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Effects of Light and Macroinvertebrate Consumers on Detrital Microbial Biofilms in Streams

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EFFECTS OF LIGHT AND MACROINVERTEBRATE CONSUMERS ON DETRITAL MICROBIAL BIOFILMS IN STREAMS

by

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ABSTRACT

In lotic freshwater systems, aquatic macroinvertebrates are key processors of biofilms that grow upon organic matter. Although macroinvertebrate effects on biofilms may depend on light availability, the combined effects of consumers and light remain unexplored. Here, I conducted experiments to test effects of presence/absence of the omnivorous shrimp *Macrobrachium ohione* and the shredding caddisfly *Pycnopsyche* sp. on *Liriodendron tulipifera* litter biofilms in experimental streams under light or darkness. I measured litter-associated algal, fungal and bacterial biomasses and production rates, as well as litter decomposition, over 49 days. Both experiments exhibited significant positive effects of light on algal productivity and interactions of *Macrobrachium* and *Pycnopsyche* presence with time and light. Light increased bacterial productivity in the *Pycnopsyche* experiment, but not in the *Macrobrachium* experiment, in which time, light, and *Macrobrachium* interactively affected bacterial production. Litter decomposition was unaffected by light or *Macrobrachium* presence, but *Pycnopsyche* presence increased decomposition rates. My results suggest that light strongly affects litter biofilms, whereas consumers primarily affect the timing and succession of periphytic microbial colonization of organic matter. Compared to omnivores, shredder-detritivores may exert stronger effects on turnover and decomposition of organic material within lotic systems.

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DEDICATION

I would like to dedicate this thesis to my family members – Rhonda Brady, Todd Brady and Betty G. Williamson – for their endless love and support, even through the long nights, lab practicals, field days, weekend work and weeklong conferences, not to mention all the rescheduled visits. Also, to Helen Weber, the best friend I've made during my time at USM: you're the greatest and I can't wait to see all the amazing ocean research you've been working on! We're two sides of, essentially, the same water equation! Thank you all for always being so understanding and kind to me as I worked my way toward this thesis. Additionally, the littlest members of my family cannot be overlooked – Bobrat, Quiche Lorraine, T'Challa, Titus and Woot, it's likely you won't be able to read this, but I hope you know that your love was seen, felt and heard. You all went above and beyond for me while I was working on this thesis, and I only hope that I can repay that over the years.

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CHAPTER I – INTRODUCTION AND REVIEW OF THE LITERATURE

In lotic freshwater systems, both shredders and grazers are considered key organisms in the processing of organic matter (Wallace & Webster 1996). Macroinvertebrates, especially shredders, assist in breakdown and processing of aquatic leaf litter as well as the constituents of heterotrophic biofilm growing therein (Cheever & Webster 2014). Decomposing litter acts as a substrate upon which biofilm can grow, in turn providing a higher-quality food source for many macroinvertebrates as it is colonized and processed (Suberkropp et al. 1976; Golladay et al. 1983; Chung & Suberkropp 2009; Cheever & Webster 2014). While detrital biofilms thus reflect both bottom-up controls by detritus and top-down effects of consumers, recent research suggests this dynamic is significantly affected by light availability (Lagrue et al. 2011). Both anthropogenic and riparian disturbances such as land use change and natural disasters can cause opening of the canopy, leading to increased light availability in streams (Kiffney et al. 2004; Dunham et al. 2007; Lagrue et al. 2011; Wasser et al. 2015). Increased light enhances growth of periphytic algae, which in turn stimulates heterotrophic activity, enhances detrital food resource quality, and may consequently alter invertebrate roles in organic matter processing (Kuehn et al. 2014; Guo et al. 2016).

Light provides a potentially significant bottom-up effect on both biofilms and leaf litter breakdown. In a stream, algal exudates have been shown to increase the mineralization of coarse particulate organic matter such as leaf litter (Danger et al. 2013). This process is known as the priming effect, and can alter competition between labile- versus recalcitrant-degrading microbes in low-nutrient streams, thus aiding in the breakdown of detritus (Guenet et al. 2010; Danger et al. 2013, Kuehn et al. 2014). Algal

activity and the priming effect can also increase the enzymatic activity and decomposition of leaf litter (Francoeur et al. 2006; Rier et al. 2014). Algae, moreover, provides an important food source for many aquatic consumers (Steinman 1996; Evans-White & Lamberti 2005), as do fungi and bacteria (Golladay et al. 1983; Suberkropp et al. 1983; Hieber & Gessner 2002). Within the biofilm and the overall ecosystem, these components come together to provide a food pathway for consumers in low-nutrient, high-detritus streams (Suberkropp et al. 1983; Holgerson et al. 2016). It is therefore plausible that a shift in autotrophic biomass and production, e.g. from increased light, should cause a corresponding increase in heterotrophic microbial biomass and activity, thus driving greater quality of detrital biofilm as a food resource for aquatic invertebrates (Danger et al. 2013; Kuehn et al. 2014).

Although detrital microbes act as critical bottom-up intermediaries to free energy and nutrients from recalcitrant material (Suberkropp et al. 1976), macroinvertebrates can also exert top-down effects on biofilms (Gulis & Suberkropp 2003; Cheever & Webster 2014). These top-down consumer effects on detrital biofilms are likely driven by selective feeding and removal of microbial biomass (Arsuffi & Suberkropp 1989; Evans-White & Lamberti 2005; Eggert & Wallace 2007). Indeed, macroinvertebrates prefer leaves containing less cellulose (Suberkropp et al. 1983), likely because this material is easier for microbial constituents to colonize and break down, thus making the substrate more palatable. Consumers have also been shown to aid in nutrient cycling, by removing biofilm and replenishing nutrients either indirectly via their egesta or directly via their excreta (Evans-White & Lamberti 2005; Liess & Haglund 2007; Cheever & Webster 2014). Invertebrate consumers act as a critical top-down influence on biofilm depending

on their feeding method (Lawrence et al. 2002), suggesting consumer identity may be an important factor to understand top-down effects on biofilm characteristics. Further studies comparing different consumer taxa are necessary to understand links between invertebrate community structure and ecosystem functions like microbial colonization and breakdown of detritus (Wallace & Webster 1996).

I employed a combination of field and laboratory techniques to examine the interactive effects of animals and light availability on microbially conditioned *Liriodendron tulipifera* leaves within flume mesocosms. Both experiments involved the manipulation of light (full versus partial shading) as well as the presence or absence of two different macroinvertebrate species (the omnivore *Macrobrachium ohione* and the shredder *Pycnopsyche* sp.). Inclusion or exclusion of either factor helped determine the strength of its impact on litter biofilms and decomposition. The interplay of these factors can improve understanding of the effects of forestry and removal of riparian vegetation on stream ecosystem processes including litter decomposition and food web interactions (Kominoski & Rosemond 2012). I hypothesized that, under dark conditions, fungi will dominate litter biofilms; under light conditions, algae will be more prevalent. Also, while microbial biomass may be decreased in the presence of invertebrates, growth of algae, and in turn fungi, should be stimulated under light conditions due to the priming effect (Danger et al. 2013; Kuehn et al. 2014). I also predicted that *Pycnopsyche*, a highly functional shredding caddisfly, will show top-down effects at a greater magnitude compared to omnivorous *Macrobrachium* (Creed et al. 2009).

CHAPTER II - METHODS

Experimental overview

This study was conducted using flume mesocosms at the Lake Thoreau Environmental Center, operated by the University of Southern Mississippi in Hattiesburg, MS. Additionally, water for the study was collected at low-nutrient forested streams (Whiskey Springs and Big Creek) in DeSoto National Forest MS, USA. The study was conducted as two separate experiments using the omnivorous shrimp *Macrobrachium ohione* and the shredding caddisfly *Pycnopsyche* sp.

Macrobrachium experiment

Both field and laboratory components were employed to investigate the effects of light and macroinvertebrate feeding on biofilm colonization of *Liriodendron tulipifera*. For the field component, 3 to 5 g of previously collected and desiccated *L. tulipifera* leaves were weighed and their petioles removed; leaf blades and petioles were next weighed separately and transferred into 1.7 mm mesh bags for use in the mesocosms. After leaves were weighed out, light (shaded or light) and consumer (present or absent) treatments, as well as sampling time and sequential order within each flume, were assigned randomly. Bags were weighed down with small stones within the flumes to prevent drifting.

A pair of recirculating flume systems, each containing 4 flumes, was constructed using 3.05-m vinyl rain gutters atop plywood constructions balanced by an adjustable sawhorse at each end. Water from Whiskey Creek was added to cattle troughs, and a pump was connected to an adjustable splitter at the opposing end to maintain even flow of approximately 1.0×10^{-5} m^3s^{-1} through all flumes (Table 1). Both shaded and light flumes had at least partial shading; for the shaded treatment, sunshade cloth and burlap were used

to block out light completely, while only burlap was used to provide partial shading in the light treatment. A preliminary reading on a sunny day indicated an average of 56% shading inside the light treatment canopy and >99% shading inside the dark canopy.

Additionally, grab samples of pre-conditioned litter were collected from Big Creek in DeSoto National Forest to inoculate the flumes; these leaves were placed in bags at the head of each flume to allow biofilm components to colonize *L. tulipifera* as it wetted and began decomposing. After an initial two-week period of conditioning, two *Macrobrachium ohione* individuals from Whiskey Creek were added to randomized bags. At 16, 23, 37, and 51 days into conditioning in April-May 2017, bags were pulled from the gutters and processed to measure litter dry mass remaining, microbial biomass and activity, and enzyme activities (detailed below).

Pycnopsyche experiment

The experiment using *Pycnopsyche* sp. was similar to the *Macrobrachium* experiment with a few modifications to methods. Individuals were collected from Chamber Springs, AR and used as a model shredder for comparison to *Macrobrachium ohione*. Flow was adjusted to measure approximately 10 mL s^{-1} across all flumes (Table 1). *Pycnopsyche* requires cooler temperatures for optimum health, so to combat rising spring temperatures, freezer packs were introduced directly into the flumes on days when air temperatures exceeded ~26° C. Additionally, to avoid pupation of individuals within the flume system, only healthy 4th-instar individuals were used, based on visual inspection of size and case material. Water was collected from Big Creek, a low-nutrient stream in DeSoto National Forest, MS. Approximately 3 grams of *Liriodendron tulipifera* litter were added to the leaf bags in order to provide both food and shelter as well as case material for *Pycnopsyche*. As

Pycnopsyche is a solitary case-building insect, this litter acted as both a means of spatial isolation as well as housing and diet. Finally, this experiment was conducted earlier in the spring (March-April 2018) to provide an optimum environment for the model organisms. Litter was conditioned for a period of 8 days, and after this initial conditioning, *Pycnopsyche* were introduced to randomized bags. At 16, 23, 33, and 44 days of conditioning, litter bags were pulled and subject to assays.

Laboratory assays for microbial biomass, growth and enzymatic activity

For the laboratory component of each experiment, biofilm microbial biomass and activity were quantified. Leaf litter was cut into 14 mm disks for quantification of algal, bacterial and fungal biomass, growth and production, using methods described by Kuehn et al. (2014) and Francouer et al. (2006).

To estimate algal biomass, chlorophyll *a* concentrations were measured using extraction in hot ethanol followed by HPLC analysis (Verma et al. 2002; Francouer et al. 2006; Kuehn et al. 2014). Leaf disks were collected and frozen at -20°C until extraction. Chlorophyll *a* was then extracted from the frozen leaf disks by boiling in 90% ethanol for a period of 5 minutes. Extracted samples were then stored overnight in the refrigerator at 4°C in darkness before quantification of chlorophyll *a* via high performance liquid chromatography with fluorescence detection at 430 nm excitation / 680 nm emission (Meyns et al. 1994).

To determine algal primary productivity, ^{14}C-bicarbonate incorporation and radioassays were used (Francouer et al. 2006; Kuehn et al. 2014). Leaf disks were placed inside autoclaved glass scintillation vials with 20 mL of filtered stream water and amended with 0.5 μCi $H^{14}CO_3^-$ (specific activity 8.4 mCi $mmol^{-1}$). Samples were then

placed in a lighted growth chamber [20°C, 400 μmol $m^{-2}s^{-1}$ photosynthetically active radiation (PAR)] to incubate for 2 hours. Killed control samples were also incubated to correct for nonbiological ^{14}C incorporation. After incubation was complete, all samples were killed with 3% formalin, filtered (0.45 μm HAWP membrane filter) and filtrate, leaf disks, and filter collected and stored frozen at -20°C until analysis. Filtrate samples were thawed, acidified to pH 2 using 1N HCl to remove inorganic ^{14}C, re-frozen, lyophilized, and resulting precipitate was suspended in 10 mL Ecolume scintillation fluid to quantify radioactivity. Leaf disk and filter samples were lyophilized, litter weighed, and then fumed with HCl for 10 minutes to remove residual ^{14}C and digested in 0.5 M NaOH for 1 hour at 80°C. 100 μL aliquots of this solution were then cleared with an equal volume of 50% hydrogen peroxide and added to scintillation vials with 10 mL of Ecolume scintillation fluid. Radioactivity was assayed on a Beckman LS 6500 Scintillation Counter, then algal production was calculated using measured alkalinity and pH to determine the pool of available dissolved inorganic C (Wetzel & Likens 2000). Algal production rates (μg C g^{-1} detrital C h^{-1}) were calculated as the sum of rates determined from dissolved and particulate fractions.

Bacterial biomass associated with litter was determined using flow cytometry after probe ultrasonication and staining. Leaf disks were preserved in a 10 mL solution of 2% formalin buffered with 0.1% sodium pyrophosphate, then refrigerated in darkness until analysis. Samples were then sonicated for 4 intervals of 20 seconds at setting 4 using a Branson probe sonifier. After sonication, 0.5 mL aliquots of each sample were filtered through a 70 μM sieve and rinsed with 4.5 mL phosphate-buffered saline into a 15-mL conical centrifuge tube to obtain proper dilution. Diluted samples were then

vortexed for 5 seconds each and 1 mL of each sample was transferred into a 1.5 mL conical microcentrifuge tube for staining. Using an Invitrogen bacterial counting kit (Thermo Fisher, Waltham, MA), each sample was stained using 1 μL of SYTO BC bacteria stain and had 10 μL of microbead suspension added. Additionally, two control tubes were made: a formalin kill with dye and only microbead suspension, and a high cell count sample with dye but no microbeads. These controls were used to correct for microbeads mis-read as bacterial cells and bacterial cells mis-read as microbeads, respectively, during flow cytometry. Samples were then counted using a Fortessa flow cytometer and BD FACSDiva software.

Bacterial production rates were estimated using [^{3}H]-leucine incorporation into bacterial protein, as described by Kuehn et al. (2014). Leaf disks were placed inside autoclaved glass scintillation vials with 4 mL of filtered stream water and 2.5 μmol/L of [4,5-^{3}H]-leucine (specific activity = 586 mCi mmol^{-1}). Vials were incubated for 30 minutes in a lighted plant growth chamber [400 μmol m^{-2}s^{-1} PAR]. Killed control samples containing 5% trichloroacetic acid were also incubated to correct for nonbiological [^{3}H]-leucine incorporation. After incubation, all samples were killed using 5% trichloroacetic acid, then digested at 80°C. Samples were then cooled, stored at 4°C and radioactivity measured using methods detailed in Gillies et al. (2006), with the exception that samples were centrifuged instead of filtered to concentrate labeled protein. Bacterial production rates were calculated as μg bacterial C g^{-1} detrital C h^{-1} using the conversion factor of 1.44 kg C produced per mole leucine incorporated (Buesing & Marxsen 2005).

For fungal biomass and growth rates, ergosterol concentrations (Gessner 2005) and rates of [1-^{14}C]-acetate incorporation into ergosterol (Suberkropp & Gessner 2005) were used, respectively. Leaf disks were placed in 20-mL autoclaved scintillation vials containing 4 mL filtered stream water and 5 mM Na[1-^{14}C]-acetate (specific activity = 1.31 mCi mmol^{-1}) and placed in the incubator at 20°C and 400 μmol m^{-2}s^{-1} PAR to measure fungal production over 5 h. Killed control samples containing 2% formalin were used to control for non-biological uptake of radiolabel. At the conclusion of assays, samples were filtered (1.2 μm glass fiber filter) and frozen at -20°C until analysis. Frozen samples were lyophilized, weighed, and their ergosterol extracted in 10 mL of 0.8% KOH in HPLC-grade methanol. Samples were then digested for 30 minutes at 80°C. The resultant digest was partitioned into *n*-pentane and evaporated to dryness in 15-mL glass conical vials under a stream of nitrogen gas (Kuehn & Suberkropp 1998). Ergosterol in dried samples was redissolved by ultrasonication in 1 mL of methanol, centrifuged, and the resultant supernatants stored in 2-mL HPLC autosampler vials at -20°C until analysis. Ergosterol content was quantified using a Shimadzu liquid chromatograph system, detected at 282 nm using a Shimadzu UV/VIS detector (retention time = ca. 8 min) and identified and quantified based on comparison with ergosterol standards. Litter ergosterol concentrations were converted to fungal biomass carbon assuming a conversion factor of 10 μg ergosterol mg^{-1} fungal C, assuming 43% C in fungal dry mass (Gessner & Newell 2002). Fungal growth rates (μ) were calculated using a conversion factor of 12.6 μg fungal biomass nmol^{-1} acetate incorporated, while fungal production was calculated by multiplying the fungal growth rate (μ) by fungal biomass.

Phenol oxidase and beta-glucosidase activity were also analyzed using 2.5 mM L-DOPA and 660 μM β-D-glucopyranoside methylumbelliferyl and filtered stream water as substrates, respectively (Francoeur et al. 2006). Litter disks were placed into autoclaved scintillation vials containing 3 mL of different concentrations of the appropriate substrate dissolved in filtered stream water. Vials with no litter served as controls for ambient absorbance or fluorescence. Vials were then incubated in a lighted growth chamber [20°C, 400 μmol m^{-2} s^{-1} (PAR)] for 1 h (phenol oxidase) or 30 minutes (beta-glucosidase). After incubation, phenol oxidase samples were subsampled, centrifuged, and aliquoted to a clear 96-well plate to measure absorbance at 480 nm using a BioTek plate reader. Beta-glucosidase samples were also subsampled from vials and boiled for 5 minutes to stop enzyme activity, then frozen. Samples were thawed, centrifuged, and 3 aliquots of 100 μL were added to 100 μL of pH 10 carbonate/bicarbonate buffer in individual wells of a black 96-well plate. Fluorescence was then measured using a BioTek plate reader.

Statistical analysis

On each sampling date, leftover leaves were saved after litter disks were cut. This remaining litter was frozen at -20°C, then each sample was lyophilized and weighed in order to compare the difference between final dry leaf mass between the sampling date and the introduction date. Litter decomposition rates k (d^{-1}) were calculated using the negative slope between log-transformed % mass remaining and time (d) over the experiment.

Algal, fungal, and bacterial biomass and production rates as well as phenol oxidase and beta glucosidase enzyme activities were analyzed using two-way repeated

measures ANOVA with consumer presence and light as fully-crossed factors and time as error. Residuals for each variable were plotted, and based on their distribution, log transformations were applied to improve normality and homogeneity of variances (Table 2; Table 3). Following ANOVA for any microbial responses that displayed interactions, a Tukey's honestly significant difference post-hoc test was used to determine any difference between pairwise means for the interaction of consumer presence and light. Decomposition rates were analyzed using two-way ANOVA, with consumer presence and light as factors. All statistical analyses were performed using R version 3.3.3 (R Core Team 2018).

Table 1 *Physico-chemical properties of stream mesocosms for the Macrobrachium and Pycnopsyche experiments.*

Variable	*Macrobrachium*		*Pycnopsyche*	
	Average	Standard Error	Average	Standard Error
Daily mean temperature (°C)	23.8	0.2	20.8	0.2
pH	5.7	0.2	6.9	0.1
Alkalinity (mg $CaCO_3$/L)	17.5	1.6	10.5	0.1
[N-NO3+NO2] (ug/L)	19.7	11.6	39.4	2.9
[P-PO4] (ug/L)	17.6	1.2	14.8	1.8
[N-NH4] (ug/L)	8.0	4.3	14.9	0.8
Flume flow rate (m^3s^{-1})	9.2×10^{-5}	2×10^{-7}	9.6×10^{-5}	1.7×10^{-7}
Survivorship (individuals remaining per sampling day)	15.5	0.5	11.25	2.8

CHAPTER III - RESULTS

Algal biomass and activity

In the *Macrobrachium* experiment, the interaction of light and time affected the colonization of algae on decomposing litter ($p<0.001$; Table 2; Fig. 1), seen as an earlier increase of algal biomass in the shaded treatment compared to the light treatment. Algal biomass also generally increased across all treatments over time ($p=0.015$), and did not differ across light or consumer treatments. In the *Pycnopsyche* experiment, no significant effects of light or consumers were observed (Table 3). However, as with the *Macrobrachium* experiment, time effects were significant across treatments ($p=0.002$), indicated by increases in algal biomass over time (Fig. 6b).

The light treatment significantly stimulated algal production rates in both experiments ($p<0.001$; Table 2; Table 3; Fig. 1). Algal production rates also generally increased over time ($p<0.001$; Table 2; Table 3; Fig. 6). During the *Macrobrachium* experiment, I observed an increase in algal production over time in the light treatment, but not in the dark treatment, reflecting a light-by-time interactive effect ($p=0.005$; Table 2; Fig. 1). Additionally, there was a light-by-time-by-consumer interactive effect in both experiments ($p<0.05$; Fig. 1). This interaction was seen most prominently in the *Macrobrachium* experiment, where algal production increased to the greatest degree and peaked on day 35 within the light present treatment, whereas the shaded present treatment exhibited the weakest temporal increases of algal production and peaked on day 49, indicating consumers trended to strengthen the contrasting light treatment effects on temporal trends in algal activity (Fig. 6c). In the *Pycnopsyche* experiment, light treatments also exhibited stronger temporal increases of algal production, with consumer

presence markedly increasing algal production in the light, but decreasing algal production in the shaded treatment, on day 33 (Fig. 6d).

Fungal biomass and activity

In the *Macrobrachium* experiment, fungal biomass was significantly higher in the shaded treatment (p=0.029), and fungal colonization of leaf litter showed a general increase over time ($p<0.001$; Table 2; Fig. 7). However, no significant effect of consumers was observed. Fungal biomass in the *Pycnopsyche* experiment showed no significant effects of light or consumers across or within flumes. A significant time-by-light interaction was present in the *Pycnopsyche* study, where the light treatment showed a greater increase of fungal biomass between the last two sampling dates than the shaded treatment (Fig. 7). However, a Tukey's honestly significant difference test showed no differences between pairwise means.

Time significantly affected fungal production rates in the *Macrobrachium* experiment ($p<0.001$), but overall, no other significant effects were observed across or within flumes (Table 2). Across each treatment, fungal production rates generally increased over time (Fig. 7). There were weak effects of a light-by-time interaction, where the light treatments showed a slightly greater increase of fungal production over time than the dark treatments (p=0.076). In the *Pycnopsyche* experiment, no significant temporal nor treatment effects on fungal production were observed (Table 3; Figs. 2, 7).

Fungal growth rates were significantly higher in the light treatment for the *Macrobrachium* experiment (p=0.018), but no significant consumer effects were observed. For the *Pycnopsyche* experiment, however, we observed a significant effect of time ($p<0.001$) and a light-by-time interaction (p=0.002). Fungal growth rates decreased

over time in all treatments, and there was a spike in fungal growth rates on Day 33 in the light treatments but not in the dark treatments, reflecting the light-by-time interaction (Fig. 7).

Bacterial biomass and activity

Light significantly increased bacterial biomass in the *Macrobrachium* experiment (p=0.005), but the presence of *Macrobrachium* did not show any significant effects. Bacterial biomass also increased over time across all treatments (p<0.001). In the *Pycnopsyche* experiment, neither light nor consumers individually affected bacterial biomass, but there was a significant light-by-consumer interactive effect (p=0.013). No treatment combinations differed significantly in the pairwise comparisons, following a Tukey's honestly significant difference test, but bacterial biomass was greatest in the shaded absent treatment, contrasted by lowest bacterial biomass in the light absent treatment, indicating *Pycnopsyche* presence reduced light-driven differences in bacterial biomass (Fig. 3; Fig. 8). There was a significant light-by-time interactive effect on bacterial biomass in the *Pycnopsyche* experiment (p=0.003; Table 3) where the shaded treatment peaked later (day 23) compared to the light treatment which peaked on the first sampling day (day 16; Fig. 8b.)

Bacterial production rates showed weak effects of light and consumer treatments for both experiments (Table 2; Table 3; Fig. 3). However, within the flumes, time significantly affected bacterial production in the *Macrobrachium* experiment (p<0.001), indicated by increases in bacterial production during the experiment. The strongest interaction was a light-by-consumer-by-time effect (p<0.001) which reflected earlier peaks of bacterial production (day 35) in all treatments compared to the shaded absent

treatment, as well as notably greater temporal variation of bacterial production in the shaded present treatment (Fig. 8). In the *Pycnopsyche* experiment, time elicited the only significant effect within flumes (p=0.009; Table 3) and each treatment showed an increase of bacterial production rates over time (Fig. 8).

Enzyme activity

Neither light nor consumers directly affected beta-glucosidase activity in the *Macrobrachium* experiment. However, time (p=0.002) and the interaction between light treatment and time ($p<0.001$) both affected beta-glucosidase activity (Table 2; Table 3; Fig. 9). The shaded treatment exhibited lower beta-glucosidase activity early in the study but increased strongly on day 49, whereas the light treatment exhibited higher activity at the beginning and decreased over time (Fig. 9). Time similarly affected beta-glucosidase activity in the *Pycnopsyche* experiment, with activity among all treatments increasing later into decomposition ($p<0.001$; Fig. 9b). Light also significantly affected beta-glucosidase activity across flumes (p=0.012) with the dark treatment significantly greater than the light treatment (Fig. 4).

In the *Macrobrachium* experiment, phenol oxidase activity showed significant changes over time ($p<0.001$), as the activity of all four treatments peaked at Day 35 and decreased afterward (Fig. 9c). There were no treatment effects on phenol oxidase activity across flumes (Table 2; Fig. 4; Fig. 9c). Likewise, the *Pycnopsyche* experiment showed no significant temporal or treatment effects overall, with activity levels remaining relatively low for all four sampling dates (Table 3; Fig. 9).

Mass loss

Neither light nor consumers showed significant effects on decomposition rates (*k*-values) in the *Macrobrachium* experiment (Table 2). As expected, litter mass decreased over time, with mean 59.7% litter mass remaining by day 49 (Fig. 5a). In the *Pycnopsyche* experiment, decomposition rates were significantly higher in the presence than in the absence of *Pycnopsyche* (p=0.007) but there were no light effects on decomposition (Table 2; Fig. 5d). Mean % litter mass remaining by day 44 was 72.1% in the absence and 63.8% in the presence of *Pycnopsyche* (Fig. 5b).

Table 2 *Results of ANOVA testing for Macrobrachium experiment. Significant p-values are denoted in bold.*

	Within-subjects (flumes)				Across-subjects (flumes)			
Response	Factor	F-value	P-value	Degrees of freedom	Factor	F-value	P-value	Degrees of freedom
Bacterial biomass*	Time	11.1	**<0.001**	15	Light	12.0	**0.005**	1, 12
	Light x Time	1.5	0.236	15	Consumer	0.2	0.634	1, 12
	Consumer x Time	0.2	0.896	15	Light x Consumer	0.8	0.387	1, 12
	Light x Consumer x Time	0.5	0.707	15				
Bacterial production*	Time	21.9	**<0.001**	15	Light	4.2	0.064	1, 12
	Light x Time	4.1	**0.014**	15	Consumer	1.0	0.332	1, 12
	Consumer x Time	4.3	**0.011**	15	Light x Consumer	3.3	0.093	1, 12
	Light x Consumer x Time	12.9	**<0.001**	15				
Phenol oxidase activity*	Time	52.2	**<.001**	15	Light	2.3	0.156	1, 12
	Light x Time	0.8	0.5	15	Consumer	0.0	0.936	1, 12
	Consumer x Time	2.3	0.090	15	Light x Consumer	0.6	0.467	1, 12
	Light x Consumer x Time	0.7	0.533	15				

Table 2 Continued

Beta-glucosidase activity	Time	6.0	**0.002**	15	Light	0.5	0.473	1, 12
	Light x Time	6.8	**<.001**	15	Consumer	0.0	0.902	1, 12
	Consumer x Time	1.7	0.191	15	Light x Consumer	0.0	0.867	1, 12
	Light x Consumer x Time	0.7	0.584	15				
Chlorophyll-a*	Time	4.7	**0.007**	15	Light	0.4	0.53	1, 12
	Light x Time	12.0	**<.001**	15	Consumer	0.3	0.599	1, 12
	Consumer x Time	0.4	0.719	15	Light x Consumer	1.3	0.281	1, 12
	Light x Consumer x Time	2.1	0.111	15				
Algal production*	Time	61.3	**<.001**	15	Light	115.5	**<.001**	1, 12
	Light x Time	5.0	**0.005**	15	Consumer	2.3	0.154	1, 12
	Consumer x Time	0.9	0.456	15	Light x Consumer	0.1	0.807	1, 12
	Light x Consumer x Time	5.7	**0.003**	15				
Fungal production*	Time	14.5	**<.001**	15	Light	4.2	0.063	1, 12
	Light x Time	0.4	0.759	15	Consumer	1.7	0.217	1, 12
	Consumer x Time	1.7	0.177	15	Light x Consumer	1.0	0.327	1, 12
	Light x Consumer x Time	1.6	0.210	15				

Table 2 Continued

Fungal growth*	Time	2.0	0.131	15	Light	10.8	**0.006**	1, 12
	Light x Time	0.9	0.435	15	Consumer	3.1	0.104	1, 12
	Consumer x Time	0.5	0.69	15	Light x Consumer	2.3	0.156	1, 12
	Light x Consumer x Time	1.3	0.296	15				
Fungal biomass*	Time	16.0	**<.001**	15	Light	6.2	**0.029**	1, 12
	Light x Time	2.4	0.079	15	Consumer	1.5	0.250	1, 12
	Consumer x Time	1.1	0.343	15	Light x Consumer	0.9	0.365	1, 12
	Light x Consumer x Time	0.0	0.999	15				
Mass loss					Light	0.0	0.943	1, 12
					Consumer	1.0	0.341	1, 12
					Light x Consumer	0.5	0.497	1, 12

*Log-transformed

Table 3 *Results of ANOVA testing for Pycnopsyche experiment. Significant p-values are denoted in bold.*

Response	Within-subjects (flumes) Factor	F-value	P-value	Degrees of freedom	Across-subjects (flumes) Factor	F-value	P-value	Degrees of freedom
Bacterial biomass	Time	31.8	**<.001**	15	Light	0.6	0.446	1, 12
	Light x Time	5.5	**0.003**	15	Consumer	1.0	0.349	1, 12
	Consumer x Time	0.6	0.616	15	Light x Consumer	0.4	**0.013**	1, 12
	Light x Consumer x Time	2.2	0.111	15				
Bacterial production	Time	4.5	**0.009**	15	Light	1.8	0.207	1, 12
	Light x Time	1.2	0.313	15	Consumer	0.0	0.871	1, 12
	Consumer x Time	0.1	0.967	15	Light x Consumer	0.3	0.600	1, 12
	Light x Consumer x Time	0.6	0.625	15				
Phenol oxidase activity*	Time	2.0	0.128	15	Light	0.2	0.662	1, 12
	Light x Time	1.3	0.3	15	Consumer	0.0	0.883	1, 12
	Consumer x Time	0.2	0.863	15	Light x Consumer	0.1	0.744	1, 12
	Light x Consumer x Time	2.0	0.130	15				

Table 3 Continued

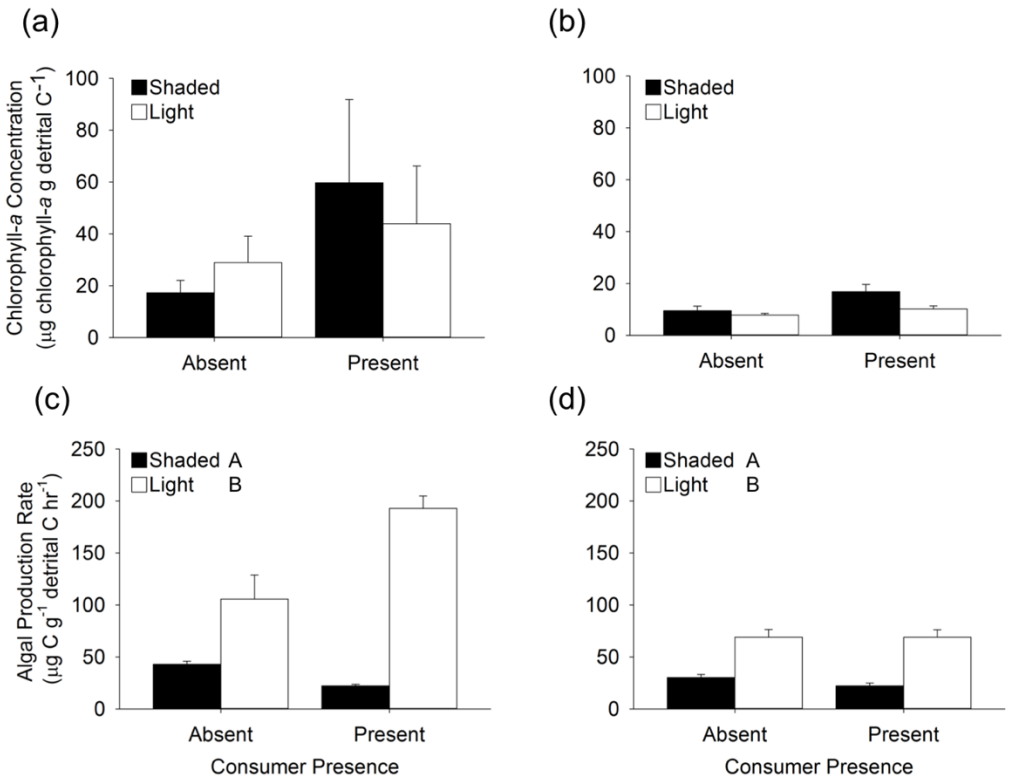
Beta-glucosidase activity*	Time	8.2	**<.001**	15	Light	8.7	**0.012**	1, 12
	Light x Time	1.1	0.344	15	Consumer	0.3	0.621	1, 12
	Consumer x Time	0.5	0.674	15	Light x Consumer	0.2	0.687	1, 12
	Light x Consumer x Time	0.9	0.430	15				
Chlorophyll-a*	Time	5.3	**0.004**	15	Light	0.6	0.439	1, 12
	Light x Time	0.0	0.999	15	Consumer	3.4	0.09	1, 12
	Consumer x Time	0.1	0.943	15	Light x Consumer	0.2	0.667	1, 12
	Light x Consumer x Time	0.7	0.962	15				
Algal production*	Time	22.0	**<.001**	15	Light	55.3	**<.001**	1, 12
	Light x Time	1.9	0.146	15	Consumer	2.2	0.163	1, 12
	Consumer x Time	0.3	0.808	15	Light x Consumer	1.8	0.205	1, 12
	Light x Consumer x Time	3.1	**0.040**	15				
Fungal production	Time	2.5	0.074	15	Light	0.0	0.983	1, 12
	Light x Time	1.4	0.260	15	Consumer	0.3	0.597	1, 12
	Consumer x Time	1.4	0.257	15	Light x Consumer	0.4	0.523	1, 12
	Light x Consumer x Time	1.5	0.243	15				

Table 3 Continued

Fungal growth*	Time	20.1	**<.001**	15	Light	3.0	0.110	1, 12
	Light x Time	6.1	**0.002**	15	Consumer	0.2	0.701	1, 12
	Consumer x Time	1.9	0.154	15	Light x Consumer	1.8	0.202	1, 12
	Light x Consumer x Time	0.4	0.761	15				
Fungal biomass*	Time	28.3	**<.001**	15	Light	2.7	0.125	1, 12
	Light x Time	4.7	**0.007**	15	Consumer	0.2	0.637	1, 12
	Consumer x Time	0.9	0.441	15	Light x Consumer	3.1	0.103	1, 12
	Light x Consumer x Time	0.7	0.574	15				
Mass loss					Light	0.3	0.601	1, 12
					Consumer	9.3	**0.010**	1, 12
					Light x Consumer	1.8	0.206	1, 12

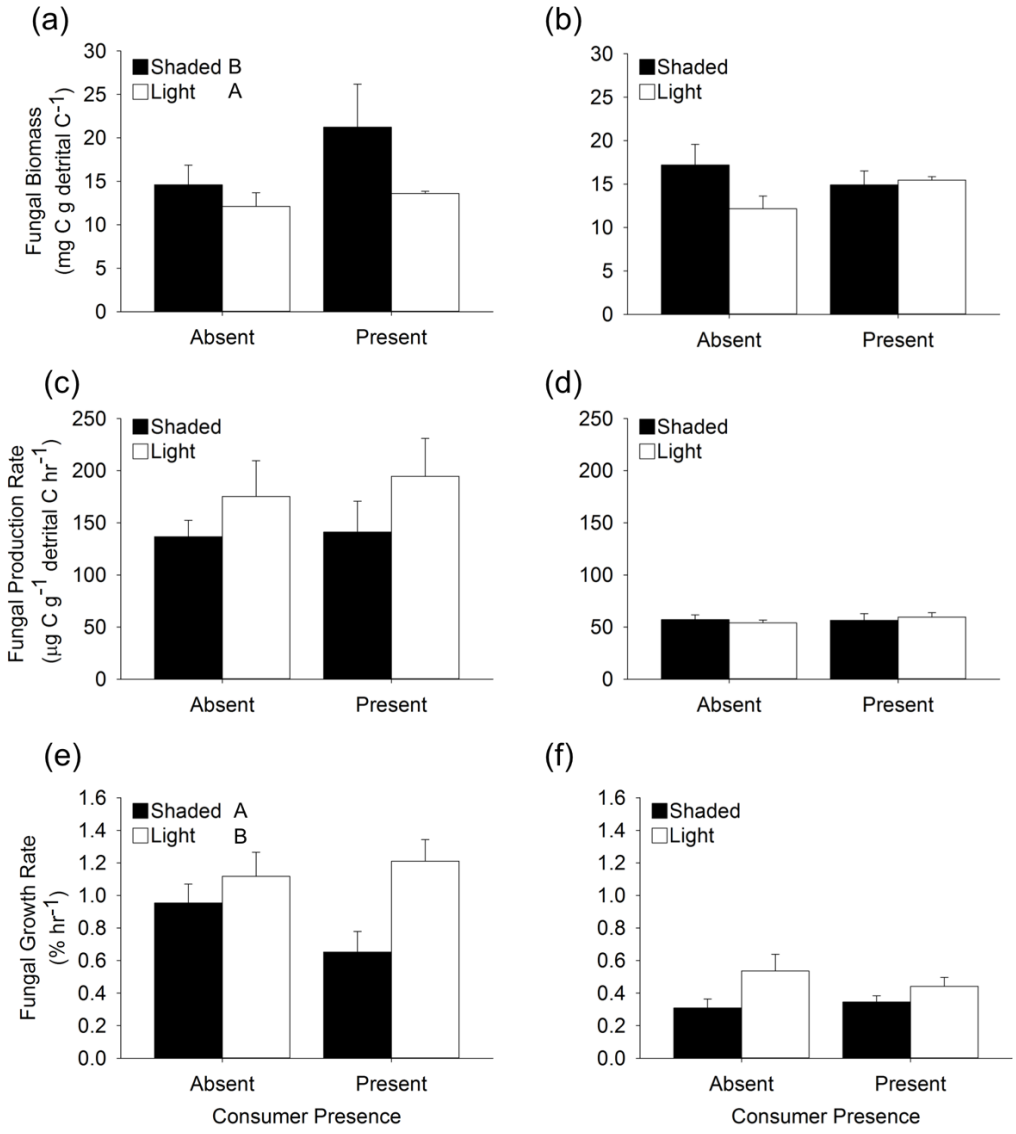
*Log-transformed

Figure 1. Mean ± SE (n=4) litter-associated algal biomass and algal production rates on litter exposed to contrasting light and consumer treatments.



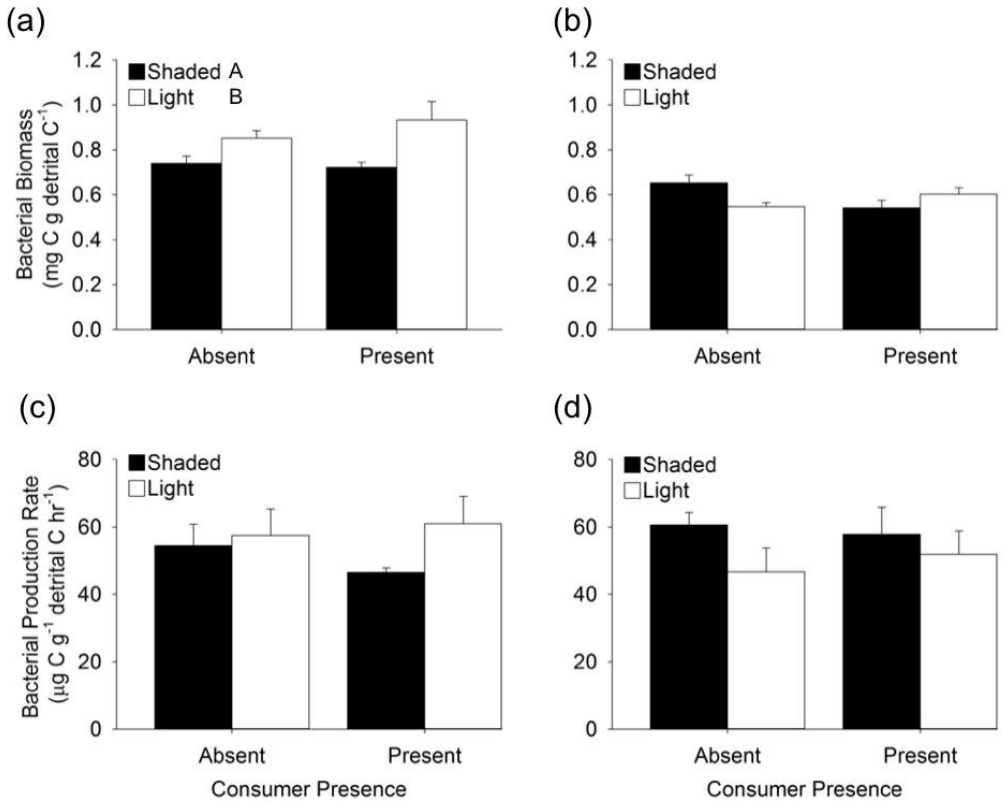
Summary means were calculated from time-pooled responses within each flume. Capital letters indicate significant treatment effects ($p<0.05$). Results of the Macrobrachium experiment are indicated in Panels (a) and (c), while results of the Pycnopsyche experiment are indicated in Panels (b) and (d). See Tables A2 and A3 for statistics, and see Figure A6 for associated variables over time.

Figure 2. Mean ± SE (n=4) litter-associated fungal biomass, fungal production rates, and fungal growth rates on litter exposed to contrasting light and consumer treatments.



Summary means were calculated from time-pooled responses within each flume. Capital letters indicate significant treatment effects ($p<0.05$). Results of the Macrobrachium experiment are indicated in Panels (a), (c) and (e), while results of the Pycnopsyche experiment are indicated in Panels (b), (d) and (f). See Tables A2 and A3 for statistics, and see Figure A7 for these variables over time.

Figure 3. Mean ± SE (n=4) litter-associated bacterial biomass and bacterial production rates on litter exposed to contrasting light and consumer treatments.



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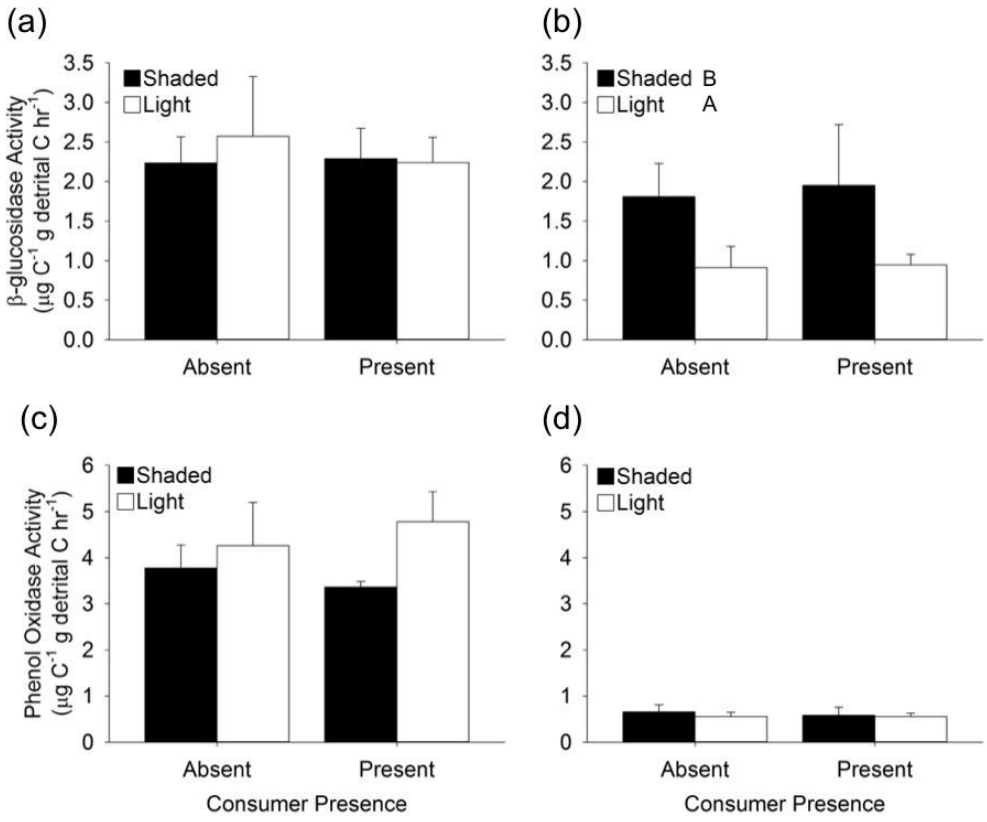
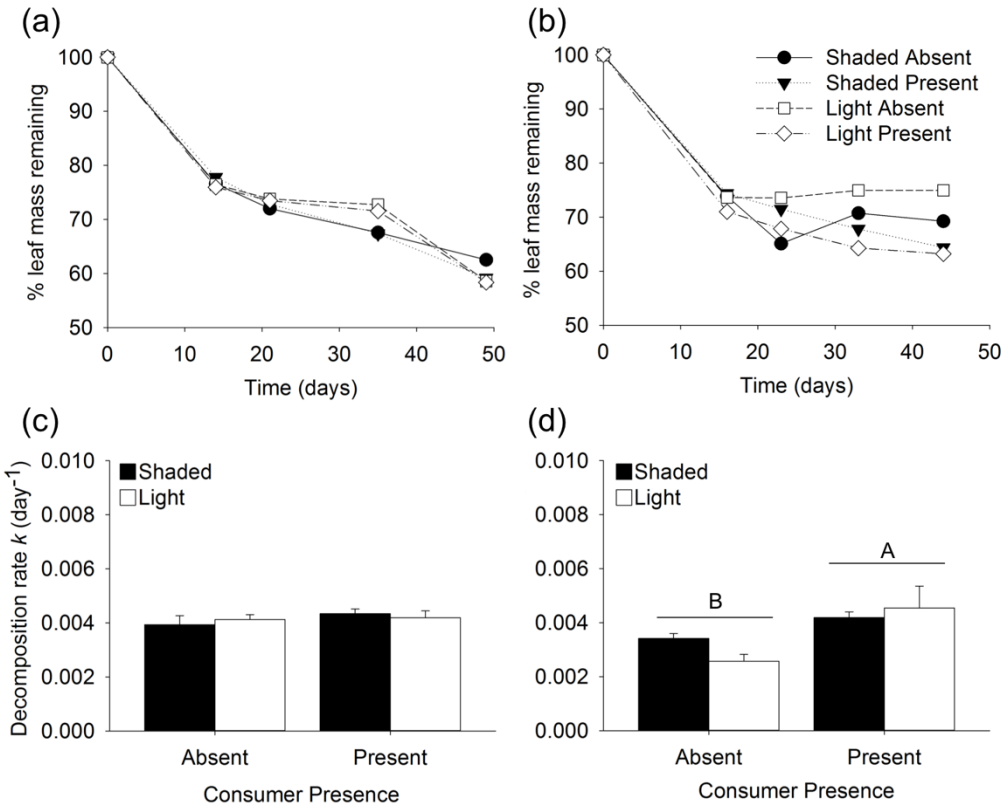
Summary means were calculated from time-pooled responses within each flume. Capital letters indicate significant treatment effects ($p<0.05$). Results of the Macrobrachium experiment are indicated in Panels (a) and (c), while results of the Pycnopsyche experiment are indicated in Panels (b) and (d). See Tables 1 and 2 for statistics, and see Figure A8 for these variables over time.

Figure 4. Mean ± SE (n=4) litter-associated beta-glucosidase and phenol oxidase activity rates on litter exposed to contrasting light and consumer treatments.



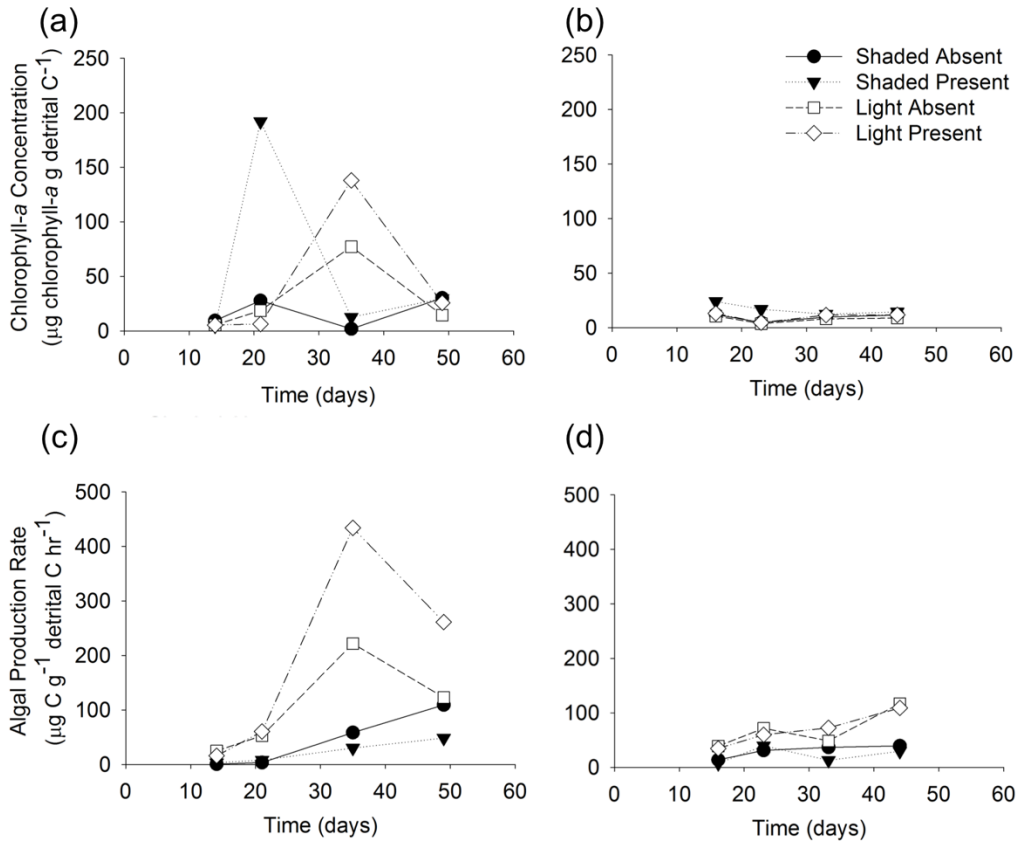
Summary means were calculated from time-pooled responses within each flume. Capital letters indicate significant treatment effects ($p<0.05$). Results of the Macrobrachium experiment are indicated in Panels (a) and (c), while results of the Pycnopsyche experiment are indicated in Panels (b) and (d). See Tables 1 and 2 for statistics, and see Figure A9 for these variables over time.

Figure 5. Summary line graphs of mass loss over time and bar graphs of timepooled *k*-values.



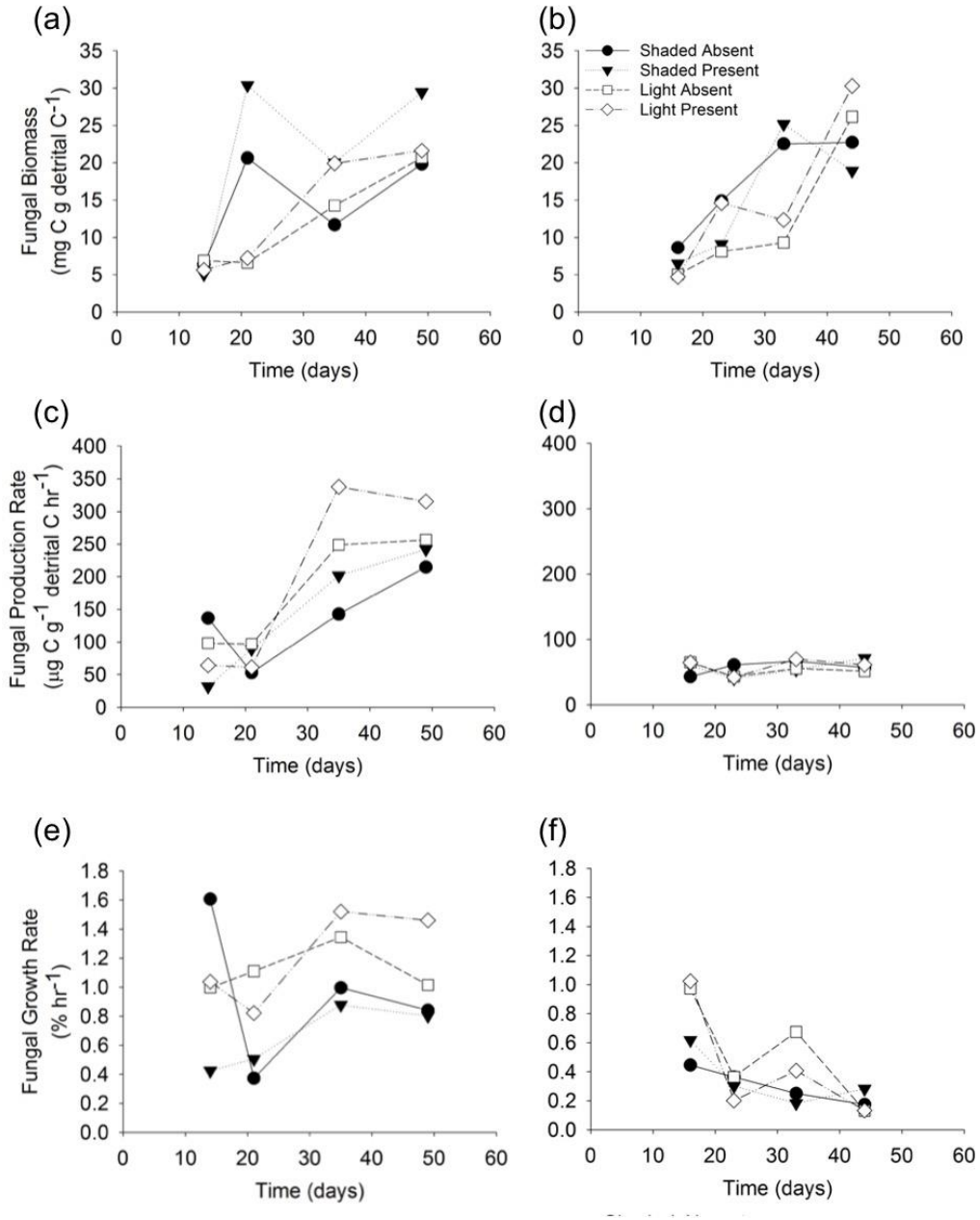
Capital letters indicate significant treatment effects ($p<0.05$). Results of the Macrobrachium experiment are indicated in Panels (a) and (c), while results of the Pycnopsyche experiment are indicated in Panels (b) and (d). See Tables 1 and 2 for statistics.

Figure 6. Summary line graphs of algal biomass and production rates over time. Results of the 2017 Macrobrachium experiment are indicated in Panels (a) and (c), while results of the 2018 Pycnopsyche experiment are indicated in Panels (b) and (d).



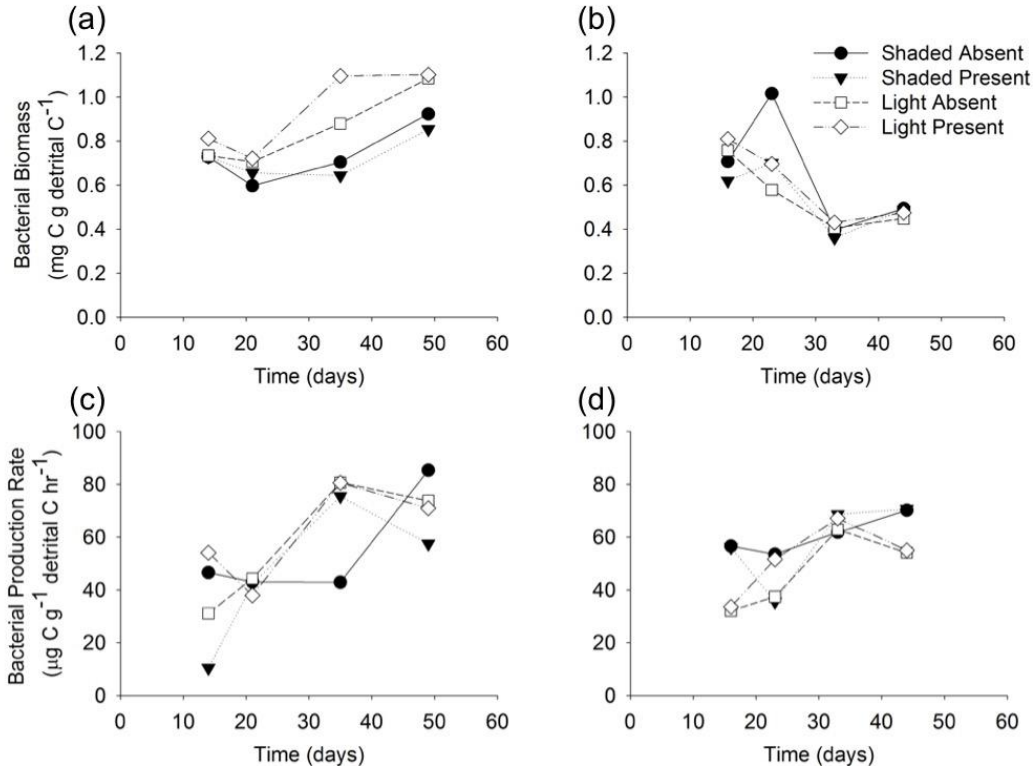
Results of the 2017 Macrobrachium experiment are indicated in Panels (a) and (c), while results of the 2018 Pycnopsyche experiment are indicated in Panels (b) and (d).

Figure 7. Summary line graphs of fungal biomass, production and growth rates over time.



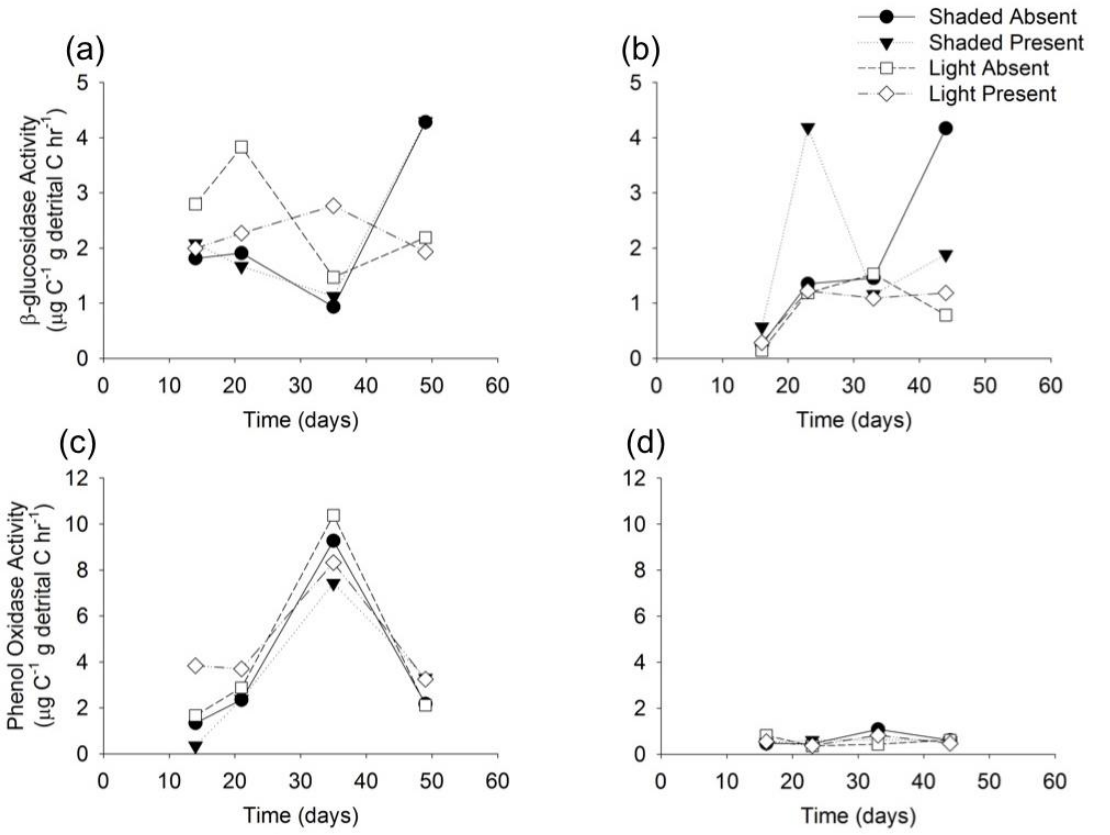
Results of the 2017 Macrobrachium experiment are indicated in Panels (a), (c) and (e), while results of the 2018 Pycnopsyche experiment are indicated in Panels (b), (d) and (f).

Figure 8. Summary line graphs of bacterial biomass and production rates over time.



Results of the 2017 Macrobrachium experiment are indicated in Panels (a) and (c), while results of the 2018 Pycnopsyche experiment are indicated in Panels (b) and (d).

Figure 9. Summary line graphs of beta-glucosidase and phenol oxidase activity rates over time.



Results of the 2017 Macrobrachium experiment are indicated in Panels (a) and (c), while results of the 2018 Pycnopsyche experiment are indicated in Panels (b) and (d).

CHAPTER IV – DISCUSSION

Overall, my study showed mixed results with respect to my hypotheses. Priming effects were weak in the *Macrobrachium* study, with algae responding positively to light, in turn stimulating fungal growth rates but reducing fungal biomass while not affecting decomposition rates. Furthermore, there was a lack of significant decomposition or overall microbial responses when *Macrobrachium* were introduced. In the *Pycnopsyche* study, light similarly stimulated algal activity, but did not alter fungal biomass or activity, nor decomposition rates; however, increased light reduced degradative enzyme activity (beta-glucosidase). When *Pycnopsyche* were introduced, *k*-values increased regardless of light treatment, suggesting that the more active shredding behavior of these consumers increased the rate of decomposition of leaf litter. Thus, in both experiments, light effectively altered algal activity on the *Liriodendron tulipifera* litter, indicating that subsequently observed effects of light on heterotrophic microbial dynamics or decomposition may be due to photolysis, but can also be attributed to contrasting algal activity and associated increases of algal exudates that stimulate heterotrophy (Kuehn et al. 2014). Low algal biomass, regardless of light treatment, indicated up-regulation of chlorophyll-*a* levels in response to shading.

In line with previous studies, my results suggest that algae can directly affect fungal growth rates and biomass accrual during leaf litter decomposition (Danger et al. 2013, Kuehn et al. 2014). Algal presence and light stimulated fungal growth rates; however, this was not necessarily associated with greater accrual of fungal biomass, nor with greater rates of decomposition, as seen in the *Macrobrachium* study, as would be expected given increased fungal activity. The fungal response observed shows a

decoupling of growth rates from biomass accrual due to algal activity, indicative of mechanisms underlying a light-induced negative priming effect, but also a shift towards biomass accumulation in the shaded treatment (Halvorson et al. 2019). We can glean two major findings from these results: in light, there is likely an alternative carbon source supporting fungal growth, such as algal exudates (Kuehn et al. 2014; Soares et al. 2017) and fungi may be investing in reproduction as opposed to hyphal biomass accrual in association with algae (Halvorson et al. 2019). The contrasting responses of fungi in both experiments suggest a potentially climate-induced plasticity of fungal processes; depending on environmental conditions, perhaps aquatic hyphomycetes can alter their allocation of resources to ensure their fitness over the long term, whether that means an increase in biomass or greater production of asexual spores.

In both experiments, bacterial production rates increased significantly over time, indicating colonization of litter in tandem with other microbes such as algae and fungi (Rier and Stevenson 2002; Halvorson et al. 2019). Bacterial biomass showed a corresponding increase and thus a greater incorporation of leucine into protein over time in the *Macrobrachium* study, whereas in the *Pycnopsyche* study there was a light-by-time interaction indicating an earlier peak of bacterial biomass in the light treatment. Thus, light can affect the timing of bacterial colonization of litter, perhaps by altering the pool of labile carbon available to support bacterial growth. Much like the effects of algae on fungi, the heterotrophic bacteria should be able to process algal exudates as a form of easily-accessible carbon. Throughout the duration of decomposition, light also significantly increased bacterial biomass in the *Macrobrachium* study, suggesting a scaffolding effect in which algal presence increases surface area and creates an

exopolymer matrix (Rier and Stevenson 2002; Carr et al. 2005). The Carr study proposes that algae and bacteria do *not* experience increased competition as they grow together, instead coexisting on the same piece of litter and sustaining the other organism's life processes (2005). As a result of this increased algal surface area and exudate, bacteria have increased area on the litter to colonize and accrue biomass. As in several recent studies, my results indicate that periphytic algae may increase litter-associated bacterial biomass, but do not as strongly increase bacterial production rates (Soares et al. 2017; Halvorson et al. 2019).

Enzymatic responses are indicative of the potential for heterotrophic degradation of organic matter (Romani et al. 2006). The activity of degradative enzymes may respond positively to light, due to algal-induced shifts in C substrates supporting growth, or alternatively due to algal-induced increases of pH within the periphyton matrix (Francoeur et al. 2006; Rier et al. 2007). In my experiments, light did not strongly affect enzyme activity, with the exception of decreasing beta-glucosidase activity in the *Pycnopsyche* study and altering temporal dynamics of beta-glucosidase activity in the *Macrobrachium* study (light-by-time interaction). Beta-glucosidase is a cellulase and is thus effective at breaking down recalcitrant organic matter during decomposition; in the *Pycnopsyche* study, perhaps activation of this enzyme is an alternative means of heterotrophic decomposition of organic matter when light-mediated photolysis or algal exudates are absent (Francoeur et al. 2006). Thus, when the priming effect is unavailable – either due to lack of light or temporal effects – beta-glucosidase could ostensibly be a mechanism by which litter is degraded. The contrasting lack of light effects on phenol oxidase activity suggests heterotrophs did not alter breakdown of comparatively

recalcitrant phenolic compounds (e.g., lignin) during either experiment. I propose that this effect is due to either the small scale of my experimental mesocosms or the lack of recalcitrant phenols within the relatively labile *Liriodendron tulipifera* litter.

My experiments also show a contrast of consumer effects during leaf litter decomposition, most notably on decomposition rates, in the *Macrobrachium* versus the *Pycnopsyche* experiments. *Macrobrachium*, as a selective omnivore (Abele and Blum 1977), did not actively interact with the *Liriodendron tulipifera* or its associated biofilm like *Pycnopsyche*, as indicated by its lack of direct effects on microbial processes or decomposition. *Macrobrachium* is a passive feeder, collecting small particles that float in the water column or sink to the stream bed. In contrast, as an obligate shredder-detritivore, *Pycnopsyche* likely stimulated decomposition rates by directly tearing pieces of leaf detritus to use as either case material or a food source (Creed et al. 2009). In addition to these direct feeding effects, consumers may indirectly affect microbial colonization through nutrient cycling as they excrete and egest wastes into the stream, a phenomenon which can vary across species (Evans-White and Lamberti 2005; Parr et al. 2018). Although consumer effects on microbial dynamics were weak in both studies, direct and indirect pathways, such as those seen in the Evans-White and Lamberti study, may explain my observation of strong consumer effects on microbial temporal dynamics during decomposition. In spite of the different consumers used in that study, there is a distinct contrast of each macroinvertebrate's feeding habits and, thus, the magnitude of their effects on the biofilm. Indeed, algal production in both of my experiments and bacterial production in the *Macrobrachium* experiment stood out as variables affected by an interaction of consumers, light and time. While all leaf packs began with similar

microbial dynamics in each study, the presence or absence of light and consumers impacted them differently over time. Interestingly, the strong consumer effects on algal and bacterial temporal dynamics, but not fungal dynamics or enzymatic activity, indicates that consumers may affect litter-associated periphytic (surface) microbial dynamics more than endogenous microbes (primarily fungi) during litter decomposition. Consumer feeding or nutrient cycling activity may thus differentially affect the long-term colonization and succession of each component of microbial biofilms during litter decomposition.

Ultimately, my experiments demonstrate that litter-associated algal activity responded positively to increased light, in turn reducing fungal biomass accrual, increasing fungal growth rates, and increasing bacterial biomass in the *Macrobrachium* study. Light also reduced beta-glucosidase activity throughout decomposition in the *Pycnopsyche* study. While these microbial responses could indicate a negative priming effect (Halvorson et al. 2019), microbial responses did not translate to significant effects on litter decomposition, indicating absence of algal priming effects on long-term litter decomposition (Elosegi et al. 2018). Algal exudates likely provided soluble inorganic compounds for the heterotrophic components of the biofilm to assimilate within light treatments, but heterotrophic use of exudates was not coupled to greater breakdown in the light (Danger et al. 2013; Kuehn et al. 2014; Soares 2017). Presence of *Pycnopsyche*, but not *Macrobrachium*, stimulated decomposition rates, but both consumers only affected the timing of microbial dynamics during leaf litter decomposition, and often in a manner that depended on light treatment. While consumer effects on fine-scale timing of microbial colonization may depend on light availability, the overall top-down effects of

consumers on litter microbial dynamics and decomposition rates are most likely independent, acting via different pathways from overall bottom-up effects of light during leaf decomposition. Thus, I continued to observe the previously-evident temporal factor of priming: after a period of time, and presumably once the limiting nutrients have been used up, most litter simply cannot maintain the priming processes long-term.

Additionally, I felt that there might be both internal and external validity issues with my study. Externally, I know that the size of the flumes is a definite limitation; a low-nutrient stream containing caddisflies, for the most part, does not exist within a 3-meter span of vinyl rain gutter, nor do said caddisflies generally prefer to be soldered within a bag made of plastic mesh with their food. "Bottle effects" applied to both the mesocosms themselves as well as the samples I took each day that I ran assays; the biofilm peeling off leaves and sticking to the Ziploc bags I used was a definite concern, regardless of how quickly I could get the samples to the lab and keep the microbes alive. Instead of a gutter mesocosm, next time I would prefer to change this experiment into a larger-scale *in situ* setup. Internally, I realize that the flumes being exposed to the elements and mostly unattended, as opposed to being housed within a building or a laboratory environment, opens them up to extraneous variable input. This input can consist of falling debris from the surrounding vegetation, rainwater inputs, changing photosynthetic radiation due to the weather, and the most noticeable of all: other organisms. Chironomids, or the larvae of non-biting midges, frequently appeared throughout both experiments, building cases on the leaf litter that I sealed within the bags. Their small body size and frequency of reproduction allowed them to infiltrate and make the litter bags their homes; their contribution to the nutrient recycling process

cannot be ignored, but is unfortunately outside the scope of this thesis. When expanding on this research in the future, I will be sure to include the chironomids as potential consumers and sources of nutrients as well.

Several other future directions result from my findings. As a whole, my stream mesocosms simulated a small segment of a low-nutrient stream, but in future experiments I would expand the volume of my mesocosms as well as the diversity of potential macroinvertebrate consumers to more accurately replicate environmental variables (e.g. temperature, dissolved nutrients, and light levels) that the litter and biofilm would be exposed to *in situ*. In-stream or streamside channel mesocosms would present a prime opportunity to further test how consumers and light affect microbial dynamics and decomposition in stream ecosystems. Additionally, I would like to examine whether algal priming effects (and the ensuing shift in palatability of the litter) varies according to the type of substrate that is colonized; a more recalcitrant species of leaf litter such as *Quercus* subjected to the same conditioning period and stream water might exhibit different results when consumers are introduced. Overall, my research contributes to the growing body of knowledge regarding the intersection of green and brown food webs within riparian streams; further research could be critical for practical applications such as land-use management, agricultural planning and forestry. If we expect to understand the large-scale ecological consequences of removing riparian vegetation, we have to examine the smaller-scale interplay of the stream's smallest organisms.

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