

Animal effects on dissolved organic carbon bioavailability in an algal controlled ecosystem

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Abstract

1. Animals exert both direct and indirect controls over elemental cycles, linking primary producer-based (green) and decomposer-based (brown) food webs through top-down trophic interactions and bottom-up element regeneration. Where animals are aggregated at high biomass, they create hotspots of elemental cycling. The relative importance of animal control on elemental cycling depends on animal biomass, species functional traits (i.e. feeding mode and stoichiometry), and their overlap.
2. We evaluated how animal community complexity affects the mechanisms regulating energy flow to the brown food web. We conducted a mesocosm experiment where we varied the biomass and overlap of animals with different life history and stoichiometric traits (stream fish and mussels) and measured how this influenced the quantity and fraction of labile carbon available to microbes. We used linear models and structural equation modelling to evaluate direct (excretion) and indirect (herbivory, nutrient availability, and nutrient stoichiometry) effects of animals on bioavailable dissolved organic carbon (BDOC) concentration.
3. In experimental stream mesocosms, we found support for both direct (DOC excretion) and indirect (grazing) animal influences on BDOC concentration. Although we found that snail, fish, and mussel biomass increased nutrient concentrations, neither nutrient concentration nor stoichiometry had a significant effect on BDOC concentration. This has been due to the high background nutrient concentration context of our stream mesocosm water. Snails, probably due to their high biomass and small body size, exerted a significant positive direct control on BDOC concentration. Fish and mussels exerted a significant negative indirect control on BDOC via their effects (grazing and bioturbation) on algal biomass.
4. Our results imply that primary consumers with different feeding strategies provide a key mechanism regulating the flow of DOC into the brown food web through direct (excretion) and indirect (grazing) controls on primary producers. This highlights that animals can provide important controls on the production of bioavailable organic energy supporting microbes in aquatic ecosystems, but the importance of these controls depends on the nutrient context and the distribution of primary producer and animal biomasses.

KEYWORDS

animal-mediated elemental cycling, biomass, dissolved organic carbon, fish, unionid mussels

1 | INTRODUCTION

Animals exert both direct and indirect controls over elemental cycles linking primary producer-based (green) and decomposer-based (brown) food webs. *Top-down* trophic interactions (e.g. herbivory, detritivory, predation) exert indirect controls by altering the demand for elements in lower trophic levels (Atwood et al., 2013; Limberger et al., 2019; Schmitz et al., 2018). Simultaneously, animal metabolism of prey produces wastes that recycle elements, exerting direct *bottom-up* controls on primary producers and microbes (Atkinson, Vaughn, Forshay, & Cooper, 2013; Frank, Groffman, Evans, & Tracy, 2000; Zou, Thébault, Lacroix, & Barot, 2016). The regeneration of inorganic nitrogen (N) and phosphorus (P) may provide a significant nexus for trophic interactions to transfer between green and brown food webs (Zou et al., 2016). Recent research highlights that animal-mediated elemental cycling also releases organic energy (dissolved organic carbon, DOC) that can stimulate microbial ecosystem functions (Frank et al., 2000; Parr, Capps, Inamdar, & Metcalf, 2019). Thus, changes in animal community abundance and complexity in either green or brown food webs may have cascading effects on the energy available to support microbial function in brown food webs.

The relative importance of indirect and direct controls that animal-mediated elemental cycling exerts on food webs depends on the total animal biomass present, its distribution among different functional traits (Hopper et al., 2018; Parr et al., 2019), as well as environmental context (Iannino, Vosshage, Weitere, & Fink, 2019; Subaluský & Post, 2018; Vaughn, Gido, & Spooner, 2004). In many ecosystems, where animals are aggregated at high biomass, they create hotspots of elemental cycling, locally concentrating nutrients and increasing productivity, such as in grazing ungulate systems (Pringle, Doak, Brody, Jocqué, & Palmer, 2010) and coral reefs (Allgeier, Layman, Mumby, & Rosemond, 2014). In stream ecosystems, fish and freshwater mussels (burrowing bivalves in the order Unionoida, hereafter *mussels*) can both occur as high biomass aggregations that have strong top-down and bottom-up effects on food webs, resulting in hotspots of elemental cycling (Atkinson & Vaughn, 2015; McIntyre et al., 2008).

Differences in traits between these two groups may further influence their ecosystem effects. Stoichiometric traits shape the metabolic demands for N and P and the stoichiometry of excreted nutrients. *Campostoma anomalum*, a common grazing minnow, excretes at an N:P ratio of 8.0–14.9 (McManamay, Webster, Valett, & Dolloff, 2011; Taylor, Back, & King, 2012). Mussels, in contrast, excrete at a higher N:P ratio (>20; Atkinson et al., 2013; Christian, Crump, & Berg, 2008). Mussels are relatively sedentary, occurring in dense aggregations or *beds* (Strayer, 2008). Mussels filter feed on suspended particles and stimulate primary and secondary production via nutrient excretion (Allen, Vaughn, Kelly, Cooper, & Engel, 2012; Atkinson et al., 2013; Spooner & Vaughn, 2012). By comparison, fish are highly mobile, typically only concentrating in hydrologically stable reaches with mussels during droughts or low flows (Hopper et al., 2018), and control primary production through both grazing and nutrient regeneration (Murdock, Dodds,

Gido, & Whiles, 2011). While hydrologically driven seasonal overlap in fish and mussel communities may alter the stoichiometry of available nutrients (Hopper et al., 2018), the effects of these overlapping animal hotspots on the availability of energy (C) have not been investigated.

Dissolved organic carbon is a primary source of organic energy for heterotrophic microbes (*microbes*—archaea and bacteria) in freshwater ecosystems. Although it is abundant in most freshwater ecosystems (Mulholland, 1997), only a relatively small fraction (<20%) of DOC is readily labile to microbes (Guillemette & del Giorgio, 2011; Søndergaard & Middelboe, 1995). In detritus-based stream ecosystems, additions of labile DOC alleviated energetic limitations on bacterial communities, increased microbial biomass, and were subsequently transferred up the food web increasing macroinvertebrate standing stocks (Wilcox, Bruce, Meyer, & Benstead, 2005). Stream insects may make significant contributions to DOC fluxes (Meyer & O'Hop, 1983) and recent research highlights that these fluxes may satisfy as much as 40% of the microbial demand for labile C (Parr et al., 2019).

Microbial capacity to decompose organic matter depends on the availability of labile organic energy, inorganic and organic nutrients, and the stoichiometry of nutrients available to microbes (Dijkstra, Carrillo, Pendall, & Morgan, 2013; Guenet, Danger, Abbadie, & Lacroix, 2010). Inorganic nutrients may increase (positive [+]) priming) or decrease (negative [-] priming) the quantity of organic matter degraded by microbes. In negative priming, added nutrients or labile C may allow microbes to degrade *recalcitrant* organic matter by alleviating nutrient or energetic constraints preventing microbial degradation. In the case of negative priming, microbes degrading organic matter to acquire organic nutrients may shift to acquiring more readily available inorganic nutrients (Guenet et al., 2010). Positive priming is most frequently observed across ecosystems (Guenet et al., 2014; Hotchkiss, Hall, Baker, Rosi-Marshall, & Tank, 2014; Kuzyakov, Friedel, & Stahr, 2000) and in aquatic ecosystems priming effects on decomposition rates average +12.6% but are highly variable (Bengtsson, Attermeyer, & Catalán, 2018). Differences in the stoichiometry of animal communities may affect the stoichiometry of nutrients released (Hopper et al., 2018). As sources of labile C, organic, and inorganic nutrients (Parr et al., 2019), animals contribute the necessary ingredients to prime the decomposition of labile organic matter. Thus, in addition to indirect trophic controls and direct excretion of labile organic matter, priming effects may provide additional indirect control on the size of the DOC pool available to microbes.

We asked how animal community complexity affects the mechanisms regulating energy flow to the brown food web. Understanding the direct and indirect roles of animal communities in regulating the coupled flows of nutrients and energy through ecosystems is a central question in ecology. We developed a conceptual model (Figure 1) predicting that animals would exert both direct and indirect controls on the availability of labile DOC. We tested our model with a mesocosm experiment that manipulated fish and mussel biomass, and their overlap, and measured inorganic nutrients, algal biomass, and DOC concentration and bioavailability. Specifically, we tested the following predictions: (1) Increasing animal biomass would

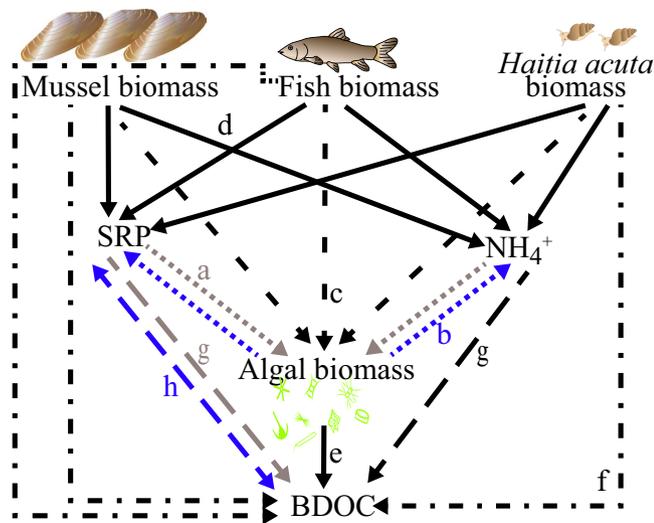


FIGURE 1 Conceptual model of animal effects on bioavailable dissolved organic carbon (BDOC). Arrows between compartments indicate a causal mechanism ($X \rightarrow$ causes an increase or decrease in Y). Line dashes indicate similar types of controls. Grey lines (a) reflect *a priori* causal structures whereby nutrient concentrations/availability control algal biomass and blue lines (b) reflects *a posteriori* causal structures whereby algal biomass controls nutrient concentrations. Black lines are causal structures tested in both *a priori* and *a posteriori* models. (c) Top-down controls: animal grazing controls standing stocks of biomass. (d) Nutrient concentrations are affected by animal-mediated nutrient recycling which increases the availability of inorganic nutrients. The quantity of BDOC available is a function of (e) direct release from algae and (f) direct release from animals. The quantity of BDOC may be further affected by (g) nutrient priming. (h) Initial results of the *a priori* model suggested collinearity between soluble reactive phosphorus (SRP) and algal biomass (g; probably due to strong algal control over SRP) leading to non-significant variance estimates and inflated R^2 values for BDOC. This was instead modelled as a covariance term in the revised *a priori* and *a posteriori* models (h; double-headed arrow)

indirectly decrease the abundance of labile organic matter through top-down controls on algal biomass and algal release of labile DOC (Figure 1c \rightarrow e). (2) Increasing animal biomass would directly increase the labile DOC pool due to the production of wastes (Figure 1f). (3) Animal-mediated nutrient recycling associated with animal biomass would indirectly increase the abundance of labile organic matter by increasing inorganic nutrient availability which microbes could use to degrade DOC (Figure 1d \rightarrow g). (4) Increasing animal biomass would indirectly increase the abundance of labile organic matter through animal-mediated nutrient recycling that stimulated algal growth and subsequently labile DOC production (Figure 1d \rightarrow a \rightarrow e). (5) The overlap of animal biomasses (fish and mussels) with different tissue stoichiometries would alter the stoichiometry of nutrients recycled and available to microbes (c) and algae (d) and subsequently the concentration of microbially bioavailable DOC (BDOC). Aggregations of consumers, i.e. *hotspots*, provide an ideal system to address this question because animal biomass, species traits, and their effects are locally concentrated and elevated compared to non-hotspot areas. In addition, mussel and fish aggregations are a particularly

good system because overall animal biomass, and the distribution of species traits, varies with the degree of spatial and temporal overlap (Hopper et al., 2018).

2 | METHODS

We investigated our questions and hypothesis as part of a larger study conducted over 16 weeks in experimental mesocosms where we measured food web responses to varying degrees of overlap in animal biomass. In this paper, we focus on a subexperiment from the 12th week of the study, where we performed additional experiments investigating the factors controlling the quantity of bioavailable DOC (BDOC; energy) available to heterotrophic microbes. The study was built around our previous work in the Kiamichi River (Atkinson et al., 2013; Hopper et al., 2018; Vaughn, Spooner, & Galbraith, 2007) where ambient nutrient concentrations are typically low ($67 \mu\text{g N-NH}_4^+/\text{L}$, $18 \mu\text{g N-NO}_3^-/\text{L}$, $14 \mu\text{g}$ soluble reactive phosphorus [SRP]/L; C.C. Vaughn unpublished data) and whose catchment remains largely forested (80–87%; Atkinson, Julian, & Vaughn, 2012).

2.1 | Experimental design

We used a simple assemblage of fish (*Camptostoma anomalum*) and mussel (*Amblema plicata* and *Actinonaias ligamentina*) primary consumers that are typical of rivers in the south-central U.S.A., frequently occur as overlapping aggregations and are known to have strong ecosystem effects. *Camptostoma anomalum* is an abundant, schooling algal grazer that has been shown to have strong top-down through grazing and bottom-up through excretion effects in stream food webs (Power, Matthews, & Stewart, 1985; Reisinger, Presuma, Gido, & Dodds, 2011; Taylor et al., 2012), and has been used successfully in multiple mesocosm experiments (Gido, Bertrand, Murdock, Dodds, & Whiles, 2010). *Amblema plicata* and *Actinonaias ligamentina* are numerical and biomass dominants in rivers in southeastern Oklahoma (Spooner & Vaughn, 2009), have been used successfully in mesocosm experiments (Allen et al., 2012), and have strong bottom-up effects in stream food webs (Atkinson et al., 2013; Spooner & Vaughn, 2012). Fish were collected from Brier Creek, Oklahoma, and held in mesocosm tanks identical to the ones used in the experiment. Mussels were collected from the Little River, Oklahoma, and returned to this location at the completion of the experiment.

Treatments consisted of: no consumer biomass, grazing fish (*Camptostoma anomalum*), mussels (equal numbers of *Amblema plicata* and *Actinonaias ligamentina*), and overlapping aggregations of fish and mussels together. Within these treatments, we varied levels of fish and mussel biomass to reflect typical biomasses and degrees of overlap between these two groups found in south-eastern Oklahoma rivers (fish biomass = c. 1–5 g dry soft tissue weight per m^2 (g DW/ m^2), mussel biomass = 40–200 g DW/ m^2 ; Hopper et al., 2018). Fish biomass treatments consisted of 0, 2.2, and 4.4 g DW/ m^2 and mussel treatments consisted of 0, 100, and 200 g DW/ m^2

in a factorial design for nine total treatments replicated five times ($n = 45$ mesocosms).

The experiment was conducted in flow-through mesocosms that have been used in multiple food web experiments with stream fishes (Martin, Gido, Bello, Dodds, & Veach, 2016; Matthews et al., 2006), that were located at the University of Oklahoma Aquatic Research Facility, Norman, OK, U.S.A. The 45 mesocosms were arrayed in eight rows of six (including three unused holding tanks) under knitted shade cloth (70% shade, 30% light transmission). Upstream to downstream, each mesocosm consisted of a shallow rectangular inflow run (1.33 m long \times 0.45 m wide \times 0.2 m deep) flowing into a deeper circular pool (1.8 m diameter \times 0.35 m deep) and out through a short rectangular outflow run (0.71 m long \times 0.45 m wide \times 0.2 m deep) opposite the inflow. The benthic substrate in mesocosms consisted of rounded and washed 1.0–1.6-cm diameter river gravel 0.05–0.10 m deep in runs and c. 0.4 m deep in pools. The total water column volume above the gravel bed was c. 1.07 m³. Water was continuously supplied to the mesocosms at a rate of c. 1 L/min (c. 2% coefficient of variations across all mesocosms) from a groundwater well and flowing into the head of the inflow run and draining out through a screen standpipe at the end of the outflow run. This provided a water turnover time of 0.74 per day.

Mesocosms were also aerated at the water inflow to prevent overnight DO stress on animals. Three 15–20-cm sections of 10-cm PVC pipe were placed in each pool that fish, if present, could use for cover. One week before beginning the experiment, mesocosms were seeded with algae from a nearby pond (Allen et al., 2012). Prior to beginning the experiment, plastic strawberry baskets (10 \times 10 \times 6 cm) were filled with gravel and buried flush with the sediment in a 3 \times 6 grid in each mesocosm for subsequent sampling of metabolism, benthic algae, and organic matter.

Temperature and light were monitored using Hobo light and temperature loggers in 24 mesocosms in an alternating grid pattern. During the week preceding the experiment, the average photosynthetic photon flux density at the water surface was $258 \pm 7 \mu\text{mol m}^{-2} \text{hr}^{-1}$. Light availability at half the depth of the water column averaged $171 \pm 9 \mu\text{mol m}^{-2} \text{hr}^{-1}$ and was negatively related to chlorophyll-*a* ($R^2 = .48$), probably due to shading by floating cladophora. The average maximum daily temperature in the week preceding the sampling was $24.8 \pm 0.6^\circ\text{C}$ and the average minimum daily temperature was $21.1 \pm 0.6^\circ\text{C}$.

2.2 | Animal biomass

Biomass of individual mussels was tracked throughout the experiment. Each mussel was uniquely tagged with a Floy shellfish tag (Floy, Seattle, Washington, U.S.A.) attached with gel adhesive on the posterior shell margin (Vaughn et al., 2007), and the maximum length and total wet weight were recorded at the beginning and end of the experiment. When a dead mussel was discovered, it was immediately replaced with another tagged mussel of the same species from a holding tank. Mussel dry masses were estimated from regression equations developed for

the taxa used in the mesocosms (Hopper et al., 2018). Total fish biomass stocked at the beginning of the experiment was estimated based on the lengths of fish using a length-weight equation. When dead fish were discovered, the length was recorded to estimate biomass loss. At the end of the experiment (week 16), fish were euthanised, preserved in accordance with the IACUC guidelines in 10% formalin, and length and wet weight were measured. Wet weight was converted to dry mass as $0.22 \times \text{weight}_{\text{wet}} = \text{weight}_{\text{dry}}$ (specific to *C. anomalum*; Hopper et al., 2018). For our analysis of processes at week 12, we assumed that relative differences in fish and mussel biomasses among treatments were comparable between weeks 16 and 12.

An invasion of two grazing snail species, *Haitia (Physa) acuta* and *Gyrulus parvus*, occurred across the experiment, which we accounted for by quantifying snail biomass. Snails were suctioned from a 25 \times 25 cm quadrat randomly placed on the bottom of the mesocosm. Snails were then separated from organic detritus, placed on a clear plastic film, and scanned at 1200 dpi optical resolution on a photographic scanner (Epson 3170). Snail length was measured in ImageJ (Abràmoff, Magalhães, & Ram, 2004) along the longest axis and mass was estimated from length–soft tissue dry mass (STDM) regressions developed from a subset of individuals.

2.3 | Sample collection

At week 12 we measured DOC concentration, microbially BDOC concentration, water column nutrients, dissolved oxygen, benthic metabolism, benthic organic matter, and algal biomass (benthic chlorophyll-*a* and visual estimates). Dissolved oxygen (DO) was measured using a handheld luminescent DO meter (Hach HQ40d, LDO101 sensor). All nutrient and DOC samples were filtered through 0.7- μm glass fibre filters, pre-combusted at 500°C. Nutrient samples were collected into 20-ml sample-rinsed polyethylene bottles and DOC samples were collected into 40-ml amber glass vials precombusted at 500°C.

2.4 | Nutrient analysis

Nutrients were measured as ammonia/ammonium as NH_4^+ , nitrate+nitrite as NO_x , SRP, total nitrogen, and total phosphorus (TP). All nutrients were analysed using colorimetric methods on an OI Analytical nutrient analyser. NO_x was analysed using the cadmium reduction method (EPA Method 353.2), NH_4^+ was analysed using the alkaline phenol method (EPA Method 350.1), and SRP was analysed using the molybdate blue method (EPA Method 365.1). Total nitrogen and TP were digested with potassium persulfate and analysed as nitrate and SRP respectively.

2.5 | Benthic metabolism

To assess the effects of our treatments on green food web production we measured benthic metabolism as the consumption

of DO during dark incubations and the production of DO during light incubations in propeller-driven recirculating chambers (Rüegg, Brant, Larson, Trentman, & Dodds, 2015). Mesocosms were sampled, in random order, by removing three baskets from each mesocosm and placing them in a chamber filled with water from the corresponding mesocosm. Chambers were sealed after removing all trapped gas bubbles and the propeller motor speed was adjusted to 260 ± 10 rpm (mean \pm standard deviation), measured with a laser tachometer (Pyle PLT26, c. 15.5 ± 0.6 cm/s, estimated by suspended particulate travel times). Dissolved oxygen was logged every 30 s from a YSI ProODO luminescent DO meter inserted through the top of each chamber. Incubations (dark then light) were conducted until we observed a c. 0.5 mg/L change in DO concentration in all chambers (c. 10–20 mins).

Metabolism ($\text{g O}_2 \text{ m}^{-2} \cdot \text{hr}^{-1}$) was calculated as $GPP = CR + NEP$ where GPP is gross primary production, CR is community respiration (dark incubation) and NEP is net ecosystem production (light incubation) (Rüegg et al., 2015). All calculations account for the specific basket areas and chamber volumes. We developed a light correction curve for one high and one low algal biomass chamber. The curve used a series of irradiance levels produced by placing layers of 1.5 mm window screen over the chambers while measuring PAR and change in DO concentration. Gross primary production was then modelled after Jassby and Platt (1976): $GPP = P_{\text{max}} \times \tanh(\alpha \times \text{PAR} \div P_{\text{max}})$. Non-linear least-squares parameter estimation determined $P_{\text{max}} = 19.16$ and $\alpha = 0.06$, which were close to the parameters reported in previous studies using similar chambers ($P_{\text{max}} = 20.16$ and $\alpha = 0.018$; Trentman, 2015). This was used to correct all metabolism chambers to a PAR of $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$, the average light correction factor applied to GPP was 1.11 (range 0.97–1.63).

2.6 | Algal biomass

Benthic organic matter and chlorophyll-*a* were measured on the same sediment-filled baskets used to measure metabolism (described above). Following metabolism measurements, baskets were placed in a bucket with 20 L of clean well water and the contents were scrubbed and homogenised to create a slurry. Triplicate subsamples of a known volume of the slurry were collected and filtered through $1.6 \mu\text{m}$ glass fibre filters. Filters were sealed in aluminium foil, stored on ice, and frozen until subsequent analysis. Chlorophyll-*a* was extracted in acetone and measured with a spectrophotometer using the acid addition method (APHA, 2017). Benthic organic matter was measured as ash-free dry mass (AFDM)—the mass of dry sample weight lost after combustion at 500°C for 4 hrs.

Additionally, we visually quantified algal biomass by regularly photographing the mesocosms from a fixed perspective and scoring the photographs. Each mesocosm was photographed using a GoPro camera mounted a fixed distance underneath a tripod spanning the mesocosms. From the resulting set of photographs, images representing high, moderate, and low algal biomass were selected as training images (Figure S1). We then used this subset to train a group of six

individuals to judge algal abundance on a scale of 1–10 with 1 representing the lowest biomass and 10 representing the highest biomass. All participants viewed photographs in random order and independently scored them. No visible identifiers (e.g. mesocosm number) indicating treatment were visible during this process. The average of all six judges' scores for each photo was then calculated; this value is reported as the *picture score*. As a consistency check, five images were repeated, and the average difference among repeat scores was ± 0.4 .

2.7 | Bioavailable dissolved organic carbon and nutrient priming assays

We conducted a BDOC assay on each of the 45 mesocosms to understand how our different consumer treatments may influence the flow of organic energy to heterotrophic microbes. We also assessed whether BDOC concentrations could be attributed to nutrients released by animals priming the decomposition of DOC. BDOC was measured in the laboratory for all mesocosms as the quantity of DOC lost during 28-day dark incubations of filtered mesocosm water (Findlay & Parr, 2017). For each mesocosm and water source, 1 L of filtered water ($0.7\text{-}\mu\text{m}$ glass fibre filter) was aliquoted into eight replicate amber glass vials. Three of the 8 vials were used to measure initial (time 0; T_0) DOC concentration. These three vials were acidified to pH 2 with concentrated HCl (c. $40 \mu\text{l}$) and refrigerated at 4°C . Two of the remaining five vials were amended with nitrogen ($112 \mu\text{g/L N}$ as NH_4NO_3) and phosphate ($40 \mu\text{g/L P}$ as KH_2PO_4) in a 7:1 molar ratio to alleviate potential nutrient limitation. This ratio was selected as it is close to microbial biomass in soils and water (Cleveland & Liptzin, 2007). These five vials were then incubated in the dark at room temperature (70°C) for 28 days, at which time they were acidified in the same way as the T_0 vials. DOC in all samples was then measured as non-purgeable organic carbon using a Shimadzu TOC-L high-temperature combustion analyser.

Priming responses were measured as the relative increase or decrease in DOC concentration following Eqn. 1.

$$BDOC_{\text{priming response ratio}} = \frac{DOC_{\text{initial}} - DOC_{\text{final nutrient amended}}}{DOC_{\text{initial}} - DOC_{\text{final unamended}}} \quad (1)$$

DOC_{initial} is the initial DOC concentration present in a sample at the start of the incubation. $DOC_{\text{final nutrient amended}}$ is the DOC concentration remaining at the end of the incubation in samples that received nutrient amendments. $DOC_{\text{final unamended}}$ is the DOC concentration remaining at the end of the incubation in samples not receiving nutrient amendments.

2.8 | Data analysis

To test our predictions and overall conceptual model, we used general linear models and structural equation models based on our conceptual

model (Figure 1). We used general linear models to test for additive and interactive effects of overlapping fish and mussel hotspots while controlling for snails as a covariate (*lm()*; R Core Team, 2019). We used the resulting significance test on linear model terms and Akaike information criterion to remove non-significant effects (interactive or additive). The best-fitting linear models are presented in Table 1. Specifically, we tested for: (1) Interactive effects of animal biomass on primary producer responses (GPP, CR, chlorophyll-*a*, AFDM, algal picture score), inorganic nutrient concentration and stoichiometry ($\text{NH}_4^+:\text{SRP}$), and BDOC concentration; (2) the effects of primary producers (GPP, CR, chlorophyll-*a*, AFDM) on BDOC concentration; (3) the additive effects of water column nutrients (NO_3^- , NH_4^+ , SRP) and nutrient stoichiometry ($\text{NH}_4^+:\text{SRP}$) on primary producers (GPP, CR, chlorophyll-*a*, AFDM) and BDOC concentration; and (4) the effects of water column nutrients, animal biomass, or primary producers on the priming response of BDOC to added nutrients (percentage increase or decrease in nutrient amended BDOC assays compared to unamended assays). All reported R^2 values are adjusted R^2 values, which account for the number of terms in the model.

We used structural equation modelling (SEM; [R] package *lavaan*; Rosseel, 2012) to assess our model as the causal structure underlying the correlative relationships observed in the general linear models. We developed and tested three models. First, an *a priori* (Figure 1) model to test our predictions based on results from previous work (Atkinson et al., 2013; Hopper et al., 2018; Vaughn, Nichols, & Spooner, 2008). Second, a revised *a priori* model that addressed collinearity issues in the initial model. Third, we developed an *a posteriori* model that addressed a theoretical inconsistency in the relationship between nutrients and algae presented in the results of the *a priori* models (Figure 1b). We used a latent variable, *algal biomass*, in all models to describe the responses of primary producer biomass in the mesocosms. As indicators for this latent variable, chlorophyll-*a* (g/m^2) and the *picture score* provided two complementary estimates of biomass.

We evaluated model fits using a combination of indicators (Table S1; see Kline (2011) or Grace (2006) for a thorough discussion of model fit statistics). We assessed the consistency of the model covariance

structure with the covariance structure (*good fit*) using χ^2 tests (Grace, 2006)—non-significant χ^2 ($p > .05$) indicated that the model covariance structure was consistent with the observed covariance structure. The comparative fit index and Tucker–Lewis index were also used to assess fit, and if these were >0.95 , this was indicative of good fit (Fan et al., 2016). Akaike information criterion and sample-size adjusted Bayesian information criterion were used to compare the fit of models with lower values indicating comparatively better fit. Root mean square error of approximation (RMSEA) was also assessed with $\text{RMSEA} < 0.06$ (Fan et al., 2016) and the 95% RMSEA confidence intervals containing 0 (Grace, 2006) indicative of an acceptable model fit. Standardised root mean square of the residual was assessed and if <0.09 , this indicated an acceptable model fit (Fan et al., 2016). We then tested the significance of our prediction of direct controls (Figure 1f) and indirect (Figure 1d \rightarrow a \rightarrow e; c \rightarrow e; d \rightarrow g) animal controls on BDOC availability.

3 | RESULTS

3.1 | Animal biomass

We observed an unplanned invasion by the snails *G. parvus* and *H. acuta*. On average, *H. acuta* accounted for $75 \pm 4\%$ (mean across mesocosms \pm standard deviation) of the total snail biomass, *G. parvus* averaged $0.97 \pm 1.01 \text{ g}/\text{m}^2$ (range $0.02\text{--}15.5 \text{ g}/\text{m}^2$) and *H. acuta* biomass averaged $3.88 \pm 2.76 \text{ g}/\text{m}^2$ (range $0\text{--}3.70 \text{ g}/\text{m}^2$) at week 12. *Hiatia acuta* biomass was unrelated to mussel biomass but negatively correlated to fish biomass (Pearson's $r = -.31$, $p < .05$; Figure 2.). The subsequent analysis focused on *H. acuta* biomass since it was the snail biomass dominant.

3.2 | Nutrient responses to animal biomass treatments

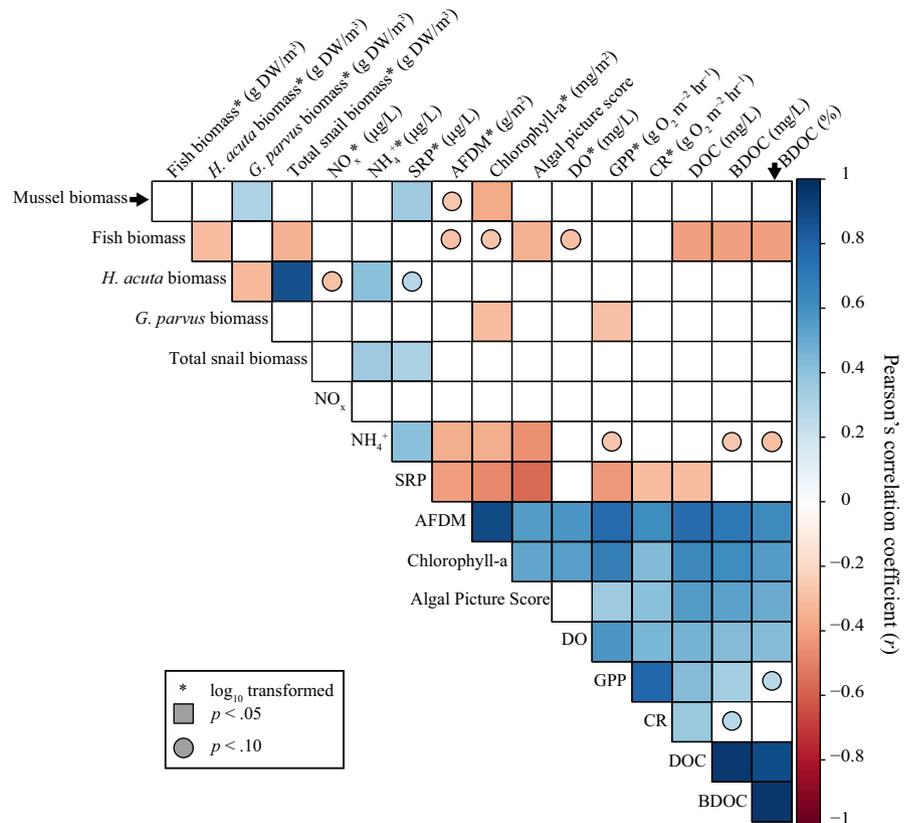
Comparison of nutrient concentrations between the groundwater supply and the mesocosms suggested a relatively high demand for P relative to N. Ambient nutrient concentrations were low in the groundwater source and mesocosms, except for NO_3^- which was naturally high (Table 1). Ammonium was not detected in the source water but averaged $16 \mu\text{g N}/\text{L}$ in mesocosms. Soluble reactive phosphorus averaged $31 \mu\text{g P}/\text{L}$ in the source water and, on average was 74% lower in mesocosms. Nitrate averaged $853 \mu\text{g N}/\text{L}$ in the source water and was 64% higher ($1,400 \mu\text{g N}/\text{L}$) in the water column across mesocosms.

Animals were associated with higher NH_4^+ and SRP concentrations but overlapping mussel and fish biomasses did not affect nutrient stoichiometry. General linear models indicated that greater mussel, fish, and *H. acuta* biomasses were additively associated with increased SRP concentrations ($R^2 = .24$, $p < .05$; Table 2), but the interaction term was not significant. Animal biomasses, except for mussels, increased NH_4^+ availability additively ($R^2 = .28$, $p < .05$; Table 2), but the interaction term was not significant. No additive or interactive effects were significant in explaining the stoichiometry of $\text{SRP}:\text{NH}_4^+$ ($p > .05$).

TABLE 1 Summary statistics for water column samples and algal response variables collected across mesocosms at week 12

	Mean (SE)	Range	
		min.	max.
DOC ($\mu\text{g C}/\text{L}$)	800 (15)	0.6	1.4
BDOC ($\mu\text{g C}/\text{L}$)	135 (18)	0	40
NH_4^+ ($\mu\text{g N}/\text{L}$)	16 (8)	2	41
NO_x ($\mu\text{g N}/\text{L}$)	1395 (345)	594	1,960
SRP ($\mu\text{g P}/\text{L}$)	8 (3)	3	16
Chlorophyll- <i>a</i> (mg/m^2)	113 (188)	15	1,152
AFDM (g/m^2)	22.7 (29.8)	5.0	201.5
GPP ($\text{g O}_2 \cdot \text{m}^{-2} \cdot \text{hr}^{-1}$)	1.1 (0.7)	0.2	2.9
CR ($\text{g O}_2 \cdot \text{m}^{-2} \cdot \text{hr}^{-1}$)	-0.4 (0.4)	0	-1.5
GPP/CR	3.9 (3.2)	1.0	13.2

FIGURE 2 Matrix of significant ($p < .05$) and marginally significant ($p < .10$) correlations (Pearson's r) among key variables considered for structural equation modelling. Along the top row of variable names, units are provided in parentheses and * indicate that the variable is log-transformed. Variables provided along the diagonal are identical in units and transformation



3.3 | Algal responses to nutrients and animal biomass

Algal biomass and AFDM varied widely among mesocosms (Table 1, chlorophyll-*a* 15–1,152 mg/m², AFDM 5.0–201.5 g/m²). Broadly consistent with top-down control by grazing, algal biomass metrics (AFDM, chlorophyll-*a*, and algal picture score) correlated negatively with animal biomasses, except *H. acuta* (Figure 2). Using general linear models, chlorophyll-*a* decreased in response to increasing fish and mussel biomass, while *H. acuta* biomass had no significant effect ($R^2 = .23$, $p < .05$; Table 2). Animal biomass had similar effects on AFDM as it did on chlorophyll-*a* (Table 2), probably due to the strong correlation between the two measures (Figure 2). The picture score, which also captures algal biomass, reflected a similar pattern with fish and *H. acuta* negatively related to increasing algal biomass ($R^2 = .20$, $p < .05$; Table 2), but the effect of mussels was not significant. Models using animal biomass did not significantly explain GPP and CR. The fish and mussel interaction term was not significant in any of the three models.

3.4 | Dissolved organic carbon responses to animals, algae, and nutrients

We assessed whether the effects of animals and primary producers on the quantity of BDOC as well as on the priming of DOC. DOC concentration and bioavailability were positively correlated with primary producers ($p < .05$; AFDM, chlorophyll-*a*, GPP, CR).

DOC concentration was negatively correlated with SRP ($p < .05$; Figure 2). Among animal biomasses, only fish biomass was significantly ($p < .05$) correlated with DOC and BDOC concentration and percent (Figure 2). Using general linear models, we found that fish and mussel biomass decreased DOC and BDOC concentrations, with fish having c. 1.7 times greater effect than mussels. Finally, no models included a significant interaction term, suggesting the effects of overlapping consumer biomass on DOC concentration and bioavailability were additive in this experiment.

We did not observe significant positive or negative nutrient priming responses among treatments with and without animals. In the BDOC incubation assay, we observed slightly higher BDOC concentrations in nutrient amended vials ($+0.02 \pm 0.68$ mg/L; mean \pm standard deviation), but this was highly variable and not significant. Neither additive nor interactive effects of animal biomass significantly explained the BDOC priming response ratios ($p > .05$). The ambient concentrations of nutrients were non-significant in explaining the priming response of DOC.

3.5 | Structural equation modelling path model

To integrate the results of linear model analysis and understand the direct and indirect causal mechanisms underlying those results, we analysed our conceptual model (Figure 1) using SEMs. Initial fitting (Figure 1) indicated that while the model provided adequate fit ($\chi^2 = 7.7$, $df = 7$, $p = .36$, Table S1), the variance estimates of BDOC were non-significant indicating high collinearity among the

Response	Predictors				Model adj. R^2	Model p
	Name	Standardised Coefficients	F	p		
SRP	PA	0.38	7.5	.01	.24	<.01
	MM	0.39	8.5	.01		
	FM	0.31	4.8	.03		
NH_4^+	PA	0.55	16.4	<.01	.28	<.01
	MM	0.17	1.8	.19		
	FM	0.41	9.3	.00		
Chlorophyll- a	PA	-0.21	2.2	.14	.23	<.01
	MM	-0.42	9.5	<.01		
	FM	-0.38	7.1	.01		
AFDM	PA	-0.22	2.1	.15	.14	.03
	MM	-0.29	4.5	.04		
	FM	-0.38	6.4	.02		
Picture score	PA	-0.35	6.0	.02	.20	<.01
	FM	-0.46	10.1	<.01		
DOC	MM	-0.27	3.9	.05	.20	<.01
	FM	-0.43	9.9	<.01		
BDOC	MM	-0.25	3.4	.07	.19	<.01
	FM	-0.42	9.4	<.01		

Note: The reported models are the best-fit consumer biomass models derived from treating *Hiatia acuta* mass (PA) as a covariate while testing for mussel biomass (MM) and fish biomass (FM) and their interactions (response = PA + MM + FM + MM \times FM). Akaike information criterion was used to determine the best-fitting model, eliminating terms that were non-significant and did not improve Akaike information criterion. Significant models could not be developed for NO_x , N:P (N- NH_4^+ /P-SRP molar ratio), CR, GPP, or BDOC nutrient priming response. All biomass predictors were \log_{10} transformed, all response variables except picture score, DOC, and BDOC were \log_{10} transformed. No significant ($p > .05$) interactions were observed and are therefore not reported in the table.

predictors. Inspection of variable relationships indicated that this was probably due to a high correlation between algal biomass and SRP. We addressed this by specifying SRP and BDOC as covariates. The revised *a priori* model covariance matrix implied by our theoretical model (Figure 3a) was sufficient to describe the covariances in the observed data ($\chi^2 = 7.7$, $df = 7$, $p = .36$, Table S1) and provide significant ($p < .05$) variance estimates for BDOC. While the positive correlations between consumers and nutrient concentrations agreed with the theoretical underpinnings of our predictions and the linear model results, negative correlations between nutrients and algal biomass resulted in the causal interpretation that nutrients decreased algae—which contradicts broader ecological theory (Figure 3a). To address this theoretical contradiction (and the high algal biomass observed in many mesocosms), we developed an *a posteriori* model identical to the revised *a priori* model except that it specified that both animal and algal biomasses controlled nutrient concentration/availability (Figure 1b). The covariance structure implied by the *a posteriori* model was consistent with the observed data covariance structure ($\chi^2 = 2.3$, $df = 7$, $p = .94$). Other indicators of model fit further supported that the model implied covariance structure was consistent with the observed covariance structure (Table

TABLE 2 General linear model analysis of replicated regression design for testing the effects of overlapping consumer biomass hotspots on ecosystem processes

S1). Thus, our subsequent analysis and interpretation focus on the *a posteriori* model (hereafter *the model*).

The results of the SEM were consistent with individual linear model results, but SEM analysis of direct and indirect causality provided further insight into the mechanisms by which animals may influence the availability of BDOC. Algal biomass was the single strongest factor controlling BDOC concentrations in our study (Figures 3b and 4). We found mixed support for our prediction that animal biomass would directly increase BDOC concentrations (Figure 1f). While all animals had positive effects on BDOC, only the effects of *H. acuta* were significant ($p < .05$, Figures 3 and 4c). In contrast, the effect of algal biomass was 3.8 times greater. We found broader support for animals influencing BDOC concentration through algal biomass. All direct paths between consumers and algal biomass were negative, but only the fish and mussel paths were significant ($p < .05$). This negative animal control on algal biomass also resulted in fish and mussels having a significant ($p < .05$) negative indirect effect on BDOC concentration. We did not find a significant influence of ambient nutrient concentration on BDOC in the SEM (Figure 3b). This was consistent with the results of the BDOC priming assays, in which the BDOC priming response ratios were not significantly different

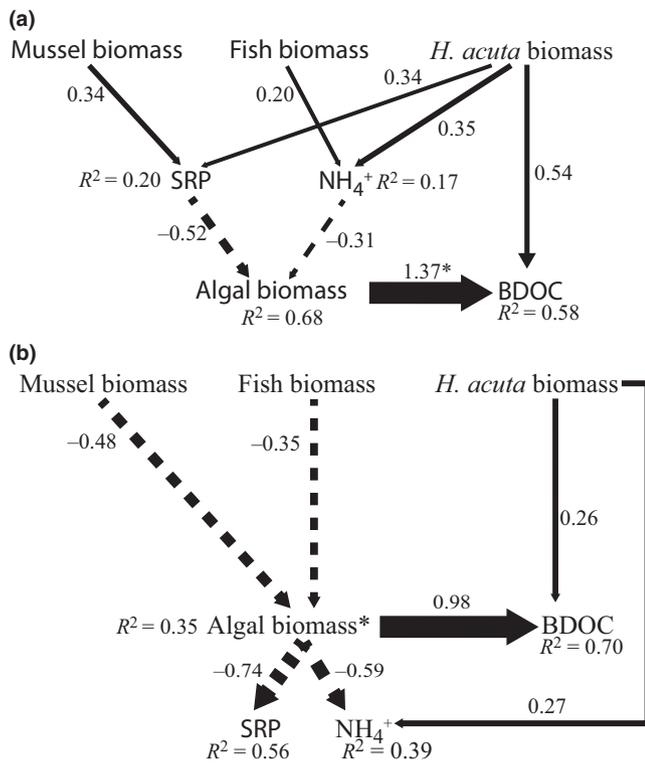


FIGURE 3 Models explaining the concentration of bioavailable dissolved organic carbon (BDOC) available to heterotrophic microbes. (a) *A priori* theoretical model that was significant ($\chi^2 = 7.7$, $p = .36$, Tucker–Lewis index = 0.97, comparative fit index = 0.99, root mean square error of approximation (RMSEA) = 0.05, $p_{\text{RMSEA}} = 0.43$, standardised root mean square of the residual = 0.07), but forced a counterintuitive interpretation of causality and whose standardised path coefficient (*) for algal biomass on BDOC suggested (>1) suggested the model was inconsistent with the data. (b) *A posteriori* model reflecting algal rather than animal control of nutrient concentrations providing better fit ($\chi^2 = 2.3$, $p = .94$, Tucker–Lewis index = 1.21, comparative fit index = 1.0, RMSEA = 0.0, $p_{\text{RMSEA}} = 0.96$, standardised root mean square of the residual = 0.03). Only significant ($p < .05$) paths are shown. Arrows between compartments indicate a causal mechanism (A → causes an increase or decrease in B). Dashed arrows indicate a negative correlation and solid arrows indicate a positive correlation. The size of each arrow is proportional to its standardised path coefficient (printed near each path) relative to the largest path coefficient in each model (e.g. paths in (a) are rescaled relative to 1.37). Algal biomass is a latent variable based on photographs of each mesocosm and chlorophyll-*a* concentration

among animal treatments ($p > .05$). Consequently, we did not find support for our prediction that animals could indirectly affect BDOC by nutrient recycling and nutrient priming.

4 | DISCUSSION

Our results highlight that direct and indirect animal controls on BDOC availability complement nutrient recycling in linking green and brown food webs allowing trophic interactions in one to affect the other (Parr et al., 2019; Zou et al., 2016). Our experiment was

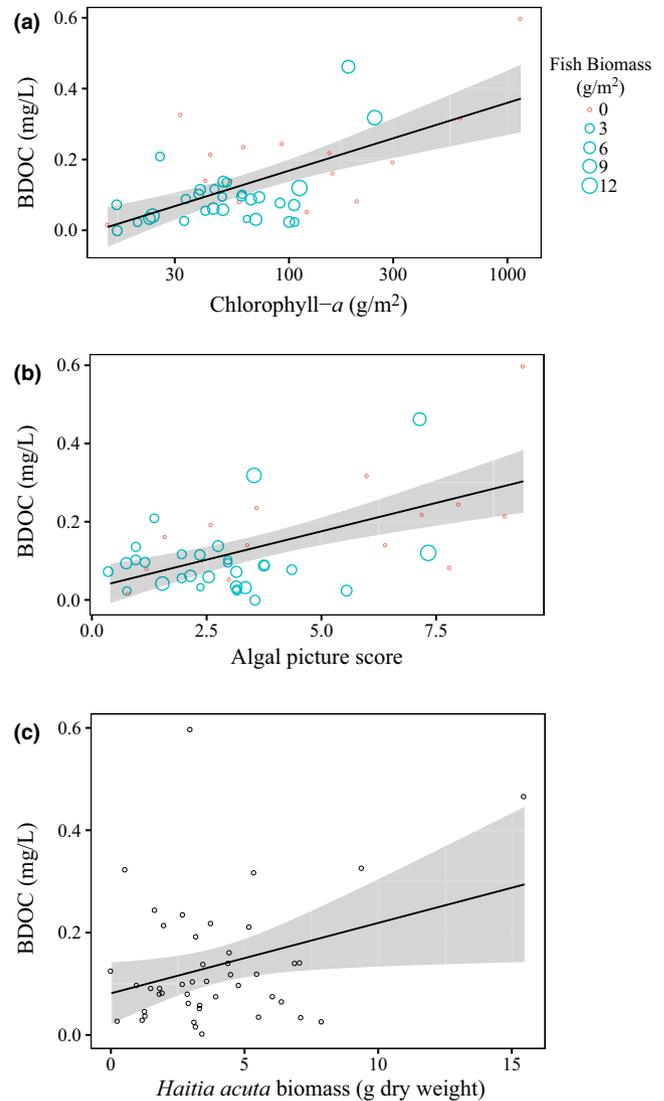


FIGURE 4 Relationships between bioavailable dissolved organic carbon (BDOC) and its primary controls. The response of BDOC to: (a) chlorophyll-*a* with fish biomass indicated by marker size and fish presence and absence indicated by colour ($R^2 = .38$, $p < .01$). (b) Algal picture score with fish biomass indicated by marker size and fish presence and absence indicated by colour ($R^2 = .31$, $p < .01$). (c) *Haitia acuta* biomass ($R^2 = .08$, $p = .03$). Shaded areas are 95% confidence bands about the regression line

designed to test the mechanisms by which animals feeding in green food webs may affect the availability of DOC to microbes in the brown food web. We found mixed support for our set of predictions using a mesocosm experiment that replicated stream ecosystem animal communities. Contrary to previously observed responses of algae and nutrients in overlapping animal biomass hotspots (Atkinson et al., 2013; Hopper et al., 2018), we did not observe significant effects of animals on inorganic nutrient stoichiometry or significant effects of nutrient stoichiometry on BDOC availability. Our results suggest that animal biomass and the effects of overlaps in animal biomass may have the least influence on BDOC bioavailability when algal biomass and ambient inorganic nutrient concentrations are high. Under these high nutrient and algal biomass conditions,

our results also suggest that nutrient and BDOC linkages between green and brown food webs may transmit trophic interactions unidirectionally (green → brown). This further supports the view that the role of animals in regulating nutrient and energy flows within and between ecosystems is context-dependent (Gido et al., 2010; Subalusky & Post, 2018).

4.1 | Direct animal influence on BDOC

Our results suggest that, in mesocosms, animals can directly influence DOC concentration available to heterotrophic microbes through direct mechanisms (excretion), but their relative importance as a source of DOC is context-dependent. Previous work measuring the DOC release by animals in excretion trials and scaling it to ecosystems proposed that animal-mediated DOC and BDOC excretion could satisfy a significant proportion of microbial DOC demand (Meyer & O'Hop, 1983; Parr et al., 2019). We found mixed support for our prediction that animal biomass would directly influence the concentration of BDOC. Algal biomass in our study was high and exerted the strongest direct control over DOC concentrations. In this context, only *H. acuta* biomass significantly increased BDOC concentrations; however, fish and mussels also exhibited positive path correlations. This result is consistent with the metabolic theory of ecology where smaller-bodied organisms have less efficient metabolisms and excrete DOC at higher rates as compared to larger-bodied organisms (Parr et al., 2019; Vanni & McIntyre, 2016).

In addition to lower rates of excretion due to their size, mussels may have exerted a lower direct influence on DOC concentrations due to low suspended food availability. While algae were highly abundant in most mesocosms, they were primarily benthic and the most abundant form was the attached filamentous algae (*Cladophora* spp.). Mussels are filter feeders requiring suspended or planktonic forms of food, thus ingestion of *Cladophora* was probably incidental and low (Vaughn et al., 2008; but see 4.2 for discussion of negative mussel control on algae). In comparison, *Cladophora* was readily available to snails and fish (Power et al., 1985). In situations of low food supply, animals may increase retention times and assimilation efficiency (DeMott, McKinney, & Tessier, 2010).

4.2 | Herbivory and bioturbation controls on BDOC concentrations

Animal activities that directly affect algal biomass provide an important control over the availability of BDOC to microbes. In our experiment, fish and mussels exerted a direct negative control over algal biomass. For the grazing minnows in the experiment, this control was probably herbivory (Power et al., 1985). Negative effects of fish on algal biomass are frequently observed in streams and mesocosms when fish biomass is low relative to algal biomass (Gido et

al., 2010). However, because *Cladophora* is unlikely to be consumed by mussels, we hypothesise this negative control reflects bioturbation. We observed *A. ligamentina* actively moved around the tanks turning over gravel (T.B. Parr, personal observation; Allen & Vaughn, 2009). This bioturbation disrupted periphyton and diatom growth and reduced algal biomass in a pattern similar to grazing. We think food limitation constrained the effects of mussels in our experiment. When assessed with SEM analysis, both the indirect effects of fish grazing and mussel bioturbation significantly reduced BDOC concentrations.

The negative control of fish herbivory on BDOC concentration is consistent with Limberger et al. (2019) who showed that zooplanktivorous fish had no effect on DOC concentration, but did change the composition by removing zooplankton grazing on algal biomass, thereby increasing the relative abundance of algal-derived DOC compositions characteristics typically correlated with greater BDOC concentration. Further research is required to understand how BDOC availability is influenced by animal community complexity and what this may mean for specific microbial ecosystem functions.

4.3 | Animals, nutrients, and priming

We predicted that animal biomasses, presumably through excretion, would prime (increase or decrease) the bioavailability of DOC by increasing NH_4^+ and SRP availability NH_4^+ :SRP stoichiometry. The results of our SEM analysis did not support this indirect mechanism of animal control over BDOC concentration. Algal biomass was the only significant control over SRP concentration, and, while NH_4^+ was significantly influenced by *H. acuta* biomass, nutrient concentration did not affect BDOC concentration. This was independently corroborated by nutrient amended BDOC incubations which exhibited both positive and negative nutrient priming responses that were on average small, not significantly correlated with animal biomass, and not correlated with ambient nutrient concentration.

The lack of an animal-nutrient control in our study may be due to relatively high background concentrations of nutrients in our experiment resulting in our ambient conditions being optimised for DOC degradation. Across aquatic systems, priming responses are typically small (12.6%) and not significantly different from zero (Bengtsson et al., 2018), which has been attributed to the relatively high dissolved organic N and P content relative to DOC (Bengtsson et al., 2018) and high background nutrient concentrations (Hotchkiss et al., 2014). Threshold elemental ratios (TER) are the elemental ratios of food resources below which organisms consuming those foods are C or energy-limited and above which they are nutrient-limited. For microbes, these ratios are variable (Scott, Cotner, & LaPara, 2012), but Sinsabaugh, Hill, and Follstad Shah (2009) estimated average C:P and C:N molar ratios of c. 186 and c. 27 respectively. In our experiment, 75% of mesocosms were below the microbial C:P TER (DOC:TP average 82.0 ± 42.3) and all mesocosms were below

the C:N TER (DOC:total nitrogen = 0.6 ± 0.2). Because nutrient stoichiometry indicated the potential for C-limitation, added nutrients from animal-mediated nutrient recycling were unlikely to increase BDOC. Thus, the importance of animal-mediated nutrient recycling as a critical nexus between green and brown food webs (Zou et al., 2016) does not appear to be significant under conditions of high nutrient availability.

4.4 | Animal-mediated green and brown food web connections in human-dominated systems

The effects of animals in making nutrients and energy (BDOC) available to microbial ecosystems may be stronger in systems where nutrient concentrations are not elevated due to anthropogenic disturbance and where animal biomass is high relative to primary producer biomass. Animal biomass is thought to play a central role in maintaining ecosystem function in ecosystems with minimal human disturbance, a role that is lost by extirpation of species and nutrient pollution (Vadeboncoeur & Power, 2017). The results from our mesocosm study suggesting that direct animal controls linking green and brown food webs through nutrient concentrations and stoichiometry are weak or non-significant should not be viewed as a contradiction of ecological theory (Cherif & Loreau, 2013; Zou et al., 2016). Rather, they may be more typical of the human-disturbed ecosystems with pyramidal trophic biomass distributions (Vadeboncoeur & Power, 2017) and elevated nutrients (Carpenter et al., 1998; Shoda, Sprague, Murphy, & Riskin, 2019) that typify freshwaters in the Anthropocene. In these ecosystems, nutrients and DOC may form less of a two-way nexus transmitting trophic interactions back and forth between green and brown food webs, and more of a one-way conduit where brown food webs merely respond to green food web processes.

In conclusion, our results highlight that animals can provide important controls on the production of bioavailable organic energy in aquatic ecosystems, but the nature of these controls depends on the nutrient context and the distribution of primary producer and animal biomasses. The relative importance of overlapping consumer hotspots may be difficult to observe in many anthropogenically modified ecosystems. In such ecosystems, human modifications may elevate background nutrients and reduce the biomass of larger-bodied animals thereby weakening their controls over nutrient availability or stoichiometry. The trophic linkages between autotrophic and heterotrophic communities in such systems probably flow directly between algal and heterotrophic communities. However, as the ratio of consumers to resources exceeds one in inverted food webs, animals may become critical mediators of energy flow between plants and animals because their indirect control over biomass may suppress biomass to levels where exudates are a relatively small quantity. Further research understanding the role of animal communities and their trophic interactions in green and brown food web-mediated biogeochemical cycles requires a deeper understanding of regional and global patterns of animal biomass and requires biomass-oriented datasets documenting both species biomass and identity.

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CONFLICT OF INTEREST

The authors declare no conflicting interests.

AUTHOR CONTRIBUTIONS

T.B.P., K.B.G., and C.C.V. designed and conducted the experiment, T.B.P. analysed the data and wrote the first draft of the manuscript. All authors revised the manuscript.

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DATA AVAILABILITY STATEMENT

Data are available from the authors upon request.

REFERENCES

- Abramoff, M. D., Magalhães, P. J., & Ram, S. J. (2004). Image processing with ImageJ. *Biophotonics International*, *11*, 36–42.
- Allen, D. C., & Vaughn, C. C. (2009). Burrowing behavior of freshwater mussels in experimentally manipulated communities. *Journal of the North American Benthological Society*, *28*, 93–100. <https://doi.org/10.1899/07-170.1>
- Allen, D. C., Vaughn, C. C., Kelly, J. F., Cooper, J. T., & Engel, M. H. (2012). Bottom-up biodiversity effects increase resource subsidy flux between ecosystems. *Ecology*, *93*, 2165–2174. <https://doi.org/10.1890/11-1541.1>
- Allgeier, J. E., Layman, C. A., Mumby, P. J., & Rosemond, A. D. (2014). Consistent nutrient storage and supply mediated by diverse fish communities in coral reef ecosystems. *Global Change Biology*, *20*, 2459–2472. <https://doi.org/10.1111/gcb.12566>
- APHA (2017). *Standard methods for the examination of water and wastewater*, (23rd ed.). Washington, DC: American Public Health Association, American Water Works Association, Water Environment Federation.
- Atkinson, C. L., Julian, J. P., & Vaughn, C. C. (2012). Scale-dependent longitudinal patterns in mussel communities: Scale-dependent patterns in mussels. *Freshwater Biology*, *57*, 2272–2284. <https://doi.org/10.1111/fwb.12001>
- Atkinson, C. L., & Vaughn, C. C. (2015). Biogeochemical hotspots: Temporal and spatial scaling of the impact of freshwater mussels on ecosystem function. *Freshwater Biology*, *60*, 563–574. <https://doi.org/10.1111/fwb.12498>
- Atkinson, C. L., Vaughn, C. C., Forshay, K. J., & Cooper, J. T. (2013). Aggregated filter-feeding consumers alter nutrient limitation: Consequences for ecosystem and community dynamics. *Ecology*, *94*, 1359–1369. <https://doi.org/10.1890/12-1531.1>

- Atwood, T. B., Hammill, E., Greig, H. S., Kratina, P., Shurin, J. B., Srivastava, D. S., & Richardson, J. S. (2013). Predator-induced reduction of freshwater carbon dioxide emissions. *Nature Geoscience*, 6, 191–194. <https://doi.org/10.1038/NGEO1734>
- Bengtsson, M. M., Attermeyer, K., & Catalán, N. (2018). Interactive effects on organic matter processing from soils to the ocean: Are priming effects relevant in aquatic ecosystems? *Hydrobiologia*, 822, 1–17. <https://doi.org/10.1007/s10750-018-3672-2>
- Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., & Smith, V. H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications*, 8, 559–568. [https://doi.org/10.1890/1051-0761\(1998\)008\[0559:NPOSW W\]2.0.CO;2](https://doi.org/10.1890/1051-0761(1998)008[0559:NPOSW W]2.0.CO;2)
- Cherif, M., & Loreau, M. (2013). Plant-herbivore-decomposer stoichiometric mismatches and nutrient cycling in ecosystems. *Proceedings of the Royal Society B: Biological Sciences*, 280, 20122453–20122453. <https://doi.org/10.1098/rspb.2012.2453>
- Christian, A. D., Crump, B. G., & Berg, D. J. (2008). Nutrient release and ecological stoichiometry of freshwater mussels (Mollusca:Unionidae) in 2 small, regionally distinct streams. *Journal of the North American Benthological Society*, 27, 440–450. <https://doi.org/10.1899/07-112.1>
- Cleveland, C. C., & Liptzin, D. (2007). C:N:P stoichiometry in soil: is there a “Redfield ratio” for the microbial biomass? *Biogeochemistry*, 85, 235–252. <https://doi.org/10.1007/s10533-007-9132-0>
- DeMott, W. R., McKinney, E. N., & Tessier, A. J. (2010). Ontogeny of digestion in *Daphnia*: Implications for the effectiveness of algal defenses. *Ecology*, 91, 540–548. <https://doi.org/10.1890/08-2103.1>
- Dijkstra, F. A., Carrillo, Y., Pendall, E., & Morgan, J. A. (2013). Rhizosphere priming: A nutrient perspective. *Frontiers in Microbiology*, 4, <https://doi.org/10.3389/fmicb.2013.00216>
- Fan, Y., Chen, J., Shirkey, G., John, R., Wu, S. R., Park, H., & Shao, C. (2016). Applications of structural equation modeling (SEM) in ecological studies: An updated review. *Ecological Processes*, 5, 19. <https://doi.org/10.1186/s13717-016-0063-3>
- Findlay, S. E. G., & Parr, T. B. (2017). Chapter 24. Dissolved organic matter. In G. A. Lamberti, & F. R. Hauer (Eds.), *Methods in Stream Ecology*, (3rd ed.) (p. 372). San Diego, CA: Elsevier Science.
- Frank, D. A., Groffman, P. M., Evans, R. D., & Tracy, B. F. (2000). Ungulate stimulation of nitrogen cycling and retention in Yellowstone Park grasslands. *Oecologia*, 123, 116–121. <https://doi.org/10.1007/s004420050996>
- Gido, K. B., Bertrand, K. N., Murdock, J. N., Dodds, W. K., & Whiles, M. R. (2010). Disturbance-mediated effects of fishes on stream ecosystem processes: Concepts and results from highly variable prairie streams. *American Fisheries Society Symposium*, 73, 593–617.
- Grace, J. B. (2006). *Structural equation modeling and natural systems*. New York, NY: Cambridge University Press, Cambridge, UK.
- Guenet, B., Danger, M., Abbadie, L., & Lacroix, G. (2010). Priming effect: Bridging the gap between terrestrial and aquatic ecology. *Ecology*, 91, 2850–2861. <https://doi.org/10.1890/09-1968.1>
- Guenet, B., Danger, M., Harrault, L., Allard, B., Jauset-Alcala, M., Bardoux, G., ... Lacroix, G. (2014). Fast mineralization of land-born C in inland waters: First experimental evidences of aquatic priming effect. *Hydrobiologia*, 721, 35–44. <https://doi.org/10.1007/s10750-013-1635-1>
- Guillemette, F., & del Giorgio, P. A. (2011). Reconstructing the various facets of dissolved organic carbon bioavailability in freshwater ecosystems. *Limnology and Oceanography*, 56, 734–748. <https://doi.org/10.4319/lo.2011.56.2.0734>
- Hopper, G. W., Gido, K. B., Vaughn, C. C., Parr, T. B., Popejoy, T. G., Atkinson, C. L., & Gates, K. K. (2018). Biomass distribution of fishes and mussels mediates spatial and temporal heterogeneity in nutrient cycling in streams. *Oecologia*, 188, 1133–1144. <https://doi.org/10.1007/s00442-018-4277-1>
- Hotchkiss, E. R., Hall, R. O., Baker, M. A., Rosi-Marshall, E. J., & Tank, J. L. (2014). Modeling priming effects on microbial consumption of dissolved organic carbon in rivers. *Journal of Geophysical Research: Biogeosciences*, 119, 982–995. <https://doi.org/10.1002/2013JG002599>
- Iannino, A., Vossage, A. T. L., Weitere, M., & Fink, P. (2019). High nutrient availability leads to weaker top-down control of stream periphyton: Compensatory feeding in *Ancylus fluviatilis*. *Freshwater Biology*, 64, 37–45. <https://doi.org/10.1111/fwb.13192>
- Jassby, A. D., & Platt, T. (1976). Mathematical formulation of the relationship between photosynthesis and light for phytoplankton: Photosynthesis-light equation. *Limnology and Oceanography*, 21, 540–547. <https://doi.org/10.4319/lo.1976.21.4.0540>
- Kline, R. B. (2011). *Principles and practice of structural equation modeling*, (3rd ed.). New York, NY: Guilford Press.
- Kuzyakov, Y., Friedel, J. K., & Stahr, K. (2000). Review of mechanisms and quantification of priming effects. *Soil Biology*, 32, 1485–1498. [https://doi.org/10.1016/S0038-0717\(00\)00084-5](https://doi.org/10.1016/S0038-0717(00)00084-5)
- Limberger, R., Birtel, J., Peter, H., Catalán, N., da Silva Farias, D., Best, R. J., ... Matthews, B. (2019). Predator-induced changes in dissolved organic carbon dynamics. *Oikos*, 128, 430–440. <https://doi.org/10.1111/oik.05673>
- Martin, E. C., Gido, K. B., Bello, N., Dodds, W. K., & Veitch, A. (2016). Increasing fish taxonomic and functional richness affects ecosystem properties of small headwater prairie streams. *Freshwater Biology*, 61, 887–898. <https://doi.org/10.1111/fwb.12752>
- Matthews, W. J., Gido, K. B., Garrett, G. P., Gelwick, F. P., Stewart, J. G., & Schaefer, J. (2006). Modular experimental riffle–pool stream system. *Transactions of the American Fisheries Society*, 135, 1559–1566. <https://doi.org/10.1577/T05-202.1>
- McIntyre, P. B., Flecker, A. S., Vanni, M. J., Hood, J. M., Taylor, B. W., & Thomas, S. A. (2008). Fish distributions and nutrient cycling in streams: Can fish create biogeochemical hotspots? *Ecology*, 89, 2335–2346. <https://doi.org/10.1890/07-1552.1>
- McManamay, R. A., Webster, J. R., Valett, H. M., & Dolloff, C. A. (2011). Does diet influence consumer nutrient cycling? Macroinvertebrate and fish excretion in streams. *Journal of the North American Benthological Society*, 30, 84–102. <https://doi.org/10.1899/09-152.1>
- Meyer, J. L., & O’Hop, J. (1983). Leaf-shredding insects as a source of dissolved organic carbon in headwater streams. *American Midland Naturalist*, 109, 175–183. <https://doi.org/10.2307/2425528>
- Mulholland, P. J. (1997). Dissolved organic matter concentration and flux in streams. *Journal of the North American Benthological Society*, 16, 131–141. <https://doi.org/10.2307/1468246>
- Murdock, J. N., Dodds, W. K., Gido, K. B., & Whiles, M. R. (2011). Dynamic influences of nutrients and grazing fish on periphyton during recovery from flood. *Journal of the North American Benthological Society*, 30, 331–345. <https://doi.org/10.1899/10-039.1>
- Parr, T. B., Capps, K. A., Inamdar, S. P., & Metcalf, K. A. (2019). Animal-mediated organic matter transformation: Aquatic insects as a source of microbially bioavailable organic nutrients and energy. *Functional Ecology*, 33, 524–535. <https://doi.org/10.1111/1365-2435.13242>
- Power, M. E., Matthews, W. J., & Stewart, A. J. (1985). Grazing minnows, piscivorous bass, and stream algae: Dynamics of a strong interaction. *Ecology*, 66, 1448–1456. <https://doi.org/10.2307/1938007>
- Pringle, R. M., Doak, D. F., Brody, A. K., Jocqué, R., & Palmer, T. M. (2010). Spatial pattern enhances ecosystem functioning in an African savanna. *PLoS Biology*, 8, e1000377. <https://doi.org/10.1371/journal.pbio.1000377>
- R Core Team (2019). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Reisinger, A. J., Presuma, D. L., Gido, K. B., & Dodds, W. K. (2011). Direct and indirect effects of central stoneroller (*Campostoma anomalum*) on mesocosm recovery following a flood: Can macroconsumers

- affect denitrification? *Journal of the North American Benthological Society*, 30, 840–852. <https://doi.org/10.1899/10-169.1>
- Rosseel, Y. (2012). lavaan: An R package for structural equation modeling. *Journal of Statistical Software*, 48, 1–36. <https://doi.org/10.18637/jss.v048.i02>
- Rüegg, J., Brant, J. D., Larson, D. M., Trentman, M. T., & Dodds, W. K. (2015). A portable, modular, self-contained recirculating chamber to measure benthic processes under controlled water velocity. *Freshwater Science*, 34, 831–844. <https://doi.org/10.1086/682328>
- Schmitz, O. J., Wilmers, C. C., Leroux, S. J., Doughty, C. E., Atwood, T. B., Galetti, M., ... Goetz, S. J. (2018). Animals and the zoogeochemistry of the carbon cycle. *Science*, 362, eaar3213. <https://doi.org/10.1126/science.aar3213>
- Scott, J. T., Cotner, J. B., & LaPara, T. M. (2012). Variable stoichiometry and homeostatic regulation of bacterial biomass elemental composition. *Frontiers in Microbiology*, 3, 1–8. <https://doi.org/10.3389/fmicb.2012.00042>
- Shoda, M. E., Sprague, L. A., Murphy, J. C., & Riskin, M. L. (2019). Water-quality trends in U.S. rivers, 2002 to 2012: Relations to levels of concern. *Science of the Total Environment*, 650, 2314–2324. <https://doi.org/10.1016/j.scitotenv.2018.09.377>
- Sinsabaugh, R. L., Hill, B. H., & Follstad Shah, J. J. (2009). Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. *Nature*, 462, 795–798. <https://doi.org/10.1038/nature08632>
- Søndergaard, M., & Middelboe, M. (1995). A cross-system analysis of labile dissolved organic carbon. *Marine Ecology Progress Series*, 118, 283–294. <https://doi.org/10.3354/meps118283>
- Spooner, D. E., & Vaughn, C. C. (2009). Species richness and temperature influence mussel biomass: A partitioning approach applied to natural communities. *Ecology*, 90, 781–790. <https://doi.org/10.1890/08-0966.1>
- Spooner, D. E., & Vaughn, C. C. (2012). Species' traits and environmental gradients interact to govern primary production in freshwater mussel communities. *Oikos*, 121, 403–416. <https://doi.org/10.1111/j.1600-0706.2011.19380.x>
- Strayer, D. L. (2008). *Freshwater mussel ecology: A multifactor approach to distribution and abundance*. Berkeley, CA: University of California Press.
- Subalusky, A. L., & Post, D. M. (2018). Context dependency of animal resource subsidies. *Biological Reviews*, 94, 517–538. <https://doi.org/10.1111/brv.12465>
- Taylor, J. M., Back, J. A., & King, R. S. (2012). Grazing minnows increase benthic autotrophy and enhance the response of periphyton elemental composition to experimental phosphorus additions. *Freshwater Science*, 31, 451–462. <https://doi.org/10.1899/11-055.1>
- Trentman, M. T. (2015). *Biotic and abiotic effects on biogeochemical fluxes across multiple spatial scales in a prairie stream network*. Manhattan, KS: Kansas State University.
- Vadeboncoeur, Y., & Power, M. E. (2017). Attached algae: The cryptic base of inverted trophic pyramids in freshwaters. *Annual Review of Ecology, Evolution, and Systematics*, 48, 255–279. <https://doi.org/10.1146/annurev-ecolsys-121415-032340>
- Vanni, M. J., & McIntyre, P. B. (2016). Predicting nutrient excretion of aquatic animals with metabolic ecology and ecological stoichiometry: A global synthesis. *Ecology*, 97, 3460–3471. <https://doi.org/10.1002/ecy.1582>
- Vaughn, C. C., Gido, K. B., & Spooner, D. E. (2004). Ecosystem processes performed by unionid mussels in stream mesocosms: Species roles and effects of abundance. *Hydrobiologia*, 527, 35–47. <https://doi.org/10.1023/B:HYDR.0000043180.30420.00>
- Vaughn, C. C., Nichols, S. J., & Spooner, D. E. (2008). Community and foodweb ecology of freshwater mussels. *Journal of the North American Benthological Society*, 27, 409–423. <https://doi.org/10.1899/07-058.1>
- Vaughn, C. C., Spooner, D. E., & Galbraith, H. S. (2007). Context-dependent species identity effects within a functional group of filter-feeding bivalves. *Ecology*, 88, 1654–1662. <https://doi.org/10.1890/06-0471.1>
- Wilcox, H. S., Bruce, W. J., Meyer, J. L., & Benstead, J. P. (2005). Effects of labile carbon addition on a headwater stream food web. *Limnology and Oceanography*, 50, 1300–1312. <https://doi.org/10.4319/lo.2005.50.4.1300>
- Zou, K., Thébault, E., Lacroix, G., & Barot, S. (2016). Interactions between the green and brown food web determine ecosystem functioning. *Functional Ecology*, 30, 1454–1465. <https://doi.org/10.1111/1365-2435.12626>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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