Summer 2022

Decomposition and Macroinvertebrate Shredder Colonization of Autumnal Shed Sycamore Leaves in Mining-Contaminated Streams

Leslie Marie Hatch
Missouri State University, Leslie614@live.missouristate.edu

DECOMPOSITION AND MACROINVERTEBRATE SHREDDER COLONIZATION OF AUTUMNAL SHED SYCAMORE LEAVES IN MINING-CONTAMINATED STREAMS

A Master's Thesis

Presented to

The Graduate College of

Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science, Biology

By

Leslie Marie Hatch

August 2022

DECOMPOSITION AND MACROINVERTEBRATE SHREDDER COLONIZATION OF AUTUMNAL SHED SYCAMORE LEAVES IN MINING-CONTAMINATED STREAMS

Biology

Missouri State University, August 2022

Master of Science

Leslie Marie Hatch

ABSTRACT

Leaf decomposition in streams is an important ecological function facilitated by bacteria, fungi, and macroinvertebrates. Metal contamination can decrease leaf decomposition rates and reduce macroinvertebrate abundance and diversity. In the current study, I focused on mining-contamination in well-buffered streams in two different Missouri mining districts with varying extents of mining contamination, Big River and Pierson Creek. I measured decomposition rates of sycamore leaves, and abundance and diversity of macroinvertebrate shredders in leaf pack experiments with a full-factorial design to determine the effects of metal contamination of leaves and stream substrates. Comparisons were made between leaf packs upstream and downstream of mining contamination sources, as well as between the origin location of leaves. In Big River, leaf decomposition rates were higher downstream of mining contamination than leaves upstream. Overall, mining contaminated leaves had higher decomposition rates than non-contaminated leaves, regardless of reach placement over the 123-day winter experiment. In Big River, macroinvertebrate shredder abundance was higher and diversity of macroinvertebrate shredders was lower upstream of mining contamination. Opposite decomposition and macroinvertebrate trends were observed in Pierson Creek over the 50-day spring experiment. Decomposition rates were higher in leaves upstream of mining contamination than leaves downstream. In Pierson Creek, macroinvertebrate shredder abundance was higher downstream of mining contamination and macroinvertebrate shredder diversity was lower downstream of mining contamination. Contaminated leaves had higher decomposition rates than non-contaminated leaves in Big River, regardless of reach placement, while decomposition rates were similar in Pierson Creek. This could be due to differences in the magnitude of mining contamination. Leaf type did not influence macroinvertebrate shredder abundance or diversity for either stream, but reach location did. This could be due to physical and chemical stream characteristics. Future studies on mining contamination effects on leaf quality and substrate characteristics and subsequent impacts on decomposition rates could lead to a better understanding of specific ecosystem interactions and inform future management decisions.

KEYWORDS: decomposition, macroinvertebrates, mining contamination, sycamore, shredders, leaves, streams, leaf pack, Big River, Pierson Creek

DECOMPOSITION AND MACROINVERTEBRATE SHREDDER COLONIZATION OF AUTUMNAL SHED SYCAMORE LEAVES IN MINING-CONTAMINATED STREAMS

By

Leslie Marie Hatch

A Master's Thesis
Submitted to the Graduate College
Of Missouri State University
In Partial Fulfillment of the Requirements
For the Degree of Master of Science, Biology

August 2022

Approved:

La Toya Kissoon-Charles, Ph.D., Thesis Committee Chair

Debra Finn, Ph.D., Committee Member

Robert Pavlowsky, Ph.D., Committee Member

Julie Masterson, Ph.D., Dean of the Graduate College

ACKNOWLEDGEMENTS

In the words of Gabriel Garcia Marquez, “Damn it, how will I ever get out of this labyrinth?” Research and academia can often feel like a labyrinth one must endlessly navigate but is made possible by those who help you along the way. I would first like to thank my advisor Dr. La Toya Kissoon-Charles for her constant support, guidance, and much needed encouragement. I would also like to thank my committee members, Dr. Debra Finn and Dr. Robert Pavlowsky for their guidance, support, and for always encouraging me to see the bigger picture, as well as Dr. Carri LeRoy for her assistance. Thank you to my family for their support, even when you were not exactly sure what I was doing. And a special thanks to Alex Beezel, I am forever grateful to have met you through graduate school and to have your unconditional friendship and support.

My research was funded by the Graduate College and Biology Department at Missouri State University and the Webster Grove Nature Study Society. I would like to thank the following people for their assistance during my graduate research; MDNR, private landowners, Indigo Tran, Hannah Whaley, Brendan Ryan, Brianne Edwards, Liz Carrol, Liz Harris, Daphne Miles, and Abby Giering.

I dedicate this thesis to my father, David Hatch. Sorry I did not go to West Point or the Naval Academy, but what a superb and far superior labyrinth to be trapped in.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

OVERVIEW

Bacterial conditioning, aquatic macroinvertebrate consumption, leaching, and physical abrasion are interactions and processes that facilitate the decomposition of leaf litter in streams. This thesis research project reports partial findings from a larger collaborative research project conducted with fellow Biology MS student Indigo Tran to study multiple components of the leaf decomposition process in mining-contaminated streams. My thesis focused on leaf decomposition rates from coarse mesh leaf packs that allowed for aquatic macroinvertebrate shredder colonization. I additionally reported on the abundance and diversity of macroinvertebrate shredders collected from the leaf packs. Tran Master's thesis will report findings of leaf decomposition from fine mesh leaf packs that restricted macroinvertebrate colonization (personal communication). Results of microbial respiration and metal concentrations of leaves in both fine and coarse mesh leaf packs were topics focused on in Indigo Tran's thesis, while I focused on macroinvertebrate shredder colonization. The development of ideas and the full experimental design and data collection were a team effort. Soon we intend to combine results and submit a manuscript for publication to the peer-reviewed journal *Water, Air, & Soil Pollution*.

INTRODUCTION

The decomposition of leaf litter in streams is facilitated by the interactions of heterotrophic microbes and aquatic macroinvertebrates converting leaf litter into animal biomass (Bastian et al., 2007; Danger et al., 2012; Martínez et al., 2015). Leaching and physical abrasion are abiotic processes that also contribute to leaf decomposition, with leaching typically occurring in the first 10 days of decomposition (Graça, 2001; Allan & Castillo, 2009). Water temperature can also influence leaf decomposition by slowing down the leaching process in cold water conditions (Chergui & Pattee, 1990). Seasonality causes a yearly cycle of increasing and decreasing water temperatures in temperate regions. The first macroinvertebrates to colonize fallen leaf litter in the earliest days of leaf submergence are typically shredders, since they primarily consume leaves and other coarse particulate organic matter (CPOM) (Whitledge & Rabeni, 1997; Allan & Castillo, 2009). Shredders prefer leaves that have been softened and conditioned by bacteria and fungi, increasing the nutritional quality of the leaves and making them easier to conusume (Cummins & Klug, 1979). Shredders tear and break apart leaves and larger detritus as they feed on them and produce fine particulate organic matter (FPOM), which other macroinvertebrates can later consume (Vannote et al., 1980; Gulis & Suberkropp, 2003; Suhaila et al., 2016). Leaf consumption increases the overall rate of leaf decomposition and the bioavailability of nutrients and carbon in the stream ecosystem (Graça & Canhoto, 2006). Macroinvertebrate shredder density tends to increase decomposition rates (Jonsson et al., 2001; McKie et al., 2008; Patrick, 2013). Crayfish, another common benthic dwelling organism, can occupy many niches in an aquatic system and are known to process just as much, if not greater

amounts, of riparian litter as other macroinvertebrate shredders (Whitledge & Rabeni, 1997; Coughlan et al., 2010).

Metal contamination tends to decrease decomposition rates, at different degrees depending on the metal contaminant and other environmental factors such as pH, conductivity, and leaf taxon (Ferreira et al., 2016). Previous leaf decomposition experiments in metal-contaminated streams reported decomposition rates of leaf litter were lower in contaminated sites with higher metal concentrations compared to sites with lower metal concentrations (Bermingham et al., 1996; Graça, 2001; Duarte et al., 2007). As reported in (Bermingham et al., 1996), a reduction in fungal colonizers on leaves downstream of mine contamination. This reduction of fungal colonizers slowed the conditioning of leaves, a precursor to shredder processing of leaves (Barnden and Harding, 2005; Bastian et al., 2007; Danger et al., 2012; Suhaila et al., 2016). Similarly, (Funck et al., 2013) also showed that high concentrations of metal nanoparticles slowed conditioning and shredder feeding on treated leaves, further lowering leaf decomposition rates. The decreased conditioning has adverse effects on subsequent macroinvertebrate leaf colonization and consumption.

Metal contamination in streams decreases the abundance of macroinvertebrate shredders, further decreasing rates of leaf decomposition (Wallace et al., 1996; Carlisle & Clements 2005; Pascoal et al., 2005). In studies examining invertebrate shredder communities, rates of leaf decomposition were lower in mining-contaminated sites without invertebrate shredders as compared to non-contaminated reference sites where shredders were present (Niyogi et al., 2001; Carlisle & Clements, 2005; Niyogi et al., 2013). Like rivers with metal-contamination, rivers contaminated with excess sediment input, agricultural activity, or increased N and P showed patterns of decreased leaf decomposition rates associated with a decrease in shredder abundance

(Sponseller & Benfield, 2001; Pascoal et al., 2005). These studies all have the same conclusion, contamination, in different forms, inhibits macroinvertebrates consumption, which in turn slows leaf decomposition.

Mining contamination from lead (Pb) is a historic problem in southern Missouri. This region has been commonly referred to as "the Old Lead Belt", in reference to its vast history of lead mining, and contains several mines, smelters, and tailings piles. The floodplain and gravel bars in mining-contaminated rivers have vegetation that can accumulate metals from the contaminated sediments. Vegetation in metal contaminated regions accumulate metals such as Pb, Zn, Cd, and As from contaminated soils (André et al., 2006; Ueno et al., 2008; Peralta-Videa et al., 2009). (Palmer & Kucera, 1980) reported Pb accumulation in leaves of American sycamores (*Platanus occidentalis*) near smelters and mines in the Big River Mine Tailings/St. Joes Minerals Corp. region. Sycamore leaves downstream of mining contamination accumulated over ten times the concentration of Pb than sycamore leaves upstream of mining contamination in the Old Lead Belt (Heiman et al., 2022). In addition to Pb, these sycamore leaves also accumulated other metals such as Cd and Zn. Vegetation growing in and along metal contaminated streams can act as secondary sources of metals either from leaching into the water or biological consumption (Shahid et al., 2017; Nugroho et al., 2021). Previous research in the Big River and the Viburnum Trend Mining District of southeast Missouri reported decreased densities of fish and crayfish populations downstream of mining contamination (Allert et al., 2008, 2013). In-situ toxicity tests with caged crayfish in these southeast Missouri mining districts showed lower crayfish survival and biomass at mining sites compared to reference sites (Allert et al., 2009, 2013). Higher metal concentrations of the water, detritus, macroinvertebrates, fish, and crayfish in the mining compared to reference sites affected the crayfish biomass and survival

(Allert et al., 2009, 2013). Despite all this work on animal responses in metal-contaminated streams in southern MO, there is little understanding of effects on important ecosystem processes such as decomposition.

My research intended to fill this gap in literature for the region through investigation of the effects of metal contamination on the rate of leaf decomposition and shredder abundance in streams in 50-day and 123-day leaf pack experiments. The objective of this study was to address the following questions: (1) how does the metal concentration in sycamore leaf litter and stream water and sediment at the reach scale affect aquatic macroinvertebrate shredder abundance; and (2) how does shredder abundance affect sycamore leaf litter decomposition? This leaf pack study focused on riparian sycamore leaf decomposition in two mining contaminated streams, Big River and Pierson Creek, in two different mining districts of the Ozark Highlands ecoregion. These questions assess the impacts of metal contamination on the invertebrate shredder colonization of sycamore leaf litter and the decomposition rate of that leaf litter. I hypothesized that (1) contaminated leaves have lower decomposition rates than non-contaminated leaves, regardless of location in the river (reference v. mining reach); (2) leaves in the mining reaches have lower decomposition rates than leaves in the reference reach, regardless of leaf type (non-contaminated v. contaminated); (3) leaves in the reference reaches have a higher abundance of macroinvertebrate shredders than leaves within the mining reach, regardless of leaf pack content (non-contaminated v. contaminated leaves); and (4) non-contaminated leaves have a higher abundance of macroinvertebrate shredders than contaminated leaves, regardless of river reach (reference v. mining).

METHODS

Experiment A: Big River

Study Sites. The study area for this experiment was comprised of two reaches within the Big River, located in the Old Lead Belt of southeast Missouri (Figure 1; Table 1). Today there are eight National Priority List (NPL) Superfund sites in southeast Missouri because of more than 200 years of mining in the Old Lead Belt (US EPA (Environmental Protection Agency), 2017). Several rivers throughout the region such as the Mississippi River, Little St. Francis River, Meramec River, and Big River have contaminated sediments in the river channel and floodplains (Allert et al., 2009; Besser et al., 2015; Pavlowsky et al., 2017). Big River and other streams in the Old Lead Belt continued to be contaminated even after mining operations in the region stopped due to tailings entering stream channels because of floodplain erosion (Newfields, 2007). I had two reaches in Big River for my study based on accessibility, proximity to mine tailings, and physical and chemical similarities of the reaches such as stream velocity, depth, pH, and conductivity. The reference reach (upstream of tailing piles) was in Washington County, MO and acted as the control site since it is upstream of the mine tailing piles and is not influenced by mining contamination (Figure 1). This reference reach is located near USGS Irondale Gage 07017200 and has been used in previous studies (Pavlowsky et al., 2010; Allert et al., 2013; Stroh et al., 2015). I chose the mining reach (downstream of tailing piles) due to Pb concentrations of the gravel bar sediments exceeding 1,000 ppm, which was 60 times above natural background levels for the region (Pavlowsky et al., 2010). The mining reach was in St. Francois County, MO and is influenced by contamination from seven upstream mine tailing piles

(Figure 1). The reference and mining reaches were used for leaf collection and placement of leaf pack grids.

Experimental Design. I collected *Platanus occidentalis* (American sycamore) leaves from contaminated and non-contaminated gravel bars in the Big River on October 3, 2020 before autumn abscission. These gravel bars were within the mining and reference reaches, adjacent to riffles where I placed leaf pack grids (Figure 2). I collected leaves from multiple trees (diameter at breast height (DBH) 12-126 cm) on the bar head, middle, and tail of each gravel bar. I cut live leaves at the base of the leaf blade (avoiding the leaf stalk) from branches at breast height, no higher than 2 m above the ground, using clean ceramic scissors to prevent metal contamination. I placed the leaves into large plastic bags separated by site to prevent cross-contamination. Leaves from individual trees were combined so that no one leaf pack would receive leaves from a single tree from one region of the gravel bar. I air dried all leaves for two weeks in paper bags after collection. Dried leaves were cut into 2.5 cm x 7.6 cm strips avoiding the main stem and large veins of the leaves (Figure 3).

I cut all leaf pack materials with ceramic scissors to limit contact with metals. I assigned each leaf pack, a mesh bag filled with leaves, its own tag number and color-coded zip tie for easy identification when being removed from the river. I filled each leaf pack (10.2 cm x 10.2 cm and bound with 13.6 kg test fishing line) with approximately 4.5 g of either contaminated or non-contaminated dried leaf strips in a modification of methods for constructing artificial leaf bags (Braioni et al., 2001). A known portion of the dried leaves were acid-digested and analyzed for metal concentrations using Inductively Coupled Plasma Mass Spectrometry. Results of the metal analysis indicated that the contaminated leaves had higher metal concentrations compared to the non-contaminated leaves (See Tran Master's thesis for metal concentration values).

I arranged 20 leaf packs in a 4 x 5 grid of randomized packs attached to each other and secured to rebar with zip-ties. Each grid contained a mixture of coarse and fine mesh packs (7 mm (coarse) and 1 mm (fine) rubber coated mesh) containing either contaminated or non-contaminated leaves. I placed 10 leaf pack grids secured by rebar hammered into the riverbed within riffles, spanning the width of the river channel of both sites (reference n = 200 leaf packs; mining n = 200 leaf packs) (Figure 2). Half of the leaf packs in each reach contained contaminated (reference n = 100 leaf packs; mining n = 100 leaf packs) and non-contaminated leaves (reference n = 100 leaf packs; mining n = 100 leaf packs). Leaf packs from the day of initial placement (November 7, 2020) included a set of packs to calculate leaf mass lost during transportation (handling loss) (n = 20) and a set of packs removed from the river after 1 hour (n = 80). Each grid contained 20 leaf packs. This thesis focused on data collected from the coarse mesh leaf packs. Leaf packs comprised of 1 mm mesh were collected and analyzed for Tran Master's Thesis.

I removed a randomized subset of the leaf packs from the leaf pack grids 6 times over a 123-day period (Nov 7, 2020, Nov 21, 2020, Dec 5, 2020, Dec 19, 2020, Jan 16, 2021, and March 9, 2021). I placed each leaf pack into its own Ziploc bag after removal from the grid. I transported the leaf packs on ice and stored them in a dark refrigerator until processing. Flooding of Big River and hazardous weather conditions resulted in irregular collection dates and extended the experiment an additional 53 days beyond a planned 70-day experiment. The extended study period included the winter months which is uncommon and allowed for me to observe effects of high flow. Due to prolonged flooding events a second mining-contaminated river was selected to conduct a similar experiment during the spring in base flow conditions (Experiment B: Pierson Creek).

Physical and Chemical Characteristics. I measured all chemical and physical water characteristics during each leaf pack collection date. Meters were placed in the center of the stream in the reference and mining reaches and held at approximately 60% of the reach depth. I measured conductivity, total dissolved solids (TDS), redox potential (ORP), and pH using a HANNA HI98194 pH/EC/DO Multiparameter Probe, dissolved oxygen (DO), air temperature, and water temperature using a YSI 6-Series Multiparameter Water Quality Meter, and turbidity using a Hach 2100Q Portable Turbidimeter. Stream width and slope at each reach were measured using a meter tape and clinometer. I measured velocity in a cross-section of the stream at 60% depth using the Marsh McBirney flow meter to calculate discharge. I calculated cumulative degree-days using the average daily temperature for each reach, using a base temperature of 0°C.

I used an 11-liter plastic bucket to collect subaqueous sediment samples (no more than 20 cm deep) in areas within each reach covered by no more than 0.5 m of water. This was repeated for a total of three samples collected across the river channel at left bank, center stream, and right bank. Sediment samples were set to the side after collection for one hour before being transferred to a Ziploc bag to allow for fine sediments to settle and be included in the sample analysis. I collected sediment samples for both study sites during each leaf pack collection date and transported them to the laboratory in a Ziploc bag on ice. Samples were dried at 60 °C for 1-4 days. I used hand sieves sized 63 mm, 31.2 mm, 16 mm, 8 mm, 4 mm, 2 mm, and >2 mm mesh to separate sediment by particle size class according to the Wentworth Grade Scale to determine particle size distribution as proportional mass.

Leaf Pack Processing. I processed all leaf packs within 7 days of retrieval from the river. A 2x3 cm rectangle was cut from random leaf strips and set aside to measure microbial respiration for Tran Master's Thesis. The mass of the rectangle leaf samples was included in the

dry mass remaining measurements for the leaf packs they were collected from. I rinsed the remaining leaves with deionized water over a 250-µm sieve to remove invertebrates, sediment, and any fine particulate organic matter (FPOM) that had accumulated on the leaves. I placed rinsed leaves into paper bags to dry at 60 °C for at least 72 hours before weighing.

Macroinvertebrate Assemblage. I rinsed macroinvertebrates remaining in sieves after leaf pack processing with 75% reagent alcohol to preserve in 8 oz jars for storage. I sorted and counted all macroinvertebrates with a dissecting microscope and identified macroinvertebrates to the family taxonomic level, using the taxonomic key by (Merritt et al., 2019). Macroinvertebrate insects were further categorized by functional feeding group to determine abundance and diversity of shredders present, using the taxonomic key by (Merritt et al., 2019). I determined pollution tolerance values for the families in each reach using the family-level biotic index (FBI) (Hilsenhoff, 1987).

Experiment B: Pierson Creek

Study Sites. The study area for this experiment comprised two reaches within Pierson Creek (a.k.a. Pearson Creek) located in the Tri-State Mining District in the southwest part of Missouri (Table 1; Figure 2). The Tri-State Mining District has three NPL Superfund sites in which Tar Creek, the Rubidoux aquifer, and Pierson Creek stream and floodplain sediments have also been contaminated because of mining in the region and the processing of Pb and Zn ore (Owen et al., 2011; Johnson et al., 2016). Pierson Creek is within the Tri-State Mining District, a Pb and Zn mining district, which operated in parts of Missouri, Oklahoma, and Kansas (Winslow, 1894). Previous studies of channel sediments along Pierson Creek reported Zn concentrations between 300 and 1000 ppm with increased metal concentrations downstream of

Lake Springfield, indicating transport of Zn throughout Pierson Creek (Womble, 2009; Kissel, 2014). The mining reach of Pierson Creek was chosen due to its proximity from a Zn-Pb mine, while the reference reach is upstream up this mine (Womble, 2009) (Figure 4). The mining reach is also located near USGS Springfield Gage 07050690. Site determination for this experiment was based on site accessibility and the ability to hold all leaf pack grids for the spring field season without impeding stream flow for the duration of the experiment. This Pierson Creek spring field season was shorter than the Big River winter study because warmer water temperatures would increase leaf decomposition rates.

Experimental Design. I collected sycamore leaves from contaminated and non-contaminated riparian zones, along two rivers in Southwest Missouri: Pierson Creek and Bull Creek on October 19, 2020 (Figure 1). I chose the second riparian location due to restrictions of river access along Pierson Creek and landowner permission. The contaminated riparian zone for sycamore leaf collection was in the headwaters of west fork Bull Creek in Christian County, MO. This region is downstream of the Tuttle Mine, an old Pb-Zn mine. The non-contaminated riparian zone for sycamore leaf collection was located along the reference reach of Pierson Creek. I used the same collection procedure as described in Experiment A. I collected leaves from multiple riparian trees (DBH with a range of 12-126 cm) within contaminated and non-contaminated riparian zones, not exceeding 3 meters from the riverbank. Leaf collection and preparation methods were the same as in Experiment A. Results of this metal analysis also indicated that the contaminated leaves used in the Pierson Creek study had higher concentrations of metals compared to the non-contaminated leaves (See Tran Master's thesis for metal concentration values).

I constructed leaf packs and leaf pack grids using the same coarse mesh (7 mm) and methods as described in Experiment A. Contaminated and non-contaminated leaves were used to filled separate leaf packs placed randomly within the grids. I placed 5 leaf pack grids in riffles at each site in Pierson Creek (reference n = 100 leaf packs; mining n = 100 leaf packs) (Figure 2). Half of the leaf packs in each reach contained contaminated (reference n = 100 leaf packs; mining n = 100 leaf packs) and non-contaminated leaves (reference n = 100 leaf packs; mining n = 100 leaf packs). Leaves from the day of initial placement (March 8, 2021) included packs to estimate handling loss (n = 20) and packs removed from the river after 1 hour (n = 40). I removed and processed a randomized subset of the leaf packs 6 times over a 50-day period (March 8, 2021, March 22, 2021, April 5, 2021, April 12, 2021, April 19, 2021, and April 26, 2021).

Physical and Chemical Characteristics. On each leaf pack collection date, I measured chemical and physical water characteristics and collected subaqueous sediment samples from the reference and mining reaches following the same procedures as described in Experiment A.

Leaf Pack Processing and Macroinvertebrate Assemblage. I processed all leaf packs within 7 days of collection and identified macroinvertebrate following the same procedures as described in Experiment A.

Data Analysis (Experiment A and Experiment B)

Physical and Chemical Characteristics. I determined the particle size distribution of each sediment sample by first collecting the total sample weight, then taking the weight of each partial sample remaining within the sieves as a percentage of the total weight. Shannon's Diversity (H') was calculated from particle size classifications to determine substrate

heterogeneity within reaches. I tested for differences between the reference and mining reach of each stream for substrate particle size, air and water temperature, flow, turbidity, pH, and conductivity using one-way ANOVA with replicates from each leaf pack collection dates (n = 6).

Leaf Mass Loss. I determined leaf biomass loss as the difference in dry mass of the leaves within each pack from day 0 to their day of collection. Handling mass loss was used to determine an accurate beginning mass for leaves at time of placement into the stream by subtracting the average mass loss from handling loss leaf packs from mass of leaves before leaving the laboratory. The rate of leaf decomposition was measured as estimated percent mass loss on each consecutive collection date, then plotted a nest-fit negative log relationship across all dates. Leaf decomposition rates (k_d) were calculated using methods described in Minshall and Rugenski, (2007) such that:

$$-k_d = \log_e(\%R/100)/t, \text{ where } \%R \text{ is the percent remaining at any time in days } (t).$$

I analyzed for differences in decomposition rates (k_d) between leaf type (contaminated and non-contaminated) and river reach (reference and mining) using Analysis of Variance (Two-way ANOVA; $\alpha = 0.05$). I used Levene's test and the Shapiro-Wilk test to check for normality and equal variance ($\alpha = 0.05$). Leaf biomass data was transformed using the Johnson transformation to meet statistical assumptions. I analyzed for differences between leaf percent mass remaining using ANCOVA with river reach (reference and mining) and leaf type (contaminated and non-contaminated) as factors, day in stream as the covariate, and mean percent dry mass remaining as the response variable.

Macroinvertebrate Analysis. I tested for differences in total macroinvertebrate and shredder abundance per leaf pack between leaf type within reference and mining reaches using two-way ANOVA with abundance representing counts of individuals collected across all taxa

present. I calculated shredder diversity (Simpson's diversity) per leaf type (contaminated and non-contaminated) and per river reach (reference and mining) for each experiment.

RESULTS

Experiment A: Big River

Physical and Chemical Characteristics. The 123-day study in Big River had 666 cumulative degree-days **(**Table 2). Discharge and conductivity were greater in the mining reach than the reference reach ($P<0.001$; Table 2). Water temperature and air temperature were similar between reaches throughout the study. Water pH, dissolved oxygen, redox potential, and turbidity were also similar between reaches.

Subaqueous substrate within the mining reach was more heterogeneous than the reference reach according to Shannon's diversity index (H') (Table 3). Coarse gravel and cobble made up 80% of substrate mass in the reference reach and 41% of substrate mass in the mining reach. Finer gravel and sand composed over 2% of substrate mass in the mining reach and 11% of substrate mass in the reference reach.

Leaf Decomposition. Contaminated leaves lost more dry mass than packs with non-contaminated leaves regardless of reach placement. Leaf packs collected from the reference reach had a mean percent dry mass remaining of 61% for non-contaminated leaves and 42% for contaminated leaves by day 123 (ANCOVA $P<0.001$; Figure 5). Leaf packs from the mining reach had a mean percent dry mass remaining of 43% for non-contaminated leaves and 41% for contaminated leaves by day 123 (Figure 5). Leaf decomposition rates in the mining reach (0.0686 k d^{-1}) were approximately 1.4 times higher than in the reference reach (0.0474 k d^{-1}) regardless of leaf type (Figure 6). In the mining reach, decomposition rates were about 1.3 times higher for the contaminated leaves compared to the non-contaminated leaves (ANOVA $P<0.001$;

Figure 6). In the reference reach, decomposition rates were 1.7 times higher in contaminated leaves than non-contaminated leaves (ANOVA $P<0.001$; Figure 6).

Macroinvertebrates. I identified 14,742 individuals representing 22 families (Appendix Ia). The total community of aquatic macroinvertebrates collected from all leaf packs over the course of the 123-day study was 50% Diptera, 33% Plecoptera, 9% Trichoptera, and 8% Ephemeroptera. Coleoptera and Megaloptera comprised less than 1% of the total population collected. The most abundant families were Chironomidae (47% of total population), Capniidae (16%), Hydropsychidae (8%), Simuliidae (6%), and Leptohyphidae (7%) (Figure 7). Functional feeding group (FFG) distribution for Big River was 36% shredders, 38% collector-gatherers/filterers, 4% predators, and 2% scrapers. A family biotic index (FBI) showed that the Big River reference reach (FBI 3.87) had higher water quality than the mining reach (FBI 4.51). Total macroinvertebrate abundance was higher in the reference reach compared to the mining reach regardless of leaf type ($P<0.001$). But leaf packs from the mining reach had greater diversity of total macroinvertebrates than the reference reach (Simpson's Diversity Index 3.24 and 5.30). Leaf packs from the reference reach had greater shredder abundance than the mining reach, regardless of leaf type ($P<0.001$). Shredder abundance was not significantly different between contaminated and non-contaminated leaves within each reach (Figure 8). The only shredder family present in the reference reach was Capniidae. Shredder families present in the mining reach were Taeniopterygidae, Capniidae, and Leuctridae.

Experiment B: Pierson Creek

Physical and Chemical Characteristics. The 50-day experiment in Pierson Creek had 663 cumulative degree-days, which was similar to that of Big River despite the substantially

shorter duration (Table 4). The mining reach of Pierson Creek had higher conductivity than the reference reach (P<0.001; Table 4). Water temperature, air temperature, and discharge were similar in both reaches throughout the experiment. Water pH, dissolved oxygen, redox potential, and turbidity were also similar between reaches.

The mining reach had higher subaqueous substrate heterogeneity than the reference reach according to Shannon's diversity index (H') (Table 5). Coarse gravel and cobble made up 66% of the substrate mass in the reference reach and 34% of the substrate mass in the mining reach. Finer gravel and sand comprised 31% of the mining reach substrate mass while the reference reach substrate mass was comprised of 17% gravel and sand.

Leaf Decomposition. Leaves in the reference reach lost more dry mass than leaves in the mining reach, regardless of leaf type (ANCOVA P<0.001; Figure 9). Leaf packs within the reference reach had an overall mean percent dry mass remaining of 64% for non-contaminated leaves and 67% for contaminated leaves by day 50. Leaf packs within the mining reach had a mean percent dry mass remaining of 71% for non-contaminated leaves and 72% for contaminated leaves by day 50 (Figure 9). Average leaf decomposition rates in the reference reach (0.0367 k d^{-1}) were approximately 1.3 times higher than in the mining reach (0.0284 k d^{-1}) (ANOVA P<0.001; Figure 10). Decomposition rates were about 1.3 times higher for the non-contaminated leaves compared to the contaminated leaves within the reference reach (ANOVA P<0.001; Figure 10). In the mining reach, decomposition rates were 1.1 times higher in contaminated leaves than non-contaminated leaves (ANOVA P<0.001; Figure 10). Patterns in decomposition rates for Experiment B were opposite to those observed in Experiment A.

Macroinvertebrates. I identified 28,186 individuals representing 23 families (Appendix Ib). The total community of aquatic macroinvertebrates collected from all leaf packs over the

course of the 50-day study was 91% Diptera, 3% Trichoptera, and 1% Plecoptera. Coleoptera and Ephemeroptera comprised less than 1% of the total population collected. The most abundant families were Chironomidae (71% of total population), Simuliidae (20%), and Hydropsychidae (3%) (Figure 11). Functional feeding group (FFG) distribution for Pierson Creek was 35% shredders, 35% collector-gatherers/filterers, 17% predators, and 13% scrapers. A family biotic index (FBI) showed that the Pierson Creek reference reach (FBI 7.00) had higher water quality than the mining reach (FBI 7.40), but both were substantially lower water quality than in Big River. Total macroinvertebrate abundance was higher in the mining reach compared to the reference reach regardless of leaf type (P<0.001). Leaf packs from the reference reach had greater diversity of total macroinvertebrates than the mining reach (Simpson's Diversity Index 2.06 and 1.68). Leaf packs from the mining reach had greater shredder abundance than the reference reach, regardless of leaf type (P<0.001; Figure 12). Shredder abundance was similar between leaf types within each reach. Shredder families present in the reference reach were Leuctridae and Nemouridae. Shredder families present in the mining reach were Asellidae, and Gammaridae.

DISCUSSION

The aim of this study was to investigate how metal contamination in streams and leaves affected (1) sycamore leaf decomposition and (2) abundance of shredders and other macroinvertebrates in Big River, Missouri. I also addressed these aims at a smaller scale in another Ozark stream, Pierson Creek. Big River is a larger order stream with a significantly longer mining history than Pierson Creek. The two studies highlighted the effects of various levels of mining contamination on leaf decomposition and macroinvertebrate abundance in different order streams with similar cumulative degree-days. This study found that while metal contamination in leaves mattered more for leaf decomposition rates, stream reach mattered more for macroinvertebrate communities, with opposing trends between Big River and Pierson Creek. Higher decomposition rates and lower shredder abundance in the mining reach of Big River indicate that leaf litter decomposition was driven by physical abrasion likely exacerbated by flooding and increased stream discharge more than consumption or breakdown by macroinvertebrates. More invertebrate shredders were found in the mining reach of Pierson Creek while leaf decomposition rates were highest in the reference reach, indicating that abiotic factors such as land use and substrate heterogeneity are more likely to have driven leaf decomposition in the Pierson Creek study.

Many studies show positive relationships between shredder abundance and litter decomposition rates (Wallace et al., 1996; Carlisle & Clements, 2005; Pascoal et al., 2005; Ferreira et al., 2016). However, in the Big River and Pierson Creek reaches, increased shredder abundance did not correspond with increased rates of leaf decomposition. In the Big River study, contaminated sycamore leaves lost more dry mass than non-contaminated leaves and the mining

reach of Big River had greater leaf decomposition rates, regardless of leaf type. In contrast to the Big River study, non-contaminated and contaminated sycamore leaves in the Pierson Creek study had similar dry mass remaining and the reference reach had greater leaf decomposition rates. These differences between the two studies could be attributed to differences in discharge, water and air temperature, substrate heterogeneity, natural disturbances, and magnitude of metal contamination. Both experiments had similarities in degree-days. The Big River study had 666 cumulative degree-days for the 123-day experiment, while the Pierson Creek study had 663 cumulative degree-days for the 50-day experiment. The mining reach of Pierson Creek was likely far less contaminated than the mining reach of Big River due to the differences in mining duration and scale between the two regions. The Pierson Creek Mining District had about 70 years of active mining (Johnson et al., 2016), while Big River and The Old Lead Belt had over 200 years of active mining, with the mining reach of Big River positioned downstream of three major tailing piles (Pavlowsky et al., 2017). Additionally, the mining reach of Pierson Creek was downstream of 8 mines, with most mining activity taking place downstream of the reach (Figure 4). Tailing piles downstream of the Pierson Creek mining reach are not as large as Big River tailing piles. Stream channel sediments in Pierson Creek downstream of mines had Zn concentrations between 300 and 1000 ppm approximately 5-18 times higher than background levels (Womble, 2009), with Big River sediments exceeding 1,000 ppm Pb, which was 60 times above natural background levels for the region (Pavlowsky et al., 2010). Land use differences between reaches in Pierson Creek likely contributed to the differences in leaf decomposition between reaches with increased urbanization in the mining reach.

Estimates of leaf mass loss and decomposition rates for the Big River study were inconsistent with primary literature and did not support my hypotheses likely due to the timing of

the study during winter high-flow conditions. Metal contaminated reaches and streams usually have decreased leaf decomposition (Bermingham et al., 1996; Cornut et al., 2012; Niyogi et al., 2013; Ferreira et al., 2016) but this was not the case in my study. I suspect that physical abrasion is primarily responsible for the higher decomposition rates in the mining reach of Big River. The mesh used to make leaf packs in both experiments (mesh holes = 7 mm) which allowed macroinvertebrates to colonize the leaf packs also provided minimal protection from the physical environment compaired to other smaller sized mesh. The higher decomposition rates may be partially due to the high discharge rate in the mining reach, which was twice the rate of the reference reach. The higher discharge increased the effects of physical abrasion as the leaf pack contents made frequent contact with the stream substrate and water flow (Heard et al., 1999; Ferreira et al., 2006; Langhans et al., 2008; Walker et al., 2020).

Seasonal flooding of Big River could have also influenced leaf decomposition rates and macroinvertebrate abundance due to increased discharge, water depths, and transport of substrate and debris downstream. A large rainfall event in 1977 resulted in a short-term decrease in macroinvertebrate abundance in nearby Logan Creek (Besser et al., 2007). During the Big River study, there were two major flooding events at near bankfull flow each lasting approximately 4 days, with peak discharge reaching 198 m^3/s and 113 m^3/s (U.S. Geological Survey, 2016). During these flood events, some leaf pack grids were either carried downstream or buried by small sized sediments. I recovered these leaf pack grids and returned them to their original riffle after flooding, but this disturbance and burial most likely increased physical abrasion and fragmentation of leaf pack contents. I expect that the effects of flooding were greater in the mining reach of Big River since it was comprised of smaller substrate than the reference reach

and thus could be more easily transported during flood events. This is supported from the increase in fine substrate that was found while processing leaf packs retrieved after flood events.

Contaminated leaves from the Big River study were more fragile than the non-contaminated leaves and were more likely to break apart while constructing leaf packs. This fragility or lower toughness of leaf litter is a likely explanation for increased fragmentation and increased decomposition rates in contaminated leaves than non-contaminated leaves. Leaf litter toughness was maintained as close to natural conditions by allowing leaves to air dry before placing them into the streams, rather than oven-drying them. Previous studies reported that oven-drying can alter the leaf cuticle structure and fracture leaf membranes, ultimately decreasing leaf toughness (Boulton & Boon, 1991; Gessner et al., 1999; Haapala et al., 2001). Shredders typically have a preference when it comes to types of leaves consumed and can increase decomposition rates of preferred leaves (Graca, 2001; Batista et al., 2012). Shredders prefer leaves that have been colonized and conditioned by microbes and fungi, as this increases the nutritional quality and makes the leaves easier to consume (Cummins & Klug, 1979; Bastian et al., 2007; Danger et al., 2012; Suhaila et al., 2016). While a decrease in leaf toughness has been found to encourage shredder feeding, even in metal contaminated leaf litter (Liu et al., 2021) shredders did not appear to prefer a particular leaf type in the Big River study. Hence the fragility of the leaves and the physical abrasion sustained by those leaves exacerbated by the hole size of the mesh, most likely contributed to the higher mass loss of the metal-contaminated leaves over time. This is most likely what we can expect to see across all streams during flow disturbance events.

Unlike the Big River study, leaf decomposition was greater in the reference than in the mining reach of Pierson Creek, supporting my hypotheses and consistent with other studies

(Cornut et al., 2012; Niyogi et al., 2013; Ferreira et al., 2016). Contaminated sycamore leaves used in the Pierson Creek study did not appear to have the same fragility as the contaminated leaves used in Big River and likely did not experience the same magnitude of physical abrasion since the stream is much smaller with a lower discharge. Contaminated and non-contaminated Pierson Creek leaves also appeared to have similar toughness throughout the air-drying and leaf pack filling processes. This lack of fragility in contaminated leaves could be a result of Christian County, the source of contaminated leaves for the Pierson Creek study, having a much shorter history of mining contamination (35 years) and less mining contamination than Big River (200 years) resulting in less prolonged and severe exposure to metal contamination. The type of mine contamination could also play a role in the fragility of leaf litter. Sediments within the Big River and the Old Lead Belt are primarily contaminated with Pb, while sediments in the Pierson Creek and the Tri-State Mining District are primarily contaminated with Zn and Cd (Besser et al., 2015; Pavlowsky et al., 2017).

Metal-contaminated sediments and subsequent toxicity of the mining reach could explain the decrease in total macroinvertebrate and shredder abundance in the mining reach of Big River (Allert et al., 2009; Besser et al., 2009; Besser et al., 2015). A test of sediment toxicity effects on benthic organisms in nearby streams within the Viburnum Trend found sediment downstream of mines to be more toxic and decreased survival rates of amphipods (Besser et al., 2009). Concentrations of Pb, Zn, and Cd of sediment in mining-contaminated reaches of Big River correlated with metal concentrations in crayfish and fish and their decreased survival (Gale et al., 2002; Besser et al., 2007; Allert et al., 2009). Mining contaminated reaches of Big River were reported to have less dense populations of crayfish and benthic fish such as stonerollers and the Missouri saddled darter compared to reference reaches upstream of mining contamination (Allert

et al., 2013). Crayfish population densities within tributaries of the Spring River in which crayfish consumed fallen leaves from the stream banks decreased downstream of metal contamination in comparison to upstream populations (Allert et al., 2012). Aquatic invertebrates that consume metal-contaminated leaves then have the potential to accumulate metals and transfer them to higher trophic levels as they are fed upon by fish or larger invertebrates. Vegetative based food webs, where algae are the main carbon source, showed an increase in Cd concentrations moving up trophic levels from benthic macroinvertebrates to largemouth bass (Croteau et al., 2005).

In the Big River study, shredders were more abundant in the reference reach compared to the mining reach. In contrast, shredders were more abundant in the mining reach compared to the reference reach of Pierson Creek. In both studies, leaf type (contaminated and non-contaminated) did not influence shredder abundance in the reference or mining reaches. Shredder abundance per leaf pack decreased after Big River flooding, but remained similar in Pierson Creek leaf packs after flooding. Differences between these two studies could be due to the increase in substrate heterogeneity in the mining reach of Pierson Creek combined with the lesser disturbance from fewer flood events, which made the mining reach a more suitable habitat for macroinvertebrates in Pierson Creek. Only one long-duration flood occurred during the Pierson Creek study lasting approximately 15 days with peak discharge reaching 25 m^3/s (U.S. Geological Survey, 2016). The two Big River floods had 7 and 4 times the amount of disharge that the Pierson Creek flood did, putting higher stress onto Big River leaf packs from flood disturbance and channel sediment turnover. Increased substrate heterogeneity has been found to increase macroinvertebrate diversity and abundance by providing protection from stream

velocity and increased foraging opportunities in sediment crevices (Laasonen et al., 1998; Allan & Castillo, 2009; Palmer et al., 2010).

Additional physical and chemical differences in Big River reaches such as conductivity and substrate heterogeneity could explain the distribution of macroinvertebrates between the reference and mining reaches. An increase of ionic concentrations as indicated by the conductivity, in streams has shown to decrease the abundance of sensitive macroinvertebrate taxa such as Ephemeroptera (Cormier et al., 2012; Clements & Kotalik, 2016). In microcosm experiments macroinvertebrates showed increased stress responses as total dissolved solids were increased within the environment (Olson & Hawkins, 2017). A decrease in substrate heterogeneity can also result in a decrease in macroinvertebrate taxa by limiting possible habitat and food sources. In the mining reach of Big River, I found that 48% of the sediment composition was 16 mm or less, which is the typical size of mine tailing chat in Big River (Pavlowsky et al., 2017), while the same sediment class made up only 21% of the reference reach substrate. An increase in smaller class sediment can lead to higher turbidity in streams. These environmental stressors decrease overall macroinvertebrate abundance and prevent sensitive macroinvertebrate families from colonizing polluted and metal-contaminated reaches (Kasangaki et al., 2008; Cormier et al., 2012; Narangarvuu et al., 2014).

Macroinvertebrate and shredder abundances were significantly higher in the reference reach than the mining reach in the Big River study. Similarly other studies reported higher macroinvertebrate shredder abundance in reference reaches of metal contaminated streams (Niyogi et al., 2001, 2002; Chaffin et al., 2005).The macroinvertebrate population of the Big River reference reach was mostly Ephemeroptera, Plecoptera, Trichoptera (EPT) with few Diptera families present which was consistent with other macroinvertebrate surveys completed in

the Viburnum Trend in 2003 and 2004 (Poulton et al., 2009). Macroinvertebrates belonging to Ephemeroptera, Plecoptera, and Trichoptera orders are typically associated with clean water conditions, with varying degrees of pollutant tollerance, while Diptera as an order are often associated with high pollution conditions (Paine & Gaufin, 1956). However, two major Diptera families (Chironomidae and Simuliidae) comprised 54% of the reference reach macroinvertebrate population and 41% of the mining reach macroinvertebrate population. Sites downstream of mining activity in previous surveys tended to support fewer total macroinvertebrates and these tended to be families that are tolerant of pollution (Poulton et al., 2009). The mining reach of Big River followed this same trend with 4 times as many pollutant tolerant Trichopteran individuals present than in the reference reach, even though the reference reach had twice the total abundance of macroinvertebrates. Unlike trends observed in Big River, the reference and mining reaches of Pierson Creek had a similar abundance in pollutant tolerant taxa.

Total macroinvertebrate and shredder abundance was higher in the mining reach of Pierson Creek than in the reference reach. In contrast, other studies reported that shredder abundance decreased downstream of metal contamination (Niyogi et al., 2001, 2002; Chaffin et al., 2005). Crayfish population densities in nearby tributaries of the Spring River were found to decrease in study sites with increased metal contamination (Allert et al., 2012). These differences could be due to reaches that I selected for the leaf pack studies. Of the approximately 40 mines in the region, only 8 are located upstream of the Pierson Creek mining reach. The remaining mines are located downstream of the mining reach, where most transportation and storage of mine waste would have taken place (DGLS, 2008; Womble, 2009). In addition, due to the short

mining history and extent of mining contamination, sediment toxicity might not be the driving factor on macroinvertebrate abundance in Pierson Creek.

Land use in the watershed surrounding the reference reach of Pierson Creek is 71% agricultural while the watershed surrounding the mining reach was 58% agricultural. Sites with increased agricultural land use are expected to have increased turbidity and conductivity (Kasangaki et al., 2008; Chase et al., 2016). Increased runoff of pesticides and fertilizers are further aspects of agricultural land that inhibit the survival of sensitive invertebrate taxa (Genito et al., 2002; Egler et al., 2012). This can be seen in the high FBI values of both the reference reach (FBI 7.00) and mining reach (FBI 7.40) in Pierson Creek. Diptera, a pollutant tolerant order-level taxon, made up 91% of the macroinvertebrate community in both the mining reach and reference reach of Pierson Creek. Conversley, FBI values in the Big River were much lower at 3.87 in the reference reach and 4.51 in the mining reach, and Diptera mdae up less than 55% of the macroinvertebrate community in both reaches. Land use can also affect stream substrate which in turn can affect macroinvertebrate assemblage and fish community structure (Hrodey et al., 2009). Clay and silt soils associated with high agricultural land use have been found to decrease substrate heterogeneity and subsequently reduce macroinvertebrate abundance and taxanomic richness (Richards et al., 1993; Hrodey et al., 2009).

Substrate heterogeneity may also be a driving factor in the increased abundance of macroinvertebrates and shredders in the mining reach of Pierson Creek. Substrate heterogeneity encourages larger macroinvertebrate populations because it provides a variety of habitat and ecological niches, allowing more taxa to occupy the same reach (Milesi et al., 2016). A common goal of stream restoration projects is to restore substrate heterogeneity (habitat heterogeneity) to reaches to promote abundance and taxa diversity (Laasonen et al., 1998; Palmer et al., 2010).

The mining reach of Pierson Creek had higher substrate heterogeneity than the reference reach, consisting of 65% sediment 16 mm and smaller while 41% of the reference reach was made up of smaller class substrate. Heterogeneous substrates provide more shelter for macroinvertebrates while collecting organic matter from riparian litter as a food source (Boyero, 2003; Hepp et al., 2012). These benefits of substrate heterogeneity are a likely cause of increased macroinvertebrate and shredder abundance in the mining reach of Pierson Creek. An increase in macroinvertebrate abundance in the downstream mining reach of Pierson Creek may simply just be a result of the longitudinal relationship of streams such that the downstream widening of streams and transport of organic matter from upstream reaches can support a higher abundance of organsims (Vannote et al., 1980).

This study, as well as previous research, shows that there are many factors that play a role in stream ecosystem functions. For example, increased precipitation or changes in land use can increase runoff and sediment deposition from floodplains into streams within those watersheds. Unfortunately, anthropogenic disturbances such as agriculture and mining tend to negatively impact streams by reducing microbial communites and decreasing macroinvertebrate shredder abundance, ultimately inhibiting leaf litter decomposition (Ferreira et al., 2016). Future work should emphasize inputs into streams as a result of landuse within the watershed. This study showed the importance of landuse as it likely contributed to a decrease of stream substrate quality, (increased metal contamination and decreased heterogeneity) further inhibiting leaf decomposition rates and macroinvertebrate community structure. Both factors result from runoff and inputs from the floodplains of the Big River and Pierson Creek. Future work should embrace environmental disturbances such as flooding in field studies and pursue field experiments during high flow seasons as performed in the Big River study. This atypical study design can provide

insight into how stream communities respond to disturbance events in real time. Future work should also address the effects of metal contamination on leaf quality and assess differences in lignin content and leaf toughness of contaminated and non-contaminated leaves. Future research in this field would provide a holistic understanding of leaf litter decomposition with the inclusion of variables such as leaf litter toughness, physical abrasion, seasonal flooding, and landuse effects.

CONCLUSION

This study showed that leaf decomposition and macroinvertebrate colonization could be influenced by both reach placement and leaf type. In the Big River study, metal-contaminated leaves lost more dry mass than non-contaminated leaves, likely due to increased physical abrasion. The Big River study took place in winter, during the high-flow season, which further contributed to understanding how natural disturbance events impact macroinvertebrate communities in non-contaminated and metal-contaminated streams. In the Pierson Creek study, leaf decomposition was influenced by reach placement but not leaf type. Leaves from the mining reach of Pierson Creek had lower decomposition rates than the reference reach, regardless of leaf type. Shredders were most influenced by metal contamination of reach substrate in Big River. A higher abundance of total macroinvertebrates and shredders were found in the reference reach of Big River, where metal concentrations were below detection limits. Substrate heterogeneity and land use appeared to be the most influential factor in the contrasting macroinvertebrate assemblage in Pierson Creek. A higher abundance of total macroinvertebrates and shredders were found in the mining reach of Pierson Creek where there was more substrate heterogeneity and less agricultural runoff. The findings of this study emphasize that disturbances such as mining contamination and high stream discharge can result in decreased leaf decomposition and macroinvertebrate abundance in streams. Designing future decomposition studies to take place during all seasons will provide a holistic view of the stream community structure and eliminate bias towards specific seasons and stream conditions.

REFERENCES

Allan, D., & Castillo, M. (2009). Stream Ecology: Structure and function of running waters. Springer, Dordrecht.

Allert, A. L., DiStefano, R. J., Fairchild, J. F., Schmitt, C. J., McKee, M. J., Girondo, J. A., Brumbaugh, W. G., & May, T. W. (2013). Effects of historical lead–zinc mining on riffle-dwelling benthic fish and crayfish in the Big River of southeastern Missouri, USA. *Ecotoxicology, 22*(3), 506–521.

Allert, A. L., DiStefano, R. J., Schmitt, C. J., Fairchild, J. F., & Brumbaugh, W. G. (2012). Effects of mining-derived metals on riffle-dwelling crayfish in Southwestern Missouri and Southeastern Kansas, USA. *Archives of Environmental Contamination and Toxicology*, *63*(4), 563–573.

Allert, A. L., Fairchild, J. F., DiStefano, R. J., Schmitt, C.J ., Besser, J. M., Brumbaugh, W. G., & Poulton, B. C. (2008). Effects of lead–zinc mining on crayfish (Orconectes hylas) in the Black River watershed. *Missouri Freshwater Crayfish,16,* 97–111.

Allert, A. L., Fairchild, J. F., Schmitt, C. J., Besser, J. M., Brumbaugh, W. G., & Olson, S. J. (2009). Effects of mining-derived metals on riffle-dwelling benthic fishes in Southeast Missouri, USA. *Ecotoxicology and Environmental Safety, 72*(6), 1642–1651.

André, O., Vollenweider, P., & Günthardt-Goerg, M. (2006). Foliage response to heavy metal contamination in Sycamore Maple (Acer pseudoplatanus L.). *Forest Snow and Landscape Research, 80*(80)*,* 275–288.

Barnden, A. R., & Harding, J. S. (2005). Shredders and leaf breakdown in streams polluted by coal mining in the South Island, New Zealand. *New Zealand Natural Sciences, 30,* 35-48.

Bastian, M., Boyero, L., Jackes, B. R., & Pearson, R. G. (2007). Leaf litter diversity and shredder preferences in an Australian tropical rain-forest stream. *Journal of Tropical Ecology, 23*(2), 219–229.

Batista, D., Pascoal, C., & Cássio, F. (2012). Impacts of warming on aquatic decomposers along a gradient of cadmium stress. *Environmental Pollution, 169*, 35–41.

Bermingham, S., Maltby, L., & Cooke, R. C. (1996). Effects of a Coal Mine Effluent on Aquatic Hyphomycetes. I. Field Study. *The Journal of Applied Ecology, 33*(6), 1311.

Besser, J. M., Brumbaugh, W. G., Allert, A. L., Poulton, B. C., Schmitt, C. J., & Ingersoll, C. G. (2009). Ecological impacts of lead mining on Ozark streams: Toxicity of sediment and pore water. *Ecotoxicology and Environmental Safety, 72*(2), 516–526.

Besser, J. M., Brumbaugh, W. G., May, T. W., & Schmitt, C. J. (2007). Biomonitoring of lead, zinc, and cadmium in streams draining lead-mining and non-mining areas, Southeast Missouri, USA. *Environmental Monitoring and Assessment, 129*(1-3), 227–241.

Besser, J. M., Ingersoll, C. G., Brumbaugh, W. G., Kemble, N. E., May, T. W., Wang, N., MacDonald, D. D., & Roberts, A. D. (2015). Toxicity of sediments from lead-zinc mining areas to juvenile freshwater mussels (Lampsilis siliquoidea) compared to standard test organisms. *Environmental Toxicology and Chemistry, 34*(3)*,* 626–639.

Boulton, A., & Boon, P. (1991). A review of methodology used to measure leaf litter decomposition in lotic environments: Time to turn over an old leaf? *Marine and Freshwater Research, 42*(1), 1.

Boyero, L. (2003). Multiscale patterns of spatial variation in stream macroinvertebrate communities. *Ecological Research, 18*(4), 365–379.

Braioni, M. G., Gumiero, B., & Salmoiraghi, G. (2001). Leaf Bags and Natural Leaf Packs: Two Approaches to Evaluate River Functional Characteristics. *International Review of Hydrobiology, 86*(4-5), 439–451.

Carlisle, D. M., & Clements, W. H. (2005). Leaf litter breakdown, microbial respiration and shredder production in metal-polluted streams. *Freshwater Biology, 50*(2), 380–390.

Chaffin, J. L., Valett, H. M., Webster, J. R., & Schreiber, M. E. (2005). Influence of elevated As on leaf breakdown in an Appalachian headwater stream. *Journal of the North American Benthological Society, 24*(3), 553–568.

Chase, J. W., Benoy, G. A., Hann, S. W. R., & Culp, J. M. (2016). Small differences in riparian vegetation significantly reduce land use impacts on stream flow and water quality in small agricultural watersheds. *Journal of Soil and Water Conservation, 71*(3), 194–205.

Chergui, H., & Pattee, E. (1990). The influence of season on the breakdown of submerged leaves. *Archiv für Hydrobiologie, 120*(1), 1–12.

Clements, W. H., & Kotalik, C. (2016). Effects of major ions on natural benthic communities: an experimental assessment of the US Environmental Protection Agency aquatic life benchmark for conductivity. *Freshwater Science, 35*(1), 126–138.

Cormier, S. M., Suter, G. W., Zheng, L., & Pond, G. J. (2012). Assessing causation of the extirpation of stream macroinvertebrates by a mixture of ions. *Environmental Toxicology and Chemistry, 32*(2), 277–287.

Cornut, J., Clivot, H., Chauvet, E., Elger, A., Pagnout, C., & Guérold, F. (2012). Effect of acidification on leaf litter decomposition in benthic and hyporheic zones of woodland streams. *Water Research, 46*(19), 6430–6444.

Coughlan, J. F., Pearson, R. G., & Boyero, L. (2010). Crayfish process leaf litter in tropical streams even when shredding insects are common. *Marine and Freshwater Research, 61*(5), 541.

Croteau, M. N., Luoma, S. N., & Stewart, A. R. (2005). Trophic transfer of metals along freshwater food webs: Evidence of cadmium biomagnification in nature. *Limnology and Oceanography, 50*(5), 1511–1519.

Cummins, K. W., & Klug, M. J. (1979). Feeding Ecology of Stream Invertebrates. Annual *Review of Ecology and Systematics, 10(1)*, 147–172.

Danger, M., Cornut, J., Elger, A., & Chauvet, E. (2012). Effects of burial on leaf litter quality, microbial conditioning and palatability to three shredder taxa. *Freshwater Biology, 57*(5), 1017–1030.

Division of Geology and Land Survey (DGLS). (2008). Inventory of Mines, Occurrences, and Prospects in Missouri (shapefile). Missouri Department of Natural Resources.

Duarte, S., Pascoal, C., Alves, A., Correia, A., & Cássio, F. (2007). Copper and zinc mixtures induce shifts in microbial communities and reduce leaf litter decomposition in streams. *Freshwater Biology, 53*, 91-101.

Egler, M., Buss, D. F., Moreira, J. C., & Baptista, D. F. (2012). Influence of agricultural land-use and pesticides on benthic macroinvertebrate assemblages in an agricultural river basin in southeast Brazil. *Brazilian Journal of Biology, 72*(3), 437–443.

Ferreira, V., Graça, M. A. S., de Lima, J. L. M. P., & Gomes, R. (2006). Role of physical fragmentation and invertebrate activity in the breakdown rate of leaves. *Archiv für Hydrobiologie, 165*(4), 493–513.

Ferreira, V., Koricheva, J., Duarte, S., Niyogi, D. K., & Guérold, F. (2016). Effects of anthropogenic heavy metal contamination on litter decomposition in streams – A meta-analysis. *Environmental Pollution, 210*, 261–270.

Funck, J. A., Clivot, H., Felten, V., Rousselle, P., Guérold, F., & Danger, M. (2013). Phosphorus availability modulates the toxic effect of silver on aquatic fungi and leaf litter decomposition. *Aquatic Toxicology, 144-145*, 199–207.

Gale, N. L., Adams, C. D., Wixson, B. G., Loftin, K. A., & Huang, Y. W. (2002). Lead concentrations In fish and river sediments in the Old Lead Belt of Missouri. *Environmental Science & Technology, 36*(20), 4262–4268.

Genito, D., Gburek, W. J., & Sharpley, A. N. (2002). Response of stream macroinvertebrates to agricultural land cover in a small watershed. *Journal of Freshwater Ecology, 17*(1), 109–119.

Gessner, M. O., Chauvet, E., & Dobson, M. (1999). A perspective on leaf litter breakdown in streams. *Oikos, 85*(2), 377.

Graça, M. A. S., & Canhoto, C. (2006). Leaf litter processing in low order streams. *Limnetica, 25*, 1–10.

Graça, M. A. S. (2001). The role of invertebrates on leaf litter decomposition in streams - a review. *International Review of Hydrobiology, 86*(4-5), 383–393.

Gulis, V., & Suberkropp, K. (2003). Effect of inorganic nutrients on relative contributions of fungi and bacteria to carbon flow from submerged decomposing leaf litter. *Microbial Ecology, 45*(1), 11–19.

Haapala, A., Muotka, T., & Markkola, A. (2001). Breakdown and macroinvertebrate and fungal colonization of alder, birch, and willow leaves in a boreal forest stream. *Journal of the North American Benthological Society, 20*(3), 395–407.

Heard, S. B., Schultz, G. A., Ogden, C. B., & Griesel, T. C. (1999). Mechanical abrasion and organic matter processing in an Iowa stream. *Hydrobiologia, 400*, 179–186.

Heiman, J. A., Tran, I. T., Behlke-Entwisle, M., Pavlowsky, R. T., & Kissoon, L. T. (2022). Metal accumulation in American sycamores in a mining-contaminated river in southeastern Missouri. *Water, Air, & Soil Pollution, 233*(4).

Hepp, L. U., Landeiro, V. L., & Melo, A. S. (2012). Experimental assessment of the effects of environmental factors and longitudinal position on alpha and beta diversities of aquatic insects in a Neotropical stream. *International Review of Hydrobiology, 97*(2), 157–167.

Hrodey, P. J., Sutton, T. M., Frimpong, E. A., & Simon, T. P. (2009). Land-use impacts on watershed health and integrity in Indiana warmwater streams. *The American Midland Naturalist, 161*(1), 76–95.

Johnson, A. W., Gutiérrez, M., Gouzie, D., & McAliley, L. R. (2016). State of remediation and metal toxicity in the Tri-State Mining District, USA. *Chemosphere, 144*(144), 1132–1141.

Jonsson, M., Malmqvist, B., & Hoffsten, P. O. (2001). Leaf litter breakdown rates in boreal streams: Does shredder species richness matter? *Freshwater Biology, 46*(2), 161–171.

Kasangaki, A., Chapman, L. J., & Balirwa, J. (2008). Land use and the ecology of benthic macroinvertebrate assemblages of high-altitude rainforest streams in Uganda. *Freshwater Biology, 53*(4), 681–697.

Kissel, J. (2014). Plant Uptake of Cr, Zn, and Pb from Historical Mining Areas, Springfield, Mo (MSc Thesis). *Missouri State University*, Springfield, MO.

Laasonen, P., Muotka, T., & Kivijärvi, I. (1998). Recovery of macroinvertebrate communities from stream habitat restoration. *Aquatic Conservation: Marine and Freshwater Ecosystems, 8*(1), 101–113.

Langhans, S. D., Tiegs, S. D., Gessner, M. O., & Tockner, K. (2008). Leaf-decomposition heterogeneity across a riverine floodplain mosaic. *Aquatic Sciences, 70*(3), 337–346.

Liu, R., Pan, Y., Fang, Y., Pang, L., Shen, J., & Tian, X. (2021). Effects of heavy metal-mediated intraspecific variation in leaf litter on the feeding preferences of stream detritivores. *Science of the Total Environment, 763*(736), 144591.

Martínez, A., Pérez, J., Molinero, J., Sagarduy, M., & Pozo, J. (2015). Effects of flow scarcity on leaf-litter processing under oceanic climate conditions in calcareous streams. *Science of the Total Environment, 503-504*(503-504), 251–257.

McKie, B. G., Woodward, G., Hladyz, S., Nistorescu, M., Preda, E., Popescu, C., Giller, P. S., & Malmqvist, B. (2008). Ecosystem functioning in stream assemblages from different regions: Contrasting responses to variation in detritivore richness, evenness and density. *Journal of Animal Ecology, 77*(3), 495–504.

Merritt, R. W., Cummins, K. W., & Berg, M. B. (2019). An introduction to the aquatic insects of North America. *Kendall Hunt Publishing Company*, Dubuque, Ia.

Milesi, S. V., Dolédec, S., & Melo, A. S. (2016). Substrate heterogeneity influences the trait composition of stream insect communities: An experimental in situ study. *Freshwater Science, 35*(4), 1321–1329.

Minshall, G. W., & Rugenski, A. (2007). Riparian Processes and Interactions, in: Hauer, F.R., Lamberti, G.A. (Eds.), *Methods in Stream Ecology*. New York: Academic Press, pp. 721–742.

Narangarvuu, D., Hsu, C. B., Shieh, S. H., Wuc, F. C., & Yang, P. S. (2014). Macroinvertebrate assemblage patterns as indicators of water quality in the Xindian watershed,Taiwan. *Journal of Asia-Pacific Entomology, 17*(3), 505-513

Newfields. (2007). Volume of Sediment in Big River, Flat River Creek, and Owl Creek-St. Francois County Mined Areas, Missouri. Report Submitted on June 29, 2007 as an Addendum to the March 2006 "*Focused Remedial Investigation of Mined Areas in St. Francois County, Missouri*."

Niyogi, D. K., Harding, J. S., & Simon, K. S. (2013). Organic matter breakdown as a measure of stream health in New Zealand streams affected by acid mine drainage. *Ecological Indicators, 24*, 510–517.

Niyogi, D. K., Lewis, W. M., & McKnight, D. M. (2001). Litter breakdown in mountain streams affected by mine drainage: Biotic mediation of abiotic controls. *Ecological Applications, 11*(2), 506–516.

Niyogi, D. K., McKnight, D. M., & Lewis, W. M. (2002). Effects of mine drainage on breakdown of aspen litter in mountain streams. *Water, Air, and Soil Pollution, 2*, 329–341.

Nugroho, A. P., Butar, E. S. B., Priantoro, E. A., Sriwuryandari, L., Pratiwi, Z. B., & Sembiring, T. (2021). Phytoremediation of electroplating wastewater by vetiver grass (Chrysopogon zizanoides L.). *Scientific Reports, 11*(1), 14484.

Olson, J. R., & Hawkins, C. P. (2017). Effects of total dissolved solids on growth and mortality predict distributions of stream macroinvertebrates. *Freshwater Biology, 62*(4), 779–791.

Owen, M. R., Pavlowsky, R. T., & Womble, P. J. (2011). Historical disturbance and contemporary floodplain development along an Ozark river, southwest Missouri. *Physical Geography, 32*(5), 423–444.

Paine, G. H., & Gaufin, A. R. (1956). Aquatic Diptera as Indicators of pollution in a Midwestern stream. *The Ohio Journal of Science, 56*(5), 291–304.

Palmer, K. T., & Kucera, C. L. (1980). Lead Contamination of Sycamore and Soil from Lead Mining and Smelting Operations in Eastern Missouri1. *Journal of Environment Quality, 9*(1), 106.

Palmer, M. A., Menninger, H. L., & Bernhardt, E. (2010). River restoration, habitat heterogeneity and biodiversity: A failure of theory or practice? *Freshwater Biology, 55*, 205–222.

Pascoal, C., Cássio, F., Marcotegui, A., Sanz, B., & Gomes, P. (2005). Role of fungi, bacteria, and invertebrates in leaf litter breakdown in a polluted river. *Journal of the North American Benthological Society, 24*(4), 784–797.

Patrick, C. J. (2013). The effect of shredder community composition on the production and quality of fine particulate organic matter. *Freshwater Science, 32*(3), 1026–1035.

Pavlowsky, R. T., Lecce, S. A., Owen, M. R., & Martin, D. J. (2017). Legacy sediment, lead, and zinc storage in channel and floodplain deposits of the Big River, Old Lead Belt Mining District, Missouri, USA. *Geomorphology, 299*, 54–75.

Pavlowsky, R. T., Owen, M. R., & Martin, D. J. (2010). Distribution, Geochemistry, and Storage of Mining Sediment in Channel and Floodplain Deposits of the Big River System in St. Francois, Washington, and Jefferson Counties, Missouri. In: *OEWRI EDR-10-002 Report Prepared for US Fish and Wildlife Service, Columbia, MO*. Ozarks Environmental and Water Resources Institute.

Peralta-Videa, J. R., Lopez, M. L., Narayan, M., Saupe, G., & Gardea-Torresdey, J. (2009). The biochemistry of environmental heavy metal uptake by plants: Implications for the food chain. *The International Journal of Biochemistry & Cell Biology, 41*(8-9), 1665–1677.

Poulton, B. C., Allert, A. L., Besser, J. M., Schmitt, C. J., Brumbaugh, W. G., & Fairchild, J. F. (2009). A macroinvertebrate assessment of Ozark streams located in lead–zinc mining areas of the Viburnum Trend in southeastern Missouri, USA. *Environmental Monitoring and Assessment, 163*(1-4), 619–641.

Richards, C., Host, G. E., & Arthur, J. W. (1993). Identification of predominant environmental factors structuring stream macroinvertebrate communities within a large agricultural catchment. *Freshwater Biology, 29*(2), 285–294.

Shahid, M., Dumat, C., Khalid, S., Schreck, E., Xiong, T., & Niazi, N. K. (2017). Foliar heavy metal uptake, toxicity and detoxification in plants: A comparison of foliar and root metal uptake. *Journal of Hazardous Materials, 325*, 36–58.

Sponseller, R. A., & Benfield, E. F. (2001). Influences of land use on leaf breakdown in southern Appalachian headwater streams: a multiple-scale analysis. *Journal of the North American Benthological Society, 20*(1), 44–59.

Stroh, E. D., Struckhoff, M. A., & Grabner, K. W. (2015). Soil lead and zinc effects on growth and survival of native plant species. *U.S. Geological Survey Administrative Report to the Missouri Trustee Council*.

Suhaila, A. H., Mohd Fakhrullah Salafusalam, M. A., & Nur Aida, H. (2016). Role of Ephemeroptera, Plecoptera and Trichoptera (Insecta) functional feeding groups in leaf decomposition in tropical river. *Journal of Biodiversity and Environmental Sciences, 9*(1), 204–213

Ueno, D., Iwashita, T., Zhao, F. J., & Ma, J. F. (2008). Characterization of Cd translocation and identification of the Cd form in xylem sap of the Cd-hyperaccumulator Arabidopsis halleri. *Plant and Cell Physiology, 49*(4), 540–548.

US EPA, R. 07. (2017). Big River Mine Tailings National Priorities List (NPL) Superfund Site, St. Francois County, Missouri - Fact Sheet, October 2017 [WWW Document]. www.epa.gov. URL https://www.epa.gov/mo/big-river-mine-tailings-national-priorities-list-npl-superfund-site-st-francois-county-missouri (accessed 2.17.22).

U.S. Geological Survey. (2016). National Water Information System data available on the World Wide Web (USGS Water Data for the Nation). URL http://waterdata.usgs.gov/nwis/ (accessed 5.15.22).

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., & Cushing, C. E. (1980). The River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Sciences, 37*(1), 130–137.

Walker, R. H., Orr, M. C., & Miller, S. W. (2020). Factors contributing to leaf decomposition vary with temperature in two montane rivers of the Intermountain West, Utah. *Aquatic Sciences, 82*(3), 49.

Wallace, J. B., Grubaugh, J. W., & Whiles, M. R. (1996). Biotic indices and stream ecosystem processes: Results from an experimental study. *Ecological Applications, 6*(1), 140–151.

Whitledge, G. W., & Rabeni, C. F. (1997). Energy sources and ecological role of crayfishes in an Ozark stream: insights from stable isotopes and gut analysis. *Canadian Journal of Fisheries and Aquatic Sciences, 54*(11), 2555–2563.

Winslow, A. (1894). Lead and Zinc Deposits (Section 2). *Missouri Geological Survey, 7*, 624-625

Womble, P. (2009). Historical Mining Contamination and Floodplain Evolution along Lower Pearson Creek, SW Missouri (MSc Thesis). *Missouri State University*, Springfield, MO.

Zhang, H., Cui, B., Xiao, R., & Zhao, H. (2010). Heavy metals in water, soils and plants in riparian wetlands in the Pearl River Estuary, South China. *Procedia Environmental Sciences, 2*(2), 1344–1354.

Table 1. Stream reach locations and descriptions for Big River and Pierson Creek, MO.

Site	Reach	Description	Latitude (°)	Longitude (°)
Big River	Reference	Bridge at St. Rte. U Irondale, MO	37.830028	-90.688944
Big River	Mining	St. Francois State Park Bonne Terre, MO	37.966499	-90.5344851
Pierson Creek	Reference	E Farm Rd 132 Springfield, MO	37.209331	-93.194447
Pierson Creek	Mining	Bridge on Farm Rd 148 Springfield, MO	37.177517	-93.198067

Table 2. Physiochemical characteristics for a reference and mining reach in Big River, MO over the 123-day leaf pack study. Table values represent the average of values (± standard deviation) collected at each leaf pack retrieval date (n = 6). *** indicates significant differences between reaches (P<0.001.)

Physiochemical Characteristics	**Reach**	
	Reference	**Mining**
Cumulative degree-day	666	666
Discharge (m^3s^{-1}) ***	2.20 (± 0.77)	5.84 (± 1.81)
Air temperature (°C)	11.05 (± 5.57)	11.1 (± 6.48)
Water temperature (°C)	8.33 (± 3.79)	8.1 (± 3.86)
pH	8.11 (± 0.15)	7.9 (± 0.42)
Dissolved oxygen (mg/L)	12.53 (±1.98)	12.4 (±1.65)
Conductivity (µS/cm) ***	315.00 (± 30.35)	452.6 (± 37.17)
Redox potential (mV)	155.80 (± 42.29)	183.5 (± 38.18)
Turbidity (NTU)	2.47 (± 2.43)	2.39 (± 1.40)

Table 3. Subaqueous sediment particle size distribution by percent composition of sample mass in Big River, MO. Table values represent the average of values (± standard deviation) collected at each leaf pack retrieval date (reference reach *n* = 4; mining reach *n* = 5).

Substrate Type	**Reach**	
	Reference	**Mining**
Substrate Heterogeneity (H’)	1.30	1.72
Cobble (63mm)	32% (± 23%)	13% (± 18%)
Coarse gravel (31.5mm	48% (± 22%)	38% (± 18%)
Pebble gravel (16mm)	10% (± 5%)	18% (± 7%)
Fine gravel (8mm)	4% (± 2%)	8% (± 6%)
Very fine gravel (4mm)	3% (± 2%)	8% (± 6%)
Coarse sand (2mm)	2% (± 1%)	7% (± 4%)
Sand(<2mm)	2% (± 1%)	7% (± 4%)

Table 4. Physiochemical characteristics of a reference and mining reach in Pierson Creek, MO over a 50-day leaf-pack study. Table values represent the average of values (± standard deviation) collected at each leaf pack retrieval date (n = 6). Discharge measurements were taken on the first and last day of study (n = 2). *** indicates significant differences between reaches (P<0.001).

Physiochemical Characteristics	**Reach**	
	Reference	**Mining**
Cumulative degree-day	663	663
Discharge (m^3s^{-1})	0.65 (± 0.64)	0.96 (± 1.03)
Air temperature (°C)	21.66 (± 4.45)	21.33 (± 4.53)
Water temperature (°C)	15.26 (± 1.57)	14.77 (± 1.87)
pH	8.39 (± 0.20)	8.2 (± 0.21)
Dissolved oxygen (mg/L)	13.91 (± 2.51)	14.62 (± 2.39)
Conductivity (µS/cm) ***	321.83 (± 17.01)	393.50 (± 23.88)
Redox potential (mV)	196.00 (± 50.43)	220.40 (± 35.84)
Turbidity (NTU)	2.42 (± 1.12)	1.79 (± 0.80)

Table 5. Subaqueous sediment particle size distribution by percent composition of sample mass in Pierson Creek, MO. Table values represent the average of values (± standard deviation) collected at each leaf pack retrieval date (n = 6).

Substrate composition	**Reach**	
	Reference	**Mining**
Substrate Heterogeneity (H')	1.41	1.57
Cobble (63mm)	12% (± 13%)	6% (± 13%)
Coarse gravel (31.5mm	54% (± 14%)	28% (± 22%)
Pebble gravel (16mm)	24% (± 6%)	34% (± 9%)
Fine gravel (8mm)	9% (± 4%)	17% (± 7%)
Very fine gravel (4mm)	5% (± 5%)	9% (± 5%)
Very coarse sand (2mm)	2% (± 3%)	3% (± 3%)
Coarse sand(<2mm)	1% (± 1%)	2% (± 2%)

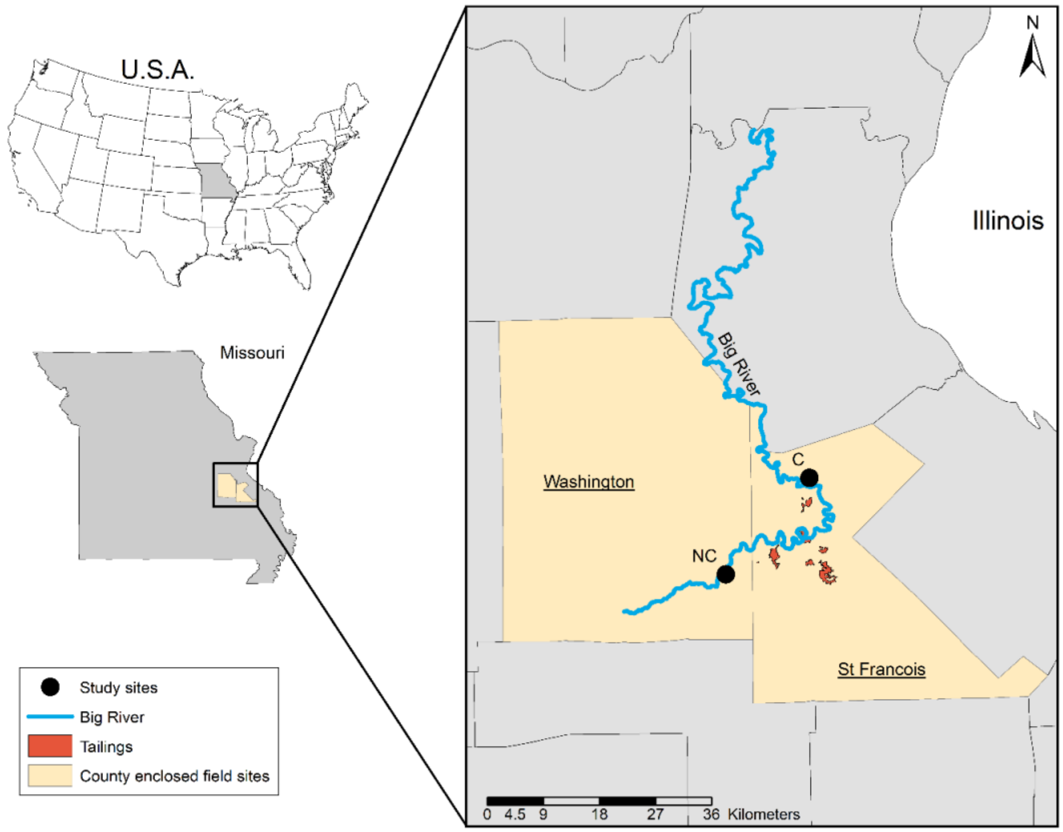

Figure 1. Map of Experiment A: Big River showing the study reaches. NC= reference reach, C = mining reach. Map created by Indigo T. Tran 2022.

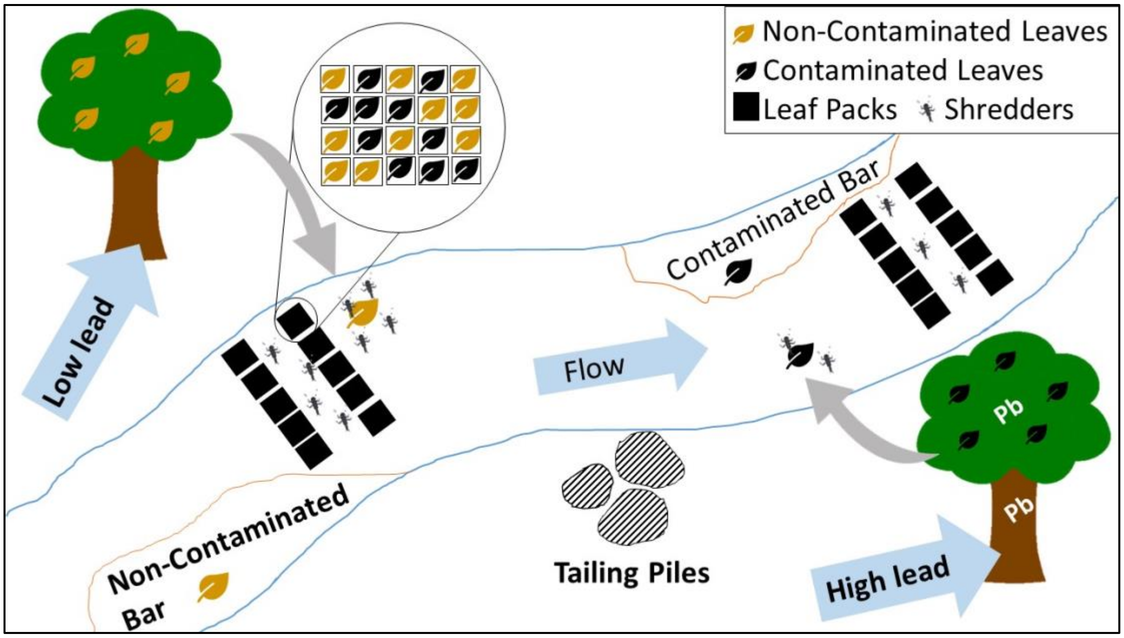

Figure 2. Diagram showing leaf pack grid composition and arrangement for Experiment A and B study sites. Figure is not to scale and is only meant to highlight the experimental setup.

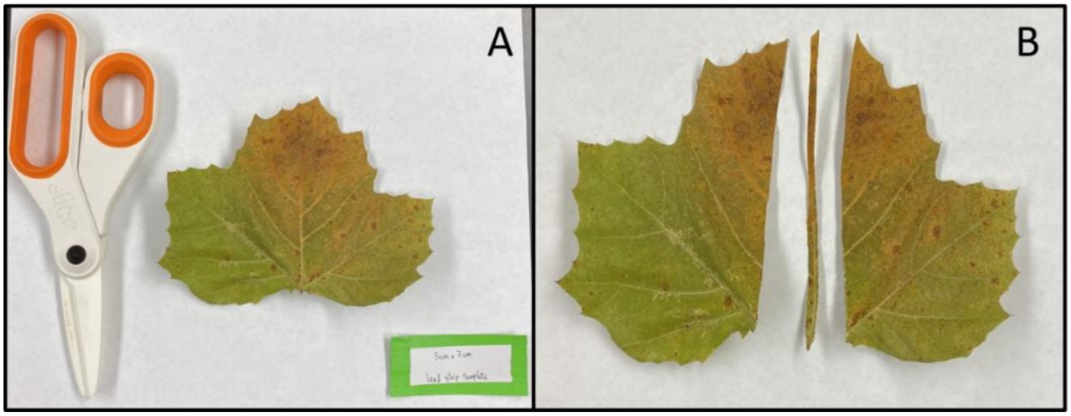

Figure 3. A) Leaf cutting materials; ceramic scissors and cutting guide B) leaf with main stem removed.

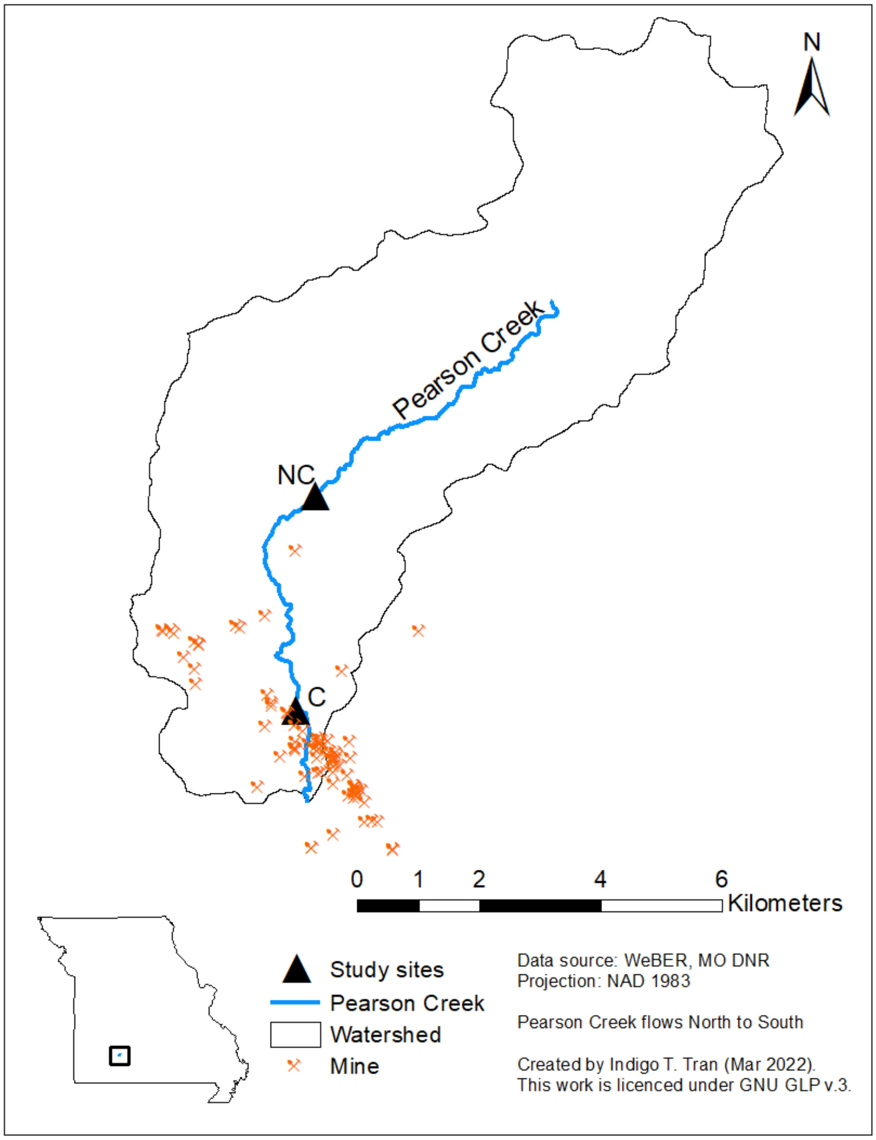

Figure 4. Map of Experiment B: Pierson Creek showing the study reaches. NC= reference reach, C= mining reach. Map created by Indigo T. Tran 2022.

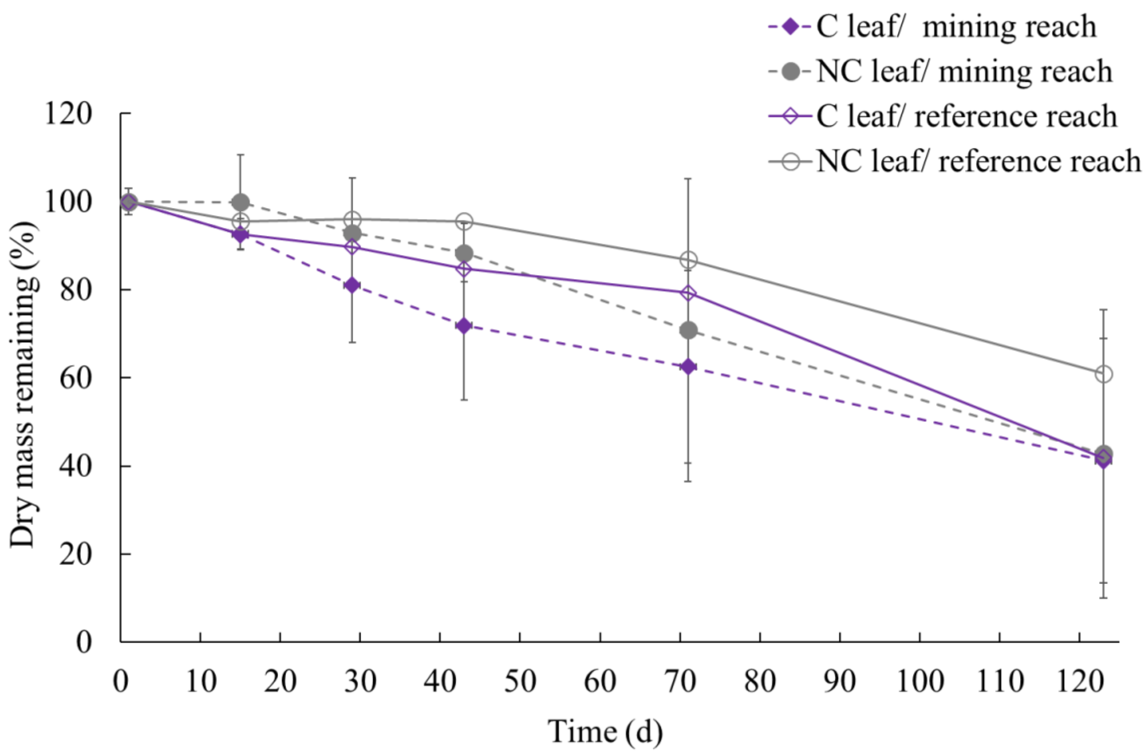

Figure 5. Dry mass remaining of contaminated (C) and non-contaminated (NC) sycamore leaf packs collected over the 123-day study in a mining and reference reach in the Big River, MO. Data represents the average (± standard error) of 3-10 leaf packs retrieved on each collection date per leaf type.

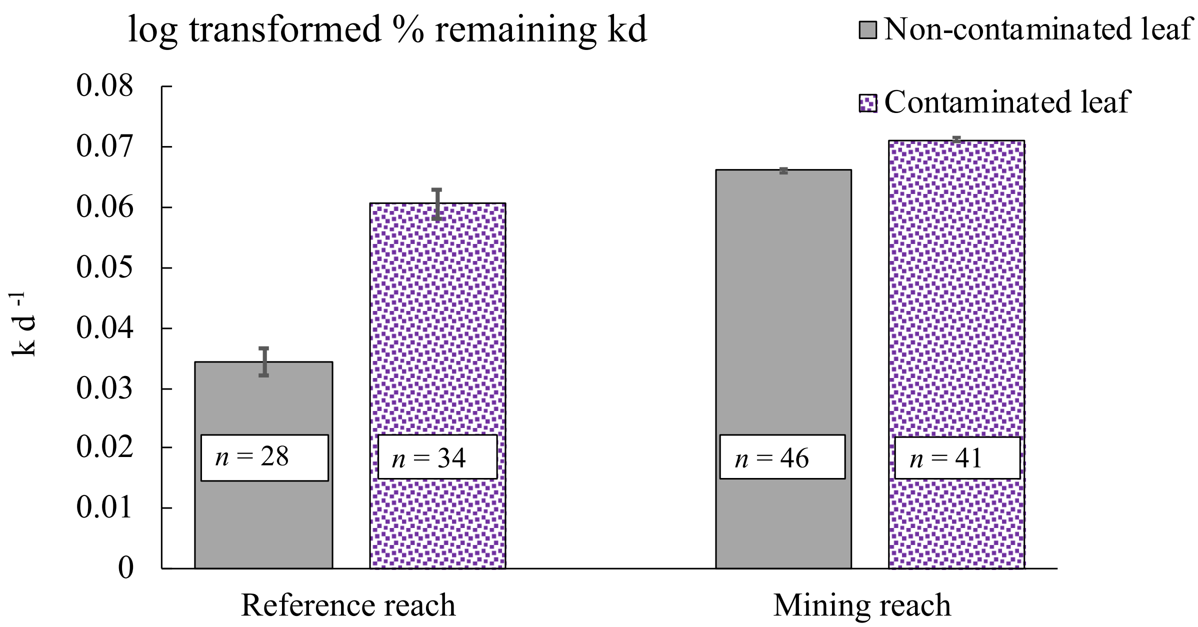

Figure 6. Comparison of sycamore leaf decomposition rates over the 123-day study expressed per day in the Big River, MO. Bars represent standard error of decomposition rates for contaminated or non-contaminated leaves in each reach. Number of leaf packs retrieved per reach and leaf type is indicated by *n*.

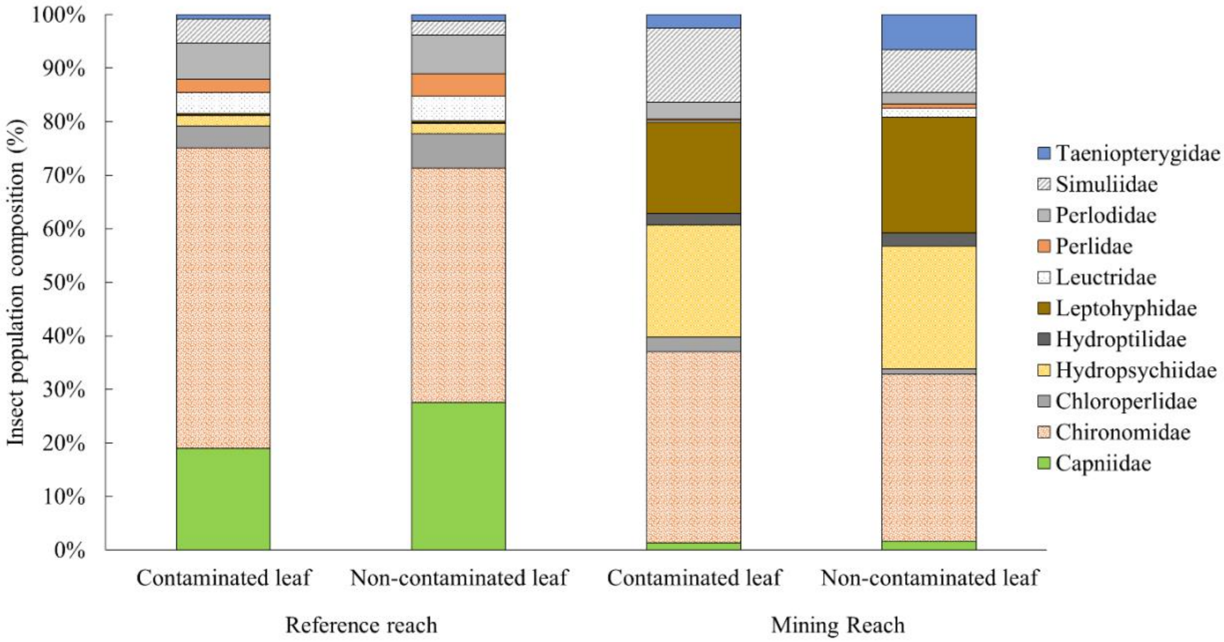

Figure 7. Percent composition of macroinvertebrate insect families collected from leaf packs in the mining and reference reaches of the Big River, MO over the 123-day study.

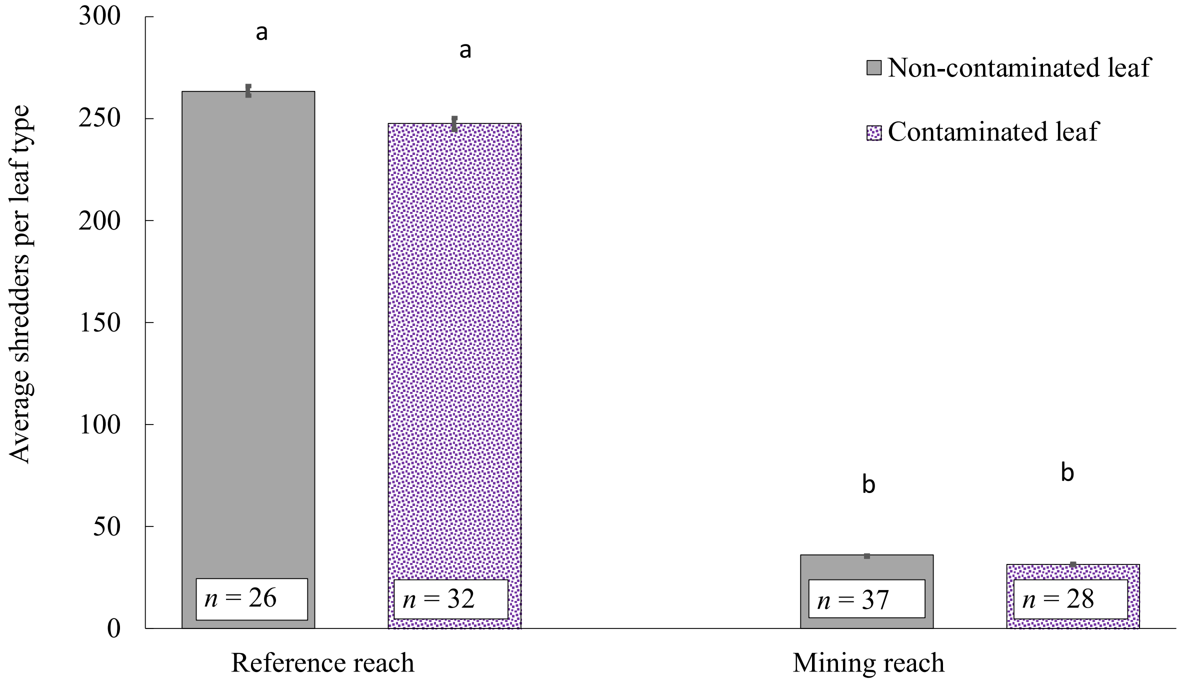

Figure 8. Average number of shredders collected from contaminated and non-contaminated leaf packs in a reference and mining reach over the 123-day study in the Big River, MO. Bars represent standard error. Different letters indicate significant differences (P<0.001) between reaches and leaf types. Number of leaf packs retrieved per reach and leaf type is indicated by n.

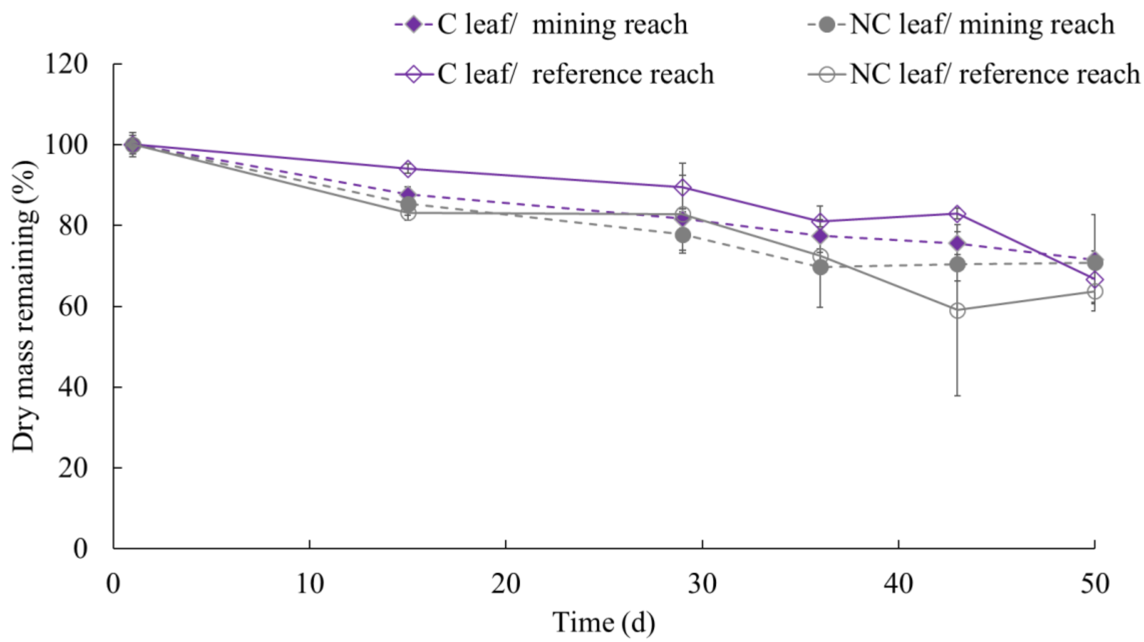

Figure 9. Dry mass remaining of contaminated (C) and non-contaminated (NC) sycamore leaf packs collected over the 50-day study in a reference and mining reach in the Pierson Creek, MO. Data represents the average (± standard error) of 5 replicate leaf packs retrieved on each collection date.

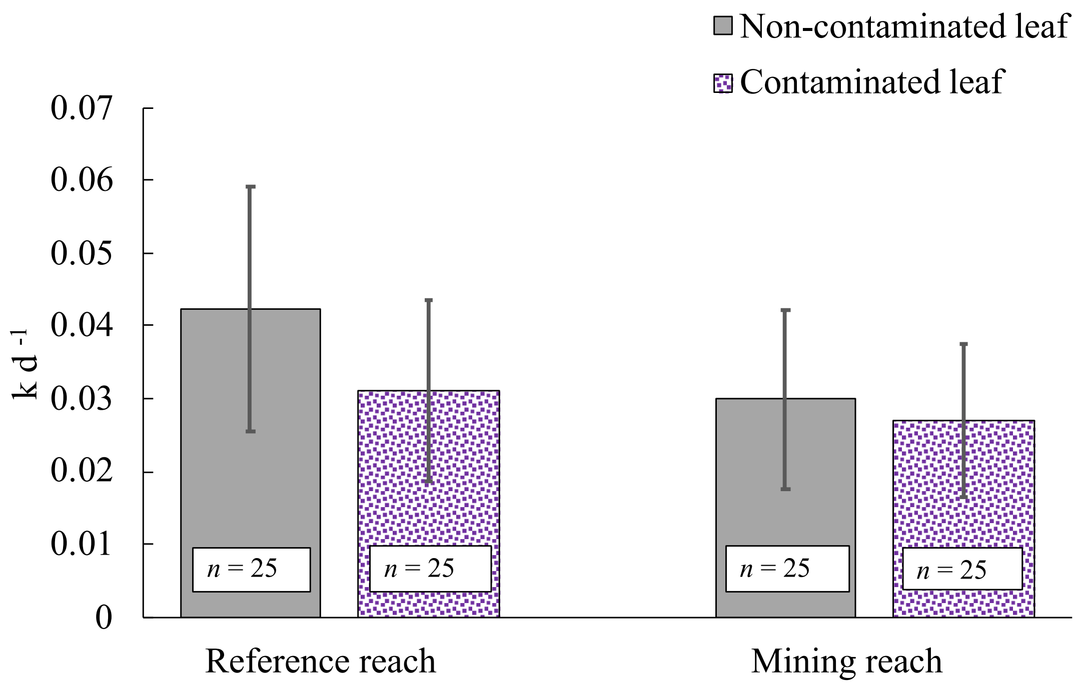

Figure 10. Comparison of sycamore leaf decomposition rates over the 50-day study expressed per day in Pierson Creek, MO. Bars represent standard error of decomposition rates for contaminated or non-contaminated leaves in each reach. Number of leaf packs retrieved per reach and leaf type is indicated by *n*.

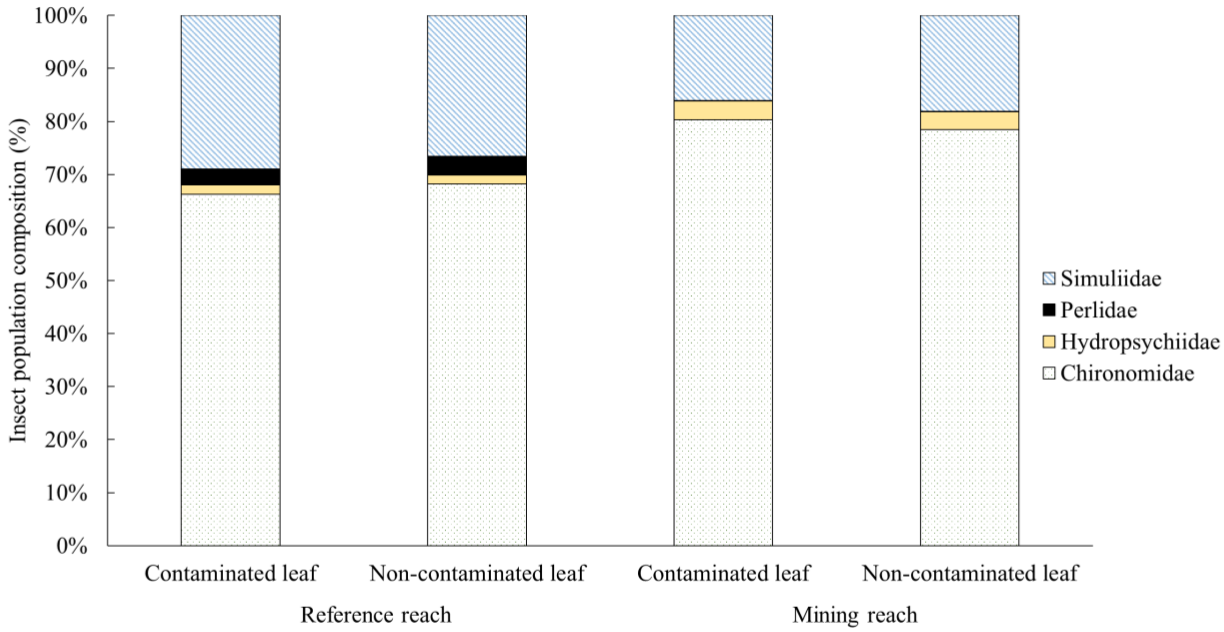

Figure 11. Percent composition of macroinvertebrate insect families collected from leaf packs in the mining and reference reaches of Pierson Creek, MO over the 50-day study.

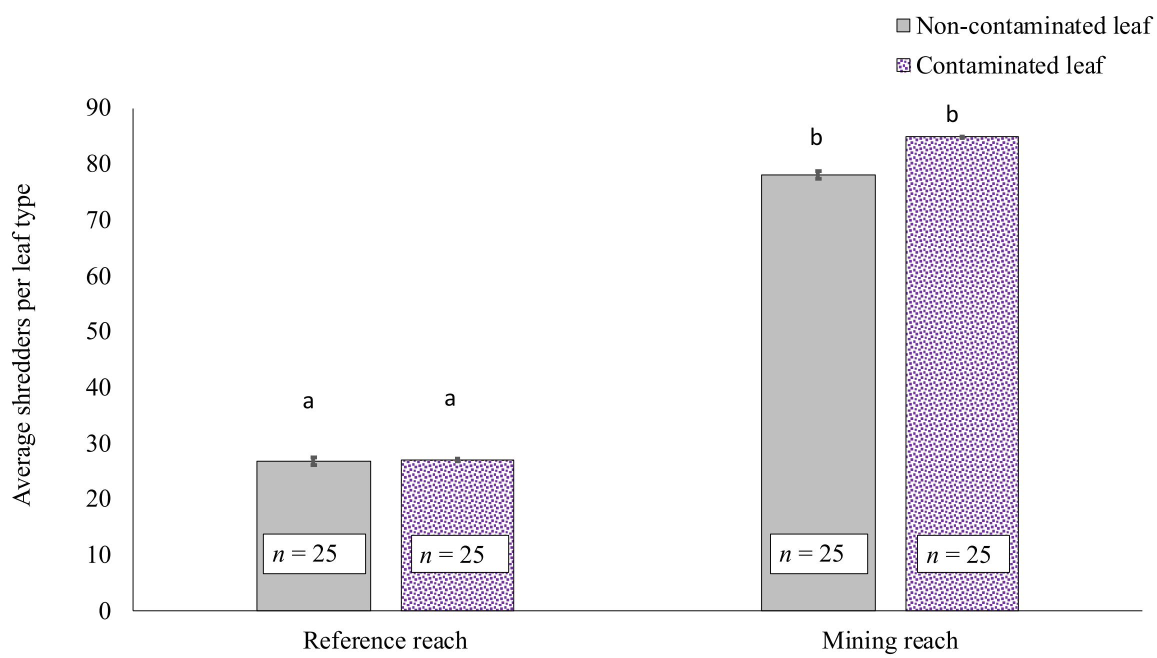

Figure 12. Average number of shredders collected from contaminated and non-contaminated leaf packs in a reference and mining reach over the 50-day study in Pierson Creek, MO. Bars represent standard error. Different letters indicate significant differences ($P<0.001$) between reaches and leaf types. Number of leaf packs retrieved per reach and leaf type is indicated by *n*.

APPENDIX

Appendix I. Descriptive information on identified aquatic macroinvertebrates.

Ia. Macroinvertebrates identified in Experiment A: Big River. "X" indicates presence within reach.

		Reach	
Order	**Family**	**Reference Reach**	**Mining Reach**
Coleoptera	Curculionidae	X	
Coleoptera	Elmidae	X	X
Coleoptera	Gyrinidae		X
Coleoptera	Hydraenidae	X	
Coleoptera	Hydrophilidae		X
Coleoptera	Psephenidae	X	
Coleoptera	Ptilodactylidae		X
Diptera	Anthericidae		X
Diptera	Ceratopogonidae	X	X
Diptera	Chironomidae	X	X
Diptera	Empididae	X	X
Diptera	Simuliidae	X	X
Diptera	Tipulidae	X	X
Ephemeroptera	Ameletidae	X	X
Ephemeroptera	Baetidae	X	X
Ephemeroptera	Ephemerellidae	X	X
Ephemeroptera	Heptageniidae	X	X
Ephemeroptera	Isonychiidae	X	X
Ephemeroptera	Leptohyphidae	X	X
Lepidoptera	Crambidae		X
Megaloptera	Corydalidae	X	X
Odonata	Calopterygidae	X	
Plecoptera	Capniidae	X	X
Plecoptera	Chloroperlidae	X	X
Plecoptera	Leuctridae	X	X
Plecoptera	Nemouridae	X	X
Plecoptera	Perlidae	X	X
Plecoptera	Perlodidae	X	X
Plecoptera	Pteronarcyidae		X

Plecoptera	Taeniopterygidae	X	X
Trichoptera	Apataniidae		X
Trichoptera	Brachycentridae		X
Trichoptera	Glossosomatidae	X	X
Trichoptera	Hydropsychiidae	X	X
Trichoptera	Hydroptilidae	X	X
Trichoptera	Leptoceridae		X
Trichoptera	Leptostomatidae	X	
Trichoptera	Philopotamidae	X	X
Trichoptera	Polycentropodidae	X	X
Trichoptera	Psychomyiidae	X	X
Trichoptera	Rhyacophilidae	X	
Trichoptera	Uenoidae	X	

Ib. Macroinvertebrates identified in Experiment B: Pierson Creek. "X" indicates presence within reach.

Order	Family	Reach	
		Reference Reach	**Mining Reach**
Ephemeroptera	Ameletidae	X	X
Amphipoda	Gammaridae	X	X
Coleoptera	Elmidae	X	X
Coleoptera	Psephenidae	X	X
Diptera	Chironomidae	X	X
Diptera	Empididae	X	X
Diptera	Pediciidae	X	X
Diptera	Simuliidae	X	X
Diptera	Tipulidae	X	X
Ephemeroptera	Ameletidae	X	X
Ephemeroptera	Baetidae	X	X
Ephemeroptera	Ephemerellidae	X	X
Ephemeroptera	Heptageniidae	X	X
Ephemeroptera	Leptohyphidae	X	
Isopoda	Asellidae	X	X
Odonata	Gomphidae	X	
Odonata	Coenagrionidae	X	
Odonata	Libellulidae	X	
Plecoptera	Chloroperlidae	X	
Plecoptera	Leuctridae	X	
Plecoptera	Nemouridae	X	X
Plecoptera	Perlidae	X	X
Trichoptera	Apataniidae	X	
Trichoptera	Glossosomatidae	X	X
Trichoptera	Hydropsychiidae	X	X
Trichoptera	Hydroptilidae	X	X
Trichoptera	Leptostomatidae	X	
Trichoptera	Odontoceridae	X	X
Trichoptera	Philopotamidae	X	X
Trichoptera	Phryganeidae	X	X
Trichoptera	Polycentropodidae	X	X
Trichoptera	Psychiomyiidae	X	X
Trichoptera	Rhyacophilidae	X	