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Species identity and diversity of filter-feeding bivalves impact green and brown food webs

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Abstract

1. In freshwater ecosystems, consumers can play large roles in nutrient cycling by modifying nutrient availability for autotrophic and heterotrophic microbes. Nutrients released by consumers directly support *green food webs* based on primary production and *brown food webs* based on decomposition. While much research has focused on impacts of consumer driven nutrient dynamics on green food webs, less attention has been given to studying the effects of these dynamics on brown food webs.
2. Freshwater mussels (Bivalvia: Unionidae) can dominate benthic biomass in aquatic systems as they often occur in dense aggregations that create biogeochemical hotspots that can control ecosystem structure and function through nutrient release. However, despite functional similarities as filter-feeders, mussels exhibit variation in nutrient excretion and tissue stoichiometry due in part to their phylogenetic origin. Here, we conducted a mesocosm experiment to evaluate how communities of three phylogenetically distinct species of mussels individually and collectively influence components of green and brown food webs.
3. We predicted that the presence of mussels would elicit a positive response in both brown and green food webs by providing nutrients and energy via excretion and biodeposition to autotrophic and heterotrophic microbes. We also predicted that bottom-up provisioning of nutrients would vary among treatments as a result of stoichiometric differences of species combinations, and that increasing species richness would lead to greater ecosystem functioning through complementarity resulting from greater trait diversity.
4. Our results show that mussels affect the functioning of green and brown food webs through altering nutrient availability for both autotrophic and heterotrophic microbes. These effects are likely to be driven by phylogenetic constraints on tissue nutrient stoichiometry and consequential excretion stoichiometry, which can have functional effects on ecosystem processes. Our study highlights the importance of measuring multiple functional responses across a gradient of diversity in ecologically similar consumers to gain a more holistic view of aquatic food webs.

KEYWORDS
decomposition, ergosterol, freshwater mussels, nutrient excretion, stoichiometry

1 | INTRODUCTION

In stream ecosystems, spatial hotspots of nutrient transformations are generally attributed to physical and microbial processes (Bernhardt et al., 2017; McClain et al., 2003). Microbes mediate nutrient processing and drive organic matter decomposition and nutrient flows to higher trophic levels (Meyer, 1994; Zeglin, 2015). However, within the past few decades, research has increasingly shown that animal consumers play a large role in driving nutrient dynamics, especially in freshwater systems (Atkinson et al., 2017; Vanni, 2002). These consumer-driven nutrient dynamics are extensive and include direct mechanisms such as the physical transformation of nutrients from one form to another via nutrient excretion and egestion, as well as indirect effects that regulate ecosystem processes such as enhancing primary productivity and decomposition (Atkinson et al., 2017).

Biogeochemical hotspots created by animals can fuel both *green food webs* (i.e., primary producers) and *brown food webs* (i.e., decomposers) with their excretion of nitrogen (N) and phosphorus (P). Previous work demonstrates that these green and brown food web effects are important pathways that maintain ecosystem stability and function (Mougi, 2020; Zou et al., 2016). Attention has focused on the cycling of N and P because they are the nutrients most likely to limit primary producers and perhaps heterotrophic microbes (Atkinson et al., 2017; Vanni, 2002). Direct excretion of N and P by animals affects the supply of bioavailable nutrients to producers and may result in differences in nutrient limitation patterns (Allgeier et al., 2013; Atkinson et al., 2013; Elser & Urabe, 1999; Hopper et al., 2018; McIntyre et al., 2008; Sterner, 1990).

Freshwater mussels (Bivalvia: Unionoida) are a guild of benthic, burrowing, filter-feeding bivalves that occur in dense aggregations in many freshwater systems (Haag, 2012; Vaughn & Hakenkamp, 2001) and regulate the aquatic environment through their bottom-up effects on nutrient cycling by acting as benthic–pelagic couplers (Atkinson et al., 2018; Atkinson & Vaughn, 2015). Mussels filter-feed across trophic levels and take in bacteria, algae, detritus, zooplankton, and dissolved organic matter (DOM) from the water column and transfer these nutrients, DOM, and energy to sediments through biodeposition of faeces and pseudofaeces (Black et al., 2017; Vaughn et al., 2008). Additionally, bioturbation of sediments through bivalve movements, increases sediment water and oxygen content and releases nutrients from the sediment to the water column, which can stimulate primary production and alleviate nutrient limitation (Vaughn & Hakenkamp, 2001). As a result of these processes and the spatial heterogeneity of their aggregations, mussels can exert control over ecosystem structure and function via generating biogeochemical hotspots (Atkinson & Forshay, 2022; Atkinson & Vaughn, 2015).

Although freshwater mussels are classified in a single functional group, different species within the same system exhibit variation in diet, nutrient excretion rates, and stoichiometry (Atkinson & Forshay, 2022; Sánchez González et al., 2023; Spooner & Vaughn, 2008; Vaughn, 2010). Differences in functional traits and effects have been linked to phylogeny across various organisms (Allgeier et al., 2021), including mussels (Atkinson, van Ee, & Pfeiffer, 2020), and are based on the hypothesis that evolutionary processes drive trait diversification and thus enhance the functional trait space of a community (Srivastava et al., 2012). Through niche complementarity or synergistic interactions, species richness may enhance ecosystem function and stability (Hopper et al., 2023; Loreau & Mazancourt, 2013). Therefore, a better understanding of functional trait diversity is key to improving our ability to understand and predict the biodiversity effects on ecosystem stability. It is especially critical to investigate trait–environment relationships within freshwater systems, where species diversity and abundance is decreasing more rapidly than in terrestrial and marine systems (Dudgeon et al., 2006; Reid et al., 2019). Freshwater mussels are among the most threatened faunal groups globally and over 70% of North American species are classified as imperilled (Dudgeon et al., 2006; Haag & Williams, 2014). This tremendous loss of biodiversity can negatively impact stream ecosystems in a multitude of ways. For example, changes in the abundance of mussel species can alter nutrient recycling, which influences both primary producers and consumers, thus biodiversity loss extends across multiple trophic levels affecting whole food webs (Vaughn, 2010).

Despite recent increased attention to biodiversity–ecosystem function relationships (Loreau & Mazancourt, 2013; Srivastava & Vellend, 2005), the congruence between phylogenetic and functional diversity is not well understood. Here, we use a mesocosm approach to investigate how phylogenetically diverse mussel communities influence ecosystem functioning including *green* and *brown* food web responses. These ecosystem functions have a large impact on the flow of energy and nutrients in stream ecosystems, thus providing a framework to investigate the influence of mussel aggregations on large-scale ecosystem responses. We hypothesised that: (1) bottom-up provisioning of N and P via excretion and egestion will vary among mussel treatments; (2) compared to no-mussel controls, the presence of mussels will elicit a positive response in both brown and green food webs; and (3) increased species richness will lead to greater ecosystem functioning (e.g., increased algal accrual, faster litter decomposition) as a result of greater trait diversity constrained by evolutionary history.

2 | MATERIALS AND METHODS

2.1 | Study design

2.1.1 | Study organisms

Mussels, sediment, and water were collected from the Sipsey River, a fifth-order tributary of the Tombigbee River in Alabama. The Sipsey River is relatively unmodified by human disturbances and harbours diverse and abundant communities of freshwater mussels (Haag & Warren, 2010). We focused on three abundant, well-studied

species in the Sipsey River: *Cyclonaias kieneriana* (Tribe Quadrulini), *Fusconaia cerina* (Tribe Pleurobemini), and *Lampsilis ornata* (Tribe Lampsilini; Table 1). These three species vary in shell morphology, life-history traits, and tissue stoichiometry (Atkinson, van Ee, & Pfeiffer, 2020; Haag, 2012; Williams et al., 2008).

We collected 183 mussels on 20–22 November 2019, from the Sipsey River and transported them back to the lab where they were held in Living Stream Systems® (Frigid Units Inc., Toledo, Ohio). Each mussel was measured, and standardised length–dry mass regressions were used to estimate dry mass (Atkinson, Parr, et al., 2020). We tagged each mussel with an individual identification tag using a piece of 15 cm long fly line attached to the posterior end of one valve of each mussel. Individual mussels of each species were randomly assigned to each of the eight treatment groups (total $n = 32$). Mussels were held at 10°C in Living Stream Systems® until the beginning of the experiment and fed with cultured algae three times weekly.

We manipulated mussel diversity by creating four replicates of eight treatments: (1) three single-species treatments (*L. ornata*, *C. kieneriana*, *F. cerina*; hereafter L, C, F); (2) three two-species treatments (*L. ornata* + *C. kieneriana*, *L. ornata* + *F. cerina*, *C. kieneriana* + *F. cerina*; hereafter LC, LF, CF); (3) one three-species treatment (*L. ornata* + *C. kieneriana* + *F. cerina*; hereafter LCF); and (4) one control treatment (no mussels; hereafter NM; Table 1). We implemented a substitutive design in which each treatment contained the same biomass of mussels (ANOVA $F_{6,21} = 0.87$, $p = 0.53$). Due to differences in mussel body sizes, it was necessary to stock mesocosms with different densities (5–10 mussels/mesocosm) to achieve the target biomass. At the start of the study, we randomly assigned treatments to each mesocosm.

2.1.2 | Mesocosm design

The 42-day mesocosm study was conducted in the greenhouse on the main campus of the University of Alabama in Tuscaloosa, AL from January to March 2020. We used 200-L recirculating stream mesocosms (81 × 51 × 48 cm) with an open-ended plastic liner placed inside a fibreglass outer tank. The inner liner was placed atop bricks to allow water recirculation as in (Nickerson et al., 2021) and the bed of the liner was filled with a mix of pea gravel and sand from the Sipsey River and supplemented with purchased pea gravel (Vigoro® Pea Gravel Pebbles). Each mesocosm was filled to a depth of 35 cm with water from the Sipsey River and used 47-W magnetic drive pumps (Dammer Mfg, Islandia, NY, U.S.A.) to recirculate the water at a rate of 2,527 L h^{-1}. We placed each pump atop the pea gravel at one end of the mesocosm so that the orientation of the pump caused water to be drawn up from the space between the tank and the internal liner and flow directly over the pea gravel. We conducted weekly water changes (15%) in which we emptied 20 L of water from each tank and replaced it with 20 L of Sipsey River water weekly for the duration of the experiment. In addition, each tank received 500 ml of a concentrated mixed algal assemblage (*c.* 750 mg chlorophyll-a [chl-a]/L) cultured from Sipsey River water twice every week. We monitored water temperature in 12 mesocosms using temperature loggers that collected records every 60 min (Hobo U20L, Onset Corp, Bourne, MA), and while water temperature varied over the course of the experiment, it did not vary between treatments (Figure S1). We sampled and filtered water samples for background nutrient concentrations on days 0, 14, 28, and 41 (Figure S2) and used standard methods on a Seal AQ300 discrete analyser (Seal Analytical, Mequon, Wisconsin, U.S.A.).

Treatment code	Species composition	
L	*Lampsilis ornata*	 *Lampsilis ornata* Tribe: Lampsilini
C	*Cyclonaias kieneriana*	*Lampsilis ornata* Tribe: Lampsilini
F	*Fusconaia cerina*	 *Cyclonaias kieneriana* Tribe: Quadrulini
LC	*Lampsilis ornata* + *Cyclonaias kieneriana*	
LF	*Lampsilis ornata* + *Fusconaia cerina*	*Cyclonaias kieneriana* Tribe: Quadrulini
CF	*Cyclonaias kieneriana* + *Fusconaia cerina*	 *Fusconaia cerina* Tribe: Pleurobemini
LCF	*Lampsilis ornata* + *Cyclonaias kieneriana* + *Fusconaia cerina*	
NM	No-mussel control	*Fusconaia cerina* Tribe: Pleurobemini

TABLE 1 Treatment code abbreviations for the mesocosm experiment and an illustration of each species (Illustration Credit: Kelly Lambert).

2.1.3 | Leaf pack assembly

To facilitate a natural decomposer microbial community in the mesocosms, we assembled 32 mixed leaf litter bags containing approximately 1.5 g of leaf tissue of *Liriodendron tulipifera* (tulip poplar), *Platanus occidentalis* (American sycamore), and *Salix nigra* (black willow), common riparian trees along the Sipsey River, and left them in the river for 12 days. Five days before mussels were added to the mesocosms, we added one of these pre-conditioned mixed-species litter bags to inoculate each mesocosm.

To assess the effect of mussel communities on leaf litter decomposition rates (i.e., brown food web), we assembled litter bags filled with dried leaves of *L. tulipifera*. Leaves that were freshly fallen and had no signs of herbivory were collected October–November 2019 and oven-dried at 40°C for 24 hr. Dried leaves were weighed, and 3.0–3.2 g placed in each of 96 20-×15-cm fibreglass mesh litter bags with 1×1.5 mm size mesh. Exact tissue weights and leaf species were labelled on aluminium tags and attached to bags via zip-ties. Three bags were secured together and placed in each mesocosm and sampled at weeks one, three, and six after the addition of mussels.

2.1.4 | Measuring consumer driven nutrient cycling

At the end of the experiment, we estimated nitrogen and phosphorus excretion for each community by randomly subsampling six mussels of each species and measuring their NH_4^+ (N) and soluble reactive phosphorus (P) excretion rates. Using a toothbrush and scour pad, we gently scrubbed each mussel to remove attached biofilm and algae and then placed them in separate excretion chambers. Depending on mussel size, we filled each container with 300 or 500 ml of filtered mesocosm water (GF/F; 0.7 μm pore size; Millipore). Three control containers without bivalves were incubated simultaneously to control for biofilm uptake. After 1 hr, we removed each mussel, filtered the chamber water (GF/F; 0.7 μm pore size; Millipore), and stored 30 ml of the sample at −20°C until analysis. Filters were retained to estimate biodeposition rates (mg dry mass [DM]/hr). We used a Seal AQ300 discrete analyser (Seal Analytical) to analyse P using the colorimetric method (Murphy & Riley, 1962) and N using the phenol method for filtered excretion samples. Mass-specific excretion rates for each mussel (μmol NH_4^+ h^{-1} g^{-1}) were calculated using the concentration of NH_4^+ in the excretion chamber (mg/L), the known volume of water in the chamber, and the amount of time the mussel incubated (1 hr) after correcting for the controls. Solid material collected on the filters was used to calculate mussel biodeposition rates (mg/hr). The filters were dried for 48 h at 50°C, weighed on an analytical balance, then combusted at 500°C for 2 hr and weighed again to calculate ash-free dry mass and the total organic matter of the biodeposits.

We used mass-specific hourly rates of excretion and biodeposition to estimate the areal community-scale rates for each of our mesocosm treatments. Following methods similar to (Atkinson & Forshay, 2022), we calculated areal excretion rates of N and P (μmol nutrient $m^{-2}hr^{-1}$) and biodeposition of C, N, and P (μmol nutrient $m^{-2}h^{-1}$) by multiplying the species-specific population biomass (i.e. mesocosm) by the per capita excretion or biodeposition rate summed across all species for each treatment.

2.2 | Green food web

2.2.1 | Benthic and leaf litter algal accrual

At the onset of the experiment, we placed four ceramic tiles (25.81 cm^2) in each mesocosm, three of which had silica discs (0.424 cm^2, Leco Corp., St. Joseph, MI, U.S.A.) attached with waterproof epoxy. The discs served as a proxy for benthic algal accrual in a mesocosm as the surface area of the discs allowed algae to colonise in a similar fashion as on the pea gravel substrate. A silica disc was removed at the end of 2, 4, and 6 weeks for determination of benthic chl-a concentration. Prior to preserving them for chl-a analysis, we gently removed the silica disc from the ceramic tile and placed it into a 50-ml centrifuge tube filled completely with filtered mesocosm water and measured the initial temperature and dissolved oxygen (DO) concentrations following the methods of Tank et al. (2006). We then placed the tubes in a light incubator for 2 h. Following incubation, temperature and DO were measured again and tubes were incubated for another 2 h in the dark. We used the change in DO between the light and dark incubations to estimate gross primary production (GPP), ecosystem respiration, and net ecosystem production on the benthic substrate. We then wrapped the discs individually in foil and immediately froze them in the dark at −20°C until analysis using methods similar to Atkinson et al. (2021). 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, we returned the samples to room temperature and analysed the extraction solution for [chl-a] on a Genesys 10S UV/Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, U.S.A.). Benthic algal accrual (μg/cm) was estimated by dividing the concentration of chl-a (μg/ml) by the known surface area of the silica disc (0.424 cm^2).

After 8 days and 3 and 6 weeks, we collected one leaf pack to assess algal biomass on leaf litter which was estimated using chl-a. We used a cork borer (2.01 cm^2) to cut two leaf discs (one from each leaf) which were stored in 15-ml polypropylene conical tubes (in the dark) and frozen at −20°C until analysis. Following similar methods to Kuehn et al. (2014) and Halvorson, Barry, et al. (2019), chl-a was extracted using the hot ethanol technique, where leaf discs were submerged in 90% ethanol (80°C for 5 min), steeped overnight in the dark (4°C), then quantified using high-performance liquid chromatography (HPLC).

2.3 | Brown food web

2.3.1 | Leaf decomposition

Litter bags were collected at the end of 1, 3, and 6 weeks and the litter in each pack was subsampled for O_2 uptake, microbial enzyme activity, and ergosterol production. We then oven-dried all bags at 50°C for 72 hr and weighed the contents of each bag to determine the percent mass loss. As litter bags started with a slightly different initial mass, all initial masses were standardised to 100%, and values for decomposition were reported as the percent of mass loss over time. To account for mass-loss from handling, we assembled 15 additional leaf litter bags of known leaf mass. These bags were submerged briefly in stream water, dried at 50°C for 72 hr, and the contents weighed to calculate tissue loss due to handling.

2.3.2 | Fungal biomass

To assess the quantitative importance of fungi in leaf litter decomposition, we used ergosterol as a measure of fungal biomass (Gessner, 2005). Leaves were cut using a cork borer (2.01 cm^2) and five leaf discs (one to three from each leaf) were placed in a 50-ml centrifuge tube and stored frozen (−20°C) until extraction. Samples were lyophilised, weighed, and ergosterol extracted in methanolic KOH (8 g KOH/L, HPLC-grade methanol, extraction volume 10 ml) for 30 min at 80°C. The resultant crude extract was partitioned into n-pentane and evaporated to dryness with nitrogen gas. Dried ergosterol residues were then dissolved in methanol and quantified by HPLC (Gessner, 2005). Ergosterol concentrations were converted to fungal C assuming 5 μg ergosterol/mg fungal dry mass and 43% fungal C (Findlay et al., 2002; Kuehn et al., 2014).

2.3.3 | Leaf litter C:N

Leaf litter was homogenised using a Wiley® Mini-Mill Grinder (Thomas Scientific, Swedesboro, NJ, U.S.A.) and a subsample of ground tissue (2.5–3.0 mg) analysed with a Carlo Erba CHNS-O EA1108-Elemental Analyser (Isomass Scientific Inc., Calgary, Alberta, Canada) to determine leaf litter C:N.

2.3.4 | Leaf disc respiration

We used respiration as an indicator of microbial activity on leaves and measured oxygen uptake on leaf discs pre- and post-incubation following the methods of Rugenski et al. (2012). Respiration measurements were taken at the end of 1, 3, and 6 weeks from one leaf litter bag from each mesocosm. Leaves were cut using a corer (2.01 cm^2) and five leaf discs (one to three from each leaf) were placed in a 50-ml centrifuge tube containing filtered mesocosm water (GF/F; 0.7 μm pore size; Millipore) and incubated for 90 min in the dark. There were three controls containing only filtered mesocosm water for each sampling event. Dissolved oxygen readings were recorded at 0 and 90 min using a YSI ProODO dissolved oxygen meter (Yellow Springs Instrument Co., Inc., Yellow Springs, OH, U.S.A.). We calculated respiration rates from change in dissolved oxygen concentrations between samples and controls, and final rates were calculated based on leaf disc DM (mg O_2 DM^{-1} hr^{-1}).

2.3.5 | Enzyme activity

Heterotrophic microbes degrade complex organic compounds by secreting extracellular enzymes into the environment. Therefore, the activities of these enzymes can serve as a proxy for rates of microbial nutrient cycling. We assayed the activity of three extracellular enzymes, β-glucosidase, β-N-acetylglucoaminidase (NAGase), and phosphatase as respective indicators of C, N, and P acquisition efforts by decomposer microorganisms. At the end of the 6-week experiment, tulip poplar leaves were cut into discs using a corer (2.01 cm^2) and four leaf discs were subsampled (one for each of the three enzymes, and one for a control). Enzyme assays were performed colorimetrically using p-nitrophenyl linked substrates following the procedures of Jackson et al. (2013). Briefly, each leaf disc was patted dry and added to 500 μL of the appropriate substrate for 0.5–2 hr. Following incubation, 150 μL of the reaction mixture was transferred to a 96-well microplate, where it received 10 μL of 1 M NaOH and 140 μL water. Absorbance of the resulting solution at 410 nm was measured on a BioTek Synergy 2 plate reader and absorbance converted to activity following Jackson et al. (2013).

2.4 | Data analyses

To test the effect of mussel species community composition on ecosystem responses, we used one- or two-way (ANOVA) tests with treatments, and sampling day as factors. Significant global tests were followed by a post hoc Tukey test. All statistical analyses were performed using the *stats* package in R (R Core Development Team, 2019) and data were log transformed (enzyme activity and ergosterol production) as necessary to meet assumptions of normality and homoscedasticity.

We used Hedge's *g* as a measure of effect size to directly compare the effects of mussel treatments relative to controls on ecosystem functions of differing units. To account for time, we calculated all effect sizes using measurements taken at the end of the experiment (6 weeks) and used tulip poplar leaves to compare all leaf-related responses. We calculated Hedge's *g* values using the R package *effsize* (Torchiano, 2020), where the difference between the mean treatment effect and mean control is divided by the pooled weighted standard deviation of the two treatments (Hedges, 1981). A large effect size is considered to be $-0.8 > g > 0.8$, a medium effect size is $-0.5 > g > 0.5$, and a small effect size is $-0.2 > g > 0.2$. Hedge's *g* in particular has been found to outperform other effect size analyses (such as Cohen's d) when dealing with small sample sizes (Grissom & Kim, 2005). In addition, Hedge's *g* uses pooled weighted standard deviations—compared to pooled standard deviations (Durlak, 2009).

3 | RESULTS

3.1 | Mussel excretion

There were significant differences in mass-specific N excretion rates among species (ANOVA $F_{2,15}=4.69$, $p<0.05$; Table 2, Figure S3A), with *L. ornata* having higher mass-specific N excretion rates than *F. cerina* (Tukey HSD, $p<0.05$). When scaled to the community level, there were significant differences in N areal excretion rates among treatments (ANOVA $F_{6,21}=215.36$, $p<0.0001$; Figure 1A). The L treatment had higher NH_4^+ areal excretion rates compared to all other treatments (Tukey HSD, $p<0.001$) followed by the CF treatment (Tukey HSD, $p<0.001$). The LC and LCF treatments did not differ in N areal excretion (Tukey HSD, $p=0.50$), but were greater than the F, LF, and L treatments (Tukey HSD, $p<0.0001$). The LF treatment did not differ from the F or L treatment (Tukey HSD, $p=0.09$), but the F treatment had greater areal excretion than the L treatment (Tukey HSD, $p<0.05$).

There were no differences in mass-specific P excretion rates among species (ANOVA $F_{2,15}=0.02$, $p=0.98$; Table 2, Figure S3B). However, when scaled to the community level, there were significant differences in areal excretion rates among treatments (ANOVA $F_{6,21}=40.04$, $p<0.00012$). Generally, treatments containing *C. kieneriana* had higher areal P excretion and treatments containing *L. ornata* had lower areal PO_4^{3-} areal excretion. The C and CF treatments did not differ between each other (Tukey HSD, $p=0.57$), but were higher than all other treatments (Tukey HSD, $p<0.0001$). There was no difference in areal excretion rates between the LC and LCF treatments (Tukey HSD, $p=0.96$), nor was there a difference between the LCF, F, and LF treatments (Tukey HSD, $p=0.20$). The LC treatment had greater areal excretion than F, LF, and L treatments (Tukey HSD, $p<0.05$).

As a result of the variation in elemental excretion across species (Table 2, Figure S3A,B), there were differences in areal N:P molar excretion among treatments (ANOVA $F_{6,21}=11.09$, $p<0.0001$; Figure 1A) with the L treatment having higher N:P than all other treatments (Tukey HSD, $p<0.0001$).

3.2 | Mussel egestion

Mass-specific elemental biodeposition rates differed significantly among mussel species for nitrogen (ANOVA $F_{2,15}=4.28$, $p<0.05$; Table 2, Figure S3D) and phosphorus (ANOVA $F_{2,15}=7.72$, $p<0.01$; Table 2, Figure 2E), but not for carbon (ANOVA $F_{2,15}=3.32$, $p=0.06$; Table 2, Figure S1C). *Fusconaia cerina* deposited higher quantities of N compared to *L. ornata* (Tukey HSD, $p<0.05$) and both *F. cerina* and *C. kieneriana* deposited higher quantities of P compared to *L. ornata* (Tukey HSD, $p<0.05$). There were also differences in total biodeposition rates of nutrients and C across species (ANOVA $F_{2,15}=3.61$, $p=0.05$; Figure S3F) with *F. cerina* having higher biodeposition rates compared to *L. ornata* (Tukey HSD, $p<0.05$).

As a result of species-specific variation in biodeposition content, when scaled to the community level, there were significant differences in stoichiometric ratios of C:N (ANOVA $F_{6,21}=9.32$, $p<0.001$; Figure 1B), N:P (ANOVA $F_{6,21}=4.18$, $p<0.01$; Table 2, Figure 1C), and C:P (ANOVA $F_{6,21}=4.35$, $p<0.01$; Figure 2D) among treatments. Generally, treatments containing *L. ornata* and or *F. cerina* had greater C:N, N:P, and C:P stoichiometric biodeposition compared to other treatments.

3.3 | Green food web

3.3.1 | Ecosystem productivity (GPP)

There were no significant mussel treatment effects; however, there was significantly different gross primary productivity between sampling weeks (ANOVA $F_{2,72}=28.98$ $p<0.0001$; Figure 2A). GPP was greatest at the end of the study (Tukey HSD, $p<0.0001$), and increased significantly from weeks 2 to 4 (Tukey HSD, $p<0.0001$), and from weeks 4 to 6 (Tukey HSD, $p<0.05$).

3.3.2 | Benthic algal biomass accrual

Chlorophyll-a biomass on the silica discs increased over the course of the experiment, but did not vary across treatments (ANOVA $F_{7,72}=1.57$ $p=0.15$; Figure 2B). Sampling week had a marginal effect on chl-a biomass accrual (ANOVA $F_{2,72}=2.79$, $p=0.07$).

3.3.3 | Leaf litter algal accrual

Chlorophyll-a biomass accrual on tulip poplar leaf litter was mainly driven by sampling week (ANOVA $F_{2,72}=6.46$, $p<0.01$) with week 6 accruing the greatest algal biomass compared to weeks 1 and 3 (Tukey HSD $p<0.0001$). There was a marginal effect of mussel community composition on chl-a production (ANOVA $F_{7,72}=1.40$,

TABLE 2 Mass-specific excretion and biodeposition rates. Values are given as means (±*SE*).

		Nutrient flux (μmol g^{-1} h^{-1})				
Species	*N*	N excretion	P excretion	C biodeposition	N biodeposition	P biodeposition
Cyclonaias kieneriana	6	1.58 (0.15)	0.08 (0.01)	0.97 (0.27)	0.14 (0.03)	0.12 (0.03)
Fusconaia cerina	6	1.35 (0.17)	0.08 (0.01)	1.30 (0.37)	0.17 (0.04)	0.11 (0.02)
Lampsilis ornata	6	2.24 (0.29)	0.07 (0.01)	0.34 (0.05)	0.05 (0.01)	0.03 (0.00)

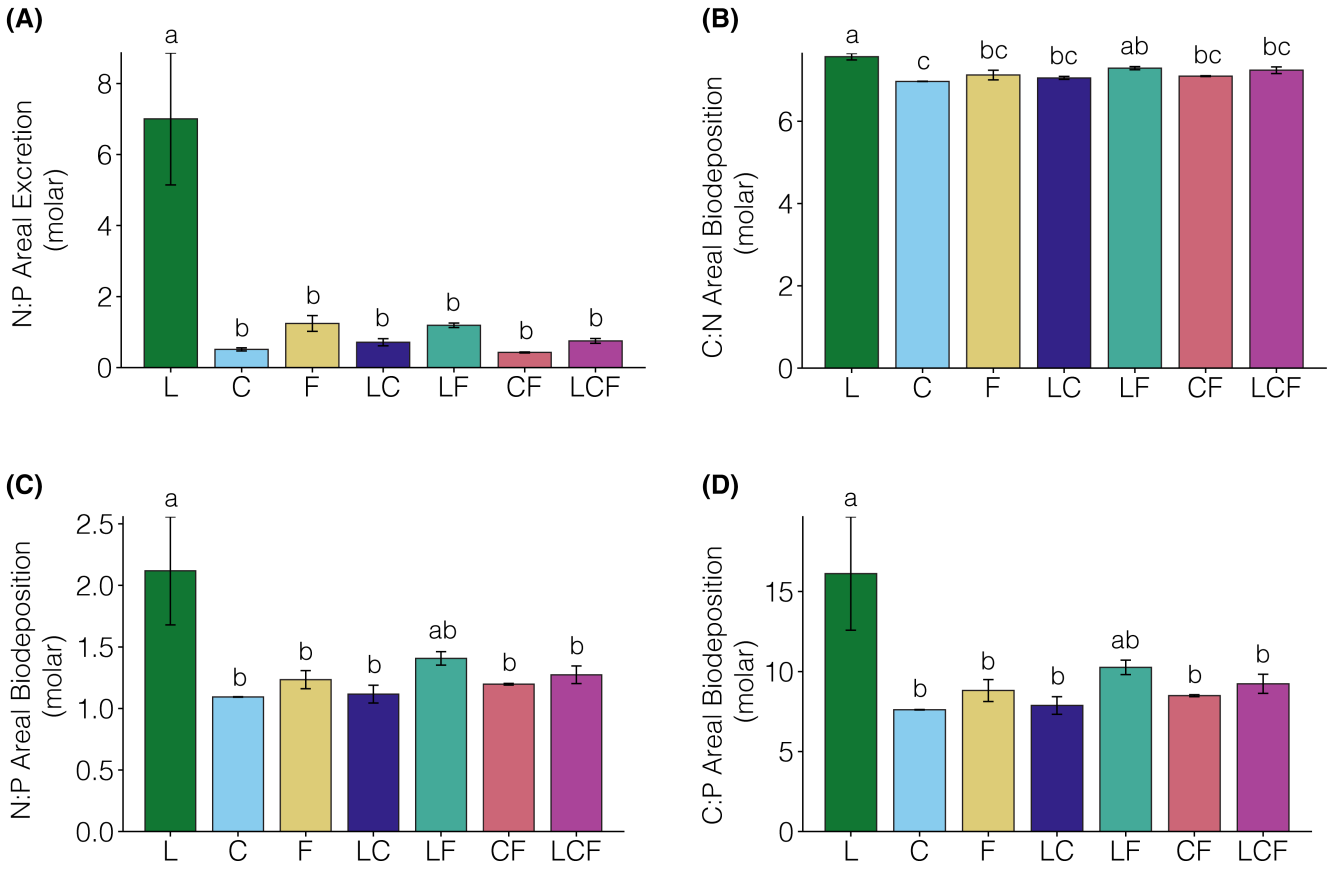

FIGURE 1 Mean (±*SE*) freshwater mussel (A) N:P areal excretion and (B) C:N, (C) N:P, and (D) C:P areal biodeposition stoichiometric ratios scaled to the community level. Different letters indicate significant differences among treatments based on Tukey's HSD.

$p = 0.08$) and post hoc pairwise comparisons revealed that compared to the no-mussel control, the L and LC treatments had greater chl-a production after 6 weeks ($p < 0.05$; Figure 2C).

3.4 | Brown food web

3.4.1 | Leaf decomposition and ergosterol production

For decomposition (Figure 3A), there was a significant effect of sampling week (ANOVA $F_{3,72} = 70.36$, $p < 0.0001$) on mass loss, but no treatment effect (ANOVA $F_{7,72} = 0.85$ $p = 0.55$). Ergosterol did not vary across treatments (ANOVA $F_{7,72} = 0.52$, $p = 0.82$; Figure 3B) or over time (ANOVA $F_{2,72} = 0.65$, $p = 0.52$; Figure 3B).

3.4.2 | Leaf C:N

There were significant differences in C:N composition among treatments (ANOVA $F_{7,72} = 2.6$, $p = 0.02$; Figure 3C) with the L and LC treatments having lower overall C:N. There were differences in stoichiometric C:N composition of tulip poplar leaves across sampling weeks (ANOVA $F_{2,72} = 47.75$, $p < 0.001$; Figure 3D), with weeks 3 and 6 having significantly lower C:N content compared to week 1 (Tukey HSD, $p < 0.001$).

3.4.3 | Leaf respiration and enzyme activity

Leaf respiration rates did not vary among treatments (ANOVA $F_{7,72} = 0.45$, $p = 0.86$; Figure S4) or sampling week (ANOVA $F_{2,72} = 0.93$, $p = 0.40$; Figure S3). Similarly, enzyme activities on leaf litter at the end of the experiment did not differ among treatments for β-glucosidase (ANOVA $F_{7,24} = 0.56$ $p = 0.78$; Figure 4), NAGase (ANOVA $F_{7,24} = 0.50$, $p = 0.83$; Figure 4), or phosphatase (ANOVA $F_{7,24} = 1.06$, $p = 0.42$; Figure 4).

3.5 | Effect size analysis

Overall, we observed that the presence of mussels had a strong positive effect on two components of the green food web by enhancing algal accrual on both organic and inorganic substrate (Figure 5). However, contrary to our predictions, we observed a mixture of positive and negative mussel effects on ecosystem productivity (GPP). We also saw mixed effects on brown food web components of litter decomposition, ergosterol, and leaf

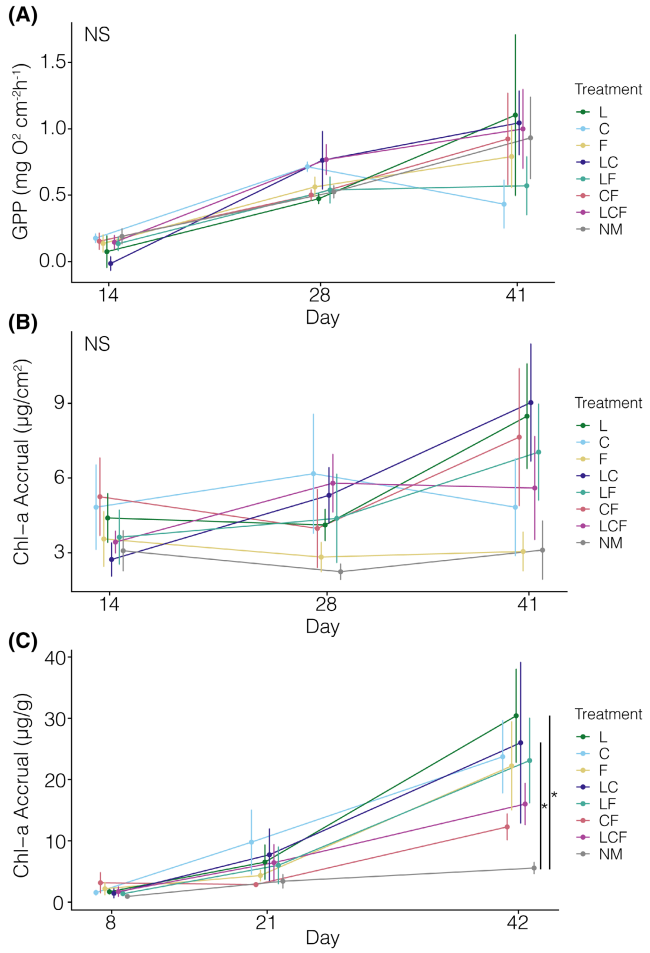

FIGURE 2 Mean (±*SE*) (A) gross primary production and algal biomass accrual on (B) inorganic benthic tiles and (C) organic tulip poplar leaf litter over 6 weeks across the mesocosm treatments ($n = 4$ per treatment). NS indicates no significant differences and single asterisks indicate $p < 0.05$.

respiration compared to the no-mussel control. Mussels did, however, decrease leaf litter C:N content and generally decreased NAGase activity relative to controls. Furthermore, for the most part the presence of mussels had a small to medium effect on increasing β-glucosidase and phosphatase activity. Even though we observed large variation within and among treatments, when taken as a whole, the presence and species composition of mussels did alter the magnitude and direction of ecosystem functions of green and brown food webs (Figure 5).

4 | DISCUSSION

Our results show that the presence of freshwater mussels and their community composition have variable effects on multiple components of brown and green food webs. This corroborates findings from previous research that mussels have varying effects on ecosystem functions (Vaughn & Hoellein, 2018). Despite weak or no treatment effects on many of the functional responses, the presence of mussels enhanced productivity of green and brown food webs. Mussels stimulated green food web components by increasing algal accrual on organic and inorganic substrata, possibly through bottom-up alleviation of nutrient limitation through mussel waste products (excretion and egestion) which stimulate productivity of autotrophic microbes (Atkinson et al., 2013). Additionally, we saw that mussels augmented components of the brown food web relative to controls. We observed significant differences among treatments in stoichiometric C:N composition of leaves, suggesting that mussels may play a key role in brown food webs based on heterotrophic microbes via microbial priming (Halvorson, Barry, et al., 2019; Halvorson, Francoeur, et al., 2019).

4.1 | Mussel excretion and egestion

We demonstrated differences in mass-specific excretion and biodeposition rates and ratios among species indicating that species may impact ecosystem functioning differentially via bottom-up nutrient release. Moreover, when scaled to the community level, variation in species-specific traits resulted in differences in areal nutrient fluxes across treatments. Previous studies have shown that mussel-mediated nutrient release (via excretion and biodeposition) can stimulate benthic productivity, alleviate nutrient limitation, enhance decomposition, and drive biogeochemical cycles (Atkinson et al., 2018, 2021; Atkinson & Forshay, 2022; Spooner & Vaughn, 2012; Vaughn et al., 2007). This bottom-up provisioning of nutrients provides a flow of energy to autotrophic and heterotrophic microbes that influences food webs. Previous studies have shown that mussels' ability to regulate ecosystem energy flow becomes more pronounced in systems where they dominate benthic biomass (Vaughn et al., 2004; Vaughn et al., 2008) and are thus able to alter nutrient dynamics and ecosystem functioning at a large scale (Atkinson & Vaughn, 2015). Taken together with previous work, our results highlight the importance of mussel species diversity and community composition on energy flow in stream food webs through bottom-up nutrient provisioning.

4.2 | Green food web impacts

As anticipated, ecosystem productivity and algal accrual on benthic substrate and leaf litter increased over the course of our 6-week experiment. Despite no significant mussel treatment effects on ecosystem productivity, our data suggested that mussels enhanced these components of the green food web as indicated by the algal accrual on leaf litter and benthic algal accrual (Figure 5). This was probably mediated by the addition of excretion and biodeposits which are rich in N, P, and labile C (Christian et al., 2008; Hopper et al., 2021; Howard & Cuffey, 2006). Controls consistently showed less chl-a over the course of the experiment compared to the mussel treatments, supporting previous work demonstrating mussels can stimulate primary productivity via bottom-up nutrient

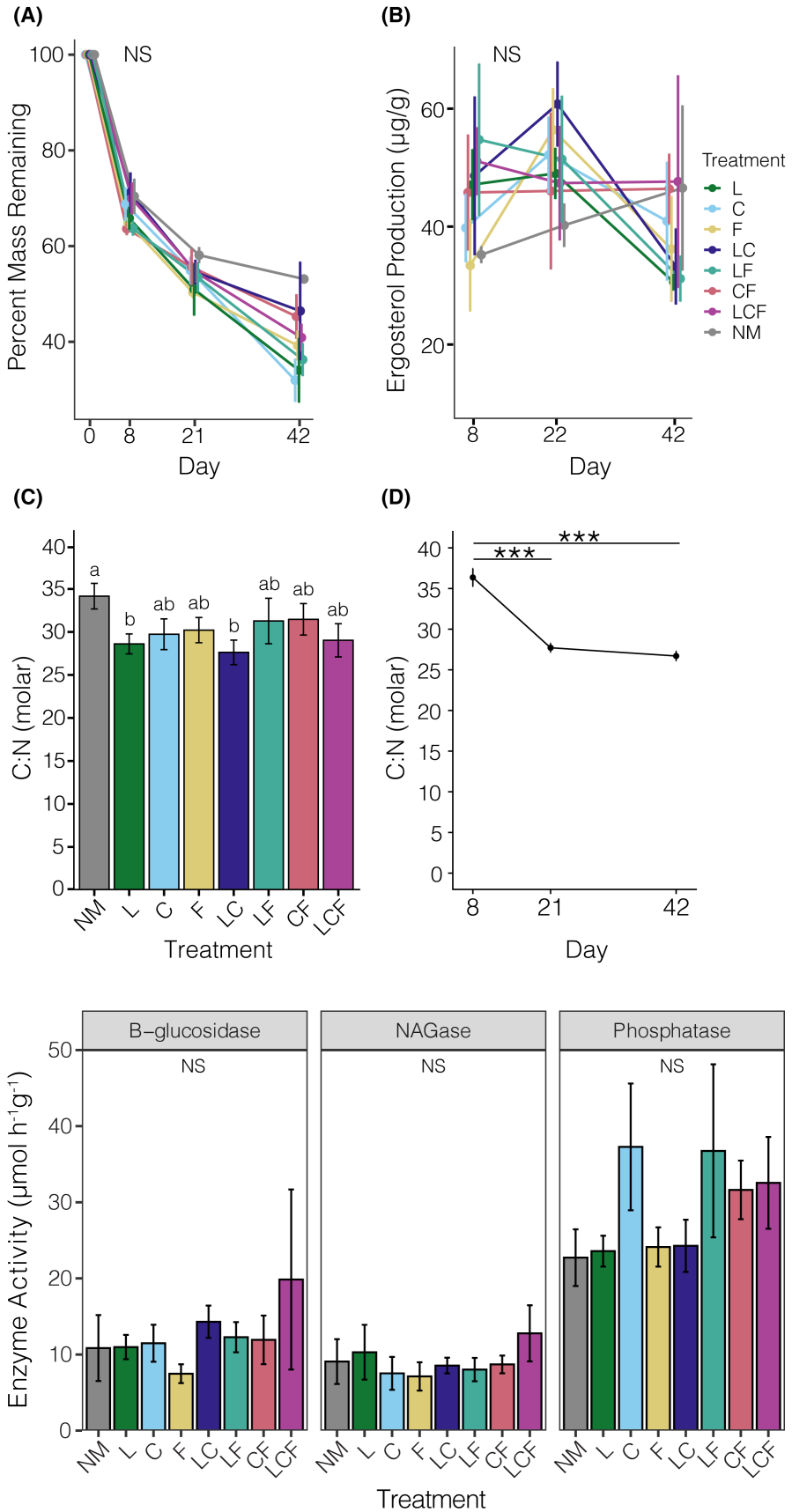

FIGURE 3 (A) Percent litter remaining leaf litter across the treatments over 6 weeks. (B) Mean (±*SE*) ergosterol activity measured on tulip poplar leaf litter over 6 weeks. (C) Average C:N of the leaf litter across our treatments and (D) average C:N across leaf litter over time. Single asterisk indicates $p < 0.05$, two asterisks indicate $p < 0.01$, and three asterisks indicate $p < 0.001$, and different letters indicate significant differences among treatments based on Tukey's HSD. NS indicates no significant differences.

FIGURE 4 Mean (±*SE*) enzyme activity measured on tulip poplar leaves. NS indicates no significant differences.

excretion. We observed similar trends with chl-a production on tulip poplar leaf litter, with the controls producing significantly less algal biomass compared to two of the mussel treatments. Specifically, these two treatments with greater chl-a accrual contained *L. ornata*, which exhibited the greatest mass-specific N:P excretion, thus the input of N may have *fuelled* this component of

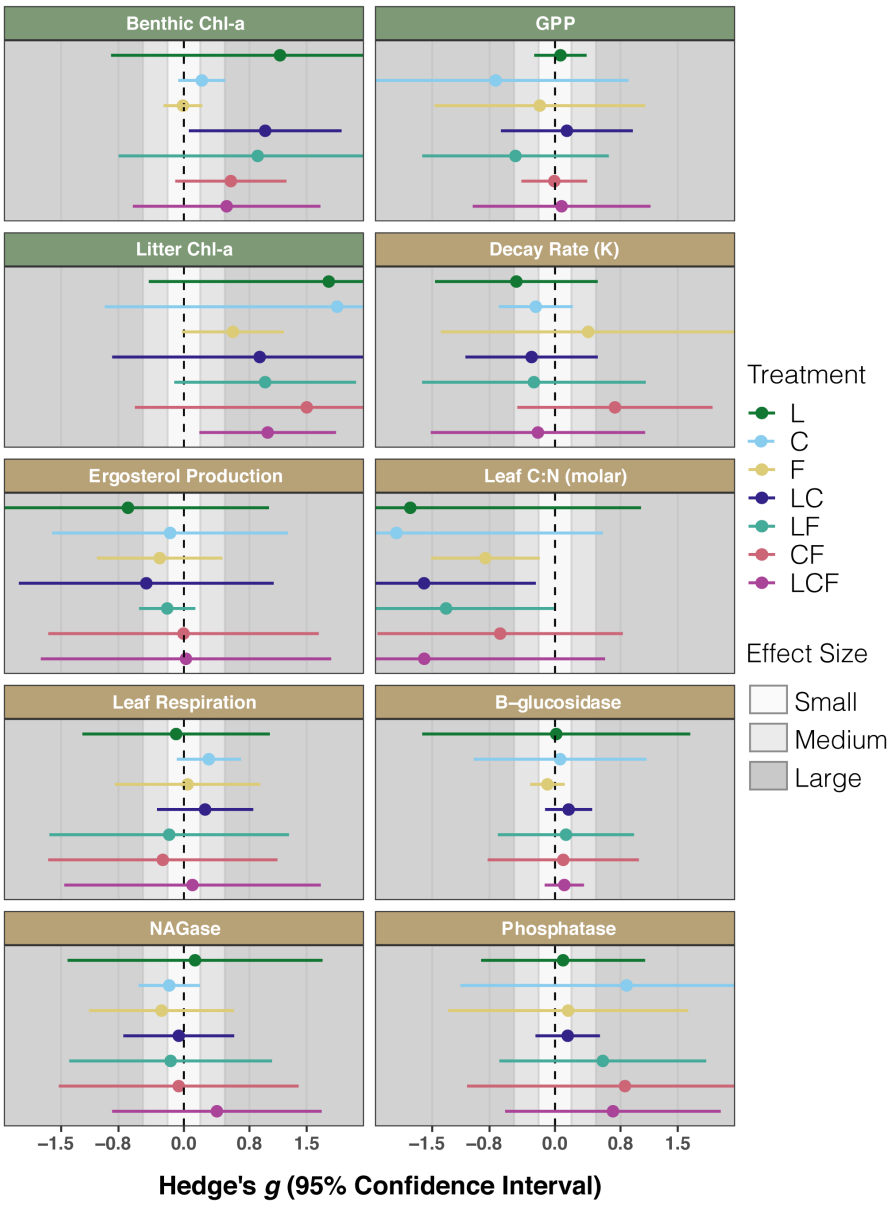

FIGURE 5 Scatterplots visualising the results of Hedge's g effect size analysis. Each plot represents one ecosystem function of interest and each treatment group within. Green and brown food web responses are represented in green and brown boxes, respectively. All leaf-related responses were calculated using tulip poplar leaves. The horizontal axis is the effect size index (Hedge's g). The vertical dashed line represents our no-mussel control and shaded regions represent thresholds for effect sizes: small ($|0.2| < g > |0.5|$); medium ($|0.5| < g > |0.8|$); large ($g > |0.8|$).

the green food web. Previous research has also found higher benthic algal biomass and increased primary production with the presence of mussels (Atkinson et al., 2018; Howard & Cuffey, 2006; Spooner & Vaughn, 2006). Overall, our results support our predictions that mussels can enhance the growth of primary producers by supplying nutrients necessary for growth via excretion and biodeposition.

4.3 | Brown food web impacts

Our results weakly support an effect of mussel driven nutrient dynamics on brown food web responses and corroborates previous research findings that suggest mussels mediate priming effects on decomposition (Nickerson et al., 2021), but litter decomposition was not different (Atkinson et al., 2021). We observed a decrease in litter C:N and an increase in decomposition rates, and leaf litter enzyme activity in some of the mussel treatments, which suggests a mussel effect on brown food web dynamics. Heterotrophic microbes synthesise and excrete degradative enzymes to acquire C, N, and P from leaf litter (Sinsabaugh et al., 1991) and thus drive decomposition and alter the nutrient ratios of the remaining litter. These microbes play key roles in aquatic systems but primarily rely on labile C for growth and enzyme production. Previous studies have shown that freshwater mussels may contribute to a priming effect by providing labile C to heterotrophic microbes in the form of biodeposition (Nickerson et al., 2021). Further, mussel-mediated DOM provides a concentrated flux of labile DOM relative to ambient pools which can be an important source of energy and nutrients for microbial communities (Hopper et al., 2021). We observed increased decomposition and microbial growth on tulip poplar leaves as indicated by increased levels of ergosterol in mussel treatments compared to controls. Over the course of the experiment, the control litter packs consistently lost less mass than

some of the mussel treatments (Figure 5), and at the end of six weeks the C, L, and LF treatments lost more mass compared to the controls; however, this was not significant. Additionally, we saw a trend that mussel treatments generally had elevated enzyme activities compared to the controls, suggesting that heterotrophic microbes may have used labile C from mussel biodeposits. We also observed significant differences in stoichiometric C:N composition of tulip poplar leaves across treatments, with the L, and LC treatments having significantly lower C:N content compared to the controls. Taken together, our results suggest that mussels affected leaf litter decomposition and subsequent stoichiometric content by enhancing microbial growth and activity.

4.4 | Interactive species effects

Even though all mussels are grouped in the same functional guild as filter-feeders, they typically occur in diverse, multi-species aggregations (Haag, 2012; Vaughn, 1997) and have evolved to vary in species-level functional traits (Atkinson, van Ee, & Pfeiffer, 2020). Thus, mussel community structure and underlying species interactions are important to consider when measuring ecosystem functions. We observed interspecific differences in mass-specific excretion and biodeposition rates, and for one species, *L. ornata*, these resulted in significant differences in nutrient fluxes when scaled to the community level. For example, at the mass-specific level, *L. ornata* excreted significantly greater N and biodeposited significantly less N compared to *F. cerina*. When we scaled these nutrient flux rates to the community level, the *L. ornata* (L) treatment had significantly greater N:P areal biodeposition compared to the *F. cerina* (F) treatment; however, when these two species are in the same treatment (LF), the N:P areal biodeposition did not differ significantly from either of the L or F treatments. This potential *counterbalancing effect* may have contributed to the weak or no treatment effects that we observed on various ecosystem responses, and may also help explain why we did not observe a link between greater species richness and functioning. Beyond the results of our experiment, this functional trait complementarity effect has implications for natural systems where many species of freshwater mussels co-occur, but do not occur at equal abundances or biomass (Hopper et al., 2021). Thus, through niche complementarity or synergistic interactions, the overall contribution to ecosystem functions may be enhanced as a result of species-specific functional traits as a result of differential biomass and resulting trait expression (e.g., Hopper et al., 2023). Such species-specific interactions means that the loss of certain species within the mussel community may affect ecosystem functioning via nutrient fluxes and stoichiometry (Benelli et al., 2019).

Using a controlled mesocosm experiment, we attempted to mimic the natural conditions in which mussels live. It is important, however, to recognise that mesocosms do differ from the natural river environment. Excretion and biodeposition rates of mussels can vary with temperature, seston quantity and quality, as well as species identity (Benelli et al., 2019; Christian et al., 2008; Nickerson et al., 2019; van Ee et al., 2022). Given that mussels can filter large volumes of water (Vaughn et al., 2004), the food quantity and quality they are filtering from the water column has a direct impact on their metabolism and thus nutrient release (both excretion and egestion). Previous work using freshwater mussels in mesocosms has found that captive animals can experience dysbiosis and a reduction in their metabolic and physiological condition (Aceves et al., 2020). In addition to potentially lower quality and quantity of added seston, the growth of algal biofilms in the mesocosms may have affected the brown food web responses as it accumulated on decomposing material and could have influenced the growth and accumulation of fungal biomass (Halvorson, Barry, et al., 2019). While excreted and egested nutrients enhance both green and brown food webs, this can lead to opposing effects on litter resource quality; nutrient additions stimulate primary producers but can also stimulate decomposition rates that reduce litter quantity (Rosemond et al., 2015). While we did not find strong, consistent effects here, our study underscores the varying responses species identity and diversity can have on ecosystem function.

4.5 | Conclusions

Our study is among the first to examine consumer driven nutrient dynamics of freshwater communities, while linking both brown and green food webs in the same study system. Our results highlight the importance of measuring multiple functional metrics across a gradient of diversity in ecologically similar consumers (Hopper et al., 2023). We observed many positive effects when mussels were present in components of both green and brown food webs. Additionally, our effect size analysis emphasises that the mussel community composition has variable effects on the direction and magnitude of a variety of ecosystem functions. Mussels occur in dense, species-rich aggregations in natural systems, with species having variable functional traits and effects (e.g., tissue stoichiometry, feeding; Atkinson, van Ee, & Pfeiffer, 2020; Sánchez González et al., 2023). We captured and quantified ecosystem effects related to this variability by implementing a substitutive experimental design (Wright et al., 2021) in order to elucidate contributions of freshwater mussels with different evolutionary histories to large-scale ecosystem functioning. As freshwater taxa are experiencing a global decline in both species richness and abundance (Dudgeon et al., 2006; Haag & Williams, 2014), future studies should address how these declines as well as shifts in community dominance alter ecosystem function.

AUTHOR CONTRIBUTIONS

Conceptualisation: C.L.A., G.W.H. Developing methods: C.L.A., G.W.H., C.R.J., K.A.K., H.M.H. Data analysis: M.E.K. Preparation of figures and tables: M.E.K., I.S.G. Conducting the research, data interpretation, writing: M.E.K., G.W.H., C.R.J., K.A.K., H.M.H., I.S.G., C.L.A.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Data available on FigShare at 10.6084/m9.figshare.23601192

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SUPPORTING INFORMATION

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