



2016

EFFECTS OF STREAM DEGRADATION ON  
ECOSYSTEM FUNCTION ARE MEDIATED  
BY LOCAL MICROBIAL AND  
MACROINVERTEBRATE LEAF LITTER  
PROCESSING

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**EFFECTS OF STREAM DEGRADATION ON ECOSYSTEM FUNCTION ARE  
MEDIATED BY LOCAL MICROBIAL AND MACROINVERTEBRATE  
LEAF LITTER PROCESSING**

A Thesis  
Presented to  
the Faculty of the Watershed Studies Institute  
Murray State University  
Murray, Kentucky

In Partial Fulfillment  
of the Requirements for the Degree  
of Master of Science

by Carla J. Rothenbuecher  
December, 2016

## ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor, Dr. Howard Whiteman, for giving me the opportunity to pursue my passion in stream ecology and restoration. I am grateful to Howard for his professional guidance, as well as his sense of humor, and coffee and beer conversations. No matter how stressed or unsure I felt going in to a weekly meeting with Howard, I always felt more positive in facing the demands of research and coursework ahead of me. I am also grateful to Howard for the opportunity to live and conduct research in western Colorado (HLR and RMBL), so that I could strive to become more than just a “fish-head”.

This research was made possible by the generous funding from the High Lonesome Ranch (HLR), and the Graduate Innovation Assistantship (2014-2016), awarded by the Jones College of Science, Engineering, and Technology, at Murray State University. I especially want to thank the ranch for a beautiful and peaceful place to spend my summers, and the opportunity to be a part of their future research and stream restoration goals. In particular, I am grateful to Scott Stewart, Maren Smith, and HLR personnel for their technical support, field assistance, and opportunity to enjoy the occasional 5-star meal with other guests at HLR headquarters.

I would also like to thank thesis committee members, Drs. Michael Flinn, Paul Gagnon, and Chris Mecklin for their support and guidance, as well as challenging my general knowledge of ecology and statistics, which have helped me to become a better scientist. I am especially thankful to Gerry Harris, Karla Johnston, and Angela Hayden, at Hancock Biological Station, whose technical support and assistance in the lab was critical to completing this research.

This research was also made possible by grad students in the Whiteman Lab, particularly Scot Peterson, Ben Tumolo, Whitney Wallet, Donald Benkendorf, Quintin Bergman, and Kaylin Boeckman. I want to thank them for their assistance in the field and lab, as well as being a sounding board to discuss my research ideas and methods. Last but certainly not least, I am forever indebted to my parents, Jeanne and Charley, for their unwavering love and support, as well providing a restful and beautiful retreat back home in Montana. Finally, I'd like to express my sincere gratitude to my friends Scot Peterson, Whitney Walleth, Mel Torres, Ben Tumolo, Leah Brown, and Kate Schaefer, for all the laughter, support, and general shenanigans that brightened my day and helped me get through graduate school.

## ABSTRACT

Riparian areas are fundamental to aquatic ecosystems by regulating temperature and light regimes, and providing allochthonous subsidies critical to the survival of aquatic and terrestrial species. On a global scale, increased rates of anthropogenic disturbance from agricultural activities and urbanization have caused the degradation of aquatic habitats. As a result, billions of dollars have been spent on stream restoration projects to protect aquatic resources; however, fundamental ecosystem processes like litter decomposition are rarely addressed. We conducted a litterbag experiment in a degraded high desert stream proposed for large-scale restoration to test the effects of reach location, canopy cover, and temperature on the rates of leaf litter breakdown attributed to microbial activity and macroinvertebrate shredders. Results from coarse mesh litterbags indicated that total leaf breakdown rates per degree-day were significantly faster in upstream, less degraded reaches, associated with higher abundance of shredders. In contrast, the main driver of leaf mass loss due to microbial activity in fine mesh litterbags was water temperature, which was significantly warmer in downstream reaches. Location effects, including temperature differences between upstream and downstream reaches, also depended on canopy cover and leaf species. Overall, the relative proportion of leaf mass loss attributed to microbial activity increased with increasing cumulative degree-days, while leaf mass loss attributed to shredders decreased. Reduced leaf processing rates by shredders in the degraded downstream reaches could further affect the timing and availability of suspended food resources to other detritivore and higher-level consumers downstream. These shifts in microbial and shredder-mediated leaf litter processing could have bottom-up effects via heterotrophic

energy pathways, especially in degraded streams subject to warming. Furthermore, the significant variation in litter decomposition we observed underscores the importance of measuring ecosystem function across multiple scales within a given stream in order to assess relative levels of degradation and guide future restoration efforts.

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## **INTRODUCTION**

Riparian areas are critical to the maintenance of terrestrial and aquatic biodiversity, while providing numerous ecosystem services such as habitat connectivity, water purification, flood control, and organic resource subsidies (Kominoski et al. 2013, Pollock et al. 2014). In stream ecosystems, allochthonous inputs from riparian vegetation are important for the flow of energy in streams, via the decomposition of litter (Allan and Castillo 2007). Mechanisms of litter decomposition mediate bottom-level processes and consumer interactions across multiple trophic levels, which are fundamental to stream ecosystem function (Gessner and Chauvet 2002, Leroux and Loreau 2008, Marcarelli et al. 2011).

On a global scale, the degradation of rivers and streams has become ubiquitous. The subsequent loss of riparian habitat and biodiversity associated with livestock grazing, conversion of land for agriculture, and increased rates of urbanization are a growing concern for the preservation of aquatic ecosystems (Grimm et al. 2000, Walsh et al. 2005, Feld et al. 2011, Batchelor et al. 2015). These human land-use practices directly alter riparian plant composition and structure, thus influencing the quantity and quality of allochthonous inputs to streams (LeRoy and Marks 2006, Richardson et al. 2007). In addition, degradation of riparian habitat can alter water temperature and light regimes, decrease species diversity, and facilitate invasion of exotic species (Pringle et al. 1988, Kaushal et al. 2010). As a result, these disturbances have cascading effects to primary

consumer community assemblages and bottom-up processes, like litter decomposition (Li et al. 1994, Kominoski et al. 2013).

In North America, billions of dollars have been spent on stream restoration to mitigate increasing rates of aquatic degradation (Burnhardt et al. 2005, Lake et al. 2007, Burchsted et al. 2010, Wohl et al. 2015). Stream restoration projects commonly include riparian plantings as a means of improving water quality, fisheries resources, and recreational opportunities (Kauffman et al. 1997, Aldridge et al. 2009, Giling et al. 2009). Yet stream and river restorations often fail to meet expectations for biological recovery while fundamental ecosystem processes like litter decomposition are frequently ignored (Ward et al. 2001, Palmer et al. 2006, Johnson et al. 2014).

The breakdown of leaf litter is a dynamic multi-step process, primarily involving microbial conditioning by bacteria and fungi, followed by mechanical fragmentation and consumption by macroinvertebrate detritivores (Anderson and Sedell 1979, Webster and Benfield 1986). Macroinvertebrate detritivores (shredders) depend on litter as a source of food and habitat, and contribute significantly to aquatic nutrient cycling in the process (Hieber and Gessner 2002, Gessner et al. 2010). In general, high quality leaves (low C:N ratio) are preferred by shredders and have been strongly correlated to local macroinvertebrate richness and colonization (Kaushik and Hynes 1971, Peterson and Cummins 1974, Layer et al. 2012). Similarly, high quality leaves are also colonized by aquatic hyphomycetes (fungi) more rapidly than nutrient-limited, low quality leaves, thus increasing leaf palatability for shredders (Pastor et al. 2014, Graça et al. 2015b, Ferreira et al. 2015a). However, species-specific leaf breakdown rates and the relative contribution of shredders to overall leaf breakdown are highly variable among streams

due to differences in seasonality (Anderson and Sedell 1979, Murphy and Giller 2000), adaptations of detritivores to locally derived riparian subsidies (Jackrel and Wootton 2014), and anthropogenic disturbance (Wenger et al. 2009, Wahl et al. 2013).

Deciphering the dominant mechanisms regulating leaf breakdown in a given stream is inherently complex (Jones and Swan 2015), and generalizations about the relative contribution of decomposers to litter decomposition across aquatic ecosystems remain inconclusive (García-Palacios et al. 2016). It is thus not surprising that few studies have addressed the effects of multiple stressors on litter decomposition in degraded streams. These few studies often show contrasting results, in part due to confounding effects of type and intensity of anthropogenic stressors like pollution, erosion, and habitat fragmentation (Hagen et al. 2006, Ferriera et al. 2015a, Nilsson et al. 2015). For example, factors such as increased water temperature or increased nutrient concentrations from organic pollution can accelerate total leaf breakdown rates as a result of decreased leaf toughness (Foucreau et al. 2016) and increased metabolic activity of litter-colonizing fungi (Fernandes et al. 2009, Ferreira et al. 2015b). In contrast, changes in water quality such as increased acidity due to low dissolved oxygen concentrations or increased rates of sedimentation often decelerate leaf breakdown rates (Young et al. 2008, Cornut et al. 2010).

The purpose of this study was to investigate potential shifts in litter decomposition processes due to riparian disturbance, as a way of evaluating current ecosystem function in a degraded stream. In addition, as part of a pre-restoration baseline-monitoring project, this research was intended to help assess the efficacy of future restoration efforts. We predicted that disturbed stream reaches with reduced

riparian canopy cover would have lower densities of shredding macroinvertebrates due to decreased litter subsidies, which would subsequently decrease leaf breakdown rates. However, we also predicted that loss of streamside canopy cover would increase irradiance and also water temperature, which could accelerate microbial metabolism and increase leaf breakdown rates (Fernandes et al. 2009). To resolve these conflicting predictions, we measured multiple biotic and abiotic parameters simultaneously, as an integrated approach to understanding shredder- and microbially-mediated leaf processing along a degradation gradient.

## **METHODS**

### *SITE DESCRIPTION*

Kimball Creek, a degraded 3<sup>rd</sup> order stream that drains into Roan Creek, is a tributary of the Colorado River (Garfield Co., Colorado, USA). Throughout the 19<sup>th</sup> and 20<sup>th</sup> centuries, Kimball Creek experienced extensive stream channel and riparian habitat degradation from heavy cattle grazing, irrigation, diversion dams, and eradication of beaver (*Castor canadensis*, R.Lee, pers.comm.). In reaction to this degradation, the High Lonesome Ranch (HLR) has proposed to restore approximately 17 km of Kimball Creek to pre-European settlement conditions, including reintroduction of native Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus*).

Cattle distribution and grazing pressure in the Kimball Creek valley vary spatially and temporally, providing a unique means of evaluating the effects of riparian disturbance on ecosystem function across multiple habitat patches along the stream. During our study period, approximately 250 cattle were present intermittently throughout the valley from April to mid November 2015. In general, lightly grazed or ungrazed areas

were characterized by dense deciduous and coniferous tree species, whereas heavily grazed areas had significantly reduced woody tree species diversity and canopy cover, with streambanks dominated by graminoid species and some willow (*Salix spp*).

We selected four stream reaches, two upstream (100 m apart) and two approximately 4 km downstream from the upper reaches (400 m apart). For each upstream and downstream pair, we selected one reach with an open streamside canopy, while the other was shaded by denser woody vegetation. Reaches were subsequently named USO (upstream, open), USS (upstream, shaded), DSS (downstream, shaded), and DSO (downstream, open). This experimental design allowed us to test the effects of location (upstream vs. downstream) and canopy (open vs. shaded) on litter decomposition along a degradation gradient. All reaches were 100 meters in length, with similar elevation (1974 – 2064 m) and gradient (3 – 11 m/100 m).

#### *LITTERBAG EXPERIMENTS*

We conducted our experiments between June 15 and July 20, 2015, based on general methods in Benfield (2006). To understand how different leaf species with known contrasting breakdown rates would affect decomposition in these disparate environments, we used two native tree species common in Kimball Creek: box-elder (*Acer negundo* L.), a “medium” decomposing species, and gamble oak (*Quercus gambelii* Nutt.), a “slow” decomposing species (Webster and Benfield 1986). In autumn 2014, leaves were collected after abscission using landscape fabric pinned to the ground at the base of three to five trees of each species. Dry senesced leaves were separated by species, placed in black trash bags, and stored indoors at ambient temperature over winter.

We constructed our litterbags in early June 2016. Stored leaves were placed into mesh laundry baskets and air-dried indoors for 10 days at ambient temperature until constant dry mass was obtained. Leaves were weighed into five-gram portions and placed into single-species coarse mesh (4 mm) litterbags measuring 20 cm x 16 cm. Half of the coarse mesh litterbags were then placed into fine mesh nylon bags (approx. 250- $\mu$ m) to exclude macroinvertebrates and estimate microbial leaf breakdown (Nelson and Anderson 2007, Tiegs et al. 2008). Once filled, each litterbag was stapled shut on the open end and marked with different color plastic zip-ties to distinguish leaf species. There were 240 litterbags, including 60 per treatment of coarse mesh box-elder (A), fine mesh box-elder (AF), coarse mesh gamble oak (Q), and fine mesh gamble oak (QF). To account for leaf mass loss due to handling, three additional litterbags of each treatment were placed in the stream, immediately removed, and later processed with all other litterbags. The average remaining leaf mass ( $n = 3$ ) of litterbags designated for handling loss was used as the initial leaf mass of each treatment (Benfield 2006).

In each reach, twelve transects were delineated in riffle habitat, which were evenly distributed 8 to 10 m apart. Transects consisted of three 61-cm metal rebar, where one rebar was placed perpendicular to stream flow and secured underwater using one rebar on each side that were anchored to the streambed. Five litterbags per treatment (A, Q, AF, QF) were randomly assigned to three of the 12 transects (i.e. blocks) within each reach. At each transect, the litterbags were evenly distributed and zip-tied to the submerged rebar and anchored several centimeters above the benthic substrate (McKie and Malmqvist 2009, LeRoy et al. 2014). Three replicate litterbags (one per transect) of each treatment were removed every week for five weeks. As litterbags were removed



from the water, a 250- $\mu\text{m}$  sieve was held underneath to collect any dislodged macroinvertebrates. Individual litterbags were placed into Whirl-Paks, transported on ice to the laboratory, and frozen for later processing (Gulis et al. 2006). During the fourth and fifth week of the experiment, beavers recolonized several small portions of the upstream reaches, resulting in the loss of one Q litterbag in the USO reach and one AF litterbag in the USS reach.

In the laboratory, frozen litterbags were thawed at room temperature and leaves were rinsed with tap water over a coarse (1mm) and fine (250  $\mu\text{m}$ ) nested sieve series to remove sediments and collect macroinvertebrates (Klemmer et al. 2012). Leaves were dried at 50°C for 48 hours to a constant mass, weighed, and homogenized with mortar and pestle while wearing latex gloves. Subsamples of ground leaves were combusted in a muffle furnace at 550°C for one hour to determine remaining ash-free dry mass (AFDM; Benfield 2006).

Macroinvertebrates were processed from litterbags collected after 7, 21, and 35 days submergence. After the leaves were washed, macroinvertebrates were removed from the coarse and fine sieve separately and preserved in 70% ethanol. All macroinvertebrates from the coarse sieve and a fractionated portion of the fine sieve (using a Folsom plankton splitter, Wildlife Supply Co.) were counted and measured to the nearest millimeter under a dissecting scope. Specimens were identified to the lowest practical taxonomic level (usually genus) and categorized by functional feeding group (using Smith 2001, Ward et al. 2002, Merritt et al. 2008, and Wiggins 2009). Biomass (mg AFDM) was estimated using length-mass relationships (as per Burgherr and Meyer 1997 and Benke et al. 1999). Adult and larval stages of aquatic beetles (Coleoptera) were

considered separate taxa given they exhibit different feeding strategies and habitat preferences at these life stages. Aquatic insect pupae were also excluded from analyses given they are a non-feeding life history stage (Huryn and Wallace 2000).

#### *ENVIRONMENTAL PARAMETERS*

Throughout the experiment, multiple water quality and habitat variables were measured at each transect (Platts et al. 1983) to assess variation in degradation between reaches. At each transect, wetted width (cm) and average water depths (cm,  $n = 4$ ) were taken every week. Streamside canopy cover (%) was also measured weekly, using a spherical densiometer held above the water's surface on the left and right streambank at each transect (Fitzpatrick et al. 1998). Incident solar radiation was estimated by measuring light level (lumens/m<sup>2</sup>, or lux) near the water surface, which was monitored continuously at 15-min intervals with two HOBO pendant data loggers (Onset, UA-002-64) secured above the water surface on a rebar post of two haphazardly selected transects within each reach. At the end of the experiment, benthic substrate composition of each reach was estimated using a pebble count (10 samples per transect), and particles were categorized into size classes using the Wentworth scale (Cummins 1962).

Water temperature was monitored continuously at 15-min intervals, using two HOBO pendant data loggers (Onset, UA-002-64), which were secured to a transect rebar post at the downstream end of each reach. Every week at the downstream end of each reach, dissolved oxygen (mg/L), pH, and specific conductivity (SPC,  $\mu\text{S}/\text{cm}$ ) was measured using a YSI Professional Plus probe, and average turbidity (NTU,  $n = 3$  readings per water sample) was measured with a LaMotte 2020wi turbidimeter. Weekly discharge (m<sup>3</sup>/s) was estimated using the average of three flow measurements, taken at

the downstream, middle, and upstream section of each reach with a Swiffer Model 2100 current velocity meter. Each week at the downstream end of each reach, grab samples of water were collected with sterilized 125 mL plastic bottles that were then transported on ice to the laboratory and frozen. At Hancock Biological Station, Murray, KY, water samples were processed for nutrients (mg/L) including ammonium ( $\text{NH}_4^+$ ), nitrite-nitrate ( $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ), and soluble reactive phosphorus (SRP), using a Lachat QuikChem Flow Injection Analyzer (Lachat Instruments, Milwaukee, USA).

### *STATISTICAL ANALYSES*

#### *Litter decomposition*

All statistical analyses were tested using  $\alpha = 0.05$ . Leaf breakdown rates per degree-day ( $k_{\text{dd}}$ ) were calculated for each litterbag treatment (A, Q, AF, QF) in each reach using linear regression of  $\log_e(x+1)$  transformed remaining AFDM (%) and cumulative degree-days of each weekly submergence period (Benfield 2006). To account for temperature effects, we used cumulative degree-days instead of days, equal to the sum of daily average water temperature over submergence period (McArthur et al. 1988, Allan and Castillo 2007). We used a general linear mixed model (GLMM) to test effects of location, canopy, and temperature (degree-days) and their interactions on leaf breakdown rates of each litterbag treatment per reach. The remaining AFDM (%) per litterbag was the response variable, location and canopy were each treated as fixed effects, and the variation among the three litterbag replicates were treated as random effects (Zuur et al. 2009). Degree-days were treated as covariates to account for heterogeneity of variance in leaf decomposition with increasing time (Bolker et al. 2008, Boyero et al. 2011). We used a GLMM rather than ANOVA because of our unbalanced

design due to the unexpected litterbag removal by beaver (Zuur et al. 2009). Analyses were performed using R v.3.1.3 (R Development Core Team, 2015).

To visualize patterns in shredder macroinvertebrates found in litterbags, we used non-metric multidimensional scaling (nMDS) with Bray-Curtis similarity matrices of log (x+1) transformed abundance and 4<sup>th</sup> root transformed biomass estimates (Clark et al. 2014, LeRoy et al. 2014). We conducted a permutational multivariate analysis of variance (PERMANOVA) to test effects of fixed factors of location, canopy, and submergence time (days) on shredder abundance and biomass, using 999 permutations to generate the sampling distribution of the pseudo-F test statistic and the permutation test p-value (Anderson et al. 2008). When significant differences in abundance or biomass occurred, pairwise comparisons were used to identify contrasts between reaches of a given factor. We performed PERMANOVA on box-elder and gamble oak separately as we were not interested in testing the effect of leaf species on macroinvertebrate abundance or biomass given their different leaf breakdown rates *a priori*. All macroinvertebrate community analyses were implemented in PRIMER v7 (Clarke and Gorely 2015).

The relative leaf mass loss attributed to shredders or microbes was estimated using mean (N = 3) AFDM (%) loss after 35 days, from coarse mesh and fine mesh litterbags. Leaf mass loss estimates were calculated as total (coarse mesh), microbial (fine mesh), and the difference between the two (shredders). The ratio of microbe to shredder leaf mass loss was calculated (Taylor and Chauvet 2014), and compared based on reach location and canopy cover, while accounting for temperature (cumulative degree-days).

### *Environmental Reach Parameters*

Multivariate Analysis of Variance (MANOVA) was used to compare environmental parameters based on reach (Bray and Maxwell 1982, Richardson et al. 2011), using weekly mean values ( $n = 5$ ) of DO, pH, SPC, turbidity, ammonium, SRP, nitrite-nitrate, water temperature, canopy, discharge, water depth, wetted width, and light level (Table 3). When significant relationships were detected of a given variable, a Tukey's HSD test was used to conduct pairwise comparisons between reaches (Rubbo and Kiesecker 2004). Benthic substrate composition was analyzed using Pearson's Chi-Square ( $\chi^2$ ) to test the effect of reach on the relative distribution of particles based on size-class (cobble, pebble, gravel, sand, silt).

## **RESULTS**

### *TOTAL LEAF BREAKDOWN*

In coarse mesh litterbags, box-elder leaf breakdown rates ( $k_{dd}$ ) differed significantly by location where leaves decomposed 1.7 to 2 times faster in upstream reaches (Table 1, Fig. 1A). This result was likely driven by several significant two-way interactions, including location\*canopy ( $F_{1,56} = 7.44$ ,  $p = 0.03$ ), location\*degree-days ( $F_{1,56} = 73.5$ ,  $p < 0.001$ ), and canopy\*degree-days ( $F_{1,56} = 4.17$ ,  $p = 0.046$ ). In contrast, coarse mesh gamble oak decomposed 4.2 to 5.5 times slower than box-elder (Table 1). While gamble oak leaf breakdown rates did not differ significantly by location (Table 1, Fig. 1B), there were significant interactions of location\*degree-days ( $F_{1,55} = 14.9$ ,  $p < 0.001$ ) and location\*canopy\*degree-days ( $F_{1,55} = 5.0$ ,  $p = 0.03$ ).

A total of 72 macroinvertebrate taxa were identified in coarse mesh litterbags, seven of which were classified as shredders (Merritt et al. 2008), including an adult and

larval beetle (*Peltodytes*), crane fly larvae (*Tipula*), stonefly larvae (*Amphinemura*), and three caddisfly larvae (*Amphicosmoecus*, *Hesperophylax*, *Psychoglypha*). Non-metric multidimensional scaling (nMDS) ordination showed that shredder abundance (Fig. 2A) and biomass (Fig. 2B) in litterbags varied based on location and canopy. These findings were supported by PERMANOVA, where shredder abundances were greater in the upstream reaches for both leaf species (Fig. 3). For box-elder, there was a significant location\*canopy interaction (pseudo- $F_{1,24} = 3.99$ ,  $p = 0.03$ ) as well as a significant main effect of location (pseudo- $F_{1,24} = 7.56$ ,  $p = 0.003$ ) on shredder abundance. However for gamble oak, there was only a significant main effect of location on shredder abundance (pseudo- $F_{1,23} = 5.89$ ,  $p = 0.01$ ).

There was no significant difference in shredder abundance in box-elder litterbags between shaded and open reaches (Fig. 3A, B). However in gamble oak litterbags, shredder abundance was significantly greater in the shaded reaches (pseudo- $F_{1,23} = 4.91$ ,  $p = 0.01$ ), which was primarily driven by greater numbers of *Amphinemura* stonefly larvae (Fig. 3C, D). Although shredder abundances were variable over time, the main effect of submergence time was only significant on gamble oak litterbags (pseudo- $F_{1,23} = 2.87$ ,  $p = 0.03$ ), where abundance of *Amphinemura* stonefly larvae was greatest at 21 days in ( $p = 0.02$ ), except in the upstream open reach (Fig. 3C, D).

Analyses of shredder biomass showed contrasting results, with no significant interactions between factors. Location had a significant main effect on shredder biomass of box-elder litterbags (pseudo- $F_{1,24} = 3.39$ ,  $p = 0.04$ ), which was greater overall in upstream reaches (Fig. 4A, B). For both leaf species, shredder biomass was significantly

greater in the open canopy reaches (both  $F > 3.84$ , both  $p < 0.03$ ), mainly due to biomass of *Tipula* larvae (Fig. 4).

#### *MICROBIAL LEAF BREAKDOWN*

Fine-mesh litterbags excluded nearly all macroinvertebrates, except some early-instar larvae (mostly chironomids [approx. 0 – 250/litterbag] and *Amphinemura* shredders [approx. 0 – 30/litterbag]). Box-elder decomposed faster than gamble oak in fine mesh bags, and there was little variation in leaf breakdown rates within each leaf species (Table 1, Fig. 1C, D). Results from the GLMM indicated significant main effects of location (both  $F > 53.7$ , both  $p < 0.0001$ ) and degree-days (both  $F > 782.2$ , both  $p < 0.0001$ ) on microbial leaf breakdown of both leaf species. However, *posthoc* analyses of box-elder and gamble oak showed that there were no significant differences in leaf breakdown rates based on location or canopy cover.

#### *MICROBIAL VERSUS SHREDDER LEAF BREAKDOWN*

Leaf mass loss (AFDM %) after 35 days submergence due to microbial activity was greater than leaf breakdown due to shredders, for both leaf species and across all reaches (Table 2). In upstream reaches, microbial leaf mass loss of box-elder was nearly two times that of leaf mass loss due to shredders, and four to nine times greater in downstream reaches. Trends were similar for gamble oak in downstream reaches, where microbial leaf mass loss was approximately three to seven times greater than leaf mass loss attributed to shredders. Additionally, for both leaf species, the ratio of microbe to shredder leaf mass loss within downstream reaches was over two times greater in the shaded reach compared to the open reach (Table 2). A comparison of these ratios with

cumulative degree-days showed a positive exponential relationship between the relative leaf mass loss due to microbial activity and water temperature (Fig. 5).

#### *DEGRADATION LEVEL BASED ON REACH CONDITIONS*

Nearly all habitat and water quality parameters differed significantly between reaches (MANOVA, Wilks'  $\lambda = 2.2 \times 10^{-8}$ ,  $F = 94.5$ ,  $p < 0.001$ ), with the exception of discharge and SRP (Table 3). *Posthoc* analyses showed that several of these parameters differed between but not within locations, where mean wetted width and nitrite-nitrate concentrations were greater in upstream reaches, and mean SPC and water temperatures were greater in downstream reaches (Table 3). Although significant differences in ammonium occurred, concentrations were either below or only slightly above the method detection limit (MDL) of 0.004 mg/L.

Both canopy cover and light level data confirmed our *a priori* designation of open and closed canopy reaches. Mean percent canopy cover in open reaches was 52.1 and 54, compared to 86.4 and 85.2 in shaded reaches ( $F_{3,15} = 91.3$ ,  $p < 0.001$ ). Similarly, average light levels in open reaches was more than twice that of shaded reaches, 48,347 to 23,414 lux, respectively ( $F_{3,15} = 8.1$ ,  $p < 0.01$ ; Table 3). Benthic substrate composition was also significantly different between reaches ( $\chi^2 = 52.5$ ,  $df = 12$ ,  $p < 0.0001$ ), where downstream reaches had greater proportions of smaller sand particles and fewer larger sized pebbles, compared to upstream reaches.

#### **DISCUSSION**

In this degraded system, reduced riparian vegetation and warmer water temperature affected litter processing across multiple trophic levels (i.e. primary consumer macroinvertebrates and microbial decomposers) in contrasting ways. The



significant variation in leaf breakdown rates was primarily driven by effects of reach location and streamside canopy cover, mediated by differences in local shredder communities and water temperature. Previous studies have examined the effects of temperature on microbial and shredder leaf litter breakdown across multiple streams along latitudinal (Irons et al. 1994, Boyero et al. 2011) and altitudinal gradients (Taylor and Chauvet 2014); however to our knowledge, this study is novel in examining within-stream variation of summer litter decomposition rates affected by alterations in water temperature and riparian canopy cover.

The relative abundance and diversity of shredder macroinvertebrates is strongly linked to the breakdown of leaf litter in temperate streams (Webster and Benfield 1986, Pomeroy et al. 2000, Sponseller and Benfield 2001, Graça et al. 2015a). The greater abundance of shredders in upstream reaches, primarily *Amphinemura* stoneflies, largely explains the faster box-elder breakdown rates in the upstream reaches. This also explains the faster leaf breakdown rates in coarse mesh litterbags compared to fine mesh litterbags of both leaf species (Table 1).

However, the distribution of macroinvertebrates in streams can be patchy, both at microhabitat and reach scales (Wright and Li 2002). Between the upstream and downstream reaches, differences in shredder communities could also have been a result of reach-specific water quality or habitat conditions. Increased amounts of fine sediments can bury leaves in the substratum, limiting access to macroinvertebrates (Cornut et al. 2010). The significantly higher proportions of smaller sand-sized particles in downstream reaches may have inhibited access of detritivores to leaves. Moreover, aquatic insect EPT taxa (orders Ephemeroptera, Plecoptera, and Trichoptera) require relatively cool

temperatures and are more sensitive to disturbance (Wallace et al. 1996, Quinn et al. 2004). The 5 to 6° C increase in water temperature observed in the downstream reaches may have limited abundance of *Amphinemura* stoneflies (order Plecoptera; Fig. 3) or other EPT shredder taxa, subsequently decreasing total leaf breakdown rates.

In our study, a 30% change in canopy cover significantly affected shredder abundance and biomass in contrasting ways. Similarly, other studies show varying results regarding the effects of reduced canopy cover on aquatic macroinvertebrate communities. For example, Hawkins et al. (1982) found that macroinvertebrate abundance was significantly lower in clearcut streams compared to forested streams. Whereas McKie and Malmqvist (2009) found no significant differences in macroinvertebrate abundance or diversity between forested and clearcut streams, and that leaf mass loss per degree-day was greater in clearcut streams. In Kimball Creek, shredder abundance in litterbags was greater in shaded reaches, yet in the downstream location, leaf mass loss due to shredders was more than two times greater in the open reach, compared to the shaded reach (Table 2). Solar radiation has been shown to negatively affect shredder abundance, such as in high desert streams (Tait et al. 1994). However our results suggest there was a positive effect of light on leaf breakdown rates in the downstream location, despite significantly lower shredder abundance. In addition, shredder biomass in litterbags was significantly greater in open reaches. Greater biomass, mainly *Tipula* larvae in open reaches, may have resulted from greater food resources due to increased light levels, which have been shown to increase algal growth and biomass of biofilm on submerged leaves (Franken et al. 2005).

Several studies have found a significant positive correlation between microbially mediated litter decomposition and temperature (Melillo et al. 1984, Fenoy et al. 2016). After 35 days submergence, microbial leaf mass loss in fine mesh litterbags was greater downstream, and was likely caused by increased microbial activity due to significantly warmer temperatures. Yet, there was little variation in fine mesh leaf breakdown rates ( $k_{dd}$ ) of either leaf species between reaches (Table 1). A global litter decomposition study by Boyero et al. (2011) had similar results, showing that the positive effect of temperature on fine mesh leaf breakdown per day was normalized when the time variable was replaced with degree-days, suggesting that temperature was the source of variation in fine mesh leaf breakdown rates. Because fine mesh  $k_{dd}$  values were relatively small (Table 1), slight variation (i.e.  $\pm 0.0001$  SE) in decomposition rates may have resulted in the statistically significant location effect, which may have minimal ecological consequence.

Interestingly, we observed a trend in higher microbial leaf mass loss in shaded reaches for box-elder in both locations, and for gamble oak only in the upstream location (Table 2, Fig. 5). Photodegradation can reduce litter decomposition rates (Austin et al. 2006), where increased irradiance can accelerate rates of nutrient loss during leaching, inhibiting microbial growth (Dieter and Vivanco 2011). The significantly higher light levels in open reaches may have thus suppressed microbial growth, reducing litter processing rates when compared to shaded reaches.

Despite these confounding interactive effects of location and canopy, our results clearly show that with increasing cumulative degree-days, leaf mass loss due to shredders decreased, and microbial leaf mass loss increased in leaves of contrasting quality (Table

2, Fig. 5). Furthermore, the relative proportion of leaf mass loss due to microbial activity was greater than shredders across all reaches. This trend was most apparent in box-elder litterbags, where microbial leaf mass loss was 60 to 79%, compared to 9.3 to 34.5% for shredders. In other temperate streams, Hieber and Gessner (2002) found that leaf mass loss due to shredders was between 51 and 64%, and only 15 to 18% and 7 to 9% for fungi and bacteria, respectively. These contrasting results can be explained by temperature, which negatively affected shredder abundance and therefore total leaf breakdown rates, while positively affecting leaf mass loss by microbial activity. Other studies have found that microbial and shredder leaf litter processing are highly sensitive to changes in water temperature (Langhens et al. 2008, Friberg et al. 2009), and that leaf breakdown responses are ultimately dependent on adaptations of aquatic insect populations to local thermal regimes (Irons et al. 1994).

Recent studies have shown that in-stream habitat and riparian organic matter inputs directly affect macroinvertebrate and microbial leaf litter processing (Casotti et al. 2015) as well as aquatic invertebrate productivity in streams (Wallace et al. 2015). In Kimball Creek, the effects of canopy cover appeared to be amplified in the degraded downstream reaches, where increased water temperature positively affected microbial leaf mass loss and shredder biomass, with negative effects on shredder abundances in litterbags. In turn, shifts in the biological processing of leaf litter could affect timing, availability, and cycling of nutrients from the bottom up (Wallace et al. 1997). Detrital resource availability can determine the spatial distribution of consumers (Tiegs et al. 2008), and decreased rates of leaf processing by shredders in degraded reaches could reduce the amount of organic matter released into the water column for other primary

consumers downstream, thus reducing prey resources for predators, such as fish and amphibians. In the case of urban streams, increased rates of microbial leaf processing decreased long-term availability of benthic organic matter, thus negatively affecting ecosystem function by altering heterotrophic energy pathways (Imberger et al. 2008). In light of predicted increases in global atmospheric temperatures (IPCC 2014), our results confirm those of other recent studies (Boyero et al. 2011, Ferreira et al. 2015b) suggesting that shifts in the relative importance of macroinvertebrate detritivores to microbes via stream warming could have cascading effects across trophic levels and ultimately carbon cycling.

Water temperature is fundamentally important to aquatic ecosystem function (Todd et al. 2008, Issak et al. 2012). Thus in Kimball Creek, water temperature in the downstream degraded reaches, ranging from 18.6 to 19.6°C, should be a primary concern for future restoration success. Moreover, maximum thermal tolerance of Colorado River cutthroat trout is 17 to 22.1°C (Todd et al. 2008), thus temperatures in Kimball Creek will likely impede survival and recovery of a native cutthroat trout fishery, especially given continual stream warming trends in the western U.S. (Isaak et al. 2012, Underwood et al. 2012). In order for successful restoration of ecosystem function and a native cutthroat trout fishery in Kimball Creek, efforts should focus on warmer reaches. Reach-scale restoration such as replanting riparian vegetation could restore nutrient inputs and shade function, as well as reduce water temperatures in degraded reaches. This process-based approach to restoration could help to reestablish the bottom-level biological processes that sustain stream ecosystems (Beechie et al. 2010).

Congruent with other studies (Bunn et al. 1999, McKie and Malmqvist 2009, Bruder et al. 2014), our experimental results further emphasize that the litterbag method is a valuable direct indicator of stream health, providing a quantitative evaluation of the relative importance of shredder and microbial-mediated litter decomposition in response to degradation by various land management practices. Our findings also suggest that using leaves with contrasting quality can be an effective way to examine multiple biological processes, as well to provide a relatively inexpensive and rapid method to monitor and evaluate restoration of degraded aquatic systems. For instance, Lepori et al. (2005) used the litterbag method to assess effectiveness of placing boulders in a channelized stream to increase organic matter retention. A long-term study by Wallace et al. (2015) showed that in forested streams where litter inputs were excluded, the addition of wood structures alone was ineffective at restoring benthic invertebrate productivity when detrital resources were depleted. Finally, results of this study could be useful in evaluating other degraded systems impacted by anthropogenic disturbance, such as in agricultural and urban streams. Future research and monitoring of ecosystem function should consider repeated experiments across multiple locations, which would capture within-stream variation in water quality and benthic communities between habitat patches in order to evaluate relative levels of reach degradation and prioritize areas most in need of restoration.

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**TABLES**

**Table 1.** Leaf breakdown rates per degree-day ( $k_{dd}$ )  $\pm$  1 standard error (SE) and R-squared values ( $R^2$ ) from linear regression analysis of litterbags (N = 60) per mesh size, leaf species, reach location [upstream (US), downstream (DS)], and canopy cover [open (O), shaded (S)]. N = 59 for Q and AF treatments, as one replicate was lost due to beaver during the final week of the experiment.

Litterbag Treatment	Location	Canopy	$k_{dd}$	$R^2$
<u>Coarse mesh:</u>				
Box-elder (A)	US	O	-0.0072 (0.0008)	0.83
	US	S	-0.0075 (0.005)	0.92
	DS	O	-0.0043 (0.0002)	0.96
	DS	S	-0.0030 (0.0003)	0.86
Gamble oak (Q)	US	O	-0.0013 (0.0002)	0.82
	US	S	-0.0018 (0.0003)	0.65
	DS	O	-0.0011 (0.0001)	0.88
	DS	S	-0.0008 (0.0001)	0.92
<u>Fine mesh:</u>				
Box-elder (AF)	US	O	-0.0021 (0.0002)	0.87
	US	S	-0.0022 (0.0001)	0.93
	DS	O	-0.0021 (0.0001)	0.94
	DS	S	-0.0020 (0.0001)	0.95
Gamble oak (QF)	US	O	-0.0006 (0.0001)	0.90
	US	S	-0.0007 (0.0001)	0.88
	DS	O	-0.0007 (0.00005)	0.93
	DS	S	-0.0006 (0.00004)	0.95

**Table 2.** Relative proportion of mean (N = 3) leaf mass loss (AFDM %) associated with microbial and macroinvertebrate shredder processing after 35 days submergence. Data are compared by leaf species (box-elder, gamble oak), location [upstream (US), downstream (DS)], and canopy cover [open (O), shaded (S)]. Leaf mass loss values are listed for coarse mesh litterbags (total), fine mesh litterbags (microbes), and the difference between them (shredders). Ratio is equal to leaf mass loss attributed to microbes over shredders.

<u>Litterbag treatment</u>				<u>Leaf mass loss</u>			
<u>Leaf species</u>	<u>Location</u>	<u>Canopy</u>	<u>Cumulative Degree-Days</u>	<u>Total</u>	<u>Microbes</u>	<u>Shredders</u>	<u>Ratio</u>
<u>Box-elder:</u>	US	O	448.5	95.3	60.8	34.5	1.8
	US	S	472.4	97.6	64.5	33.1	1.9
	DS	O	668.4	96.4	76.7	19.7	3.9
	DS	S	704.3	88.7	79.4	9.3	8.5
<u>Gamble oak:</u>	US	O	448.5	47.0	24.9	22.1	1.1
	US	S	472.4	60.9	31.4	29.6	1.1
	DS	O	668.4	50.6	38.5	12.1	3.2
	DS	S	704.3	43.5	38.2	5.3	7.2

**Table 3.** Mean ( $N = 5$ )  $\pm$  1 standard error (SE) of water quality and habitat parameters measured weekly in each reach (USO, USS, DSO, DSS), where \* denotes statistical significance of MANOVA.<sup>1</sup>  $N = 4$  for the USO reach, as one water sample was processed incorrectly in the laboratory. Substrate composition is categorized by percent particle size, which was estimated by conducting a pebble count in each reach ( $N = 120$ ), where \*\* denotes statistical significance of Pearson's Chi-Square ( $\chi^2$ ) test.

Parameter	USO	USS	DSO	DSS
<u>Water Quality:</u>				
Ammonium (mg/L)*	0.007 (0.002) <sup>1</sup>	0.006 (0.001)	0.002 (0.001)	0.003 (0.009)
Dissolved oxygen (mg/L)*	9.20 (0.12)	8.90 (0.23)	8.09 (0.15)	8.12 (0.13)
Nitrite-nitrate (mg/L)*	0.085 (0.018) <sup>1</sup>	0.088(0.014)	0.024 (0.003)	0.004 (0.001)
pH*	8.33 (0.03)	8.44 (0.02)	8.54 (0.02)	8.57 (0.02)
Soluble reactive phosphorus (mg/L)	0.007 (0.0004) <sup>1</sup>	0.007 (0.0005)	0.007 (0.0005)	0.008 (0.0007)
Specific conductivity ( $\mu$ S/cm)*	765 (5.78)	741 (21.9)	878 (3.26)	879 (5.46)
Temperature ( $^{\circ}$ C)*	12.5 (0.14)	13.1 (0.17)	18.6 (0.25)	19.6 (0.25)
Turbidity (NTU)*	5.23 (0.70)	10.2 (1.82)	5.99 (1.01)	5.99 (1.93)
<u>Habitat:</u>				
Canopy cover (%)*	52.1 (0.02)	86.4 (0.01)	54.0 (0.03)	85.2 (0.01)
Discharge ( $m^3/s$ )	0.022 (0.005)	0.015 (0.003)	0.018 (0.003)	0.018 (0.003)
Light level (lux)*	48,187 (2,337)	20,295 (992)	48,506 (1,800)	26,533 (1,302)
Water depth (cm)*	14.8 (0.85)	9.3 (0.49)	12.1 (0.23)	12.3 (0.31)
Wetted width (cm)*	143 (6.47)	141 (4.87)	116 (5.06)	119 (2.93)
<u>Substrate (%): **</u>				
Cobble (64 - 256 mm)	3	8	2	8
Pebble (16 - 64 mm)	59	68	47	55
Gravel (2 - 6 mm)	18	3	17	6
Sand (0.063 - 2 mm)	10	8	24	23
Silt (< 0.063 mm)	11	13	11	8

## FIGURES

**Figure 1.** Mean ( $N = 3$ ) AFDM remaining (%) per degree-day, compared between reaches by leaf species and mesh-size litterbag treatment: box-elder coarse mesh (A), gamble oak coarse mesh (B), box-elder fine mesh (C), and gamble oak fine mesh (D). Litterbag treatment regression lines are distinguished by upstream (blue) and downstream location, and open (hollow) and shaded (solid) canopy cover. Error bars represent  $\pm 1$  SE.

**Figure 2.** Non-metric multidimensional scaling (nMDS) ordination on Bray-Curtis similarity of mean ( $N = 3$ )  $\log(x + 1)$  transformed shredder abundance (A) and 4<sup>th</sup>-root transformed shredder biomass (B) in coarse mesh litterbags after 7, 21, and 35 days submergence. Data are compared by leaf species [box-elder (triangles), gamble oak (circles)], location [upstream (blue), downstream (red)], and canopy cover [open (hollow), shaded (solid)].

**Figure 3.** Mean ( $N = 3$ ) total shredder abundance in coarse mesh litterbags after 7, 21, 35 days submergence. Data are compared by location and leaf species: box-elder upstream (A), box-elder downstream (B), gamble oak upstream (C), and gamble oak downstream (D). Mean shredder abundance is also compared by canopy cover, open (left x-axis) and shaded (right x-axis).

**Figure 4.** Mean ( $N = 3$ ) total shredder biomass in coarse mesh litterbags after 7, 21, 35 days submergence. Data are compared by location and leaf species: box-elder upstream (A), box-elder downstream (B), gamble oak upstream (C), and gamble oak downstream (D). Mean shredder biomass is also compared by canopy cover, open (left x-axis) and shaded (right x-axis).

**Figure 5.** The ratio of leaf mass loss (AFDM %) attributed to microbes and shredders per cumulative degree-days ( $^{\circ}\text{C day}^{-1}$ ), estimated from box-elder (triangle) and gamble oak (circle) litterbags after 35 days submergence. Ratios are compared by location [upstream (blue), downstream (red)] and canopy cover [open (hollow), shaded (solid)].



## FIGURES

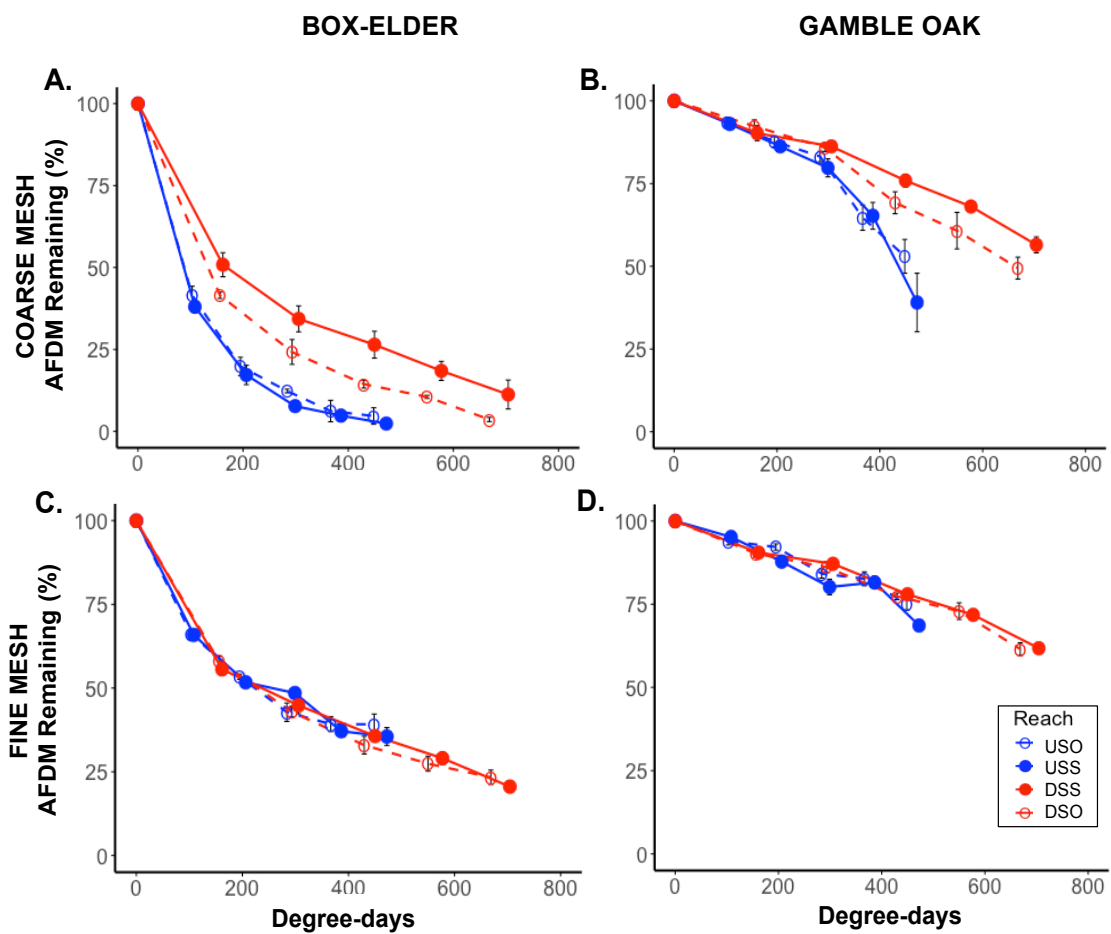
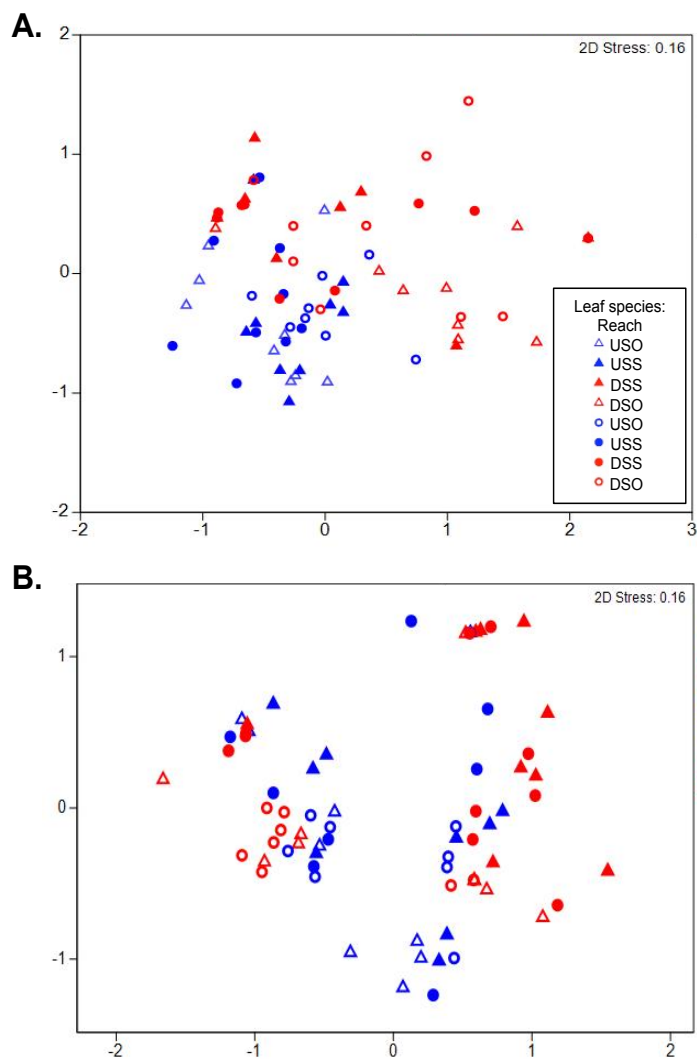


Figure 1.



**Figure 2.**

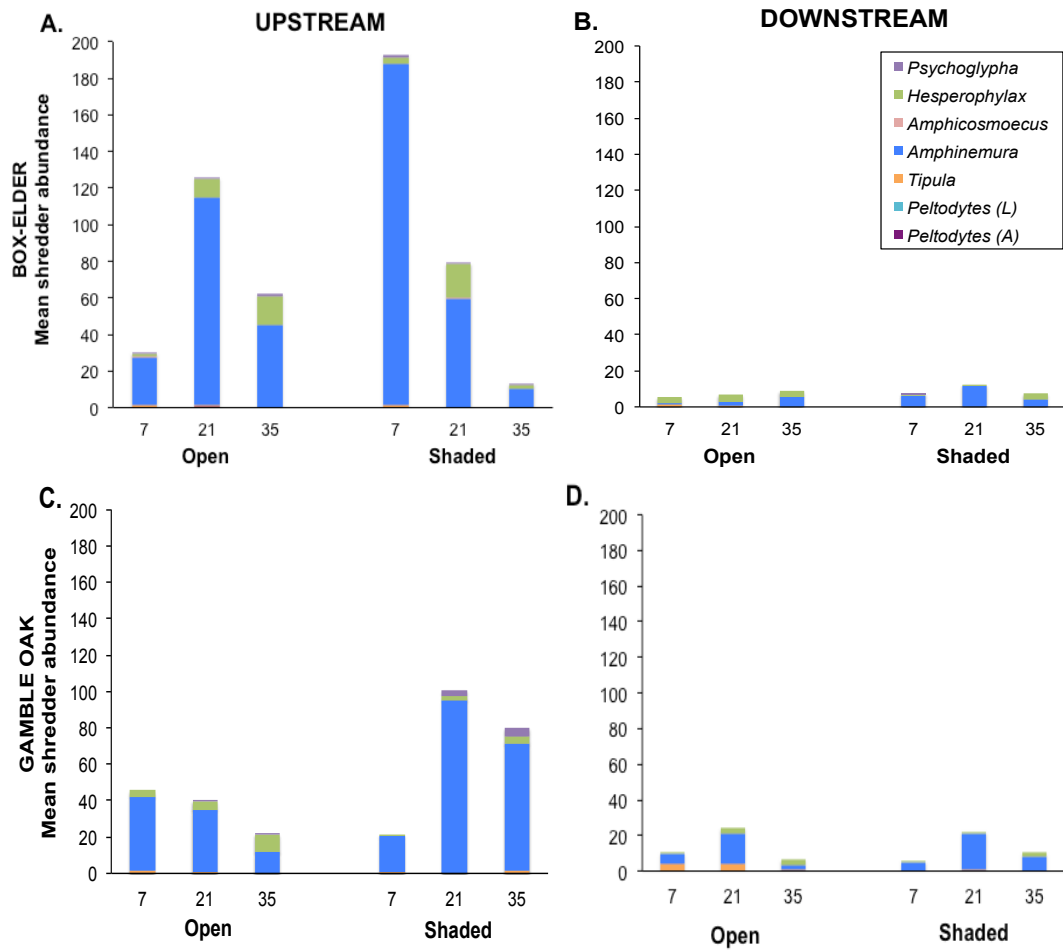


Figure 3.

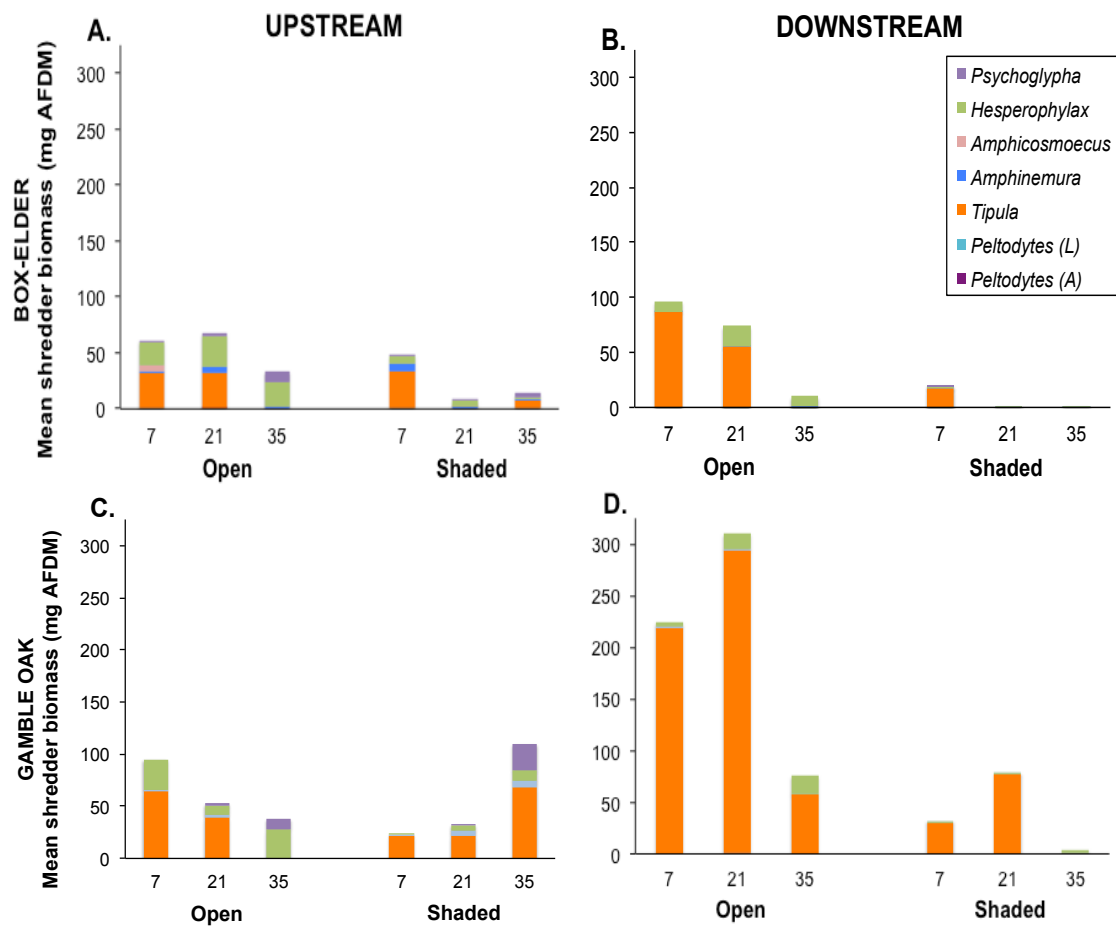


Figure 4.

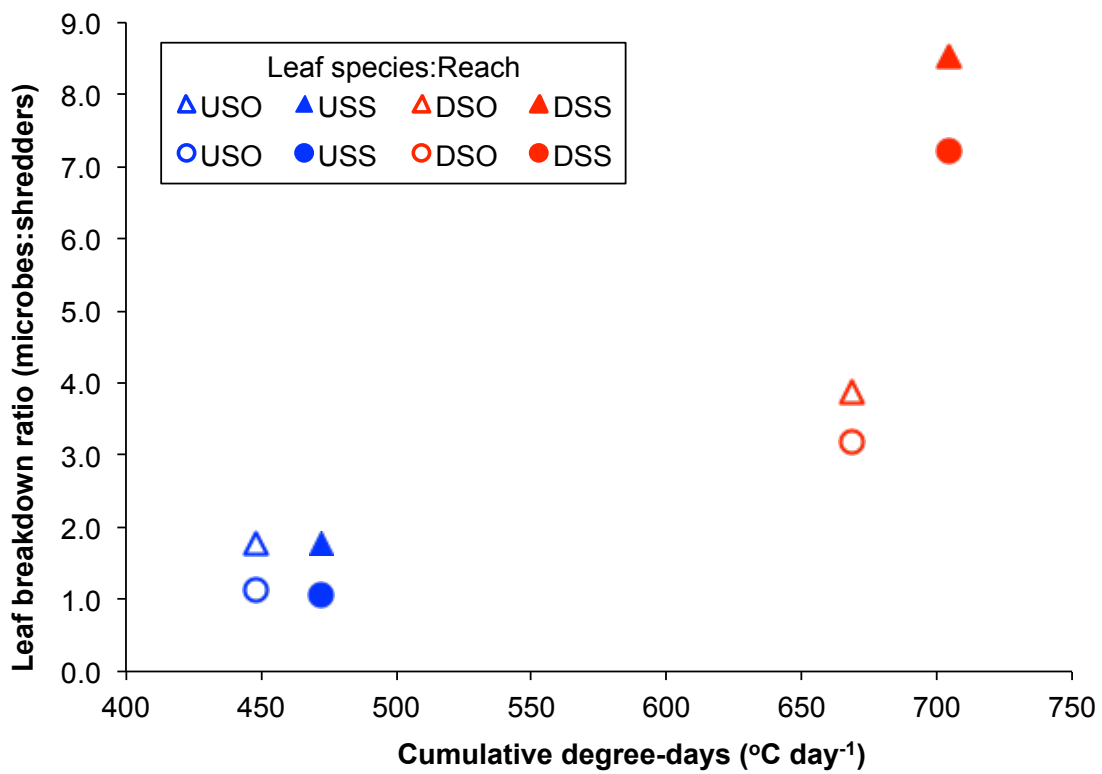


Figure 5.

