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Aggregated filter-feeding consumers alter nutrient limitation: consequences for ecosystem and community dynamics

Carla L. Atkinson,[1,4] Caryn C. Vaughn,[1] Kenneth J. Forshay,[2] and Joshua T. Cooper[3]

[1]*Oklahoma Biological Survey, Department of Biology, and Ecology and Evolutionary Biology Graduate Program, University of Oklahoma, Norman, Oklahoma 73019 USA*
[2]*Robert S. Kerr Environmental Research Center, Office of Research and Development, Environmental Protection Agency, Ada, Oklahoma 74820 USA*
[3]*Department of Microbiology and Plant Biology, University of Oklahoma, Norman, Oklahoma 73019 USA*

Abstract. Nutrient cycling is a key process linking organisms in ecosystems. This is especially apparent in stream environments in which nutrients are taken up readily and cycled through the system in a downstream trajectory. Ecological stoichiometry predicts that biogeochemical cycles of different elements are interdependent because the organisms that drive these cycles require fixed ratios of nutrients. There is growing recognition that animals play an important role in biogeochemical cycling across ecosystems. In particular, dense aggregations of consumers can create biogeochemical hotspots in aquatic ecosystems via nutrient translocation. We predicted that filter-feeding freshwater mussels, which occur as speciose, high-biomass aggregates, would create biogeochemical hotspots in streams by altering nutrient limitation and algal dynamics. In a field study, we manipulated nitrogen and phosphorus using nutrient-diffusing substrates in areas with high and low mussel abundance, recorded algal growth and community composition, and determined in situ mussel excretion stoichiometry at 18 sites in three rivers (Kiamichi, Little, and Mountain Fork Rivers, south-central United States). Our results indicate that mussels greatly influence ecosystem processes by modifying the nutrients that limit primary productivity. Sites without mussels were N-limited with ~26% higher relative abundances of N-fixing blue-green algae, while sites with high mussel densities were co-limited (N and P) and dominated by diatoms. These results corroborated the results of our excretion experiments; our path analysis indicated that mussel excretion has a strong influence on stream water column N:P. Due to the high N:P of mussel excretion, strict N-limitation was alleviated, and the system switched to being co-limited by both N and P. This shows that translocation of nutrients by mussel aggregations is important to nutrient dynamics and algal species composition in these rivers. Our study highlights the importance of consumers and this imperiled faunal group on nutrient cycling and community dynamics in aquatic ecosystems.

Key words: algae; mussel; nitrogen; nonmetric multidimensional scaling; nutrient limitation; nutrient translocation; spatial heterogeneity; stoichiometry; unionid.

Introduction

Biogeochemical cycling controls nutrient availability in ecosystems and is often a major driver of ecosystem processes and community dynamics such as trophic interactions and food chain length (Post 2002), decomposition (Elwood et al. 1981), and production (Davis et al. 2010). While nutrient resources are often set by a geologic and climatic template that bounds ecosystem processes (Kaspari and Yanoviak 2009, Small and Pringle 2010), nutrient cycling by organisms can support a substantial proportion of nutrient demand (Vanni 2002). Biogeochemical cycling is driven by organisms that have specific nutritional requirements (Sterner and Elser 2002). Excretion by organisms influences nutrient dynamics in both aquatic and terrestrial systems, and the effects are often associated with dominant taxa (i.e., high biomass) rather than spatiotemporal variation among individual excretion rates (Caraco et al. 1997, Vanni 2002). Translocation and transformation of nutrients by animals is an influential biogeochemical process that enhances primary production across ecosystems and can have large effects on community composition and ecosystem function (Vanni 2002, McIntyre et al. 2008).

Biogeochemical cycling is particularly important in streams because nutrients are taken up quickly and availability is influenced by unidirectional downstream flow. Availability of essential elements controls rates of primary productivity and decomposition in streams (Meyer et al. 1998), and nutrient concentrations in streams can vary substantially across short distances

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[4] Present address: Oklahoma Biological Survey, 111 East Chesapeake Street, Norman, Oklahoma 73019 USA.
E-mail: carlalatkinson@gmail.com

(e.g., Peterson and Grimm 1992). Ecological stoichiometry predicts that biogeochemical cycles of different elements are interdependent because the organisms that drive these cycles require fixed ratios of nutrients (Sterner and Elser 2002, Elser et al. 2007). Variation in nutrient availability depends on surface and subsurface hydrologic exchanges (Dent et al. 2001) as well as spatial variation in microbial and algal activity (Malard et al. 2002). However, excretion by animals at high densities may cause heterogeneity in nutrient availability and dominate nutrient cycling (e.g., Vanni 2002, McIntyre et al. 2008, Small et al. 2009). Freshwater ecosystems are often limited by phosphorus (P) and nitrogen (N), so the ratio at which animals excrete nutrients is potentially important in determining the relative degree of N vs. P limitation and algal species composition in streams (Sterner and Elser 2002). Fish aggregations and migratory fish such as salmon can alleviate nutrient limitation and control in-stream nutrient dynamics (Moore et al. 2007, McIntyre et al. 2008). Sedentary consumers that occur in dense patches in streams may also strongly influence biogeochemical processes and community assemblages.

Freshwater mussels (Bivalvia: Unionidae) are large, long-lived (6–100 years) filter-feeding mollusks that occur in dense, speciose aggregations in river ecosystems (Strayer 2008). Mussels perform important ecological functions in rivers by altering energy pathways and providing habitat (Vaughn and Hakenkamp 2001, Vaughn 2010). As they filter-feed, they remove nutrients and particulates from the water column and make them locally available, reducing rates of downstream loss (Vaughn and Hakenkamp 2001). Mussel excretion facilitates algal growth through nutrient remineralization, which is an important subsidy in nutrient-limited streams. This transfer of energy and nutrients generates spatial heterogeneity in rivers (Vaughn and Spooner 2006) and fuels adjacent terrestrial ecosystems (Allen et al. 2012). Therefore, high-density consumers, like mussels, have the potential to influence stream nutrient dynamics through differential excretion of limiting and non-limiting nutrients (Vanni 2002, Small et al. 2009). Mussels typically excrete and biodeposit materials with low C : nutrient ratios (Christian et al. 2008, Atkinson et al. 2010). Due to their high densities, patchy distribution, and influence on nutrient composition, mussels provide an opportunity to test the predictions of stoichiometric theory that consumers not only alter nutrient availability but also indirectly control downstream primary producer community structure.

Here we investigate how freshwater mussels influence nutrient limitation and algae community composition. In streams we have studied, mussels occur at high densities and increase primary and secondary production (Vaughn and Spooner 2006, Vaughn et al. 2007, Spooner et al. 2012). We hypothesized that increases in production (e.g., Vaughn and Spooner 2006, Vaughn et al. 2007) are due to nutrient translocation by mussels creating biogeochemical hotspots through their filtering and concurrent excretion. Additionally, we hypothesize that due to their high biomass, mussels have the potential to alter the availability and ratios of nutrients (C, N, and P) and alter nutrient limitation and algae species composition locally. We predict that aggregations of mussels alter the direction of nutrient limitation and consequentially affect algal assemblages. Here we combine field observations, experimental manipulation, and statistical modeling to determine whether natural, patchy aggregations of filter feeders in streams give rise to biogeochemical hotspots through nutrient translocation and alteration of the community structure of primary producers.

Methods

Study area

We studied three mid-sized rivers in the south-central United States (Kiamichi, K; Little, L; and Mountain Fork, M [see Plate 1]) where previous work suggests mussels play an important role in supporting primary and secondary production (Vaughn and Spooner 2006, Spooner and Vaughn 2009). Here mussel beds are diverse; they can contain over 20 mussel species at densities up to 100 mussels/m^2 and biomass exceeding 200 g dry tissue mass/m^2. Mussel beds are often separated by large distances within streams (500–5000 m). We selected 18 sites for this study (Fig. 1): nine sites with dense mussel aggregations and nine sites with no or few mussels. All sites were ~1500 m^2. We chose sites based on visual surveys done prior to the experiments and sampling. All sites were located upstream of in-channel reservoirs, and mussel and no-mussel sites were similar in size and water chemistry (Appendix A).

Nutrient diffusing substrates

We used nutrient diffusing substrates (NDS) to address whether nutrient limitation varied as a consequence of mussel filtration and excretion. Prior to placing the NDS in the stream, we qualitatively sampled all sites for mussels using 30-minute timed searches to determine mussel presence (Strayer and Smith 2003). We made NDS with 30-mL plastic cups filled with 2% agar amended with four treatments: nitrate (N, 0.25 mol/L $NaNO^{3-}$), phosphate (P, 0.25 mol/L KH_2PO^{4-}), a combined treatment containing 0.25 mol/L of both N and P (NP), and a control cup of agar alone (C) (Tank et al. 2006). Cups were capped with fritted glass discs that allowed diffusion of nutrients from the agar to the surface. We deployed 12 replicates of each treatment type at each site ($n = 48$ NDS per site, $n = 864$ total) during the summer of 2010 (22 June–6 July). We attached the NDS randomly to a plastic L-bar (three replicates of each treatment per L-bar) and secured four L-bars to the streambed at each site with rebar. After an 18-day incubation, we removed the NDS from the stream and the discs were immediately removed, wrapped in foil, placed on ice, and then frozen for later

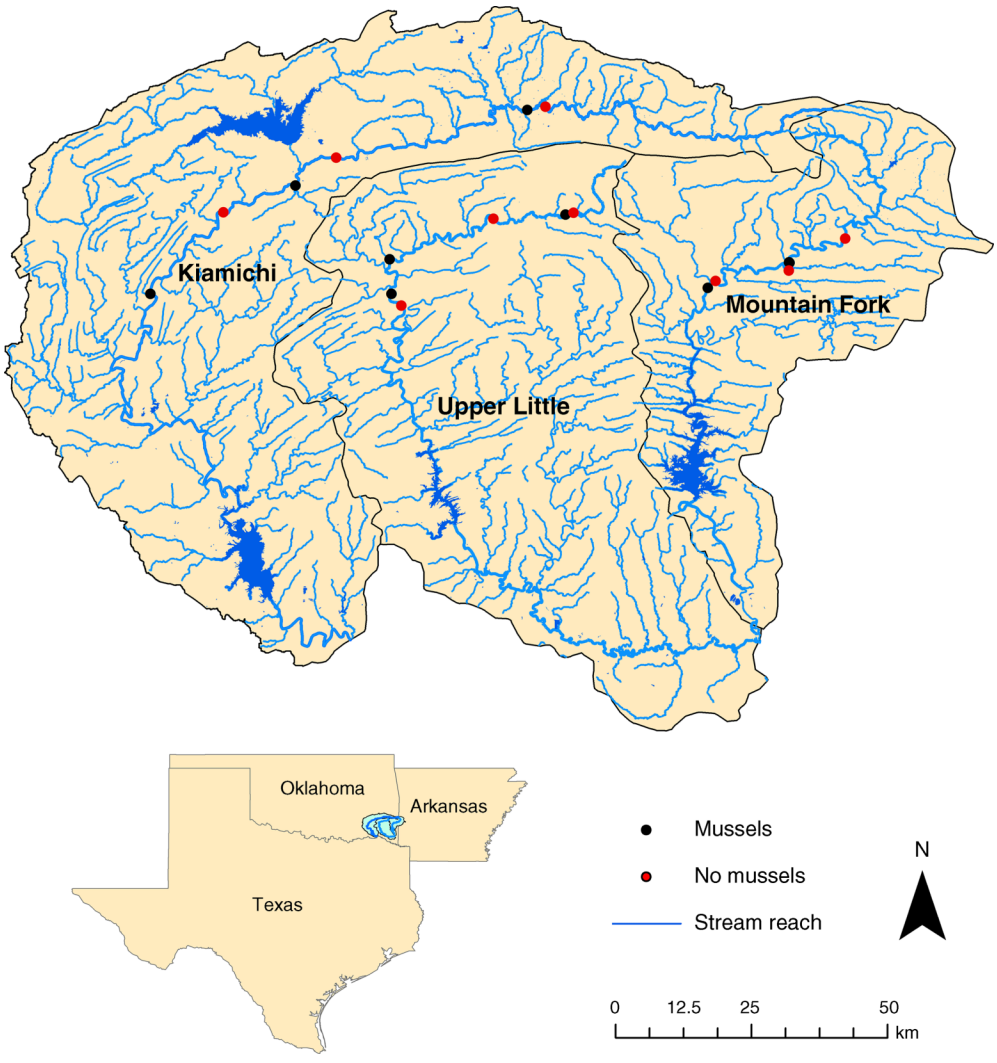

FIG. 1. Map depicting the study area in the Kiamichi, Little, and Mountain Fork Rivers, south-central United States. "No mussels" sites had no mussels or very low densities of mussels (<0.8 mussels/m^2), while mussel sites contained an abundance of mussels.

processing. Nutrient diffusion through NDS is constant through 17 days and declines slightly to day 21 (Tank et al. 2006); thus our treatments encompassed the most constant diffusion time. Whole discs were placed in 60-mL Nalgene bottles, and chlorophyll *a* was cold-extracted in 90% high performance liquid chromatography (HPLC)-grade acetone for 24 h before measurement. Chlorophyll *a* concentrations were measured with a TD-700 laboratory fluorometer (Wetzel and Likens 2000).

Water chemistry and canopy cover

Prior to NDS placement and following retrieval, we measured background temperature, pH, conductivity (μS), and dissolved oxygen (mg/L) with a Hydrolab MiniSonde 4a (Hach Company, Loveland, Colorado, USA). Turbidity was measured with a Turner Designs Aquafluor Handheld fluorometer (Turner Designs, Sunnyvale, California, USA). Samples for total dissolved nitrogen and phosphorus were collected from the middle of the stream channel at each site, field-filtered, acidified, and analyzed (following persulfate digestion) within 28 days of collection using a Lachat QuikChem FIA +8000 Series flow injection analyzer (Hach Company, Loveland, Colorado, USA) for determination of water column N:P. Total dissolved carbon was determined from filtered (GF/F) samples collected in 40-mL volatile organic analysis vials using a Phoenix 8000 carbon analyzer (Teledyne Tekmar, Mason, Ohio, USA). We estimated stream shading using a spherical densiometer to quantify riparian forest canopy cover over the stream (Appendix A).

Benthic algal community

At each site five rocks were haphazardly selected along a transect perpendicular to the stream flow. Rocks were scrubbed with a brush in water, and the resulting slurry was collected and preserved in 3% glutaraldehyde. To describe the benthic algal communities at these locations, algal cells were counted and identified to genus in five fields of view at 200× magnification (>150 cells identified for each sample). Further observation of cells was done at 400× for identification. Counts were used to calculate relative abundances (proportions) of algal genera and the distribution of algal groups (green algae, Chlorophyta; diatoms, Bacillariophyceae; and blue-green algae, Cyanobacteria) at each site.

Mussel surveys and excretion experiments

After NDS were removed, all sites were quantitatively surveyed for mussels by excavating 10 0.25-m^2 quadrats randomly placed within each study site. Quadrats were excavated to a depth of 15 cm and all mussels were removed and identified to species. Excretion experiments were done at each site using five individuals of the most common species (often more than one species at each site; Appendix B). Five control containers filled with 1000 mL of filtered river water were used for all treatments. Empty mussel shells collected from the stream were used as a control for the presence of an object in the chambers and the potential of associated algae and bacterial fauna passing through the filter. Mussels and shells were removed from containers after an hour and then the water from each container was filtered through a GF/F filter (1.0-μm pore size) to separate egestion products (i.e., biodeposits) collected on the filter, from excretion products (i.e., the filtrate, nutrients returned to the water column). Excretion stoichiometry was calculated based on differences in dissolved nutrient concentrations (DOC, TN, TP) in the controls and mussel treatments. We collected three replicates of seston (suspended matter in the water column), the food resource for mussels, at all sites when the NDS were deployed and removed from the stream.

Tissue stoichiometry (%C, %N, and %P) was determined for all of the mussels used in the excretion experiments. Following the excretion experiments, mussels were placed on ice and returned to the laboratory. Length, total wet mass, and tissue dry mass were determined for each individual. Foot muscle tissue was sampled from each individual and dried at 60°C until mass remained constant. Seston, mussel tissue, and biodeposit samples were analyzed on a Finnigan Delta Plus mass spectrophotometer (Thermo-Finnigan, Bremen, Germany) in the University of Georgia's Analytical Laboratory for the determination of %C and %N. For %P, samples were weighed, combusted at 550°C for 2 h, and analyzed with HSO_4 digestion followed by soluble reactive phosphorus analysis (Solorzano and Sharp 1980). Excretion samples (filtrate) were analyzed for total dissolved N (TN), P (TP), and dissolved organic carbon (DOC) as for the water chemistry samples. The carbon, nitrogen, and phosphorus composition was then converted to molar ratios to express stoichiometric ratios. Body nutrient composition was measured for 105 individuals and the nutrient composition of egestion and excretion were measured for 85 of those individuals of six different species (Appendix B).

Statistical analyses

NDS and excretion experiments.—Using the Tank et al. (2006) protocol for NDS analyses, limitation was indicated when NO^{3-} or PO_4^{3-} alone initiated a positive response of chlorophyll *a* growth without a significant interaction. Co-limitation was indicated when two treatments independently affected the response, or when a combined treatment affected the response. To determine if the presence of mussels altered nutrient limitation, we analyzed chlorophyll data from all sites using a two-way ANOVA (mussel vs. no-mussel and nutrient treatment were the main effects) followed by Tukey's HSD multiple comparisons. To test whether water column N:P influenced the response of the NDS treatments, we used an ANOVA to test if there was a significant difference in water column N:P across the sites grouped based on their NDS responses (i.e., N-limitation, co-limitation, no significant difference). To test whether mussel excretion altered N:P, we used a Wilcoxon ranked sum test to determine if there was a difference between the control and mussel treatments in the excretion experiments. All analyses were done in R 2.14.0 (R Development Core Team 2011).

Modeling the influence of mussels.—Water column N:P is likely both directly and indirectly influenced by mussel activity, and the relationship between mussels and water N:P likely includes both strong and weak interactions in stream systems. To explore the effects of mussels on nutrient pathways, we used path analysis to model the stoichiometric relationships among mussels (tissue, excretion, and biodeposits), mussel food (seston), and water column N:P. This analysis was by necessity restricted to sites with mussels. All available data were included in the model: tissue N:P for individual mussels, biodeposit N:P for individual mussels, excretion N:P (means for species by sites, corrected for controls), seston N:P (means by site), and water column N:P (means by site). We created five hypothesized models to examine stoichiometric relationships that affect water column N:P (Appendix C) using R version 2.14.0 with package sem version 2.1-0 (R Development Core Team 2011). We treated a path model as "valid" only if the model's χ^2 was nonsignificant, an indication that the actual and model correlation matrices do not differ (Mitchell 1993). In the case of multiple valid models, we accepted the most parsimonious one (lowest AIC_c). Resultant models are not a full explanation of cause-and-effect relationships; rather they are simplified models for the system. Following the path analysis, we used a linear regression to examine

the difference in water column N:P between the paired mussel and no-mussel sites by comparing the difference to the average excretion from the site.

Benthic algae.—We examined the differences in both algal functional groups and composition between mussel and no-mussel sites. Algae were grouped into broad functional categories (i.e., diatoms, green algae, or blue-green algae). Following this classification, a *t* test was performed on arcsin, square-root transformed proportions for each algal group with mussel vs. no-mussel being the predictor using R 2.14.0. Because algae can differ in their nutrient response to nutrient limitation (Stelzer and Lamberti 2001), we tested for a difference in algal community composition among rivers (K, L, M) or mussel presence (mussel vs. no-mussel) using a nonparametric permutation MANOVA (PerMANOVA) with 999 random permutations using the vegan package (Oksanen et al. 2011) in R 2.14.0. PerMANOVA only assumes independence and similar multivariate distribution of data making it ideal for comparisons of community assemblages that generally violate the assumptions of parametric MANOVA (Anderson 2001). The test computes a multivariate pseudo-*F* statistic by comparing the variation among groups and the variation within groups and generates *P* values through permutation of the data. After testing for main effects on the algal communities, we conducted a nonmetric multidimensional scaling (NMDS) ordination using the algae relative abundance data at each site to compare community assemblages across the sites. NMDS is the most robust unconstrained ordination method (Minchin 1987) and uses species-occurrence data alone to identify the axes that best explain variation. NMDS seeks an ordination in which the distances between all pairs of sample variables are in rank order agreement with their dissimilarities in species composition (McCune and Melford 1999). We used the metaMDS function in the vegan package (version 2.0-2; Oksanen et al. 2011) for R (version 2.14.0) with community dissimilarities based on the Bray-Curtis index. This function produces ordinations based on multiple random starts to avoid local minima, and rotates the resulting axes in such a way that the variance of sites is maximized along the first axis. A joint plot of secondary variables (i.e., water chemistry variables in Appendix A) was superimposed on the ordination map (setting the minimum R^2 value to 0.15) to illustrate associations among these variables and algal assemblages.

Results

Mussel surveys

Initial qualitative surveys verified the absence of mussels at sites classified as "not having mussels." Following the more rigorous quantitative surveys, some mussels were found at "no-mussel" sites, but at very low densities (0–0.8 mussels/m^2 with mussels found at four of the sites). In contrast, densities at mussel sites were 6.8–20.2 mussels/m^2.

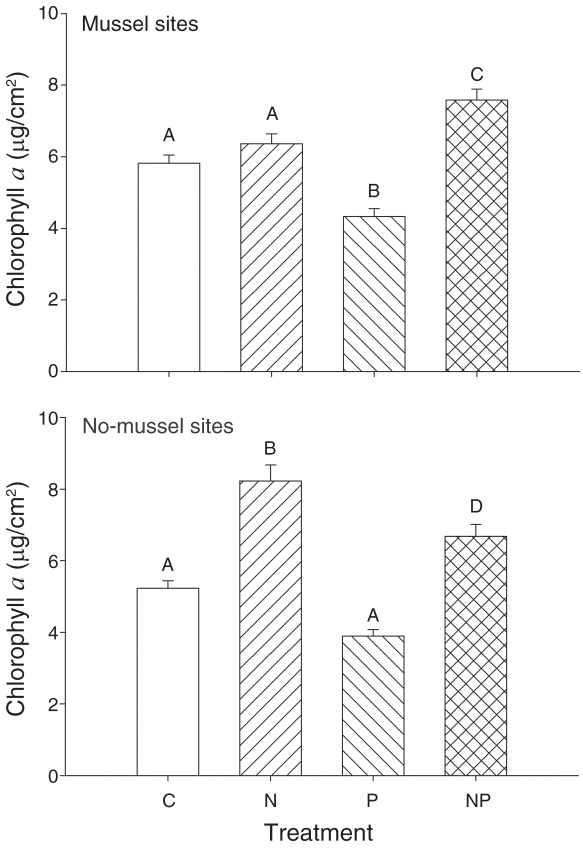

FIG. 2. Algal standing crop (chlorophyll *a*) from the nutrient-diffusing substrate experiments. No-mussel sites had no mussels or very low densities of mussels (<0.8 mussels/m^2), while mussel sites contained an abundance of mussels. Means + SE are shown for all the sites combined (nine mussel sites and nine no-mussel sites). Different uppercase letters denote significant differences between the groups means ($\alpha = 0.05$). Abbreviations are: C, control; N, nitrogen amended treatment; P, phosphorus amended treatment; and NP, nitrogen and phosphorus amended treatment.

Nutrient diffusing substrates

Chlorophyll *a* standing stocks at mussel and non-mussel sites responded differently to nutrient treatments, indicated by a significant interaction between site type and the nutrient treatment (two-way ANOVA, interaction, $F_{3,781} = 9.41$, $P < 0.0001$; Fig. 2). Therefore, to assess nutrient limitation, we examined chlorophyll *a* standing stocks at sites with and without mussels with separate individual one-way ANOVAs. Sites without mussels were N-limited, having higher chlorophyll growth on the N treatments (ANOVA, $F_{3,402} = 36.23$, $P < 0.0001$; Tukey's HSD, $P < 0.01$), while sites with mussels were co-limited (ANOVA, $F_{3,379} = 25.94$, $P < 0.0001$; Tukey's HSD, $P < 0.01$). High mussel densities resulted in a greater response to the +NP treatments

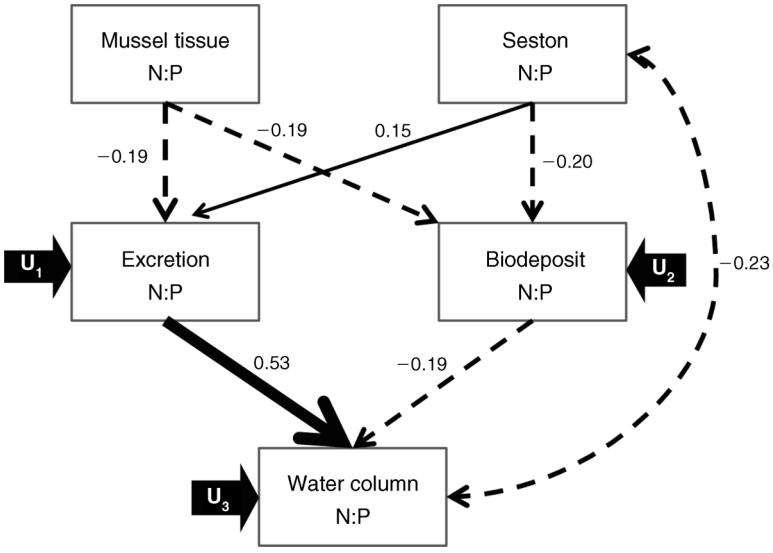

FIG. 3. Path analysis of the effects of mussel N:P and their food source, seston N:P, and water column N:P. The model provided a good fit for the data. The width of the postulated cause–effect path corresponds to the strength of the relationship, with negative relationships indicated by dashed lines. U_i refer to unknown sources of variation (i.e., not explained by the model). Correlation coefficients are shown for each path.

(approximately 1.2× higher chl *a* growth) than sites without mussels, although this difference was not statistically significant (*t* test, $t_{194} = -1.86$, $P = 0.065$). Sites without mussels had a significantly greater response (approximately 1.3× higher chl *a* growth) to N addition than sites with mussels (*t* test, $t_{196} = 3.50$, $P = 0.005$). Water column N:P did not have a significant influence on the NDS response ($P > 0.10$).

Stoichiometry

Mussel tissue C:N was 4.23–4.94 (4.45 ± 0.06, mean ± SE, $N = 105$), and an N:P was 10.2–42.1 (24.6 ± 1.09) with little variation within species across sites. During the excretion experiments, the N:P ratios in the mussel treatments were significantly higher than those in the controls (Wilcoxon test, $W = 1946$, $P < 0.001$). On average, mussels increased N:P in the excretion chambers by 11.73 ± 1.3 in comparison to the control chambers. After correcting for the control, excretion C:N was 8.15 ± 0.23 and N:P was 24.70 ± 1.16 ($N = 85$). Mussel excretion caused a significant decrease in C:N and an increase in N:P mostly mediated by high N excretion in comparison to the control. Mussel biodeposits (egestion) were similar in C:N (8.12 ± 0.12, $N = 85$), but N:P (8.05 ± 0.39) was lower in comparison to mussel excretion due to a higher %P content.

Path analysis

Our hypothesized model was a plausible model to describe how mussel feeding and excretion mediates differences in limiting nutrients across sites based on food (seston) and mussel tissue stoichiometry (Fig. 3). The χ^2 of our path analysis was not significant ($\chi^2 = 3.72$, $AIC_c = 9.4$, df = 3, $P > 0.30$), indicating good model fit. Only one other hypothesized model (Appendix C) had a nonsignificant χ^2, but a much higher AIC_c score ($AIC_c = 12.66$, df = 3, $P > 0.05$). The resultant best fit model indicated that N:P of the water column (field data) was positively correlated with N:P of mussel excretion. Seston N:P and mussel tissue N:P were not correlated, but both slightly influenced excretion and egestion (biodeposits) N:P. Mussel excretion was positively correlated to the N:P of the seston and negatively correlated to N:P of mussel tissue, while N:P of biodeposits was negatively affected by both of those variables. Our regression analysis examining the influence of mussel excretion N:P on the difference in water column N:P between paired sites (mussel vs. no-mussel) indicated that mussel excretion N:P was positively associated with higher water column N:P, but this relationship was not significant ($R^2 = 0.28$, $P = 0.13$).

Algae

We collected and identified 38 genera of algae, and overall algal functional group representation differed significantly between the site types (Fig. 4). Sites without mussels (41.1% ± 20.7%) had a significantly greater relative abundance of blue-green algae than sites with mussels (14.9% ± 11.1%) (Fig. 3; $t_{16} = -3.326$, $P = 0.004$), whereas sites with mussels had a higher relative abundance of diatoms (mussel, 64.8% ± 7.6%; no mussels, 42.0% ± 6.7%) ($t_{16} = 2.236$, $P = 0.04$). There was no significant difference in the relative abundance of green algae between sites with and without mussels ($t_{16} = 0.471$, $P = 0.64$).

Variation in algal assemblages was explained both by river and mussel density. Algal assemblages differed due to river (PerMANOVA, $F_{1,14} = 2.68$, $P = 0.001$) and the

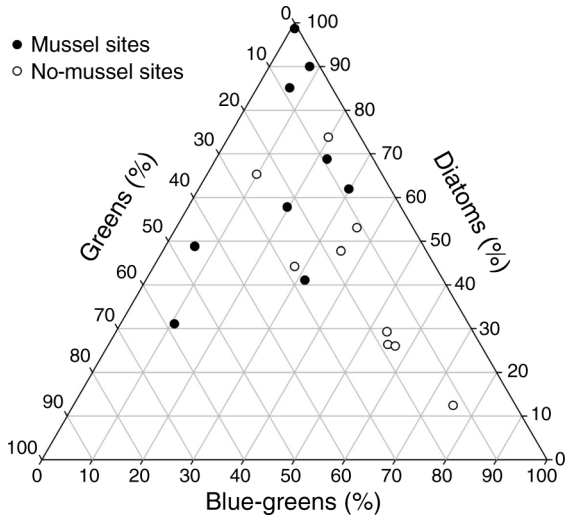

FIG. 4. Triangle plot illustrating the relative abundances of algae in the three functional groups (blue-green, diatoms, and green) represented in the periphyton samples.

interaction between site type (mussel vs. non-mussel) and river ($F_{1,14} = 1.64$, $P = 0.04$), but did not differ based on site type alone ($F_{1,14} = 0.98$, $P = 0.48$). Two NMDS axes explained 98.5% of the variation in algae community composition. Although NMDS does not give factor loadings, examination of the data indicates that axis 1 was strongly correlated to algal functional groups: diatoms (especially *Gomphonema*, *Frustulia*, *Nitszchia*, and *Stauroneis*) negatively correlated to axis 1 and blue-green algae (*Anabaena*, *Aphanizomenon*, and *Gloeocapsa*) and *Epithemia* positively correlated to axis 1. Interestingly, algae within the family Epithemiaceae, all containing N-fixing cyanobacterial endosymbionts, were also positively correlated to NMDS axis 1 and were only found at four sites, all without mussels. Algal assemblages had some partitioning due to site type, but algal assemblages from sites within the same river also tended to cluster (Fig. 5). Our joint plot of environmental variables suggested that axis 1 was negatively correlated to water column nitrogen concentrations, while axis 2 was positively correlated to both percent canopy cover and pH and negatively correlated to conductivity.

DISCUSSION

Our study is among the first to show that aggregations of filter-feeding organisms alter nutrient limitation and community composition in river ecosystems. Other studies have shown that nonnative, invasive zebra mussels shift food webs and energy flow from pelagic to benthic energy pathways (Caraco et al. 2006), and invasive mud snails dominated carbon and nitrogen fluxes primarily due to their high biomass (Hall et al. 2003). Our work supports and extends these previous studies by showing that excretion by dense aggregations of filter-feeders can change which nutrients are limiting in a system and alter algal community composition. We demonstrate how consumer-mediated changes in water chemistry alter community composition and dominance patterns among algal functional groups. These results suggest that filtering consumers, i.e., freshwater unionid mussels, have a profound impact on ecosystem and community dynamics. Areas with high mussel densities showed different patterns of nutrient limitation and algae community assemblages than areas with low densities. Elser et al. (2007) showed in a meta-analysis that there is usually a synergistic effect of N and P addition; that adding N and P together boosts primary productivity more than does adding either one separately; and suggested that the stoichiometry of N and P supply and demand must be in close balance in most ecosystems. Our results suggest that in the rivers we studied, mussels help to maintain this balance in N and P stoichiometry. Our findings suggest that the mechanism behind this change is nutrient excretion by dense mussel communities; excretion experiments showed that mussels increased water column N:P. This evidence,

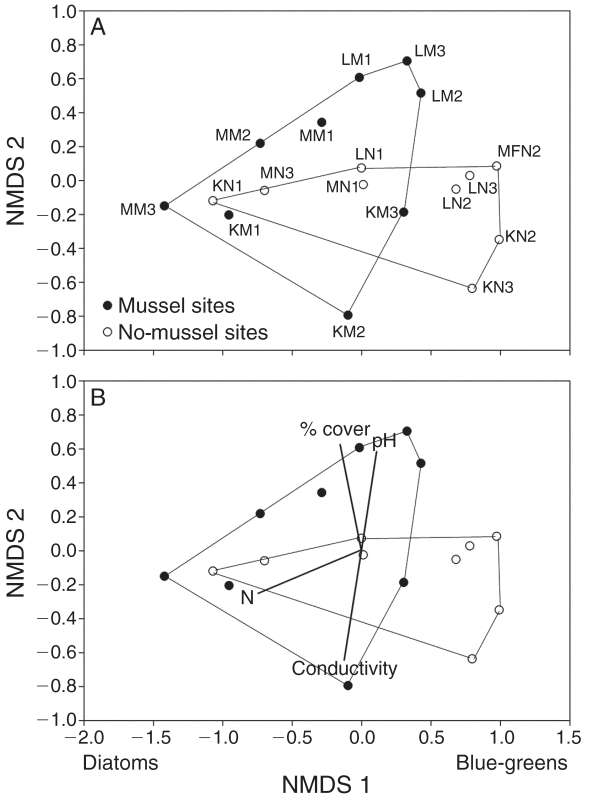

FIG. 5. Nonmetric multidimensional scaling (NMDS) ordination of algae genera. Mussel sites are coded with M, while no-mussel sites are coded with N. NMDS axis 1 differentiated sites with high blue-green vs. diatom dominance. Plots show the NMDS scores for the sites in relation to the ordination of the algae with (A) sites labeled and convex hulls drawn to differentiate mussel and no-mussel sites; (B) a joint plot indicating how environmental drivers are correlated to the NMDS plot (minimum R^2 was set at 0.15).

PLATE 1. Most downstream study site on the Little River, Oklahoma, USA. Photo credit: C. L. Atkinson.

coupled with our path analysis results, indicates that nutrient translocation and nutrient remineralization by mussels alleviates strict N-limitation in these streams and causes a consequent change in algae communities.

Freshwater mussels translocate nutrients and energy from the water column to the benthic compartment (Vaughn and Hakenkamp 2001); thus large aggregations of these animals can cause tight coupling of nutrient dynamics between these compartments. This process should shorten nutrient spiraling in streams by taking nutrients that would otherwise flow downstream (Newbold et al. 1982) and concentrating them in the benthic food web. This concentration may represent a shortening of spiraling length that may allow streams to be more efficient per unit area. Here, translocation of nutrients by dense communities of freshwater mussels potentially led to alteration of nutrient limitation through an incremental change in the availability of nutrients. Even more striking, this alteration of nutrient limitation led to differences in algae community composition.

The potential effects of mussels on nutrient limitation we observed are consistent with stoichiometric theory (Sterner and Elser 2002). Elemental demand (driven by body stoichiometry and constrained by phylogeny) combines with diet nutrient content to control the nutrient ratios of excretion (Vanni 2002, Torres and Vanni 2007). Our path analysis showed that N:P of nutrient excretion was negatively correlated to tissue N:P and positively correlated to seston N:P, which is consistent with stoichiometric theory. Further, higher mussel excretion N:P was associated with higher water column N:P. Changes in the ratios of available nutrients can drive changes in species composition (Kutka and Richards 1997, Sterner and Elser 2002). We know from previous work that mussel aggregations stimulate benthic algal production (Vaughn et al. 2007). Here we show that mussels alter water column stoichiometry, which leads to changes in algal functional groups. N-fixing algae (i.e., blue-green algae and *Epithemia*) were more common in N-limited sites lacking mussels. Other studies have found that Epithemiacean diatoms often dominate periphyton communities in environments where nitrogen concentrations are low (Mulholland et al. 1991, Peterson and Grimm 1992). *Epithemia* contain cyanobacterial endosymbionts that enable these diatoms to fix atmospheric nitrogen (Geitler 1977). Mussel aggregations altered water column stoichiometry that corresponded to differences in algal assemblages (more N-fixers at N-limited sites), which is consistent with stoichiometric theory.

Mussels are spatially heterogeneous at our study sites and in many rivers; thus, their effects on river function are spatially heterogeneous. Spatial heterogeneity influences population dynamics, community structure, and ecosystem function (Zerba and Collins 1992, McIntyre et al. 2008). Our results highlight that nutrient dynamics can vary within a system based upon patch dynamics of organisms that function as ecosystem engineers through modification of the physical habitat and availability of nutrients and food, but that the impact of the organisms is a function of their behavior, size, and density (Moore 2006). For example, variations in fish densities and species composition altered the availability of nutrients and created biogeochemical hotspots in a tropical stream (McIntyre et al. 2008). Mollusks are well known as structural engineers (Gutierrez et al. 2003), but the influence of native freshwater mussels (see *Results*), invasive freshwater mussels (Goedkoop et al. 2011), and marine mussels on nutrient dynamics is only beginning to be appreciated. For instance, Aquilino et al. (2009) showed that higher mussel densities among intertidal areas caused differences in nutrient recycling rates and increased the abundance of a seaweed species. Vaughn and Spooner (2006) found increased abundance and richness of insect larvae in mussel aggregations, which could be in response to the enhanced biogenic habitat caused by mussels, but also in response to the enhanced algae production and quality of algae (diatoms are a high quality food resource) stimulated by mussel activity. Translocation of nutrients and materials by mussels as a function of patch dynamics is important to ecosystem processes through increasing habitat heterogeneity.

Our study demonstrates the influence of a functional group of consumers, filter-feeding mussels, on ecosystem processes across three rivers in which background organismal densities and abiotic factors varied. Some of the differences we saw across the mussel sites are likely due to species identity effects (Evans-White and Lamberti 2006, Spooner and Vaughn 2008, Spooner et al. 2012) and differences in background conditions (e.g., elevated nutrients; Evans-White and Lamberti 2006). Within these rivers not all patches are equivalent because mussel density and species composition vary both within and among rivers (Spooner and Vaughn 2009) and are influenced by a hierarchy of factors including local environmental conditions, fish host abundance and dispersal, and biogeographic history (Vaughn and Taylor 2000, Strayer 2008). Functional traits of mussels, such as filtration and excretion rates, also vary among species (Spooner and Vaughn 2008). Thus, some of the observed differences in the strength of nutrient limitation across sites are likely due to a combination of different species-specific excretion rates, richness/biomass differences among rivers and sites, and unmeasured environmental correlates. The ratio of N to P has frequently been used as a predictor of nutrient limitation in aquatic systems (Tank and Dodds 2003), yet we did not see a strong relationship between N:P of the water and limitation when evaluating both mussel and non-mussel sites. However, in lotic systems, continuous unidirectional flow may cause deviation from expected relationships between nutrient limitation and concentration (Tank and Dodds 2003). For example, if there is a continuous flux of nutrients, nutrient requirements can be met despite low nutrient concentrations in stream water. While we observed differences between sites with and without mussels, some differences in benthic algal community composition were correlated to water chemistry and canopy cover. The interaction of species effects and background nutrient conditions on the influence of animals on nutrient dynamics is an important avenue for future research. Nonetheless, the strong effect of mussels that we observed among our sites across three rivers with varying background conditions underscores the important role of mussels in river ecosystems.

There has been increased recognition of the importance of animals in shaping ecosystems (Polis et al. 2004, Moore 2006). Our study of freshwater mussels demonstrates how a distinct group of organisms can fundamentally alter ecosystem processes and associated communities through the translocation of nutrients and materials. Loss of species has the potential to drastically alter nutrient recycling and other ecosystem functions (McIntyre et al. 2007). The North American freshwater mussel fauna is diverse with approximately 308 native species, but is also North America's most threatened aquatic faunal group (Bogan 2008). Entire assemblages of mussels have been extirpated from rivers due to a variety of anthropogenic causes (e.g., dams, dredging, sedimentation; Strayer 2008, Vaughn 2010). Our results demonstrate that nutrient translocation by a biodiverse group influences nutrient limitation and community assemblages. The full ramifications of past and future losses are not known, but our results suggest that loss of species would change community composition and ecosystem properties of riverine ecosystems.

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Supplemental Material

Appendix A

Physiochemical parameters of all the sample sites used in the study (*Ecological Archives* E094-120-A1).

Appendix B

Mussel species used in the excretion experiments (*Ecological Archives* E094-120-A2).

Appendix C

The five candidate models used in the path analysis selection ordered by AIC_c scores (*Ecological Archives* E094-120-A3).

Appendix D

The nutrient diffusing substrate (NDS) response at each study site (*Ecological Archives* E094-120-A4).

Appendix E

Underwater photograph of the nutrient-diffusing substrates in the stream (*Ecological Archives* E094-120-A5).