Oecologia
https://doi.org/10.1007/s00442-020-04821-7

COMMUNITY ECOLOGY – ORIGINAL RESEARCH

Filter-feeders have differential bottom-up impacts on green and brown food webs

Carla L. Atkinson[1] · Halvor M. Halvorson[2,3] · Kevin A. Kuehn[2] · Monica Winebarger[1] · Ansley Hamid[1] · Matthew N. Waters[4]

Received: 9 April 2020 / Accepted: 4 December 2020

Abstract

Nutrient recycling by consumers can strongly impact nutrient availability for autotrophic and heterotrophic microbes, thus impacting functions such as primary production and decomposition. Filter-feeding freshwater mussels form dense, multi-species assemblages in aquatic ecosystems and have been shown to play a critical role in nutrient cycling. Mussel excretion can enhance benthic primary production and influence algal species composition. However, the role of mussels in brown or detritus-based food webs and species-specific differences has received considerably less attention. Here, using mesocosm experiments, we assessed how three species of freshwater mussels that occupy three different phylogenetic tribes influenced benthic algal accrual, ecosystem metabolism, cotton strip decomposition, leaf litter (*Acer saccharum*) decomposition, and litter-associated fungal biomass measured as ergosterol. Additionally, we measured mussel excretion and biodeposition rates and assessed the stoichiometry (C:N, C:P, and N:P) of the benthic algae, cotton strips, and leaf litter. In comparison to controls without mussels, generally, mussel treatments had higher benthic algal biomass composed of more diatoms, higher gross primary productivity and net ecosystem production rates, and higher cotton strip tensile strength loss, but there was not a difference in ecosystem respiration rates, leaf litter decomposition rates, or fungal biomass. Benthic algae had lower C:N and higher N:P in mussel treatment tanks and cotton strip C:N was lower in mesocosms with mussels. Our results suggest that nutrient regeneration by mussels most strongly regulates green food webs, with some impacts to brown food webs, suggesting that consumers have interactive effects on microbial functioning in freshwaters.

Keywords Species traits · Excretion · Decomposition · Ergosterol · Unionid

Introduction

Nutrient availability significantly influences ecosystem processes and has cascading effects on community dynamics as most ecosystems are limited by energy (carbon, C) or nutrients such as nitrogen (N), phosphorus (P), or both N and P (Cross et al. 2006; Harpole et al. 2011; Tank and Dodds 2003). Nutrient capture and release by consumers can strongly impact nutrient availability for autotrophs and microbes in terrestrial, marine, and freshwater ecosystems (Covich et al. 1999; Doughty et al. 2016; McNaughton et al. 1997; Parr et al. 2019; Roman and McCarthy 2010; Turner 2015). Across ecosystems there is substantial evidence indicating that abundant or dense aggregations of consumers can significantly alter nutrient dynamics (Atkinson et al. 2017; McIntyre et al. 2008; McNaughton et al. 1988; Sitters et al. 2017). In particular, high-density consumers have been shown to alter limiting nutrients (Allgeier et al. 2013), alter

Communicated by Maarten Boersma.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00442-020-04821-7.

✉ Carla L. Atkinson
carlalatkinson@gmail.com

[1] Department of Biological Sciences and the Center for Freshwater Studies, University of Alabama, 2109 Bevill Building, Tuscaloosa, AL 35487, USA

[2] School of Biological, Environmental, and Earth Sciences, University of Southern Mississippi, 118 College Dr. #5018, Hattiesburg, MS 39402, USA

[3] Department of Biology, University of Central Arkansas, 180 Lewis Science Center 201 Donaghey Ave, Conway, AR 72035, USA

[4] Department of Crop, Soil and Environmental Sciences, Auburn University, Auburn, AL 36849, USA

Published online: 02 January 2021

primary producer community composition (Spooner et al. 2013; van der Waal et al. 2011), and enhance gross primary production (Atkinson et al. 2018; Hall et al. 2003; Sitters et al. 2017). Despite the emphasis on consumer-driven nutrient dynamic (CND) effects on "green" food webs based on algae and living plant material, little research has been done to assess the bottom-up impact of species-specific CND effects on "brown", detrital-based food webs (Atkinson et al. 2017; Evans-White and Halvorson 2017).

Similar to green food webs, CND may affect processes in brown food webs because nutrients like N and P also limit major processes of heterotrophy including heterotrophic respiration, production of degradative enzymes, and biomass accrual (Jabiol et al. 2018; Moore et al. 2004; Suberkropp et al. 2010). Prior studies indicate that heterotrophic bacteria and fungi assimilate dissolved nutrients excreted by animals, often stimulating microbial growth and enhancing respiration and/or decomposition rates (Cheever and Webster 2014; Ramamonjisoa and Natuhara 2018; Rugenski et al. 2012). However, studies still rarely link CND effects on organisms outside of those animals' direct feeding channels. For example, animals excrete both inorganic and organic forms of nutrients, and the former are most available to both autotrophs and heterotrophs whereas the latter are more available to heterotrophs like bacteria (Parr et al. 2019). Consideration of simultaneous CND effects on both green and brown processes will provide a broader understanding of animals' roles in ecosystem functions, especially given that many animals do not feed exclusively in green or brown food webs (Wolkovich et al. 2014; Zou et al. 2016).

In many river ecosystems, freshwater mussels are often the dominant macroconsumer and their filter-feeding can significantly influence ecosystem structure and function by facilitating the cycling of nutrients and creating habitat for other benthic organisms (Vaughn and Hakenkamp 2001). Mussels occur as spatially heterogeneous, dense aggregations that remove nutrients from the pelagic zone and excrete or egest them into the benthos, translocating them from one habitat of the stream to another resulting in reduced nutrient transport downstream (Atkinson et al. 2013; Atkinson and Vaughn 2015). Because mussels often occur in dense, speciose aggregations, they can exert strong bottom-up controls on aquatic systems by creating biogeochemical hotspots (Atkinson and Vaughn 2015) that alleviate nutrient limitation and stimulate primary production (Atkinson et al. 2013). Recent research has increased our awareness of the importance of mussels in ecosystem structure and function, particularly in green food webs (Atkinson et al. 2013, 2018), but they are still understudied relative to other aquatic taxa, in particular their role in brown food webs (Vaughn and Hoellein 2018). At the same time, freshwater mussel diversity and abundance is declining globally (Lopes-Lima et al. 2018) raising concerns about how ecological structure and function may be changing in aquatic ecosystems worldwide (Vaughn and Hoellein 2018).

Previous work has shown that the tissue and excretion stoichiometry (C:N:P) of mussels differ between species as a result of phylogenetic history and differences in assimilation (Atkinson et al. 2020a). Thus, community composition even within a single functional feeding group has the potential to alter stream nutrient availability differentially and have subsequent bottom-up influences on ecosystem processes such as primary production, stream metabolism, and decomposition. Because these ecosystem processes reflect dynamics of both green and brown food webs, there is a need to understand the potential effects of CND in both green and brown food webs, the interplay between these two major energy flow pathways, and the indirect interactions within ecosystems (Buchkowski et al. 2019; Evans-White and Halvorson 2017; Zou et al. 2016). For example, differences in CND across filter-feeders, such as proportions or ratios of nutrients released as organic vs. inorganic forms, may result in divergent effects on autotrophic vs. heterotrophic processes, indicating species-specific effects on the trophic state of ecosystems (Parr et al. 2019). Specifically, consumers such as mussels may play a strong role in mediating microbial conditioning of leaf litter and smaller detrital particles.

In the present study, we investigated the effects of mussel species identity and excretion stoichiometry on ecosystem functioning using controlled mesocosm experiments. In particular, we used three species that occupy distinct phylogenetic tribes (Lampsilini, Pleurobemini, and Quadrulini) and stoichiometric niches where Lampsilini have lower tissue N:P resulting in higher excretion N:P than the other two groups (Atkinson et al. 2020a). The objectives of our study were to determine if the presence of mussels and the species identity of mussels altered green and/or brown food webs by examining variation in (1) the biomass and stoichiometry of benthic algae; (2) stream benthic metabolism; (3) litter decomposition rates and stoichiometry; and (4) fungal biomass accrual on leaf litter between species treatments and between live vs sham mussels. We hypothesized that, due to the stimulatory effects of mussel CND, mussel treatments would have higher benthic algal accrual, rates of primary production, cotton strip decomposition rates, natural leaf litter decomposition rates and litter fungal biomass. In addition, we hypothesized algal stoichiometry, cotton strip and leaf litter stoichiometry would partially be driven by the distinct excretion stoichiometries of the mussel species treatments.

Methods

Study organism and system

The Sipsey River in west-central Alabama, USA is a fifth-order tributary of the Tombigbee River in the Mobile Basin, which has rich and stable mussel communities throughout (Haag and Warren 2010). We utilized three common and abundant unionid species from the Sipsey River: *Cyclonaias asperata* (common name: Alabama orb; Tribe Quadrulini), *Elliptio arca* (common name: Alabama spike; Tribe Pleurobemini), and *Obovaria unicolor* (common name: Alabama hickorynut; Tribe: Lampsilini) (Haag and Warren 2010). These species vary in shell morphology and life history (Williams et al. 2008), and we anticipated these species may vary in their ecological roles as they vary in their tissue stoichiometry (Atkinson et al. 2020a), making these species ideal for assessing variation in functional effects related to CND.

Mesocosm design

We conducted a 28-day mesocosm experiment in a greenhouse with natural light using a total of 24 recirculating stream mesocosms (122 × 48 × 28 cm) that consisted of two tanks, a fiberglass outer tank into which an open-ended plastic inner tank was placed (see Nickerson et al. In press for design and Appendix, Fig. S1). The inner tank was raised to separate the bottom of the inner tank from the bottom of the outer tank to allow for water recirculation. The inner plastic tanks were filled with sifted pea gravel mixed with sand collected from the Sipsey River, which served as benthic habitat for the mussels. Each tank was filled with 60 L of water collected from the Sipsey River where mussels were collected, then filled with an additional 19 L of deionized water to completely fill the tanks. We also inoculated each tank with natural microbial communities. First, microbes were naturally present in the Sipsey water we used to fill the tanks, but we also inoculated each tank with benthic algae by scraping rocks from the river into a bucket full of river water to create a slurry and added 300 mL of this slurry to each tank. Weekly water changes of mesocosm tanks were conducted, where 20 L of water was emptied and 20 L of fresh Sipsey water was added to each tank. In addition, every other day each tank received 300 mL of a concentrated mixed algal assemblage (C:N:P ~ 82:9:1) cultured in a conical tube in the greenhouse from natural river water. We also deployed ten mesh bags of sugar maple (*Acer saccharum*) leaves in the Sipsey River for 5 days to facilitate natural colonization of microbes and then divided those leaves equally among the mesocosms to inoculate them with a natural microbial assemblage. Water was circulated at a rate of 2527 L h^{-1} with 47-W magnetic drive pumps (Danner Mfg., Islandia, NY) that were placed atop the gravel at one end of the mesocosm to stimulate stream flow. The mesocosms were acclimated for a week prior to the addition of leaf litter and mussels. We placed three mesh bags of pre-weighed sugar maple leaves (~ 2.5 g) into each of the mesocosms (72 leaf packs in total) at the onset of the experiment (see details below).

A total of 108 live mussels were collected from the Sipsey River (36 of each species) in September 2018 and housed together in a single living stream for approximately 2 weeks prior to the start of the experiment. Mussels were fed a mixed live-cultured algal assemblage daily (as above) prior to allocating them to experimental tanks. We manipulated species composition by creating four treatments: control treatment without mussels and three experimental single-species treatments containing the common mussel species *Cyclonaias asperata*, *Elliptio arca*, and *Obovaria unicolor*. Treatments were randomized across our mesocosms and each treatment mesocosm was replicated six times and stocked with six individuals of a single species (~ 24 indiv m^{-2}; a common density in the Sipsey River; Haag and Warren 2010). We measured the length of each individual to estimate total biomass stocked into each tank using previously generated species-specific length dry mass equations (Appendix, Table S1; Atkinson et al. 2020b).

Species-specific excretion rates

We estimated mass-specific and total community biodeposition rates, NH_4^+, and PO_4^{3-} excretion rates (hereafter NH_4 and PO_4), and N:P excretion by randomly subsampling mussels of each species at the end of the experiment ($N = 18$ per species; see Table S1 for mussel size range used). Each mussel was gently scrubbed and placed individually in separate excretion chambers that were filled with 500 mL of filtered (GF/F filter, 0.7 µm pore size) stream water. Additionally, we used three water-only chambers as controls. The mussels were incubated in the excretion chambers for 1-h at 25.5 ± 0.3 °C, similar to the mesocosm temperatures and previous work on other aquatic species (McIntyre et al. 2008; Atkinson et al. 2013), after which we gently removed them and filtered the chamber water (GF/F filter, 0.7 µm pore size). Filters were retained to estimate biodeposit dry mass, biodeposit rates (mg DM^{-1} h^{-1}), mass-specific biodeposit rates (mg DM g^{-1} h^{-1}) and the %C, %N, and %P of mussel biodeposits. The resulting filtrate was collected in 45 mL sample bottles and stored at − 20 °C until analyzed for NH_4 and PO_4 concentrations using a Seal AQ300 discrete analyzer (Seal Analytical, Mequon, Wisconsin, USA). Using the concentration of nutrients in the excretion chamber (g

L^{-1}), the known volume in the excretion chamber, and the amount of time the mussels incubated in the chamber (1 h), we determined the NH_4 and PO_4 excretion rates for each mussel (µg nutrient hr^{-1}) by subtracting the nutrient concentrations found in the control chambers. Further, we estimated mussel mass-specific excretion rates (µg nutrient g^{-1} h^{-1}). To estimate the areal excretion rate (µg m^{-2} h^{-1}) in each mesocosm, we calculated the product of the estimated mass-specific excretion and biodeposition rates for each species and by the estimated mussel dry mass per m^{-2} in each mesocosm unit.

Benthic algae and metabolism

After mussel communities were assigned, we placed two 100 cm^2 ceramic tiles in each mesocosm for algal accrual. One of these tiles had five porous silica discs attached (Leco Corp., St. Joseph, MI) facing upwards using waterproof epoxy. A silica disc was collected in weeks 2 and 4 from each mesocosm tank and immediately frozen in the dark at – 80 °C for determination of benthic chlorophyll-a concentrations. We extracted the chl-a from each disc in a black-plastic film canister with 15 mL acetone–bicarbonate extraction reagent (90 vol% acetone + 1 g $MgCO_3$) and incubated the discs for 18-h at − 20 °C. After 24 h, samples were brought to room temperature and the extracted chl-a concentration analyzed on a Genesys 10S UV/Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Using the concentration of chl-a determined colorimetrically (µg mL^{-1}), the volume of the extraction solution (15 mL) and the known surface area of the silica disc (0.424 cm^2), we estimated benthic algal accrual for each experimental community (µg cm^{-2}). Two additional silica discs in weeks 2 and 4 were also collected for estimating mesocosm metabolism using methods similar to Tank et al. (2006). Briefly, we placed a silica disc into a 50-mL centrifuge tube filled completely with filtered Sipsey stream water and measured the initial temperature and dissolved oxygen (DO) concentrations using a YSI ProODO probe (Yellow Springs Incorporated, Yellow Springs, OH) prior to placing the tubes in an incubator at 25 °C for 2 h in the light. Following 2 h, temperature and DO were measured. Then, the tubes were emptied and refilled with filtered Sipsey water and the incubations were repeated at the same temperature, but in the dark. The change in DO between the light and dark incubations was used to estimate gross primary production (GPP), ecosystem respiration (ER), and net ecosystem production (NEP) on the benthic substrate.

Using the tile without silica discs, we collected one benthic algae biofilm sample per tank in week 4 by scraping each tile into 150 mL of filtered water to create a slurry. This benthic biofilm slurry was split into three filtered subsamples (pre-ashed 47-mm glass fiber filters; pore size = 0.7 µm; MilliporeSigma, Burlington, MA) for determination of ash-free dry mass (AFDM), the %C, %N, and %P of biofilms, and benthic algal pigments (i.e., Chlorophyta, Bacillariophyceae, and Cyanobacteria) using high-performance liquid chromatography (HPLC). All filters were immediately frozen at -20˚C following collection. For AFDM, filters were dried for 48 h at 50 °C and weighed to the nearest 0.00001 g to determine dry mass (DM). Filters were then ashed in a muffle furnace for 2 h at 500 °C, wetted, and dried for 48 h at 50 °C, reweighed, and ash-free dry mass (AFDM) was determined. To determine %C, %N, and %P of the benthic biofilm, we subsampled the filter. The %C and %N was determined using a Carlo Erba CHNS-O EA1108-Elemental Analyzer (Isomass Scientific Inc., Calgary, Alberta, Canada). For %P, dry samples were weighed, combusted at 500 °C for 2 h, and digested and dissolved with HCl followed by soluble reactive P analysis (Solorzano and Sharp 1980) using a Seal AQ300 discrete analyzer (Seal Analytical, Mequon, Wisconsin, USA). The C, N, and P composition was then converted to molar ratios to express stoichiometric ratios. Photosynthetic pigments on filters were measured using an HPLC system following the methods of Leavitt and Hodgson (2001) and Waters et al. (2012). Briefly, filters containing a known volume of particulate material were extracted with a solvent mixture of acetone, methanol and water in an 80/15/5 ratio, which contains an internal standard (Sudan II; Sigma Chemical Corp., St. Louis, MO). The filters and extraction solution were sonicated with a wand to break cell walls of intact primary producers. Samples were extracted for 16–24 h in a − 20 °C freezer. Samples were injected into a Shimadzu HPLC system following the mobile phase and time sequence of Leavitt and Hodgson (2001). Chlorophylls and carotenoids were separated by passing through a Phenomenex Luna C18 column and measured using a photodiode array detector coupled with a fluorescence detector. Pigment identification was made using retention times and pigment-specific spectra of known standards (DHI Lab Products, Denmark). Pigment concentrations are expressed as µg pigment L^{-1} and calculated by comparing peak areas against standards of known concentration.

Decomposition

We employed two methods to assess the impact of different mussel species on the brown food web in our experiment. First, 7 days into the experiment, we placed one cotton strip (as in Tiegs et al. 2013) into each mesocosm as a standardized measure of decomposition that has been used to compare across systems. After 3 weeks of incubating, the cotton strips were removed, cleaned by lightly squirting ethanol to remove debris, dried for 48 h at 50 °C and tensile strength was determined using a tensiometer (Mark-10 brand, Model #MG100, Copiague, NY, USA) mounted on a motorized test stand as in Tiegs et al. (2013). Tensile loss was expressed as

$$\%\ \text{Tensile Loss} = 1 - \left[\left(\frac{Tensile\ Strength_{treatment}}{Tensile\ Strength_{reference}}\right) \times 100\right] \Big/ Incubation\ Time, \quad (1)$$

where $Tensile\ Strength_{treatment}$ is the maximum tensile strength for the strips that were incubated in the mesocosms, $TensileStrength_{reference}$ is the mean tensile strength of five strips that were not incubated in the mesocosms, but also cleaned and stored in the same way, and *Incubation Time* was the time in which the strips were incubated (21 days).

In addition, we constructed leaf packs that consisted of ~ 2.5 g of senesced sugar maple (*Acer saccharum*) leaves. Three leaf litter packs were placed into the downstream end and adhered near the benthic substrate of each mesocosm and one leaf pack was removed following one, two, and four weeks after mussels were added to mesocosms. All collected leaf packs were placed into separate prelabeled bags and frozen at − 20 °C in darkness until leaf litter was lyophilized and weighed. Using the bulk leaf dry mass, we calculated litter dry mass decomposition rates k (day^{-1}) based on the exponential decay model (Barlocher 2005):

$$M_t = M_0 \times e^{-kt},$$

where Mt is bulk leaf dry mass (mg) at time t (days), and k is the exponential decay coefficient (day^{-1}). Immediately after lyophilization and weighing, leaf litter was ground into powder using a Wiley Mill (Thomas Scientific, Swedesboro, NJ) and ca. 100 mg leaf powder was subsampled and preserved from each leaf pack for ergosterol determination following methods of Gessner (2005) to estimate fungal biomass. Briefly, ergosterol in samples were preserved and subsequently extracted in methanolic KOH (8 g L^{-1} KOH, HPLC-grade methanol, total volume 10 mL) for 30 min at 80 °C. The resultant extract was the partitioned into n-pentane and evaporated to dryness under a stream of nitrogen gas. Ergosterol in dried sample residues was redissolved by sonication in 1 mL of methanol and stored tightly capped in 1.5 mL screw cap HPLC vials at − 20 °C in darkness until quantified by HPLC. Ergosterol concentrations were converted to fungal biomass C assuming 5 µg ergosterol/mg fungal dry mass and 43% fungal C (Findlay et al. 2002; Kuehn et al. 2014). Cotton strips and the leaf litter powder were also subsampled for carbon and nutrient contents (%C, %N, and %P) as above for algal biofilms.

Statistical analyses

We used one-way analysis of variance (ANOVA) to test for differences in mass-specific excretion rates among mussel species. A Kruskal–Wallis ANOVA on ranks was performed on total biomass and tank areal excretion rates across the treatments as the data were not normally distributed. Two-way ANOVAs were used to examine if chl-a biomass, GPP, ER, and NPP varied across our two sampling times and across treatments on the silica discs. One-way ANOVAs were used to determine if there were differences in algal pigment biomass, biofilm stoichiometry, percent tensile loss of the cotton strips, cotton strip C:N, and decomposition rates (k values) across the treatments. Significant ANOVAs were followed by Tukey's post hoc tests. We used repeated-measures ANOVAs to examine differences in % leaf litter mass loss, fungal biomass, and leaf litter stoichiometry across treatments over time. Statistical analyses were performed using the packages car and nlme and base R-software v3.3.1 (R Development Core Team 2015).

Results

Mussel biomass, excretion, and egestion

Mussel biomass varied among the mussel treatments ($H_2 = 0.006$; $P < 0.01$), with Tukey's multiple comparisons showing that *Elliptio arca* treatments had higher total soft tissue biomass than *Obovaria unicolor* treatments. There were significant differences in NH_4 excretion rates among species (Fig. 1a; ANOVA, $F_{2,51} = 13.48$, $P < 0.0001$), with *O. unicolor* having higher mass-specific NH_4 excretion rates than both of the other species (Tukey HSD; $P < 0.002$). Mass-specific PO_4 excretion rates did not vary across species (Fig. 1b; ANOVA; $F_{2,51} = 2.58$, $P = 0.09$). Excretion stoichiometry (N:P) did vary across species (Fig. 1c; ANOVA, $F_{2,51} = 6.62$, $P = 0.003$), with *O. unicolor* excreting at higher N:P than both *E. arca* (Tukey HSD; $P = 0.005$) and *Cyclonaias asperata* (Tukey HSD; $P = 0.01$). Mass-specific biodeposit rates varied 0.2–37.6 mg DM g^{-1} h^{-1}, but did not vary across mussel species (ANOVA, $F_{2,51} = 0.76$). Total areal NH_4 excretion rates in the mesocosms also varied across the treatments (Fig. 1d; $H_2 = 11.56$, $P = 0.003$); this pattern is likely given the higher biomass of *E. arca* and the higher mass-specific excretion rates of *O. unicolor* as both these species treatments resulted in higher areal NH_4 excretion rates than the *C. asperata* treatments (Tukey HSD; $P < 0.05$). Total areal PO_4 excretion rates also varied across treatments (Fig. 1e; $H_2 = 14.0$, $P < 0.001$) with *E. arca* treatments having higher rates than the *O. unicolor* treatments (Tukey HSD, $P < 0.001$), but were not higher than the *C. asperata* treatments (Tukey HSD, $P = 0.09$).

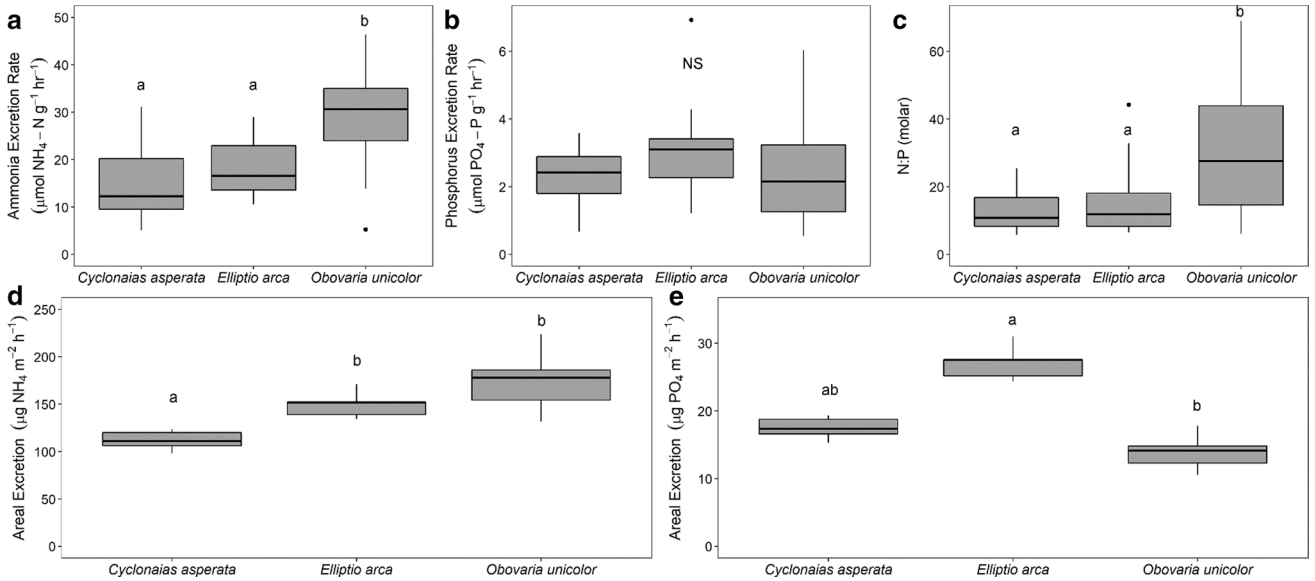

Fig. 1 Mass-specific **a** ammonia and **b** phosphorus excretion rates and **c** molar N:P excretion for our three study taxa ($N = 18$ per species). Estimated mussel biomass was used to estimate mean areal **d** ammonia and **e** phophorus excretion rates across the treatments. Different letters indicate significant differences among treatments based on Tukey's HSD and ns indicates no significant differences

Algal biomass and production

Overall, chl-a biomass on the silica discs were higher in the mussel versus control treatments (Fig. 2a; ANOVA, $F_{3,44} = 4.16$, $P = 0.01$), but did not vary between the two sampling time points (ANOVA, $F_{1,44} = 0.75$, $P = 0.79$). Only the *E. arca* treatments had significantly higher chl-a biomass than the control treatment (Tukey HSD; $P < 0.01$). When examining algal pigments on the tiles, only diatom abundances varied among the treatments (Fig. 2b; ANOVA, $F_{3,20} = 4.41$, $P = 0.02$) with the *C. asperata* treatments having higher diatom pigment biomass than the controls (Tukey HSD; $P < 0.001$). Biofilm N:P on tiles also varied (Fig. 2c; ANOVA, $F_{3,20} = 4.03$, $P = 0.02$), with *C. asperata* treatments having higher biofilm N:P. Biofilm C:N and C:P did not vary across the treatments (Appendix Fig. S2, ANOVA; $P > 0.05$). The *O. unicolor* treatment never had a significant effect on algal biomass or stoichiometry (Fig. 2a–c).

GPP on the silica discs was higher in all of the mussel species treatments than in the controls (Fig. 3a; ANOVA, $F_{3,40} = 4.37$, $P < 0.01$) and was higher during the first sampling event in comparison to the second sampling event (Tukey HSD; $P = 0.001$). ER did not significantly vary across the treatments or across the two sampling points (Fig. 3b; ANOVA, $P > 0.05$). Similar to GPP, NEP also varied across the treatments (Fig. 3c; ANOVA, $F_{3,40} = 5.66$, $P = 0.002$) and across the two sampling points (ANOVA, $F_{1,40} = 12.03$, $P = 0.001$), with all mussel treatments having higher rates of NEP during the first sampling period (Tukey HSD; $P < 0.05$).

Decomposition

The cotton strips that were deployed in the mesocosms had greater loss in tensile strength in the *C. asperata* and *E. arca* tanks than the control tanks (Fig. 4a; ANOVA, $F_{3,20} = 6.71$, $P = 0.003$), while tensile strength loss was intermediate in the *O. unicolor* treatments and not different from the control or other mussel treatments (Tukey, $P > 0.1$). In addition, cotton strip C:N was significantly lower in the *C. asperata* treatments (Fig. 4b; ANOVA, $F_{3,20} = 4.65$, $P = 0.01$), but did not differ from the control in the other two mussel treatments (Tukey HSD, $P > 0.05$). Cotton strip C:P was lower in both the *C. asperata* and *E. arca* treatments, but the *O. unicolor* treatment did not vary from the control or the other two mussel treatments (Fig. 4c; ANOVA, $F_{3,20} = 4.54$, $P = 0.01$). Cotton strip N:P did not vary across the control or treatments (Fig. 4d; ANOVA, $F_{3,20} = 1.74$, $P = 0.2$).

In contrast to tensile strength, maple litter decomposition rates did not vary across mussel treatments (repeated-measures ANOVA; $F_{3,64} = 2.2$, $P = 0.1$), but *A. saccharum* litter biomass declined significantly over time at k values of 0.008–0.152 day^{-1} (Fig. 5a; $F_{1,64} = 110.2$, $P < 0.001$). Litter decomposition rates measured as k values did not differ across treatments (ANOVA, $F_{3,20} = 1.3$, $P = 0.29$). Fungal biomass on the maple litter was 50.2–172.7 mg g^{-1} detritus and varied over time (Fig. 5b; $F_{1,64} = 10.5$; $P = 0.001$), but did not vary across the treatments ($P > 0.05$). In addition, maple litter C:N (range 24.6–39.5), C:P (range 810.8–3252), and N:P (range 30.1–99.7) did not vary across the treatments or over time ($P > 0.05$ in all cases; Appendix Fig. S3).

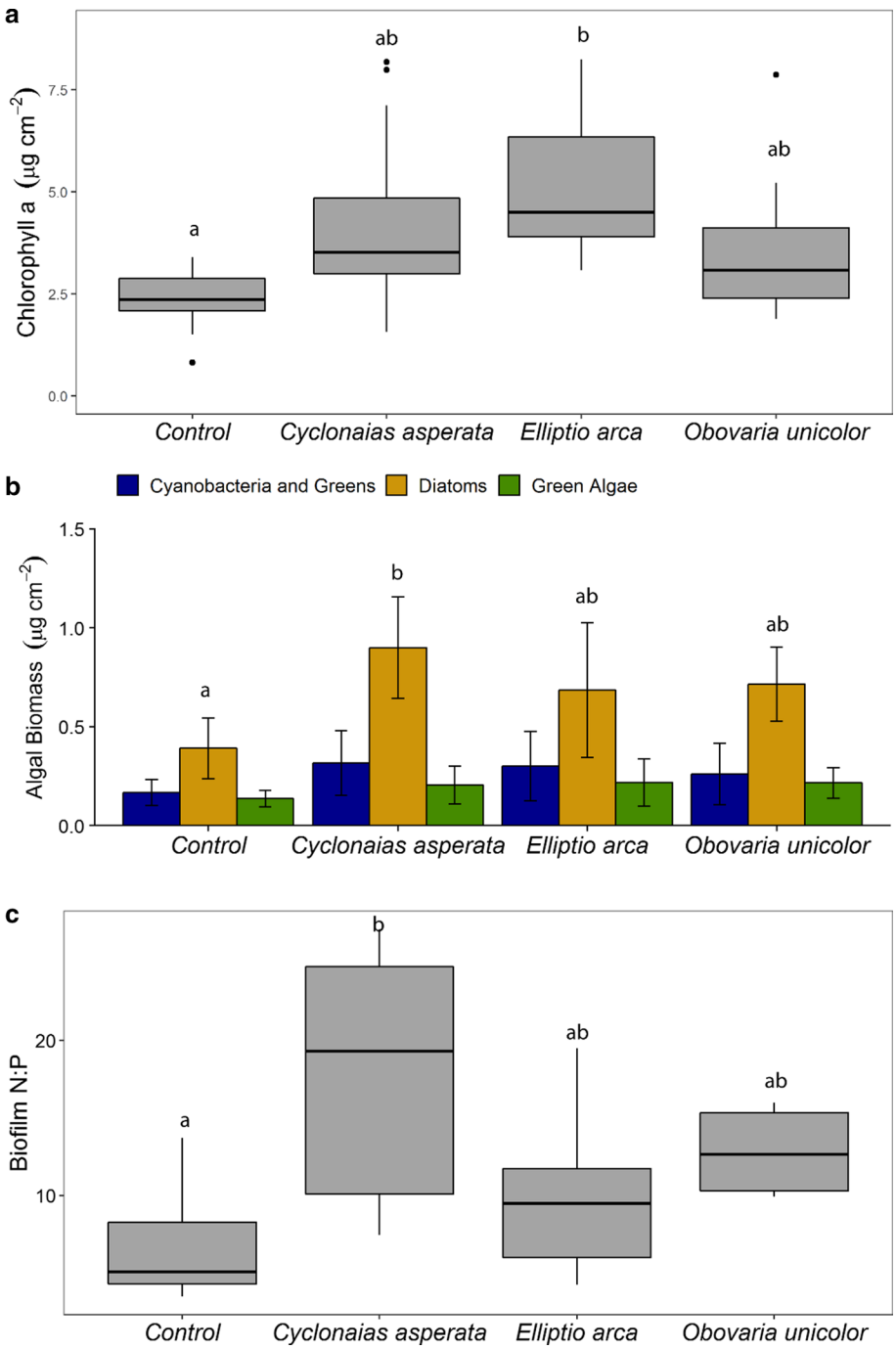

Fig. 2 **a** Chlorophyll-a biomass on glass fritts in the mesocosms across the treatments ($N = 12$ per treatment). **b** Algal biomass from tiles of the three major algal groups across our treatments as determined by HPLC ($N = 6$ per treatment). Only diatom biomass varied among treatments. **c** Algal biofilm N:P ratios from tiles placed in the mesocosms ($N = 6$ per treatment). Different letters indicate significant differences among treatments based on Tukey's HSD

Discussion

Our results show that the presence of filter-feeding consumers and their species identity play important roles in determining the magnitude and direction of ecosystem function provisioning. We found that freshwater mussels enhanced benthic algal concentrations, particularly diatoms, which led to higher gross primary production and net ecosystem production. This was particularly evident early on in the experiment as GPP and NEP were higher during the first sampling period. We also found that the presence of mussels led to higher rates of cotton strip decomposition in our mesocosms as indicated by higher tensile loss rates in two of our treatments (i.e., *Cyclonaias asperata* and *Elliptio arca*). Conversely, we saw no difference in leaf decomposition rates, fungal biomass, or leaf litter stoichiometry as a result of mussel presence. Our study indicates that ecosystem function provisioning within a single guild of filter-feeders varies based on the function of interest, indicating differential bottom-up functional effects initiated by CND.

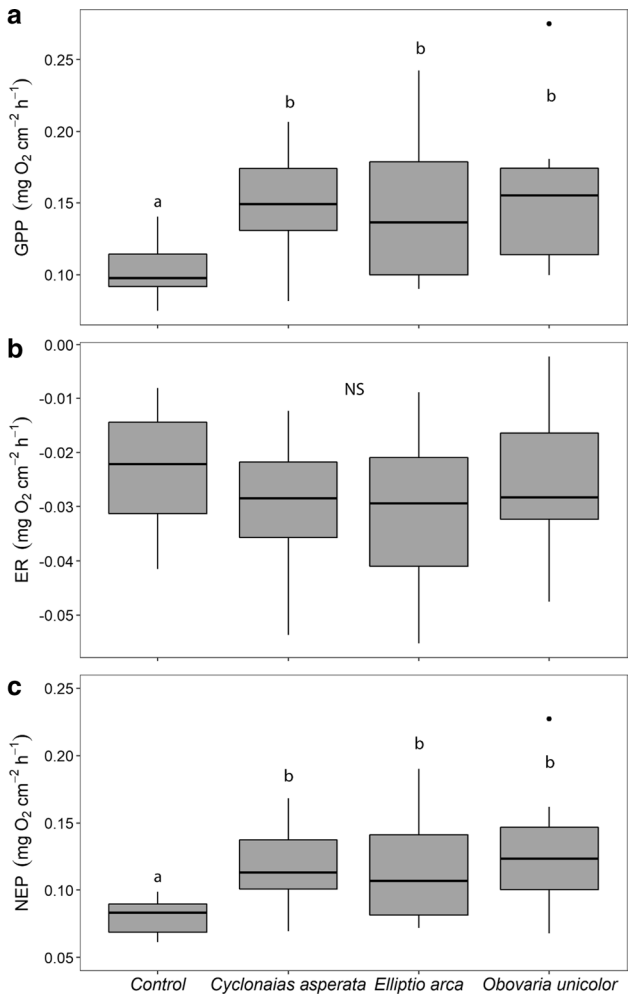

Fig. 3 **a** Gross primary production, **b** ecosystem respiration, and **c** net ecosystem production across the mesocosm treatments ($N = 12$ per treatment). Different letters indicate significant differences among treatments based on Tukey's HSD while NS indicates no significant differences

Our experimental results corroborates previous research findings indicating that the presence of mussels enhances benthic chlorophyll biomass (Spooner and Vaughn 2006; Vaughn et al. 2007) and algal production (Atkinson et al. 2018), which can fuel "green" food webs (Atkinson et al. 2014; Vaughn and Spooner 2006). Specifically, the presence of all species of mussels used in this study increased both gross primary production and net ecosystem production. However, we only observed greater algal biomass in the *E. arca* treatments, while the algae growing on the tiles indicated an increase in diatom biomass in only the *C. asperata* treatments. This suggests a disconnect between structural and functional indicators as well as differences in response based on substrate composition.

Our experiment supports a direct effect of mussel CND on "brown" food web processes, but mussel effects were only evident in cotton strip assays compared to leaf litter decomposition. These findings underscore that more research is needed to tease apart the influence of consumers, specifically filter-feeders, on brown food web processes (Vaughn and Hoellein 2018). As predicted, mussel CND enhanced cotton strip decomposition rates (measured as tensile strength) and lowered cotton strip C:N and C:P, suggesting mussels stimulated heterotrophic microbial activity and accrual of N- and P-rich biomass. However, these trends were not apparent in the leaf litter fungal biomass or decomposition responses. Mussels may have weakly affected leaf litter decomposition because heterotrophic microbes can acquire litter-derived nutrients, providing a source alternative to dissolved N and P which could be derived from mussels (Cheever et al. 2013; Manzoni et al. 2010). In contrast, cotton strips are 95% cellulose and contain minimal internal N and P to support heterotrophic microbes, which may enhance the apparent sensitivity to mussel CND. Algae may have also differently affected the brown food web responses, by accumulating on decomposing material, suppressing the accumulation of fungal biomass, and slowing decomposition rates by directly adding biomass (Halvorson et al. 2019; Kuehn et al. 2014). Indeed, mussel treatments had higher benthic algae concentrations which may have led to overall biomass accrual on the leaves, slowing the overall rate of mass loss and also reducing fungal biomass and degradative activity, a key driver of leaf decomposition consistent with a negative algal 'priming' effect facilitated by mussels (Halvorson et al. 2019; Romaní et al. 2006). In contrast, cotton strip decomposition may have been enhanced in our mussel treatments because decomposition was measured as tensile strength, and thus was not directly affected by mussel-stimulated accrual of algal biomass on the strips. Despite weak treatment effects on litter decomposition, both cotton strip and leaf litter decomposition rates in our study were similar to values reported from natural streams (Gulis and Suberkropp 2003a, b; Tiegs et al. 2013; Webster and Benfield 1986) and span a gradient representing forested and agricultural streams (Paul et al. 2006). While biodeposition rates did not vary across the species studied here, mussel biodeposits also likely play a strong role in fueling the brown food web as the nutrient release can be more substantial than excretion and can enhance microbial activity and serve as a potential food sources for other invertebrates (Wotton and Malmqvist 2001; Halvorson et al. 2017; Halvorson and Atkinson 2019) Based on our results, further work should investigate how CND may affect multiple measures of heterotrophic activity, heterotrophic acquisition of N and P from organic matter, autotrophic interactions with heterotrophs and mussel effects on other macroinvertebrates that play strong roles in the brown food web.

We noted different stoichiometric responses in our functional metrics. In particular, the presence of mussels resulted

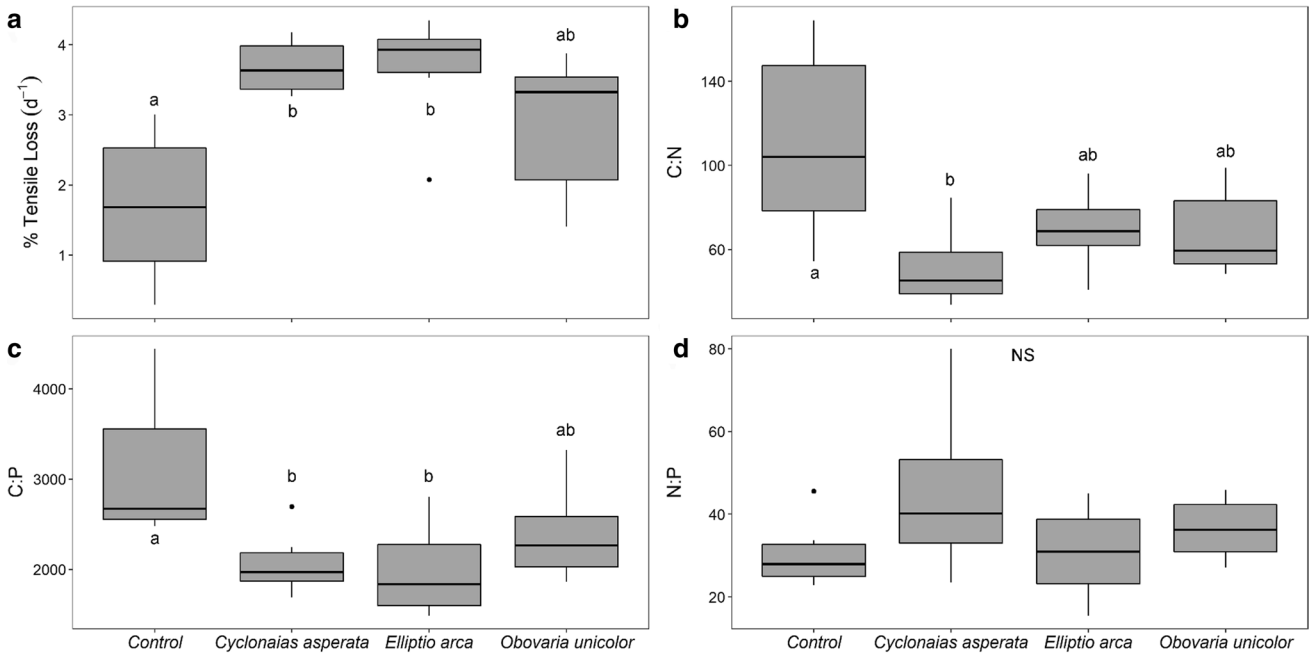

Fig. 4 **a** Cotton strip percent tensile loss and the **b** C:N, **c** C:P, and **d** N:P of the cotton strips across all treatments ($N=6$ per treatment). Different letters indicate significant differences among treatments based on Tukey's HSD while NS indicates no significant differences

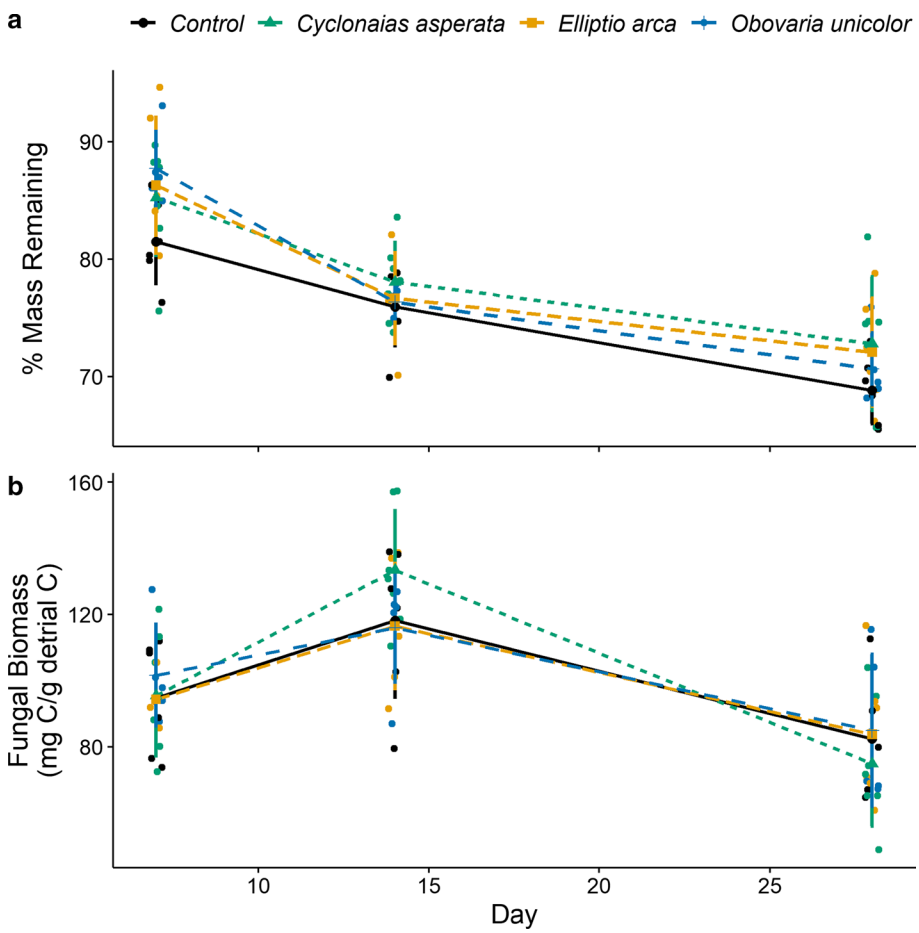

Fig. 5 **a** The percent maple (*Acer saccharum*) litter remaining in comparison to the initial leaf mass over time in each of the treatments ($N=6$ per treatment per day). **b** Fungal biomass over time in the leaf packs in each of the treatments ($N=6$ per treatment per day). Each point represents a treatment and the bars represent the mean (± standard error). There were no significant differences among the treatments

in higher biofilm N:P primarily as a result of algal %N being higher in the mussel treatments, but it did not track mussel species-specific excretion N:P as anticipated. Previous work on tadpoles has shown that the bottom-up effects of consumers increased both biofilm and particulate organic matter N:P (Rugenski et al. 2012). Cotton strips had lower C:N and C:P values in the presence of mussels with %N and %P being higher in mussel treatments, but cotton strip N:P did not respond. The observed reduction in cotton strip C:N and C:P suggests that the mussel excretion enhanced both dissolved N and P availability. However, maple litter stoichiometry was not different across our treatments. Our experiment may have been too short for a nutrient response on the leaf litter as previous research examining algal priming has shown a stoichiometric response in litter is lagged (Halvorson et al. 2016). Our results suggest that biofilms may be more sensitive to CND on surficial surfaces (e.g. tiles) than when they grow on organic substrates (e.g., leaf litter) that contain internal N and P (Cheever et al. 2013; Manzoni et al. 2010).

While all freshwater bivalves are commonly classified within the same guild or functional feeding group (i.e., filter-feeding bivalves), they can vary dramatically in their excretion stoichiometries (Atkinson et al. 2018; Atkinson and Vaughn 2015) and resulting ecosystem-level effects (Atkinson et al. 2013; Spooner et al. 2013). As shown here, these species vary in overall excretion rates and to some degree excretion stoichiometry, which can exert varying bottom-up effects depending on nutrient limitation status. The two species that we observed having the strongest effects on both green and brown food webs here, *Cyclonaias asperata* and *Elliptio arca*, both have lower excretion N:P ratios suggesting that the higher availability of P relative to N may have elicited a stronger affect. Our work generally shows that there is a positive bottom-up effect on the green food web and varying impacts to the brown food web, which has implications for other primary consumer groups. For example, previous studies have indicated that bottom-up provisioning by freshwater mussels positively influences aquatic insect abundances (Howard and Cuffey 2006; Spooner and Vaughn 2006; Vaughn and Spooner 2006), which can be traced up the food web (Allen et al. 2012; Atkinson et al. 2014). Our study is among one of the first to link bottom-up provisioning by consumers, particularly filter-feeders, to the brown food web. However, our study only employed single-species treatments while freshwater mussels typically occur as multi-species assemblages. Given the mussel species studied here typically occur together in dense aggregations (Haag and Warren 2010) and have distinct stoichiometric niches (Atkinson et al. 2020a), some of our results may be due to a reduction in trait complementarity within treatments (Loreau and Hector 2001; Spooner et al. 2012). A related study that examined single and multispecies communities of mussels showed that multispecies communities enhanced *Justicia americana* litter decomposition more than single-species treatments (Nickerson et al. *Accepted*). More studies need to be conducted to examine the role of multi-species assemblages of filter-feeding consumers on decomposition processes, particularly using various species of natural leaf litter that vary in recalcitrance while studying the decomposer community (i.e., both microbial and macroinvertebrate shredders).

Our study highlights the need for multiple metrics of ecosystem function to be employed to understand the bottom-up effects of animals giving us a more holistic view of ecosystems. Collectively, autochthonous and allochthonous resources fuel stream food webs. While excretion tends to enhance the quality of both green and brown food webs, there are contrasting effects on resource quantity because nutrients stimulate autotroph growth, while nutrients increase decomposition rates and therefore reduce resource quantity in brown food webs (Manning et al. 2016; Rosemond et al. 2015). While organic matter budgets have been constructed to examine the role of autochthonous or allochthonous resources in stream energetics, few studies have explicitly determined the interactions between these two food webs (green and brown) and how animal consumers both enhance and utilize these basal resources (but, see Wolkovich et al. 2014). Future work should aim to disentangle the role of consumer-driven nutrient cycling on algal accrual and algal-mediated priming and heterotrophic activity and the resulting impacts to secondary production.

Acknowledgements This work would not have been possible without assistance from Brian van Ee, Lucas Edgren, Maya Owens-Freeman, Stephanie Koury, Tori Hebert and Jane Kundberger. We appreciate comments and advice on previous versions of this manuscript from Garrett Hopper, Arial Shogren and Irene Sanchez Gonzalez. The Alabama Department of Natural Resources and Conservation and the National Science Foundation DEB-1831512 provided support for this project. Freshwater mussel collection was conducted under ALCDNR Permit # 2016077745468680.

Author contribution statement CLA and MNW conceived and designed the experiments. CLA, MW, and AH performed the experiments. MW conducted the chlorophyll-a analysis. CLA conducted the algae stoichiometry analysis. HMH and KAK conducted the ergosterol and leaf litter stoichiometry analysis. MNW conducted HPLC analysis. CLA performed the statistical analysis. CLA and HMH wrote the manuscript with all other authors providing editorial advice.

References

Allen DC, Vaughn CC, Kelly JF, Cooper JT, Engel M (2012) Bottom-up biodiversity effects increase resource subsidy flux between ecosystems. Ecology 93:2165–2174

Allgeier JE, Yeager LA, Layman CA (2013) Consumers regulate nutrient limitation regimes and primary production in seagrass ecosystems. Ecology 94:521–529
Atkinson CL, Vaughn CC (2015) Biogeochemical hotspots: temporal and spatial scaling of the impact of freshwater mussels on ecosystem function. Freshw Biol 60:563–574
Atkinson CL, Vaughn CC, Forshay KJ, Cooper JT (2013) Aggregated filter-feeding consumers alter nutrient limitation: consequences for ecosystem and community dynamics. Ecology 94:1359–1369
Atkinson CL, Kelly JF, Vaughn CC (2014) Tracing consumer-derived nitrogen in riverine food webs. Ecosystems 17:485–496
Atkinson CL, Capps KA, Rugenski AT, Vanni MJ (2017) Consumer-driven nutrient dynamics in freshwater ecosystems: from individuals to ecosystems. Biol Rev 92:2003–2023
Atkinson CL, Sansom BJ, Vaughn CC, Forshay KJ (2018) Consumer aggregations drive nutrient dynamics and ecosystem metabolism in nutrient-limited systems. Ecosystems 21:521–535
Atkinson CL, van Ee BC, Pfeiffer J (2020b) Evolutionary history drives aspects of stoichiometric niche variation and functional effects within a guild. Ecology 101:e03100
Atkinson CL et al (2020a) Length-mass equations for freshwater unionid mussel assemblages: implications for estimating ecosystem function. Freshw Sci 39:377–390
Barlocher F (2005) Freshwater fungal communities. Mycol Ser 23:39
Buchkowski RW, Leroux SJ, Schmitz OJ (2019) Microbial and animal nutrient limitation change the distribution of nitrogen within coupled green and brown food chains. Ecology 100:e02674
Cheever BM, Webster JR (2014) Effects of consumers and nitrogen availability on heterotrophic microbial activity during leaf decomposition in headwater streams. Freshw Biol 59:1768–1780
Cheever BM, Webster JR, Bilger EE, Thomas SA (2013) The relative importance of exogenous and substrate-derived nitrogen for microbial growth during leaf decomposition. Ecology 94:1614–1625
Covich AP, Palmer MA, Crowl TA (1999) The role of benthic invertebrate species in freshwater ecosystems—zoobenthic species influence energy flows and nutrient cycling. Bioscience 49:119–127
Cross WF, Wallace JB, Rosemond AD, Eggert SL (2006) Whole-system nutrient enrichment increases secondary production in a detritus-based ecosystem. Ecology 87:1556–1565
Doughty CE et al (2016) Global nutrient transport in a world of giants. Proc Natl Acad Sci 113:868–873
Evans-White MA, Halvorson HM (2017) Comparing the ecological stoichiometry in green and brown food webs—a review and meta-analysis of freshwater food webs. Front Microbiol 8:1184
Findlay SE, Dye S, Kuehn KA (2002) Microbial growth and nitrogen retention in litter of *Phragmites australis* compared to *Typha angustifolia*. Wetlands 22:616–625
Gulis V, Suberkropp K (2003b) Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. Freshw Biol 48:123–134
Gulis V, Suberkropp K (2003a) Interactions between stream fungi and bacteria associated with decomposing leaf litter at different levels of nutrient availability. Aquat Microb Ecol 30:149–157
Haag WR, Warren ML (2010) Diversity, abundance, and size structure of bivalve assemblages in the Sipsey River, Alabama. Aquat Conserv Mar Freshw Ecosyst 20:655–667
Hall RO, Tank JL, Dybdahl MF (2003) Exotic snails dominate nitrogen and carbon cycling in a highly productive stream. Front Ecol Environ 1:407–411
Halvorson HM, Atkinson CL (2019) Egestion versus excretion: a meta-analysis examining nutrient release rates and ratios across freshwater fauna. Diversity 11:189
Halvorson HM, Scott EE, Entrekin SA, Evans-White MA, Scott JT (2016) Light and dissolved phosphorus interactively affect microbial metabolism, stoichiometry and decomposition of leaf litter. Freshw Biol 61:1006–1019
Halvorson HM, Hall DJ, Evans-White MA (2017) Long-term stoichiometry and fates highlight animal egestion as nutrient repackaging, not recycling, in aquatic ecosystems. Funct Ecol 31:1802–1812
Halvorson HM, Barry JR, Lodato MB, Findlay RH, Francoeur SN, Kuehn KA (2019) Periphytic algae decouple fungal activity from leaf litter decomposition via negative priming. Funct Ecol 33:188–201
Harpole WS et al (2011) Nutrient co-limitation of primary producer communities. Ecol Lett 14:852–862
Howard JK, Cuffey KM (2006) The functional role of native freshwater mussels in the fluvial benthic environment. Freshw Biol 51:460–474
Jabiol J, Cornut J, Tlili A, Gessner MO (2018) Interactive effects of dissolved nitrogen, phosphorus and litter chemistry on stream fungal decomposers. FEMS Microbiol Ecol 94:fly151
Kuehn KA, Francoeur SN, Findlay RH, Neely RK (2014) Priming in the microbial landscape: periphytic algal stimulation of litter-associated microbial decomposers. Ecology 95:749–762
Loreau M, Hector A (2001) Partitioning selection and complementarity in biodiversity experiments. Nature 412:72–76
Manning DW, Rosemond AD, Gulis V, Benstead JP, Kominoski JS, Maerz JC (2016) Convergence of detrital stoichiometry predicts thresholds of nutrient-stimulated breakdown in streams. Ecol Appl 26:1745–1757
Manzoni S, Trofymow JA, Jackson RB, Porporato A (2010) Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter. Ecol Monogr 80:89–106
McIntyre PB, Flecker AS, Vanni MJ, Hood JM, Taylor BW, Thomas SA (2008) Fish distributions and nutrient cycling in streams: can fish create biogeochemical hotspots? Ecology 89:2335–2346
McNaughton S, Ruess R, Seagle S (1988) Large mammals and process dynamics in African ecosystems. Bioscience 38:794–800
McNaughton S, Banyikwa F, McNaughton M (1997) Promotion of the cycling of diet-enhancing nutrients by African grazers. Science 278:1798–1800
Moore JC et al (2004) Detritus, trophic dynamics and biodiversity. Ecol Lett 7:584–600
Nickerson ZL, Green PAM, Shouse LN, Atkinson CL (accepted) Enhanced species richness of functionally similar stream consumers results in higher ecological function. Freshw Sci
Parr TB, Capps KA, Inamdar SP, Metcalf KA (2019) Animal-mediated organic matter transformation: aquatic insects as a source of microbially bioavailable organic nutrients and energy. Funct Ecol 33:524–535
Paul MJ, Meyer JL, Couch CA (2006) Leaf breakdown in streams differing in catchment land use. Freshw Biol 51:1684–1695
Ramamonjisoa N, Natuhara Y (2018) Contrasting effects of functionally distinct tadpole species on nutrient cycling and litter breakdown in a tropical rainforest stream. Freshw Biol 63:202–213
Roman J, McCarthy JJ (2010) The whale pump: marine mammals enhance primary productivity in a coastal basin. PLoS ONE 5:e13255
Romaní AM, Fischer H, Mille-Lindblom C, Tranvik LJ (2006) Interactions of bacteria and fungi on decomposing litter: differential extracellular enzyme activities. Ecology 87:2559–2569
Rosemond AD et al (2015) Experimental nutrient additions accelerate terrestrial carbon loss from stream ecosystems. Science 347:1142–1145
Rugenski AT, Murria C, Whiles MR (2012) Tadpoles enhance microbial activity and leaf decomposition in a neotropical headwater stream. Freshw Biol 57:1904–1913

Sitters J, Bakker ES, Veldhuis MP, Veen GF, Olde Venterink H, Vanni MJ (2017) The stoichiometry of nutrient release by terrestrial herbivores and its ecosystem consequences. Front Earth Sci 5:32

Solorzano L, Sharp JH (1980) Determination of total dissolved phosphorus and particulate phosphorus in natural waters. Limnol Oceanogr 25:754–757

Spooner DE, Vaughn CC (2006) Context-dependent effects of freshwater mussels on stream benthic communities. Freshw Biol 51:1016–1024

Spooner DE, Vaughn CC, Galbraith HS (2012) Species traits and environmental conditions govern the relationship between biodiversity effects across trophic levels. Oecologia 168:533–548

Spooner DE, Frost PC, Hillebrand H, Arts MT, O. P, Xenopoulos MA, (2013) Nutrient loading associated with agriculture land use dampens the importance of consumer-mediated niche construction. Ecol Lett 16:1115–1125

Suberkropp K, Gulis V, Rosemond AD, Benstead JP (2010) Ecosystem and physiological scales of microbial responses to nutrients in a detritus-based stream: results of a 5-year continuous enrichment. Limnol Oceanogr 55:149–160

Tank JL, Dodds WK (2003) Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. Freshw Biol 48:1031–1049

Tiegs SD, Clapcott JE, Griffiths NA, Boulton AJ (2013) A standardized cotton-strip assay for measuring organic-matter decomposition in streams. Ecol Ind 32:131–139

Turner JT (2015) Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump. Prog Oceanogr 130:205–248

van der Waal C et al (2011) Large herbivores may alter vegetation structure of semi-arid savannas through soil nutrient mediation. Oecologia 165:1095–1107

Vaughn CC, Hakenkamp CC (2001) The functional role of burrowing bivalves in freshwater ecosystems. Freshw Biol 46:1431–1446

Vaughn CC, Hoellein TJ (2018) Bivalve impacts in freshwater and marine ecosystems. Annu Rev Ecol Evol Syst 49:183–208

Vaughn CC, Spooner DE (2006) Unionid mussels influence macroinvertebrate assemblage structure in streams. J N Am Benthol Soc 25:691–700

Vaughn CC, Spooner DE, Galbraith HS (2007) Context-dependent species identity effects within a functional group of filter-feeding bivalves. Ecology 88:1654–1662

Webster J, Benfield E (1986) Vascular plant breakdown in freshwater ecosystems. Annu Rev Ecol Syst 17:567–594

Williams JD, Bogan AE, Garner JT (2008) Freshwater mussels of Alabama and the mobile basin in Georgia, Mississippi, and Tennessee. University of Alabama Press, Tuscaloosa

Wolkovich EM, Allesina S, Cottingham KL, Moore JC, Sandin SA, de Mazancourt C (2014) Linking the green and brown worlds: the prevalence and effect of multichannel feeding in food webs. Ecology 95:3376–3386

Wotton RS, Malmqvist B (2001) Feces in aquatic ecosystems. Bioscience 51:537–544

Zou K, Thébault E, Lacroix G, Barot S (2016) Interactions between the green and brown food web determine ecosystem functioning. Funct Ecol 30:1454–1465