ABSTRACT

Title of Dissertation: LEAF-ASSOCIATED PERIPHYTON IN HETEROTROPHIC STREAMS: EFFECT ON MACROINVERTEBRATE ASSEMBLAGES AND GROWTH

Rebecca A. Eckert, Doctor of Philosophy, 2020

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Temperate headwater streams are often shaded, limiting autochthonous production, and therefore energetically supported by allochthonous material, e.g., leaves, via fungal and bacterial decomposition. Macroinvertebrate shredders feed on this leaf matrix, providing food for other organisms. Recent work indicates that periphyton (e.g., diatoms, green algae, cyanobacteria; hereafter, algae) interacts with microbial decomposers and provides higher quality food. Little work has, however, examined these interactions in natural

settings. I investigated leaf-associated algae's impact on macroinvertebrate leaf colonization in the field, followed by measuring growth and food preferences in the lab based on field results. First, I manipulated leaf light availability in high- and low-nutrient streams in winter and spring. Leaf-associated algal and fungal biomass were positively correlated in winter. Leaf C:N negatively correlated to algae in winter and fungi in spring, while N:P and C:P negatively correlated to fungi in winter and algae in spring. These factors predicted functional feeding guild biomass and abundance, e.g., predator biomass by algal and fungal biomass and spring shredder biomass by leaf stoichiometry. Algal biomass elicited differential taxon responses; e.g., *Ephemerella* (Ephemeroptera:Ephemerellidae) and *Stenonema* (Ephemeroptera:Heptageniidae) responded positively while *Tipula* (Diptera:Tipulidae) responded negatively. Second, I fed light- and dark-conditioned leaves to *Ephemerella invaria* and *Caecidotea communis* (Isopoda:Asellidae), which both consumed leaves and algae. *C. communis* experienced greater growth on light-conditioned leaves, indicating a high-quality resource, while *E. invaria* had no growth differences between treatments. Third, light- and dark-conditioned leaves were offered to five taxa, *Amphinemura* (Plecoptera:Nemouridae), *Tipula*, *Stenonema*, *Lepidostoma* (Trichoptera:Lepidostomatidae), and *Caecidotea communis*. *Tipula* alone demonstrated a preference which was for dark-conditioned leaves. These results indicate that leaf-associated algae are a food resource and attractant for some macroinvertebrates and a deterrent to others. Natural headwater streams are heterogeneous with leaves exposed to varying light levels, altering leaf-associated algae and providing differential food resources. Anthropogenic impacts often homogenize these streams. Although restoration seeks to restore heterogeneity, headwater stream algae are

largely ignored. This work demonstrates the important role algae play in macroinvertebrate interactions with senescent leaves, highlighting the need to incorporate allochthonous and autochthonous resources into stream restoration and management efforts to support biodiversity.

LEAF-ASSOCIATED PERIPHYTON IN HETEROTROPHIC STREAMS: EFFECT ON MACROINVERTEBRATE ASSEMBLAGES AND GROWTH

by

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Dedication

This dissertation is dedicated in part to my advisor, Bill Lamp, without whom none of this would have been possible. You believed in my ideas about algae on leaves from the very beginning, even when others suggested it might not be a fruitful topic and provided me everything I could have ever needed or wanted to pursue it. You have fostered and created the best of environments to pursue science within and taught me so much more than just how to do the best science, and I will always be grateful for my time in the Lamp Lab.

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Abstract

Temperate headwater streams traditionally have been considered heterotrophic with little primary production. Recent work, however, suggests algae on leaves in these streams may play a greater role than previously thought through interactions with microbial decomposers like fungi. Algae also may be important for macroinvertebrates colonizing leaves in streams. Algae are a more nutritious food resource for shredders than fungi and bacteria and provide a food resource for non-shredder macroinvertebrates. In a field experiment, I manipulated light in three low-nutrient and three high-nutrient streams using leaf packs filled with red maple leaves in winter and spring. Algal and fungal biomass, leaf stoichiometry, and macroinvertebrate abundance and biomass associated with the leaf packs were measured after four weeks. I identified the macroinvertebrate community and examined differences in functional feeding guilds and taxa under ambient- and shaded-light treatments and low- and high-nutrient concentrations in relation to measured leaf characteristics. Leaf-associated algal biomass was greatest in

high-nutrient streams and ambient-light treatments in both seasons. Fungal biomass on leaves was greatest in high-nutrient streams and had a moderate marginally significant positive correlation with algae during the winter. Leaf C:N was negatively correlated to algae in winter and fungi in both seasons, while leaf N:P and C:P were negatively correlated to fungi in winter and algae in spring. Interactions between fungi and algae on leaves and the nutritional importance of each for macroinvertebrates likely change across seasons, potentially impacting macroinvertebrate community composition. Macroinvertebrate diversity did not differ, but biomass was significantly greater in shaded-light treatments during spring. Abundance was highest in the high-nutrient ambient-light conditions in both seasons, corresponding to greatest algal biomass. Functional feeding guild biomass and abundance were related to different leaf characteristics by season and guild. Higher algal biomass was an important factor for colonization of certain macroinvertebrates (e.g., *Ephemerella* (Ephemeroptera: Ephemerellidae) and *Stenonema* (Ephemeroptera: Heptageniidae)), while others were more abundant under shaded treatments with lower algal biomass (e.g., *Tipula* (Diptera: Tipulidae)), indicating taxon-specific responses. Leaf-associated algae may be an important factor mediating macroinvertebrate communities associated with leaves in temperate headwater streams. These results demonstrate that green and brown food webs intersect within leaf packs, and they cannot be easily disentangled. We therefore should consider both autochthonous and allochthonous resources within headwater streams when examining their communities or developing water management strategies.

Introduction

Temperate headwater streams have traditionally been considered heterotrophic brown food webs, where energy is derived from organic matter decomposition, such as leaves (Vannote, 1980; Abelho, 2001). Organic matter is colonized by fungal and bacterial decomposers, and this leaf matrix is fed upon by macroinvertebrate shredders (Webster & Benfield, 1986; Gessner et al., 1999; Abelho, 2001). These basal microbes and the organisms feeding on them provide energy and nutrients for higher trophic levels, supporting headwater stream food webs (Wallace & Webster, 1996). Temperate headwater streams are often heavily shaded and experience low light availability, restricting primary production (Richardson & Danehy, 2007; Richardson, 2019). As a result, primary producers such as algae are considered to have an insignificant role in temperate headwater streams where there is little to no autotrophic green food web present (Richardson, 2019).

Recent work has challenged this view, suggesting that algae play a disproportionate role in relation to its biomass in shaded headwater streams (e.g., Lagrue et al., 2011; Guo et al., 2016a). Conclusions in the literature have been mixed, indicating the mechanisms and magnitude of these interactions are still unclear (Brett et al., 2017; Bengtsson et al., 2018). For instance, algae may prime decomposition by providing labile carbon (C) exudates for microbial decomposers to utilize prior to obtaining C from organic matter decomposition (e.g., leaves), ultimately increasing decomposition rates (Danger et al., 2013). Algal biomass also has been linked to enzyme activity within leaf litter (Rier et al., 2007), and fungal and algal biomass are often positively related (e.g., Rier et al., 2007; Kuehn et al., 2014). Other work, however, has shown that algae may

stimulate fungal growth directly and decouple fungi from leaf decomposition (e.g., Halvorson et al., 2019a). Leaf-associated algae also are consumed directly by macroinvertebrates feeding on the leaves. Guo et al. (2016b) showed that algal polyunsaturated fatty acids were incorporated preferentially into the tissues of the shredder *Anisocentropus bicoloratus* (Trichoptera: Calamoceratidae) and when more algae were present, growth was greater. In addition, a number of other studies have indicated increased growth with algae or preference for algae by macroinvertebrate shredders (e.g., Friberg & Jacobsen, 1994; Franken et al., 2005; Leberfinger & Bohman, 2010). Algae on leaves thus appear to have a much greater role than traditional headwater stream paradigms indicate.

Within shaded headwater streams, organic matter often accumulates into natural leaf packs, providing discrete food resources for shredders, but leaves also may provide resources for other macroinvertebrate functional groups. Leaves provide refugia from disturbance and predators as well as a colonization surface for non-shredding macroinvertebrates (Richardson, 1992; Malmqvist, 1993; Dobson, 1994). The entrapment of other food sources like fine particulate organic matter within leaves provides food for non-shredders such as collectors, and the aggregation of macroinvertebrates provides food for predators (Richardson, 1992; Dobson, 1994; Dangles et al., 2001; Tonin et al., 2014). Scrapers/grazers also colonize leaf packs and feed primarily on algal components of biofilms (e.g., Dobson, 1994; Carrick et al., 2012). Algae, particularly diatoms, provide a more nutritious food than fungi and bacteria within the leaf biofilm for macroinvertebrates (Brett & Müller-Navarra, 1997; Guo et al., 2016b). Algal presence on

leaves therefore may be an important factor influencing macroinvertebrate community structure associated with leaves in headwater streams.

Environmental factors such as light and nutrients can influence benthic algae and macroinvertebrate colonizers on leaves. Algal productivity increases with greater light (Minshall, 1978; Hill et al., 2009) and nutrient availability (Smith et al., 1999; Dodds et al., 2002; Dodds, 2006). Algal species composition also may vary with light and nutrients and affect the colonization of macroinvertebrates. Diatoms, the most nutritious group of algae (Brett & Müller-Navarra, 1997; Guo et al., 2016b), tend to dominate lotic communities (Wehr & Sheath, 2015); species composition and proportion of diatoms may, however, shift with changes in nutrient and light availability (Borchardt, 1996; Hill, 1996). Macroinvertebrate communities also can shift with increasing nutrients from intolerant species to those tolerant of high nutrient concentrations (Wang et al., 2007) and from species with lower to higher nutrient demands. Growth is enhanced in those with higher nutrient demands due to decreased stoichiometric imbalances in their food with increased nutrient availability (e.g., Evans-White et al., 2009). Leaf incubation in natural streams and stream mesocosms with varied nutrient concentrations results in decreases in nutrient ratios (Scott et al., 2013; Tant et al., 2013), often through changes in associated microbial biomass (France, 2011; Connolly & Pearson, 2013). Further, elemental imbalances may respond to light as algae also alter leaf nitrogen (N) and phosphorus (P) contents during decomposition, shifting leaf C:N and C:P ratios (Halvorson et al., 2019b). As such, light may affect the colonization of certain macroinvertebrates or functional groups. For instance, in a manipulative light study, Kiffney et al. (2004) found that the biomass of the collector-gatherer families Chironomidae and Baetidae was positively

related to light levels likely owing to a bottom-up effect on periphyton. Thus, light availability and nutrients may moderate relationships between algae and macroinvertebrates associated with leaves.

To date, there is limited information regarding the role of algae on leaves in headwater streams, particularly in reference to macroinvertebrates, and so there is an open question as to whether and how leaf-associated algae may impact macroinvertebrate assemblages, and whether these impacts are related to algal interactions with other leaf-associated microbes and/or algae's influence on leaf characteristics (e.g., stoichiometry). To examine this question, I performed a manipulative light experiment in three high-nutrient and three low-nutrient headwater streams in winter and spring to investigate relationships between macroinvertebrates, algae, and fungi associated with leaves. I hypothesized that greater algal, fungal, and macroinvertebrate biomass as well as macroinvertebrate diversity (taxonomic and functional) would be present on leaves in ambient-light and high-nutrient conditions than shaded-light and low-nutrient conditions. I also predicted that algal and fungal biomass would be positively related to each other but negatively related to leaf stoichiometry (C:N:P). I further predicted that the macroinvertebrate community associated with each leaf factor combination would differ as reflected by taxonomic and functional measures due to changes in leaf characteristics, particularly changes in algae. Lastly, I hypothesized that macroinvertebrate functional feeding guilds colonizing the leaves would each exhibit different relationships to leaf-associated algal and fungal biomass and leaf stoichiometric ratios.

Methods

Field sites

A manipulative field experiment for light was carried out in six Piedmont first order headwater streams in Maryland, USA, in December 2016-January 2017 (winter) and March-April 2017 (spring). Winter was chosen to capture a period of high shredder activity (Graça et al., 2001) while spring was chosen to capture a peak in algal productivity (Halliday et al., 2016). Streams were selected based upon nutrient concentrations, location, ease of access, and protection from vandalism; three streams had lower and three higher nutrient concentrations consistently throughout the experiment, providing clear nutrient categories without experimental nutrient additions. Two streams were selected in Little Bennett Regional Park in Clarksburg, Montgomery County, MD: Browning Run, a low-nutrient stream, and Tobacco Barn, a high-nutrient stream. Two streams were selected in Rachel Carson Conservation Park in Brookeville, Montgomery County, MD: Zion Road and Fern Valley, both low-nutrient streams. Two streams were selected at Central Maryland Research and Education Center in Clarksville, Howard County, MD: South Stream and Forest Stream, both high-nutrient streams. The furthest of the six reaches were 32 km apart. Browning Run, Tobacco Barn, and Fern Valley reaches were located upstream of natural surface hiking trails, out of view of the trails to avoid vandalism. Zion Road reach was located downstream of a road and culvert within Rachel Carson Conservation Park. South Stream and Forest Stream reaches were located within forested areas surrounded by University of Maryland farm fields. Initial nutrient concentrations were obtained from water samples taken in August 2016, except for South Stream for which data was already available. According to Dodds et al. (1998), the low-

and high-nutrient streams can be classified as oligo-mesotrophic and eutrophic, respectively. Sensitive taxa were assessed via percent Ephemeroptera, Plecoptera, and Trichoptera (% EPT) and the Hilsenhoff Biotic Index, which measures the average pollution tolerance value of taxa within a sample, using the experiment's leaf packs. All streams were embedded in a forested matrix surrounded by more agricultural than urban land. Location information, watershed land-use, initial nutrient concentrations, and data on sensitive taxa are in Table 1.1.

Stream characteristic measurements were taken at experiment deployment and retrieval in both seasons. Water samples were taken from midstream of the experimental reach and frozen at -20°C for subsequent analysis of TP, total nitrogen (TN), SRP, and NO_3^-. TP and TN samples were first oxidized via the persulfate method (APHA, 2012). TP and SRP were measured using the ascorbic acid method, and TN and NO_3^- were measured using cadmium reduction following standard protocols (APHA, 2012). Canopy photographs were taken at downstream, midstream, and upstream along the experimental reach; images were converted to black and white and the number of sky vs. canopy pixels counted to estimate canopy cover via ImageJ (National Institutes of Health, Bethesda, MD). Pictures were taken prior to full leaf-out in the spring. At the same three locations, dissolved oxygen, conductivity, water temperature, and pH were measured using YSI sondes (YSI, Yellow Springs, OH); average depth and wetted width were measured; and flow was measured via a Marsh-McBirney FloMate (Hach, Loveland, CA). In-stream habitat was approximately the same in all reaches, containing sand and silt benthos with minimal cobble and some trapped organic debris. Algal blooms, when observed, occurred by growing on the benthos and not cobble or organic debris.

Table 1.1. Stream and watershed characteristics. Values represent means±SEM where available.

Stream	Location	Watershed Land Use Percent and Area		Initial Nutrient Concentrations (mg/L)[‡]		Distance to Closest Road (m)	% EPT		Hilsenhoff Index	
Browning Run (Low Nutrient)	39.2735, -77.2792	*Forested*	72.15% 248,393.5 m^2	*TP*	0.013		*Winter*	17.47±3.13	*Winter*	5.75±0.24
		Agricultural	20.42% 70,317.3 m^2	*SRP*	0.006	185	*Spring*	43.45±8.38	*Spring*	4.12±0.45
		Developed	7.43% 25,569.9 m^2	NO_3^-	0.40		*Total*	30.46±5.27	*Total*	4.93±0.31
Zion Road (Low Nutrient)[†]	39.2145, -77.0895	*Forested*	20.35% 437,507.8 m^2	*TP*	0.070		*Winter*	26.60±5.87	*Winter*	5.26±0.19
		Agricultural	68.93% 1,481,968 m^2	*SRP*	0.052	20	*Spring*	8.77±1.55	*Spring*	5.64±0.06
		Developed	6.96% 149,720.9 m^2	NO_3^-	0.51		*Total*	17.68±3.59	*Total*	5.45±0.11
Fern Valley (Low Nutrient)	39.2144, -77.0796	*Forested*	92.83% 195,851.9 m^2	*TP*	0.027		*Winter*	36.64±9.41	*Winter*	5.03±0.26
		Agricultural	7.17% 15,134.0 m^2	*SRP*	BD	800	*Spring*	40.66±4.46	*Spring*	4.15±0.20
		Developed	0% 0 m^2	NO_3^-	0.70		*Total*	38.65±5.09	*Total*	4.59±0.19
Tobacco Barn (High Nutrient)	39.2787, -77.2828	*Forested*	63.95% 84,550.8 m^2	*TP*	0.024		*Winter*	16.56±3.21	*Winter*	5.35±0.36
		Agricultural	36.05% 47,672.3 m^2	*SRP*	0.045	600	*Spring*	49.85±4.54	*Spring*	3.51±0.22
		Developed	0% 0 m^2	NO_3^-	4.74		*Total*	33.21±4.68	*Total*	4.43±0.30
South Stream (High Nutrient)	39.2412, -76.9240	*Forested*	12.98% 22,203.6 m^2	*TP*	0.135		*Winter*	61.11±5.42	*Winter*	3.81±0.25
		Agricultural	87.02% 148,912.4 m^2	*SRP*	0.028	500	*Spring*	38.37±3.15	*Spring*	4.57±0.10
		Developed	0% 0 m^2	NO_3^-	2.70		*Total*	49.74±4.01	*Total*	4.19±0.16

Stream (cont.)	Location (cont.)	Watershed Land Use Percent and Area (cont.)		Initial Nutrient Concentrations (mg/L) (cont.)		Distance to Closest Road (m) (cont.)	% EPT (cont.)		Hilsenhoff Index (cont.)	
Forest Stream (High Nutrient)	39.2417, -76.9283	*Forested*	51.15% 79,789.0 m^2	*TP*	0.073		*Winter*	19.53±4.33	*Winter*	4.98±0.22
		Agricultural	36.05% 47,672.3 m^2	*SRP*	0.122	500	*Spring*	8.22±1.23	*Spring*	5.59±0.07
		Developed	0% 0 m^2	*NO_3^-*	3.40		*Total*	13.88±2.55	*Total*	5.28±0.13
Low Nutrient Average	-	*Forested*	61.78±21.56%	*TP*	0.037±0.021		*Winter*	26.90±3.98	*Winter*	5.35±0.14
		Agricultural	32.18±18.77%	*SRP*	0.019±0.016	-	*Spring*	30.96±4.25	*Spring*	4.64±0.21
		Developed	4.80±2.40%	*NO_3^-*	0.54±0.09		*Total*	28.93±2.90	*Total*	4.99±0.13
High Nutrient Average	-	*Forested*	42.69±15.31%	*TP*	0.077±0.032		*Winter*	32.40±4.51	*Winter*	4.71±0.20
		Agricultural	57.31±15.31%	*SRP*	0.065±0.029	-	*Spring*	32.15±3.73	*Spring*	4.56±0.18
		Developed	0.00±0.00%	*NO_3^-*	3.61±0.60		*Total*	32.27±2.90	*Total*	4.63±0.13

†Zion Road's watershed also contains area from other land use categories, totaling 3.75% and 80,687.9 m^2. These other land use categories include wooded wetland, open water, and herbaceous land.

‡TP is total phosphorus in mg P/L, SRP is soluble reactive phosphorus in mg P/L, TN is total nitrogen in mg N/L, and NO_3^- is nitrite-nitrate in mg N/L.

Sampling structures

Within each stream, I erected square 30 cm x 30 cm structures using 30.5 cm length nails and 16-gauge wire and placed them at least one meter apart (n=10/stream; total reach length about 50 m). All structures were placed so that leaves would be submerged throughout the experimental time period and were within a run in the stream; spacing was greater than one meter where necessary but never less. In each stream, the ten structures were randomly assigned to one of ten experimental units, five of which were shaded and five open to ambient light. The shaded structures were covered by 50 cm x 50 cm square pieces of weed cloth cable-tied to the wire. Cloth edges were left loose over the side of the wire to prevent sunlight from reaching underneath and stopped just above the streambed. Photosynthetically active radiation was reduced on average by >98.5% under each structure as measured by a light meter (LI-185B, LI-COR Biosciences, Lincoln, NE) above and below the cloth. Within each structure, one coarse mesh bag (opening: 6 mm x 6 mm square) of ~4.0 g of red maple leaves (*Acer rubrum*) collected from three locales around Prince George's County, MD, were secured to the nails.

After a 28-day incubation, leaf packs were collected and returned to the lab on ice for processing. Macroinvertebrates were removed from the leaves and preserved in 80% ethanol for identification. Five leaf disk punches (18 mm diameter) were each removed for algal biomass and algal class identifications, and three were removed for fungal biomass measurements. The remaining leaves were ground for stoichiometric measurements of C:N:P. Leaf discs were removed from throughout the leaf pack to obtain representative values to which the macroinvertebrates were naturally exposed.

Leaf disks for algal and fungal biomass were frozen at -20°C until analysis. Leaf disks for algal class identification were vortexed in 10 mL of deionized water for one minute to remove attached algae and preserved with 2% formaldehyde (Goldsborough & Robinson, 1986). Although community heterogeneity is common in lotic systems, site selection both at the reach and leaf pack level was chosen to minimize these differences, and the use of the same tree species and amount of leaf helps minimize taxonomic differences between sites (e.g., Heino, et al. 2003; Heino, et al., 2004). A previous study in the same area showed more similarity between rural headwater streams than between other streams, supporting the site selection criteria used here to minimize differences (Smith & Lamp, 2008) and suggesting these results may be applicable to other non-urban headwater streams in the area.

Leaf measurements

I analyzed leaf-associated algal biomass on frozen leaf disks using chlorophyll-a as a proxy by extracting chlorophyll-a in a mixture of 50:50 dimethylsulfoxide:90% acetone for two hours at 4°C (Shoaf & Lium, 1976) and measuring fluorescence with a narrow-band pass filter using a non-acidification module on a Trilogy fluorometer (Turner Designs, San Jose, CA). Chlorophyll-a values were normalized to total area sampled accounting for five leaf disks, each with two surfaces for algal growth. Chlorophyll-a was additionally measured within abscissed, non-incubated leaf disks from the same sources at the time of each experiment to obtain a background chlorophyll-a value. Measurements from stream-incubated leaves were normalized to this background chlorophyll-a by subtracting it from calculated values; background chlorophyll-a was 0.07 mg/m^2 in winter and 0.08 mg/m^2 in spring.

Fungal biomass was measured using contents of the fungal sterol ergosterol (Gessner, 2005). Samples were lyophilized, weighed, and lipids saponified in methanolic potassium hydroxide for 30 min at 80˚C. Ergosterol was partitioned into n-pentane, pentane evaporated to dryness, and sample re-suspended in methanol prior to ergosterol quantification by High-Performance Liquid Chromatography following Gessner (2005). Ergosterol contents were then converted to fungal biomass assuming 5 µg ergosterol/mg fungal dry mass and reported as mg fungal dry mass/g detritus. Fungal measurements were performed by the Kuehn Lab at the University of Southern Mississippi.

Leaf tissue remaining after removing leaf disks was oven-dried at 60°C and ground using an IKA A10 Basic Analytical Mill (IKA Works, Wilmington, NC). Ground tissue was stored frozen at -20°C and placed into a drying oven overnight before weighing for stoichiometric analysis. C and N were determined via combustion with a LECO CN628 (LECO Corporation, Saint Joseph, MI) by the University of Maryland Environmental Science and Technology Elemental Analysis lab. P was determined by ashing for 4 h at 500°C followed by 30 minutes in 1N hydrochloric acid (Rosemond et al., 1993). Samples were subsequently analyzed via the ascorbic acid method (APHA, 2012). All stoichiometric values are reported as molar ratios.

Identification

Due to low densities at 10 mL, I concentrated algal identification samples by centrifugation and examined a subset by choosing two random samples from each light/stream/season combination. Samples were placed into a Palmer-Maloney counting chamber and identified as Bacillariophyta, Chlorophyta, Cyanobacteria, Rhodophyta, or other. Due to low densities, standard protocols were modified to enable a representative

community assessment by identifying cells to a minimum of 100 natural units or 24 fields at 400x (Charles et al., 2002). I then calculated the proportion of diatoms in each sample.

Macroinvertebrates were identified to lowest possible taxonomic level, typically genus (subfamily/tribe for Chironomidae; Merritt et al., 2008), and the length of the first ten individuals of every taxon encountered was measured to estimate biomass via published length-biomass relationships (Meyer, 1989; Smock, 1980; Benke et al., 1999; Johnston & Cunjak, 1999; Miyasaka et al., 2008; Mährlein et al., 2016). Macroinvertebrates were assigned to functional feeding guilds following Merritt et al. (2008). I calculated sample diversity using Hill numbers (order q=1) to determine effective number of taxa and functional feeding guilds. A Hill number of q=1 is a transformation of Shannon diversity (entropy) that converts from entropy measure to true diversity measure and represents the number of taxa or guilds that would be present if all taxa or guilds were equally common in the sample (rather than a value of entropy without desirable mathematical properties); q=1 provides low sensitivity to rare taxa (Hill, 1973; Jost, 2006).

Data analysis

Winter and spring experiments were treated independently in all data analyses as I expected seasonal differences, and season was not a factor of interest. Differences between stream characteristics were assessed via independent Student's *t*-tests after testing for homoscedasticity via Levene's test. Measured leaf pack variables were tested via a linear mixed effects model; light (ambient, shade) was treated as a sub-plot within main plot stream nutrient level (high, low), and stream was treated as a random factor to control for unmeasured differences between streams. An ANOVA was performed on the

model to test for significance of light, nutrient, and their interaction, producing an analysis of deviance table with Type II Wald's Chi-Square values. Data were $\log_{10}$, $\log_{10}(x+1)$, or square root transformed as necessary to meet assumptions for normality and homoscedasticity.

The macroinvertebrate taxonomic and functional communities were analyzed via NMDS ordination using Bray-Curtis dissimilarity. I used PERMANOVA to test for differences in each combination of factor levels (ambient-light low-nutrient, ambient-light high-nutrient, shaded-light low-nutrient, shaded-light high-nutrient). SIMPER (similarity percentage) analysis was also performed to highlight key taxa contributing to community dissimilarities, focusing on common taxa. Functional feeding guilds were examined via generalized additive mixed models (GAMM) to determine whether measured leaf characteristics (algal biomass, fungal biomass, C:N, C:P) influenced biomass and abundance; models included a random effect of stream to account for unmeasured differences between streams. The tested leaf characteristics are each key factors related to leaf quality as a food resource but do not always exhibit linear relationships to each other or to macroinvertebrates; GAMM does not assume linearity, and a spline fit was used for each predictor/model with a maximum likelihood approach as I was assessing differences in fixed factors between models. I tested abundance and biomass separately in each season; although there was a significant correlation between the two in winter (Pearson r=0.50, p<0.001), there was no relationship in the spring as some organisms are much larger than others (e.g., *Tipula* vs. *Leuctra*), and their abundance therefore does not reflect their biomass contribution. Biomass models used a Gaussian distribution while abundance models used a Poisson distribution. Nine models

were examined using leaf characteristic predictors both separately and as combinations assessing different aspects of food quality. Model 1 examined algal biomass; model 2 examined fungal biomass; model 3 examined C:N of the leaf tissue; model 4 examined C:P of the leaf tissue; model 5 examined algal and fungal biomass; model 6 examined C:N and C:P of the leaf tissue; model 7 examined algal biomass, fungal biomass, and C:N; model 8 examined algal biomass, fungal biomass, and C:P; and model 9 included all predictors of interest: algal biomass, fungal biomass, C:N, and C:P. Model AIC scores, Δ_i (AIC normalized to the lowest AIC within candidate model set), Akaike weights, and model likelihoods were computed to determine the best single or set of models. The Akaike weights were summed from lowest AIC to highest until the weights were ≥ 0.95, resulting in a 95% confidence set of models that best fit the data (Burnham & Anderson, 2002). After this set of models was computed, pseudo-R^2 values were considered to assess how much of the data was explained by the selected models.

All data analyses were performed using R software v. 3.6.0 (R Core Team, 2019). The lme4 (Bates et al., 2015), mgcv (Wood, 2011) and vegan (Oksanen et al., 2019) packages were used for analyses, and ggplot2 (Wickham, 2016) and cowplot (Wilke, 2019) packages were used for visualization. Values are reported as significant if $p<0.05$ and marginally significant if $p<0.10$.

Results

Stream characteristics

Land use within watersheds and presence of sensitive macroinvertebrate taxa generally did not vary between low- and high-nutrient streams (Table 1.1). The watersheds of low- and high-nutrient streams did not statistically differ in percent of

forested, agricultural, or developed land. The % EPT collected in leaf packs in low- and high-nutrient streams during the experiments did not statistically differ. The Hilsenhoff Biotic Index indicated good water quality with some organic pollution in the high-nutrient streams (values between 4.26 and 5.00) while the low-nutrient streams were on average of fair quality in winter (5.01-5.75) and good quality in spring. The Hilsenhoff Biotic Index was significantly higher in the low-nutrient streams (Student's t=-2.593, df=58, p=0.012) in the winter, but there were no differences between low- and high-nutrient streams in spring.

During the experiments in each season, low- and high-nutrient streams were similar to each other in most measured characteristics (Table 1.2; Appendix I). pH was marginally higher in the high-nutrient streams in spring, although absolute differences were approximately half a pH unit (Student's t=2.40, df=4, p=0.074). Temperature was marginally higher in the spring in the high-nutrient streams (Student's t=2.45, df=4, p=0.070). Although TP and SRP were on average higher in the high-nutrient streams, P was generally quite low, often below detection. TP was, however, significantly greater in the high-nutrient streams in spring (Student's t=3.64, df=4, p=0.022). TN and NO_3^- were about five times higher in the high-nutrient streams in both seasons (winter TN: Student's t=6.68, df=4, p=0.003; spring TN: Student's t=4.60, df=4, p=0.010; winter NO_3^-: Student's t=12.67, df=4, p<0.001; spring NO_3^-: Student's t=13.31, df=4, p<0.001).

Table 1.2. Stream characteristics during winter and spring experimental periods. Values represent means† ± SEM for the low and high nutrient streams in each season. Values are provided for the low and high nutrient streams on average. Full table by stream can be found in Appendix I.

Season	Stream	Dissolved Oxygen (mg/L)	Temperature (°C)	Specific Conductivity (µS/cm)	pH	Depth (cm)	Width (cm)	Flow (m/s)	Canopy Cover (%)	TP‡ (mg/L)	SRP‡ (mg/L)	TN‡ (mg/L)	NO_3^-‡ (mg/L)
Winter	Low Nutrient	11.61 ±0.56	4.72 ±1.05	71.26 ±22.55	6.12 ±0.23	6.25 ±1.52	104.22 ±14.45	0.13 ±0.05	28.64 ±1.66	0.05 ±0.02	0.005 ±0.023	0.84 ±0.36	0.65 ±0.13
	High Nutrient	11.84 ±0.30	6.02 ±0.43	89.09 ±6.19	6.70 ±0.24	5.17 ±0.38	102.08 ±20.40	0.14 ±0.05	28.01 ±2.84	0.08 ±0.03	0.021 ±0.015	5.31 ±1.13	4.62 ±0.26
Spring	Low Nutrient	10.28 ±0.33	10.79 ±0.54	130.07 ±45.02	6.61 ±0.10	6.01 ±1.10	119.56 ±6.61	0.17 ±0.04	39.49 ±4.57	0.04 ±0.01	B.D.	0.91 ±0.12	0.63 ±0.11
	High Nutrient	10.71 ±0.45	12.14 ±0.13	133.07 ±10.74	7.02 ±0.13	4.83 ±0.69	92.28 ±10.97	0.10 ±0.02	45.14 ±3.38	0.07 ±0.02	0.022 ±0.017	4.52 ±0.54	4.35 ±0.22

†Measurements were taken at the beginning and end of each experiment at three locations in each stream, with the exception of nutrient concentrations which were measured at one location in the stream (middle of reach).

‡TP is total phosphorus in mg P/L, SRP is soluble reactive phosphorus in mg P/L, TN is total nitrogen in mg N/L, and NO_3^- is nitrite-nitrate in mg N/L.

Leaf characteristics: Algae, fungi, and stoichiometry

Leaf-associated algae responded to experimental conditions in both experiments, while fungal biomass responded only in winter. Algal biomass responded to light and nutrients, with significantly greater biomass in high-nutrient (p=0.001) and ambient-light conditions (p<0.001) in winter and greatest biomass in high-nutrient ambient-light leaves in spring (p=0.002; Table 1.3; Figure 1.1; Appendix I). Diatoms dominated leaf algal communities, ranging from 75.2-99.3% of the community, and comprised a significantly greater proportion of the community in ambient-light treatments in both the winter and spring experiments (p<0.001; p=0.001, respectively) and in high-nutrient streams during the spring (p=0.022; Table 1.3, Appendix I). Common genera included *Nitsczhia*, *Navicula*, *Aulacoseira*, *Meridion*, *Ulnaria*, and *Achnanthes*. Fungal biomass was significantly greater in high-nutrient streams in winter (p=0.017), but there were no differences in spring (Table 1.3; Figure 1.1; Appendix I). Fungal and algal biomass exhibited a moderate marginally significant positive correlation with each other during the winter (Spearman's ρ=0.531, p=0.079) but not spring (Spearman's ρ=0.048, p=0.115) (Appendix I).

Incubation in the streams altered leaf stoichiometric ratios from their original values (background leaf values: C:N=64.29±2.24; C:P=1160.73±121.07; N:P=18.16±2.28), and these changes were related to light, nutrients, and microbial biomass (Figure 1.1; Table 1.3; Appendix I). Across light and nutrient conditions, ambient-light resulted in significantly lower leaf C:N, C:P, and N:P ratios during the winter experiment (p=0.002; p=0.003; p=0.0037, respectively), and high-nutrient streams resulted in significantly lower C:N during winter but only marginally lower C:N during

spring (p=0.022; p=0.069, respectively); C:P and N:P showed no responses to nutrients or light during the spring experiment (Table 1.3; Figure 1.1; Appendix I). Algal biomass was significantly negatively related to C:N and marginally negatively related to C:P during the winter experiment (p=0.017; p=0.089, respectively) and significantly negatively related to C:P and N:P during spring (p=0.007; p=0.013, respectively; Appendix I). In contrast, fungal biomass was significantly negatively related to C:N during the winter and spring experiments (p<0.001; p=0.006, respectively) and significantly negatively related to C:P and marginally to N:P during winter (p=0.024, p=0.084, respectively).

Table 1.3. Results of two-way ANOVAs testing leaf characteristics against nutrient (low or high) and light (ambient or shaded) conditions in winter and spring. Significant *p*-values are bolded.

Response Variable Tested	Season	Factor	Wald χ^2	*p*-value
Algal Biomass (mg/m^2)	Winter	Nutrient (N)	10.34	**0.001**
		Light (L)	16.30	**<0.001**
		NxL	0.49	0.484
	Spring	Nutrient	3.15	0.076
		Light	3.45	0.063
		NxL	9.80	**0.002**
Fungal Biomass (mg fungal dry mass/g detritus)	Winter	Nutrient	5.72	**0.017**
		Light	2.12	0.146
		NxL	0.11	0.744
	Spring	Nutrient	2.48	0.116
		Light	0.37	0.544
		NxL	0.06	0.807
Proportion of Diatoms	Winter	Nutrient	1.75	0.186
		Light	31.52	**<0.001**
		NxL	0.05	0.819
	Spring	Nutrient	7.45	**0.006**
		Light	10.65	**0.001**
		NxL	0.07	0.793
Leaf C:N	Winter	Nutrient	5.27	**0.022**
		Light	10.05	**0.002**
		NxL	0.07	0.786
	Spring	Nutrient	3.31	0.069
		Light	1.55	0.214
		NxL	1.39	0.239
Leaf C:P	Winter	Nutrient	1.29	0.257
		Light	8.67	**0.003**
		NxL	2.90	0.089
	Spring	Nutrient	1.20	0.274
		Light	0.01	0.942
		NxL	0.78	0.378
Leaf N:P	Winter	Nutrient	0.62	0.433
		Light	4.37	**0.037**
		NxL	2.09	0.148
	Spring	Nutrient	0.35	0.557
		Light	0.39	0.533
		NxL	0.30	0.583

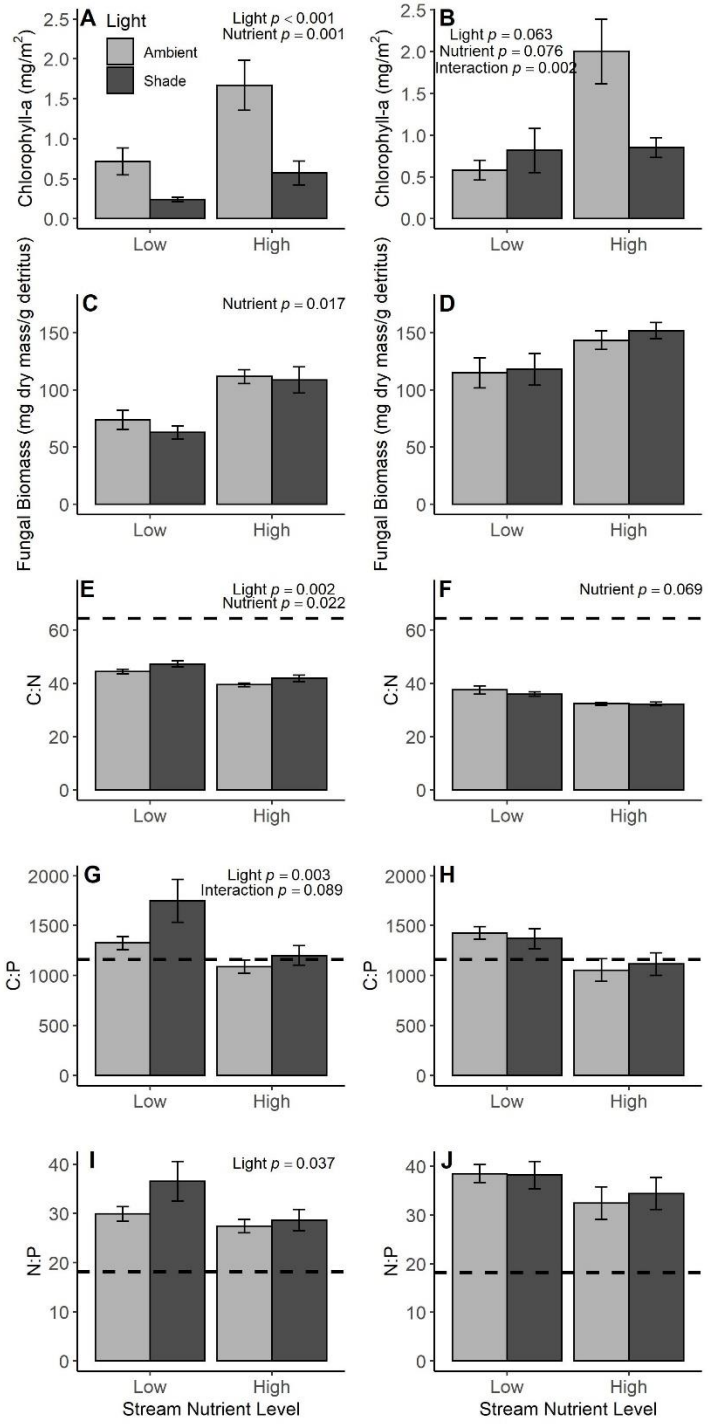

Figure 1.1. Leaf characteristic measurements from leaves incubated in winter (left) and spring (right) in streams of low- and high-nutrient concentrations and under either ambient- or shaded-light treatments. Values represent means with SEM error bars. Dashed lines represent stoichiometric ratio of leaves prior to incubation in streams. (A, B) Algal biomass (chlorophyll-a per m2 of leaf area). (C, D) Fungal biomass (dry mass of fungus per g of detritus). (E, F) C:N ratio of leaf tissue. (G, H) C:P ratio of leaf tissue. (I, J) N:P ratio of leaf tissue.

Macroinvertebrate biomass, abundance, taxa richness, and taxonomic diversity

Some coarse measures of the macroinvertebrate community responded to experimental conditions, but others did not. Macroinvertebrate biomass associated with leaves showed no response to light or nutrients during the winter experiment, however, there was a significant increase in biomass under shaded-light treatments during the spring (p=0.030; Table 1.4; Figure 1.2; Appendix I). High-nutrient and ambient-light interacted significantly to increase macroinvertebrate abundance during both winter and spring experiments (p=0.006 in both seasons; Table 1.4; Figure 1.2; Appendix I). Taxa richness showed no relationship to either factor in either season, while taxonomic diversity showed no response during the winter but responded marginally to nutrients during the spring, with higher diversity in high-nutrient streams (p=0.067; Table 1.4; Figure 1.2; Appendix I).

Table 1.4. Results of two-way ANOVAs testing coarse macroinvertebrate measures against nutrient (low or high) and light (ambient or shaded) conditions in winter and spring. Significant *p*-values are bolded.

Response Variable Tested	Season	Factor	Wald χ^2	*p*-value
Macroinvertebrate Biomass (mg DM/leaf pack)	Winter	Nutrient (N)	2.55	0.110
		Light (L)	0.66	0.416
		NxL	0.11	0.736
	Spring	Nutrient	2.64	0.104
		Light	4.71	**0.030**
		NxL	0.56	0.455
Macroinvertebrate Abundance (number of individuals)	Winter	Nutrient	0.91	0.340
		Light	2.38	0.123
		NxL	7.46	**0.006**
	Spring	Nutrient	0.15	0.694
		Light	0.14	0.709
		NxL	7.56	**0.006**
Taxa Richness	Winter	Nutrient	0.98	0.323
		Light	0.08	0.778
		NxL	1.50	0.221
	Spring	Nutrient	0.06	0.805
		Light	1.62	0.203
		NxL	0.30	0.585
Taxonomic Diversity	Winter	Nutrient	0.16	0.686
		Light	0.14	0.706
		NxL	0.52	0.473
	Spring	Nutrient	3.35	0.067
		Light	1.34	0.247
		NxL	1.58	0.208
Functional Diversity	Winter	Nutrient	0.54	0.464
		Light	0.02	0.894
		NxL	5.21	**0.022**
	Spring	Nutrient	0.40	0.528
		Light	6.43	**0.011**
		NxL	0.46	0.497

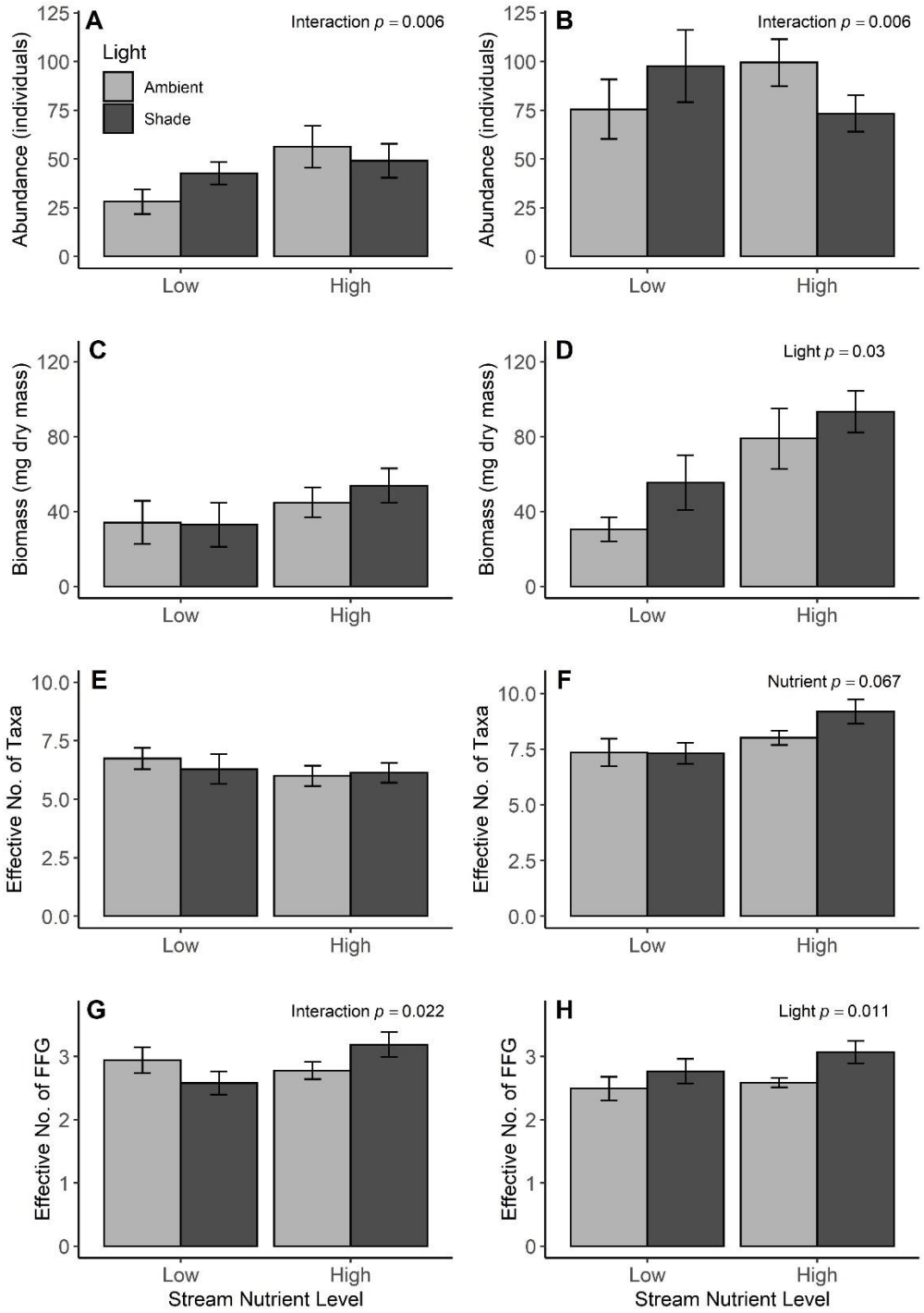

Figure 1.2. Macroinvertebrate variables measured in winter (left) and spring (right) within leaf packs incubated in streams of low- and high-nutrient concentrations and under either ambient- or shaded-light treatments. Values represent means with SEM error bars. (A, B) Abundance of macroinvertebrates in each leaf pack (individuals per leaf pack). (C, D) Biomass of macroinvertebrates in each leaf pack (mg dry mass per leaf pack). (E, F) Diversity, as Hill number (sensitivity q=1), of leaf packs. (G, H) Diversity of functional feeding guilds (FFG) as Hill number (sensitivity q=1) in leaf packs.

Macroinvertebrate functional feeding guilds and functional feeding guild diversity

Overall functional diversity and individual guilds responded to experimental conditions, but these responses were variable. Light and nutrient interacted significantly on functional diversity during winter (p=0.022), with lowest diversity in low-nutrient streams and shaded-light treatments while during spring there was a significant effect of light, with highest diversity in shaded-light treatments (p=0.011; Table 1.4; Figure 1.2; Appendix I). Functional feeding guilds responded differently to nutrient and light conditions across guilds and seasons, and between abundance and biomass (Table 1.5; Figures 1.3-4; Appendix I). Predator and collector-filterer abundance and predator biomass were significantly higher in high-nutrient streams in winter (p=0.009; p=0.039; p=0.008, respectively). Significantly lower collector-filterer winter and spring abundance, winter biomass, and shredder spring biomass were measured in ambient-light conditions (p=0.002; p=0.013; p<0.001; p=0.004, respectively). Conversely, ambient-light resulted in significantly higher winter and spring biomass of collector-gatherers and spring abundance and biomass of scrapers (p=0.024; p=0.042; p=0.020, p=0.014, respectively). The low-nutrient ambient-light combination resulted in the highest scraper biomass and lowest predator abundance during spring (p=0.033; p=0.021, respectively), while the high-nutrient ambient-light combination had the highest collector-gatherer abundance in both seasons and biomass in winter (winter: p<0.001; spring: p=0.008; p<0.001, respectively).

Table 1.5. Results of two-way ANOVAs testing functional feeding guild biomass (mg dry mass) and abundance (number per leaf pack) against nutrient (low or high) and light (ambient or shaded) conditions in winter and spring. Significant *p*-values are bolded.

Response Variable Tested	Season	Factor	Abundance		Biomass	
			Wald χ^2	*p*-value	Wald χ^2	*p*-value
Collector-filterer	Winter	Nutrient (N)	4.27	**0.039**	3.31	0.069
		Light (L)	9.50	**0.002**	6.21	**0.013**
		NxL	0.00	0.991	0.11	0.743
	Spring	Nutrient	0.09	0.765	0.58	0.446
		Light	18.68	**<0.001**	3.61	0.058
		NxL	0.63	0.426	0.01	0.920
Collector-gatherer	Winter	Nutrient	0.12	0.725	1.94	0.163
		Light	0.23	0.634	5.09	**0.024**
		NxL	14.36	**<0.001**	12.03	**<0.001**
	Spring	Nutrient	0.19	0.664	0.77	0.381
		Light	3.40	0.065	4.14	**0.042**
		NxL	7.00	**0.008**	1.20	0.274
Scraper	Winter	Nutrient	0.35	0.551	0.55	0.458
		Light	2.92	0.087	0.01	0.903
		NxL	0.24	0.623	0.01	0.924
	Spring	Nutrient	0.45	0.502	0.97	0.324
		Light	5.42	**0.020**	6.08	**0.014**
		NxL	1.80	0.180	4.52	**0.033**
Shredder	Winter	Nutrient	0.11	0.744	0.28	0.597
		Light	0.05	0.821	0.34	0.559
		NxL	0.24	0.623	1.12	0.290
	Spring	Nutrient	0.25	0.618	0.02	0.889
		Light	2.60	0.107	8.42	**0.004**
		NxL	3.04	0.081	1.06	0.303
Predator	Winter	Nutrient	6.82	**0.009**	7.02	**0.008**
		Light	0.65	0.421	0.82	0.365
		NxL	0.02	0.890	0.22	0.636
	Spring	Nutrient	0.18	0.669	3.79	0.052
		Light	0.36	0.551	2.81	0.094
		NxL	5.37	**0.021**	0.11	0.738

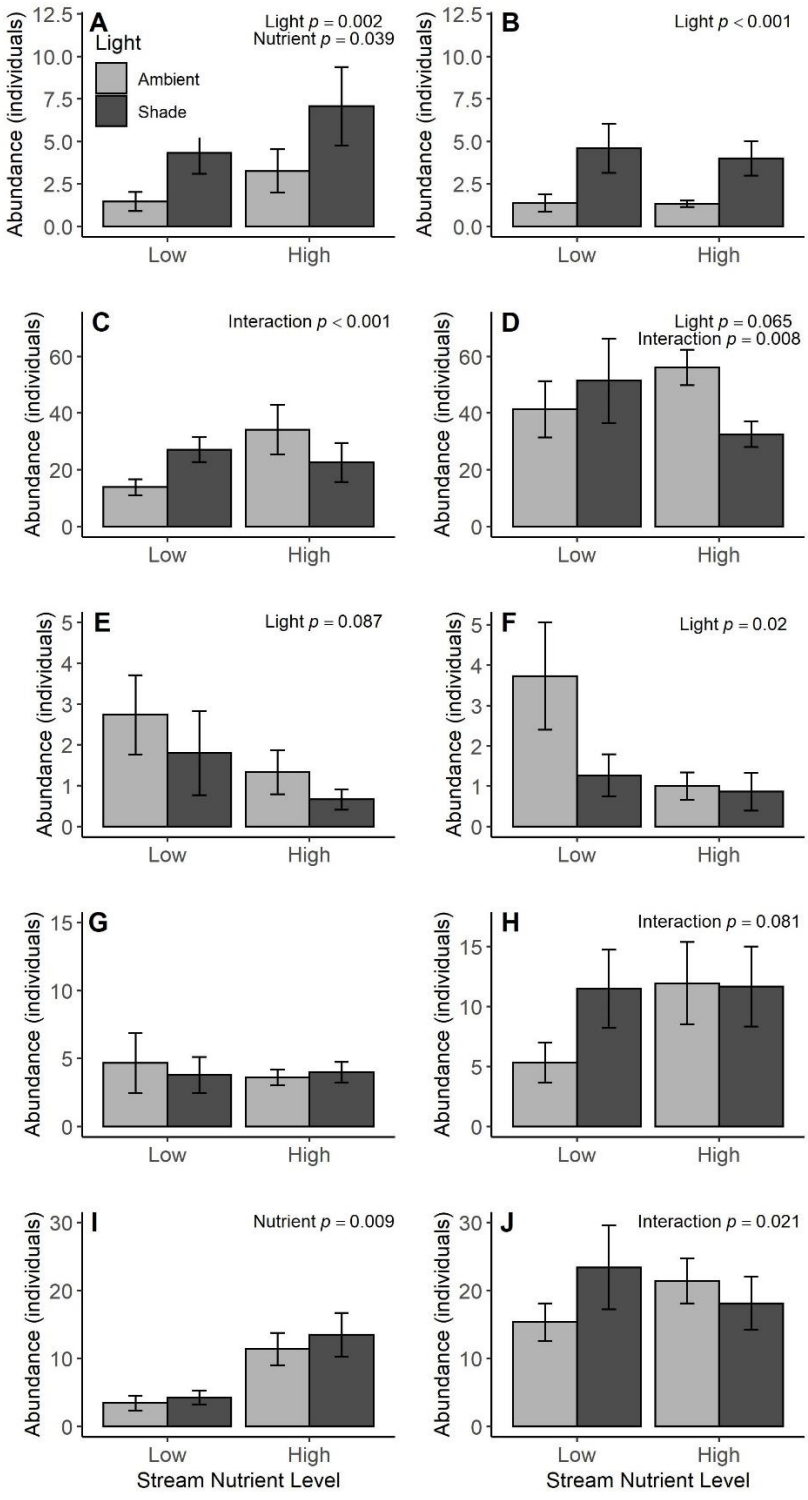

Figure 1.3. Abundance of functional feeding guilds in winter (left) and spring (right) within leaf packs incubated in streams of low- and high-nutrient concentrations and under ambient- or shaded-light conditions. Values represent means with SEM error bars. Abundances are in individuals per leaf pack. (A, B) Collector-filterers. (C, D) Collector-gatherers. (E, F) Scrapers. (G, H) Shredders. (I, J) Predators.

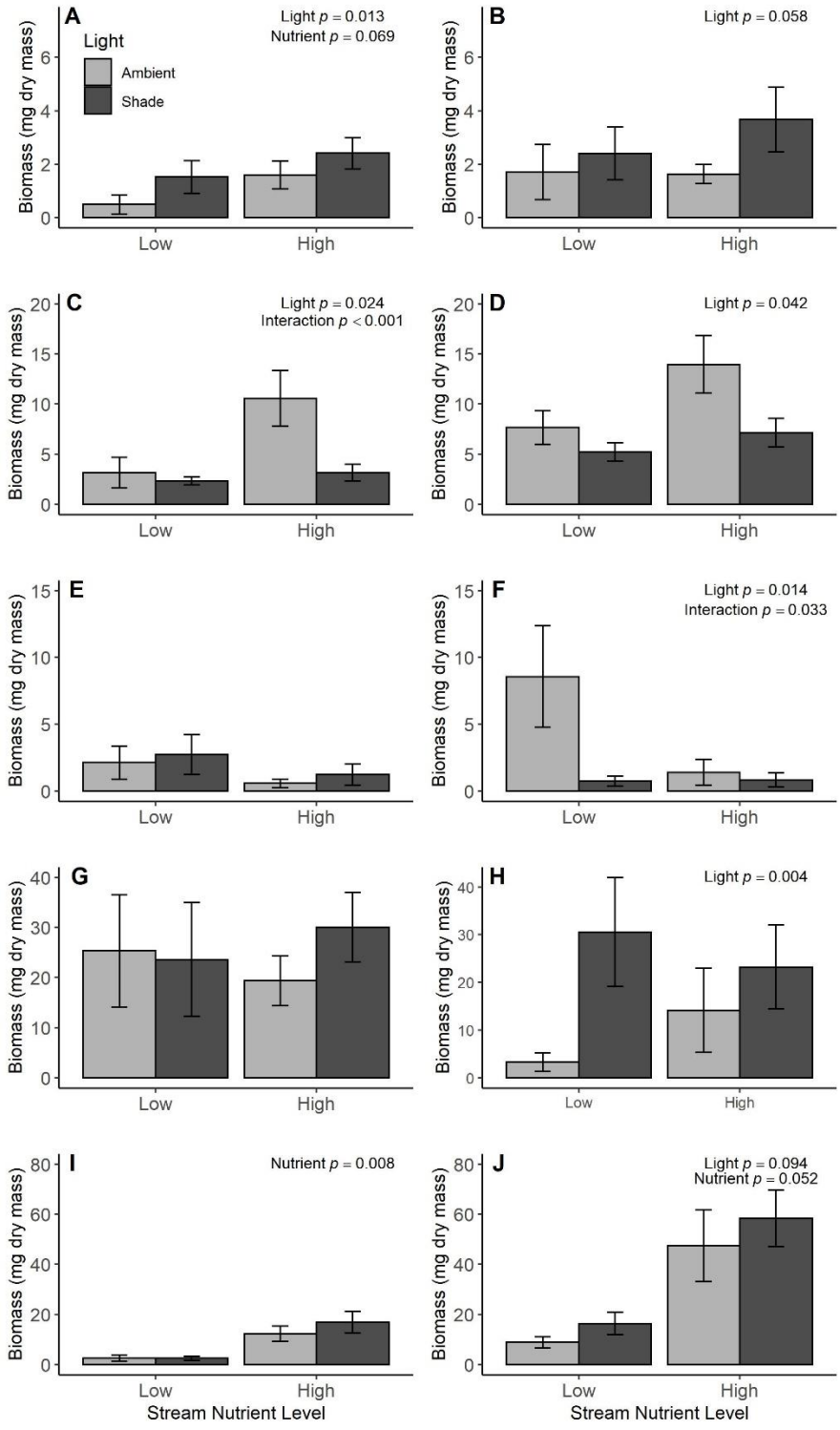

Figure 1.4. Biomass of functional feeding guilds in winter (left) and spring (right) within leaf packs incubated in streams of low- and high-nutrient concentrations and under either ambient- or shaded-light treatments. Values represent means with SEM error bars. Biomass is in mg dry mass per leaf pack. (A, B) Collector-filterers. (C, D) Collector-gatherers. (E, F) Scrapers. (G, H) Shredders. (I, J) Predators.

Macroinvertebrate Community

Ordination of Bray-Curtis dissimilarity showed high levels of overlap in both seasons for taxonomic and functional community compositions (Figure 1.5). PERMANOVA indicated experimental factor combinations were significantly dissimilar between winter taxonomic community compositions, but the percentage of dissimilarity explained was low ($F_{3,59}$=2.41, R^2=11.42%, p=0.001) with more explained by comparing streams ($F_{5,54}$=6.43, R^2=37.33%, p=0.001). Between factors, nutrients explained greater variance than light (R^2=6.60 vs. 2.85%; p=0.001, p=0.054, respectively). Winter functional community composition showed similar results, with experimental factor combinations, although significant, explaining low amounts of variance ($F_{3,56}$=3.24, R^2=14.81%, p=0.001), and this was again less than stream alone ($F_{5,54}$=5.25, R^2=32.72%, p=0.001). There was no effect of light on community functional dissimilarity and minimal variance explained by nutrients ($F_{1,58}$=4.19, R^2=6.74%, p=0.005).

Spring results were similar. Taxonomic community composition showed greater dissimilarity within low-nutrient samples than high-nutrient samples (Figure 1.5). Little variance was explained by factor combinations ($F_{3,56}$=2.17, R^2=10.42%, p=0.004) while nearly half could be explained by stream ($F_{5,54}$=10.65, R^2=49.66%, p=0.001). Again, light did not explain the dissimilarity, and minimal amounts were explained by nutrient ($F_{1,58}$=4.12, R^2=6.64%, p=0.001). Similarly, minimal variance within the functional community was explained by factor combinations but almost half was explained by stream ($F_{3,56}$=1.81, R^2=8.86%, p=0.045; $F_{5,54}$=10.11, R^2=48.35%, p=0.001, respectively). Neither nutrient nor light affected the dissimilarity.

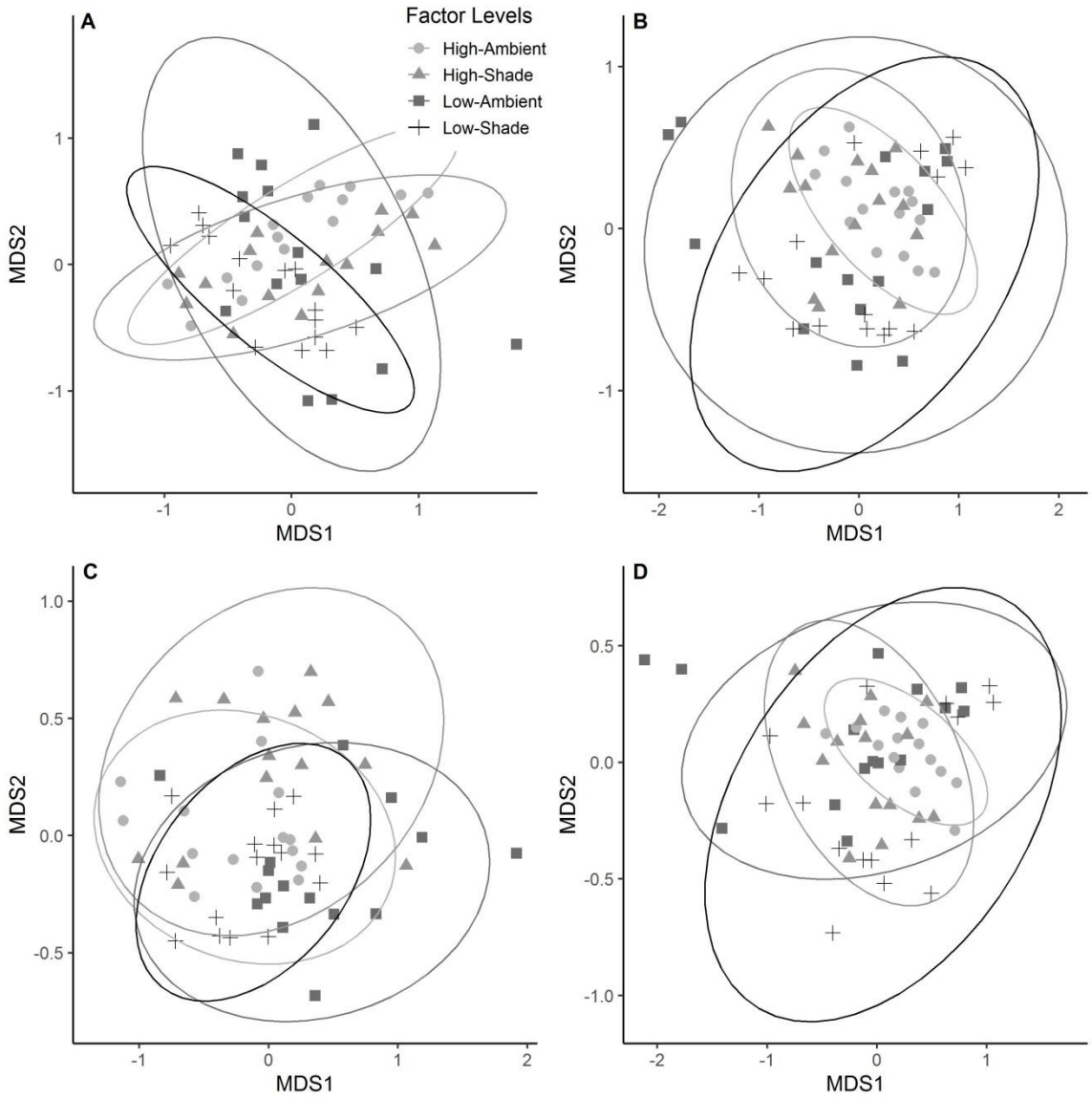

Figure 1.5. NMDS ordinations of Bray-Curtis dissimilarities measured between leaf packs incubated in streams of low- and high-nutrient concentrations and under either ambient- or shaded-light treatments. Ellipses show 95% confidence intervals for treatment combinations, and each dot represents a leaf pack. (A) Taxonomic composition ordination of winter leaf packs. Stress = 0.2104. (B) Taxonomic composition ordination of spring leaf packs. Stress = 0.1627. (C) Functional feeding guild composition ordination of winter leaf packs. Stress = 0.1311. (D) Functional feeding guild composition ordination of spring leaf packs. Stress = 0.1107.

SIMPER results indicated that specific common taxa contributed to differences between experimental conditions. Chironomidae larvae were key contributors to differences. In particular, Orthocladiinae, the most abundant taxon collected, was the top taxon in proportion of dissimilarity explained in all contrasts during both seasons' experiments (approximately 20% in each). Generally across comparisons, Orthocladiinae was found in greater abundance within high-nutrient streams and shaded-light treatments, where nutrient contributed more to dissimilarity during the winter and light during the spring. The predatory Tanypodinae were also key contributors and followed similar patterns to orthoclads. Ephemerellid mayflies (collector-gatherers) accounted for approximately 2-15% of dissimilarity. In particular, *Ephemerella* and *Eurylophella* were more abundant under ambient-light and in high-nutrient streams, with stronger contributions by *Ephemerella*. In contrast, *Serratella* was often more important in driving dissimilarity in low-nutrient streams and shaded-light treatments. The dipteran shredder *Tipula* appeared as a key taxon during winter associated with shaded-light treatments and high-nutrient streams accounting for about 3% of the dissimilarity. In the spring, the stonefly shredders *Leuctra* and *Amphinemura* accounted for at least 5% dissimilarity across contrasts, and the caddisfly shredder *Lepidostoma* accounted for about the same in most cases. *Lepidostoma* was more prevalent in the high-nutrient streams and often in the shaded-light treatments while the stoneflies varied. The predatory stoneflies *Diploperla* and *Isoperla* also contributed around 5% to dissimilarity. Overall, a number of common taxa across different orders, families, and functional feeding guilds appear to drive macroinvertebrate differences between experimental conditions.

Functional feeding guild models

GAMM models supported the importance of leaf characteristics for some functional feeding guild biomass and abundance measures within these experiments. Model AIC, Δ_i, Akaike weight, model likelihood, and pseudo-R^2 (adjusted) are displayed in Table 1.6 for abundance and 1.7 for biomass, with the top model/set of models bolded. In many of the models, pseudo-R^2 was negative, indicating low explanatory power regardless of model fit and weight; for these models, either tested predictors were not important or there was insufficient data. About half of the models, however, explained at least 10% of data variance, with a few explaining 25% or more. Scrapers were not well-explained by any set of models in any season for biomass or abundance. Of the well-performing model sets, biomass tended to have more positive pseudo-R^2 values than abundance. Abundance model sets often included the more complex models, with both microbial biomass and stoichiometric factors; for instance, collector-gatherer and shredder abundance in winter included models 7 (algal biomass, fungal biomass, and C:N) and 9 (algal biomass, fungal biomass, C:N, and C:P) as having great weight. In contrast, shredder and collector-gatherer winter biomass model sets included more models overall, and they tended to be the simpler models such as model 1 (algal biomass), model 5 (algal and fungal biomass), and model 3 (C:N) along with model 7 for collector-gatherers. In addition to different model sets explaining abundance and biomass, different model sets explained the same parameter across seasons for groups like shredders and predators. Overall, model results for abundance and biomass were often related to different factors which varied seasonally and by guild.

Table 1.6. Results of general additive mixed models for each functional feeding guild's abundance in each season showing AIC, pseudo-R^2 (adjusted), and factors included in model. Models are sorted by Δi, and models comprising a 95% confidence set are bolded.

Variable Tested	Season	Model	AIC	Δ_i	Model Likelihood	Akaike Weight	Pseudo-R^2 (adj.)	Model Factor(s)
Collector-filterer Abundance	Winter	**9**	**285.94**	**0.00**	**1.000**	**0.64**	**-2.370**	**A, F, C:N, C:P**
		7	**287.10**	**1.16**	**0.560**	**0.36**	**-1.320**	**A, F, C:N**
		8	300.91	14.97	0.001	0.00	-1.950	A, F, C:P
		5	320.30	34.36	0.000	0.00	-0.306	A, F
		2	322.80	36.86	0.000	0.00	-0.303	F
		1	355.14	69.20	0.000	0.00	-0.003	A
		6	369.46	83.52	0.000	0.00	-0.273	C:N, C:P
		3	369.67	83.74	0.000	0.00	-0.219	C:N
		4	403.85	117.92	0.000	0.00	-0.043	C:P
	Spring	**5**	**212.32**	**0.00**	**1.000**	**0.59**	**0.193**	**A, F**
		1	**213.51**	**1.20**	**0.549**	**0.32**	**0.143**	**A**
		7	**217.58**	**5.26**	**0.072**	**0.04**	**0.202**	**A, F, C:N**
		8	**218.45**	**6.14**	**0.046**	**0.03**	**0.209**	**A, F, C:P**
		6	219.02	6.70	0.035	0.02	0.372	C:N, C:P
		4	222.44	10.12	0.006	0.00	0.175	C:P
		9	241.35	29.04	0.000	0.00	0.532	A, F, C:N, C:P
		3	254.98	42.66	0.000	0.00	0.048	C:N
		2	257.12	44.81	0.000	0.00	-0.007	F
Collector-gatherer Abundance	Winter	**7**	**299.67**	**0.00**	**1.000**	**0.87**	**0.101**	**A, F, C:N**
		9	**303.41**	**3.74**	**0.154**	**0.13**	**0.076**	**A, F, C:N, C:P**
		8	327.18	27.51	0.000	0.00	-0.259	A, F, C:P
		5	345.97	46.30	0.000	0.00	-0.011	A, F
		6	353.74	54.07	0.000	0.00	-0.170	C:N, C:P
		3	396.93	97.26	0.000	0.00	0.050	C:N
		4	403.00	103.33	0.000	0.00	-0.289	C:P
		1	428.17	128.50	0.000	0.00	-0.090	A
		2	432.95	133.28	0.000	0.00	-0.014	F
	Spring	**9**	**315.92**	**0.00**	**1.000**	**0.98**	**-2.160**	**A, F, C:N, C:P**
		7	324.16	8.23	0.016	0.02	-0.452	A, F, C:N
		8	334.09	18.17	0.000	0.00	-0.559	A, F, C:P
		5	338.45	22.53	0.000	0.00	-0.205	A, F
		1	343.69	27.77	0.000	0.00	-0.072	A
		6	395.78	79.85	0.000	0.00	-1.270	C:N, C:P
		4	442.24	126.32	0.000	0.00	-0.464	C:P
		3	455.33	139.40	0.000	0.00	-0.056	C:N
		2	498.41	182.49	0.000	0.00	-0.140	F
Scraper Abundance	Winter	**1**	**262.81**	**0.00**	**1.000**	**0.97**	**-0.065**	**A**
		5	271.15	8.34	0.016	0.02	-0.070	A, F
		7	272.64	9.83	0.007	0.01	-0.092	A, F, C:N
		3	273.82	11.01	0.004	0.00	-0.022	C:N
		8	274.36	11.56	0.003	0.00	-0.083	A, F, C:P
		2	277.82	15.01	0.001	0.00	-0.062	F
		4	278.17	15.36	0.001	0.00	-0.012	C:P
		9	281.63	18.83	0.000	0.00	-0.125	A, F, C:N, C:P
		6	283.98	21.17	0.000	0.00	-0.043	C:N, C:P

Variable Tested (cont.)	Season (cont.)	Model (cont.)	AIC (cont.)	Δ_i (cont.)	Model Likelihood (cont.)	Akaike Weight (cont.)	Pseudo-R^2 (adj.) (cont.)	Model Factor(s) (cont.)
Scraper Abundance	Spring	**1**	**241.63**	**0.00**	**1.000**	**0.74**	**-0.013**	**A**
		5	**245.62**	**3.98**	**0.137**	**0.10**	**-0.031**	**A, F**
		3	**246.74**	**5.10**	**0.078**	**0.06**	**-0.021**	**C:N**
		4	**248.13**	**6.50**	**0.039**	**0.03**	**-0.018**	**C:P**
		2	**248.16**	**6.53**	**0.038**	**0.03**	**-0.018**	**F**
		7	249.10	7.47	0.024	0.02	-0.043	A, F, C:N
		8	249.66	8.03	0.018	0.01	-0.050	A, F, C:P
		6	250.66	9.02	0.011	0.01	-0.041	C:N, C:P
		9	253.30	11.67	0.003	0.00	-0.059	A, F, C:N, C:P
Shredder Abundance	Winter	**7**	**179.51**	**0.00**	**1.000**	**0.76**	**0.525**	**A, F, C:N**
		9	**182.91**	**3.40**	**0.183**	**0.14**	**0.481**	**A, F, C:N, C:P**
		5	**183.57**	**4.06**	**0.131**	**0.10**	**0.324**	**A, F**
		3	194.80	15.29	0.001	0.00	0.041	C:N
		6	198.07	18.56	0.000	0.00	-0.013	C:N, C:P
		1	211.51	32.00	0.000	0.00	0.181	A
		2	220.08	40.57	0.000	0.00	-0.004	F
		8	227.43	47.92	0.000	0.00	-0.032	A, F, C:P
		4	254.60	75.09	0.000	0.00	-0.011	C:P
	Spring	**9**	**187.51**	**0.00**	**1.000**	**0.99**	**-0.529**	**A, F, C:N, C:P**
		6	198.48	10.98	0.004	0.00	-0.423	C:N, C:P
		8	202.28	14.78	0.001	0.00	-0.723	A, F, C:P
		7	203.54	16.04	0.000	0.00	-0.332	A, F, C:N
		3	208.51	21.00	0.000	0.00	-0.134	C:N
		4	246.01	58.50	0.000	0.00	-0.347	C:P
		5	252.97	65.47	0.000	0.00	-0.092	A, F
		2	257.84	70.33	0.000	0.00	-0.065	F
		1	291.09	103.59	0.000	0.00	0.011	A
Predator Abundance	Winter	**9**	**258.36**	**0.00**	**1.000**	**0.98**	**-0.052**	**A, F, C:N, C:P**
		1	267.40	9.04	0.011	0.01	0.021	A
		5	269.08	10.71	0.005	0.00	0.045	A, F
		7	270.26	11.90	0.003	0.00	0.032	A, F, C:N
		8	271.35	12.99	0.002	0.00	-0.113	A, F, C:P
		4	272.89	14.53	0.001	0.00	-0.045	C:P
		6	275.56	17.19	0.000	0.00	-0.048	C:N, C:P
		2	304.28	45.92	0.000	0.00	-0.021	F
		3	307.97	49.60	0.000	0.00	0.015	C:N
	Spring	**8**	**201.69**	**0.00**	**1.000**	**0.64**	**-5.430**	**A, F, C:P**
		9	**202.85**	**1.16**	**0.560**	**0.36**	**-4.540**	**A, F, C:N, C:P**
		7	217.76	16.06	0.000	0.00	-1.100	A, F, C:N
		5	255.23	53.53	0.000	0.00	-0.254	A, F
		6	264.56	62.87	0.000	0.00	-1.340	C:N, C:P
		4	283.97	82.28	0.000	0.00	-1.310	C:P
		2	300.19	98.49	0.000	0.00	0.032	F
		1	314.96	113.27	0.000	0.00	-0.116	A
		3	318.84	117.15	0.000	0.00	-0.276	C:N

Table 1.7. Results of general additive mixed models for each functional feeding guild's biomass in each season showing AIC, pseudo-R^2 (adjusted), and factors included in model. Models are sorted by Δi, and models comprising a 95% confidence set are bolded.

Variable Tested	Season	Model	AIC	Δ_i	Model Likelihood	Akaike Weight	Pseudo-R^2 (adj.)	Model Factor(s)
Collector-filterer Biomass	Winter	**1**	**262.69**	**0.00**	**1.000**	**0.35**	**0.019**	**A**
		3	**263.71**	**1.02**	**0.601**	**0.21**	**0.067**	**C:N**
		7	**264.25**	**1.56**	**0.458**	**0.16**	**0.193**	**A, F, C:N**
		2	**264.92**	**2.24**	**0.326**	**0.11**	**0.009**	**F**
		4	**265.92**	**3.23**	**0.199**	**0.07**	**-0.005**	**C:P**
		5	**266.65**	**3.96**	**0.138**	**0.05**	**0.013**	**A, F**
		6	**267.63**	**4.94**	**0.085**	**0.03**	**0.052**	**C:N, C:P**
		9	268.22	5.54	0.063	0.02	0.179	A, F, C:N, C:P
		8	270.22	7.53	0.023	0.01	0.019	A, F, C:P
	Spring	**3**	**320.46**	**0.00**	**1.000**	**0.24**	**0.107**	**C:N**
		1	**320.56**	**0.10**	**0.951**	**0.23**	**-0.03**	**A**
		2	**320.75**	**0.29**	**0.865**	**0.21**	**0.049**	**F**
		5	**322.19**	**1.73**	**0.421**	**0.10**	**0.037**	**A, F**
		4	**322.30**	**1.84**	**0.399**	**0.10**	**0.000**	**C:P**
		7	**324.10**	**3.64**	**0.162**	**0.04**	**0.127**	**A, F, C:N**
		6	**324.43**	**3.97**	**0.137**	**0.03**	**0.088**	**C:N, C:P**
		8	324.45	3.99	0.136	0.03	0.057	A, F, C:P
		9	327.36	6.90	0.032	0.01	0.115	A, F, C:N, C:P
Collector-gatherer Biomass	Winter	**1**	**400.61**	**0.00**	**1.000**	**0.77**	**0.138**	**A**
		5	**404.26**	**3.65**	**0.161**	**0.12**	**0.083**	**A, F**
		3	**406.58**	**5.97**	**0.051**	**0.04**	**0.105**	**C:N**
		7	**407.51**	**6.90**	**0.032**	**0.02**	**0.128**	**A, F, C:N**
		8	408.20	7.59	0.023	0.02	0.079	A, F, C:P
		4	409.61	9.00	0.011	0.01	0.021	C:P
		2	410.06	9.45	0.009	0.01	0.019	F
		6	410.55	9.94	0.007	0.01	0.090	C:N, C:P
		9	411.51	10.90	0.004	0.00	0.112	A, F, C:N, C:P
	Spring	**1**	**413.73**	**0.00**	**1.000**	**0.26**	**-0.012**	**A**
		4	**413.81**	**0.08**	**0.961**	**0.25**	**-0.012**	**C:P**
		2	**414.29**	**0.55**	**0.760**	**0.20**	**-0.019**	**F**
		3	**414.34**	**0.60**	**0.741**	**0.19**	**-0.017**	**C:N**
		5	**417.66**	**3.92**	**0.141**	**0.04**	**-0.031**	**A, F**
		6	**417.69**	**3.96**	**0.138**	**0.04**	**-0.018**	**C:N, C:P**
		8	419.78	6.05	0.049	0.01	0.044	A, F, C:P
		7	421.65	7.92	0.019	0.01	-0.052	A, F, C:N
		9	423.54	9.81	0.007	0.00	0.058	A, F, C:N, C:P
Scraper Biomass	Winter	**2**	**342.66**	**0.00**	**1.000**	**0.25**	**0.005**	**F**
		1	**342.68**	**0.02**	**0.990**	**0.25**	**0.004**	**A**
		4	**343.06**	**0.40**	**0.819**	**0.21**	**-0.012**	**C:P**
		3	**343.14**	**0.48**	**0.787**	**0.20**	**-0.021**	**C:N**
		5	**346.23**	**3.57**	**0.168**	**0.04**	**0.000**	**A, F**
		6	347.01	4.35	0.114	0.03	-0.033	C:N, C:P
		7	350.15	7.49	0.024	0.01	-0.006	A, F, C:N
		8	350.18	7.52	0.023	0.01	-0.019	A, F, C:P
		9	354.02	11.36	0.003	0.01	-0.020	A, F, C:N, C:P

Variable Tested (cont.)	Season (cont.)	Model (cont.)	AIC (cont.)	Δ_i (cont.)	Model Likelihood (cont.)	Akaike Weight (cont.)	Pseudo-R^2 (adj.) (cont.)	Model Factor(s) (cont.)
Scraper Biomass	Spring	**4**	**418.70**	**0.00**	**1.000**	**0.25**	**-0.056**	**C:P**
		1	**418.74**	**0.04**	**0.980**	**0.24**	**0.000**	**A**
		3	**418.94**	**0.24**	**0.887**	**0.21**	**-0.007**	**C:N**
		2	**419.04**	**0.34**	**0.844**	**0.21**	**-0.020**	**F**
		5	**422.46**	**3.76**	**0.153**	**0.04**	**-0.018**	**A, F**
		6	422.62	3.92	0.141	0.03	-0.062	C:N, C:P
		8	425.44	6.74	0.034	0.01	-0.074	A, F, C:P
		7	426.00	7.31	0.026	0.01	-0.011	A, F, C:N
		9	429.39	10.70	0.005	0.00	-0.078	A, F, C:N, C:P
Shredder Biomass	Winter	**2**	**598.97**	**0.00**	**1.000**	**0.31**	**0.033**	**F**
		1	**599.63**	**0.67**	**0.715**	**0.22**	**0.000**	**A**
		5	**599.75**	**0.79**	**0.674**	**0.21**	**0.058**	**A, F**
		4	**601.39**	**2.42**	**0.298**	**0.09**	**-0.007**	**C:P**
		3	**601.51**	**2.54**	**0.281**	**0.09**	**-0.017**	**C:N**
		7	**603.41**	**4.44**	**0.109**	**0.03**	**0.060**	**A, F, C:N**
		8	603.75	4.78	0.092	0.03	0.042	A, F, C:P
		6	605.34	6.37	0.041	0.01	-0.018	C:N, C:P
		9	607.19	8.22	0.016	0.01	0.058	A, F, C:N, C:P
	Spring	**4**	**578.34**	**0.00**	**1.000**	**0.85**	**0.258**	**C:P**
		6	**582.28**	**3.94**	**0.140**	**0.12**	**0.242**	**C:N, C:P**
		8	586.04	7.70	0.021	0.02	0.240	A, F, C:P
		1	589.80	11.46	0.003	0.00	0.014	A
		9	589.97	11.63	0.003	0.00	0.224	A, F, C:N, C:P
		3	590.65	12.31	0.002	0.00	-0.008	C:N
		2	590.72	12.38	0.002	0.00	-0.021	F
		5	593.75	15.41	0.001	0.00	-0.005	A, F
		7	597.83	19.49	0.000	0.00	-0.018	A, F, C:N
Predator Biomass	Winter	**2**	**454.27**	**0.00**	**1.000**	**0.83**	**0.317**	**F**
		5	**458.27**	**4.00**	**0.135**	**0.11**	**0.305**	**A, F**
		8	**460.96**	**6.70**	**0.035**	**0.03**	**0.310**	**A, F, C:P**
		7	461.98	7.72	0.021	0.02	0.301	A, F, C:N
		9	464.95	10.69	0.005	0.00	0.298	A, F, C:N, C:P
		4	466.82	12.55	0.002	0.00	0.056	C:P
		3	468.06	13.80	0.001	0.00	0.051	C:N
		1	468.58	14.31	0.001	0.00	-0.025	A
		6	470.68	16.41	0.000	0.00	0.058	C:N, C:P
	Spring	**1**	**559.44**	**0.00**	**1.000**	**0.70**	**0.212**	**A**
		7	**562.39**	**2.95**	**0.229**	**0.16**	**0.317**	**A, F, C:N**
		5	**563.37**	**3.93**	**0.140**	**0.10**	**0.211**	**A, F**
		9	566.17	6.73	0.035	0.02	0.273	A, F, C:N, C:P
		8	566.43	6.99	0.030	0.02	0.398	A, F, C:P
		4	579.19	19.75	0.000	0.00	0.409	C:P
		3	579.41	19.97	0.000	0.00	0.100	C:N
		2	580.84	21.39	0.000	0.00	0.009	F
		6	582.43	22.99	0.000	0.00	0.411	C:N, C:P

Discussion

Despite recent work investigating algal influence on leaf decomposition and its potential as a food resource for macroinvertebrates (e.g., Halvorson et al., 2019a; Guo et al., 2016b), the role of leaf-associated algae in temperate headwater streams remains poorly understood. A particularly underexplored area is the impact of leaf-associated algae on macroinvertebrate assemblages colonizing leaves. This experimental design manipulated light availability in headwater streams of contrasting nutrient levels in two seasons to assess relationships between leaf-associated algae on leaf fungal biomass and stoichiometry and whether any of these changes related to macroinvertebrate assemblages. Algal biomass, fungal biomass, and leaf stoichiometry were altered by light and nutrient conditions, and these leaf characteristics were correlated. While there were no differences in macroinvertebrate taxa richness or diversity among the light and nutrient conditions, there were differences in biomass and abundance among functional feeding guilds and individual taxa. Functional feeding guild abundance and/or biomass was further explained for most guilds by leaf characteristics, including algal biomass, and the importance of these leaf characteristics often changed seasonally and varied between biomass and abundance. Macroinvertebrate assemblages therefore respond to leaf-associated algae and its interactions with other leaf characteristics such as fungal biomass and stoichiometry. Temperate headwater streams have generally been considered to be dominated by brown food web characteristics, with little influence of algal primary producers. These results indicate that green food webs, fueled by algae, thoroughly interact with brown food webs within leaf packs.

Microbial biomass and leaf characteristics

Algal biomass responded to light and nutrient concentrations during the winter experiment, with higher biomass in high-nutrient streams and reduced biomass due to shading, consistent with my predictions. This follows previous studies demonstrating that light availability and nutrients are important drivers of algal growth (e.g., Hill et al., 2009; Dodds 2006). In the spring experiment, however, the algal biomass relationship was not as clear: low-nutrient streams exhibited lower than expected algal biomass in the ambient-light treatments compared to the shaded-light treatments. Algae can photoacclimate to low irradiance and increase the amount of chlorophyll-a they produce with increasing shade (Beale & Appleman, 1971; Quinn et al., 1997; Ferreira et al., 2016); because chlorophyll-a was used as a proxy for biomass, the relatively greater amounts of algal biomass in the shaded-light treatment in the low-nutrient streams during the spring may be due to greater accumulation of chlorophyll-a pigment in comparison to the ambient-light treatment. Flow was relatively higher in the low-nutrient streams during the spring experiment as well, especially in one stream in which flow doubled compared to winter. Hydrological disturbances, which are particularly flashy in headwater streams, can reduce algal biomass (Biggs & Smith, 2002). Given the 28-day incubation period, it is possible a scouring event abraded algal biomass from ambient-light treatment leaves and resulted in lower algal biomass.

Fungal biomass did not follow the responses of algal biomass; rather, high-nutrient concentrations increased fungal biomass, consistent with previous studies showing fungal biomass increases with NO_3^- and SRP concentrations (e.g., Cheever et al., 2012) and partially consistent with my hypothesis. Light level did not directly impact

fungal biomass, contradicting my prediction, but during the winter experiment there was a moderate positive relationship between algal and fungal biomass. This suggests that, as seen in other studies and hypothesized here, there is a positive relationship between algae and fungi within leaf packs, at least at certain points in time (Rier et al., 2007; Kuehn et al., 2014). Much of the current debate has centered on the role of leaf-associated algae in leaf decomposition particularly in relation to fungi, and specifically as to whether algae decouples fungi from decomposition or promotes fungal decomposition through exchange of nutrients such as labile C exudates (e.g., Danger et al., 2013; Bengtsson et al., 2018; Halvorson et al. 2019a; Halvorson et al. 2019b). I did not measure decomposition in this study, so it is difficult to determine if these effects would have an impact on decomposition rates either positively (priming; e.g., Danger et al., 2013) or negatively (e.g., Halvorson et al., 2019a), but these results lend support to the existing literature that algae do interact with fungi at the leaf surface. Experiments examining fungal-algal relationships in streams have largely been conducted in the laboratory or in mesocosms (Bengtsson et al., 2018), and while these field results support laboratory data that a relationship exists in headwater streams, more experimental field studies are needed to examine the strength and direction of this microbial interaction and its impact on leaf decomposition rates in natural streams.

Microbial interactions between leaf-associated fungi and algae can have impacts beyond decomposition through changes in production rates via exchange of nutrients (e.g., Halvorson et al., 2019a) and alterations of leaf litter quality, often measured as leaf stoichiometry (e.g., Connolly & Pearson, 2013; Halvorson et al., 2019b). Here, as hypothesized, leaf stoichiometry was significantly impacted by algal and fungal

colonization, although this relationship differed between seasons. Colonization of microbes can decrease leaf nutrient ratios (France, 2011; Connolly & Pearson, 2013) dependent upon stream water conditions (Scott et al., 2013; Tant et al., 2013). C:N ratios decreased in a pattern mirroring algal biomass during the winter experiment, indicating algae were important drivers of winter C:N. In the spring experiment, the C:N pattern more closely matched that of fungal biomass, suggesting seasonal differences between key microbes impacting stoichiometric ratios and how they interact with leaves. These seasonal differences are further supported by correlations between stoichiometry and algae/fungi. C:N was related to both algae and fungi in winter and to fungi in spring, but fungi were related to N:P and C:P ratios during winter while algae were related to them during spring. Algae are capable of taking up and storing P, even in low concentrations as in the streams here (Price & Carrick, 2016). Algal productivity should peak in shaded streams in the spring due to increased light availability and higher water temperatures (e.g., Halliday et al., 2016), and this growth requires nutrients like P. Although overall C:P and N:P ratios were higher in the spring compared to the winter, the negative correlation to algae and not fungi during the spring experiment suggests that algae were likely immobilizing more P and therefore having greater impacts on C:P and N:P than fungi.

I focused on fungi and algae in this study and did not assess any direct contributions from bacteria, however, they also could have influenced leaf characteristics. Bacteria are known to alter the quality of leaves, increasing the palatability of leaves for macroinvertebrates (Gessner et al., 1999; Scott et al., 2013). Measurements of bacterial versus fungal contributions to leaf litter loss have, however, varied, with some studies

showing bacterial contributions are only about half that of fungi (e.g., Hieber & Gessner, 2002), and others indicating much greater fungal than bacterial contribution (e.g., Pascoal & Cássio, 2004). These contributions to leaf litter loss by bacteria are disproportionate to their measured biomass (<1-5% of heterotrophic biomass associated with leaf litter, ~5-15% leaf litter loss contribution; Hieber & Gessner, 2002; Pascoal & Cássio, 2004; Gulis & Bärlocher, 2017), indicating a potentially greater impact on leaf litter loss than biomass would suggest. In studies where algae, fungi, and bacteria were measured on leaf litter, there has generally been a positive relationship between algal and bacterial biomass (e.g., Kuehn et al., 2014) and production (Halvorson et al., 2019a), and algae also can influence microbial enzyme activity, including that of bacteria (Rier et al., 2007). While direct comparisons were possible between fungi and algae in this study, direct comparisons are not possible with bacteria. Based upon previous findings, however, it is likely that algae and bacteria interacted. Just as fungi and bacteria both impact leaf quality, this study indicates that algae also can impact leaf quality, whether through direct (e.g., production, exudation, nutrient immobilization) or indirect methods (e.g., providing nutrients, altering enzyme activity of fungi and bacteria). These results further suggest that the nutritional importance of different microbial colonizers may vary by season and alter food quality in contrasting ways. As such, changes in leaf stoichiometric ratios, algal biomass, fungal biomass, and (unmeasured) bacterial biomass can affect taxa feeding within leaves.

Macroinvertebrate leaf-associated community

In response to changes in leaf characteristics, especially algae, I expected the macroinvertebrate community to shift similarly with lights and nutrients. Metrics of the leaf-colonizing macroinvertebrate community, with the exception of abundance, did not,

however, exhibit strong responses to experimental factors at a coarse level. Abundance responded positively to ambient-light and high-nutrient concentrations, which corresponds to highest algal biomass and high fungal biomass. The greater availability of both algal and fungal biomass may support more individuals in these leaf packs, as previously demonstrated by positive effects of heterotrophic microbial and algal biomass on macroinvertebrate biomass and abundance in streams (Quinn et al., 1997; Gulis et al., 2006; Greenwood et al., 2007). Macroinvertebrate biomass did not follow the same trend as abundance and only responded during the spring experiment, with greater biomass in shaded-light treatments. This is likely due to the presence of a few large *Tipula* contributing greater biomass to shaded- than ambient-light leaf packs—this same trend was measured in shredder biomass during the spring experiment. Examining both biomass and abundance can provide different insights into communities as contrasting patterns may be at work and be driven by different organisms within the community (Tonin et al., 2014). There were also no changes in taxa richness and diversity within the experiments, though diversity trended as higher in high-nutrient streams during the spring. Although diversity often follows unimodal responses along productivity gradients (e.g., Mittelbach et al., 2001), I exclusively examined incubated leaf packs rather than the whole stream, and relationships may have been more apparent if I had sampled throughout the year as well as throughout the whole stream.

Functional feeding guild abundance and biomass were altered by experimental factors and predicted by leaf characteristics in GAMM models. Explanatory power of about half of the models was low and/or indicated very poor model fit, and those models will not be discussed. In general, the models are not meant to be predictive, but rather to

illuminate potentially important factors and guide future avenues for research with respect to leaf-associated algae and other leaf quality factors, particularly for those models with higher levels of variance explained. Abundance and biomass were often better predicted by different key model parameters; abundance and biomass are not always directly related to each other or aspects of leaf litter breakdown, and so each may provide different insights into macroinvertebrate assemblages and their functioning in stream dynamics (e.g., Tonin et al., 2014). Both microbial biomass and leaf stoichiometry were important, indicating separate roles for each in influencing macroinvertebrate colonization (Hladyz et al., 2009); importance of microbial biomass over stoichiometry may reflect the acquisition of essential nutrients, like specific polyunsaturated fatty acids (e.g., Guo et al., 2016b), while importance of stoichiometry may reflect a need to maintain elemental balances (e.g., Cross et al., 2005; Frost et al., 2006).

Collector-filterer abundance and biomass were generally well-supported in the models. Collector-filterers rely on fine particulate organic matter (FPOM) comprised in part of broken-down leaf material. Its nutritional quality is determined by a number of leaf-associated factors, including algal and fungal colonization (Farrell et al., 2018) and water column nutrients (Halvorson et al., 2015); its stoichiometric quality is also affected by organismal feeding, such as shredders (Halvorson et al., 2015). FPOM can be entrapped by leaves (Dangles et al., 2001) and promote collector-filterer colonization based upon its quality. Given the greater biomass and abundance measured in shaded-light conditions, it is likely that the inclusion of algae in both seasons and C:P in spring in the model results reflects a negative relationship with collector-filterers, while C:N and fungi reflect positive relationships.

Collector-gatherers tended to have highest abundance and biomass where algal biomass was also highest; while algal biomass was important for biomass, the models indicated that fungal biomass and leaf stoichiometry also were important for abundance. This may reflect taxon-specific feeding preferences. Some collector-gatherers can consume high amounts of algae (Erdozain et al. 2019) while others are influenced by nutrient conditions affecting leaf stoichiometry (Demi et al., 2019). The importance of more complex models to explain abundance suggests a relationship with numerous collector-gatherer taxa, while the simpler models supporting biomass may more strongly reflect the requirements of organisms present in greatest biomass even if they were less abundant. For instance, two key collector-gatherer groups were non-Tanypodinae chironomids and ephemerellid mayflies. While in many cases chironomids were numerically dominant, ephemerellids contributed more towards biomass, and so biomass models may incorporate the preferences of ephemerellids more strongly while the abundance models may reflect numerous non-tanypod chironomid taxa.

Scrapers were not well-predicted by the models, possibly due to low abundance and biomass in most samples. Experimental factors did, however, relate to scraper abundance and biomass, which were greatest in the ambient-light treatments where algal biomass was greater. Although scrapers feed on algae (Erdozain et al., 2019), and so this result is not unexpected, they can also feed on allochthonous material (Collins et al. 2016). Given their feeding mode of removing biofilm from surfaces, they can ingest allochthonous material; as the models were unable to provide additional insights, further experimentation could support whether their increased colonization where greater leaf-

associated algal biomass is present reflects a food preference or an attractant to a location where they then incorporate and feed on multiple resources in addition to algae.

Shredder abundance and biomass were impacted by major size differences between *Tipula* and other shredders, with biomass responses driven by *Tipula* in regards to light treatments. Models of shredders during the spring experiment indicated a greater role of leaf stoichiometry, while microbial biomass and stoichiometry appeared more important during the winter experiment. Similar to collector-gatherer results, taxon-specific responses are likely at work; previous work has shown that some shredder genera respond to leaf stoichiometry (Demi et al., 2019) while others appear to selectively feed on algae and/or microbial heterotrophs (Rosi-Marshall & Wallace, 2002; Guo et al., 2016b).

Predator biomass and abundance were generally higher in high-nutrient streams, a trend observed in other temperate headwater streams (e.g., Demi et al., 2019). Predator biomass was well-explained by the models, with a seasonal switch from inclusion of single parameter models with fungal biomass in winter to algal biomass in the spring in addition to both microbial biomasses with C:N in winter to both with C:P in spring. Although predators are not expected to be feeding directly on leaves, they are feeding on organisms colonizing and feeding on the leaves. Their response may therefore reflect changes in prey between seasons and a cumulative effect, and it suggests that leaf quality represents a bottom-up effect on predators colonizing leaf packs through their prey. Stable isotope work supports this idea; for instance, Erdozain et al. (2019) showed that a stonefly predator incorporated carbon and nitrogen from algae, leaves, and FPOM into its tissues.

I expected that at least some taxa also may show distinct responses to experimental conditions, which could drive the macroinvertebrate results if they were common taxa. SIMPER analysis of Bray-Curtis dissimilarities, although an imperfect analysis method, highlighted a number of taxa that were abundant and likely leading to differences in communities, and these taxa responded to experimental conditions. The subfamilies/tribes of chironomids were the most abundant taxa, particularly Orthocladiinae, classified here as collector-gatherers. Orthoclads showed a disparate response where greatest abundances were in high-nutrient ambient-light and low-nutrient shaded-light factor combinations. In tropical streams, Orthocladiinae were associated with higher amounts of algae on leaves (Dudgeon & Wu, 1999) while other work has seen increases in non-Tanypodinae chironomids with nutrient enrichment (Demi et al., 2019); the high abundances measured in the high-nutrient ambient-light combination suggest similar responses. Grubbs et al. (1995) recorded a number of different species of orthoclads, including shredders, associated with leaves in a Pennsylvania stream. The contrasting response measured here may reflect a difference at the species level with different orthoclad communities in high-nutrient ambient-light vs. low-nutrient shaded-light leaves. Predatory Tanypodinae chironomids were also abundant and showed the same responses as orthoclad larvae. Tanypod larvae primarily feed on smaller macroinvertebrates (such as other chironomids) or meiofauna, but in early instars or at smaller sizes they also feed on algae and detritus (Baker & McLachlan, 1979). It is difficult at the subfamily level to determine whether their responses are due to tracking chironomid prey, algal biomass, or a combination of factors. Given chironomid

dominance here and in other leaf pack studies, the chironomid community should be assessed at a finer scale in relation to leaf characteristics to clarify these relationships.

Tipula was a common shredder in both winter and spring associated with shaded-light treatments (p=0.057; p=0.004, respectively). *Tipula* was shown to have few diatoms and more fungi within its gut after consuming biofilms from wood (Eggert & Wallace, 2007), and in a gut-analysis study across streams (orders 5-7), *Tipula* consumed greater leaf than diatom material regardless of resource availability (Rosi-Marshall & Wallace, 2002). *Tipula* may therefore prefer organic matter with lower algal biomass, leading to its colonization within the shaded-light leaf packs and suggesting its response here is food preference rather than behavioral. In contrast, the collector-gatherer ephemerellid mayflies, including *Ephemerella*, *Eurylophella*, and *Serratella*, were more common in ambient-light treatments in the winter and spring experiments (p<0.001; p=0.039, respectively) and were positively correlated with algal biomass in the winter experiment (abundance: Spearman's ρ=0.719, p=0.008; biomass: Spearman's ρ=0.762, p=0.006). *Ephemerella* was particularly abundant. Previous work indicates they feed on both diatoms and leaf material (Rosi-Marshall & Wallace, 2002), and algae may comprise ~50% of their diet in some streams (Erdozain et al., 2019). Algal colonization of leaves may therefore mediate leaf colonization by ephemerellid larvae. *Stenonema* was the most common scraper and was more abundant in ambient-light treatments in spring (p=0.042), corresponding to greater algal biomass. In a previous study, as stream algal biomass increased, *Stenonema* abundance within salamander guts also increased, lending further support to algal importance for *Stenonema* (Bumpers et al., 2017). *Stenonema* can, however, feed on biofilms that are not exclusively algae. Collins et al. (2016) found that

as canopy cover increased, *Stenonema* consumed greater amounts of allochthonous material. *Stenonema* may therefore choose leaves with higher algal biomass when available, only consuming leaf material when necessary.

Although I focused on patterns of macroinvertebrates related to food resources given my hypotheses, it is possible that some taxa are responding to the light treatments due to other factors. For instance, the dipteran *Dixa* was more common in shaded- than ambient-light treatments yet they filter algae from the water, and some species have been found to prefer dark or shaded conditions (Elliott & Tullett, 1977). For some of these taxa, colonization responses may be reflective of not just food choice but also avoidance of visual predators (e.g., Kohler & McPeek, 1989; Haddaway et al., 2014), but untangling these responses requires further experimentation. For taxa discussed here, food choice appears to play a part in their responses through the measured relationships. The highlighted taxa provide a snapshot into macroinvertebrate communities colonizing leaves in temperate headwater streams and indicate that a number of leaf characteristics, including algal biomass, can mediate these assemblages.

Conclusions

Until recently, much of the work examining leaf packs in streams has focused on brown food webs. Studies have begun to provide evidence that algae have a role in leaf pack dynamics, whether in leaf litter decomposition (e.g., Danger et al., 2013; Bengtsson et al., 2018) or as an important resource for essential nutrients (e.g., Guo et al., 2016b), which suggests that brown food webs do not exist in isolation. It is still unclear, however, in what direction or at what magnitude some of these algal interactions occur. Further, very little work has specifically focused on how algal interactions with leaf litter, e.g.,

with fungi, may directly or indirectly impact associated macroinvertebrate assemblages. As such, this study examined interactions between green and brown food webs within leaf packs in first order streams and provided insight into factors, particularly algae, that can influence macroinvertebrate communities utilizing leaves. Algae and fungi colonized leaves, interacted with each other, and impacted leaf stoichiometry, and these relationships changed seasonally. C:N was more strongly related to algae in winter and fungi in spring, while N:P and C:P were more strongly related to fungi in winter and algae in spring. The relationship between algae and fungi is one that has been of particular interest for researchers, as some studies have shown positive impacts on leaf decomposition (e.g., Danger et al., 2013) while others have shown negative impacts (Halvorson et al., 2019a). As nearly all of these measurements associated with streams have come from lab or mesocosm studies (Bengtsson et al., 2018), this study provided insight into natural stream relationships between algae and fungi which can direct future studies.

Macroinvertebrate diversity did not change in response to experimental conditions, although community composition did. Biomass and abundance of macroinvertebrate functional feeding guilds often responded differently to light and nutrient availability and were impacted by different leaf characteristics. For example, collector-gatherer biomass responded positively to light in both seasons and was influenced by algal biomass. In contrast, collector-gatherer abundance was impacted by microbes and leaf stoichiometry, and light and nutrients interacted to affect abundance. Macroinvertebrates can be highly mobile and likely choose specific leaf packs, and these results suggest that the choice of colonizing one patch of leaves over another may be

based in part on leaf-associated algae, which in turn can influence other leaf characteristics such as fungi and leaf stoichiometry. Higher leaf-associated algal biomass was important for some taxa (e.g., *Ephemerella*, *Stenonema*) while for others, lower leaf-associated algal biomass appeared preferable (e.g., *Tipula*). Thus, algae, although only present in low biomass on leaves measured here, were important for macroinvertebrates within leaves. This low biomass may be enough to provide essential nutrition macroinvertebrates cannot obtain from other microbes (e.g., Guo et al., 2016b). Leaf packs therefore may represent a convergence of green and brown food webs within streams. Allochthonous leaf inputs to streams comprise a complex matrix within which abiotic and biotic factors shape interactions between microbes and macroinvertebrates (Abelho, 2001; Graça, 2001). Changes in the riparia of streams due to anthropogenic activities (e.g., clearing for development or planting for restoration) can impact algal colonization and may have complicated effects on communities utilizing leaf resources (e.g., Kiffney et al., 2004; Lagrue et al., 2011). As we develop sustainable water management strategies, we should therefore consider the importance of autochthonous food resources such as algae for macroinvertebrates in addition to allochthonous resources like leaves.

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Chapter 2 -- Contribution of leaf-associated algae to growth of a shredder, *Caecidotea communis*, and a collector-gatherer, *Ephemerella invaria*

Abstract

Allochthonous material is generally an unpalatable food resource to macroinvertebrate shredders in streams without microbial conditioning. Traditionally, fungi and bacteria have been considered important microbes increasing leaf nutritional quality to shredders, with less work focusing on leaf-associated algae. Algae are increasingly recognized as important members of the leaf microbial community which interact with fungi, bacteria, and macroinvertebrates. Limited research, however, has been conducted on macroinvertebrate growth in regards to leaf-associated algae, with mixed results. I conditioned red maples leaves in light and dark treatments and measured the growth and consumption of a common shredder, *Caecidotea communis* (Isopoda: Asellidae), and a collector-gatherer associated with leaves and algae, *Ephemerella invaria* (Ephemeroptera: Ephemerellidae) on the leaves. Over four weeks, *C. communis* and *E. invaria* significantly decreased leaf mass and algal biomass through consumption. *C. communis* consumed and grew significantly more on light-conditioned leaves, indicating they were a higher quality food resource than dark-conditioned leaves. *E. invaria* consumed more area of light-conditioned leaves but similar mass in both leaf treatments and there were no growth differences between treatments. Stable isotope signatures indicated the microbial community varied between leaf treatments for *C.*

communis, with potentially greater high-quality algae like diatoms on light-conditioned leaves providing a nutritional benefit, although *C. communis* primarily assimilated an unmeasured carbon source. *E. invaria* stable isotope signatures showed assimilation of both algal and leaf resources supported growth. These results indicate that although algal biomass is low in headwater streams, small amounts of high-quality algae present on leaves can support macroinvertebrate growth.

Introduction

In temperate headwater streams, terrestrial organic matter entering the water represents the major energy source to the food web, and this allochthonous matter, primarily comprised of leaves, is fed upon by shredding macroinvertebrates (Vannote et al., 1980; Abelho, 2001). Leaves entering the water tend to be poor quality resources due to, e.g., high lignin content (Melillo et al., 1982) and high carbon:nitrogen ratios (C:N) (Cross et al., 2003). These leaves, often already colonized by some fungi prior to entering the water (Marks, 2019), are quickly colonized by aquatic fungi and bacteria that function as microbial decomposers (Abelho, 2001). Shredders preferentially feed upon leaves that have been conditioned by fungi and bacteria (Bärlocher & Kendrick, 1975; Bärlocher, 1985; Friberg & Jacobsen, 1994; Graça, 2001), and it is this microbial "peanut butter" that is preferential to the leaf "cracker" (Cummins, 1974), though the quality of the "peanut butter" can vary (Marks, 2019). Shredder leaf palatability tends to increase with microbial conditioning due to multiple mechanisms, including microbial enzymatic breakdown of the leaf (Bärlocher & Kendrick, 1975) and the nutrient content of the microbes and their exudates (Bärlocher, 1985). Some work has further shown that microbial biomass alone can contribute to shredder growth (Chung & Suberkropp, 2009).

This consumption of microbial decomposers by shredders serves as an important link to higher trophic levels (Marks, 2019).

Upon entering the stream, organic matter also is exposed to algal primary producers. Temperate headwater streams are often shaded, leading to light limitation for algal primary production; consequently, because of low biomass, algae have largely been disregarded as important contributors to trophic dynamics in these systems (Vannote et al., 1980; Richardson, 2019). Within headwater streams, algal biomass can peak in spring when increased light availability and higher water temperatures support increased growth rates (e.g., Halliday et al., 2016) and is measurable throughout the year (personal observations). The primary algal community members in shaded headwater streams are often Bacillariophyta (diatoms), but Rhodophyta (red algae) can also be abundant, and the community includes some Chlorophyta (green algae) and Cyanobacteria (blue-green algae; prokaryotes) (Stevenson, 1996; Wehr & Sheath, 2015; Eckert et al., 2020). Algae, particularly diatoms, have higher nutritive quality than fungi or bacteria (Brett & Müller-Navarra, 1997; Guo et al., 2016; Grieve & Lau, 2018) and recent work indicates algae interact with fungi and bacteria colonizing leaves (e.g., Danger et al., 2013; Kuehn et al., 2014; Halvorson et al., 2019a) and impact colonization of macroinvertebrates within leaves (e.g., Eckert et al., 2020). Most studies have not explicitly measured algae and their role in altering leaf quality (but see Halvorson et al., 2019b). A few studies, however, have shown shredders select for fresh algae in addition to microbially conditioned leaf tissue (Friberg & Jacobsen, 1994; Leberfinger & Bohman, 2010) and that algal consumption promotes growth of shredders (Guo et al. 2016; Grieve & Lau, 2018).

Leaf quality is altered by the microbial community through changes to, e.g., nutrient stoichiometry and fatty acid composition, with impacts on macroinvertebrate growth and survival (e.g., Anderson & Cummins, 1979; Brett & Müller-Navarra, 1997; Sterner & Elser, 2002; Frost et al., 2006; Torres-Ruiz et al., 2007). Stoichiometric ratios of carbon, nitrogen, and phosphorus (P) are often used as food quality indicators as these macronutrients are acquired from food and are major components in molecules such as nucleic acids, amino acids, and lipids (Sterner & Elser, 2002). A number of factors can decrease C:N:P ratios, including increases in water nutrient concentrations (e.g., Morse et al., 2012), increases in microbial biomass on leaves (e.g., Cross et al., 2003), and interactions between the two (e.g., Tant et al., 2013; Connolly & Pearson, 2013). Many macroinvertebrates maintain a homeostatic stoichiometric ratio; for these organisms, it is less energetically expensive to feed on resources with similar ratios to themselves, so similar stoichiometric resources are of higher quality (e.g., Sterner & Elser, 2002; Frost et al., 2006). Other macroinvertebrates, however, do not appear to follow these types of diet ratio requirements (e.g., Halvorson et al., 2015), with greater flexibility in optimizing growth across varying food resources, and so quality is more difficult to determine by stoichiometry alone. More recent work has investigated the nutritional role of fatty acids in determining food quality. Fatty acid profiles of resources have indicated that algae, especially diatoms, provide high quality polyunsaturated fatty acids (PUFAs) such as ω3s, especially eicosapentaenoic acid (EPA; 20:5ω3) and docosahexaenoic acid (DHA; 22:6ω3) among others (Brett & Müller-Navarra, 1997; Torres-Ruiz et al., 2007; Guo et al., 2018). EPA and DHA are present in higher amounts in diatoms and cryptophytes than in other aquatic microbes and are essential dietary nutrients that cannot be synthesized by

some macroinvertebrates and only minimally by others (Brett & Müller-Navarra, 1997; Torres-Ruiz et al., 2007; Guo et al., 2018). Higher diatom presence on leaves consequently results in higher quality food resources.

Although we can infer food quality based upon microbial biomass or nutrient content and stoichiometry, organismal growth is the definitive test of food quality as it quantitatively indicates the assimilation of a food resource towards tissue building after excretion and other nutritional losses (Graça et al., 1993; Flores et al., 2014). Assimilation depends upon ingested food resources which can be selected for by macroinvertebrates. Shredders selectively feed by choosing higher quality patches of leaves (e.g., Arsuffi & Suberkropp, 1985; Motomori et al., 2001), which can include choosing areas that have certain fungi and bacteria of higher quality (Marks, 2019), better matched stoichiometric ratios (Cross et al., 2005; Frost et al., 2006), and/or greater algal biomass harboring essential nutrients and potentially lower stoichiometric ratios (Cross et al., 2003; Guo et al., 2016). Feeding choices can, however, be complex. For instance, in the absence of high-quality food, shredders may exhibit compensatory feeding to meet nutritional needs (Swan & Palmer, 2006a; Flores et al., 2014). Further, although gut analyses and consumption studies can provide insight into feeding preferences, consumption does not directly infer use towards growth, as ingested material can be assimilated, respired, or egested. Stable isotopes can, however, indicate which food resources are assimilated into organisms and aid in unveiling relationships within food webs (Middelburg, 2014), and their use has demonstrated that algae are often incorporated into organisms in higher than expected amounts (e.g., Guo et al., 2016; Neres-Lima et al., 2016). Tools like stable isotopes can therefore provide greater insight

into the growth of organisms on food resources of differing quality and help untangle the role of leaf-associated algae in relation to macroinvertebrates.

Research has begun to unravel the role of leaf-associated algae in headwater streams, particularly in respect to microbial interactions (e.g., Danger et al., 2013; Kuehn et al., 2014; Halvorson et al., 2019a). Limited research has, however, investigated the importance of leaf-associated algae in the nutrition of macroinvertebrates, particularly of shredders, and this research has generated unclear conclusions. Some results suggest algae are important components of shredder diets for growth (Franken et al., 2005; Guo et al., 2016; Grieve & Lau, 2018), while others report mixed results or negative responses (Carvalho & Graça, 2007; Albariño, et al., 2008). As such, more work is necessary to elucidate the importance of leaf-associated algae to macroinvertebrate growth, especially as it may vary by functional feeding guild and/or species (Eckert et al., 2020). In this study, I measured the growth and consumption of two common headwater stream species with respect to algal biomass on leaves: *Caecidotea communis* (Isopoda: Asellidae), a shredder, and *Ephemerella invaria* (Ephemeroptera: Ephemerellidae), a collector-gatherer. Algae was found to be important in the diet of another isopod, *Asellus aquaticus* (Grieve & Lau, 2018), and ephemerellid mayflies often feed on and are associated with algae (Bird & Kaushik, 1988; Rosillon, 1988; Eckert et al., 2020), suggesting *C. communis* and *E. invaria* may also utilize algae on leaves. Thus, I fed leaves conditioned under light and dark conditions to each species in separate experiments and compared changes to control treatments without macroinvertebrates. I predicted that (1) algal biomass would be greatest in treatments without macroinvertebrates and in the light treatments (light-conditioned leaves without macroinvertebrates > light-conditioned

leaves with macroinvertebrates > dark-conditioned leaves without macroinvertebrates > dark-conditioned leaves with macroinvertebrates). I expected that, due to the predicted differences in algal biomass, (2) highest leaf consumption would occur on dark-conditioned leaves for *E. invaria* due to compensatory feeding while consumption would not vary between light- and dark-conditioned leaves for *C. communis*. I predicted that (3) stable isotope signatures of *C. communis* would indicate assimilation of both leaf and algal resources in both leaf treatments, as seen in other isopod studies (e.g., Grieve & Lau, 2018), while *E. invaria* signatures would indicate assimilation of algal and not leaf resources under both leaf treatments, given *Ephemerella* relationships with algae (e.g., Rosillon, 1988; Eckert et al., 2020). Lastly, I expected that (4) leaves conditioned in the light would promote growth in both *C. communis* and *E. invaria* due to the consumption and assimilation of high-quality algal resources (e.g., Guo et al., 2016; Grieve & Lau, 2018).

Methods

Organisms

Two species, *Caecidotea communis* and *Ephemerella invaria*, were used in growth experiments to test hypotheses regarding macroinvertebrate growth with respect to algae on leaves. *Caecidotea communis* (Isopoda: Asellidae) is a widespread surface water dwelling isopod commonly encountered in both lentic and lotic systems throughout the eastern United States and Canada as well as Washington and Colorado (Williams, 1972). Although they can be collected throughout the course of a year, studies in New Jersey and Wisconsin suggest a univoltine life cycle, with reproduction occurring in the spring (Jass & Klausmeier, 1997; Hernandez & Sukhdeo, 2008). As with other isopods,

reproduction occurs around the time of female molts, and development is direct. Females oviposit into a ventral marsupium, where embryos develop until hatching and emerging as free-swimming juveniles (Wellborn et al., 2015). *C. communis* is a common shredding detritivore in Maryland (Swan & Palmer, 2006b) with high survivorship within the laboratory and therefore provided a model shredder for testing my hypotheses. *C. communis* individuals were collected using a D-net from Folly Quarter Creek, CMREC, Clarksville, MD (39° 15' 14.60" N, 76° 55' 37.18" W), and maintained in an aerated tank of moderately hard synthetic stream water (US EPA, 2002) with abundant detritus until the beginning of the experiment. Moderately hard synthetic stream water mimics stream conditions from the collection site by the addition of $MgSO_4$, $NaHCO_3$, KCl, and $CaSO_4*2H_2O$ to reverse osmosis (RO) water to obtain similar conductivity and pH. Survivorship within the tank was high, and reproduction occurred.

Ephemerella invaria (Ephemeroptera: Ephemerellidae) is a widely distributed mayfly in the eastern and central United States and Canada (Alexander et al., 2011). *E. invaria* has a univoltine life cycle, with emergence as an adult occurring in the study area from approximately mid-April through May (Alexander et al., 2011). *E. invaria* often clings to root wads and other vegetation at stream margins (Alexander et al., 2011). I selected *E. invaria* based upon results from a prior field experiment where ephemerellid mayflies colonizing leaves exhibited a strong positive correlation with algal biomass on leaves (abundance: Spearman's correlation ρ=0.719, p=0.008; biomass: Spearman's correlation ρ=0.762, p=0.006; Eckert et al., 2020). Of the ephemerellids, *Ephemerella*, including *E. invaria*, was the most common genera collected from leaf packs and are collector-gatherers that feed on algae as at least part of their diet (e.g. Bird & Kaushik,

1988; Rosillon, 1988; Merritt et al., 2008; Erdozain et al., 2019). Because of this, I expected that *E. invaria* should perform best when provided with algal resources and included them as a comparison to the shredder. Individual *E. invaria* were collected from South Stream, CMREC, Clarksville, MD (39° 14' 28.06" N, 76° 55' 26.17" W), using a D-net and bags of moss given its tendency to cling to root wads with similar textures. They were maintained in an aerated tank of moderately hard synthetic stream water with abundant food including leaves, moss from collection packs, and rocks collected from the stream until the experiment start. Survivorship within the tank was low to moderate for *E. invaria*.

Leaf conditioning

For each experiment, senescent red maple leaves (*Acer rubrum*) collected from three locations around Prince George's County, MD, were conditioned under light and dark conditions with equal contribution from each leaf collection locale. Leaves were leached for a week in aerated RO water while covered to prevent light. Water was changed frequently, and leaves were rinsed and stirred at each water change to limit microbial colonization. After leaching, leaf disks (diameter=18 mm) were removed from the leaves and conditioned for two weeks in open rectangular bins filled with 10 L of water comprised of 90% moderately hard synthetic stream water and 10% natural stream water from South Stream to seed microbes from a natural stream community prior to being fed to macroinvertebrates. Stream water was filtered through a 250 µm mesh sieve before addition; this sieve size prevented addition of macroinvertebrates but did not prevent introduction of meiofauna. The light treatment was amended with 10 mL of algal slurry scraped from rocks collected in South Stream; during the experiment with *C.*

communis, this slurry had a concentration of 907.8 µg chlorophyll-a/L while the slurry for the experiment with *E. invaria* had a concentration of 4492.9 µg chlorophyll-a/L (areal chlorophyll-a = 16.73 and 43.54 mg/m^2, respectively). The dark treatment was kept completely covered to prevent light from reaching the leaf disks. Water within the bins was aerated and circulated via a submersible pump (Figure 2.1).

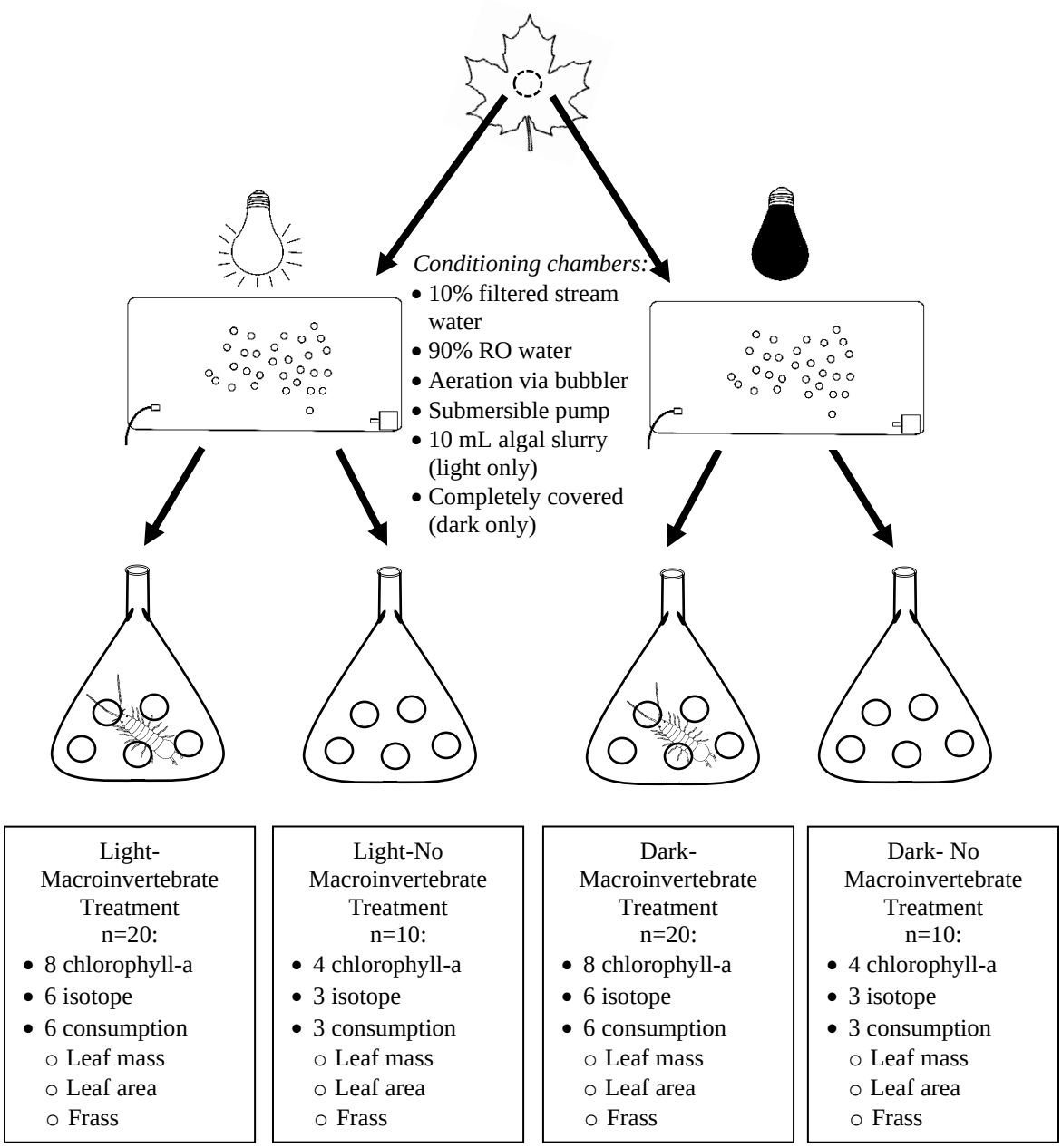

Figure 2.1. Diagram of methods used in experiments lasting four weeks. Experiment 1 tested *Caecidotea communis*, and experiment 2 tested *Ephemerella invaria*. Leaf disks were removed from red maple leaves and incubated under light or dark conditions. After two weeks, five leaf disks were placed into flasks in a 2x2 factorial design for light- or dark-conditioned leaves and macroinvertebrate presence or absense (one per flask). Flasks within each treatment combination were designated for specific measurements: algal biomass as chlorophyll-a, stable isotope analysis of carbon and nitrogen, or consumption, measured as frass produced by macroinvertebrate, area of leaf disk consumed, and change in leaf mass over the course of one week. Measurements were made weekly when leaf disks and water were replaced.

Leaf conditioning and experiments took place in a 10°C chamber under a 10h:14h light:dark cycle to mimic average mid-fall/winter light conditions; ambient light for light treatments was measured at 48 µmol/m^2/s during the *C. communis* experiment and 142 µmol/m^2/s during the *E. invaria* experiment while levels were 0 µmol/m^2/s in the dark treatments. For comparison, average light level measured at the stream surface in South Stream in mid-December was 106.5 µmol/m^2/s (personal observation), and other feeding experiments manipulating algae have used ranges from ~0-150 µmol/m^2/s (Franken et al., 2005, Danger et al., 2013; Guo et al., 2016); these light levels resulted in abundant diatom colonization beginning around 25 µmol/m^2/s when measured across a light gradient (Franken et al., 2005), suggesting that levels in these experiments were sufficient for algal production. Bins were checked for leaf entrapment on pumps, leaf disks were mixed to disorder to prevent microbial colonization effects due to leaf position, and evaporated water was replaced with RO water regularly. Leaves continued to leach tannins into conditioning bins, so water was completely replaced in both treatments by repeating the initial setup, including adding more algal slurry to the light treatment, two and eight days after the start of conditioning in each experiment. Preliminary conditioning tests run for two weeks indicated these treatments provided differential algal growth; algal biomass was greater in light treatments supplemented with lower additions of algal slurry than used in the experiments than it was in dark treatments (~1.5x greater) or light treatments without added algal slurry (~2x greater).

Feeding experiment

The feeding experiment was set up as a completely randomized 2x2 factorial unbalanced design; factor one was leaf treatment with two levels, light and dark, and

factor two was macroinvertebrate treatment, present or absent (Figure 2.1). Each level of leaf treatment consisted of thirty 250 mL Erlenmeyer flasks topped with a rubber stopper and a plastic pipette connected to a central aeration system; twenty of these flasks contained a single macroinvertebrate and ten did not. Prior to macroinvertebrate introduction, flasks were filled with moderately hard synthetic water and aerated overnight. All flasks were provided with five leaf disks from the respective light treatment, enough for *ad libitum* feeding for the macroinvertebrates. Every seven days over the course of the 28-day experiment, leaf disks and water were removed and replaced with new conditioned disks and water, and flask locations were rerandomized to prevent chamber effects. The replacement leaves were conditioned for two weeks plus the time of the growth experiment (e.g., at two weeks into the growth experiment, the leaves had been conditioned for four weeks), in order to maintain a gradient of algal conditioning without compromising behavioral responses by maintaining macroinvertebrates in constant dark or light-limited conditions within the flasks.

Growth was measured for each of the twenty macroinvertebrates per leaf treatment level. Macroinvertebrates were photographed at the beginning and end of the 28-day experiment for body length and head width measurements via ImageJ (National Institutes of Health, Bethesda, MD) and wet-weighed after blotting on a paper towel. After initial measurements, macroinvertebrates were placed into individual experimental flasks. After final measurements, macroinvertebrates were placed into a drying oven to obtain final dry mass. An additional 30 *C. communis* and 25 *E. invaria* were photographed for measurements, wet-weighed, and oven-dried at 60°C to obtain dry masses to construct size and wet mass vs. dry mass regressions estimating initial dry

mass for experimental macroinvertebrates. For both organisms, dry mass vs. wet mass provided the best estimate of initial dry mass (*C. communis*: Dry Mass = 0.2000 * Wet Mass + 0.0513, R^2=85.9%; *E. invaria*: Dry Mass = 0.2547 * Wet Mass + 0.3079, R^2=86.0%). Measurements were used to determine changes in size, mass, and growth rate. Relative growth rate was calculated as percent growth per day for each size variable using the equation:

$$\% \text{ Growth/Day} = \frac{F_v - I_v}{I_v * 28 \text{ days}} * 100$$

where F_v is the final measurement of wet mass, dry mass, body length, or head width, and I_v is the initial measurement of the same variable.

Algal biomass, consumption, and stable isotope signatures were measured on flask leaf disks each week, with subsets of flasks assigned to each variable within each factor combination (Figure 2.1). Algal biomass was measured on leaf disks from eight flasks with and four flasks without macroinvertebrates per leaf treatment. Algal biomass was also measured on leaves from the conditioning chambers beginning on day 0 of the experiment and once per week to the last day of the experiment (five total sampling periods; termed background leaves throughout) to provide estimates of algal biomass on leaves fed to macroinvertebrates in flasks (n=3 per treatment per week). All algal biomass was measured using chlorophyll-a as a proxy by extracting chlorophyll-a in a mixture of 50:50 dimethylsulfoxide:90% acetone for two hours at 4°C (Shoaf & Lium, 1976) and measuring fluorescence with a narrow-band pass filter using a non-acidification module on a Trilogy fluorometer (Turner Designs, San Jose, CA). Chlorophyll-a values were normalized to total area sampled accounting for five leaf disks, each with two surfaces for algal growth. Chlorophyll-a remaining in abscissed leaf disks from the same leaf

sources was measured and subtracted from all experimental leaf disks to account for non-algal chlorophyll-a within the leaves; this value was 0.017 ± 0.002 mg/m^2.

Consumption was measured on leaf disks from six flasks with and three flasks without macroinvertebrates per leaf treatment. Consumption was measured in three ways: leaf area consumed, frass production, and change in leaf mass. Leaf area consumed was calculated in Image J (National Institutes of Health, Bethesda, MD) using digital images of the leaf disks taken at the beginning and end of each week (O'Neal et al., 2002). Consumption, normalized to the mass of the macroinvertebrate, was calculated for leaf disks in each consumption-designated flask by leaf treatment using the equation:

$$\text{Leaf Area Consumed} = \frac{(A_{IML}-A_{FML})-\overline{(A_{INML}-A_{FNML})}}{DM}$$

where A_{IML} and A_{FML} are the leaf areas at the beginning and end of the week in the macroinvertebrate present treatment (M), respectively; $\overline{(A_{INML}-A_{FNML})}$ is the average leaf area difference between the beginning of the week (A_{INML}) and the end of the week (A_{FNML}) in the macroinvertebrate absent treatment (NM); L is the leaf treatment, either light or dark; and DM is the initial dry mass of the macroinvertebrate. Leaf area consumed was computed for each week individually and summed to obtain total consumption over the course of the experiment. Frass production was measured by pouring water in the flasks containing macroinvertebrates through a pre-dried and pre-weighed filter paper. The filter with frass was then oven-dried and weighed to obtain frass mass. Frass was normalized to the size of the organism by dividing by the initial dry mass and no coprophagy was assumed. Similar to leaf area consumption, frass production was measured on a weekly basis and summed to obtain total frass produced over the course of the experiment.

Changes in leaf mass were measured by comparing differences in mass between the start and end of each week. Leaf disks were wet-weighed after gently blotting the disks on a paper towel. The wet-weighed leaves at the end of the week were placed into a drying oven to obtain dry mass; this dry mass showed no relationship to the wet-weighed leaves, preventing an estimate of initial dry mass and was not analyzed further. Wet mass was used for two measurements. For each consumption-designated flask, change in leaf wet mass per day on a weekly basis was calculated by subtracting the initial total mass of all five leaf disks from the final mass and dividing by seven days. Leaf disk wet masses were also used to calculate the change in mass due to macroinvertebrate consumption each week by leaf treatment, normalized to mass of the macroinvertebrate, using the equation:

$$\text{Change in leaf mass} = \frac{(M_{FML}\text{-}M_{IML})\text{-}(\overline{M_{FNML}\text{-}M_{INML}})}{DM}$$

where M_{FML} is the final mass of leaves in the macroinvertebrate treatment; M_{IML} is the initial mass of leaves in the macroinvertebrate present treatment; $\overline{M_{FNML}\text{-}M_{INML}}$ is the average of the differences of the final wet mass (M_{FNML}) and the initial wet mass (M_{INML}) of leaves in the macroinvertebrate absent treatment; L is the leaf treatment, either light or dark; and DM is the initial dry mass of the macroinvertebrate.

Leaf disks were oven-dried at 60°C for stable isotope analysis (SIA) of $\delta^{13}C$ and $\delta^{15}N$ from six flasks with and three flasks without macroinvertebrates per leaf treatment. Stable isotope analysis was performed by the UC Davis Stable Isotope Facility (Davis, CA) for natural abundance of $\delta^{13}C$ and $\delta^{15}N$ within initial macroinvertebrates collected at the time of the experiment, experimental macroinvertebrates, algal slurry used to seed the conditioning chambers, unconditioned leached leaves, and flask leaf disks from each

week. Isotopes were measured via combustion on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Carbon values are reported with respect to the Vienna Pee Dee Belemnite standard, and nitrogen values are reported with respect to air. Ten initial macroinvertebrates from each experiment were encapsulated whole after drying to obtain dry mass; gut clearance was not performed prior to encapsulation, although all were without food for a few hours prior to entering the drying oven. Experimental macroinvertebrates were placed into a flask with clean, moderately hard synthetic water at the end of the experiment for a three-day starvation period to allow time for gut clearance (Grieve & Lau, 2018). All macroinvertebrates surviving to the end of the experiment were encapsulated whole and sent for analysis. Three replicates from each experiment were taken from a homogenized algal slurry and dried down for analysis. Leaf disks from flasks designated for SIA were dried at the end of each week and sent for analysis, but only week 4 results are reported here. Beginning in week two of the *E. invaria* experiment, one of the macroinvertebrate light-conditioned treatment flasks was dropped as a SIA replicate and used for chlorophyll-a analysis due to mortality, resulting in five replicates instead of six for the final three weeks for the light treatment. Three replicates from leaves after leaching were also dried for SIA analysis. All leaf samples were ground using a mortar and pestle prior to weighing to obtain a representative sample.

Data Analysis

Three-way ANOVAs for leaf treatment, macroinvertebrate treatment, and week were used to assess differences in algal biomass on leaves and leaf wet mass. Two-way

ANOVAs for leaf treatment and week were used to assess differences in macroinvertebrate leaf area consumed, frass produced, leaf mass consumed, and algal biomass of background leaves. Student's *t*-tests were used to analyze differences in initial body length, head width, wet mass, and dry mass between leaf treatments, percent growth rates of each size variable, total leaf area consumed, and total frass produced. All data were checked for normality and homoscedasticity, and chlorophyll-a values were $\log_{10}$ transformed to meet assumptions. Grubb's test was used to check for outliers; if identified as significantly different, only the largest/smallest value was dropped. Tukey's Honestly Significant Difference (HSD) was computed for significant ANOVA results. All analyses were performed using R v. 3.6.0 (R Core Team, 2019) and the packages ggplot2 (Wickham, 2016), lme4 (Bates et al. 2015), car (Fox & Weisberg, 2019), effects (Fox, 2003), cowplot (Wilke, 2019), outliers (Komsta, 2011), agricolae (de Mendiburu, 2019), plyr (Wickham, 2011), and dplyr (Wickham et al., 2019). Values of $p<0.05$ are reported as significant and $p<0.10$ are reported as marginally significant.

Results

Leaf-associated algal biomass

During the *C. communis* experiment, variation in leaf-associated algal biomass was high across replicates, and the leaves post-leaching had comparatively high chlorophyll-a measurements (mean±SEM: 0.166±0.010 mg/m^2 vs. 0.017±0.002 mg/m^2 background levels in leaves) prior to introduction to the conditioning chambers. Background algal biomass on conditioned leaf disks did not differ significantly between weeks or leaf treatments ($p=0.716$, $p=0.542$, respectively; Table 2.1; Figure 2.2; Appendix II). Over the course of the experiment, algal biomass on leaves within

treatment flasks was significantly lower in flasks with *C. communis* than without (p=0.012; Table 2.1; Figure 2.3; Appendix II), and there was marginally less algal biomass on light-conditioned leaves than dark-conditioned leaves (p=0.076).

Variation was high across replicates in the *E. invaria* experiment as well, although the leaves post-leaching had lower chlorophyll-a measurements prior to introduction to the conditioning chambers than in the *C. communis* experiment (mean±SEM: 0.098±0.015 mg/m^2). As in the *C. communis* experiment, background algal biomass did not differ significantly between weeks or leaf treatments (p=0.589, p=0.681, respectively; Table 2.1; Figure 2.2; Appendix II). Over the course of the experiment, there was marginally more algal biomass on light-conditioned leaves in flasks (p=0.096) and a marginal three-way interaction between leaf treatment, macroinvertebrate treatment, and week on algal biomass on leaves in flasks (p=0.083; light-week 1-macroinvertebrate present > dark-week 2 & 4-macroinvertebrate present & light-week 3-macroinvertebrate present; Table 2.1; Figure 2.3; Appendix II-III). A significant interaction between macroinvertebrate presence and week affected algal biomass on leaves (p=0.035; Table 2.1; Figure 2.4; Appendix III), where there was less algal biomass with macroinvertebrates present in week 3 and 4 versus week 1 (Tukey's HSD: p=0.285, p=0.141, respectively); although the interaction was significant, Tukey's HSD did not pinpoint any significant comparisons.

Table 2.1. Results of ANOVAs on response variables for *Caecidotea communis* and *Ephemerella invaria*. Significant *p*-values ($p<0.05$) are bolded.

Response Variable	Factor	*Caecidotea communis*		*Ephemerella invaria*	
		***F*-value$_{df}$**	***p*-value**	***F*-value$_{df}$**	***p*-value**
Algal Biomass† on Background Leaves (mg/m^2)	Leaf Treatment (L)	$0.38_{1,20}$	0.542	$0.17_{1,20}$	0.681
	Week (W)	$0.53_{4,20}$	0.716	$0.72_{4,20}$	0.589
	LxW	$1.27_{4,20}$	0.314	$0.35_{4,20}$	0.841
Algal Biomass† on Leaves in Flasks (mg/m^2)	Leaf Treatment (L)	$3.24_{1,75}$	0.076	$2.83_{1,81}$	0.096
	Macroinvertebrate Treatment (M)	$6.66_{1,75}$	**0.012**	$1.88_{1,81}$	0.175
	Week (W)	$1.23_{3,75}$	0.305	$1.00_{3,81}$	0.398
	LxM	$1.37_{1,75}$	0.246	$0.06_{1,81}$	0.805
	LxW	$0.63_{3,75}$	0.596	$1.08_{3,81}$	0.364
	MxW	$0.64_{3,75}$	0.590	$3.00_{3,81}$	**0.035**
	LxMxW	$1.79_{3,75}$	0.156	$2.31_{3,81}$	0.083
Change in Leaf Wet Mass (mg/day)	Leaf Treatment	$0.13_{1,56}$	0.720	$0.26_{1,56}$	0.616
	Macroinvertebrate Treatment	$38.94_{1,56}$	**<0.001**	$157.95_{1,56}$	**<0.001**
	Week	$24.10_{3,56}$	**<0.001**	$33.45_{3,56}$	**<0.001**
	LxM	$1.81_{1,56}$	0.184	$0.01_{1,56}$	0.917
	LxW	$2.77_{3,56}$	0.050	$0.25_{3,56}$	0.862
	MxW	$2.35_{3,56}$	0.082	$3.01_{3,56}$	**0.037**
	LxMxW	$0.06_{3,56}$	0.979	$0.69_{3,56}$	0.564
Leaf Area Consumed (mm^2/mg DM‡)	Leaf Treatment	$5.72_{1,38}$	**0.022**	$6.77_{1,32}$	**0.014**
	Week	$3.96_{3,38}$	**0.015**	$8.61_{3,32}$	**<0.001**
	LxW	$0.15_{3,38}$	0.930	$4.80_{3,32}$	**0.007**
Frass Produced (mg/mg DM‡)	Leaf Treatment	$23.72_{1,40}$	**<0.001**	$0.52_{1,30}$	0.475
	Week	$41.97_{3,40}$	**<0.001**	$2.85_{3,30}$	0.054
	LxW	$3.19_{3,40}$	**0.034**	$0.05_{3,30}$	0.984
Leaf Wet Mass Change due to Consumption (mg/mg DM‡)	Leaf Treatment	$16.01_{1,40}$	**<0.001**	$0.15_{1,29}$	0.699
	Week	$6.20_{3,40}$	**0.001**	$2.43_{3,29}$	0.085
	LxW	$0.13_{3,40}$	0.939	$1.35_{3,29}$	0.278

†Algal biomass was $\log_{10}$-transformed prior to analysis and measured as chlorophyll-a.
‡Normalized to initial dry mass (DM) of macroinvertebrate and to no macroinvertebrate treatment.

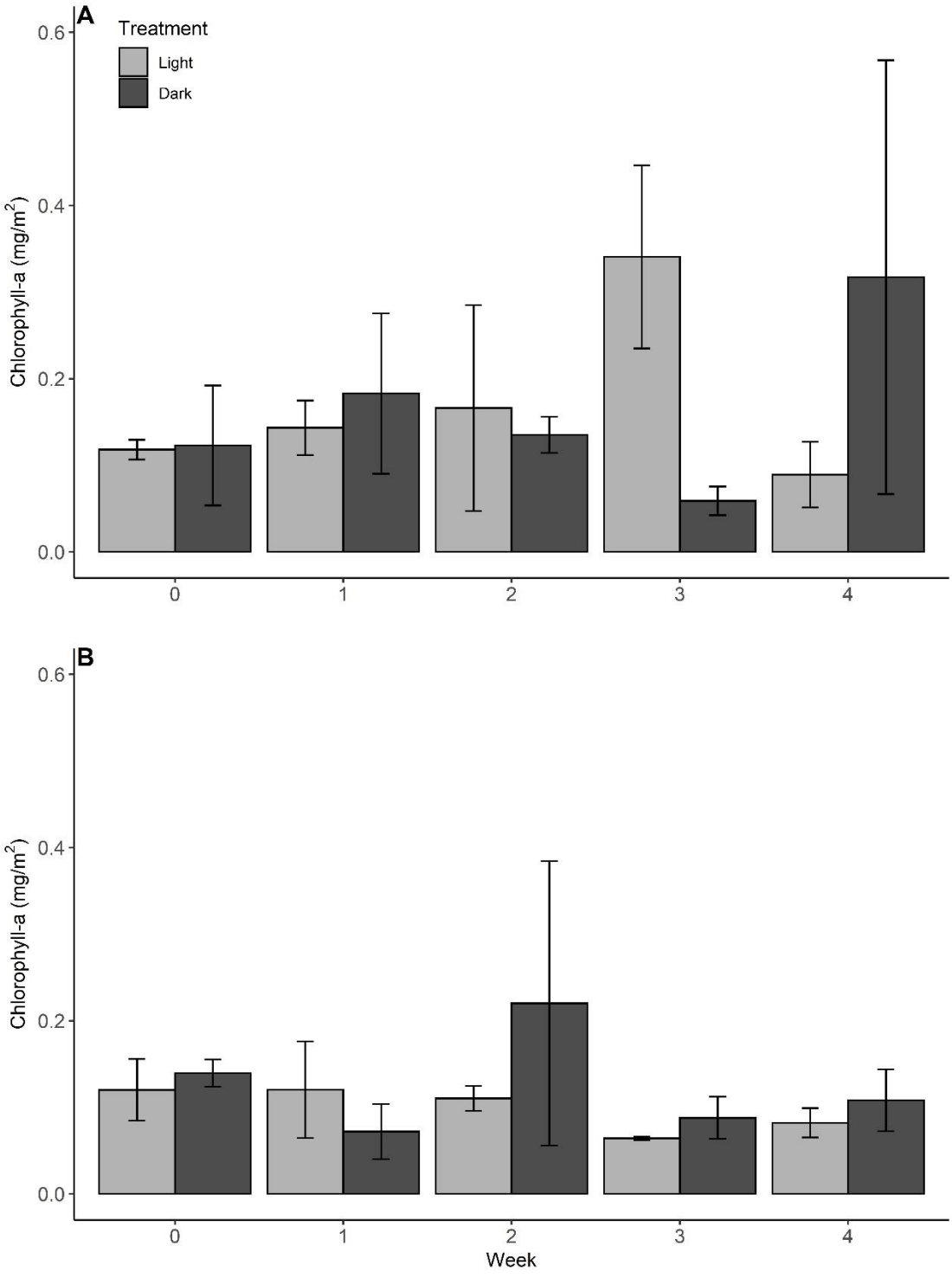

Figure 2.2. Algal biomass (chlorophyll-a) on background leaf disks in conditioning chambers measured each week starting with day 0 of the experiment (week 0) and ending on the final day of the experiment (week 4) during the (A) *Caecidotea communis* experiment and (B) *Ephemerella invaria* experiment.

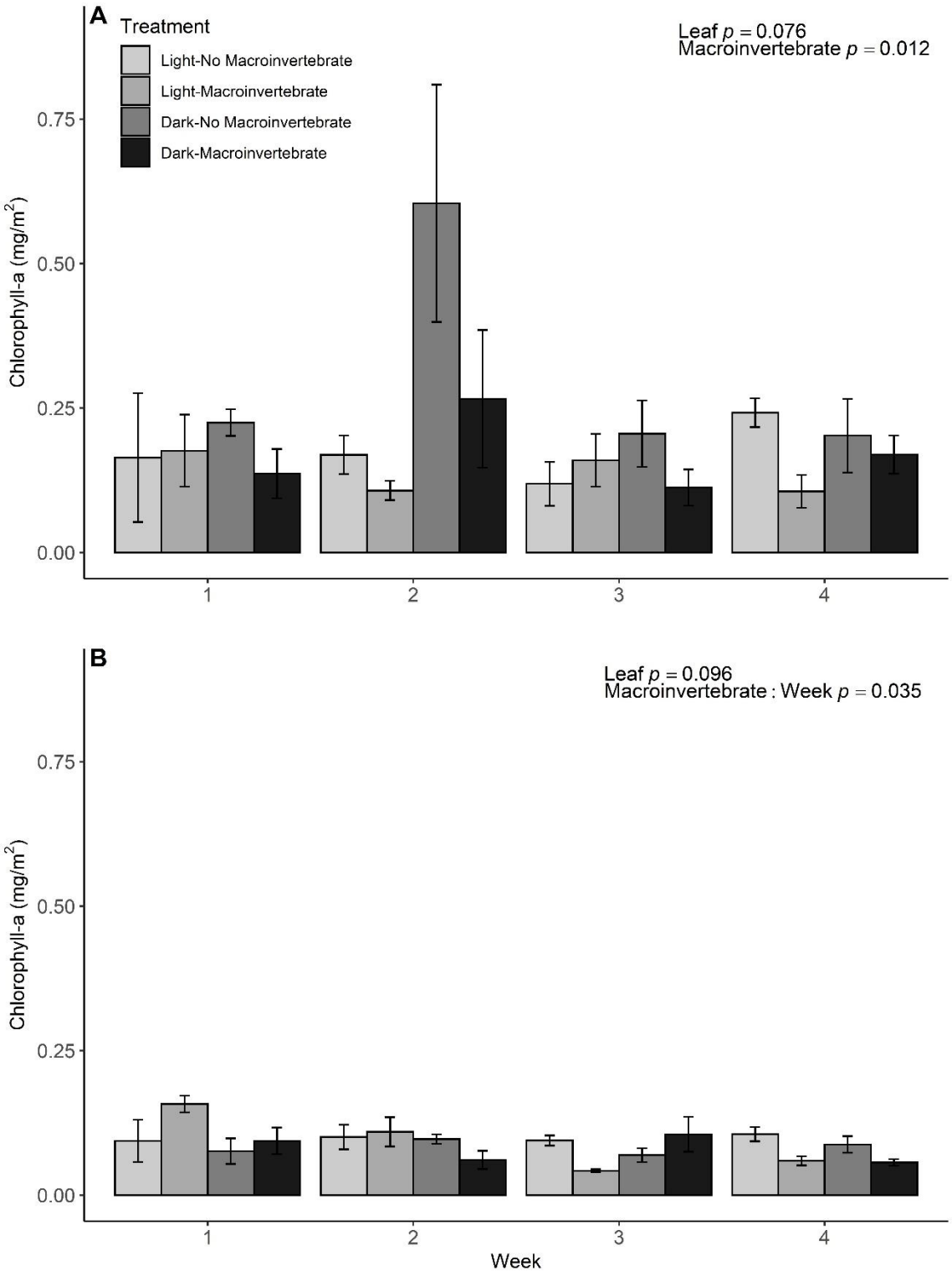

Figure 2.3. Algal biomass (chlorophyll-a) on leaves within the flasks measured at the end of each week during the (A) *Caecidotea communis* experiment and (B) *Ephemerella invaria* experiment.

C. communis *and* E. invaria *leaf consumption*

Leaf disks in the *C. communis* experiment showed clear skeletonization indicating shredding activity which altered leaf measurements. Leaf wet mass during the *C. communis* experiment decreased significantly more when macroinvertebrates were present ($p<0.001$), and every week had a greater mass loss than the previous week ($p<0.001$; Table 2.1; Figure 2.4; Tukey's HSD: week 1<2 $p=0.005$; week 1<3 $p<0.001$; week 1<4 $p<0.001$; week 2<3 $p=0.047$; week 2<4 $p<0.001$; week 3<4 $p=0.008$). Leaf treatment and week marginally interacted ($p=0.050$), with generally greater mass losses in later weeks than in the first week and greatest mass loss in week 4 (Table 2.1; Figure 2.4; Appendix III). Additionally, macroinvertebrate presence and week marginally interacted, with later weeks having greater mass loss changes than early weeks, and this was particularly pronounced with macroinvertebrates present ($p=0.082$; Table 2.1; Figure 2.4; Appendix III).

Although not classified as a shredder, *E. invaria* also skeletonized the leaf disks, impacting leaf characteristics. Leaf wet mass change was significantly greater when macroinvertebrates were present ($p<0.001$) and every week had a significantly greater decrease in mass than the previous week ($p<0.001$), except for between weeks 2 and 3 (Table 2.1; Figure 2.4; Tukey's HSD: all $p<0.001$, except week 2 and 3 $p=1.000$). Macroinvertebrate and week also interacted significantly, with greater changes in leaf wet mass when macroinvertebrates were present and greater mass lost each successive week ($p=0.037$; Table 2.1; Figure 2.4; Appendix III).

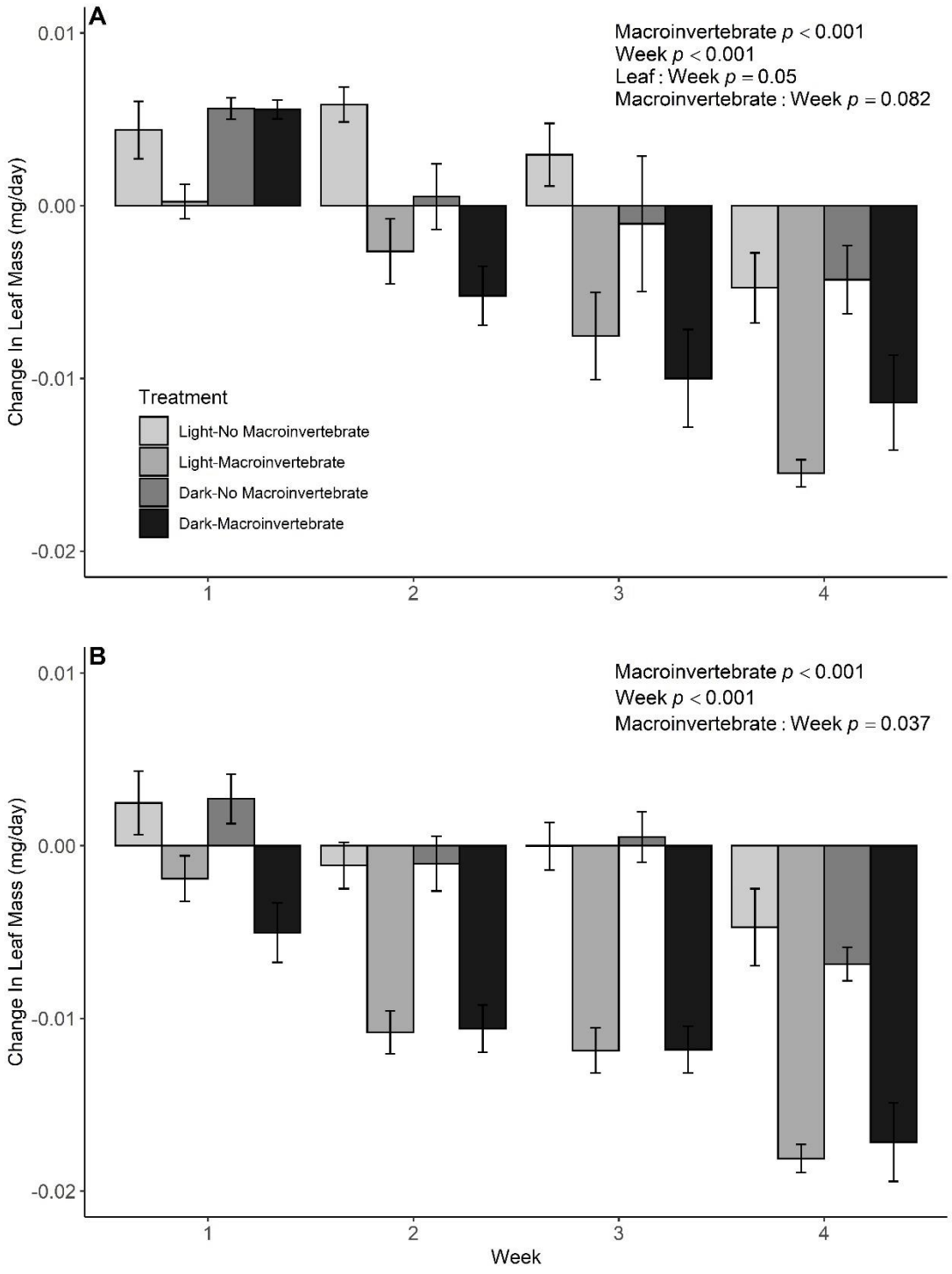

Figure 2.4. Change in leaf wet mass measured on leaves within the flasks over one week for each week during the (A) *Caecidotea communis* experiment and (B) *Ephemerella invaria* experiment.

Leaf area consumed by *C. communis* was significantly greater in the light-conditioned leaf treatments (p=0.022) and changed between weeks (p=0.015) with more consumption in week 4 compared to weeks 1 and 3 (Tukey's HSD: p=0.034 and p=0.021, respectively; Table 2.1; Figure 2.5; Appendix II). The total leaf area consumed over all four weeks was significantly greater in the light-conditioned leaf treatment (p=0.045; Table 2.2). Frass production was significantly greater in the light-conditioned leaf treatment (p<0.001) and changed between weeks (p<0.001) with more in week 3 compared to week 1 (Tukey's HSD: p=0.002) and week 4 compared to weeks 1, 2, and 3 (Tukey's HSD: all p<0.001; Table 2.1; Figure 2.5). Additionally, leaf treatment and week interacted significantly (p=0.034), with generally greater frass production in the light-conditioned leaf treatment and increasing frass production over time (Appendix III). Total frass produced over the course of the experiment was significantly greater in the light-conditioned leaf treatment (p=0.005; Table 2.2). Leaf wet mass change normalized to macroinvertebrate mass was significantly greater in the light-conditioned leaf treatment (p<0.001) and changed across weeks (p=0.001), with a greater decrease in week 3 and 4 compared to week 1 (Tukey's HSD: p=0.002, p=0.006, respectively; Table 2.1; Figure 2.5).

The leaf area consumed by *E. invaria* was significantly greater in the light-conditioned leaf treatment (p=0.014) and changed between weeks (p<0.001) with greater consumption in week 4 than week 1 or week 3 (Tukey's HSD: p<0.001, p=0.009, respectively; Table 2.1; Figure 2.5; Appendix II). Leaf treatment and week also significantly interacted impacting leaf area consumed (p<0.001), driven by high consumption on the light-conditioned leaves in weeks 2 and 4 (Appendix III). The total

leaf area consumed summed over all four weeks was marginally greater in the light- than dark-conditioned leaf treatment (p=0.070; Table 2.2). Frass production was marginally different between weeks (p=0.054), with highest frass production in week 3 (Tukey's HSD: p=0.046; Table 2.1; Figure 2.5; Appendix II). Total frass produced over the course of the experiment did not differ between leaf treatments (p=0.957; Table 2.2). Change in leaf wet mass due to macroinvertebrate consumption was also only marginally different between weeks (p=0.085), with the greatest decrease in week 3 vs. week 1 (Tukey's HSD: p=0.074; Table 2.1; Figure 2.5; Appendix II).

Table 2.2. Initial body size characteristics, total consumption values, and growth rates for *Caecidotea communis* and *Ephemerella invaria* from each leaf treatment over the course of the experiment. Additionally, results of Student's *t*-tests are shown, and significant *p*-values are bolded. Treatment values represent mean±SEM.

Parameter	*Caecidotea communis*					*Ephemerella invaria*				
	Treatment[†]		***t*-value**	**df**	***p*-value**	Treatment[†]		***t*-value**	**df**	***p*-value**
	Light	*Dark*				*Light*	*Dark*			
Initial Body Length (mm)	5.67±0.12 n=20	6.01±0.09 n=20	-2.26	38	**0.030**	7.50±0.29 n=20	7.32±0.24 n=20	0.47	38	0.640
Initial Head Width (mm)	0.92±0.02 n=20	0.99±0.02 n=19	-2.68	37	**0.011**	1.34±0.04 n=20	1.43±0.03 n=19	-1.41	37	0.166
Initial Wet Mass (mg)	6.96±0.39 n=20	7.95±0.33 n=20	-1.95	38	0.058	13.65±1.28 n=20	13.07±1.05 n=20	0.35	38	0.728
Initial Dry Mass (mg)	1.44±0.08 n=20	1.64±0.07 n=20	-1.95	38	0.058	3.78±0.33 n=20	3.64±0.27 n=20	0.35	38	0.728
Total Leaf Area Consumed (mm^2/mg DM[‡])	101.24±19.87 n=6	47.54±12.36 n=6	2.30	10	**0.045**	112.98±22.24 n=5	64.68±6.11 n=5	2.09	8	0.070
Total Frass Produced (mg/mg DM[‡])	6.58±0.51 n=6	4.51±0.25 n=6	3.63	10	**0.005**	3.18±0.33 n=4	3.21±0.42 n=5	-0.06	7	0.957
Percent Growth/Day of Body Length	0.38±0.07 n=18	0.04±0.05 n=19	4.23	35	**<0.001**	0.48±0.07 n=13	0.35±0.09 n=15	1.14	26	0.265
Percent Growth/Day of Head Width	0.44±0.09 n=18	0.20±0.06 n=18	2.08	34	**0.046**	0.40±0.06 n=11	0.36±0.11 n=16	0.31	25	0.762
Percent Growth/Day of Wet Mass	0.62±0.16 n=18	0.25±0.16 n=19	1.63	35	0.112	0.67±0.08 n=12	0.70±0.08 n=16	-0.29	26	0.772
Percent Growth/Day of Dry Mass	1.00±0.31 n=18	-0.15±0.19 n=18	3.12	34	**0.004**	-1.4±0.07 n=13	-1.27±0.07 n=16	-1.43	27	0.165

[†]Treatment refers to leaves conditioned under ambient light (light) or completely covered (dark).
[‡]Values were normalized to the initial dry mass (DM) of the macroinvertebrates.

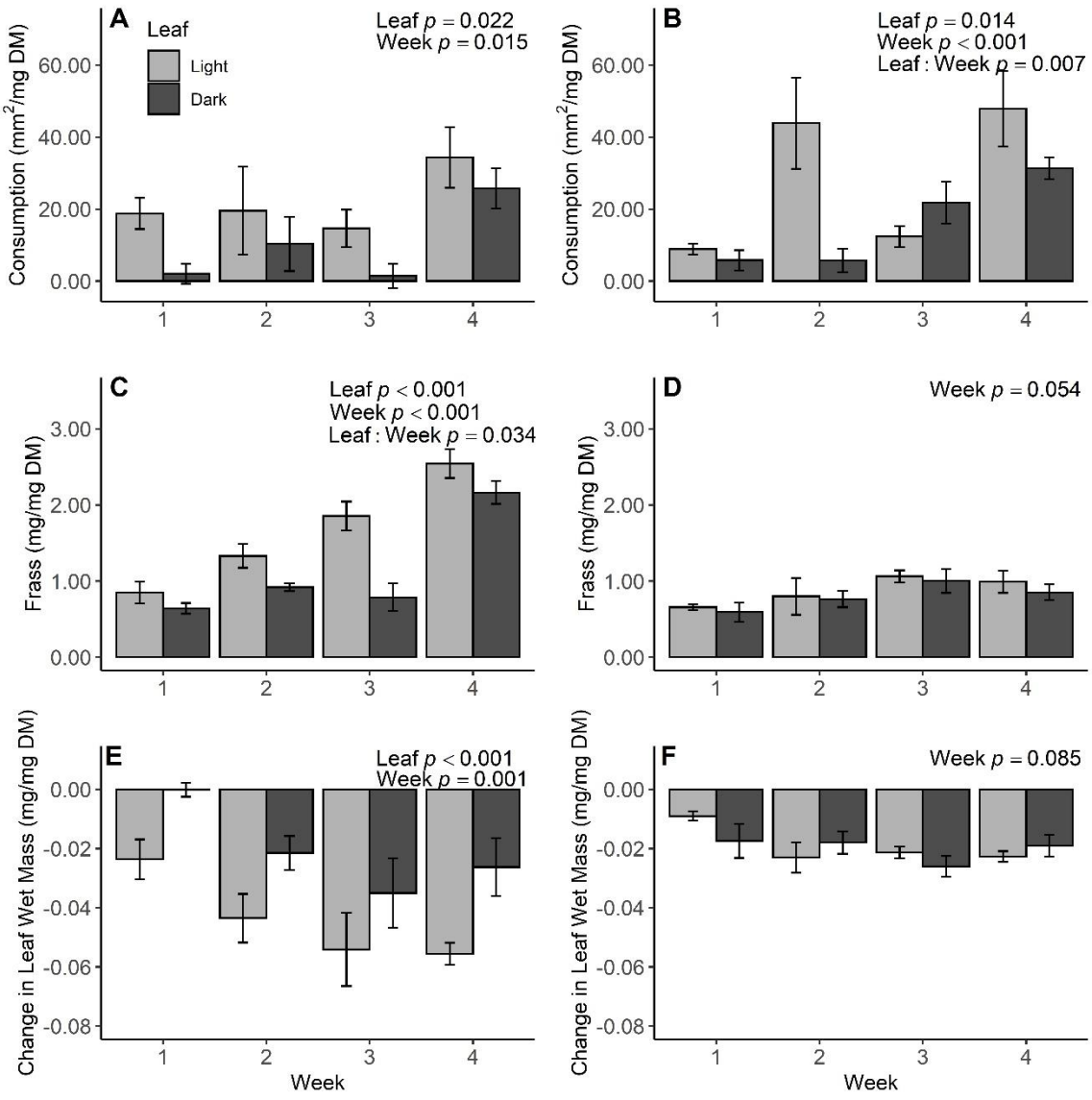

Figure 2.5. Consumption variables measured for *Caecidotea communis* (left) and *Ephemerella invaria* (right) each week. DM refers to the initial dry mass of the macroinvertebrate. (A, B) Leaf area consumed. (C, D) Frass produced. (E, F) Leaf wet mass change

Stable isotope analysis

Stable isotope signatures during the *C. communis* experiment were distinct for $\delta^{13}C$ and $\delta^{15}N$ in the background leaves, algal slurry, and *C. communis* fed on dark- and light-conditioned leaves (Figure 2.6; Appendix II). The background leaves overlapped in $\delta^{13}C$ with leaf disks incubated in flasks at the end of the experiment (week 4), and $\delta^{15}N$

signals were highly variable across leaf sources. Leaf disks in flasks without macroinvertebrates exhibited $\delta^{15}N$ that were generally more depleted than those in flasks with macroinvertebrates, and the dark-conditioned leaves and the macroinvertebrates fed on them were generally more enriched in $\delta^{15}N$. The $\delta^{13}C$ measured for *C. communis* was much less depleted than the potential food resources, and $\delta^{15}N$ were less than typical trophic enrichment factors in comparison to measured food resources. *C. communis* signatures prior to the experiment showed similar $\delta^{13}C$ signatures to experimental isopods and $\delta^{15}N$ that were intermediate between the light- and dark-conditioned leaf treatment individuals.

There were distinct $\delta^{13}C$ and $\delta^{15}N$ signals for background leaves, algal slurry, and *E. invaria* both pre- and post-experiment. *E. invaria*'s $\delta^{13}C$ after the experiment had higher variation and was between leaf and algal signatures while $\delta^{15}N$ signals overlapped between light and dark treatments (Figure 2.6; Appendix II). Similar to the *C. communis* experiment, background leaves overlapped in $\delta^{13}C$ and $\delta^{15}N$ with the week 4 leaves incubated in flasks. Leaves with and without macroinvertebrates in the dark-conditioned leaves were nearly identical in signature, and leaf disks from the light-macroinvertebrate present treatment overlapped with dark-conditioned leaves while the light-no macroinvertebrate treatment was similar to background leaves. Initial *E. invaria* signatures were more depleted in $\delta^{13}C$ than other sources, with a shift during the experiment to between the algal and leaf resources.

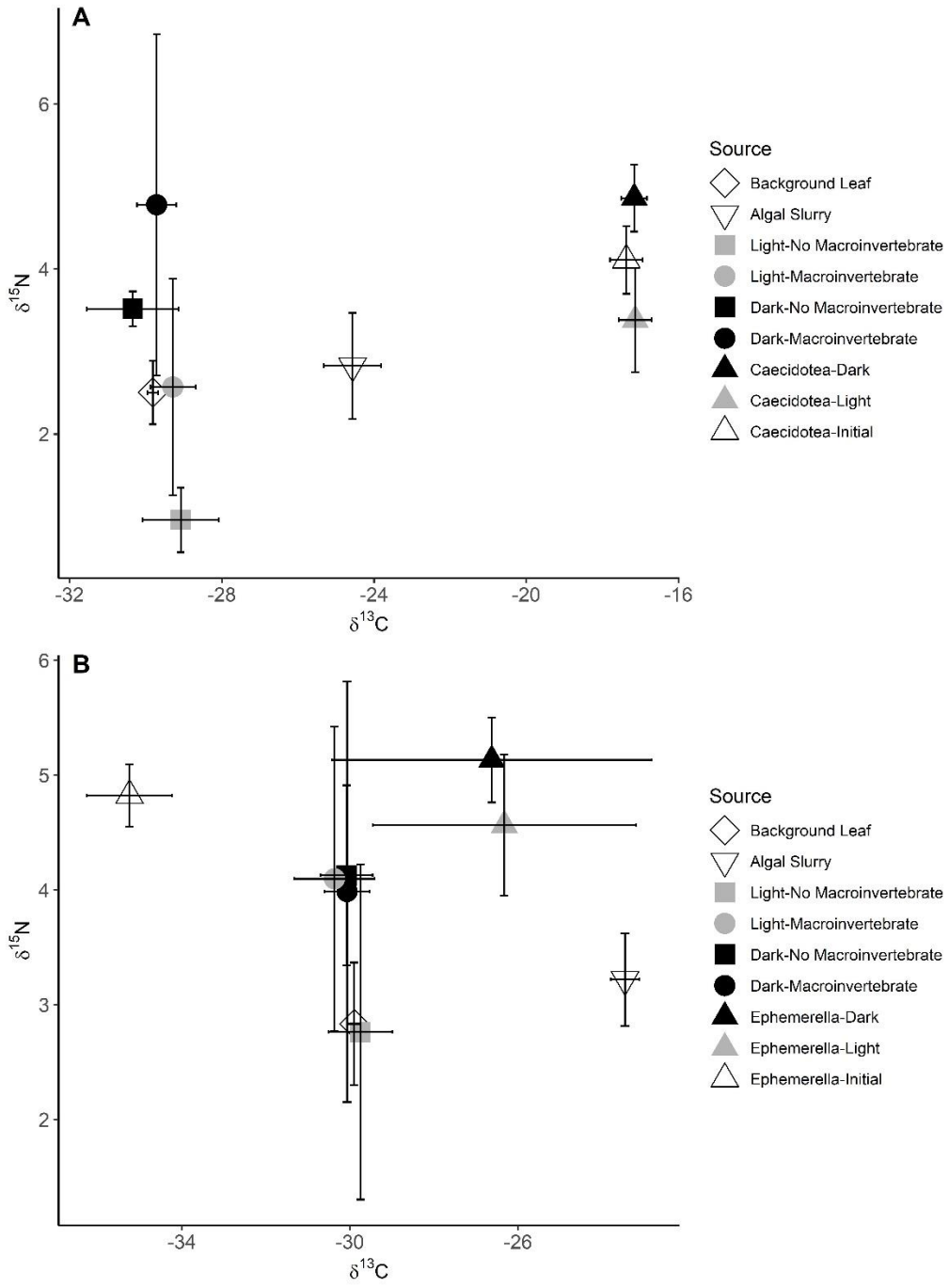

Figure 2.6. Plot of $\delta^{13}C$ and $\delta^{15}N$ for sources measured from the (A) *Caecidotea communis* and (B) *Ephemerella invaria* experiment. Background leaves were leached but not conditioned. Algal slurry was scraped from rocks and used to seed the light treatment. Light-No macroinvertebrate, Light-Macroinvertebrate, Dark-No Macroinvertebrate, and Dark-Macroinvertebrate were conditioned leaves incubated in flasks during the last week (week 4) of the experiment. The species-dark and -light are incubated individuals at the end of the experiment, while species-initial were individuals prior to the experiment.

C. communis *and* E. invaria *growth*

The *C. communis* individuals used in the experiment were larger in the dark-conditioned leaf treatment at the beginning of the experiment (Table 2.2); absolute differences were small and appeared driven by one to two small individuals in the light-conditioned leaf treatment. Values used elsewhere were normalized to account for this difference. Mortality of *C. communis* during the experiment was low, with one dead in each leaf treatment. Twenty-one individuals molted during the course of the experiment, eleven individuals in the dark-conditioned leaf treatment and ten individuals, one twice, molted in the light-conditioned leaf treatment. Light-conditioned leaf treatment *C. communis* grew significantly more than those in the dark-conditioned leaf treatment in dry mass, body length, and head width but not in wet mass (p=0.004, p<0.001, p=0.046, p=0.112, respectively), and light-conditioned leaf treatment growth rates were at least double dark-conditioned leaf treatment rates (Table 2.2; Figure 2.7).

There were no differences in size between the individuals placed into leaf treatment flasks for *Ephemerella invaria* (Table 2.2). Mortality for *E. invaria* was much higher than that for *C. communis*. Greatest mortality occurred in the first week, with most dying within the first two days of the experiment; after this, only two more individuals, one per leaf treatment, died. Total mortality was eleven individuals, with seven dying within the light-conditioned leaf treatment and four within the dark-conditioned leaf treatment. Of those surviving to the end of the experiment, seven individuals molted in the light-conditioned leaf treatment and eight in the dark-conditioned leaf treatment. There were no significant differences in growth rates for *E. invaria* between the two treatments (Table 2.2; Figure 2.7).

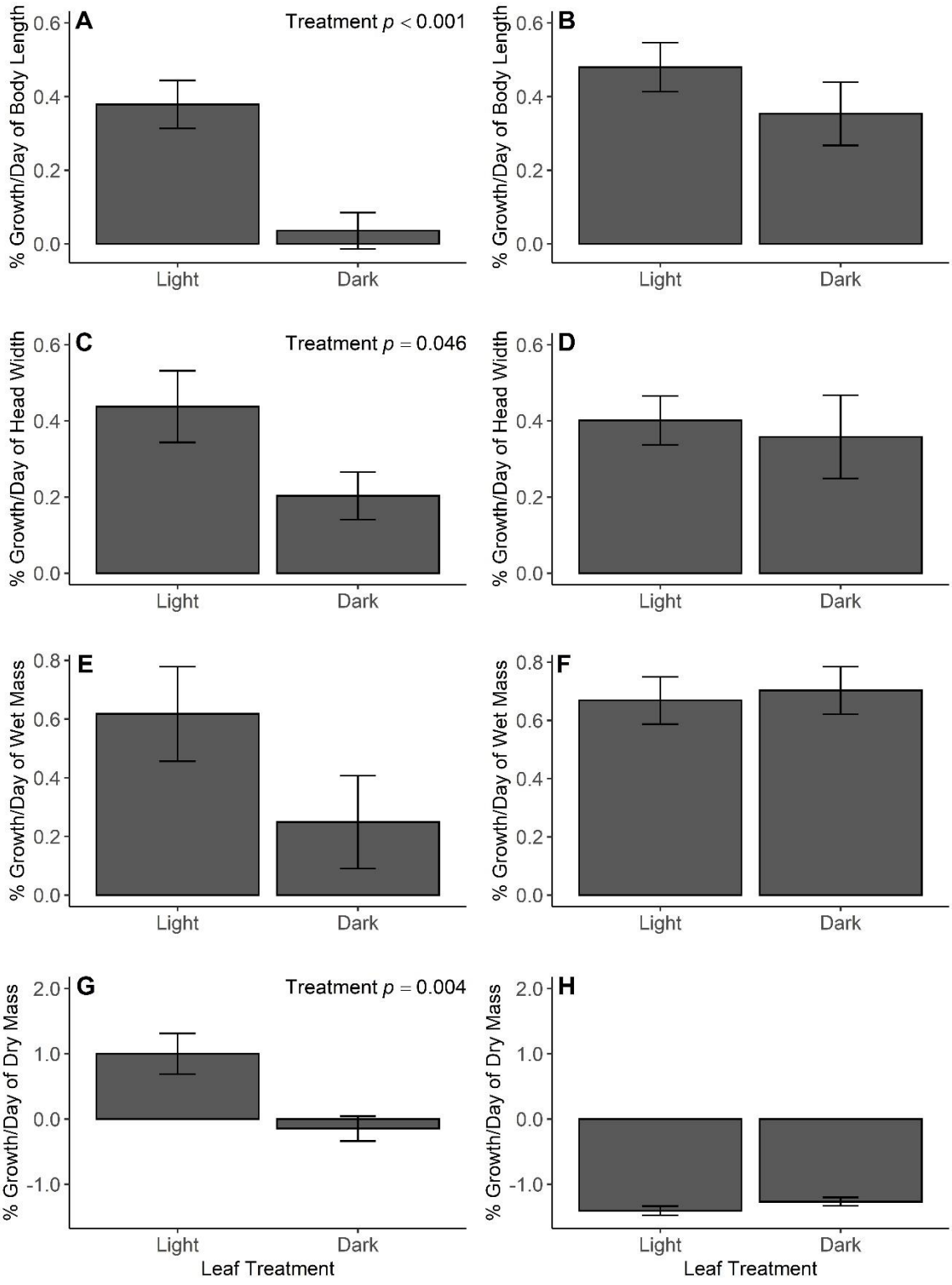

Figure 2.7 Percent growth rates within each treatment for *Caecidotea communis* (left) and *Ephemerella invaria* (right), where leaves were conditioned under ambient light or in the dark, over the course of the 28-day experiment. (A, B) Percent growth per day of body length. (C, D) Percent growth per day of head width. (E, F) Percent growth per day of wet mass. (G, H) Percent growth per day of dry mass.

Discussion

Algae has been recognized as a higher quality food resource (Brett & Müller-Navarra, 1997; Guo et al., 2016) and a potential food resource for macroinvertebrate shredders in tests where it is offered as a distinct food choice from leaf detritus (e.g., Friberg & Jacobsen, 1994; Leberfinger & Bohman, 2010). Little work, however, has investigated leaf-associated algae as a food resource for shredders or non-shredders, and results have been mixed where tested (e.g., Sweeney & Vannote, 1981; Rosillon, 1988; Franken et al., 2005; Albariño, et al. 2008; Guo et al., 2016). Here, I provided leaves conditioned in either the light or dark to two taxa to measure whether algae were consumed and promoted growth: a shredder, *Caecidotea communis*, and a collector-gatherer, *Ephemerella invaria*. *C. communis* significantly decreased algal biomass on the leaf disks in all weeks, with greater decreases on light-conditioned leaves, while *E. invaria* decreased algal biomass in later weeks; both decreased leaf disk mass compared to controls. *C communis* consumption was greater on light-conditioned leaves, while only greater area of light-conditioned leaves was consumed by *E. invaria*. Stable isotope signatures of *C. communis* and conditioned leaves without macroinvertebrates were different between leaf treatments, suggesting microbial community differences between light- and dark-conditioned leaves which may be related to the algal community. Conversely, there were no stable isotope differences between leaf treatments for *E. invaria* or the conditioned leaves. There was, however, a strong shift in *E. invaria* signatures during the experiment which indicated incorporation of both algal and leaf material; there were no differences in growth between treatments, and growth of *E. invaria* was similar to that of *C. communis* within the light-conditioned treatment. Within

C. communis, isotope differences and higher light-conditioned leaf consumption rates translated to a higher growth rate on light-conditioned leaves than on dark-conditioned leaves while there was no difference in growth rates for *E. invaria* between leaf treatments. These results indicate that the leaf microbial community composition can be impacted by light availability and have impacts on macroinvertebrate growth. Further, small amounts of high-quality algae like diatoms (e.g., <1 mg/mm^2) can impact the growth of some organisms, supporting previous studies that the presence of at least some high quality algae is important for growth of organisms feeding on detritus (e.g., Franken et al., 2005; Guo et al., 2016; Grieve & Lau, 2018).

Algal colonization of leaves

The conditioning treatment was not effective in altering algal biomass on leaves in either the *C. communis* or the *E. invaria* experiment as background algal biomass did not vary between leaf treatments. Probable chlorophyll-a contamination related to the leaching process appears to have influenced the leaf algal biomass prior to introduction to the conditioning chambers in both experiments. One potential source is residual contamination from the building's water source which is contaminated with filamentous cyanobacteria (personal observation). Although filtered and purified RO water from an uncontaminated source was used to leach the leaves, it is possible that cyanobacterial resting cells persisted in materials other than the leaves, e.g., the leaching container, and were transferred and reactivated during leaching. A second potential source is that the leaves themselves were harboring subaerial or aeroterrestrial algae suspended in resting stages. There are a number of algal taxa, especially green algae and cyanobacteria, which live within subaerial habitats such as soil, rocks, and tree bark and can form resting stages

when experiencing desiccation or other unfavorable environmental conditions (e.g., Potts et al., 1999; Holzinger & Karsten, 2013; Wehr & Sheath, 2015). The leaves used in these experiments were collected from the ground beneath red maple trees and may have harbored these resting cells. Recent findings have shown that prior to entering the water, leaves already contain many of the fungi associated with leaf decomposition (Marks, 2019), and this may be true of some algal colonizers as well. Upon rehydration, these cells could have been reactivated and subsequently captured in the initial chlorophyll-a measurements, which then resulted in competition with the algal communities introduced by treatment conditions, limiting biomass differences between leaf treatments.

Algal biomass within the flasks differed even though background measurements did not. In line with predictions, flasks with *C. communis* throughout the experiment and flasks with *E. invaria* in later weeks had less algal biomass than flasks without macroinvertebrates. The effect of leaf treatment on algal biomass was, however, more complicated; patterns followed expectations during the *E. invaria* experiment with marginally greater algal biomass in the light-conditioned treatment, but in the *C. communis* experiment, algal biomass was marginally greater on dark-conditioned leaves with lowest algal biomass on light-conditioned leaves in flasks with *C. communis*. The algal biomass results from the *C. communis* experiment could be due to two non-mutually exclusive mechanisms. First, consumption measures indicated the light-conditioned leaves were fed upon more than dark-conditioned leaves by *C. communis*, and macroinvertebrate presence decreased algal biomass. Higher feeding on light-conditioned leaves would therefore also result in the measured lower algal biomass on light-conditioned leaves in flasks with *C. communis* due to higher consumption of algae.

Second, during the *C. communis* experiment, background algal contamination was nearly two-fold higher than in the *E. invaria* experiment. Within the dark-conditioned leaf treatment, the contaminating cells along with algal cells from the stream water could have increased internal cellular chlorophyll-a concentrations as a means to photoacclimate to low irradiance (e.g., Beale & Appleman, 1971; Quinn et al., 1997; Ferreira et al., 2016), which would have resulted in overestimates of algal biomass by chlorophyll-a in the dark-conditioned leaf treatment and no background differences. Once exposed to light in the flasks, these dark treatment leaf disk communities would be poised to quickly proliferate and increase algal biomass to levels greater than in the light-conditioned leaf treatment.

Alternatively, some algal species, including both diatoms and cyanobacteria, are known to shut down parts of their photosystems during prolonged darkness and limit growth; upon reexposure to light, they quickly (hours to days) begin to photosynthesize, produce more chlorophyll-a, and proliferate (Evans et al., 1978; Peters & Thomas, 1996). Cells on dark-conditioned leaf disks utilizing these mechanisms would therefore quickly respond to being placed in flasks where light is now available, with measurable increases in biomass that could be greater than that in the light-conditioned leaf treatment. In the *E. invaria* experiment, this same pattern was not observed, but background contamination was lower, the algal slurry had higher concentrations of chlorophyll-a, and light availability was 3x higher. While the same mechanisms could be at work in the dark-conditioned leaf treatment, the algal community on light-conditioned leaves may have been better poised to keep ahead of the dark-conditioned leaves once placed into flasks, resulting in the expected treatment difference in algal biomass.

Macroinvertebrate consumption and leaf stable isotope signatures

In addition to having an impact on algal biomass, macroinvertebrate presence resulted in greater leaf mass loss than in treatments without macroinvertebrates for both *C. communis* and *E. invaria*. In both experiments, leaf tissue exhibited signs of skeletonization, indicating these leaf mass losses were due at least in part to consumption. There were also greater decreases in leaf mass in later weeks, suggesting increased decomposition rates over time, as is typical in breakdown curves, that were enhanced by the shredding activity of the macroinvertebrates (Abelho, 2001; Graça, 2001). Macroinvertebrate consumption as measured by area consumed, frass production, and changes in leaf mass due to macroinvertebrate presence did not follow predictions. *E. invaria* showed no signs of compensatory feeding in the dark-conditioned leaf treatment as changes in leaf mass and frass production were similar between leaf treatments, and greater areal consumption occurred in the light-conditioned leaf treatment. In contrast, *C. communis* fed significantly more on the light-conditioned leaves by all measures. This greater feeding is also not related to compensatory feeding mechanisms as similar growth rates would be necessary between food resources. It therefore appears that feeding activities were sufficient to support growth without compensatory feeding (e.g., Flores et al., 2014)

The effects of macroinvertebrate feeding on leaves likewise extended to the stable isotope signatures of the leaves in the *C. communis* experiment. Leaves in both leaf treatments without macroinvertebrates present were distinct from each other, with a more depleted $\delta^{15}N$ in the light-conditioned leaf treatment than the dark treatment. Although not distinct from each other, the $\delta^{13}C$ of the leaves were trending towards opposite

directions, with light-conditioned leaves moving towards the algal slurry signal and the dark becoming more depleted. These signals suggest that the dark-conditioned leaves were more highly influenced by fungi than the light-conditioned leaves, as colonization by fungi depletes $\delta^{13}C$ and enriches $\delta^{15}N$ (Costantini et al., 2014). These differences further indicate that the microbial communities were different between the light- and dark-conditioned leaves, which can promote differences in food quality. With feeding, $\delta^{15}N$ became more enriched and variation increased, so that leaves were no longer distinct. Macroinvertebrates can actively discriminate amongst specific food resources, including amongst microbial species and locations within a food resource like a leaf surface (e.g., Arsuffi & Suberkropp, 1985; Graça, 2001). The shifts in $\delta^{15}N$ and discrete patches of skeletonization observed on leaves suggest that *C. communis* was feeding selectively, altering the microbial community as measured by the stable isotope signature. During the *E. invaria* experiment, the leaf signals were not as distinct as in the *C. communis* experiment and had greater variation. While a similar pattern was measured for the light-conditioned leaves, where $\delta^{15}N$ signals were more enriched when *E. invaria* was present, there were no differences between the dark-conditioned leaves. *E. invaria* therefore may have been consuming without selection and feeding randomly, especially within the dark-conditioned leaf treatment.

Macroinvertebrate growth and stable isotope signatures

Consumption of a food resource alone does not indicate that it is high quality; rather, growth is a more definitive test (Graça et al., 1993; Flores et al., 2014) as it incorporates assimilation without egestion and respiration. In these experiments, the growth response of *C. communis* and *E. invaria* was mixed. As predicted, *C. communis*

grew best on leaves that had been conditioned in the light, while, contrary to my prediction, there were no differences in growth rates for *E. invaria*. Further, growth rates were similar between *E. invaria* and the light-conditioned leaf treatment *C. communis*, while the dark-conditioned leaf treatment *C. communis* growth rates were lower. For *C. communis*, these growth results indicate that the dark-conditioned leaves were of lower quality than the light-conditioned leaves; as mentioned above, given the differences in stable isotope signatures and lack of differences in leaf stoichiometry, this is likely driven by the microbial community on the leaves. Although the leaf-associated communities were not directly examined in this study, data collected here and in other studies suggest a few non-mutually exclusive possibilities. While some studies have seen increases in fungal biomass in the light (e.g., Kuehn et al., 2014), others have seen the opposite pattern (e.g., Halvorson et al., 2019a). As also discussed above, the $\delta^{15}N$ of the dark-conditioned leaves without macroinvertebrates suggests greater influence of fungi than algae (e.g., Costantini et al., 2014), indicating there may have been greater fungal biomass on the dark-conditioned leaves. Although shredders directly consume and can select for fungal species on leaves (e.g., Arsuffi & Suberkropp, 1985), fungi lack essential PUFAs that are present within diatoms and support macroinvertebrate growth and are therefore of lower quality (e.g., Brett & Müller-Navarra, 1997; Guo et al., 2016; Grieve & Lau, 2018; Guo et al., 2018). Greater levels of fungi relative to algae on the dark-conditioned leaves subsequently may have limited growth.

Heterotrophic bacteria may also vary between the light- and dark-conditioned leaf treatments. Bacterial production can be much greater than its perceived biomass on leaf litter, as its standing stock biomass is typically much less than that of fungi (Hieber &

Gessner, 2002; Pascoal & Cássio, 2004; Gulis & Bärlocher, 2017). Research investigating the impact of algae on heterotrophs have found that increases in algal biomass typically promote increases in bacteria as well, both in production and biomass, and these are generally greater than impacts on fungi, suggesting facilitation between algae and bacteria (e.g., Rier et al., 2007; Kuehn et al., 2014; Halvorson et al., 2019a). Bacteria can be an important carbon source in macroinvertebrate consumer diets, deriving both from its presence on detritus and within epilithic biofilms (e.g., Hall & Meyer, 1998). Other work, however, has found that bacterial fatty acids associated with leaf litter may not support the growth of shredders (Guo et al., 2016). If bacteria were stimulated by algal growth in this experiment, as appears to be typical, they may have contributed carbon but provided limited other nutrition to the macroinvertebrates in support of growth.

In addition to heterotrophs, previous work has shown that the algal community composition is important for growth, with greater proportions of diatoms indicative of higher quality (e.g., Brett & Müller-Navarra, 1997; Guo et al., 2016; Grieve & Lau, 2018; Guo et al., 2018). Here, data suggests the community on light- and dark-conditioned leaves were not equal in the *C. communis* experiment. There is limited data on algal communities associated with leaves, particularly under different light regimes. Diatom abundance, however, generally increases with light availability while the proportion of cyanobacteria can increase with shading, although these relationships also may be altered by nutrient availability (Franken et al., 2005; Guo et al., 2016; Eckert et al., 2020). These trends along with observational data suggest that the dark-conditioned leaf algal community was dominated by cyanobacteria, which tend to limit shredder growth in

comparison to diatoms (Guo et al., 2016). In contrast, the light-conditioned leaves likely had greater proportions of diatoms, supporting growth through incorporation of essential PUFAs (e.g., Guo et al., 2016; Grieve & Lau, 2018). The water and algal source in this experiment came from a stream where algal-associated leaf communities were dominated by diatoms (Eckert et al., 2020), and the introduction of the algal slurry likely further promoted the growth of diatoms in the light-conditioned leaf treatment. Greater diatom proportions on light-conditioned leaves would result in higher quality for macroinvertebrates, supporting increased growth rates.

The $\delta^{13}C$ of *C. communis*, contrary to expectations, does not provide any further insights into what foods were assimilated, although it supports selective feeding on some resource. No differences were found between isopods prior to the experiment and after feeding on the conditioned leaves, although observed molting indicated growth occurred and therefore changes in $\delta^{13}C$ should have been measurable. The $\delta^{13}C$ does not match any of the tested food resources, and so the main source of carbon is unknown; it is therefore impossible to make determinations as to what proportion of *C. communis*'s diet was comprised of algae, leaf, and this unknown source. It can be concluded, however, that given the growth differences, the light-conditioned leaves were of higher quality than the dark-conditioned leaves, and this difference in quality has to do with the microbial community. Other studies manipulating algae on leaf surfaces have shown increased growth even with incorporation of small amounts of high-quality algae, particularly diatoms. Franken et al. (2005) measured greater growth of *Asellus aquaticus*, another isopod, corresponding to diatom presence on leaves. Similarly, Grieve & Lau (2018) fed varying ratios of leaf to algae to *A. aquaticus* and found that growth was maximized with

90:10 leaf:algae proportions; there was very high assimilation (≥94%) of this algal resource, indicating that these small additions of algae are sufficient and necessary to support the optimal growth of the isopod *A. aquaticus*. Further, Guo et al. (2016) found that shredder growth was optimized when greater proportions of the leaf-associated algae were comprised of diatoms, and diatom PUFAs were selectively incorporated into a trichopteran shredder's tissues. As discussed above, light-conditioned leaves likely had greater proportions of diatoms which would support the growth of *C. communis* on the leaf tissue with minimal incorporation, even if neither algae nor leaf was the primary carbon source. Future work should consider observations of microbial community composition and biomass of all components in tandem with other measurements to provide greater insight into microbial effects on growth.

Contrary to expectations, both resources provided equal quality for growth of *E. invaria*, and the microbial community did not differ as strongly as in the *C. communis* experiment. *E. invaria* skeletonized leaves—a feeding mode expected from a shredder—in patches that were often were larger than those from *C. communis*'s feeding; this skeletonization may, however, be due to a lack of other food resources more typical of their diet. Consumption of the whole leaf included both algal and leaf material, and stable isotope signatures indicated both of these resources were assimilated equally in each treatment, although the high variation in $\delta^{13}C$ suggests that on an individual basis, assimilation of one or the other may have been greater. Other *Ephemerella* species have also been capable of growth on both leaves and periphyton. Experiments on *E. subvaria* fed maple leaf disks and periphyton, among other foods, found highest growth rates on periphyton, but survival over time was lower than on maple leaf disks (Bird & Kaushik,

1984). In another experiment using *E. ignita*, mayflies exhibited a preference for a diet of diatoms over detritus, and they grew and survived better on the diatom diet (Rosillon, 1988). Natural diets of various *Ephemerella* species seem to incorporate both (e.g., Erdozain et al., 2019), and this appears true for *E. invaria*. The proportion of incorporated detritus and algae may, however, change temporally and spatially based upon availability and growth needs (Sweeney & Vannote, 1981; Collins et al., 2016; Erdozain et al., 2019), and food resources with higher algal biomass may be preferentially selected (e.g., Rosillon, 1988; Eckert et al., 2020). It therefore appears that it is both algae and leaf tissue, including associated fungi and bacteria, that are important for *E. invaria*, even if that algal biomass is present in low amounts, similar to other observations (e.g., Guo et al., 2016; Grieve & Lau, 2018). Given these results, for *E. invaria*, and perhaps for many other *Ephemerella* species, a mixed diet can be adequate for growth, with detritus sufficient to support growth as long as some algae are present.

Conclusions

The importance of fungal and bacterial colonizers of leaves has been studied and accepted for nearly half a century, particularly in relation to shredder feeding and growth (e.g., Abelho, 2001; Graça, 2001). It is only within recent years that leaf-associated algae have been recognized as higher quality food resources than fungi and bacteria for shredders (e.g., Guo et al., 2016; Grieve & Lau, 2018); as such, information gaps remain regarding the ecological implications and contribution of leaf-associated algae to macroinvertebrates. I manipulated the leaf microbial community via light and dark leaf treatments and evaluated the importance of algae to the growth of a shredder, *Caecidotea communis*, and a collector-gatherer, *Ephemerella invaria*. Both organisms fed on leaf

tissue and algal biomass by skeletonizing the leaves. *C. communis* consumed more of the light-conditioned leaves and the associated algae, which harbored a distinct microbial community, likely dominated by diatoms, from that of the dark-conditioned leaves. Greater feeding on light-conditioned leaves translated to greater growth for *C. communis*, indicating it is a high-quality food resource for the shredder, and that, similar to other studies on isopods, high-quality algae provide a nutritional benefit (Franken et al., 2005; Grieve & Lau, 2018). *E. invaria* consumed more area of light-conditioned leaves but similar mass between leaf treatments and had the same growth rates on both food resources; both leaf treatments therefore provided high quality food resources. Stable isotope signatures indicated assimilation of both detritus and algae, supporting previous studies in various *Ephemerella* species (e.g., Bird & Kaushik, 1984; Sweeney & Vannote, 1981; Erdozain et al., 2019). It therefore appears that mixed diets of detritus and algae are adequate to support growth of *E. invaria*.

Although algal biomass is low in headwater streams, its presence on leaves can play an important nutritional role for some species, whether by facilitating assimilation of other food resources or by providing essential nutrition unobtainable from detritus alone (e.g., Guo et al., 2016; Grieve & Lau, 2018). The strength of its relationship to macroinvertebrates may depend upon the algal community composition, however, and this is still poorly understood. Mixed conclusions regarding algae's role in shredder diets (this study; Franken et al., 2005; Carvalho & Graça, 2007; Albariño, et al. 2008; Guo et al., 2016; Grieve & Lau, 2018) may be due in part to this lack of knowledge. Future work should therefore examine the algal community composition in addition to its biomass. Due to the highly heterogeneous nature of natural headwater streams, food resources like

leaves are unevenly distributed throughout the stream and exposed to varying light levels which can impact algal colonization and community composition, and, in turn, macroinvertebrate colonization of leaves (Guo et al., 2016; Eckert et al., 2020). Loss of heterogeneity as occurs through anthropogenic changes may lead to loss of diverse food resources as well (e.g., Palmer et al., 2014). Thus, in management and restoration efforts, maintenance of or return to natural stream conditions should consider the need to support differential algal growth on leaves in addition to other food resources.

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Chapter 3 -- Feeding preferences of four macroinvertebrate shredders and a scraper in relation to leaf-associated algae and clumped or dispersed leaves

Abstract

The primary source of energy within temperate headwater streams is the decomposition of organic matter such as leaves, driven by microbial decomposition and macroinvertebrate shredder feeding. Recent work indicates that algae colonizing leaves also can be an important energetic resource and provide essential nutrients, thereby supporting increased growth rates for macroinvertebrates. Higher growth rates do not, however, always equate to preference for a food resource, as macroinvertebrates need to be able to locate and discriminate between food of higher and lower quality and selectively feed on that resource to demonstrate preference. In this experiment, four shredders, *Amphinemura* sp. (Plecoptera: Nemouridae), *Tipula* sp. (Diptera: Tipulidae), *Lepidostoma* sp. (Trichoptera: Lepidostomatidae), and *Caecidotea communis* (Isopoda: Asellidae), and a scraper, *Stenonema* sp. (Ephemeroptera: Heptageniidae) were placed into arenas with leaves conditioned to have higher or lower algal biomass in a clumped or dispersed arrangement and their consumption rates were measured and compared. No preferences were found for either leaf arrangement, indicating mobility did not impact feeding. *Tipula* exhibited a preference for leaves with lower algal biomass while *Amphinemura*, *Lepidostoma*, *Stenonema*, and *Caecidotea* exhibited no preferences for

either leaf type. These results indicate that the presence of algae on leaves can be important for some but not all species in choosing a food resource, and its presence can be a feeding deterrent. Leaf-associated algae should therefore be considered as a factor that impacts the feeding, growth, and colonization of macroinvertebrates in headwater streams.

Introduction

Temperate headwater streams are often highly shaded and therefore supported energetically by allochthonous organic matter inputs (Vannote, et al., 1980; Abelho, 2001), as light limitation prevents high production by primary producers such as algae (Richardson et al., 2019). Leaves are the most abundant type of organic matter entering these systems, primarily through autumnal fall (Pozo et al., 1997), but their palatability for consumption by macroinvertebrate shredders is often low given, e.g., high lignin content (Melillo et al., 1982) and naturally high carbon (C) to nitrogen (N) and phosphorus (P) ratios that are further exacerbated by resorption of foliar nutrients prior to leaf abscission (Aerts, 1996; Cross et al., 2003). After entering the stream, leaves are colonized by fungal and bacterial microbial decomposers which directly provide nutritional content and increase leaf nutritional quality by enzymatic breakdown of the leaf (e.g., Bärlocher & Kendrick, 1975; Bärlocher, 1985; Abelho, 2001). Recent work indicates leaves also are colonized by algae which interact with microbial decomposers, often by increasing heterotroph production and altering decomposition rates (e.g., Danger et al., 2013; Kuehn et al., 2014; Halvorson et al., 2019a). These algae, largely diatoms with some red algae, green algae, and cyanobacteria, provide higher nutritive quality to consumers than microbial decomposers (Brett & Müller-Navarra, 1997; Guo et al., 2016;

Grieve & Lau, 2018) and appear to play more important roles in headwater stream dynamics than their low biomass would suggest (e.g., Guo et al., 2016; Eckert et al., 2020). This colonization by fungi, bacteria, and algae alters leaf nutritional quality for macroinvertebrate consumers in combination with leaf characteristics.

Leaf quality can be defined in a number of ways including leaf toughness (e.g., Graça, 2001), the presence of secondary defense compounds (e.g., Graça, 2001), leaf stoichiometry (e.g., Cross et al., 2003), and the concentration and presence of polyunsaturated fatty-acids (PUFAs; e.g., Guo et al., 2016; Grieve & Lau, 2018), and different measures of quality may be important for different shredders (Motomori et al., 2001). As leaf toughness increases, macroinvertebrates have a decreased ability to pierce through the leaf tissue, resulting in decreased leaf quality (Graça, 2001). Similarly, some plant secondary compounds remain active in abscissed leaves, and these chemicals can inhibit feeding or be toxic to macroinvertebrates, decreasing leaf quality (Graça, 2001). Lower C:N:P ratios support higher leaf quality as N and P are necessary for building important biochemical molecules such as proteins and nucleic acids (Sterner & Elser, 2002). Microbes colonizing leaves have lower C:N:P than the leaves, and leaf C:N:P further decreases as microbes grow and assimilate nutrients, increasing leaf quality (Connolly & Pearson, 2013; Tant et al., 2013; Danger et al., 2016). Long-chain PUFAs are increasingly considered important indicators of food quality for macroinvertebrates (Guo et al., 2018). In particular, ω3s such as eicosapentaenoic acid (EPA; 20:5ω3) and docosahexaenoic acid (DHA; 22:6ω3) are dietary essentials used in, e.g., growth, development, and emergence that generally cannot be synthesized by aquatic macroinvertebrates (Stanley-Samuelson, 1994; Guo et al., 2018). Although a variety of

PUFAs are found in aquatic microbes, those considered essential such as EPA and DHA are found in diatoms but not cyanobacteria, green algae, or heterotrophs; resources with higher diatom abundance are therefore of higher quality (Brett & Müller-Navarra, 1997; Guo et al., 2018).

The ultimate test of quality for a macroinvertebrate, however, is not measurement of any of these factors, but empirical tests via growth experiments with organisms (e.g., Franken et al., 2005; Flores et al., 2014). While algae, especially diatoms, represent a higher quality food resource, few studies have explicitly tested for the impact of algae on shredder growth, and in those that have, results have been mixed across taxa. Some studies have shown increased growth with greater amounts of high-quality algae (e.g., Franken et al., 2005; Guo et al., 2016; Grieve & Lau, 2018; Eckert, unpublished data). On the other hand, Carvalho & Graça (2007) found lower growth rates of the trichopteran *Sericostoma vittatum* on biofilm than leaves, and leaves incubated in shade produced higher growth rates in the stonefly *Klapopteryx kuscheli* than leaves incubated in the sun harboring higher algal biomass (Albariño et al., 2008).

Although growth may be greater on one type of food resource over another, indicating higher quality, an organism may not preferentially feed on that resource. For instance, although *K. kuscheli* grew better on leaves incubated in the shade, consumption was similar between leaves incubated in the shade and in the sun, and the individuals showed no preference for either (Albariño et al., 2008). Similarly, although few studies have tested leaf litter in addition to benthic algae for shredder preferences, in those that have, shredders often preferentially feed on algae in addition to or in higher quantities than leaf litter (Friberg & Jacobsen, 1994; Leberfinger & Bohman, 2010). Preference is

driven by the ability of shredders to feed selectively, which can be at the scale of a single food resource, such as a leaf patchily colonized by different microbes, or involve discrimination between different types of food resources, such as leaf species that differ in toughness or stoichiometric ratios (Graça, 2001). Shredders have demonstrated preferences amongst types of fungi colonizing leaves (Arsuffi & Suberkropp, 1985; Aβmann et al., 2011) but also may switch preferred food sources over time, altering growth patterns between food resources temporally (e.g., Hutchens et al., 1997). Further, a preferred food resource or one that provides optimal growth in the lab may not provide the highest nutritional value over the long term (Marks, 2019). Feeding selectively also relies on the ability of an organism to actively forage for a food resource and can therefore be altered by non-nutritional factors such as predation risk and mobility (e.g., Arsuffi & Suberkropp, 1989; Kohler & McPeek, 1989). Responses to food quality and foraging ability vary across taxa and time (e.g., Arsuffi & Suberkropp, 1989; Marks, 2019); while growth studies and measurements of food quality can provide insights into feeding choice and behavior, preference studies can help elucidate other factors that also impact macroinvertebrate food choices.

The importance of leaf-associated algae has been understudied in relation to shredder growth and preference compared to other microbes, but in the few studies that have investigated it, results have been mixed (e.g., Franken, et al., 2005; Albariño et al., 2008; Guo et al., 2016; Grieve & Lau, 2018). In this study, I primarily sought to determine whether leaf-associated algae impacted the consumption of leaf tissue. I therefore measured the preference of four shredder and one scraper taxa across five orders (shredders: *Amphinemura* sp. (Plecoptera: Nemouridae), *Tipula* sp. (Diptera:

Tipulidae), *Lepidostoma* sp. (Trichoptera: Lepidostomatidae), *Caecidotea communis* (Isopoda: Asellidae); scraper: *Stenonema* sp. (Ephemeroptera: Heptageniidae)) for leaves conditioned with higher or lower amounts of leaf-associated algae. Secondarily, based upon observations of these taxa indicating some taxa were highly active while others tended to remain hidden or camouflaged within leaf packs, I investigated whether greater feeding would occur across two leaf arrangements: dispersed which required greater mobility, or clumped near a refuge. Based on field observations of movement and prior experiments in the field and laboratory, I expected that *Amphinemura* sp. would exhibit no preference for leaf type and prefer a clumped arrangement, *Tipula* sp. would prefer dark-conditioned leaves in a clumped arrangement, *Lepidostoma* sp. would exhibit no preference for leaf type and prefer a clumped arrangement, and *Caecidotea communis* would prefer light-conditioned leaves with no preference for leaf arrangement. As a scraper which feeds on algal biofilms and which I've observed agilely moving across the stream benthos and out of leaf packs, I expected that *Stenonema* sp. would prefer light-conditioned leaves and have no preference for leaf arrangement.

Methods

Macroinvertebrate collections

Macroinvertebrates were collected from headwater streams at the Central Maryland Education and Research Center in Clarksville, Howard County, MD. *Amphinemura* sp. (hereafter, *Amphinemura*) were collected using a D-net and organic matter sorting from South Stream (39° 14' 28.06" N, 76° 55' 26.17" W) just before use to minimize mortality. *Tipula* sp. (hereafter, *Tipula*) were collected using mesh bags filled

with moss (Alexander et al., 2011) from South Stream and Forest Stream (39° 14’ 30.03” N, 76° 55’ 42.77” W) and maintained in a tank with leaves from the streams and moderately hard synthetic water (mimicking conductivity and pH of natural stream water by addition of $MgSO_4$, $NaHCO_3$, KCl, and $CaSO_4*2H_2O$ to reverse osmosis water; US EPA, 2002). *Stenonema* sp. (hereafter, *Stenonema*; previously *Maccaffertium*; Zembrzuski & Anderson, 2018) were collected from rocks and D-net sampling in South Stream and were maintained in an aerated bucket with stream water and rocks until use. *Lepidostoma* sp. (hereafter, *Lepidostoma*) were collected from South Stream using leaf packs, D-nets, and organic matter sorting. They were maintained in a bucket with aeration, leaves, and stream water until use. *Caecidotea communis* (hereafter, *Caecidotea*) were collected from Folly Quarter Creek, CMREC, Clarksville, MD (39° 15' 14.60" N, 76° 55' 37.18" W), using a D-net and maintained in a bucket with stream water and leaves. Experiments were performed in late April through early June 2019.

Experimental process

The experimental process was repeated for each of the five taxa using leaves conditioned under a light or dark treatment. Red maple leaves (*Acer rubrum*) were collected after abscission from one location in Prince George’s County to minimize differences outside of treatment conditions and leached for one week in reverse osmosis water with frequent water changes and stirring. Leaf disks (diameter = 18 mm) were removed from leaves, avoiding the midvein, using a corkborer and placed in open plastic circular containers (diameter = 25.4 cm) filled with 900 mL of moderately hard synthetic stream water and 100 mL of stream water from South Stream passed through a 250 µm mesh sieve to seed a natural microbial community; this mesh size prevented the addition

of macroinvertebrates and debris but did not prevent the addition of meiofauna. Containers were aerated and maintained in a 10°C chamber under a 10:14h light:dark cycle (light level ~130 µmol/m^2/s) mimicking average mid-fall/winter light conditions and temperatures. The light treatment was amended with 4 mL of algal slurry scraped from rocks obtained from South Stream while the dark treatment was kept completely covered to prevent light and limit algal growth. Conditioning proceeded for two weeks prior to preference experiments. During conditioning, leaching occurred, leading to darkened water that could limit algal growth in the light treatment. Water was therefore changed in both treatments by repeating the setup process three days after the initial conditioning setup. Conditioning leaves were frequently stirred, and evaporated water was replaced with RO water.

Ten arenas were set up in open, shallow, plastic circular containers (25.4 cm diameter) with pre-rinsed and autoclaved sand on the bottom and an autoclaved natural stream rock of approximately the same size and height in the center of each arena. This design mimics the bottom of the streams from which the macroinvertebrates were collected, which are primarily sandy-bottomed with some cobble. Arenas were filled with 1 L of moderately hard synthetic water and aerated using a plastic pipette attached to a centralized air pump. The arenas were arranged in a completely randomized split-plot experimental design for macroinvertebrates with an additional three control experimental units to measure leaf changes without macroinvertebrates present. Sub-plot was leaf conditioning treatment, either light or dark. Main plots were leaf arrangement, either clumped, with six total leaf disks near the center of the arena in two rows alternating light and dark leaves next to each other, or dispersed, with six total leaf disks alternating light

and dark leaves equally spaced near the edge of the arena, for three dark and three light leaves per arena (Figure 3.1). Arenas were randomly assigned to a leaf arrangement, and the control arenas utilized the dispersed arrangement.

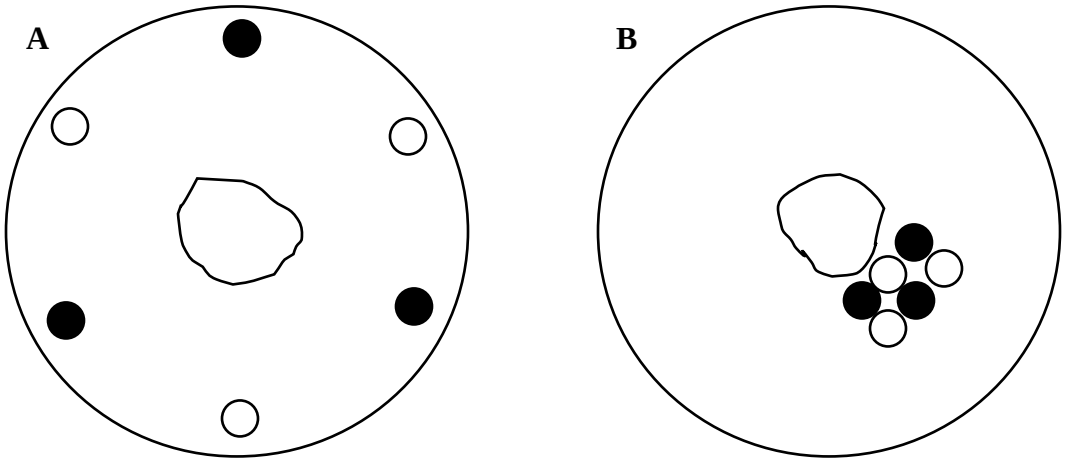

Figure 3.1. Experimental design used in preference tests. Three leaf disks from each of two conditioning treatments, light (open circles) and dark (black circles) were placed into arenas with a sand bottom and a rock in the center. Two arrangements were used, (A) dispersed and (B) clumped. Leaf disks were evenly distributed around the edge of the arena in the dispersed arrangement and placed in two rows in the clumped arrangement, alternating light and dark treatment disks. Controls utilized the dispersed setup in (A).

Macroinvertebrates were placed into arenas 24 hours prior to leaf disk introduction for an acclimatization and starvation period. The densities of macroinvertebrates were similar to those measured in natural leaf packs in the same streams (*Amphinemura*: seven individuals/arena, natural densities of 5.05/leaf pack; *Tipula*: two individuals/arena, natural densities of 2.41/leaf pack; *Stenonema*: six arenas with five individuals/arena, four arenas with four individuals/arena including one large individual due to pre-experiment mortality, natural densities of 4.79/leaf pack; *Lepidostoma*: six individuals/arena, natural densities of 7.90/leaf pack; *Caecidotea*: five

arenas with six individuals/arena, five arenas with five individuals/arena due to pre-experiment mortality, natural densities of 6.38/leaf pack). After 24 hours, conditioned leaf disks were wet-weighed by blotting on a paper towel, labeled with a pin, and secured in the arenas by sticking pins into poster board buried in the sand. After 72 hours of feeding, leaves were removed and wet-weighed using the same process, oven-dried at 60°C for at least 48 hours to obtain dry masses, and, except in the *Amphinemura* experiment, ashed for 8 hours in a muffle furnace at 500°C to determine ash free dry mass (AFDM). After the *Amphinemura* experiment, it was evident that leaves removed from the arenas often had a small amount of sand attached, and therefore AFDM was measured in the other experiments to account for the excess mass of sand. Macroinvertebrates were removed from each arena, wet-weighed by blotting, and dry-weighed after at least 48 hours in a 60°C drying oven.

Initial leaf dry masses (DM) and AFDM were determined from initial wet masses (WM) using another set of leaves that underwent two weeks of conditioning. ANCOVA testing indicated that while the slopes of the light and dark treatment relationships were the same (DM: p=0.9403, AFDM: p=0.6821), intercepts were significantly different (DM: p=0.007; AFDM: p=0.010); I therefore estimated a separate equation for initial DM and AFDM for each conditioning treatment using linear regression. DM of the dark conditioned leaves was calculated by DM = WM * 0.35219 - 4.16978 (R^2=0.80; n=39), AFDM of the dark conditioned leaves was calculated by AFDM = WM * 0.3208 - 3.6668 (R^2=0.79; n=39), DM of the light conditioned leaves was calculated by DM = WM * 0.35522 - 5.21109 (R^2=0.81; n=39), and AFDM of the light conditioned leaves was calculated by AFDM = WM * 0.33650 - 5.17867 (R^2=0.80; n=39). Consumption rates

were calculated within each arena following Canhoto et al. (2005) for each leaf conditioning treatment and both DM and AFDM using the equation:

$$C=\frac{(M_I-M_F)*F}{I*3\ \text{days}}$$

where C is consumption, M_I is initial leaf mass (DM or AFDM), M_F is the final leaf mass (DM or AFDM), F is a correction factor based on controls, and I is macroinvertebrate dry mass within an arena. F was calculated as the average ratio of the initial to final leaf mass of the control leaf disks, estimated separately for light- and dark-conditioned leaves. Macroinvertebrate dry mass included only individuals surviving to the end of the experiment in each arena.

Estimation of algal biomass

A subset of leaves (three replicates, each consisting of five leaf disks) from each conditioning treatment were frozen for later chlorophyll-a analysis as a proxy of algal biomass at the time of leaf introduction to arenas. Chlorophyll-a was measured by extracting leaves in a mixture of 50:50 dimethylsulfoxide:90% acetone for two hours at 4°C (Shoaf & Lium, 1976) and measuring the extract's fluorescence with a narrow-band pass filter using a non-acidification module on a Trilogy fluorometer (Turner Designs, San Jose, CA). Chlorophyll-a values were normalized to total area sampled accounting for five leaf discs, each with two surfaces for algal growth. Chlorophyll-a remaining in abscissed leaves to be used in the experiment was measured at 0.031±0.002 and subtracted from all chlorophyll-a values to account for background levels.

Data analysis

All data were checked for normality and homoscedasticity prior to analysis and met assumptions. Outliers in consumption rates were tested for using Grubb's test; in the case of a significant test, only the largest/smallest value was dropped. The difference in algal biomass between light and dark treatments was analyzed via a two-way ANOVA for leaf treatment and taxa. Consumption data also was analyzed using a two-way ANOVA for a split-plot design where leaf arrangement was the main plot and leaf treatment was the subplot for each taxon. The Friedman test was used to further test for preference between light- vs. dark-conditioned leaves (Canhoto et al., 2005). All analyses were conducted in R v. 3.6.0 (R Core Team, 2019) and used the packages ggplot2 (Wickham, 2016), lme4 (Bates et al. 2015), car (Fox & Weisberg, 2019), effects (Fox, 2003), cowplot (Wilke, 2019), outliers (Komsta, 2011), and dplyr (Wickham et al., 2019).

Results

Leaf treatment differences

Algal biomass was significantly higher on leaves conditioned in the light treatment than in the dark treatment in each experiment ($p<0.001$; Figure 3.2), and generally algal biomass in the dark treatment was near zero. Experiments for some taxa had greater differences in light and dark treatment algal biomass than others ($p=0.011$, interaction: $p=0.002$).

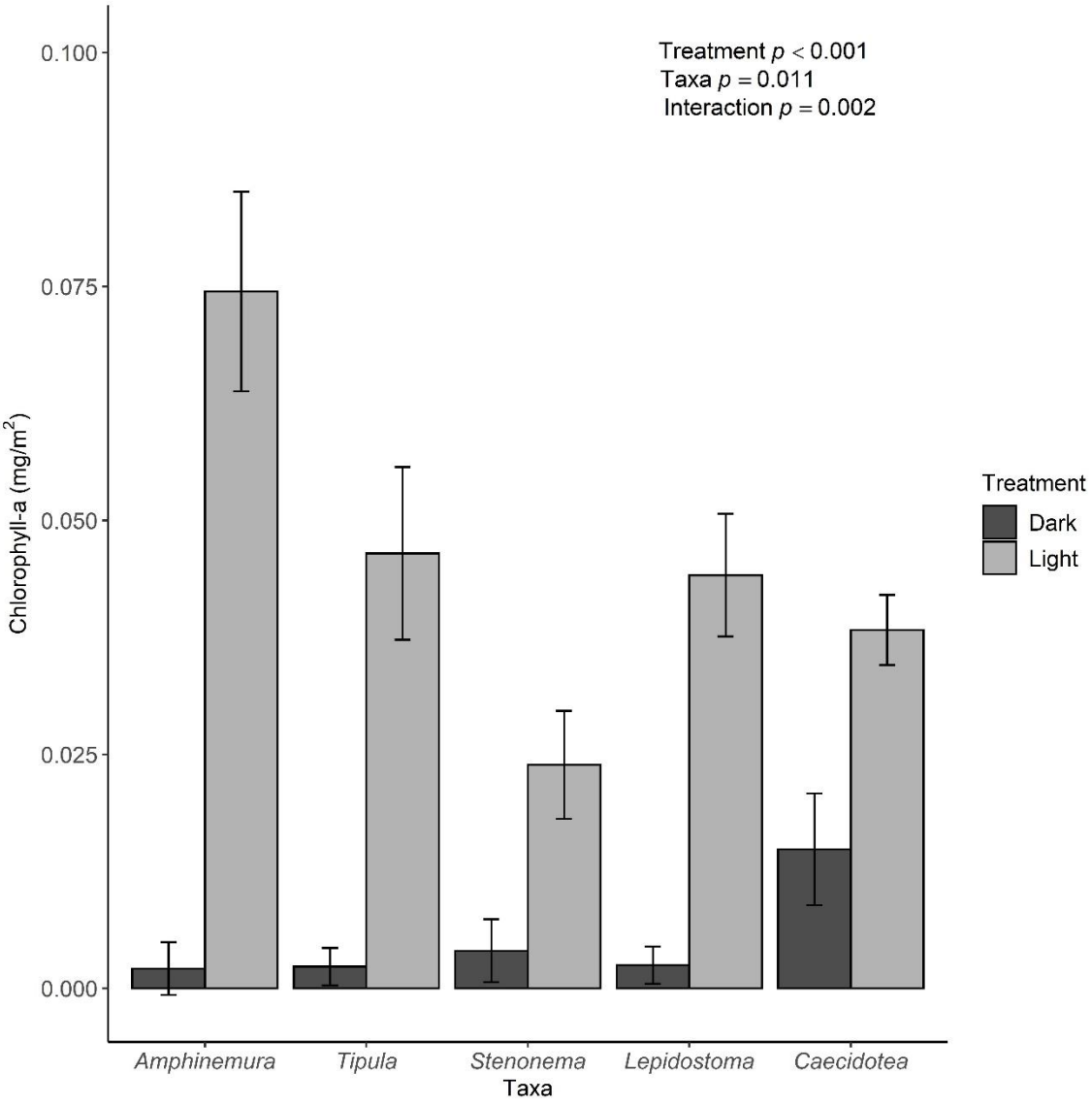

Figure 3.2. Algal biomass measured as chlorophyll-a (mg/m^2) at the beginning of each experiment on a subset of leaves incubated in light or dark conditions.

Consumption rates

There were no differences in DM consumption rates between leaf treatment or arrangement for any of the taxa tested (Table 3.1; Figure 3.3). Similarly, there were no differences in consumption rates of AFDM between leaf arrangement or treatment for any of the taxa (Table 3.2; Figure 3.4).

Table 3.1. Results of two-way ANOVAs on leaf dry mass consumption for each experiment.

Taxa	Factor	F-value	df numerator	df denominator	*p*-value
Amphinemura	Leaf Treatment (LT) †	0.310	1	16	0.586
	Leaf Arrangement (LA) †	0.063	1	8	0.808
	LT x LA	2.476	1	16	0.135
Tipula	Leaf Treatment	1.151	1	16	0.299
	Leaf Arrangement	0.907	1	8	0.369
	LT x LA	0.011	1	16	0.919
Stenonema	Leaf Treatment	2.430	1	16	0.139
	Leaf Arrangement	0.092	1	8	0.770
	LT x LA	0.001	1	16	0.972
Lepidostoma	Leaf Treatment	2.149	1	16	0.162
	Leaf Arrangement	0.000	1	8	0.992
	LT x LA	0.034	1	16	0.855
Caecidotea	Leaf Treatment	1.416	1	16	0.251
	Leaf Arrangement	0.743	1	8	0.414
	LT x LA	0.261	1	16	0.616

†LT refers to whether leaves were conditioned in light or dark conditions. LA refers to the arrangement of leaves within an arena, either clumped or dispersed.

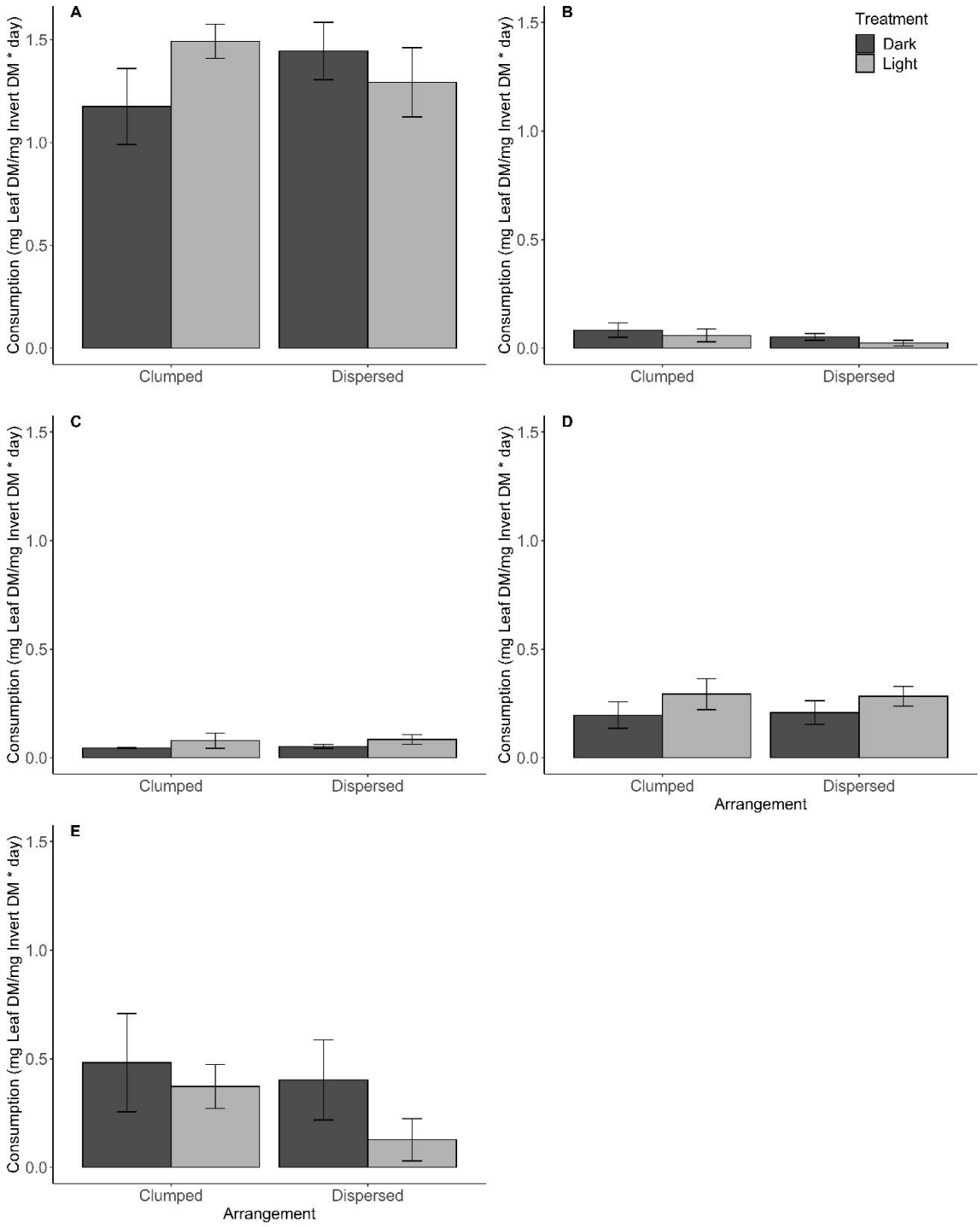

Figure 3.3. Leaf dry mass (DM) consumed within each experiment. Leaves were conditioned under light and dark treatments and arranged in arenas in an either clumped or dispersed arrangement. (A) Experiment with *Amphinemura*. (B) Experiment with *Tipula*. (C) Experiment with *Stenonema*. (D) Experiment with *Lepidostoma*. (E) Experiment with *Caecidotea*.

Table 3.2. Results of two-way ANOVAs on leaf ash free dry mass consumption within each experiment.

Taxa	Factor	F-value	df numerator	df denominator	*p*-value
Tipula	Leaf Treatment (LT) †	2.121	1	16	0.165
	Leaf Arrangement (LA) †	0.840	1	8	0.386
	LT x LA	0.025	1	16	0.878
Stenonema	Leaf Treatment	1.962	1	16	0.180
	Leaf Arrangement	0.008	1	8	0.931
	LT x LA	0.012	1	16	0.913
Lepidostoma	Leaf Treatment	1.962	1	16	0.180
	Leaf Arrangement	0.001	1	8	0.971
	LT x LA	0.012	1	16	0.913
Caecidotea	Leaf Treatment	0.011	1	16	0.919
	Leaf Arrangement	0.173	1	8	0.688
	LT x LA	0.000	1	16	0.997

†LT refers to whether leaves were conditioned in light or dark conditions. LA refers to the arrangement of leaves within an arena, either clumped or dispersed.

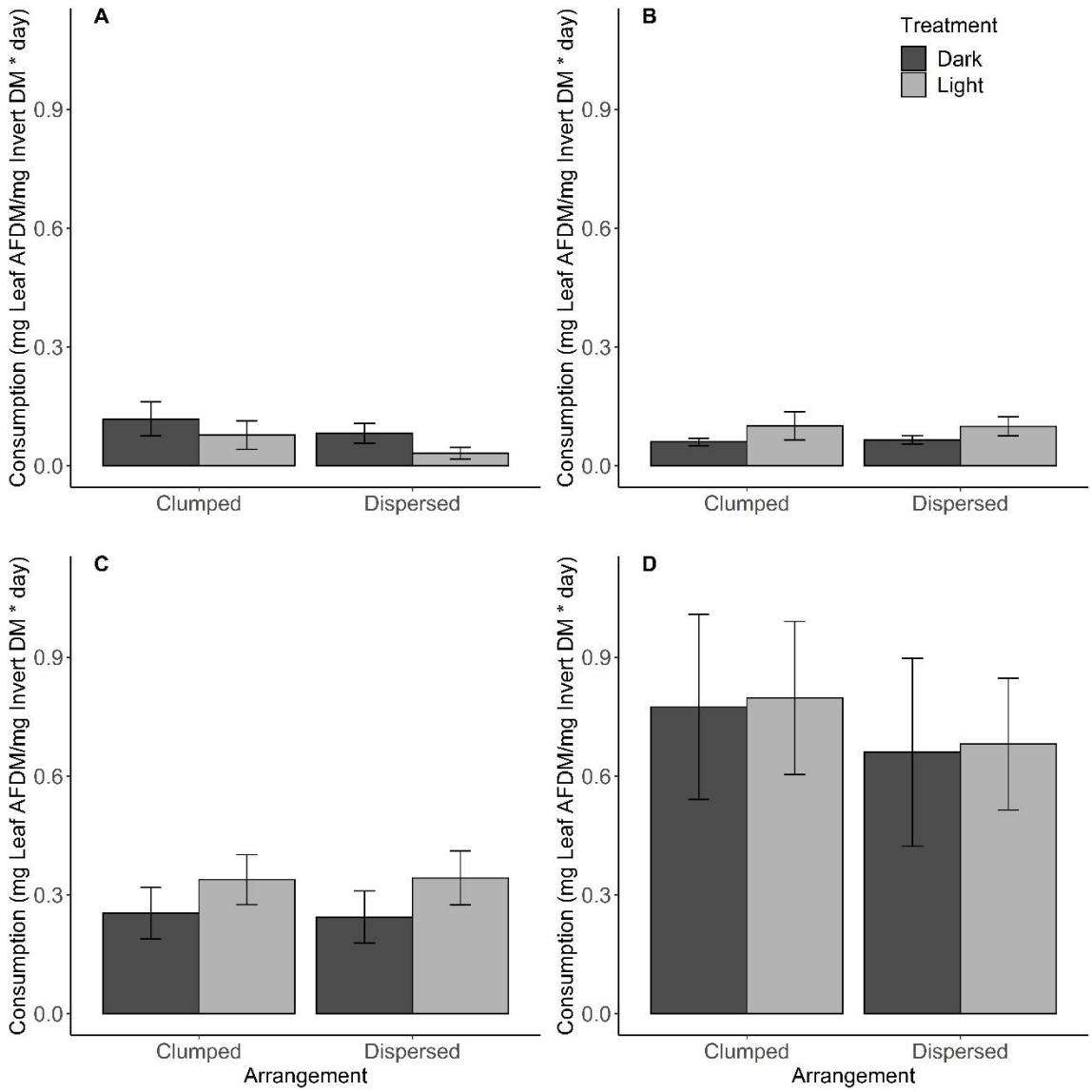

Figure 3.4. Leaf ash free dry mass (AFDM) consumed within each experiment. Leaves were conditioned under light and dark treatments and arranged in arenas in an either clumped or dispersed arrangement. (A) Experiment with *Tipula*. (B) Experiment with *Stenonema*. (C) Experiment with *Lepidostoma*. (D) Experiment with *Caecidotea*.

Preference tests

Light- vs. dark-conditioned leaf consumption was ranked in each arena, and the number of arenas where each were ranked highest are shown in Figure 3.5 (values in

Appendix IV). Friedman tests indicated no preference in DM consumed for *Amphinemura* (*p*=1.000), *Stenonema* (*p*=1.000), *Lepidostoma* (*p*=0.206), and *Caecidotea* (*p*=0.527). *Tipula* exhibited a significantly greater consumption of dark-conditioned leaves *(p*=0.002). Similarly, there were no preferences in AFDM consumed for *Stenonema* (*p*=0.527), *Lepidostoma* (*p*=0.206), and *Caecidotea* (*p*=0.527), while *Tipula* again showed a significant preference for dark-conditioned leaves (*p*=0.002).

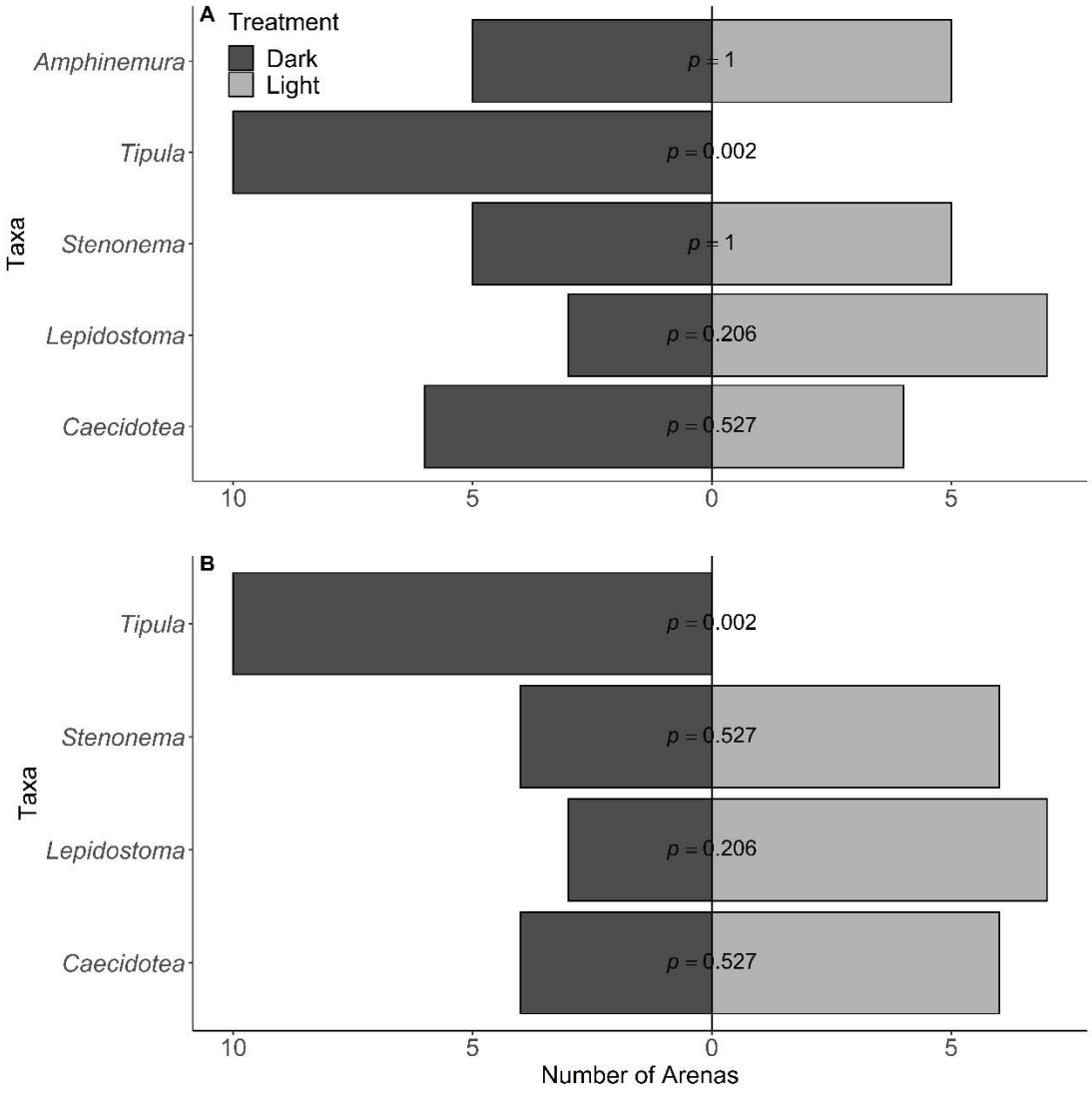

Figure 3.5. Number of arenas in which consumption rates of light- or dark-conditioned leaves were higher for (A) dry mass or (B) ash free dry mass measurements for each taxon. The *p*-values for Friedman tests are shown on each bar, indicating significance of preference.

Discussion

Few studies have directly examined how algae versus leaves impact macroinvertebrate shredder growth, and even fewer have examined impacts on feeding preferences. Where preference has been assessed, results have been mixed. Some studies have shown shredders will select for fresh algae at similar rates as they do conditioned leaves (e.g., Friberg & Jacobsen, 1994; Leberfinger & Bohman, 2010), and at least one study has shown no selection preference amongst leaves with varied algal biomass (Albariño et al., 2008). Here, I tested the feeding preferences of four shredders and one scraper for leaves conditioned in light or dark treatments across dispersed or clumped leaf arrangements to examine whether algae impacted feeding preferences and if it varied by mobility. In my previous work, these taxa were common and/or showed relationships to algae, both positive and negative (Eckert et al., 2020; Eckert, unpublished data). Algal biomass was consistently higher on light-conditioned leaves in each experiment. Consumption rates did not vary between leaf treatment or leaf arrangement for any taxon tested. Overall consumption therefore was not affected by algal biomass or mobility differences between taxa. Preference tests, however, indicated that, as hypothesized, *Tipula* fed more on dark-conditioned leaves. Algal biomass associated with leaves appears to be a deterrent for feeding by *Tipula* and have no impact on the feeding of *Amphinemura*, *Stenonema*, *Lepidostoma*, and *Caecidotea*, resulting in indiscriminate consumption of leaf tissue that can include algae necessary for growth (Webb & Merritt, 1987; Benstead & Pringle, 2004; Grieve & Lau, 2018). Algal biomass can therefore impact the colonization of some taxa (e.g., *Tipula*) within leaf packs due to feeding preferences.

Taxon-specific leaf preferences

As expected, *Amphinemura* showed no response to leaf treatment, with similar feeding rates between light- and dark-conditioned leaves. Previous studies have shown *Amphinemura* feeds on detritus with no strong relationships to algae. Madsen (1974) investigated the food resources available to *Amphinemura sulcicollis* in relation to gut contents and preferences and found *A. sulcicollis* fed extensively on detritus and preferred high amounts of fungal material as opposed to leaf material, bacteria, or algae. In another gut content study, Dangles (2002) found detritus to be most common in *A. sulcicollis* across multiple streams, with minimal amounts of benthic algae. Similarly, no relationship was found between algal biomass and densities of *Amphinemura* colonizing substrates (Clifford et al., 1992) or leaves (Eckert, unpublished data). Associations in the literature are borne out by this preference study, in that algae do not affect leaf preferences of *Amphinemura* and any algal consumption is likely incidental.

Stenonema showed no preference although it was expected to feed on leaf biofilm and prefer light-conditioned leaves because of its scraping feeding guild, although there was a trend of higher consumption on light-conditioned leaves. *Stenonema* can be opportunistic in its feeding choices and feed on both allochthonous and autochthonous materials (Rosi-Marshall & Wallace, 2002; Collins et al., 2016), and the results here suggest that they will feed on either resource indiscriminately if both are available. While other studies have shown increased abundances of *Stenonema* with increasing algal biomass (Bumpers et al., 2017; Eckert et al., 2020) and higher growth on algae than leaf detritus (Webb & Merritt, 1987), algal biomass on light-conditioned leaves was lowest during the *Stenonema* experiment, and it may have been too low to or may not elicit a

preference response. Further, the higher variability in consumption on light-conditioned leaves may reflect differences in feeding on individual light-conditioned leaves that varied in algal biomass. These results suggest that *Stenonema* will feed on any leaf resource available, regardless of algal biomass, although the consumption of some algal biomass may be required for high growth rates (Webb & Merritt, 1987; Guo et al., 2016).

Lepidostoma also appears to be indiscriminate in preference for leaf-associated algae, as predicted, yet, similar to *Stenonema*, the highest consumption rates were measured on the light conditioned leaves. *Lepidostoma* may choose leaves not only as a food resource but also to build cases, and case-building using the leaves was observed during this study. Intriguingly, they also chose to sometimes include pieces of the labels on the pins into their cases but only from the light-conditioned leaves (green labels vs. orange labels). It is therefore unclear whether higher consumption rates and the greater number of arenas where consumption was higher on light-conditioned leaves may be due to actual consumption or to use of the leaves in cases. More experimentation to differentiate between feeding and case building preferences would provide greater insight into this dynamic. In other studies, *Lepidostoma* has also shown no preference for algal biomass, although algae have been found within its gut (Mayer & Likens, 1987), and carbon and nitrogen stable isotope signatures have indicated algal incorporation into its tissues even when guts were almost exclusively filled with leaf litter (Benstead & Pringle, 2004). The consumption measured here indicates that, although they will feed on both light- or dark-conditioned leaves, at least a small amount of algae may be important in their diet, similar to studies where small amounts of algae elicited disproportionate assimilation in shredders (e.g., Guo et al., 2016; Grieve & Lau, 2018).

Caecidotea also demonstrated no preference for light- or dark-conditioned leaves contrary to predictions, and similar consumption rates were found between both leaf treatments. In a growth study, *Caecidotea communis* exhibited higher growth and consumption on light-conditioned leaves likely harboring a higher quality diatom community compared to dark-conditioned leaves (Eckert, unpublished data). The isopod *Asellus aquaticus* also has shown increased growth when provided with algal resources along with detritus (Franken et al., 2005; Grieve & Lau, 2018), and small amounts of algae (i.e., 90:10 leaf:algae) have been shown to provide greatest growth with high incorporation of algal PUFAs into isopod tissues regardless of increases in algal availability (Grieve & Lau, 2018). Across arenas, about half showed higher consumption of light-conditioned leaves and half showed higher consumption of dark-conditioned leaves. This seemingly random feeding suggests that some algal biomass is likely to be consumed by chance, and given the small amounts of algal biomass necessary to support *A. aquaticus* growth, this may be enough to support growth of *Caecidotea* as well by, e.g., providing essential algal PUFAs (e.g., Brett & Müller-Navarra, 1997; Guo et al., 2016). Consumption of algae by *Caecidotea* may therefore be incidental, similar to the apparent feeding habits of *Lepidostoma*, and appears not to correspond to growth rates (Eckert, unpublished data), similar to the stonefly *Klapopteryx kuscheli* (Albariño et al., 2008).

Consumption rates of *Tipula* did not vary between light- and dark-conditioned leaves but there was a strong preference for dark-conditioned leaves, as predicted, with more of the dark-conditioned than light-conditioned leaves consumed in every arena. Gut studies have shown *Tipula* to generally have greater leaf or fungal matter in its gut than

algal material, regardless of algal availability (Dangles, 2002; Rosi-Marshall & Wallace, 2002; Eggert & Wallace, 2007), which suggests targeted consumption of low-algal biomass leaves. Further, *Tipula* were significantly more common within leaf packs containing lower algal biomass due to shading (Eckert et al., 2020). In the Eckert et al. (2020) study, this association could have been due to the shading structures to limit algal growth providing refuge rather than reflective of food choices. However, given the results here indicating strong preference for dark-conditioned leaves with minimal algal biomass, it is likely that colonization of low-algal biomass leaves was in fact related to feeding and not to behavioral choices. The preference for dark-conditioned leaves here in combination with previous studies indicate that *Tipula* actively feeds on leaf material with lower algal biomass.

General feeding preference responses

The accumulation of leaves in streams results in discrete but patchy food resources for macroinvertebrates, and these leaves may not be equal in quality within or between leaf packs resulting in differential colonization (e.g., Richardson, 1992; Palmer et al., 2000; Kobayashi & Kagaya, 2004; Eckert et al., 2020). Because of the patchy distribution and differential food quality of leaf packs, macroinvertebrates must be able to locate and determine the quality of a given resource. However, spending more time searching for high-quality resources that are further apart can increase energy costs and predation risk (e.g., Schoener et al., 1971; Arsuffi & Suberkropp, 1989). Macroinvertebrates may therefore limit movement depending upon the availability of adequate food and the threat of predators (e.g., Kohler & McPeek, 1989; Haddaway et al., 2014). For shredders, this can relate to selectivity amongst leaves (e.g., Arsuffi &

Suberkropp, 1989). Here, leaf disks were arranged in two ways, one which required greater mobility and movement away from a refuge to find resources and one with clumped resources near a refuge to see whether consumption rates may vary across taxa when greater mobility is required to find food. While, as expected, *Stenonema* and *Caecidotea* showed no preferences for leaf arrangement as active macroinvertebrates, *Amphinemura*, *Tipula*, and *Lepidostoma* showed no preferences across these leaf distributions for the clumped distribution as predicted even though observations indicated they tend to be clustered within leaves, suggesting that this setup did not limit mobility or searching ability for them.

Leaf-specific factors can influence the feeding and growth of macroinvertebrates, and for shredders these include the presence of fungi, including different fungal species, on the leaf surface (e.g., Arsuffi & Suberkropp, 1985; Aβmann, et al., 2011); leaf species, driven by characteristics such as toughness or secondary defense compounds within the leaf (e.g., Graca, 2001; Swan & Palmer, 2006); nutrient content of the leaves, including carbon, nitrogen, phosphorus, and lignin (e.g., Gessner & Chauvet, 1994; Motomori et al., 2001; Hyadlz et al., 2009); and algae on the leaf surface (e.g., Franken et al., 2005; Albariño et al., 2008; Guo et al., 2016). These factors seem to differ in importance across species (e.g., Motomori et al., 2001) as well as temporally as resources change over time via greater conditioning (e.g., Hutchens et al., 1997) or availability (e.g., Fuller et al., 2015; Siders et al., 2018). For these leaves, many influences were kept constant by the use of water from one stream to seed the microbial communities and using the same leaf species collected from one location where leaves were exposed to similar conditions prior to abscission. There was significantly greater algal biomass on the light-conditioned

leaves providing the opportunity for responses to vary by leaf-associated algae. Algae likely impacted the nutritional quality of the leaves through providing essential PUFAs (Guo et al., 2016; Guo et al., 2018) or impacts on C:N:P and influences on the rest of the microbial community, including alterations of the fungal community (Rier et al., 2007; Kuehn et al., 2014; Halvorson et al., 2019a; Halvorson et al., 2019b), but this did not translate into preferential feeding. Although algal biomass differences were clear on leaves, only *Tipula* exhibited significant preferences, and this preference was for dark-conditioned leaves with lower algal biomass. For *Amphinemura*, *Stenonema*, *Lepidostoma*, and *Caecidotea*, algae are not preferred or avoided and appear to play no role in leaf feeding preferences.

Conclusions

In these experiments, I investigated whether four shredders and one scraper across five orders demonstrated preferences for feeding on light- versus dark-conditioned leaves or for the arrangement of leaves as clumped near a refuge or dispersed around an arena. No taxa exhibited responses to leaf arrangement, suggesting mobility did not limit their feeding behaviors. Only *Tipula* exhibited a significant response to leaf conditioning treatment, preferring to feed on dark-conditioned leaves, and these responses are likely due to preferences for lower algal biomass associated with leaves as suggested in other studies (e.g., Dangles, 2002; Rosi-Marshall & Wallace, 2002; Eggert & Wallace, 2007; Eckert et al., 2020). In contrast, *Amphinemura*, *Stenonema*, *Lepidostoma*, and *Caecidotea* exhibited no preferences for either leaf treatment. *Amphinemura* has not been found to have relationships with algae in other studies (e.g., Madsen, 1974; Clifford, et al., 1992; Dangles et al., 2002; Eckert, unpublished data), and these results support the lack of algal

importance to their feeding choices. *Stenonema*, *Lepidostoma*, and *Caecidotea* showed no preferences, but previous work has indicated algae may be important in their diet for growth (Mayer & Likens, 1987; Webb & Merritt, 1987; Benstead & Pringle, 2004; Franken et al., 2005; Bumpers et al., 2017; Grieve & Lau, 2018). For these taxa, algal consumption can occur incidentally while consuming leaves, and small amounts of algae may be sufficient to support growth, similar to studies finding small amounts of algae are disproportionately assimilated by macroinvertebrates (Guo et al., 2016; Grieve & Lau, 2018); these taxa would therefore not need to detect algal presence in selecting food. Natural streams are inherently heterogenous in many aspects, including in food resources such as leaves. In temperate headwater streams, organic matter tends to accumulate in certain areas which can be exposed to higher or lower light levels. These changes in light can alter the algal biomass and community on the leaves, providing differential food resources to macroinvertebrates affecting their colonization (Eckert et al., 2020). Anthropogenic changes to streams such as channelization, riparian alterations like clear-cutting, and flow regime changes alter this natural stream heterogeneity (e.g., Palmer et al., 2014; Palmer & Ruhi, 2019). Stream restoration often seeks to restore natural stream heterogeneity, but this does not promote the return of macroinvertebrate communities (Palmer et al., 2010). These results indicate that algal biomass on leaves can be an important negative factor for *Tipula*, and it may also impact other taxa not tested here (Eckert et al., 2020). As such, when restoring a stream, food resources, including leaves and algae that may colonize those leaves, should also be considered in order to promote the greatest biodiversity within the stream.

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Chapter 4 – Conclusions, future directions, and implications

Traditional stream paradigms characterize temperate headwater streams as brown food web dominated, with decomposition of allochthonous material (e.g., leaf detritus) serving as a primary energy source (Vannote et al., 1980; Abelho, 2001). Leaf detritus is colonized by fungal and bacterial decomposers after entering streams, and it is this matrix of heterotrophic microbes and leaf material that is consumed by macroinvertebrate shredders (Cummins & Klug, 1979; Abelho, 2001, Graca, 2001), with shredders preferring the microbial "peanut butter" to the leaf "cracker" (Cummins, 1974). This paradigm was established because low light levels in headwater streams limit primary production (Abelho, 2001; Richardson, 2019); researchers have, however, described diatoms on collected leaves (Suberkropp & Klug, 1974), compared between algal and fungal colonization of wood, leaves, and glass slides (Golladay & Sinsabaugh, 1991; Sinsabaugh et al. 1991) and found positive correlations with macroinvertebrate abundance and leaf-associated algal but not fungal biomass (Hax & Golladay, 1993). Further, shredder preference studies have indicated fresh algae are preferred food alongside conditioned leaf tissue for some macroinvertebrate shredders (e.g., Friberg & Jacobsen, 1994; Leberfinger & Bohman, 2010), and algae on leaves can promote shredder growth (Franken et al., 2005; Guo et al., 2016). Although these studies supported a role for leaf-associated algae, it remained understudied within headwater streams until recently.

In 2010, Guenet et al. proposed that algae might prime stream leaf decomposition, sparking new research into the role of leaf-associated algae. These studies have supported

the premise that algae, although typically a minor portion of microbial biomass associated with leaves, can affect various aspects of headwater stream dynamics including fungal and bacterial biomass and production (e.g., Rier et al., 2007, Halvorson et al., 2019a), decomposition rates (e.g., Danger et al., 2013; Halvorson et al., 2019a), and leaf stoichiometry (e.g., Halvorson et al., 2019b; Eckert et al., 2020). Algae also provide nutrition in the form of essential polyunsaturated fatty acids (PUFAs) such as ω3s like eicosapentaenoic acid (EPA; 20:5ω3) and docosahexaenoic acid (DHA; 22:6ω3) which support macroinvertebrate growth (e.g., Guo et al., 2016; Grieve & Lau, 2018; Guo et al., 2018). This influx of research has focused mainly on relationships between microbial leaf colonizers, especially fungi and algae, with some limited work on the incorporation of algal PUFAs into macroinvertebrates and their effects on growth (Guo et al., 2016; Grieve & Lau, 2018), and has primarily been conducted in the laboratory or mesocosms (Bengtsson et al., 2018).

Research is still lacking on how leaf-associated algae may impact macroinvertebrate assemblages associated with leaves in headwater streams, and there is limited information regarding effects on growth and preference across taxa. I therefore sought to investigate how leaf-associated algae impact macroinvertebrate assemblages within natural streams and to compare taxon growth and preferences. I conducted preliminary experiments and observations in the field in the development of hypotheses and experiments including examinations of algae colonizing leaves, measurement of algae on leaves and the variation between samples, the spatial variation of algae within one leaf pack, and general observations of macroinvertebrate movement and behavior within streams. Observations of leaf-associated algae from leaves incubated in a

Piedmont headwater stream in spring of 2015 indicated abundant algal colonization mainly comprised of diatoms with some cyanobacteria and green algae. Diatoms included *Nitzschia, Ulnaria, Meridion, Fragilaria, Navicula, Cymbella, Gomphoneis,* and *Aulacoseira.* Paired fine- and coarse-mesh leaf packs were incubated in four streams of differing physiographic provinces and nutrient content in winter 2015-2016 to test algal procedures, examine colonizing macroinvertebrates, and compare variation in measures between replicates. Rocks were also collected to compare leaf-associated algae with epilithon. Variances were used in power analyses for future replication. Results indicated measurable algal biomass on leaves as chlorophyll-a, although algal biomass was significantly greater on rocks than on leaves from either coarse- or fine-mesh leaf packs (Figure 4.1; one-way ANOVA: $F_{2,89}=247.5$, $p<0.001$; Tukey's HSD, rock vs. coarse and rock vs. fine: both $p<0.001$). In August of 2016, algal variation within leaf packs was measured by incubating leaf packs under both open and closed canopy conditions in a Piedmont stream. Leaf disks were taken from the top, middle, and bottom of the leaf pack for chlorophyll-a measurements. Two-way ANOVA indicated no differences between canopy coverage ($F_{1,22}=1.900$, $p=0.182$) but significant differences between locations within the leaf pack ($F_{2,22}=5.451$, $p=0.012$) with the top having significantly greater biomass than the middle (Tukey's HSD: $p=0.011$) and marginally greater biomass than the bottom (Tukey's HSD: $p=0.068$) (Figure 4.2).

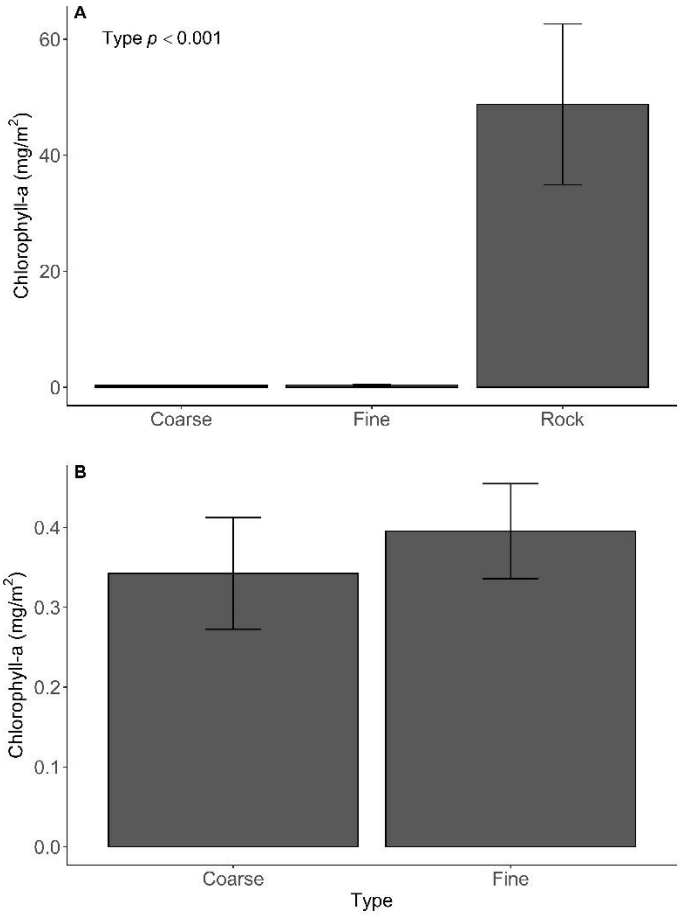

Figure 4.1. Average algal biomass measured on leaves from coarse and fine leaf packs (A, B) and on rocks (A) collected in four streams during a preliminary experiment.

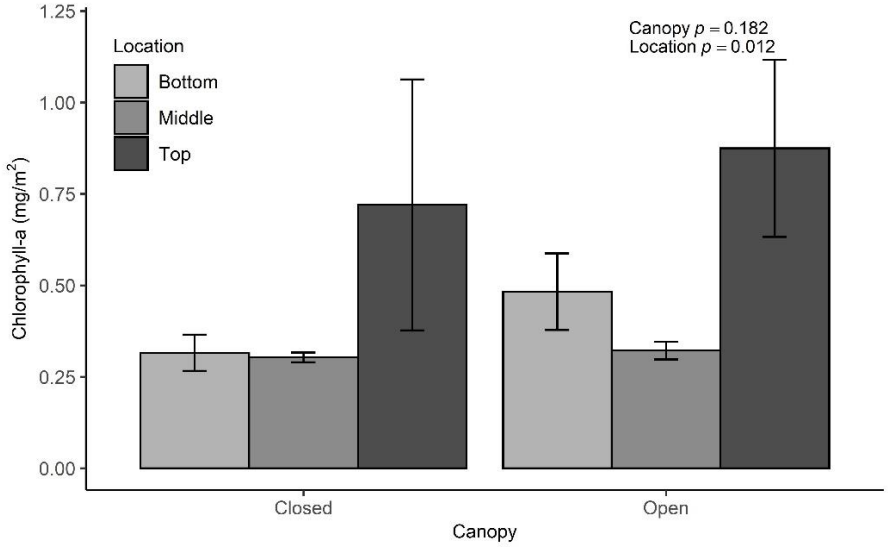

Figure 4.2. Algal biomass measured on leaves taken from the bottom, middle, and top of leaf packs incubated under open and closed canopy conditions.

Based upon the literature and these preliminary experiments and observations, I therefore hypothesized that leaf-associated algae in temperate headwater streams are important for macroinvertebrates and would have an impact on their colonization, growth, and food preferences. Specifically, I hypothesized that leaf-associated algae on leaves would impact macroinvertebrates assemblages colonizing leaf packs through changes to leaf characteristics, and that these differences would be reflected in functional feeding guild differences and the responses of specific taxa (Chapter 1). Secondly, I hypothesized that leaf-associated algae would promote the growth of macroinvertebrates feeding on leaves conditioned in the light and dark to alter the microbial community (Chapter 2). Lastly, I hypothesized that leaf-associated algae would affect the feeding preferences of different taxa of macroinvertebrates, but that these responses would be taxon specific (Chapter 3).

In Chapter 1, I carried out a manipulative light experiment in low- and high-nutrient streams to test my hypotheses regarding leaf-associated algae's impact on leaf characteristics and how these leaf characteristics in turn affected macroinvertebrate assemblages colonizing leaves in winter, when shredders are highly active, and spring, when algal biomass peaks. Leaf-associated algal biomass was greater under ambient-light conditions and in high-nutrient streams. In support of previous work, fungi and algae were positively correlated in winter (Rier et al., 2007; Kuehn et al., 2014). Further, both microbes impacted leaf stoichiometry, but there were seasonal differences. Leaf C:N was negatively correlated to fungi in both seasons and algae in winter. N:P and C:P were negatively correlated to fungi in winter and algae in spring. These influences on leaf characteristics indicate that there may be seasonal differences in nutritional importance of

different portions of the microbial community for macroinvertebrates. Macroinvertebrate diversity did not differ across factor combinations, but taxonomic differences were apparent, which may be due to differences in feeding requirements including feeding to meet stoichiometric needs versus feeding on specific members of the microbial community. Models investigating leaf characteristics across seasons and functional feeding guilds supported these differences. Common taxa exhibited trends in relation to algal biomass. *Stenonema* and *Ephemerella* mayflies were more abundant with greater algal biomass. In contrast, *Tipula* was more common where algal biomass was lower. In sum, following predictions, algal biomass associated with leaves impacted the macroinvertebrate assemblage within leaf packs through alterations to leaf characteristics and effects on specific taxa.

In Chapter 2, I conducted growth experiments in the laboratory on *Caecidotea communis*, a shredder, and *Ephemerella invaria*, a collector-gatherer, using leaves incubated in light or dark conditions to test whether leaf-associated algae promoted macroinvertebrate growth. *C. communis* is common in streams in the area and was abundant in leaf packs in preliminary experiments. *E. invaria* is also common in streams in the area and, given its significant correlation to leaf-associated algal biomass in Chapter 1, expected to respond to algal biomass. Leaf mass and algal biomass decreased due to consumption by both macroinvertebrates. *C. communis* consumed more leaf mass and algal biomass and had higher growth rates on light-conditioned leaves, although they were primarily assimilating an unmeasured carbon source. Stable isotope measurements of the leaves indicated that the microbial community differed between leaf treatments, and previous studies suggest the light-conditioned leaves may have harbored a

community comprised of greater diatom abundance compared to greater lower quality algae (e.g., cyanobacteria) on dark-conditioned leaves. *E. invaria* generally consumed similar amounts and grew at the same rate on both leaf treatments, although greater area was consumed of light-conditioned leaves. Stable isotope analysis indicated they assimilated both leaf and algal material in similar amounts in both leaf treatments. Previous work has indicated that at least small amounts of high-quality algae, e.g., diatoms, are required to support growth of macroinvertebrates by providing essential PUFAs (e.g., Guo et al. 2016; Grieve & Lau, 2018), and it appears that even the small amounts of algae available here was sufficient. These results were only partially in line with predictions, as *C. communis* grew better on light-conditioned leaves, but there were no differences in growth for *E. invaria*, although they assimilated algae from both leaf treatments. Additionally, it is not clear what aspect of the light-conditioned leaves supported higher growth in *C. communis* as algal community analysis was not performed and there is a missing carbon source(s).

In Chapter 3, to test predictions regarding leaf-associated algae on food preferences, I conducted laboratory feeding preference studies on four shredder and one scraper macroinvertebrate taxa for light- and dark-conditioned leaves, where light-conditioned leaves had significantly greater algal biomass, and for a clumped or dispersed leaf arrangement, examining effects of mobility on feeding based upon observations in the field. These taxa were common taxa found in leaf packs in my previous work. *Caecidotea communis*, as mentioned above, is common in leaf packs and grew significantly more on light-conditioned leaves in Chapter 2 and tends to actively move around on and off of collected leaves. *Amphinemura* sp. and *Lepidostoma* sp. were

abundant within leaf packs in Chapter 1 but did not show a relationship to algal biomass and tend to stay within collected leaves. *Tipula* sp. was abundant and showed a negative relationship to algae in Chapter 1 and tends to stay within collected leaves. *Stenonema* sp., the only scraper tested, was significantly more abundant with greater algal biomass in Chapter 1 and tends to actively move around on and off collected leaves. No tested taxon showed a preference for leaf arrangement or greater overall consumption of light- or dark-conditioned leaves, but Friedman tests indicated *Tipula* sp. fed more on dark-conditioned leaves in every arena, indicating a preference for lower algal biomass in line with results in Chapter 1. These results only partially support my hypotheses; *Tipula* sp. exhibited a strong preference but no other taxa had a preference for leaf treatment. Additionally, only some followed predictions for leaf arrangement (*Caecidotea communis*, *Stenonema* sp.). These results indicated that within this experimental setup, all taxa were equally mobile. Further, while algae appear to be important as a deterrent for feeding by *Tipula* sp., other tested taxa showed no responses. For these other taxa, although algae can support growth (e.g., *Caecidotea communis*), it may not be necessary for them to distinguish between high- and low-algal leaf resources in order to obtain enough essential nutrition to support growth, as only small amounts may be required (e.g., Grieve & Lau, 2018).

These experiments naturally lead to further questions. While more about the algae-fungi relationship on leaves continues to be explored, less is known about the algae-bacteria relationship in the field, although previous literature would suggest that this relationship is positive (e.g., Rier et al., 2007; Kuehn et al. 2014; Halvorson et al., 2019a). Bacteria were not measured in Chapter 1, so it is not possible to obtain an

estimate of carbon contribution to total leaf carbon, but estimates from Chapter 1 indicate that algal carbon compared to fungal carbon comprised on average about 6.6% of measured microbial carbon and was greatest under high-nutrient and ambient-light conditions in both seasons (~11%; Appendix V). Previous studies have shown that while bacteria comprise only <1-5% of heterotrophic biomass associated with leaves, they can have a disproportionate effect on leaf decomposition, contributing about 5-15% towards leaf litter loss, although this is less than fungal contributions (Hieber & Gessner, 2002; Pascoal & Cássio, 2004; Gulis & Bärlocher, 2017). These carbon estimates indicate that algal carbon contributions are similar to or higher than bacteria, but more work is needed to determine how this relates to leaf litter decomposition and its relative importance to macroinvertebrates. Teasing apart microbial effects on macroinvertebrates by isolating different microbial components can provide greater insights into leaf decomposition and the specifics of colonization and feeding responses in relation to fungi, bacteria, and algae and into, e.g., the response of *Caecidotea communis* measured here.

More work is also needed to better characterize the algal community colonizing leaves as little is currently known. Interactions between algae and fungi are more likely on leaves than they are on, e.g., epilithic surfaces where less fungi tend to colonize (Sinsabaugh et al., 1991), and there may therefore be competition or facilitation affecting community composition of both groups. Since macroinvertebrates can actively differentiate between different microbes, such as between fungal species (Arsuffi & Suberkropp, 1985; Aβmann et al., 2011), the specifics of the community composition may elucidate differences in preference, growth, and colonization. While laboratory tests to continue to tease these variables apart are invaluable, more field tests are also needed

to confirm laboratory results and better understand relationships within the complexity of natural conditions.

The experiments conducted here provided insights into leaf-associated algae and macroinvertebrate dynamics. Although traditional headwater stream paradigms characterize temperate headwater streams as primarily supported by brown food webs (Vannote et al., 1980; Abelho 2001), these results support the interconnectedness of green and brown food webs within leaf detritus. Although leaf-associated algal biomass is lower than epilithic algal biomass, it is enough to affect macroinvertebrate leaf pack colonization, feeding preference, and growth, and so, while questions remain, it should not be disregarded in temperate headwater streams. Indeed, as the fungal/bacterial "peanut butter" has been shown to be more important than the leaf "cracker" for macroinvertebrates, algal "jelly" also influences their choices. Leaf-associated algae-macroinvertebrate interactions vary by taxon and can be positive, neutral, or negative. Algae deter *Tipula*, which are more likely to be present within leaves in a stream where algae are less abundant. For *C. communis*, algal presence may not affect preference, but the community on a leaf surface may impact its growth over the long term. Although the exact algal community composition and the main carbon source are not known within Chapter 2, it appears that microbial differences can impact *C. communis* growth, even when they do not selectively choose to feed on leaves with greater algal biomass. Ephemerellid mayflies, including *E. invaria*, colonized leaves with greater algal biomass; their growth was not, however, impacted by differential leaf conditioning, and they are capable of feeding and growing on both leaf and algal material. Other common taxa did not show specific colonization relationships or exhibit preferences for leaves with higher

or lower algal biomass. Responses therefore span a large range, suggesting heterogeneity within streams may be important in supporting diverse macroinvertebrate communities as related to leaf-associated algal biomass in addition to factors typically considered such as differences in flow, substrate texture and size, and distribution of habitat patches (Figure 4.3; e.g., Mackay, 1992; Palmer et al., 2000; Boyero, 2003).

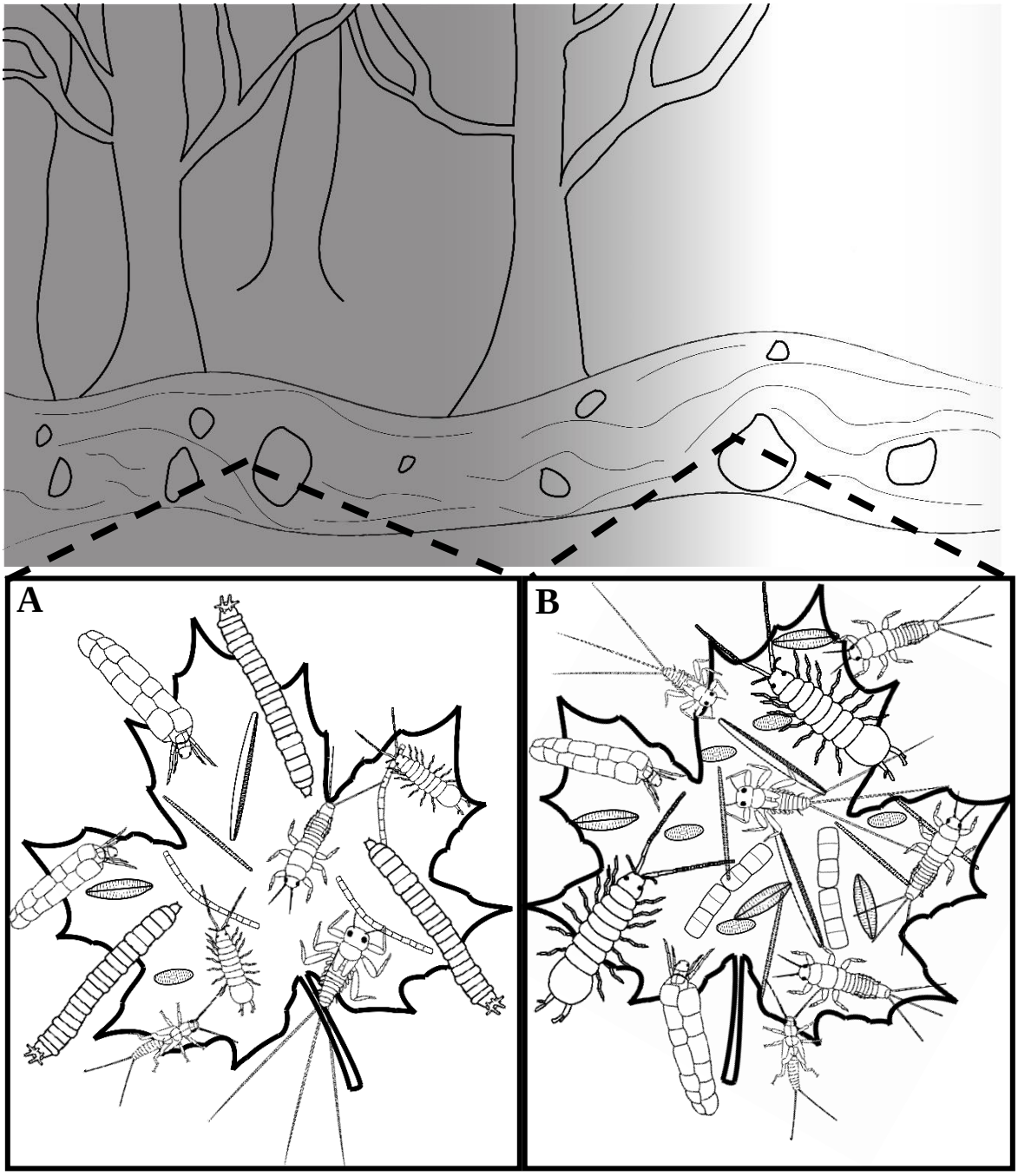

Figure 4.3. Headwater stream canopy cover alters light availability which alters leaf-associated algae. Greater light availability increases algal biomass and proportion of diatoms (B). Macroinvertebrates respond to algal biomass. Some prefer lower algal biomass (e.g., *Tipula*) (A). Others are more abundant with higher algal biomass (e.g., *Stenonema*, *Ephemerella*) or grow better on light-conditioned leaves (*Caecidotea communis*) (B). Some exhibit no preferences and are equally common between leaves (e.g., *Lepidostoma*, *Amphinemura*) (A, B). Nutrient availability can further modify these relationships, and differences between leaf microbial communities support whole-stream macroinvertebrate diversity associated with leaves.

These results also indicate that nutrients and light availability alter leaf-associated biomass, both of which are altered by anthropogenic factors degrading streams (Dudgeon et al., 2006). For instance, clearing of riparian trees by, e.g., logging, increases light availability within a stream and promotes algal growth (e.g., Minshall, 1978; Kiffney et al., 2004; Hill et al., 2009). This also impacts the availability of leaf detritus within the stream (e.g., Reid et al., 2008), and changes in riparian species and coverage can affect both microbes and macroinvertebrates (Kiffney et al., 2004; Lecerf et al., 2005; Swan & Palmer, 2006). Nutrient runoff similarly affects algal biomass, with greater nutrient availability promoting algal growth (Smith et al., 1999; Dodds et al., 2002). Nutrients also promote heterotrophic biomass increases (Connolly & Pearson, 2013) and increase leaf decomposition by both microbes and macroinvertebrates (e.g., Rosemond et al., 2015). Stream restoration endeavors to return a stream to its prior physical, chemical, and biological conditions, by reversing changes in, e.g., light and nutrient availability. Restoration has largely focused on restoring heterogeneity within streams by, e.g., adding woody debris and boulders, replanting riparia, and reducing runoff (Palmer et al., 2014), but this has generally not been successful in restoring macroinvertebrate assemblages in streams (Palmer et al., 2010; Palmer et al., 2014). Greater consideration should be given to how changes in light and nutrient availability might affect the distribution of leaf-associated algae with resultant direct and indirect effects on macroinvertebrate assemblages. Considering leaf-associated algae in combination with other factors can provide a more holistic approach to restoration that may prove more successful in supporting macroinvertebrate diversity in headwater streams.

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Appendices

Appendix I.

Correlation plots, stream characteristics, average values measured in leaf packs, and full GAMM model results for Chapter 1: Macroinvertebrate community patterns in relation to leaf-associated periphyton under contrasting light and nutrient conditions in headwater streams.

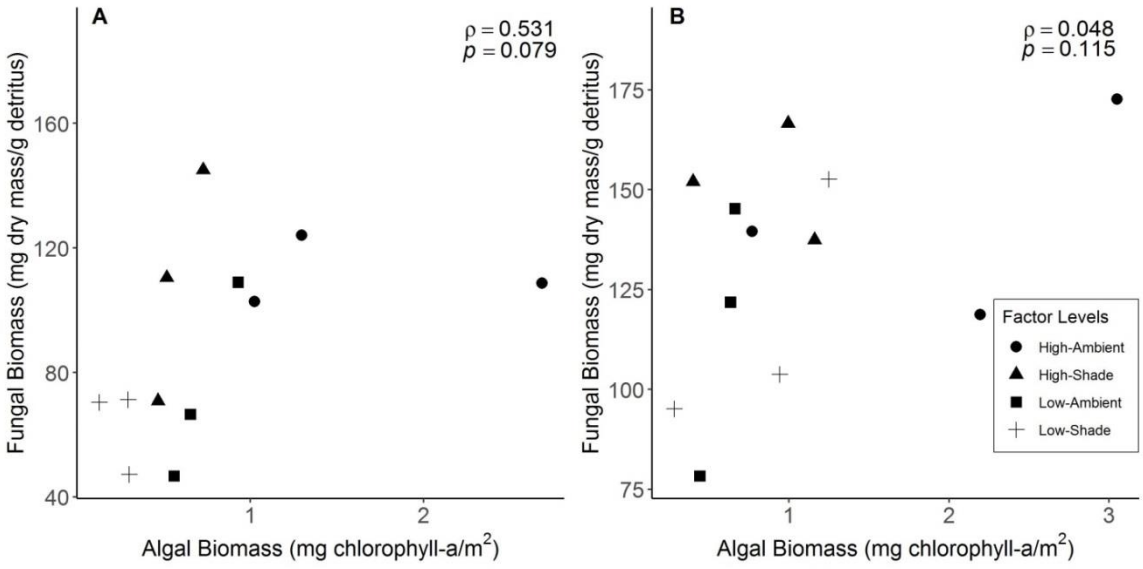

Figure I.1 Spearman correlations (ρ) and associated *p*-values (*p*) between algal (chlorophyll-a) and fungal (ergosterol) biomass measured from leaves incubated in streams of high and low nutrient concentrations and under either ambient light or shaded conditions. (A) Spearman correlation in the winter. (B) Spearman correlation in the spring.

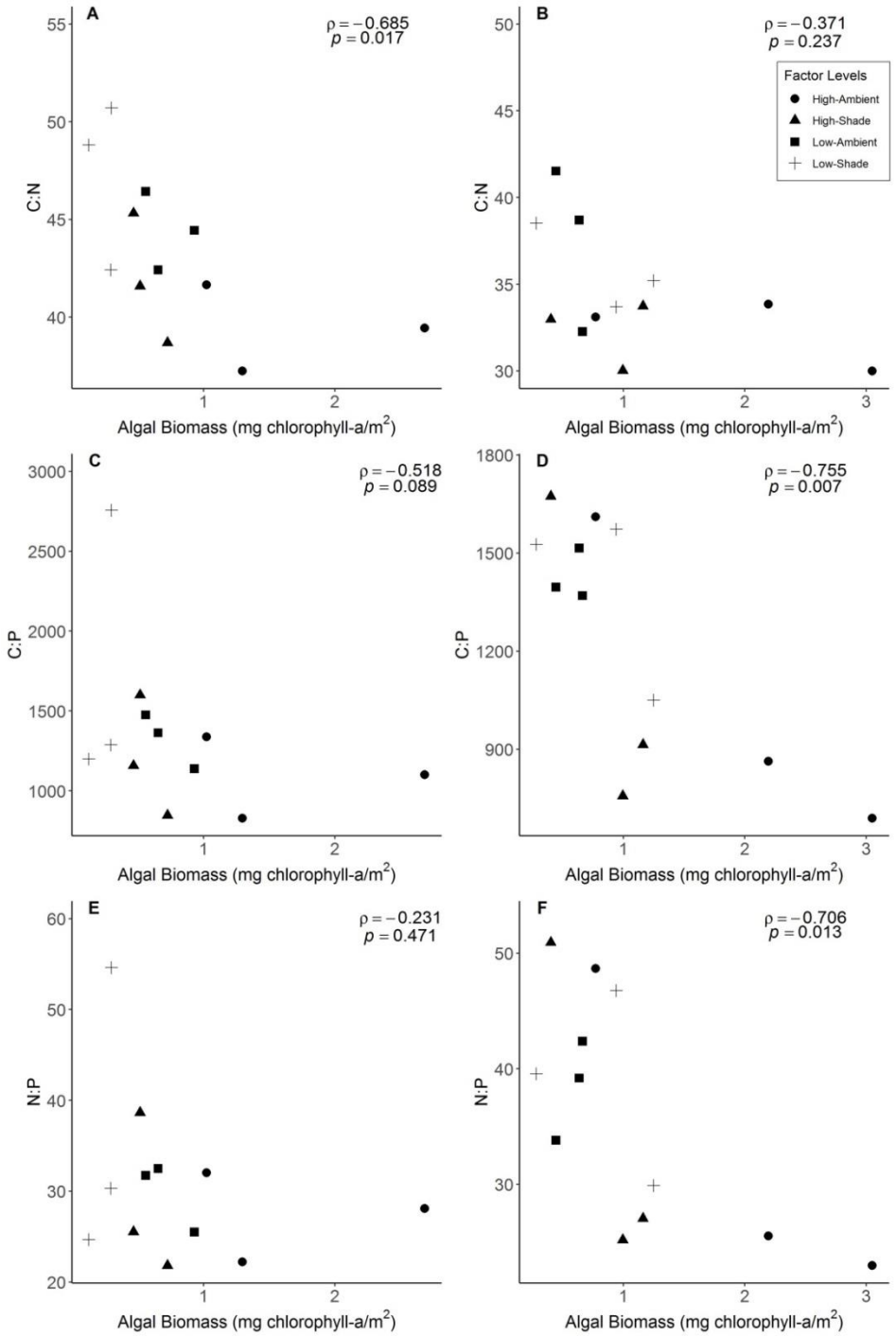

Figure I.2. Spearman correlations (ρ) and associated *p*-values (*p*) between leaf stoichiometric ratios and algal (chlorophyll-a) biomass (mg chlorophyll-a/m^2 leaf surface) measured from leaves incubated in winter (left) and spring (right) in streams of high and low nutrient concentrations and under either ambient light or shaded conditions. (A, B) Spearman correlation between leaf C:N and algal biomass. (C, D) Spearman correlation between leaf C:P and algal biomass. (E, F) Spearman correlation between leaf N:P and algal biomass.

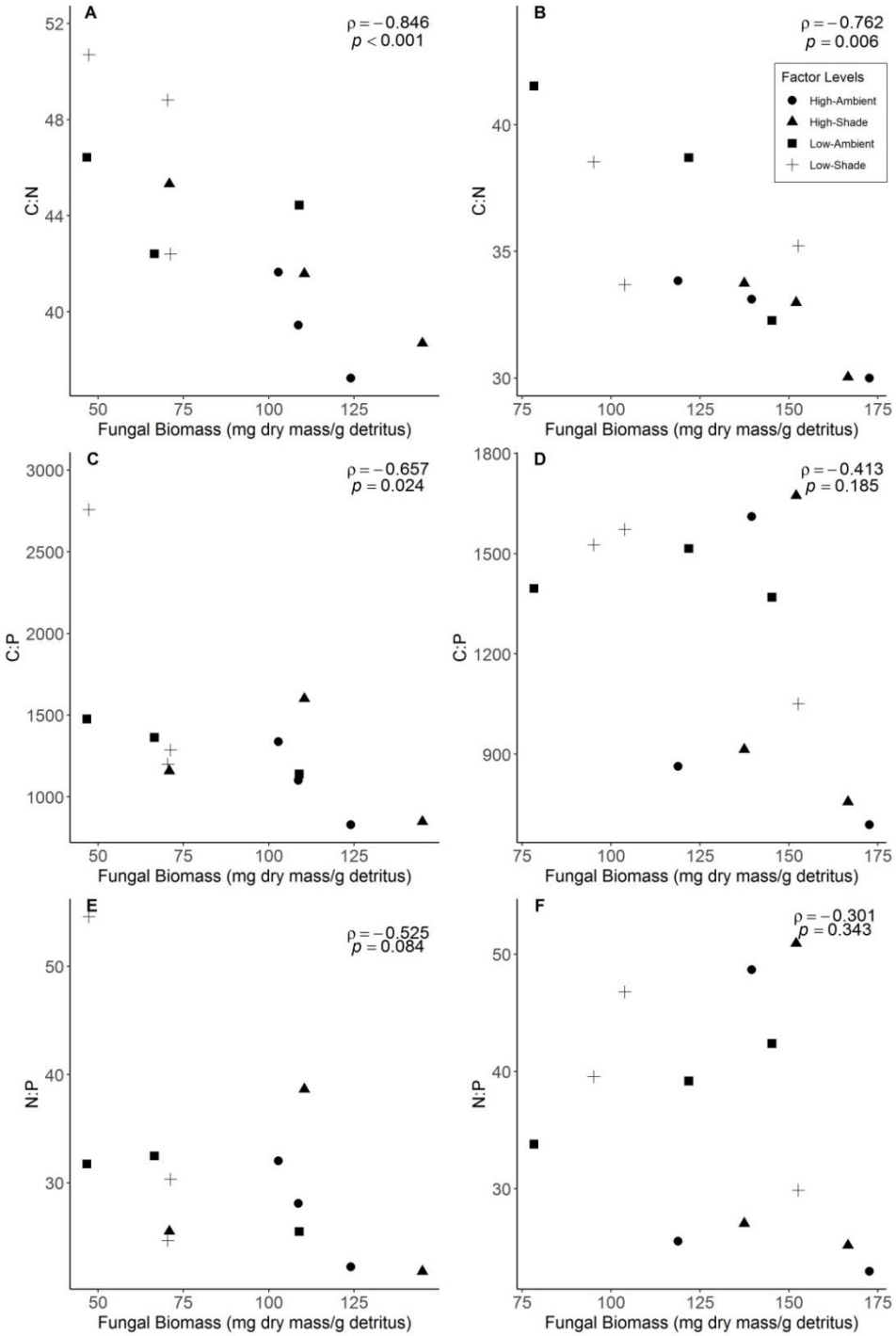

Figure I.3. Spearman correlations (ρ) and associated *p*-values (*p*) between leaf stoichiometric ratios and fungal (ergosterol) biomass (mg dry fungal mass/g leaf detritus) measured from leaves incubated in winter (left) and spring (right) in streams of high and low nutrient concentrations and under either ambient light or shaded conditions. (A, B) Spearman correlation between leaf C:N and fungal biomass. (C, D) Spearman correlation between leaf C:P and fungal biomass. (E, F) Spearman correlation between leaf N:P and fungal biomass.

Table I.1. Stream characteristics during winter experimental period. Values represent means† ± SEM. Values are provided for each stream along with their nutrient classification as well as on average for the low- and high-nutrient streams.

	Stream	Dissolved Oxygen (mg/L)	Temp§ (°C)	Specific Conductivity (µS/cm)	pH	Depth (cm)	Width (cm)	Flow (m/s)	Canopy Cover (%)	TP‡ (mg/L)	SRP‡ (mg/L)	TN‡ (mg/L)	NO_3^-‡ (mg/L)
Low	Browning Run	10.59 ±0.22	6.77 ±0.14	83.35 ±1.78	5.77 ±0.06	3.58 ±0.88	75.33 ±10.22	0.08 ±0.03	26.66 ±4.28	0.005 ±0.020	0.053 ±0.052	0.47 ±0.18	0.37 ±0.00
	Zion Road	12.66 ±0.29	3.27 ±0.75	102.84 ±2.10	6.55 ±0.04	8.83 ±0.95	118.17 ±9.60	0.22 ±0.05	27.33 ±3.61	0.053 ±0.004	B.D.	0.86 ±0.10	0.53 ±0.11
	Fern Valley	11.58 ±0.30	4.13 ±1.28	27.58 ±0.36	6.05 ±0.03	6.33 ±0.92	119.17 ±8.32	0.09 ±0.02	31.93 ±2.47	0.093 ±0.020	B.D.	1.20 ±0.06	1.04 ±0.09
High	Tobacco Barn	11.24 ±0.23	6.83 ±0.54	78.82 ±0.78	6.23 ±0.02	4.50 ±0.22	62.58 ±1.70	0.15 ±0.02	23.60 ±1.84	0.029 ±0.068	B.D.	6.58 ±0.14	4.93 ±0.67
	South Stream	12.16 ±0.48	5.38 ±1.65	100.20 ±2.48	7.02 ±0.03	5.83 ±0.17	113.00 ±9.40	0.22 ±0.10	27.12 ±2.47	0.121 ±0.001	0.063 ±0.034	4.72 ±0.06	4.16 ±0.38
	Forest Stream	12.14 ±0.07	5.83 ±1.46	88.27 ±1.24	6.86 ±0.04	5.17 ±0.48	130.67 ±14.46	0.06 ±0.02	33.30 ±1.76	0.105 ±0.074	0.012 ±0.007	4.63 ±0.88	4.79 ±0.29
Average	Low Nutrient	11.61 ±0.56	4.72 ±1.05	71.26 ±22.55	6.12 ±0.23	6.25 ±1.52	104.22 ±14.45	0.13 ±0.05	28.64 ±1.66	0.05 ±0.02	0.005 ±0.023	0.84 ±0.36	0.65 ±0.13
	High Nutrient	11.84 ±0.30	6.02 ±0.43	89.09 ±6.19	6.70 ±0.24	5.17 ±0.38	102.08 ±20.40	0.14 ±0.05	28.01 ±2.84	0.08 ±0.03	0.021 ±0.015	5.31 ±1.13	4.62 ±0.26

† Measurements were taken at the beginning and end of each experiment at three locations in each stream, with the exception of nutrient concentrations which were measured at one location in the stream (middle of reach).

‡TP is total phosphorus in mg P/L, SRP is soluble reactive phosphorus in mg P/L, TN is total nitrogen in mg N/L, and NO_3^- is nitrite-nitrate in mg N/L.

§Temp= temperature

Table I.2. Stream characteristics during spring experimental period. Values represent means[†] ± SEM. Values are provided for each stream along with their nutrient classification as well as on average for the low- and high-nutrient streams.

	Stream	Dissolved Oxygen (mg/L)	Temp[§] (°C)	Specific Conductivity (µS/cm)	pH	Depth (cm)	Width (cm)	Flow (m/s)	Canopy Cover (%)	TP[‡] (mg/L)	SRP[‡] (mg/L)	TN[‡] (mg/L)	NO_3^-[‡] (mg/L)
Low	Browning Run	10.36 ±0.43	9.82 ±0.83	158.15 ±16.47	6.67 ±0.10	5.00 ±0.80	132.67 ±16.80	0.19 ±0.09	38.25 ±3.20	0.041 ±0.017	0.024 ±0.019	0.70 ±0.27	0.51 ±0.21
	Zion Road	10.80 ±0.72	11.67 ±1.77	190.12 ±0.18	6.75 ±0.09	8.21 ±1.24	111.50 ±13.51	0.24 ±0.06	32.28 ±2.22	0.055 ±0.007	B.D.	0.83 ±0.14	0.45 ±0.08
	Fern Valley	9.68 ±0.36	10.88 ±0.92	41.93 ±0.37	6.42 ±0.12	4.83 ±0.54	114.50 ±9.00	0.09 ±0.02	47.95 ±5.34	0.036 ±0.013	B.D.	1.19 ±0.01	0.93 ±0.00
High	Tobacco Barn	10.17 ±0.38	12.22 ±0.69	112.78 ±1.15	6.75 ±0.07	4.13 ±0.35	72.00 ±3.01	0.14 ±0.03	36.08 ±4.31	0.067 ±0.003	B.D.	5.99 ±0.02	4.82 ±0.47
	South Stream	10.35 ±0.30	11.88 ±0.56	149.35 ±2.75	7.17 ±0.02	6.21 ±0.49	95.17 ±5.57	0.11 ±0.02	34.18 ±5.28	0.070 ±0.031	0.060 ±0.010	3.38 ±0.86	4.14 ±0.31
	Forest Stream	11.62 ±0.39	12.32 ±0.39	137.07 ±0.39	7.13 ±0.05	4.17 ±0.35	109.67 ±8.89	0.07 ±0.02	45.14 ±5.17	0.062 ±0.025	0.010 ±0.045	4.19 ±0.08	4.10 ±0.23
Average	Low Nutrient	10.28 ±0.33	10.79 ±0.54	130.07 ±45.02	6.61 ±0.10	6.01 ±1.10	119.56 ±6.61	0.17 ±0.04	32.95 ±2.81	0.04 ±0.01	B.D.	0.91 ±0.12	0.63 ±0.11
	High Nutrient	10.71 ±0.45	12.14 ±0.13	133.07 ±10.74	7.02 ±0.13	4.83 ±0.69	92.28 ±10.97	0.10 ±0.02	30.15 ±1.81	0.07 ±0.02	0.022 ±0.017	4.52 ±0.54	4.35 ±0.22

† Measurements were taken at the beginning and end of each experiment at three locations in each stream, with the exception of nutrient concentrations which were measured at one location in the stream (middle of reach).

‡TP is total phosphorus in mg P/L, SRP is soluble reactive phosphorus in mg P/L, TN is total nitrogen in mg N/L, and NO_3^- is nitrite-nitrate in mg N/L.

§Temp= temperature

Table I.3. Measured leaf pack variables incubated in winter and spring in streams of low- and high-nutrient concentrations under either ambient- or shaded-light treatments.

Factor†	Winter				Spring			
	Low Nutrient-Ambient	Low Nutrient-Shaded	High Nutrient-Ambient	High Nutrient-Shaded	Low Nutrient-Ambient	Low Nutrient-Shaded	High Nutrient-Ambient	High Nutrient-Shaded
Algal Biomass (mg chl-a/m^2)	0.72 ±0.17	0.24 ±0.03	1.67 ±0.31	0.57 ±0.15	0.58 ±0.12	0.82 ±0.26	2.00 ±0.39	0.85 ±0.12
Percent Diatoms	90.06 ±1.11	83.55 ±1.87	93.11 ±1.76	86.26 ±0.84	91.05 ±1.13	87.42 ±1.74	94.67 ±0.86	91.40 ±0.92
Fungal Biomass (mg DM/g detritus)	74.06 ±8.52	62.97 ±5.71	111.86 ±6.12	108.79 ±11.36	115.13 ±13.08	118.14 ±13.96	143.67 ±8.17	152.03 ±7.18
C:N	44.43 ±0.92	47.31 ±1.11	39.44 ±0.68	41.86 ±1.23	37.50 ±1.51	35.96 ±0.78	32.32 ±0.51	32.25 ±0.63
C:P	1326.09 ±66.50	1748.04 ±216.13	1089.35 ±65.99	1201.98 ±95.91	1426.87 ±64.67	1369.74 ±102.23	1054.44 ±114.21	1114.98 ±111.52
N:P	29.91 ±1.49	36.53 ±4.06	27.46 ±1.37	28.66 ±2.18	38.46 ±1.83	38.17 ±2.82	32.39 ±3.33	34.38 ±3.30
Macroinvertebrate Abundance	28.13 ±6.30	42.73 ±5.69	56.40 ±10.75	49.07 ±8.69	75.53 ±15.26	97.73 ±18.47	99.47 ±12.01	73.33 ±9.38
Macroinvertebrate Biomass	34.16 ±11.56	32.91 ±11.73	44.86 ±8.07	53.83 ±9.15	30.46 ±6.31	55.34 ±14.60	78.94 ±16.12	93.33 ±11.03
Macroinvertebrate Diversity	6.74 ±0.45	6.29 ±0.64	6.00 ±0.43	6.14 ±0.43	7.35 ±0.62	7.31 ±0.47	8.01 ±0.32	9.18 ±0.54
Macroinvertebrate Taxa Richness	9.20 ±0.73	9.87 ±0.80	11.87 ±1.01	10.80 ±0.78	13.20 ±1.39	14.53 ±0.94	14.20 ±0.55	14.73 ±0.74
Functional Feeding Guild Diversity	2.94 ±0.21	2.58 ±0.18	2.78 ±0.14	3.19 ±0.20	2.49 ±0.19	2.77 ±0.19	2.59 ±0.07	3.06 ±0.18
Collector-filterer Abundance	1.47 ±0.57	4.33 ±1.23	3.27 ±1.27	7.07 ±2.32	1.40 ±0.51	4.60 ±1.44	1.33 ±0.19	4.00 ±1.02
Collector-gatherer Abundance	13.93 ±2.80	27.13 ±4.37	34.20 ±8.71	22.53 ±6.89	41.40 ±9.92	51.40 ±14.80	56.07 ±6.22	32.53 ±4.47
Shredder Abundance	4.67 ±2.20	3.80 ±1.32	3.60 ±0.57	4.00 ±0.75	5.33 ±1.67	11.47 ±3.24	11.93 ±3.44	11.67 ±3.32
Scraper Abundance	2.73 ±0.98	1.80 ±1.03	1.33 ±0.54	0.67 ±0.25	3.73 ±1.33	1.27 ±0.52	1.00 ±0.34	0.87 ±0.47
Predator Abundance	3.47 ±1.10	4.27 ±1.04	11.40 ±2.36	13.47 ±3.21	15.33 ±2.77	23.40 ±6.15	21.40 ±3.32	18.13 ±3.94
Collector-filterer Biomass	0.50 ±0.35	1.52 ±0.62	1.60 ±0.52	2.41 ±0.59	1.71 ±1.03	2.40 ±0.99	1.63 ±0.36	3.67 ±1.20
Collector-gatherer Biomass	3.16 ±1.52	2.37 ±0.40	10.59 ±2.78	3.16 ±0.85	7.66 ±1.69	5.22 ±0.90	13.97 ±2.87	7.15 ±1.44
Scraper Biomass	2.15 ±1.23	2.76 ±1.49	0.59 ±0.30	1.26 ±0.79	8.56 ±3.80	0.76 ±0.39	1.42 ±0.96	0.84 ±0.53
Shredder Biomass	25.34 ±11.18	23.63 ±11.36	19.38 ±4.96	30.06 ±6.96	3.35 ±1.98	30.58 ±11.36	14.13 ±8.80	23.24 ±8.80
Predator Biomass	2.67 ±1.22	2.55 ±0.90	12.31 ±3.07	16.92 ±4.39	8.96 ±2.19	16.37 ±4.47	47.60 ±14.29	58.38 ±11.27

†Percent diatoms was estimated from a subset of leaf packs. Algal biomass is in units of mg chlorophyll-a per m^2, while fungal biomass is in units of mg dry mass per g detritus Macroinvertebrate biomass, both total and by functional feeding guild, is in mg dry mass. Macroinvertebrate abundance, both total and by functional feeding guild, is in individuals per leaf pack. Values represent means (± SEM).

Appendix II. Average algal biomass values on background leaves and on leaves within flasks, consumption values by week, and average stable isotope values for the growth experiments in Chapter 2: Contribution of leaf-associated algae to growth of a shredder, *Caecidotea communis*, and a collector-gatherer, *Ephemerella invaria*.

Table II.1. Algal biomass (as chlorophyll-a; mg/m^2) on leaves from conditioning chambers (background) across weeks, from the first day of the experiment (week 0) to the last day of the experiment (week 4) for both experiments, listed by species tested. Values represent mean±SEM.

Species	Leaf Treatment†	Week 0	Week 1	Week 2	Week 3	Week 4
Caecidotea communis	Light	0.118±0.011 n=3	0.143±0.031 n=3	0.167±0.119 n=3	0.341±0.106 n=3	0.089±0.038 n=3
	Dark	0.123±0.069 n=3	0.183±0.093 n=3	0.135±0.021 n=3	0.059±0.017 n=3	0.317±0.250 n=3
Ephemerella invaria	Light	0.120±0.036 n=3	0.120±0.056 n=3	0.110±0.014 n=3	0.064±0.002 n=3	0.082±0.017 n=3
	Dark	0.140±0.016 n=3	0.072±0.032 n=3	0.220±0.164 n=3	0.088±0.024 n=3	0.108±0.036 n=3

†Leaf treatment refers to leaves conditioned under ambient light (light) or completely covered (dark).

Table II.2. Algal biomass on leaves (as chlorophyll-a; mg/m^2) measured each week within the flasks during both experiments. Values represent mean±SEM.

Species	Leaf †	Macro ‡	Week 1	Week 2	Week 3	Week 4
Caecidotea communis	Light	No	0.164±0.112 n=4	0.169±0.033 n=3	0.119±0.038 n=4	0.242±0.025 n=3
		Yes	0.176±0.062 n=8	0.107±0.017 n=8	0.160±0.046 n=7	0.106±0.029 n=8
	Dark	No	0.225±0.023 n=4	0.604±0.205 n=4	0.206±0.057 n=5	0.202±0.064 n=5
		Yes	0.137±0.043 n=8	0.266±0.119 n=8	0.113±0.031 n=6	0.170±0.033 n=6
Ephemerella invaria	Light	No	0.094±0.037 n=4	0.100±0.021 n=7	0.095±0.009 n=9	0.106±0.012 n=9
		Yes	0.158±0.014 n=8	0.109±0.025 n=5	0.042±0.002 n=3	0.059±0.008 n=4
	Dark	No	0.076±0.022 n=5	0.097±0.008 n=5	0.069±0.012 n=5	0.088±0.014 n=6
		Yes	0.094±0.023 n=8	0.061±0.016 n=6	0.105±0.030 n=7	0.057±0.006 n=6

†Leaf=Leaf treatment; refers to leaves conditioned under ambient light (light) or completely covered (dark).
‡Macro=Macroinvertebrate treatment; refers to whether macroinvertebrates were present in the flask or absent from the flask.

Table II.3. Consumption variables measured for both species each week during the experiments. Values represent mean±SEM.

Species	Leaf Treatment[†]	Week	Areal Leaf Consumption (mm^2/mg DM[‡])	Frass Production (mg/mg DM[‡])	Change in Leaf Mass (mg WM[§]/mg DM[‡])
Caecidotea communis	Light	*Week 1*	18.79±4.31 n=6	0.85±0.14 n=6	-0.024±0.007 n=6
		Week 2	19.56±12.19 n=6	1.33±0.16 n=6	-0.044±0.008 n=6
		Week 3	14.64±5.22 n=5	1.86±0.19 n=6	-0.054±0.012 n=6
		Week 4	34.33±8.44 n=6	2.54±0.19 n=6	-0.056±0.004 n=6
	Dark	*Week 1*	2.08±2.77 n=6	0.64±0.07 n=6	-0.000±0.002 n=6
		Week 2	10.32±7.52 n=6	0.92±0.05 n=6	-0.021±0.006 n=6
		Week 3	1.45±3.40 n=5	0.79±0.18 n=6	-0.035±0.012 n=6
		Week 4	25.74±5.62 n=6	2.16±0.15 n=6	-0.027±0.010 n=6
Ephemerella invaria	Light	*Week 1*	8.89±1.46 n=5	0.66±0.04 n=4	-0.009±0.002 n=4
		Week 2	43.86±12.68 n=5	0.80±0.24 n=5	-0.023±0.005 n=5
		Week 3	12.39±2.94 n=5	1.07±0.08 n=4	-0.021(0.002) n=4
		Week 4	47.84±10.52 n=5	0.99±0.15 n=5	-0.023±0.002 n=4
	Dark	*Week 1*	5.81±2.81 n=5	0.59±0.13 n=5	-0.017±0.006 n=5
		Week 2	5.73±3.30 n=5	0.76±0.11 n=5	-0.018±0.004 n=5
		Week 3	21.78±5.87 n=5	1.00±0.16 n=5	-0.026±0.003 n=5
		Week 4	31.37±3.03 n=5	0.85±0.10 n=5	-0.019±0.004 n=5

[†]Leaf treatment refers to leaves conditioned under ambient light (light) or completely covered (dark).
[‡]DM refers to the initial dry mass of the macroinvertebrate
[§]WM refers to the wet mass of the leaves

Table II.4. Stable isotope signatures measured from food resources and macroinvertebrates during both experiments. Values represent means±SD.

Source	*Caecidotea communis*			*Ephemerella invaria*		
	n	**$\delta^{13}C$**	**$\delta^{15}N$**	**n**	**$\delta^{13}C$**	**$\delta^{15}N$**
Background Leaves[†]	3	-29.81±0.14	2.50±0.38	3	-29.90±0.15	2.83±0.54
Algal Slurry[‡]	3	-24.57±0.75	2.83±0.65	3	-23.46±0.34	3.22±0.40
Light-No Macroinvertebrate[§]	3	-29.08±1.00	0.96±0.39	4	-29.75±0.75	2.76±1.46
Light-Macroinvertebrate[§]	6	-29.29±0.59	2.57±1.31	4	-30.37±0.96	4.10±1.33
Dark-No Macroinvertebrate[§]	3	-30.34±1.21	3.52±0.21	4	-30.08±0.62	4.13±0.78
Dark-Macroinvertebrate[§]	6	-29.71±0.52	4.78±0.85	5	-30.07±0.53	3.99±1.83
Species-Dark[¶]	19	-17.16±0.34	4.86±0.09	16	-26.63±3.80	5.13±0.37
Species-Light[¶]	19	-17.14±0.43	3.38±0.15	13	-26.33±3.13	4.56±0.61
Species-Initial[¶]	10	-17.37±0.42	4.11±0.41	10	-35.25±1.02	4.82±0.27

[†]Background leaves were unconditioned but leached for one week in RO water prior to isotope analysis.
[‡]Algal slurry is algal biofilm scraped off of rocks and amended into the light treatment.
[§]These values were obtained from leaves in flasks after the fourth week of the experiment. Light and dark refer to the leaf conditioning treatment and No Macroinvertebrate and Macroinvertebrate refer to whether the macroinvertebrate was present or absent in the flask.
[¶]Species refers to measurements taken from either *C. communis* or *E. invaria*, for each respective experiment. Dark and light refer to whether the macroinvertebrates were kept in flasks with light or dark conditioned leaves during the duration of the experiment and were ground up after a 3 day starvation period post-experiment. Initial refers to individuals analyzed before the experiment without starvation period.

Appendix III.

Tukey's HSD post-hoc test results for significant and marginally significant interactions in growth experiments in Chapter 2: Contribution of leaf-associated algae to growth of a shredder, *Caecidotea communis*, and a collector-gatherer, *Ephemerella invaria*.

Table III.1. Tukey's HSD post-hoc comparisons for changes in leaf wet mass during the *Caecidotea communis* experiment for the interaction between leaf treatment (light or dark) and week (1-4). Significant *p*-values are bolded. Leaf:Week interaction: p=0.050.

Comparison	Difference	*p*-value
Light:1-Dark:1	-0.004	0.567
Dark:2-Dark:1	-0.009	**0.002**
Light:2-Dark:1	-0.005	0.191
Dark:3-Dark:1	-0.013	**0.000**
Light:3-Dark:1	-0.010	**0.001**
Dark:4-Dark:1	-0.015	**0.000**
Light:4-Dark:1	-0.018	**0.000**
Dark:2-Light:1	-0.005	0.293
Light:2-Light:1	-0.001	0.997
Dark:3-Light:1	-0.009	**0.003**
Light:3-Light:1	-0.006	0.147
Dark:4-Light:1	-0.011	**0.000**
Light:4-Light:1	-0.014	**0.000**
Light:2-Dark:2	0.003	0.713
Dark:3-Dark:2	-0.004	0.646
Light:3-Dark:2	-0.001	1.000
Dark:4-Dark:2	-0.006	0.137
Light:4-Dark:2	-0.009	**0.003**
Dark:3-Light:2	-0.007	**0.024**
Light:3-Light:2	-0.004	0.483
Dark:4-Light:2	-0.009	**0.001**
Light:4-Light:2	-0.012	**0.000**
Light:3-Dark:3	0.003	0.849
Dark:4-Dark:3	-0.002	0.978
Light:4-Dark:3	-0.005	0.296
Dark:4-Light:3	-0.005	0.277
Light:4-Light:3	-0.008	**0.010**
Light:4-Dark:4	-0.003	0.866

Table III.2. Tukey's HSD post-hoc comparisons for changes in leaf wet mass during the *Caecidotea communis* experiment for the interaction between macroinvertebrate presence (yes[Y] or no[N]) and week (1-4). Significant *p*-values are bolded. Macroinvertebrate:Week interaction: p=0.082.

Comparison	Difference	*p*-value
Y:1-N:1	-0.002	0.981
N:2-N:1	-0.002	0.997
Y:2-N:1	-0.009	**0.004**
N:3-N:1	-0.004	0.766
Y:3-N:1	-0.014	**0.000**
N:4-N:1	-0.010	**0.011**
Y:4-N:1	-0.018	**0.000**
N:2-Y:1	0.000	1.000
Y:2-Y:1	-0.007	**0.009**
N:3-Y:1	-0.002	0.987
Y:3-Y:1	-0.012	**0.000**
N:4-Y:1	-0.007	**0.031**
Y:4-Y:1	-0.016	**0.000**
Y:2-N:2	-0.007	**0.045**
N:3-N:2	-0.002	0.988
Y:3-N:2	-0.012	**0.000**
N:4-N:2	-0.008	0.074
Y:4-N:2	-0.017	**0.000**
N:3-Y:2	0.005	0.376
Y:3-Y:2	-0.005	0.158
N:4-Y:2	-0.001	1.000
Y:4-Y:2	-0.010	**0.000**
Y:3-N:3	-0.010	**0.001**
N:4-N:3	-0.005	0.413
Y:4-N:3	-0.014	**0.000**
N:4-Y:3	0.004	0.555
Y:4-Y:3	-0.005	0.190
Y:4-N:4	-0.009	**0.004**

Table III.3. Tukey's HSD post-hoc comparisons for frass production during the *Caecidotea communis* experiment for the interaction between leaf treatment (light or dark) and week (1-4). Significant *p*-values are bolded. Leaf:Week interaction: p=0.034.

Comparison	Difference	*p*-value
Light:1-Dark:1	0.208	0.975
Dark:2-Dark:1	0.279	0.888
Light:2-Dark:1	0.689	**0.044**
Dark:3-Dark:1	0.144	0.997
Light:3-Dark:1	1.215	**0.000**
Dark:4-Dark:1	1.522	**0.000**
Light:4-Dark:1	1.902	**0.000**
Dark:2-Light:1	0.071	1.000
Light:2-Light:1	0.481	0.336
Dark:3-Light:1	-0.063	1.000
Light:3-Light:1	1.007	**0.001**
Dark:4-Light:1	1.314	**0.000**
Light:4-Light:1	1.695	**0.000**
Light:2-Dark:2	0.410	0.539
Dark:3-Dark:2	-0.135	0.998
Light:3-Dark:2	0.936	**0.002**
Dark:4-Dark:2	1.243	**0.000**
Light:4-Dark:2	1.623	**0.000**
Dark:3-Light:2	-0.545	0.199
Light:3-Light:2	0.526	0.235
Dark:4-Light:2	0.833	**0.007**
Light:4-Light:2	1.213	**0.000**
Light:3-Dark:3	1.070	**0.000**
Dark:4-Dark:3	1.378	**0.000**
Light:4-Dark:3	1.758	**0.000**
Dark:4-Light:3	0.307	0.829
Light:4-Light:3	0.688	**0.045**
Light:4-Dark:4	0.380	0.630

Table III.4. Tukey's HSD post-hoc comparisons for changes in algal biomass on leaves during the *Ephemerella invaria* experiment for the interaction between macroinvertebrate presence (yes[Y] or no[N]) and week (1-4). Marginally significant *p*-values are bolded. Macroinvertebrate:Week interaction: p=0.035.

Comparison	Difference	*p*-value
Y:1-N:1	0.191	0.556
N:2-N:1	0.104	0.976
Y:2-N:1	0.010	1.000
N:3-N:1	0.066	0.998
Y:3-N:1	-0.037	1.000
N:4-N:1	0.114	0.950
Y:4-N:1	-0.071	0.998
N:2-Y:1	-0.087	0.981
Y:2-Y:1	-0.181	0.546
N:3-Y:1	-0.125	0.846
Y:3-Y:1	-0.228	**0.285**
N:4-Y:1	-0.077	0.986
Y:4-Y:1	-0.262	**0.141**
Y:2-N:2	-0.094	0.982
N:3-N:2	-0.039	1.000
Y:3-N:2	-0.141	0.870
N:4-N:2	0.010	1.000
Y:4-N:2	-0.175	0.691
N:3-Y:2	0.056	0.999
Y:3-Y:2	-0.047	1.000
N:4-Y:2	0.104	0.958
Y:4-Y:2	-0.081	0.994
Y:3-N:3	-0.102	0.969
N:4-N:3	0.048	0.999
Y:4-N:3	-0.136	0.871
N:4-Y:3	0.151	0.789
Y:4-Y:3	-0.034	1.000
Y:4-N:4	-0.185	0.573

Table III.5. Tukey's HSD post-hoc comparisons for changes in algal biomass on leaves during the *Ephemerella invaria* experiment for the three-way interaction between leaf treatment (light or dark), macroinvertebrate presence (yes[Y] or no[N]), and week (1-4). Significant and marginally significant *p*-values are bolded. Leaf:Macroinvertebrate:Week interaction: p=0.083.

Comparison	Difference	*p*-value
Light:N:1-Dark:N:1	0.100	1.000
Dark:Y:1-Dark:N:1	0.078	1.000
Light:Y:1-Dark:N:1	0.396	0.251
Dark:N:2-Dark:N:1	0.192	0.996
Light:N:2-Dark:N:1	0.152	0.999
Dark:Y:2-Dark:N:1	-0.087	1.000
Light:Y:2-Dark:N:1	0.205	0.993
Dark:N:3-Dark:N:1	0.031	1.000
Light:N:3-Dark:N:1	0.174	0.995
Dark:Y:3-Dark:N:1	0.069	1.000
Light:Y:3-Dark:N:1	-0.164	1.000
Dark:N:4-Dark:N:1	0.123	1.000
Light:N:4-Dark:N:1	0.214	0.966
Dark:Y:4-Dark:N:1	-0.047	1.000
Light:Y:4-Dark:N:1	-0.027	1.000
Dark:Y:1-Light:N:1	-0.022	1.000
Light:Y:1-Light:N:1	0.295	0.820
Dark:N:2-Light:N:1	0.092	1.000
Light:N:2-Light:N:1	0.052	1.000
Dark:Y:2-Light:N:1	-0.187	0.998
Light:Y:2-Light:N:1	0.105	1.000
Dark:N:3-Light:N:1	-0.070	1.000
Light:N:3-Light:N:1	0.073	1.000
Dark:Y:3-Light:N:1	-0.031	1.000
Light:Y:3-Light:N:1	-0.265	0.986
Dark:N:4-Light:N:1	0.022	1.000
Light:N:4-Light:N:1	0.113	1.000
Dark:Y:4-Light:N:1	-0.147	1.000
Light:Y:4-Light:N:1	-0.127	1.000
Light:Y:1-Dark:Y:1	0.318	0.393
Dark:N:2-Dark:Y:1	0.114	1.000
Light:N:2-Dark:Y:1	0.074	1.000
Dark:Y:2-Dark:Y:1	-0.165	0.996
Light:Y:2-Dark:Y:1	0.127	1.000
Dark:N:3-Dark:Y:1	-0.048	1.000
Light:N:3-Dark:Y:1	0.095	1.000
Dark:Y:3-Dark:Y:1	-0.009	1.000

Comparison (cont.)	Difference	*p*-value
Light:Y:3-Dark:Y:1	-0.243	0.981
Dark:N:4-Dark:Y:1	0.045	1.000
Light:N:4-Dark:Y:1	0.136	0.999
Dark:Y:4-Dark:Y:1	-0.125	1.000
Light:Y:4-Dark:Y:1	-0.105	1.000
Dark:N:2-Light:Y:1	-0.204	0.982
Light:N:2-Light:Y:1	-0.243	0.845
Dark:Y:2-Light:Y:1	-0.482	**0.031**
Light:Y:2-Light:Y:1	-0.190	0.991
Dark:N:3-Light:Y:1	-0.365	0.378
Light:N:3-Light:Y:1	-0.222	0.871
Dark:Y:3-Light:Y:1	-0.327	0.404
Light:Y:3-Light:Y:1	-0.560	**0.067**
Dark:N:4-Light:Y:1	-0.273	0.763
Light:N:4-Light:Y:1	-0.182	0.972
Dark:Y:4-Light:Y:1	-0.443	**0.073**
Light:Y:4-Light:Y:1	-0.423	0.259
Light:N:2-Dark:N:2	-0.040	1.000
Dark:Y:2-Dark:N:2	-0.279	0.865
Light:Y:2-Dark:N:2	0.013	1.000
Dark:N:3-Dark:N:2	-0.162	0.999
Light:N:3-Dark:N:2	-0.019	1.000
Dark:Y:3-Dark:N:2	-0.123	1.000
Light:Y:3-Dark:N:2	-0.357	0.805
Dark:N:4-Dark:N:2	-0.069	1.000
Light:N:4-Dark:N:2	0.022	1.000
Dark:Y:4-Dark:N:2	-0.239	0.956
Light:Y:4-Dark:N:2	-0.219	0.992
Dark:Y:2-Light:N:2	-0.239	0.917
Light:Y:2-Light:N:2	0.053	1.000
Dark:N:3-Light:N:2	-0.122	1.000
Light:N:3-Light:N:2	0.021	1.000
Dark:Y:3-Light:N:2	-0.083	1.000
Light:Y:3-Light:N:2	-0.317	0.868
Dark:N:4-Light:N:2	-0.030	1.000
Light:N:4-Light:N:2	0.061	1.000
Dark:Y:4-Light:N:2	-0.199	0.981
Light:Y:4-Light:N:2	-0.179	0.998
Light:Y:2-Dark:Y:2	0.292	0.819
Dark:N:3-Dark:Y:2	0.117	1.000
Light:N:3-Dark:Y:2	0.260	0.792
Dark:Y:3-Dark:Y:2	0.156	0.998

Comparison (cont.)	Difference	*p*-value
Light:Y:3-Dark:Y:2	-0.078	1.000
Dark:N:4-Dark:Y:2	0.209	0.979
Light:N:4-Dark:Y:2	0.300	0.581
Dark:Y:4-Dark:Y:2	0.040	1.000
Light:Y:4-Dark:Y:2	0.060	1.000
Dark:N:3-Light:Y:2	-0.175	0.999
Light:N:3-Light:Y:2	-0.032	1.000
Dark:Y:3-Light:Y:2	-0.136	1.000
Light:Y:3-Light:Y:2	-0.370	0.760
Dark:N:4-Light:Y:2	-0.083	1.000
Light:N:4-Light:Y:2	0.008	1.000
Dark:Y:4-Light:Y:2	-0.253	0.933
Light:Y:4-Light:Y:2	-0.232	0.986
Light:N:3-Dark:N:3	0.143	0.999
Dark:Y:3-Dark:N:3	0.039	1.000
Light:Y:3-Dark:N:3	-0.195	0.999
Dark:N:4-Dark:N:3	0.092	1.000
Light:N:4-Dark:N:3	0.183	0.992
Dark:Y:4-Dark:N:3	-0.078	1.000
Light:Y:4-Dark:N:3	-0.057	1.000
Dark:Y:3-Light:N:3	-0.104	1.000
Light:Y:3-Light:N:3	-0.338	0.759
Dark:N:4-Light:N:3	-0.051	1.000
Light:N:4-Light:N:3	0.040	1.000
Dark:Y:4-Light:N:3	-0.221	0.931
Light:Y:4-Light:N:3	-0.200	0.991
Light:Y:3-Dark:Y:3	-0.234	0.989
Dark:N:4-Dark:Y:3	0.054	1.000
Light:N:4-Dark:Y:3	0.145	0.998
Dark:Y:4-Dark:Y:3	-0.116	1.000
Light:Y:4-Dark:Y:3	-0.096	1.000
Dark:N:4-Light:Y:3	0.287	0.946
Light:N:4-Light:Y:3	0.378	0.589
Dark:Y:4-Light:Y:3	0.117	1.000
Light:Y:4-Light:Y:3	0.138	1.000
Light:N:4-Dark:N:4	0.091	1.000
Dark:Y:4-Dark:N:4	-0.170	0.997
Light:Y:4-Dark:N:4	-0.150	1.000
Dark:Y:4-Light:N:4	-0.261	0.790
Light:Y:4-Light:N:4	-0.241	0.951
Light:Y:4-Dark:Y:4	0.020	1.000

Table III.6. Tukey's HSD post-hoc comparisons for changes in leaf wet mass during the *Ephemerella invaria* experiment for the interaction between macroinvertebrate presence (yes[Y] or no[N]) and week (1-4). Significant *p*-values are bolded. Macroinvertebrate:Week interaction: p=0.037.

Comparison	Difference	*p*-value
Y:1-N:1	-0.006	**0.011**
N:2-N:1	-0.004	0.438
Y:2-N:1	-0.013	**0.000**
N:3-N:1	-0.002	0.881
Y:3-N:1	-0.014	**0.000**
N:4-N:1	-0.008	**0.000**
Y:4-N:1	-0.020	**0.000**
N:2-Y:1	0.002	0.755
Y:2-Y:1	-0.007	**0.000**
N:3-Y:1	0.004	0.228
Y:3-Y:1	-0.008	**0.000**
N:4-Y:1	-0.002	0.775
Y:4-Y:1	-0.014	**0.000**
Y:2-N:2	-0.010	**0.000**
N:3-N:2	0.001	0.992
Y:3-N:2	-0.011	**0.000**
N:4-N:2	-0.005	0.099
Y:4-N:2	-0.017	**0.000**
N:3-Y:2	0.011	**0.000**
Y:3-Y:2	-0.001	0.994
N:4-Y:2	0.005	**0.048**
Y:4-Y:2	-0.007	**0.000**
Y:3-N:3	-0.012	**0.000**
N:4-N:3	-0.006	**0.012**
Y:4-N:3	-0.018	**0.000**
N:4-Y:3	0.006	**0.006**
Y:4-Y:3	-0.006	**0.005**
Y:4-N:4	-0.012	**0.000**

Table III.7. Tukey's HSD post-hoc comparisons for leaf area consumed during the *Ephemerella invaria* experiment for the interaction between leaf treatment (light or dark) and week (1-4). Significant *p*-values are bolded. Leaf:Week interaction: p=0.007.

Comparison	Difference	*p*-value
Light:1-Dark:1	3.082	1.000
Dark:2-Dark:1	-0.079	1.000
Light:2-Dark:1	38.051	**0.006**
Dark:3-Dark:1	15.974	0.674
Light:3-Dark:1	6.588	0.996
Dark:4-Dark:1	25.561	0.143
Light:4-Dark:1	42.035	**0.002**
Dark:2-Light:1	-3.161	1.000
Light:2-Light:1	34.969	**0.014**
Dark:3-Light:1	12.891	0.856
Light:3-Light:1	3.505	1.000
Dark:4-Light:1	22.479	0.266
Light:4-Light:1	38.953	**0.004**
Light:2-Dark:2	38.130	**0.006**
Dark:3-Dark:2	16.053	0.669
Light:3-Dark:2	6.666	0.996
Dark:4-Dark:2	25.640	0.141
Light:4-Dark:2	42.114	**0.002**
Dark:3-Light:2	-22.078	0.286
Light:3-Light:2	-31.464	**0.035**
Dark:4-Light:2	-12.490	0.874
Light:4-Light:2	3.984	1.000
Light:3-Dark:3	-9.386	0.969
Dark:4-Dark:3	9.587	0.966
Light:4-Dark:3	26.062	0.129
Dark:4-Light:3	18.974	0.470
Light:4-Light:3	35.448	**0.012**
Light:4-Dark:4	16.474	0.641

Appendix IV. Average algal biomass values on leaves for each experiment and leaf consumption rates for *Amphinemura*, *Tipula*, *Stenonema*, *Lepidostoma*, and *Caecidotea communis* in Chapter 3: Feeding preferences of four macroinvertebrate shredders and a scraper in relation to leaf-associated algae and clumped or dispersed leaves.

Table IV.1. Algal biomass (as chlorophyll-a; mg/m^2) measured on leaves for each experiment.

Leaf Treatment	*Amphinemura*	*Tipula*	*Stenonema*	*Lepidostoma*	*Caecidotea*
Dark	0.002±0.003	0.002±0.002	0.004±0.003	0.002±0.002	0.015±0.006
Light	0.074±0.011	0.046±0.009	0.024±0.006	0.044±0.007	0.038±0.004

Table IV.2. Consumption rates (mg leaf tissue/mg DM macroinvertebrate*day) for each organism in each treatment.

Taxa	Leaf Mass	Dispersed-Dark[†]	Dispersed-Light[†]	Clumped-Dark[†]	Clumped-Light[†]
Amphinemura	DM[‡]	1.444±0.139	1.293±0.168	1.175±0.185	1.492±0.082
Tipula	DM	0.053±0.016	0.023±0.013	0.083±0.034	0.059±0.030
	AFDM[‡]	0.083±0.025	0.032±0.015	0.118±0.043	0.077±0.036
Stenonema	DM	0.052±0.009	0.084±0.022	0.045±0.003	0.079±0.035
	AFDM	0.066±0.011	0.100±0.024	0.060±0.009	0.101±0.035
Lepidostoma	DM	0.208±0.054	0.283±0.045	0.193±0.061	0.293±0.071
	AFDM	0.244±0.066	0.343±0.068	0.254±0.066	0.339±0.063
Caecidotea	DM	0.402±0.185	0.127±0.097	0.482±0.226	0.372±0.101
	AFDM	0.660±0.237	0.681±0.166	0.775±0.234	0.797±0.193

[†]Light and dark refers to leaf conditioning treatment, where leaves were conditioned in either the light or dark. Dispersed and clumped refers to the leaf arrangement treatment, either equally spread out around an arena or near a refuge.
[‡]DM refers to the consumption of leaf dry mass. AFDM refers to the consumption of leaf ash free dry mass.

Table IV.3. Results of Friedman tests for ranked preference of dark- or light-conditioned leaves for each experiment and measured factor. In addition, the number of arenas where the dark arenas had higher consumption is displayed in sum and by leaf arrangement.

Taxa	Factor	Friedman χ^2	*p*-value	Total Number of Dark[†]	Dispersed Number of Dark[†]	Clumped Number of Dark[†]
Amphinemura	DM[‡]	0	1.000	5	3	2
Tipula	DM	10	0.002	10	5	5
	AFDM[‡]	10	0.002	10	5	5
Stenonema	DM	0	1.000	5	2	3
	AFDM	0.4	0.527	4	2	2
Lepidostoma	DM	1.6	0.206	3	2	1
	AFDM	1.6	0.206	3	1	2
Caecidotea	DM	0.4	0.527	6	4	2
	AFDM	0.4	0.527	4	2	2

[†]Number of dark refers to the number of arenas in which the consumption values for dark-conditioned leaves are higher than the light-conditioned leaves. Total indicates the number out of all ten arenas, dispersed indicates the number within the dispersed leaf arrangement arenas out of five, and clumped indicates the number within the clumped leaf arrangement arenas out of five.
[‡]DM refers to the consumption of leaf dry mass. AFDM refers to the consumption of leaf ash free dry mass.

Appendix V. Calculation and estimated values for algal and fungal carbon associated with leaves in Chapter 1.

Calculation of carbon estimates from Chapter 1 samples

Total leaf carbon (C) was measured via CN analysis of leaves, fungal biomass was estimated via ergosterol as mg fungal dry mass/mg dry weight of detritus, and algal biomass was estimated as mg chlorophyll-a/m^2 as detailed in Chapter 1 Methods. Total leaf carbon was normalized to the known leaf dry mass of CN samples. Conversions were used to determine algal and fungal C. Ergosterol was normalized to the dry mass of leaves from which it was measured, and fungal dry mass was converted to fungal C assuming that 44% of fungal dry mass is carbon (Zhang & Elser, 2017). The exact leaf dry mass from which chlorophyll-a was measured is unknown given the extraction process. Chlorophyll-a leaf dry mass was therefore estimated by averaging the mass of ten leaf disks from each treatment combination in each stream and season; this value was multiplied by five to account for the five leaf disks from which chlorophyll-a was measured. Extraction of chlorophyll-a initially results in total ug chlorophyll-a per samples; this value was converted to mg of algal C using a factor of 11.1 ug chlorophyll-a/mg algal C (Halvorson et al., 2019) and divided by the estimated dry leaf mass to normalize to dry leaf mass. The percent of total C comprised of algal and fungal C was computed by comparing algal and fungal C to the total amount of C measured within the leaves. The percent of microbial C comprised of algal and fungal C was computed by summing algal and fungal C and determining the proportion contributed by each.

Note: Three initial leaf replicates were measured for CN, and final leaf carbon and final total leaf carbon values were known, but due to differences in leaf decomposition across streams and time in combination with microbial colonization, it is not possible to determine how much of the total carbon was attributable to the leaf alone and therefore impossible to determine the bacterial component of total carbon without direct measures of bacteria.

Table V.1. Average carbon values estimated from leaves and associated fungal and algal biomass in Chapter 1 across treatments in each season.

Factor	Winter				Spring			
	Low Nutrient-Ambient	Low Nutrient-Shaded	High Nutrient-Ambient	High Nutrient-Shaded	Low Nutrient-Ambient	Low Nutrient-Shaded	High Nutrient-Ambient	High Nutrient-Shaded
Total Carbon (mg C/g DM)	427.58 ±8.43	445.51 ±8.30	462.19 ±5.54	454.70 ±6.30	419.26 ±11.96	399.32 ±13.86	436.44 ±6.75	454.70 ±6.30
Algal Carbon (mg C/g DM)	2.51 ±0.64	0.78 ±0.09	6.64 ±1.38	2.09 ±0.53	1.95 ±0.45	3.06 ±1.02	8.59 ±2.15	3.67 ±0.62
Fungal Carbon (mg C/g DM)	32.59 ±3.75	27.71 ±2.51	49.22 ±2.69	47.87 ±5.00	50.66 ±5.75	51.98 ±6.14	63.22 ±3.59	66.89 ±3.16
Percent Algal Carbon (Total)	0.58 ±0.14	0.17 ±0.02	1.42 ±0.28	0.45 ±0.12	0.51 ±0.15	0.75 ±0.24	1.92 ±0.47	0.81 ±0.14
Percent Fungal Carbon (Total)	7.60 ±0.82	6.31 ±0.64	10.68 ±0.61	10.37 ±1.18	12.16 ±1.32	13.14 ±1.55	14.51 ±0.80	14.71 ±0.64
Percent Algal Carbon (Microbial)	7.15 ±1.69	3.06 ±0.43	11.40 ±2.17	4.54 ±1.12	4.62 ±1.17	5.28 ±1.34	11.21 ±2.17	5.53 ±1.08
Percent Fungal Carbon (Microbial)	92.85 ±1.69	96.94 ±0.43	88.60 ±2.17	95.46 ±1.12	95.38 ±1.17	94.72 ±1.34	88.79 ±2.07	94.47 ±1.08

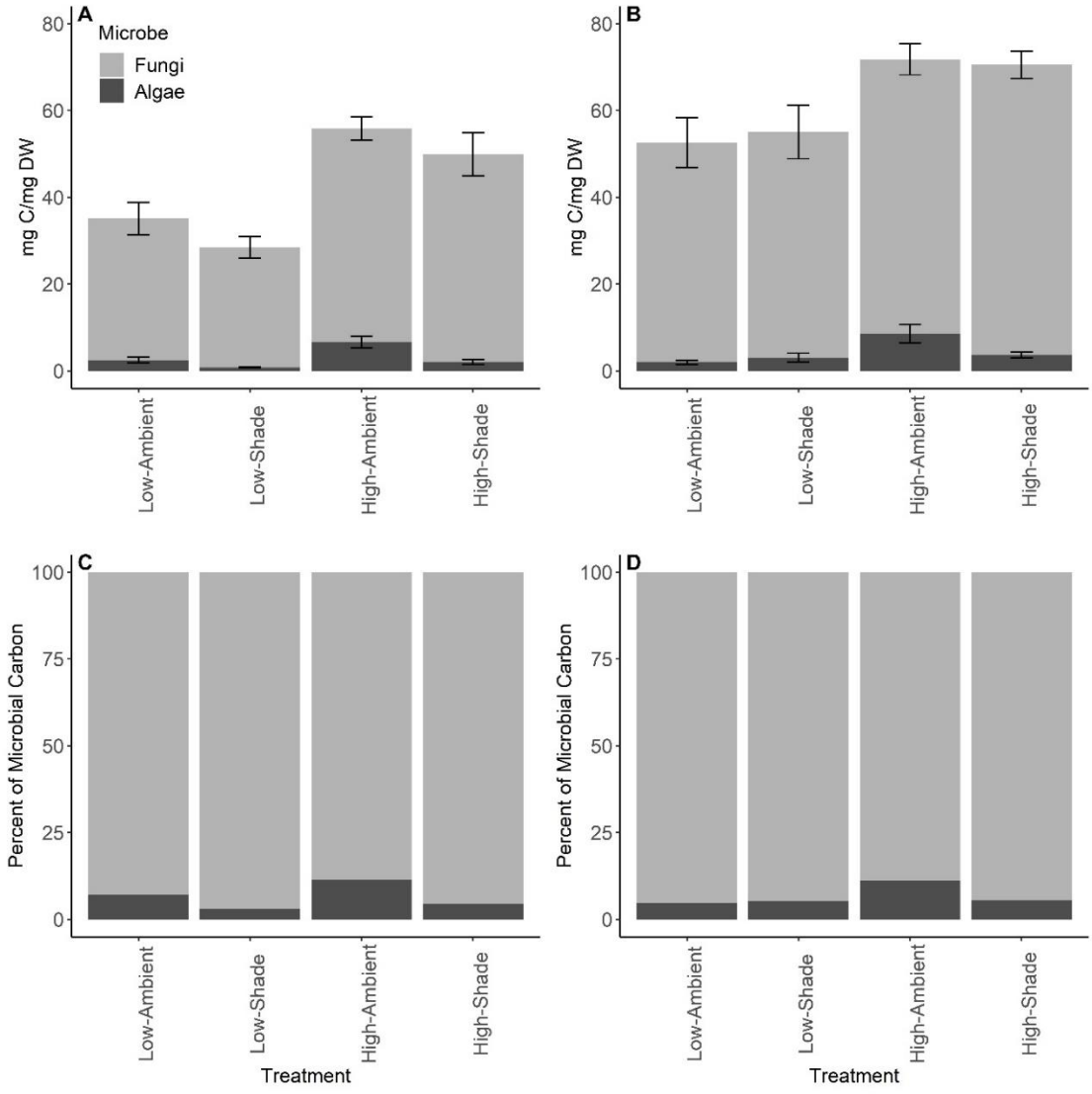

Figure V.1. Fungal and algal carbon associated with leaves in winter (A) and spring (B) within each treatment from Chapter 1. Percent of microbial (algal + fungal) carbon comprised of algal and fungal carbon associated with leaves in winter (C) and spring (D) within each treatment from Chapter 1. Values are estimated from biomass measured on leaves.

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