchanged by short-term exposure to As except in the 1.0 mg/L treatment where the average rate was 1.3 times higher than in control flasks. Biofilm respiration on red maple leaves (0.38 ± 0.04 μg O_2 mg $AFDM^{-1}$ h^{-1}) was significantly higher (species main effect, $p = 0.005$) than on white oak disks (0.22 ± 0.03 μg O_2 mg $AFDM^{-1}$ h^{-1}) across all levels of As exposure.

Chronic.—Microbial respiration rates on chestnut oak leaves differed with leaf source (2-way ANOVA, leaf source main effect, $p < 0.0001$; Fig. 9). Mean respiration rate for leaves collected in the reference reach (0.37 ± 0.01 μg O_2 mg $AFDM^{-1}$ h^{-1}) was 28% higher than respiration recorded for leaf biofilms from the mine-influenced reach (0.29 ± 0.01 μg O_2 mg $AFDM^{-1}$ h^{-1}). Respiration rates did not differ

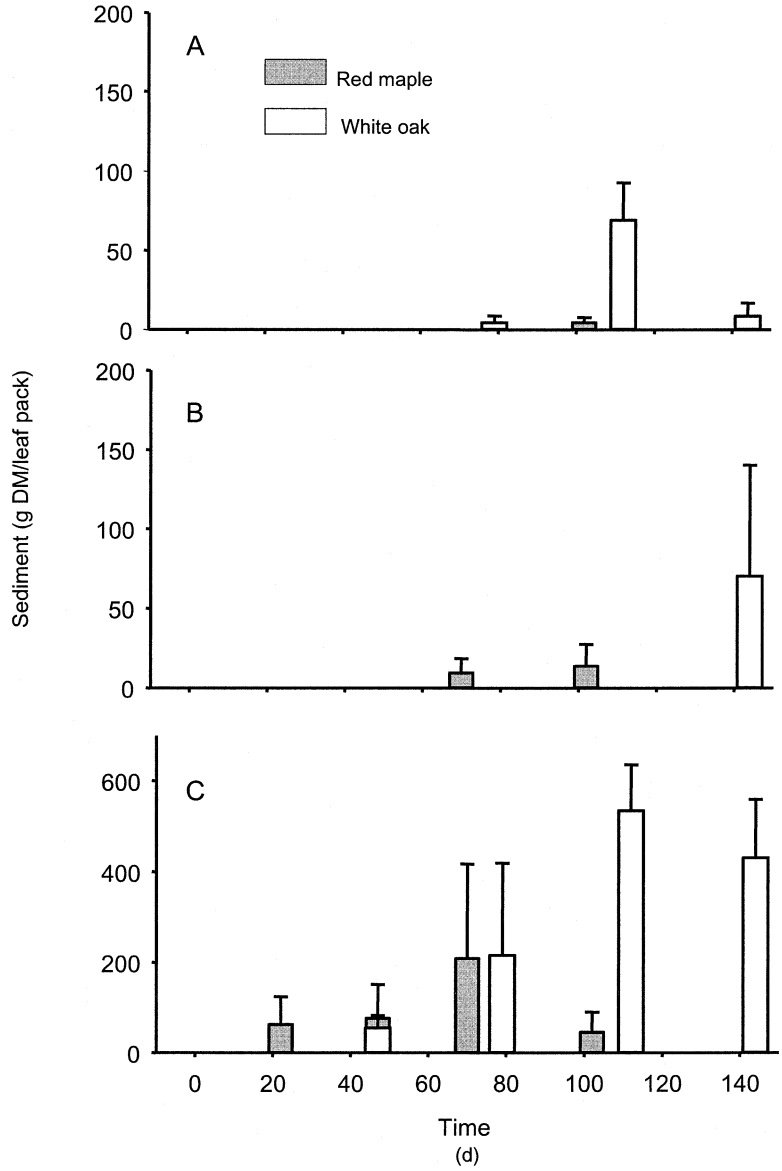

FIGURE 5. Mean (+ SE) sediment accumulation in red maple and white oak leaf packs during breakdown assays for (A) LB1 (upstream reference), (B) LB2 (upper portion of mine-influenced reach), and (C) LB3 (downstream portion of mine-influenced reach). Leaf packs for both species were collected on 5 occasions. Superimposed bars on day 47 in panel C illustrate that sediments were collected in leaf packs from both species on that date. DM = dry mass.

significantly between flasks filled with upstream or downstream water (water source main effect, $p = 0.61$; Fig. 9), and this response did not depend on leaf origin (i.e., water source $\times$ leaf source interaction, $p = 0.064$). Lower mass-specific respiration rates for leaf disks from the mine-influenced reach were, in part, a result of the fact that AFDM of leaf disks from that reach (28.6 $\pm$ 0.001 mg AFDM) was significantly greater (t-test, $p = 0.05$) than for the reference leaf disks (25.3 $\pm$ 0.001 mg AFDM).

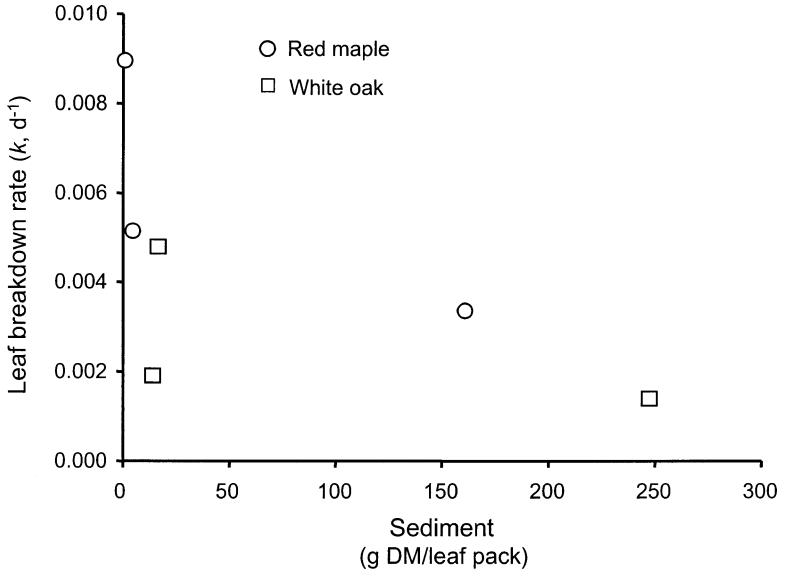

FIGURE 6. Breakdown (k) rate vs leaf pack sediment content. Data are ks derived from leaf pack assays and mean sediment in leaf packs (n = 12) used in each regression for red maple and white oak.

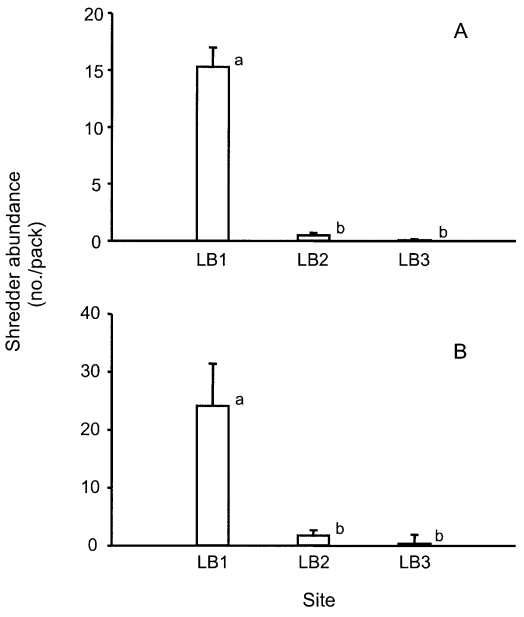

FIGURE 7. Mean (+ SE) shredder abundance for red maple (A) and white oak (B) leaf packs. Leaf breakdown (LB) sites as in Fig. 1. Within a panel, bars with similar letters were not significantly different ($p > 0.05$).

Discussion

Leaf breakdown

Leaf breakdown rates within the study stream were significantly lower in the downstream reach exposed to elevated As concentrations in comparison to the upstream reference reach. This observation was true for both red maple and white oak leaves despite their very different propensities for breakdown. For example, red maple breaks down at intermediate rates relative to other leaf species found in streams (Webster and Benfield 1986). Breakdown rates in the upstream reference reach were typical for this species. However, rates in the mine-influenced reach were more like those reported for leaf species considered to be in the slow-breakdown category (Webster and Benfield 1986). White oak breakdown rates in the reference reach were also similar to those reported by Webster and Benfield (1986). Conversely, breakdown rates in the mine-influenced reach were at the lower end of the range reported elsewhere for this leaf species (Webster and Benfield 1986). The decrease in breakdown rates was evident for leaf packs placed in the more upstream area of the mine-influenced reach (i.e., LB2), even though mean As concentration observed at that site was only 2/3 that of the USEPA chronic

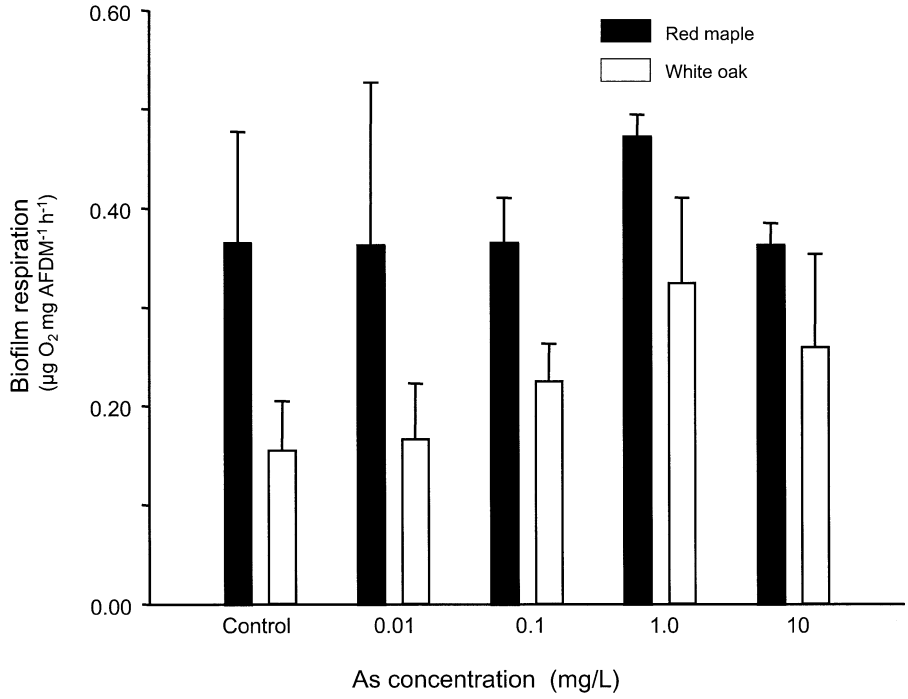

FIGURE 8. Mean biofilm respiration rates (+ SE, $n = 5$) on red maple and white oak disks exposed to upstream ambient (i.e., control) or experimentally enhanced As concentrations for ~9 h. For a given leaf species, respiration rates did not differ significantly ($p > 0.05$) among As concentrations.

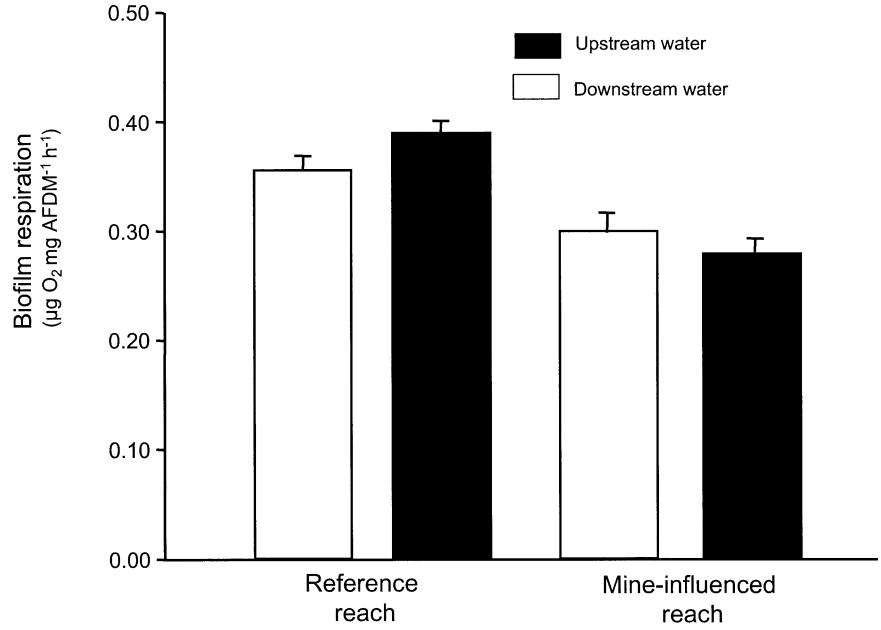

FIGURE 9. Mean biofilm (+ SE, $n = 4$) respiration rates on leaves collected from either the upstream reference or downstream mine-influenced reaches exposed to water from upstream or downstream.

level criterion value of 150 μg/L (USEPA 2002). This concentration was associated with significantly reduced organic matter processing as indicated by slower rates of leaf breakdown.

Sedimentation from land development or disturbance of riparian zones can slow the rate of detritus processing by burying leaf packs (Webster and Waide 1982, Sponseller and Benfield 2001). In our investigation, mine waste piles were a local source of sediment to the stream that may result in leaf pack burial and contributed to decreased leaf breakdown rates. However, use of sediment mass as a covariate demonstrated that site was a strong determinant of breakdown despite potential sediment influence. Direct assessment showed that sedimentation rates in LB1 and LB2 were similar, whereas breakdown rates were significantly lower at LB2. Furthermore, breakdown rates within the mine-influenced reach (i.e., LB2 and LB3) did not differ despite large (i.e., more than 10-fold) differences in the sediment mass found in leaf packs.

Stream biota responsible for leaf breakdown

The initial stages of leaf breakdown involve colonization and conditioning of leaf litter by fungal and bacterial assemblages (Peterson and Cummins 1974, Findlay et al. 2001). Growth of these microbial populations conditions leaf litter and renders it available as food for shredding macroinvertebrates that significantly enhance breakdown. Toxicants may influence leaf breakdown in a variety of ways. For example, Cu has been identified as a cause of slower leaf breakdown rates through toxicity to microorganisms (Leland and Carter 1985, Schultheis and Hendricks 1999). Similarly, Newman et al. (1987) and Newman and Perry (1989) found that high doses of Cl and Cl + NH_3 reduced litter breakdown rates by initially reducing microbial conditioning. In addition, Mulholland et al. (1987) found that lower rates of leaf breakdown in acidic streams were a result of reduced rates of microbial activity. However, although Newman and Perry (1989) found that microbial processing was decreased by exposure to toxicants, they also found that the primary cause for reduced litter breakdown was a reduction in the number of leaf-shredding amphipods.

In our study, elevated As concentrations appeared to have a significant effect on the invertebrate community with a strong influence on shredder abundance. Red maple and white oak leaf packs within the mine-influenced reach had 10 times fewer shredders than were present in the reference reach. The close association between increased As concentrations and drastic reduction in invertebrate abundance suggests that As may be a toxicant to these animals, resulting in an associated decline in the number of individuals belonging to the shredder functional group. Further, the close relation between shredder abundance and leaf breakdown rate suggests that As contamination may be altering rates of organic matter processing by reducing or eliminating macroinvertebrate species that are central to the later stages of leaf breakdown (e.g., Wallace et al. 1982). Niyogi et al. (2001) reached a similar conclusion for elevated Zn concentrations when addressing the influence of mine drainage on litter breakdown in Rocky Mountain streams.

We found that As contamination did not alter breakdown by reducing rates of microbial processing. Comparison of our microbial respiration rates with others using similar methods of assessment suggests that assayed leaf discs supported active microflora both in reference and mine-influenced reaches. Average respiration rates for chestnut oak in the mine-influenced reach were nearly 4-fold greater than Fuss and Smock (1996) reported for mixed species from a coastal plain stream (0.29 vs 0.08 μg O_2 mg $AFDM^{-1}$ h^{-1}). As part of a study addressing leaf decomposition in a forested Appalachian stream, Tank et al. (1993) measured a maximum respiration rate for birch leaves of 0.14 μg O_2 mg $AFDM^{-1}$ h^{-1}, ~½ the rate we observed for leaves from the mine-influenced reach. Average rates for both upstream reference leaves and those collected in the downstream mine-influenced reach were bracketed within the 0.26 to 0.45 μg O_2 mg $AFDM^{-1}$ h^{-1} measured by Stelzer et al. (2003) for sugar maple leaves in Norris Brook, New Hampshire. Furthermore, respiration rates in our study system were lower than rates for aspen, birch, and dogwood leaves (0.75–1.5 μg O_2 mg $AFDM^{-1}$ h^{-1}) recorded under conditions of nutrient enrichment (Royer and Minshall 2001).

Short-term exposure to As concentrations as high as 10 mg/L failed to depress biofilm respiration rates on leaves collected from the upstream reference reach. Biofilms typically in-

clude extracellular polysaccharide components that may provide a degree of protection during acute exposure to toxicants (Costerton et al. 1995). Mean respiration rates were slightly higher in 5 of 8 treatments with elevated As concentrations. These results are consistent with the finding that short-term exposure to elevated As can stimulate microbial activity (Speir et al. 1999), a response that may be related to the chemical similarity between AsO_4^{-3} and PO_4^{-2} in freshwater ecosystems. Chronic exposure to elevated As did lower mass-specific respiration rates on chestnut oak leaves. However, the decline in mean respiration rate for leaves from the mine-influenced reach was relatively small (i.e., 28% reduction for leaves collected from downstream sites) and, in part, related to the significantly higher amounts of organic matter present per unit area for downstream leaves. Respiration rates on an areal basis for leaf disks from the downstream site were only 15% lower than the mean value for the upstream sites (data not shown). Thus, As influence on microbial processing of leaf material may be both direct by altering microbial physiology and indirect by altering the mass of organic matter per unit area for leaves and, presumably, the total surface area available for colonization by microflora. However, we have no direct assessment of the extent or composition of the extant biofilms on leaf litter in either the reference or mine-influenced reaches and, thus, are not able to address activity per unit biomass.

Microbial biofilms are natural metal-immobilizing matrices (Ferris et al. 1989), and tolerance of some bacteria to As has been well established (Oremland and Stolz 2003). Brunskill et al. (1980) found no inhibition of microbial degradation of organic matter in lake microcosms treated with As. Furthermore, Leblanc et al. (1996) found microbial uptake of As to be an important control on As concentrations in acidic mine waters. Several studies (Newman et al. 1988, Ahmann et al. 1994, Stolz and Oremland 1999, Langner and Inskeep 2000) have shown that many resistant bacterial strains reduce As^{+5} to As^{+3} while simultaneously experiencing cell growth, suggesting a link between AsO_4^{-3} reduction and cellular energy generation. There is an established link between As tolerance and bacteria, but less is known about how these links cascade into ecosystem effects such as geochemical cycling of As in natural waters. Our work demonstrates that leaf biofilms in this lotic ecosystem are resistant to As even at concentrations nearly 70 times greater than those set as chronic toxic levels for metazoans (USEPA 2002).

Ecosystem implications

Arsenic in the stream water of this catchment appears to influence ecosystem process by eliminating macroinvertebrates, including shredders and their important role as detritus processors. Anthropogenic loading of different chemical pollutants may influence leaf breakdown rates by reducing microbial colonization and activity, reducing shredders, or a combination of both. Effects of toxic chemicals on benthic communities are complex and require extensive observational and experimental studies to develop causation in ecological assessments (Clements et al. 2002, Courtney and Clements 2002). Gessner and Chauvet (2002) have argued that leaf breakdown is a good parameter for assessing the functional integrity of streams at the ecosystem level. In our study, As appears to have impaired stream function through reduced rates of organic matter processing, not by influencing detrital primary consumers but through foodweb alterations at higher trophic levels. Instead of reducing the development and activity of microbial flora, As appears to eliminate macroinvertebrate shredders that consume these biofilms and play a key role in the breakdown of allochthonous inputs in deciduous forested headwater streams.

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