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# HIERARCHICAL CONTROLS ON THE IMPACT OF CONSUMER STOICHIOMETRIC REGULATION: FROM SPECIES TRAITS TO ECOSYSTEM LEVEL CONSEQUENCES

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HIERARCHICAL CONTROLS ON THE IMPACT
OF CONSUMER STOICHIOMETRIC REGULATION:
FROM SPECIES TRAITS TO ECOSYSTEM LEVEL CONSEQUENCES

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF BIOLOGY

BY

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**ABSTRACT**

As natural ecosystems become increasingly changed due to habitat alteration, species loss, introduction of non-native species, and climate change, understanding the functional significance of communities to ecosystem function has become imperative. Consequently, recent research has focused on how landscape scale processes influence the distribution of organisms and the influence of organisms on ecosystem function. Freshwater ecosystems are especially sensitive to changes on the landscape because everything that occurs on the land is reflected in the receiving watershed and because of this these ecosystems are subject to declines in native biodiversity that far exceed most terrestrial ecosystems. Approximately half of North American freshwater mussels, a third of crayfishes, a fourth of amphibians, and one fifth of freshwater fishes and gastropods are considered imperiled. This makes research in aquatic freshwater ecosystems essential for understanding the biodiversity of this planet and the linkages between biodiversity and ecosystem function. Linkages between spatial distributions of animals and ecological stoichiometric theory provide a framework for understanding and predicting these linkages. This dissertation shows that examination of spatial patterns in community composition and examination of the role of these communities reveals unknown patterns, interactions, and linkages within stream communities and biogeochemical cycling. Chapter one examined the variables that impact patterns of mussel community composition and showed that stream size and watershed slope are both predictive of community assemblage patterns. The linkages between food webs and nutrient cycles are heterogeneous and often influenced by human activities. Chapter two shows that long-lived mussels integrate agricultural land use in the basin in their

tissue as reflected by enriched $\delta^{15}N$ of their tissue with increasing agriculture. Stoichiometric theory can be extended to ecosystems, such as streams, to predict the role of consumers in food web and nutrient cycles. Chapters three, four, and five show the importance of mussels in influencing nutrient dynamics and their bottom-up impact on stream food webs. Specifically, chapter 3 shows that mussels influence the nutrients that limit primary productivity and mussels shift the system from N-limitation to co-limitation by N and P. This alleviation of strict N-limitation leads to varied algal assemblages in areas with and without mussels with diatoms dominating in areas with mussels and blue-green algae dominating in areas without mussels. This alteration of nutrient limitation and algal assemblages leads to increased heterogeneity within streams. Chapter 4 demonstrates the importance of mussel-derived nitrogen (MDN) to the food web. By enriching mussels with $\delta^{15}N$ in the lab and then placing them in a stream, I was able to trace the N leaving mussels and entering the stream food web. Chapter 5 investigated the impact of a drought on mussel communities and ecosystem functions (i.e. nutrient cycling and storage). I documented a large loss of mussels between 2010 and 2012 and a consequential decline in nutrient cycling and storage. Furthermore, the loss of specific species (thermally sensitive) led to a change in the N:P excreted by the mussel communities. Stoichiometric theory can be extended to ecosystems, such as streams, to predict the role of consumers in food web and nutrient cycles. Taken together, this dissertation demonstrates that examination of spatial patterns in communities and stoichiometric assumptions improves our understanding of consumer-resource dynamics, food webs, the role of consumers in nutrient cycles, and the potential impacts of species loss.

# CHAPTER 1

## Scale-dependent longitudinal patterns in mussel communities

Keywords:

unionid, GIS, channel slope, Bray-Curtis ordination, community composition

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**Abstract**

1. Species richness and assemblage patterns of organisms are dictated by numerous factors, likely operating at multiple scales. Freshwater mussels (Unionidae) are an endangered, speciose faunal group, making them an interesting model system to study the influence of landscape features on organisms. In addition, landscape features that influence species distributions and the scale in which the factors have the greatest impact are important issues that need to be answered to conserve freshwater mussels.

2. In this study, we quantified freshwater mussel communities at 16 sites along three mid-sized rivers in the south-central United States. We addressed the following questions: (1) Are there predictable longitudinal changes in mussel community composition?; (2) What landscape variables best explain shifts in community composition?; and (3) At what scale do landscape variables best predict mussel community composition?

3. After controlling for the influence of longitudinal position along the stream, we compared mussel distributions to a suite of hypothesized explanatory landscape variables across multiple scales -- watershed scale (entire drainage area), buffer scale (100 m riparian buffer of the entire watershed), and reach scale (100 m riparian buffer extending 1 km upstream from the sampling site).

4. We found a significant and consistent longitudinal shift in dominant mussel species across all three rivers, with community composition strongly related to distance from the headwaters, which is highly correlated to stream size. After accounting for stream size, variables at the buffer scale were the best predictors of mussel community

composition. After accounting for watershed position, mean channel slope was the best explanatory variable of community composition and appeared in all top candidate models at the watershed and buffer scales. Coverage of wetland and urban area were also correlated to community composition at the watershed and buffer scales.

5. Our results suggest that landscape-scale habitat factors influence mussel community composition. Landscape features at the buffer scale performed best at determining community composition after accounting for position in the watershed, thus further protection of riparian buffers will help conserve mussel communities.

## Introduction

Species richness and community composition are often dictated by numerous factors operating at multiple spatial scales. In stream ecosystems, both abiotic and biotic attributes are closely related to watershed geology, land-use, and climate, especially at the interface between land and water (Hynes, 1975; Burcher, Valett, & Benfield, 2007). Recent research has focused on regional- and landscape-scale factors (e.g., watershed area, land use, geology) that influence stream communities (Allan, 2004; Hopkins, 2009). Stream communities are strongly influenced by hydrologic factors that shape habitat suitability (Richards, Johnson, & Host, 1996; Galbraith, Vaughn, & Meier, 2008) and resource availability (Golladay, 1997; Atkinson *et al.*, 2009). Runoff patterns are determined primarily by longitudinal location in a watershed, thus spatial patterning in streams is primarily linear. However, landscape alterations such as conversion of forests to urban or agricultural areas typically lead to degraded stream conditions and consequently altered species distributions (Paul & Meyer, 2001; Roy *et al.*, 2003; Riva-Murray *et al.*, 2010). Few studies have examined how the combination of linear location in a watershed and land use structure lotic communities.

Freshwater mussels are a diverse faunal group, particularly in North America (with >300 species), but are also a highly threatened faunal group (Bogan, 2008). They occur in most freshwater habitats with mussel abundance and diversity being greatest in medium to large rivers where they typically occur as dense, multi-species communities called mussel beds (Strayer, 2008). Within mussel beds, biomass can exceed that of other benthic organisms by an order of magnitude and annual production (in dry

biomass) can equal that of other macrobenthos (Strayer *et al.*, 1994). Mussels play important roles in aquatic ecosystems by filtering suspended materials, transferring energy and nutrients from the water column to the sediment, biodepositing organic matter, excreting nutrients and providing biogenic habitat for other organisms (Vaughn, Gido, & Spooner, 2004; Vaughn, Nichols, & Spooner, 2008; Atkinson *et al.*, 2010). Because mussels are long lived (i.e., in comparison to most stream invertebrates; Haag & Rypel, 2011) and relatively immobile as adults they integrate stressors occurring at multiple temporal and spatial scales – from local to watershed.

The mechanisms that lead to species shifts in aquatic insect communities along longitudinal gradients in rivers have been integrated into conceptual models (e.g., Vannote *et al.*, 1980), but less is known about how mussel communities change along gradients and the formation of a conceptual model to describe shifts in mussel community composition is very recent (Haag, 2012). Previous descriptive studies have discussed succession in mussel community composition due to stream size (Ortmann, 1913; Coker *et al.*, 1921), but only a few studies have quantified this pattern (Strayer, 1983; Haag & Warren, 1998). Distribution patterns of freshwater mussels may be influenced by environmental variables operating at multiple spatial and temporal scales (Strayer *et al.*, 1994; Strayer, 2008), but most quantitative studies of habitat influences on mussel community composition have been performed at local stream-reach scales (e.g., Strayer & Ralley, 1993; Steuer, Newton, & Zigler, 2008). Recent studies have begun to examine broader spatial scales, particularly with regard to the distribution of endangered mussels (Hopkins, 2009; Brown, George, & Daniel, 2010), but few have addressed patterns of community structure. Potential watershed-scale effects on mussel

diversity and abundance include physiography (Arbuckle & Downing, 2002) and anthropogenic disturbance in riparian areas (McRae, Allan, & Burch, 2004; Newton, Woolnough, & Strayer, 2008). Overall, the mechanisms underlying how the structure of mussel communities changes along longitudinal gradients in streams are poorly understood.

Here we address how landscape-scale variables influence shifts in mussel communities along three rivers within the same physiographic province. This region of exceptionally high mussel biodiversity allowed us to examine composition and distribution patterns of mussel communities, and answer the following questions: (1) Are there predictable longitudinal changes in mussel community composition?; (2) What landscape variables best explain shifts in community composition?; and (3) At what scale do landscape variables best predict mussel community composition?

## Methods

### *Study Area*

The Ouachita Mountains ecoregion, which covers 46,500 $km^2$ in central Arkansas and southeastern Oklahoma (U.S.), is characterized by a sub-humid subtropical climate, mixed forests/woodlands, rugged mountains, broad valleys, and several large gravel-bed rivers (OEAT, 2003). This region is a center of speciation for both terrestrial and aquatic organisms, with a large number of endemic species (Mayden, 1985). Mussel diversity is noteworthy with >60 species, including 4 federally threatened or endangered species (Vaughn & Taylor, 2000). The three rivers used in this study (Kiamichi, Little, and Mountain Fork; Fig. 1) are all tributaries of the Red River and share regional species pools. Furthermore, these rivers support healthy and

diverse mussel communities primarily due to relatively low anthropogenic impacts compared to other areas in the U.S. (Vaughn & Taylor, 1999). Land cover is primarily forest and pasture, however extensive logging does occur (OEAT, 2003). The rivers are very similar hydrologically and geomorphically (Table 1). Mussel beds in the Kiamichi, Little, and Mountain Fork Rivers can contain over 20 mussel species at densities up to $100/m^2$, with biomass exceeding 20 $kg/m^2$ (Spooner & Vaughn, 2009).

***Mussel Sampling***

We sampled mussels by excavating 10 to 20, 0.25-$m^2$ quadrats along 100 m study reaches at each site (Fig. 1) and by conducting semi-quantitative timed searches (Strayer and Smith 2003, Vaughn et al. 1997), which allowed us to more fully assess species composition. Previous work in this system showed that 10 quadrats provided accurate estimates of the abundance of most mussel species within beds (Vaughn, Taylor, & Eberhard, 1997). Mussel sampling was confined to high-density (8.6-86.4 mussels $m^{-2}$) mussel beds. Sampling occurred during the summers of 1994 [Little River (LM) sites 2 and 5, Mountain Fork River (MFM) site 3], 2003 - 2005 [Kiamichi River (KM) sites 1, 2, 3, 5, and 6], and 2010 [KM site 4; LM sites 1, 3, and 4; MFM sites 1, 2, 4, 5]. We repeated timed searches at LM2, LM5, and MFM3 during 2010-2011 to insure there were no major species composition changes between the 1994 quantitative survey and the 2010 semi-quantitative survey.

***Landscape analysis***

Mussel survey data for each site were compared to geospatial data across multiple spatial scales as suggested by Allan (2004). The spatial scales analyzed for

each sample point were: (1) watershed scale (entire drainage area); (2) buffer scale (100 m riparian buffer of the entire watershed); and (3) reach-scale (100 m riparian buffer extending 1 km upstream from the sampling site; Fig. 2). Watersheds for each sampling point were derived using the Spatial Analyst Toolkit in ArcMap 9.3.2 (*Environmental System Research Institute*, Redlands, CA) with a 30 m digital elevation model (DEM) from the National Elevation Dataset. Mean channel slope was calculated by extracting elevations and distances from the DEM along the National Hydrology Dataset (NHD) flowlines. Mean channel slope for each spatial scale were: (1) mean of the slope for the entire drainage upstream for the watershed scale; (2) mean channel slope 10 km upstream of the site for the buffer scale; and (3) mean channel slope 1 km upstream of the site for the site scale. NHD flowlines were also used to generate a 100 m buffer around the stream channels. Flowlines from the NHD were compared to the National Agricultural Inventory Program (NAIP) 2008 aerial photographs to verify channel locations. We used SSURGO soil data (National Resources Conservation Service, 2006) to assess connectivity of the river to the floodplain, specifically by quantifying the area that is frequently flooded (water is ponded >50% chance in any year, or >50 times in 100 years). Soils that were classified as being frequently flooded were considered to have high connectivity to the floodplain. Land cover (30-m resolution) was obtained from the 2001 National Land Cover Database (Homer *et al.*, 2004).

***Data Analyses***

Relative abundance (% of total species composition) was used to describe mussel community structure at each site. We used polar ordination with a Sorenson distance measure to describe community structure for each river and then all sites

collectively (Bray & Curtis, 1957). The distance between communities indicates the degree to which mutual species similarity factors determine structure (Bray & Curtis, 1957), and allows for community structure to be dissected apart from environmental data. We performed polar ordinations with PC-ORD (Version 6.0, McCune & Melford, 1999) using the variance-regression endpoint selection method. The solution generated by the ordination was one-dimensional. Ordinary-least squares linear regression was used to determine if there was a relationship between distance from the headwaters and the ordination score for the individual rivers and all rivers collectively. To remove the influence of longitudinal position (distance from the headwaters), the residuals from the linear regression performed on all the sites was used as a response variable in the following model building.

Explanatory variables for mussel community composition patterns were evaluated using an information-theoretic approach (Akaike Information Criterion, AIC) to determine which landscape variables (mean channel slope, land cover, floodplain connectivity) at each scale (reach, buffer, watershed) were most strongly correlated to mussel community composition. We used the residuals (values that represent community composition after accounting for variation due to stream position) from the linear regression describing the correlation between distance from the headwaters and the Bray-Curtis score for each site as the response variable. Similar ordination approaches have been successfully used to examine relationships between biological assemblage data and environmental factors elsewhere (e.g., Roy *et al.*, 2003; Vaughn *et al.*, 2008; Riva-Murray *et al.*, 2010). We derived several multiple linear regression models and compared them using AIC. Based on maximum-likelihood estimates and

the number of model parameters, AIC provides a measure for selecting among competing models of a given data set (Anderson, Burnham, & Thompson, 2000). The model having the lowest AIC is selected because it identifies the main explanatory variables while providing the best compromise between predictive power and model complexity (Johnson & Omland, 2004). Models with $\Delta_i$ less than 2 are generally considered to have substantial support (Burnham & Anderson, 2002). The $\Delta i$ is the difference between the AIC of the best fitting model and that of model *i.* We evaluated the relative strengths of models with Akaike weights ($w_i$), which indicate the strength of evidence that a particular model is the best model, given the data and the set of candidate models being compared. This allowed us to determine which set of landscape variables explain the most variation in composition among mussel communities after controlling for distance from the headwaters. We analyzed each spatial scale separately using AIC to determine the variables that best described community composition at each scale and then compared models from each scale. Multiple linear regressions and the AIC analyses were done in SAS v9.2 (*SAS Institute*, Cary, NC).

***Post-hoc substrate test***

Substrate (or bed sediment) size is often highly correlated with position within a watershed (Ferguson *et al.*, 1996). Additionally, maximizing substrate heterogeneity in ecological communities has been suggested to promote temporally stable and diverse communities (Williams, 1980; Brown, 2003). To test whether substrate characteristics had an effect on mussel community composition at our sites, we conducted pebble counts at all sites (using multiple transects distributed across the mussel bed), with at

least 100 pebbles measured at each site (Kondolf et al. 2003). From these pebble counts, we derived texture distribution ($D_{10}$, $D_{50}$, and $D_{90}$) and heterogeneity (D60/D10; Williams, 1980). We performed Spearman rank correlations in SAS v9.2 to test relationships (Spearman rho > 0.51, $\alpha = 0.05$) between mussel community composition (Bray-Curtis score) and substrate metrics, as well as between landscape and substrate metrics. Local substrate metrics were not included in the multivariate models described in the previous section because they are not measured at multiple scales.

## Results

### *Mussel community structure*

Species composition and dominance varied across sample sites (Fig 1). Overall, 18 species were detected at our sites in the Kiamichi River, 16 in the Little River, and 18 in the Mountain Fork River (Fig 3). Headwater sites were generally dominated by small-bodied mussels in the Lampsilini tribe (*Lampsilis siliquoidea, Villosa iris*, and *Villosa lienosa*) that decreased in abundance downstream. *Fusconaia flava* (Pleurobemini tribe) and *Quadrula verrucosa* (Quadrulini tribe) tended to inhabit the mid-reaches. *Amblema plicata* (Amblemini tribe) became increasingly prevalent with increasing distance downstream excluding the most downstream Kiamichi site. *Actinonaias ligamentina, Potamilus purpuratus,* and *Obliquaria reflexa* (all larger bodied mussels in the Lampsilini tribe) only occurred in the furthest downstream sites of the Kiamichi River.

The distributions described above reveal that mussel species composition was structured along a longitudinal gradient, which was also strongly supported by the polar ordination. The polar ordination explained 40% of the variation in mussel communities.

Not surprisingly, sites that were geographically closer tended to have more similar communities, and community structure was more similar at sites that were closer in longitudinal position (Fig 4; Little River, $R^2 = 0.86$, $p = 0.01$; Kiamichi River, $R^2 = 0.66$, $p = 0.05$; Mountain Fork River, $R^2 = 0.53$, $p = 0.16$). Additionally, mussel communities occupying similar longitudinal positions in different watersheds were more similar than communities within the same watershed that were far apart in longitudinal distance (All rivers; $R^2 = 0.77$, $p < 0.001$). Drainage area was also a good a predictor ($R^2 = 0.73$, $p < 0.001$), but was highly correlated to distance from the headwaters. Across all rivers, mussel community composition changed predictably as the distance from the headwaters increased.

***Landscape variables***

The three rivers and their respective watersheds were similar in physiography and hydrology (Table 1). Watershed area of our sites ranged from 73.5 - 2044 $km^2$. Drainage density was similar among the three watersheds, ranging from 0.93 to 1.4 $km/km^2$. Channel slope was variable with headwater locations being the steepest (15.1 m/km, maximum). However, mean channel slope across the watersheds was not highly variable (range of the most downstream sites: 2.3 - 4.3 m/km). Land cover varied across sites with among-site variation increasing with decreasing spatial scale (see Supporting Information). Forest was the dominant land cover at all three scales; however, its relative percentage decreased from watershed (70.9-87.1%) to reach scale (15.3-78.3%). Forest coverage was the only variable that was strongly correlated to distance from headwaters ($|r| = -0.75$). Water coverage varied little among the watersheds, but was

more variable at the reach and buffer scales. Wetland coverage was more variable at the reach and buffer scales and was highest in the Kiamichi River. Water (0-26.1%) and wetland (0-56.8%) percentages were particularly high at the reach scale. There were also differences in land cover among the watersheds, including greater agricultural and urban cover in the Kiamichi (8-15.4% and 2.6-3.1%, respectively) and Mountain Fork (8.8-15.3% and 3.8-4.1%, respectively) watersheds compared to the Little River (1.2-3.2% and 1.6-3.3%, respectively) watershed. The rivers varied in the area that was flooded frequently (5.1-11.4%), with the Mountain Fork River (5.1-5.9%) having the least amount of frequently flooded area at the watershed scale.

The variables retained for the AIC models were: mean channel slope, % water, % urban, % agriculture, % grassland/shrub, % forest, % wetland, and % area frequently flooded. Correlation matrices indicated that multi-colinearity was low among this subset of independent variables ($|r| < 0.60$).

***Mussel community composition vs. landscape variables***

After accounting for distance from the headwaters, the residual variation in freshwater mussel community composition (23% remaining variation) was best described by watershed- and buffer-scale predictors (Table 1). At the watershed scale, a model including channel slope, % wetland, and % urban best predicted mussel community composition ($w_m = 0.111$, $R^2 = 0.43$), however this was not significant at $\alpha < 0.05$ ($p = 0.07$). Overall, channel slope accounted for over 23% of the residual variation in species composition at the watershed scale. The remainder of the variability was explained by % wetland and % urban land use, with both variables in the top three

models (however those models had a $p > 0.05$). At the buffer scale, channel slope and % wetland were in the top model ($w_m = 0.124$, $R^2 = 0.47$). Channel slope was also the primary explanatory variable at the buffer scale accounting for over 26% of the residual variation in community composition. Percent wetland was included in the top three models and explained 9% of the residual variation in species composition, while other land cover variables (% forest, % urban, and % open water) had lower explanatory power in the models. Reach scale did the poorest job of describing mussel community composition with no single variable being in the top models (Table 5). Percent wetland coverage was also influential at the reach scale and was included in the top 2 models. Post-hoc substrate analyses revealed that community composition was not significantly correlated (Table 2) to minimum ($D_{10}$; rho = 0.09, $p = 0.73$), median ($D_{50}$; rho = -0.18, $p = 0.30$), or maximum ($D_{90}$; rho = 0.22, $p = 0.41$) substrate size, or heterogeneity ($D_{60}/D_{10}$; rho = -0.27, $p = 0.34$). The only landscape and substrate metrics that were significantly correlated to one another (among all scales) were $D_{90}$ and channel slope at the site scale (rho = 0.57, $p = 0.02$). Overall, land cover variables at the watershed and buffer scales did a better job describing mussel community composition after accounting for longitudinal position within the watersheds.

## Discussion

### *Longitudinal gradients and landscape drivers*

We found that mussel community composition was influenced foremost by longitudinal position in the watershed or stream size and by landscape factors after accounting for stream size. In addition, there was a predictable downstream shift in

mussel community composition that was influenced by a few variables at the buffer scale. Sites in different watersheds that were comparable distances from headwaters were more similar in mussel community composition than sites within the same watershed that were farther apart (Fig. 4), showing that species turnover is attributable to longitudinal position and suggesting that similar factors are regulating species compositions in these rivers. Higher species turnover with increasing longitudinal distance between sites can reflect dispersal patterns, increasing habitat heterogeneity over broader spatial scales, or both (Balvanera *et al.*, 2002; Brown, 2003; Maloney & Munguia, 2011). Overall, headwater communities were more variable and were composed of smaller, shorter-lived species, which may indicate that these communities experience greater environmental variability than more downstream sites, as shown by Haag (2012).

Mean channel slope at both the watershed and buffer scale influenced mussel community composition. Changes in slope may lead to a more variable stream-reach habitat and may be a driver of longitudinal shifts in community composition. Our results corroborate findings of Arbuckle and Downing (2002) who showed that channel slope was important in determining density and species richness of mussel beds in an agriculturally- influenced drainage. Channel slope has been shown to influence species compositions of other aquatic organisms, including shrimp and fish (Covich *et al.*, 1996; McGarvey & Hughes, 2008). Sites located closer to the headwaters tend to be more variable because they undergo more frequent high shear stress events during high flows and more drying down conditions during low flows. While headwater streams often are in high elevations with greater slopes, they are also smaller which influences

pool size and permanence. Depths and volumes of pool habitats generally decrease with increasing elevation, making headwater habitats less stable during drought (Sabo *et al.*, 2010). High water temperature is associated with drought in these rivers and some species have been found to be more sensitive to high temperatures (e.g. *Actinonaias ligamentina*) than others (e.g., *Amblema plicata*) (Spooner & Vaughn, 2008). Larger volumes of water lead to habitats that are better buffered against thermal extremes, likely contributing to the community composition we observed. Additionally, high shear stress, which is often associated with headwater streams, has been shown to be associated with lower abundances of mussels (Gangloff & Feminella, 2007; Allen & Vaughn, 2010). Highly variable habitats are often considered to be suboptimal for aquatic organisms whereas more stable habitats likely allow for higher survivorship and reproductive success (e.g., Hutchinson, 1957; Brown, 1984). Life history of these organisms may be closely tied to the habitats in which certain species are successful (Haag, 2012). Thus, communities located closer to the headwaters may be better adapted than downstream communities to deal with stress, both dewatering associated with drought (Galbraith et al. 2010) and high shear stress associated with spates.

Wetland coverage also seemed to influence mussel community composition at the watershed and buffer scales. Wetland coverage was positively correlated to distance from the headwaters, and was still an influential explanatory factor to community composition after accounting for longitudinal position in the watershed. Inundation of wetlands provides water storage allowing attenuation of floods that mitigates the influence of high-flow pulse events on downstream sites (Mitsch & Gosselink, 2000; Zedler, 2003). The shift in community composition due to % wetland coverage is likely

due to some species being more tolerant of high flow events. Smaller, shorter-lived species (e.g., *Villosa lienosa*) that occupied the headwater sites may have greater turnover allowing them to be better suited to high stress environments. Rypel, Haag, and Findlay (2009) found that mussel growth was negatively correlated to the annual flood pulse count. In our study, the Kiamichi River had higher percentages of wetland coverage while the Mountain Fork had the least. The Mountain Fork sites had higher abundances of *Ptychobranchus occidentalis*, *Strophitus undulatus*, and *Fusconaia flava*, indicating that these species are not associated with wetland coverage. Species that were associated with the lower Kiamichi sites such as *Actinonaias ligamentina*, likely need more stable flows that are associated with higher wetland coverage. Wetlands help reduce the frequency and magnitude of flooding which contributes to greater habitat stability. Our results suggest that the protection of riparian wetlands may contribute to maintaining freshwater mussel communities.

Although we found a minor influence of urban land coverage at the buffer scale on mussel species composition, all sites had <4.2% urban coverage. Further research is necessary to understand the influence of urbanization on mussel communities (see Brown *et al.*, 2010). Previous studies have shown shifts in aquatic insect assemblages in watersheds with >10% impervious surface cover (Paul & Meyer, 2001; Roy *et al.*, 2003; Utz, Hilderbrand, & Boward, 2009), which suggests that changes in hydrology, increased nutrient loads, and increased sediment loads from urbanization may alter mussel community composition (Gangloff *et al.*, 2009). Because the rivers in this study are threatened by planned municipal water extractions (Oklahoma Water Resources Board, 2011) and further dam construction (Vaughn & Taylor, 1999; Galbraith,

Spooner, & Vaughn, 2010), an understanding of factors influencing mussel community composition is critical to future river management plans.

***Scale-dependency of mussel community composition***

We found a predictable longitudinal shift in mussel community composition across the broad watershed scale (as influenced by position in the watershed), but the influence of landcover variables were best explained at the buffer scale. Previous studies have found correlations between riparian buffer condition and mussel communities (McRae *et al.*, 2004; Poole & Downing, 2004; Brown *et al.*, 2010). The effect of buffer condition on mussel communities is not definitive, but our results and others suggest that natural buffers maintain healthy mussel populations better than modified buffers (Poole & Downing, 2004), likely due to their mitigation on watershed disturbances (Jones *et al.*, 2010).

Stream organisms are influenced by factors at various temporal and spatial scales, including impacts at the watershed scale (McRae *et al.*, 2004; Andrew & Wulder, 2011). The temporal scale at which an organism experiences environmental factors can have a large influence on which spatial scale is most explanatory. For example, the presence and community structure of short-lived aquatic insects has been successfully predicted from local scale variables, while the composition of longer lived aquatic insects and fishes is better explained by watershed scale variables (Morley & Karr, 2002; Yates & Bailey, 2011). Because mussels are long-lived and sedentary, their community structure should be reflective of factors that may change temporally at small spatial scales, but that are integrated over time at larger spatial scales. For example,

reach-scale land use measured recently may not reflect reach-scale conditions 20 or 30 years ago when a mussel bed was colonized, but such patchiness in land use should be apparent over time at the watershed scale. Variability measured at broad spatial scales may serve as a coarse filter on community composition because it influences aspects of local habitat suitability (Poff, 1997). This suggests that impacts at the watershed scale influence reach scale processes, which can then have a consequential affect on biotic communities.

Reach-scale factors were not predictive of mussel community composition in this study. While several reach-scale studies have found that shear stress influences the location and structure of mussel beds (Gangloff & Feminella, 2007; Allen & Vaughn, 2010), most studies focusing solely on local factors, such as substrate size, substrate heterogeneity, and water chemistry, have not been shown to be good predictors of mussel community composition (Strayer, 2008). This is likely because mussel community compositions should be governed by a hierarchy of factors including spatial variability (biogeographic history, biological attributes of species), dispersal (fish hosts dispersing mussels among patches, see below) and habitat (including both biotic and abiotic factors) (Vaughn & Taylor, 2000; Daraio, Weber, & Newton, 2010). Thus, local factors are likely important, but are influenced by factors at a broader spatial scale (Burcher *et al.*, 2007). The watershed and buffer scales are likely better predictors because they encompass this hierarchy.

Our study provides empirical evidence of factors associated with mussel community composition, but does not investigate the mechanisms behind these patterns. There are broader scale mechanistic variables that may influence mussel community

composition that we were unable to include in our study, such as the distribution and assemblage structure of fishes. Adult mussels are sedentary and movement of mussels between habitat patches is through dispersal of larval mussels (glochidia) attached to the gills and fins of fishes (Vaughn & Taylor, 2000). Mussel species vary in the type and number of suitable fish hosts, mechanisms employed in infecting the host(s), and timing of glochidial development and release (Barnhart et al. 2008). This variation has consequences for mussel dispersal abilities and population dynamics; thus, mussel distribution and abundance can be strongly influenced by the composition of the co-occurring fish assemblages (Haag & Warren, 1998; Vaughn & Taylor, 2000; Schwalb, Garvie, & Ackerman, 2010; Schwalb *et al.*, 2011). Fish of the Ouachita Highlands are distinct and speciose, and the rivers we studied contain similar fish faunas (Mayden, 1985). Fish assemblages can also be influenced by factors operating at the buffer and watershed scale (Andrew & Wulder, 2011; Yates & Bailey, 2011) and species turnover of fish, as was found in our study with mussels, occurs as a function of be influenced by the same set of watershed characteristics longitudinal stream distance (Maloney & Munguia, 2011). Thus, the occurrence of mussels and fishes may be influenced by the same set of watershed characteristics (Vaughn & Taylor, 2000; Rashleigh, 2008).

### *Conclusions*

Mussels are sedentary and relatively long-lived (typically 10-25 y, but up to 190 y; Haag & Rypel, 2011) and thus likely respond slowly to landscape changes. In our study region, long-term habitat stability has aided in the persistence of mussel communities, but new stressors may be causing shifts in species composition because

land use alters watersheds and riparian areas (Spooner & Vaughn, 2008; Jones *et al.*, 2010). Although their life history traits such as immobility and dependence on fish hosts for dispersal render them poorly adapted to deal with landscape change (Strayer et al. 2004), this is not always evident because relict, non-reproducing populations of adults can survive for many decades in degraded areas (Haag, 2009). Thus, freshwater mussels are likely subject to a large extinction debt (Haag, 2012) where there may be a long time lag between landscape alteration and final species extinctions (Spooner *et al.*, 2011; Vaughn, 2012). Therefore, effects of landscape disturbances such as increased sedimentation, introduced species, or high nutrient loads, may be slow and in some cases irreversible (Allan, 2004; Newton *et al.*, 2008). Our study indicates that mussel community composition is structured by a hierarchy of factors governed at the watershed and riparian buffer scale, but due to their long life spans, the full effects of landscape change on mussels may not be fully realized for a long time. However, because watershed and riparian scale factors are important, protecting riparian buffers and associated wetland habitats should support healthier mussel populations and help lessen the potential extinction debt.

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**Table 1** AIC model selection results. The best 3 models for each scale are shown. Models are shown in order of predictability and are bolded for $p < 0.05$. K is the number of variables in the model. The $\Delta i$ is the difference between the AIC of the best fitting model and that of model *i.* The $w_m$ is the normalized relative likelihood values known as the model weights. The variable slope refers to mean channel slope.

| Scale | Parameters in Model | K | F-value | $R^2$ | *p* value | AIC | $\Delta i$ | $w_m$ |
|---|---|---|---|---|---|---|---|---|
| | Slope, %Urban, %Wetland | 3 | 3 | 0.43 | 0.07 | -74.55 | 0 | 0.11 |
| | Slope, %Urban, %Agriculture, %Wetland | 4 | 2.69 | 0.49 | 0.088 | -73.5 | 1.06 | 0.11 |
| Watershed | **Slope** | **1** | **4.35** | **0.24** | **< 0.05** | **-72.91** | **1.64** | **0.08** |
| | **Slope, %Wetland** | **2** | **5.73** | **0.47** | **0.016** | **-76.71** | **0** | **0.12** |
| | **Slope, %Urban, %Forest, %Wetland** | **4** | **3.75** | **0.58** | **0.037** | **-76.36** | **0.35** | **0.1** |
| Buffer | **Slope, %Open Water, %Wetland** | **3** | **4.32** | **0.52** | **0.028** | **-76.31** | **0.39** | **0.1** |
| | %Forest, %Grassland/Shrubs, %Wetland | 3 | 4.54 | 0.39 | 0.323 | -72.77 | 0 | 0.08 |
| | %Forest, %Wetland | 2 | 4.42 | 0.32 | 0.308 | -72.71 | 0.06 | 0.08 |
| Reach | %Agriculture | 1 | 3.32 | 0.13 | 0.466 | -72.46 | 0.3 | 0.06 |

**Table 2** Substrate size and heterogeneity from pebble counts. None of these variables had significant Spearman rank correlations (rho > 0.51) with the Bray-Curtis score.

| River | Site | $D_{10}$ (mm) | $D_{50}$ (mm) | $D_{90}$ (mm) | Substrate Heterogeneity (D60/D10) |
|---|---|---|---|---|---|
| Kiamichi | KM1 | 2 | 25 | 100 | 22.5 |
| | KM2 | 2 | 15 | 115 | 12.5 |
| | KM3 | 2 | 15 | 50 | 10.0 |
| | KM4 | 2 | 30 | 145 | 17.5 |
| | KM5 | 10 | 50 | 172 | 7.0 |
| | KM6 | 9 | 29 | 95 | 4.0 |
| Little | LM1 | 4 | 30 | 85 | 10.0 |
| | LM2 | 2 | 40 | 255 | 32.5 |
| | LM3 | 10 | 35 | 80 | 4.0 |
| | LM4 | 0.5 | 45 | >256 | 80.0 |
| | LM5 | 10 | 40 | >256 | 5.5 |
| Mt. Fork | MFM1 | 5 | 28 | >256 | 8.0 |
| | MFM2 | 2 | 22 | >256 | 20.5 |
| | MFM3 | 11 | 52 | 114 | 5.5 |
| | MFM4 | 3 | 31 | 82 | 13.0 |
| | MFM5 | 1 | 22 | 77 | 28.0 |

## Figure Legends

**Figure 1** Sample site locations and relative species compositions for the three study rivers.

**Figure 2** Scales used for analyses: watershed, buffer, and reach. Reach scale is the buffer area 1 km upstream from the sample site. The NHD stream network is provided for reference. The example given is for the most upstream site in the Little River (LM1).

**Figure 3** Ordered matrix illustrating the presence and absence of species at all of the sites. The sites are ranked by the Bray-Curtis ordination score from lowest to highest. The most upstream site in the Little River (LM1) represents one pole in the ordination, while the most downstream site in the Kiamichi River (KM6) represents the other pole.

**Figure 4** Relationships between distance from the headwaters and the Bray-Curtis ordination value for the 3 rivers (A – C) and all sites combined (D). The Bray-Curtis ordination value is indicative of community structure; values that are more similar are sites that have more species in common and are similar in which species are dominant. Overall, sites that were closer together within a watershed had more similar species compositions, while sites across all watersheds that were approximately the same distance from the headwaters had more similar species compositions.

Figure 1

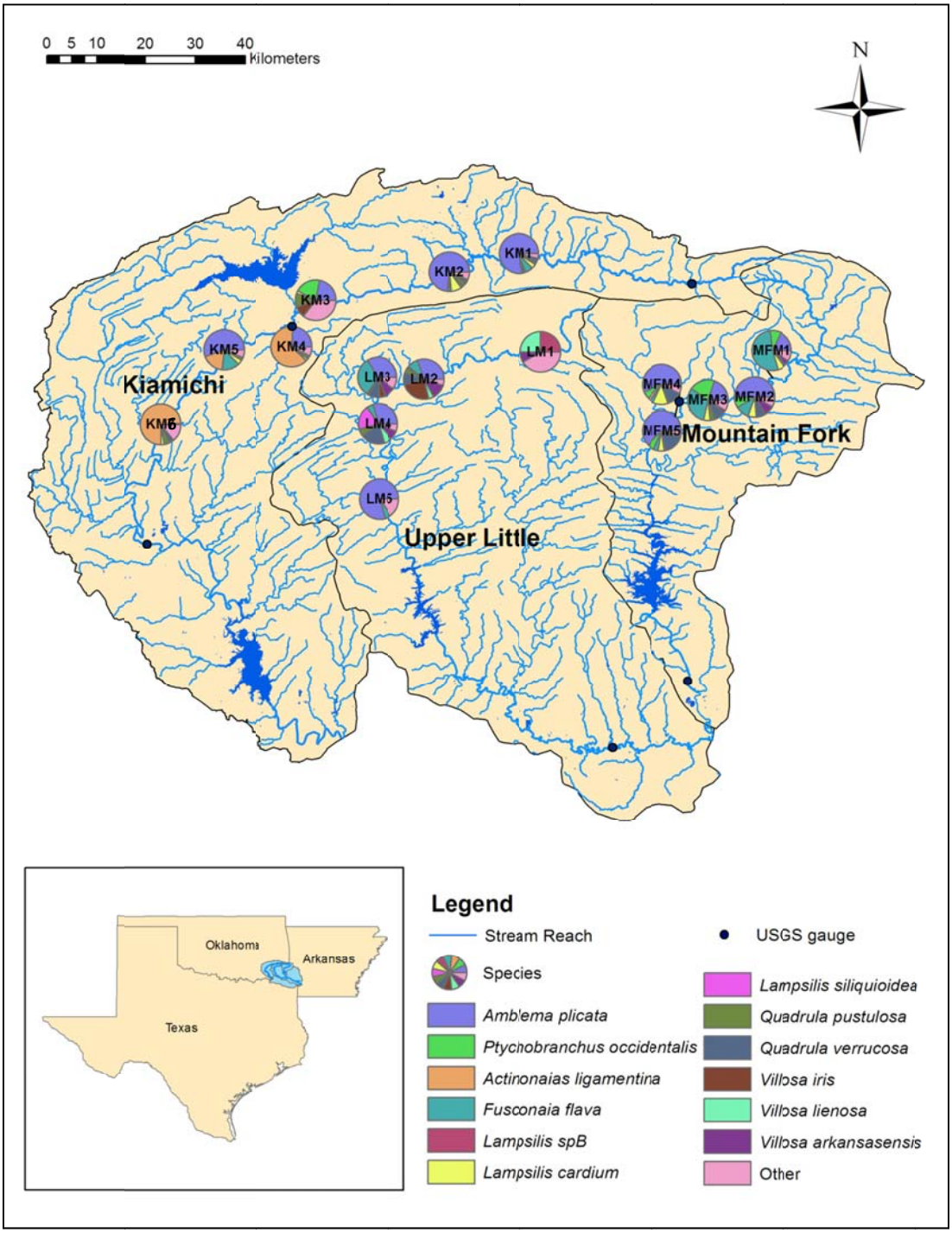

0 5 10 20 30 40
kilometers
N
KM1
KM2
KM3
KM4
KM5
KM6
Kiamichi
LM1
LM2
LM3
LM4
LM5
Upper Little
MFM1
MFM2
MFM3
MFM4
MFM5
Mountain Fork
Oklahoma
Arkansas
Texas
Legend
Stream Reach
USGS gauge
Species
Amblema plicata
Ptychobranchus occidentalis
Actinonaias ligamentina
Fusconaia flava
Lampsilis spB
Lampsilis cardium
Lampsilis siliquioidea
Quadrula pustulosa
Quadrula verrucosa
Villosa iris
Villosa lienosa
Villosa arkansasensis
Other

Figure 2

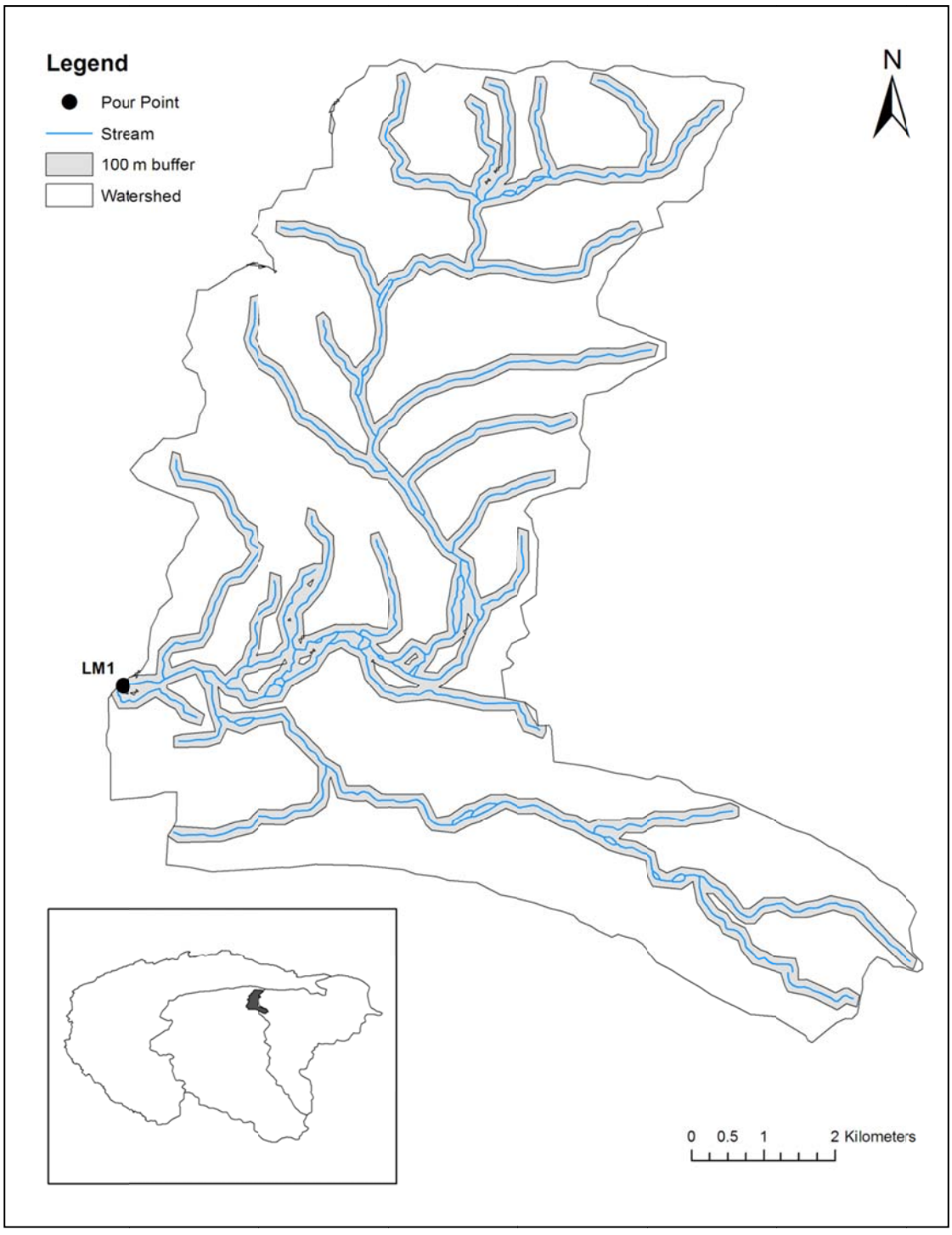

Legend
Pour Point
Stream
100 m buffer
Watershed
N
LM1
0 0.5 1 2 Kilometers

Figure 3

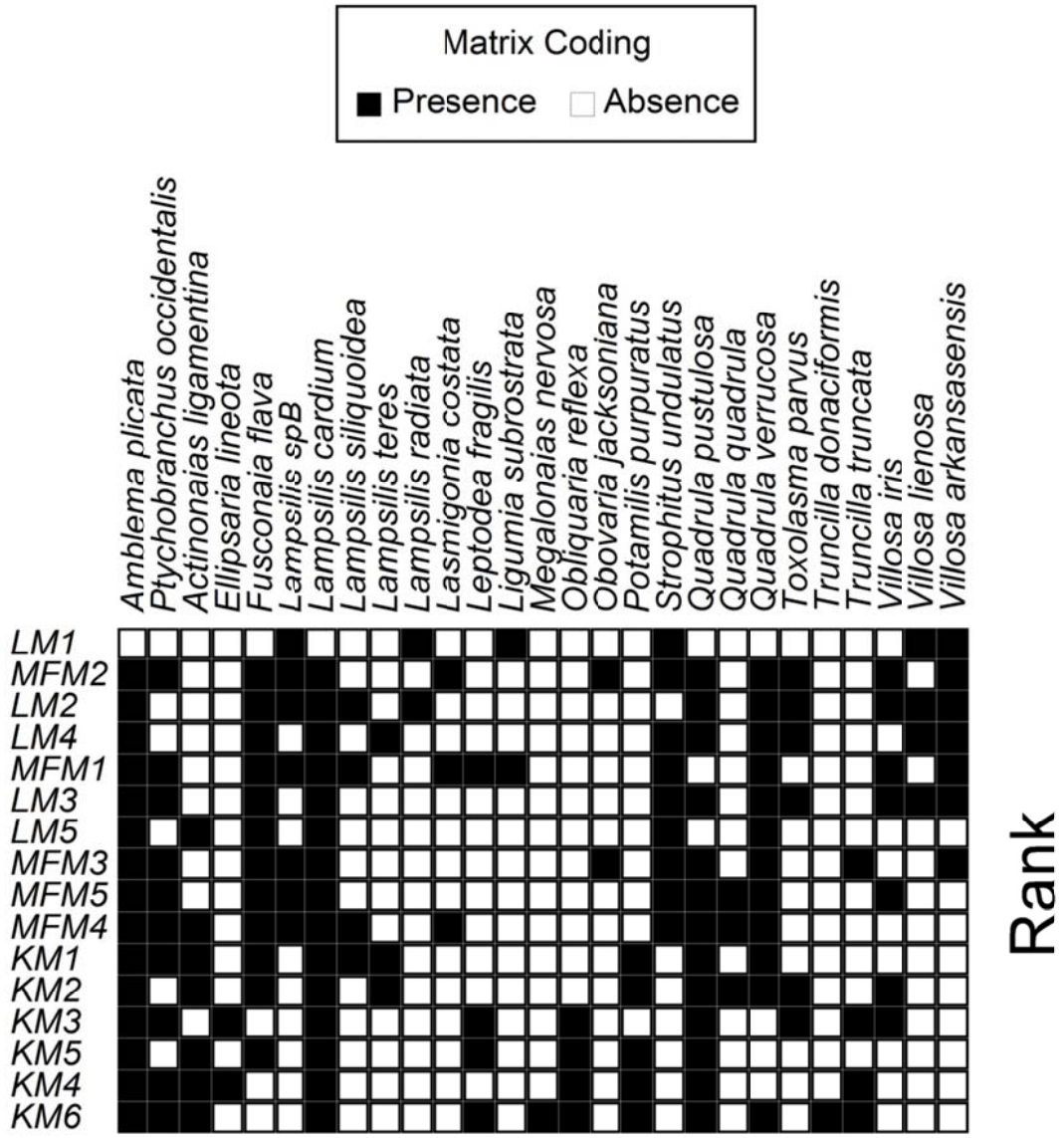
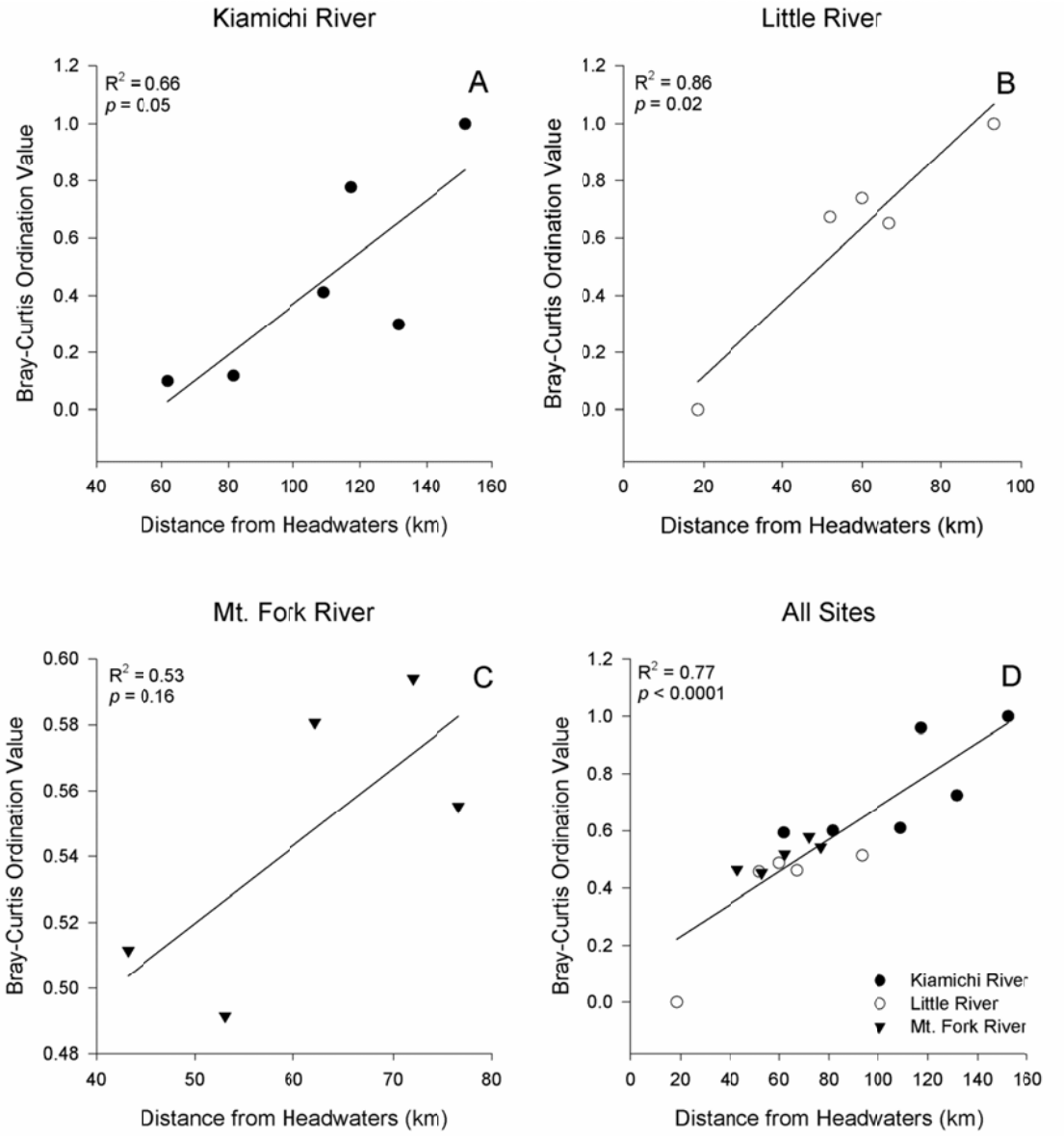

Matrix Coding
Presence
Absence
Amblema plicata
Ptychobranchus occidentalis
Actinonaias ligamentina
Ellipsaria lineota
Fusconaia flava
Lampsilis spB
Lampsilis cardium
Lampsilis siliquoidea
Lampsilis teres
Lampsilis radiata
Lasmigonia costata
Leptodea fragilis
Ligumia subrostrata
Megalonaias nervosa
Obliquaria reflexa
Obovaria jacksoniana
Potamilis purpuratus
Strophitus undulatus
Quadrula pustulosa
Quadrula quadrula
Quadrula verrucosa
Toxolasma parvus
Truncilla donaciformis
Truncilla truncata
Villosa iris
Villosa lienosa
Villosa arkansasensis
LM1
MFM2
LM2
LM4
MFM1
LM3
LM5
MFM3
MFM5
MFM4
KM1
KM2
KM3
KM5
KM4
KM6
Rank

Figure 4


Kiamichi River
A
$R^2$ = 0.66
p = 0.05
Bray-Curtis Ordination Value
Distance from Headwaters (km)
Little River
B
$R^2$ = 0.86
p = 0.02
Bray-Curtis Ordination Value
Distance from Headwaters (km)
Mt. Fork River
C
$R^2$ = 0.53
p = 0.16
Bray-Curtis Ordination Value
Distance from Headwaters (km)
All Sites
D
$R^2$ = 0.77
p < 0.0001
Bray-Curtis Ordination Value
Distance from Headwaters (km)
Kiamichi River
Little River
Mt. Fork River

# CHAPTER 2

## Long-lived organisms provide an integrative footprint of agricultural land use

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Keywords:

stable isotopes, $\delta^{15}N$, bioindicator, biomonitoring, baseline,

nitrogen management tools, NANI, geographic information systems, unionid

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ABSTRACT

Nitrogen (N) fertilizer runoff into rivers is linked to nutrient enrichment, hydrologic alteration, habitat degradation and loss, and declines in biotic integrity in streams. Nitrogen runoff from agriculture is expected to increase with population growth, so tracking these sources is vital to enhancing biomonitoring and management actions. Unionid mussels are large, long-lived, sedentary primary consumers that transfer particulate material and nutrients from the water column to the sediments through their filter feeding. Because of these traits, mussels may provide a temporal integration of nitrogen inputs into watersheds. Our goals were to (1) establish a baseline $\delta^{15}N$ signature for unionid mussels in watersheds not heavily influenced by agriculture for use in comparative analyses and (2) determine if mussels provide an integrative measure of N sources in watersheds with varying percentages of agriculture across large spatial scales. We compiled tissue $\delta^{15}N$ data for 20 species of mussels from seven geographic areas, including 23 watersheds and 42 sample sites that spanned varying degrees of agricultural intensification across the eastern U.S. and Canada. We used GIS to determine land cover within the study basins and we estimated net anthropogenic nitrogen inputs (NANI) entering these systems. We then determined the relationship between mussel tissue $\delta^{15}N$ and % agriculture and net anthropogenic N loading. The $\delta^{15}N$ of mussel tissue could be predicted from both % agriculture and net anthropogenic N loading and one component of NANI, the amount of N fertilizer applied, was strongly related to the $\delta^{15}N$ of mussel tissue. Based on our results, mussels occupying a system not affected by agricultural land use would have a baseline $\delta^{15}N$ signature of approximately 2.0‰, whereas mussels in high basins with heavy agriculture had $\delta^{15}N$

signatures of 13.6‰. Our results demonstrate that mussels integrate anthropogenic N input into rivers at a watershed scale and could be a good bioassessment tool for tracking agriculture N sources.

## INTRODUCTION

Nitrogen is an important limiting resource for primary production, but has become increasingly prevalent due to anthropogenic inputs (Vitousek 1997, Elser 2011). Reactive N is responsible for biodiversity losses, eutrophication, hypoxia, habitat degradation, and acidification (with sulfur) of both marine and freshwater habitats (Vitousek 1997, Howard 2000). Agriculture has a large effect on stream nutrient concentrations, sediment load, water flow, and stream channel placement (Gordon et al. 2008) and is the largest single contributor to anthropogenic nitrogen in many rivers (Boyer et al. 2002, Howarth et al. 2012). Even though rivers have the capacity to process much of the N entering a catchment (Boyer et al. 2002, Galloway et al. 2003), N entering coastal areas from rivers is a large pollution problem because it stimulates algal blooms and subsequent oxygen depletion (Dodds 2006, Elser 2011). Although management practices to decrease the amount of N entering waterways have been instigated (e.g. riparian buffers, wetland protection), the success of these practices in mitigating N pollution varies regionally or is largely unknown (Riseng et al. 2011). The ability to biologically track N loading to watersheds in a way that complements predictive modeling has become of great interest in recent years (e.g., Lefebvre et al. 2007, Hong et al. 2011), and further tools are needed to determine watershed N loading across large spatial scales and multiple time scales.

Nitrogen stable isotopes reflect both the nitrogen source and the outcomes of processes that transform N (Robinson 2001, Vander Zanden et al. 2005, Diebel and Vander Zanden 2009). Consequently, N isotope ratios have been suggested as surrogate measures of nutrient loading and processing in stream watersheds (Lefebvre et al.

2007). Differences in land use among watersheds is correlated with the source $\delta^{15}N$ signal of macrophytes (Cole et al. 2004), marine plants (Costanzo et al. 2001), invertebrates, and fish (Fry and Allen 2003, Anderson and Cabana 2005, Fertig et al. 2009). Nitrogen from both animal manure and synthetic fertilizers can be transformed by processes such as volatilization and denitrification (Groffman et al. 2006), leading to gaseous losses of N that fractionate N isotopes and result in elevated $\delta^{15}N$ values of the remaining N. For this reason, manure and synthetic fertilizer both often have enriched $\delta^{15}N$ values relative to background values (Hogberg 1990, Kendall 1998). Consequently, food web components become more enriched in $\delta^{15}N$ in areas receiving high fertilizer inputs. The $\delta^{15}N$ value of primary consumer tissue reflects an integration of the N assimilated by the consumer over a particular time period, which varies as a function of the life span and tissue turnover of the consumer (Peterson and Fry 1987). Thus, $\delta^{15}N$ provides an integrated temporal and spatial measure of N sources and land use rather than a one-time snapshot of N concentrations. The information gained by isotope analyses may allow managers to collect fewer samples while still obtaining information on the N entering a stream reach over a time relevant to an animal's tissue turnover.

Freshwater mussels (Mollusca: Bivalvia: Unionidae) are large, long-lived (typically 10-25 y, but up to 190 y; Haag and Rypel 2011) primary consumers that may provide a temporal integration of nitrogen inputs into watersheds. Freshwater mussels play an important ecological role through their filter feeding. This feeding activity transfers organic materials and nutrients from the water column to the surrounding benthic area and stimulates increased primary and secondary production (Howard and

Cuffey 2006, Vaughn et al. 2007). Adult mussels can ingest and assimilate a wide range of suspended fine particulate organic matter (FPOM), ranging in size from 1 μm up to at least 40 μm (Brönmark and Malmqvist 1982, Atkinson et al. 2011). Thus, mussels are able to assimilate a wide variety of particulates originating from both aquatic and terrestrial sources. Despite some differences in diet, mostly due to particle size preferences (Leff et al. 1990, Galbraith et al. 2009, Atkinson et al. 2011), different taxa of unionids tend to have similar isotopic signatures within a site allowing cross-species comparisons (Christian et al. 2004, Atkinson et al. 2010). Additionally, adult mussels are sedentary and rarely move further than a few meters laterally per year (Kappes and Haase 2012) and less than a half meter in a week (Allen and Vaughn 2009), so the isotopic signatures of their tissues should provide a good representation of N inputs into a specific stream reach.

Our goals were to (1) establish a baseline $\delta^{15}N$ signature for unionid mussels in watersheds not heavily influenced by agriculture for use in comparative analyses and (2) determine if freshwater mussels provide an integrative measure of N sources in watersheds with varying percentages of agriculture across large spatial scales. We compiled $\delta^{15}N$ data for a total of 20 species of freshwater mussels from seven geographic areas, including 23 watersheds and 42 sample sites that spanned varying degrees of agricultural intensification across the eastern United States and Canada. We then determined the relationship between mussel tissue $\delta^{15}N$ with % agriculture and net anthropogenic N loading across this broad geographic scale, and used these data to estimate the $\delta^{15}N$ of mussels occupying a watershed with little to no agricultural land use.

METHODS

*Study areas and sample collection:*

We compiled $\delta^{15}N$ tissue data for 20 species of freshwater mussels from seven geographic areas, including 23 watersheds and 42 sample sites that spanned varying degrees of agricultural intensification across the eastern United States and Ontario, Canada (Fig. 1, Table 1, Appendix A). These data included samples that we collected ourselves (Red River, Buffalo, Darby, Ouachita, Ichawaynochaway, and Ontario) and published studies (Neuse Basin).

*Isotope sample processing:*

Foot-muscle or mantle tissue samples were collected (Naimo et al. 1998) from each individual mussel, dried (45° C) and ground. Isotope ratios are expressed in the delta ($\delta$) notation: $\delta^{15}N$ (units of ‰) = $(R_{sample} - R_{standard} / R_{standard}) \times 1000$, where R is the $^{15}N$:$^{14}N$ ratio. A bovine protein (peptone) lab standard was referenced against an international standard and precision averaged to 0.1‰ or less. Stable isotope analyses were performed as follows: Red River drainage and Ichawaynochaway Creek, University of Georgia Stable Isotope Facility, Finnigan Delta Plus mass spectrometer; Buffalo River, Darby Creek basin, and Ouachita River, University of Alaska Fairbanks Stable Isotope Facility, Europa 20–20 continuous flow-isotope ratio mass spectrometer; Ontario study sites, Trent University Water Quality Center, ICP IRMS.

*GIS analysis:*

We derived watershed areas for each sampling point using the Spatial Analyst Toolkit in ArcMap 10.0 (Environmental System Research Institute, Redlands, CA) with a 30-m digital elevation model (DEM) from the National Elevation Dataset. We obtained land cover (30-m resolution) for the U.S. from the 2006 National Land Cover Database (Homer et al. 2004). Land cover for the Ontario sites was obtained from the Ontario Land Cover (OLC) data base. Land cover was delineated for each individual sample site.

*Net Anthropogenic Nitrogen Inputs:*

We assessed net anthropogenic nitrogen inputs (NANI) to the most downstream sampling point of each individual U.S. watershed ($n$ = 10) in our database using the NANI Calculator Toolbox (Hong et al. 2011). This model has been used to estimate total riverine N flux from landscapes to coastal ecosystems (Galloway et al. 2004, Howarth et al. 2012). Databases included in the NANI Toolbox are county-level Agricultural Census data for the Agricultural Census years 1987, 1992, 1997, 2002, and 2007 (http://www.agcensus.usda.gov/), county-level Census data for the population in Census years 1990 and 2000 (http://www.census.gov/), county-level USGS nutrient input estimates for annual fertilizer application in years 1987 to 2001 ( http://pubs.usgs.gov/sir/2006/5012), and 36 $km^2$ grid-scale of the US Environmental Protection Agency's Community Multi-scale Air Quality (CMAQ) data for nitrogen deposition annually available from 2002 to 2006. We were unable to obtain net anthropogenic nitrogen input data for Ontario, thus those watersheds were not used to determine the relationship between NANI and mussel tissue $\delta^{15}N$.

*Statistical Analyses:*

We investigated spatial autocorrelation among sites in the dataset using Moran's *I*. We used Spatial Analysis in Macroecology (SAM 4.0, Rangel et al. 2010) to assess spatial autocorrelation between the percentage of agriculture in the watershed and $\delta^{15}N$ of mussel tissue. To determine if the percentage of agriculture and $\delta^{15}N$ of mussel tissue followed the same spatial patterns, we used an ordinary-least squares regression (OLS) to determine the relationship of their Moran's *I* values. To avoid problems of spatial autocorrelation in further analyses, we grouped data by watershed ($n = 24$) and used average % agriculture and $\delta^{15}N$ values of the watersheds. The watersheds used in the following analyses included one point each for the Buffalo, Kiamichi, Little, Mountain Fork, Big Darby, Little Darby, Ouachita, and Neuse rivers, and 14 separate watersheds in Ontario. Prior to analysis, percent agricultural use (% agriculture) was arc sine-square root transformed to meet assumptions of normality (Gotelli and Ellison 2004). Because error in predictors was high in relation to the response, we used reduced major axis regression (RMA) to evaluate the relationship between the arc-sine square-root of agriculture and the $\delta^{15}N$ of mussel tissue. Because most streams are impacted by agriculture, the y-intercept was used to investigate what the $\delta^{15}N$ of mussel tissue may be in a watershed without agriculture. RMA was performed in the lmodel2 package in R 2.14.1 (R Core Development Team). Ordinary least-squares (OLS) regression was used to determine the relationship between agriculture and both NANI and total N in fertilizer applied to the landscape. OLS regression also was used to determine the relationship between NANI and $\delta^{15}N$ of mussel tissue. To determine what components

of NANI (non-food crop N (e.g. cotton and tobacco), N fertilizer applied, N deposition, agricultural N fixation, and/or food crop N (e.g. wheat, corn) were important in influencing the $\delta^{15}N$ of mussel tissue, a backwards stepwise multiple linear regression with AIC selection was used to examine significant predictors using the MASS package using R 2.14.1 (R Core Development Team). Prior to multiple linear regressions, we checked for multicolinearity using Pearson correlations in the Hmisc package using R 2.14.1 (R Core Development Team). Significant predictors of $\delta^{15}N$ found with AIC selection were then used in an OLS regression to determine their univariate influence on $\delta^{15}N$.

## RESULTS

### *Spatial Patterns*

For both % agriculture and $\delta^{15}N$ values, our data exhibited positive spatial autocorrelation for sites geographically closer to each other (positive Moran's *I* values) and negative correlation for sites further apart (negative Moran's *I* values) (Fig. 2). The patterns of spatial autocorrelation of these two variables were similar and our regression results suggested that the Moran's I for $\delta^{15}N$ increased as Moran's I for % agriculture increased ($R^2 = 0.94$, $p < 0.0001$, Fig 2).

### *Mussel tissue $\delta^{15}N$ and % Agriculture and NANI*

Our results show that mussel tissue $\delta^{15}N$ was positively related to percent agriculture in watersheds (Fig. 3; $R^2 = 0.74$, $y = 11.79x + 1.05$, $p < 0.0001$). The y-intercept suggests that mussels occupying a watershed without agriculture would have a

$\delta^{15}N$ value around 1.05 ± 0.92‰ (± stdev). Additionally, NANI ($R^2 = 0.58$, $y = 33.4x + 2171$, $p < 0.001$) and the amount of N applied as fertilizer ($R^2 = 0.90$, $y = 84.4x - 573$, $p < 0.001$) in a watershed were both significantly positively predicted by percent agriculture in a watershed (Fig. 4). Furthermore, net NANI was a significant predictor of $\delta^{15}N$ in watersheds (Fig. 5; $R^2 = 0.60$, $y = 0.002x + 2.09$, $p < 0.01$), demonstrating a signal of human application of nitrogen to the landscape. The y-intercept of the net NANI relationship suggests that mussels in a watershed not affected by anthropogenic nitrogen would have a $\delta^{15}N$ value around 2.09 ± 1.6 ‰. Collectively, the relationship of $\delta^{15}N$ to the percentage of agriculture in a watershed and NANI indicate that a $\delta^{15}N$ value ranging from 0.13 to 3.69‰ would represent a mussel in a system not influenced by agriculture. Among the components of NANI, only the amount of nitrogen fertilizer applied to a watershed appeared as a strong predictor of $\delta^{15}N$ ($t_5 = 3.87$, $p = 0.01$, backward stepwise regression). Accordingly, it was closely and positively related to mussel $\delta^{15}N$ ($R^2 = 0.83$, $y = 0.0009x + 5.4$, $p < 0.0001$).

## DISCUSSION

Our results show that primary consumers, especially freshwater mussels, are a good integrator of land use influences and should be a focal component of stream biomonitoring. Continuous monitoring of water quality can be time-consuming and expensive and results are often difficult to summarize in an ecologically meaningful way (Olden and Poff 2003). We found that the nitrogen isotope ratio of freshwater mussel tissue could be predicted from both the percent agriculture in the watershed and NANI. Our results indicate that mussels biogeochemically integrate nitrogen loading

on the landscape and are good indicators of anthropogenic N inputs. We suggest that biomonitoring isotope ratios of mussels would be an efficient way to assess agricultural runoff into streams.

Our results combined with other studies demonstrate that the $\delta^{15}N$ signature of primary consumers nicely reflects variation in anthropogenic N loading. For example, in a study of 82 streams (Anderson & Cabana, 2006) demonstrated a significant curvilinear relationship between stream N concentration and primary consumer $\delta^{15}N$. Other studies confirm a large range in primary consumer $\delta^{15}N$ across gradients of land use (Fry and Allen 2003, Anderson and Cabana 2005) and nutrient enrichment (Bergfur et al. 2009, Diebel and Vander Zanden 2009). While the relationship between agricultural land use and stream water nitrogen loads has been well established (Vitousek 1997, Lefebvre et al. 2007), the relationship between agricultural land use and $\delta^{15}N$ is not as clear (Diebel and Vander Zanden 2009). However, based on our results, we would expect riverine mussels to have a $\delta^{15}N$ of approximately 2.0 ‰ without agriculture, allowing us to establish a theoretical baseline $\delta^{15}N$ signature for future bioassessments.

Better predictions about how much N is entering watersheds at a variety of spatial and temporal scales and the effects of these N subsidies on ecosystem processes would be valuable. Models to calculate NANI can be used, however real-time measurements are necessary to understand amounts of N actually reaching streams. While the NANI toolbox allows prediction of the amount of N entering a watershed from various sources, it may not be very sensitive to how much N actually enters a stream over a variety of temporal and spatial scales (e.g., stream buffers may mitigate

some N). Also, some of the data layers used by NANI are several years old, so the analyses may not reflect which N sources are currently influencing the river. In contrast, the $\delta^{15}N$ of various tissue compartments within mussels, assuming tissue-specific nitrogen turnover times, are a better representation of N that is entering a given area over a specific time period (dependent on the turnover time of the tissue). For example, Howarth et al. (2012) suggested a NANI value of 1070 kg N $km^{-2}$ $yr^{-1}$ or lower as a threshold of N that rivers can process without exporting excess amounts to coastal waterways. Our regression analysis suggests that this would be a $\delta^{15}N$ value of 3.8‰ ± 1.6 in freshwater mussels. Collectively, the use of NANI as a predictive tool in conjunction with field monitoring tools, such as $\delta^{15}N$ in consumer tissues, will be useful for future N management.

In this study we only considered the influence of agriculture on freshwater mussel tissue N isotope signatures. There was some scatter in our data and other anthropogenic land uses, such as higher urban cover, are also likely to drive the $\delta^{15}N$ signatures of aquatic organisms. For example, previous studies have shown that high $\delta^{15}N$ values of inorganic N derived from sewage (Kendall 1998) can be traced in aquatic food webs influenced by urban development (e.g., Cabana and Rasmussen 1996, Steffy and Kilham 2004, Vander Zanden et al. 2005). Additionally, land cover such as forest and wetlands may mitigate the influence of N loading (Zedler 2003). While human and animal derived wastes have $\delta^{15}N$ values that are elevated (Tucker et al. 1999, Vander Zanden et al. 2005) and inorganic fertilizers typically have lower values (0 ‰) of $\delta^{15}N$ (Kendall 1998), N from both synthetic fertilizers and animal manure can be transformed in watersheds by processes (e.g. assimilation, nitrification,

denitrification) leading to fractionation of N through gaseous loss of $^{14}N$ and disproportionate retention of $^{15}N$ within the watershed (Robinson 2001, Groffman et al. 2006). These differing initial signatures of fertilizer and the varying usage of fertilizer across different types of agriculture could have led to the variability in $\delta^{15}N$ values across locations, yet the $\delta^{15}N$ signature of freshwater mussels was correlated strongly with the percentage of agriculture in watersheds, suggesting that mussel tissue signatures are good indicators of human disturbance.

The linkage between land use, anthropogenic nitrogen, and the assimilation of this nitrogen into food webs shows a direct connection between the influence of humans on watersheds and the biochemical makeup of organisms. These relationships can be complex because of system-specific differences in background $\delta^{15}N$, the type of N inputs, and hydrology (Fry et al. 2003, Hoffman et al. 2012, Howarth et al. 2012). Although this study does not completely agree with existing N isotope cycling models (Fry 2006, Diebel and Vander Zanden 2009), our results are similar to other recent studies that show that $\delta^{15}N$ content of organisms track anthropogenic N inputs (Lefebvre et al. 2007, Hoffman et al. 2012, Spooner et al. 2013). We found that freshwater mussels reflect watershed scale changes in N entering rivers as indicated by the biochemical makeup of nitrogen ($\delta^{15}N$) in their tissue. Thus, mussels should be a useful future monitoring tool for riverine N because they integrate N entering the river across both time and space. For example, threshold nitrogen concentrations of 0.3-1.0 mg N/L alter species composition of algae (specifically diatoms, Black et al. 2011), chlorophyll concentrations in streams (Dodds et al. 2002b) and N uptake capacity (Dodds et al. 2002a). However, such thresholds can be hard to identify because N

concentrations can vary widely over time such that typical water quality samples of N in stream water may not represent the total N loading to the stream. There is often a time lag between N loading to the watershed and when the N enters the stream, usually linked to discharge (Golladay and Battle 2002). Freshwater mussels are long-lived and sedentary; thus, individual populations could be sampled over time as part of long-term monitoring across multiple watersheds. Plus, different tissue types from freshwater mussels, such as mantle tissue (Berg et al. 1995) and hemolymph (Gustafson et al. 2007), can be sampled non-lethally allowing for continual monitoring without compromising mussel populations. Additionally, different tissue types have varying turnover times (Raikow and Hamilton 2001, Gustafson et al. 2007); thus, there is a potential to examine N loading expressed in mussel tissue across different seasons and time scales.

Excessive nitrogen loading to water bodies is responsible for loss of biodiversity, eutrophication, hypoxia, and habitat degradation in coastal ecosystems globally (Turner and Rabalais 1994, Howard 2000, Dodds 2006, Riseng et al. 2011). Thus, adequate monitoring and mitigation of N loading is essential. Our results show that the N signature in primary consumer tissue can be used as a bioassessment tool that integrates watershed-level land use change with incoming stream nitrogen fluxes. We suggest setting baseline N signatures (for example $\delta^{15}N$ of 3.8‰) and resampling populations over time in sensitive rivers to assess management outcomes. Freshwater mussels and other long-lived primary consumers may be an ideal tool to achieve these biomonitoring objectives.

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Table 1. Description of datasets used in the analyses.

| **Study Area (number of sites) and Data Sources** | **Major Drainage Basin** | **Dominant Land Use** | **Watershed Points (no.) used in Analyses** | **Range of Site Watershed** | **Species Sampled (no. indiv. per site)** |
|---|---|---|---|---|---|
| Buffalo River (6)<br>A.D. Christian - Unpublished | White River, Arkansas | Forest and Pasture | Buffalo River (1) | 482-3,037 | *Actinonaias ligamentina* (5)<br>*Cyclonaias turbiculata* (5)<br>*Lampsilis reeviania* (5)<br>*Ptychobranchus occidentalis* (5) |
| Darby Creek Basin (4)<br>Christian et al. (2004)<br>A.D. Christian - Unpublished | Scioto River, Ohio | Agriculture (corn and soybeans) (Ohio EPA 1987) | Big Darby, Little Darby (2 total) | 179-399 | *Elliptio dilatata* (3-10)<br>*Ptychobranchus fasciolaris* (3-10) |
| Ichawaynochaway Basin (5)<br>Atkinson et al. (2010) | Flint River, Georgia | Agriculture (cotton, corn, peanuts), Forest | Ichawaynochaway, Chickasawhatchee (2 total) | 773-2,557 | *Elliptio crassidens* (5-10) |
| Neuse Basin (2)<br>Bucci et al. (2011) | Neuse River, North | Agriculture | Neuse (1) | 74-240 | *Elliptio complanata* |
| Ouachita River (2)<br>A.D. Christian - Unpublished | Ouachita River, Arkansas | Forest, some cattle and chickens | Ouachita (1) | 982-1,040 | *Actinonaias ligamentina* (3-5)<br>*Elliptio dilatata* (3-5)<br>*Ptychobranchus occidentalis* (3-5) |
| Ontario, Canada (14)<br>Spooner et al. (2013) | Lakes Erie and Huron | Agriculture (row crop corn and soybean) | Beaverton, Uxbridge, Nottawasaga, Fleetwood, East Cross, Nonquon, Fish Creek, Sydenham, Thames, Cayanville, Indian, Humber, Ausable, Teeswater (14 | 128-2,123 | *Lasmigona costata/compressa* (5-10)<br>*Elliptio dilatata/complanata* (5-10) |
| Kiamichi, Little, and Mountain Fork Rivers (9)<br>C.L. Atkinson - Unpublished | Red River, Oklahoma | Forest, some cattle and chickens (Matthews et al. 2005) | Kiamichi, Little, Mt. Fork (3) | 74-2,044 | *Amblema plicata* (5)<br>*Actinonaias ligamentina* (5)<br>*Fusconaia flava* (5)<br>*Lampsilis spB* (2-5)<br>*Quadrula pustulosa* (4-5)<br>*Quadrula verrucosa* (4-5) |

FIG. 1. Maps of study areas. Black dots indicate individual sampling sites. Watersheds with more than 2 sampling sites within them are shown with the land use in that basin. All the sites sampled within Ontario are within individual watersheds.

FIG. 2. Correlogram of Moran's *I* with the data used in the analyses. Moran's *I* for % agriculture and $\delta^{15}N$ of mussels overlapped significantly. The distance on the x-axis was determined by SAM 4.0 and represents the straight line distance between sites. The insert shows the ordinary least squares regression for the Moran's *I* values ($R^2 = 0.94$, $p < 0.0001$).

FIG. 3. (A) Percent agriculture versus mussel tissue $\delta^{15}N$ values (±SE) for all of the sites in the 7 watersheds used in this study. (B) Watershed level grouped data (arc sign square root transformed % agriculture and average mussel tissue $\delta^{15}N$) and regression lines for both reduced major axis (RMA) regression (solid line; $y = 11.79x + 1.05$, $p < 0.0001$) and ordinary least squares (OLS) regression (dashed line; $y = 10.21x + 2.13$, $p < 0.0001$).

FIG. 4. Relationship between % agriculture and (A) the amount of NANI and mussel $\delta^{15}N$ and (B) N fertilizer applied within the watershed and mussel $\delta^{15}N$. The slope of both NANI and N fertilizer are both similar to the slope of $\delta^{15}N$.

FIG. 5. Relationship between (A) NANI and (B) average N fertilizer applied versus $\delta^{15}N$ of mussel tissue with 95$^{th}$% confidence intervals.

FIG. 1.

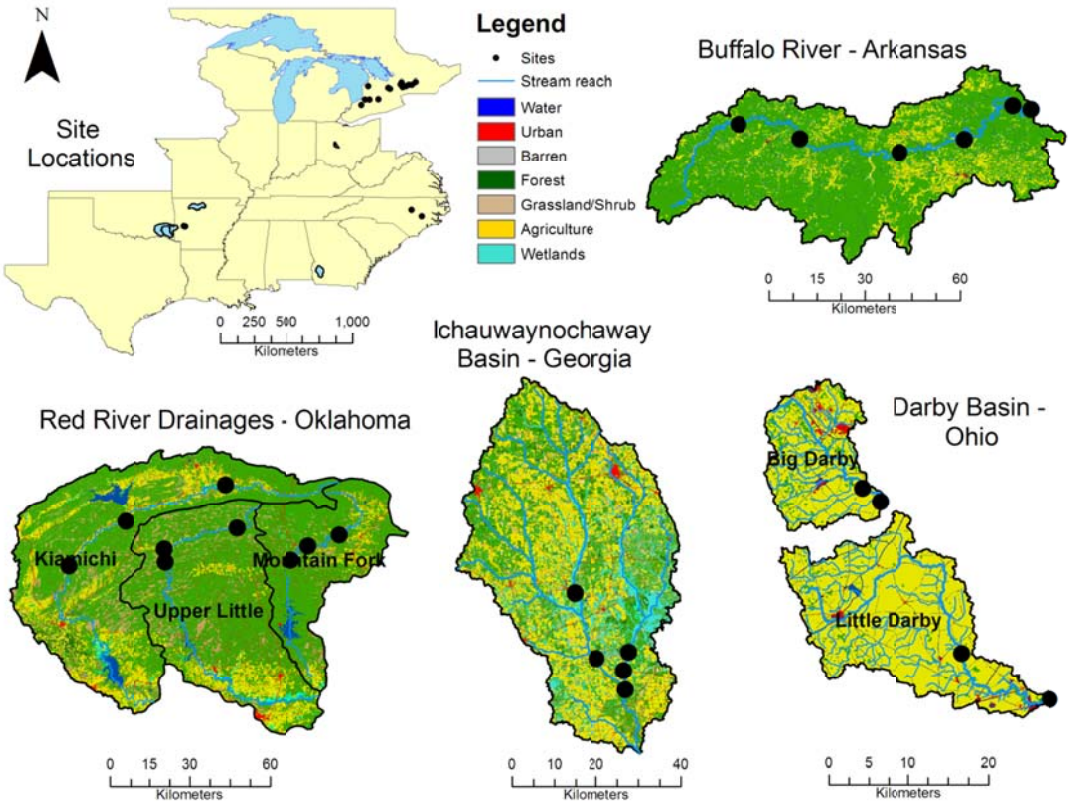

N
Site Locations
0 250 500 1,000
Kilometers
Legend
Sites
Stream reach
Water
Urban
Barren
Forest
Grassland/Shrub
Agriculture
Wetlands
Buffalo River - Arkansas
0 15 30 60
Kilometers
Ichauwaynochaway Basin - Georgia
0 10 20 40
Kilometers
Red River Drainages - Oklahoma
Kiamichi
Mountain Fork
Upper Little
0 15 30 60
Kilometers
Darby Basin - Ohio
Big Darby
Little Darby
0 5 10 20
Kilometers

FIG. 2.

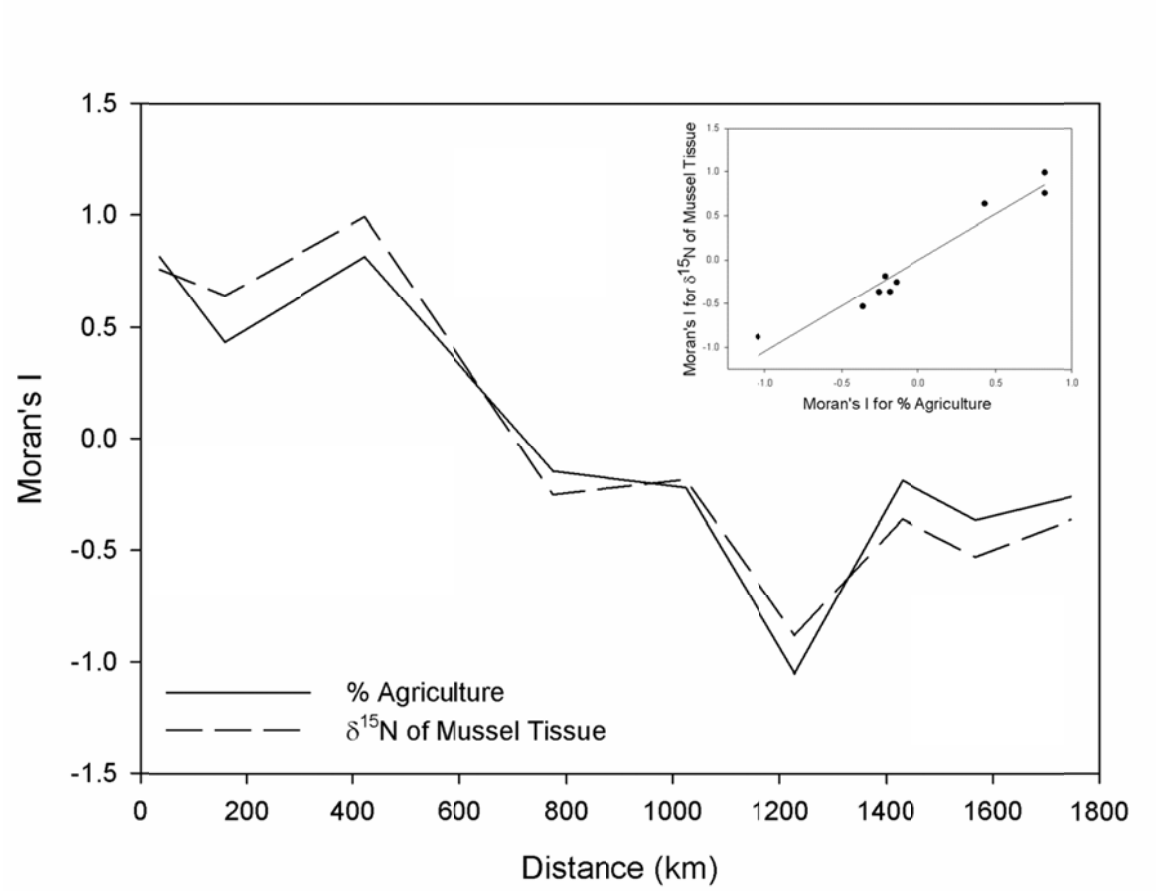

Moran's I
Distance (km)
% Agriculture
δ15N of Mussel Tissue
Moran's I for δ15N of Mussel Tissue
Moran's I for % Agriculture

FIG 3.

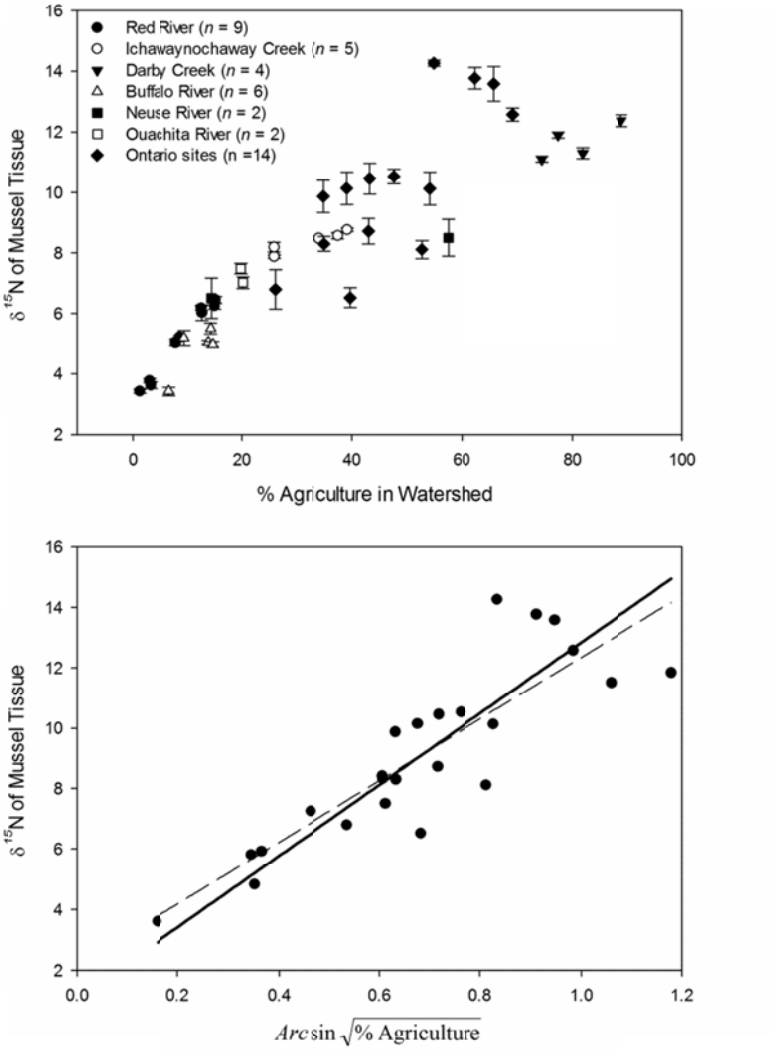

Red River (n = 9)
Ichawaynochaway Creek (n = 5)
Darby Creek (n = 4)
Buffalo River (n = 6)
Neuse River (n = 2)
Ouachita River (n = 2)
Ontario sites (n =14)
δ15N of Mussel Tissue
% Agriculture in Watershed
δ15N of Mussel Tissue
Arcsin √% Agriculture

FIG. 4.

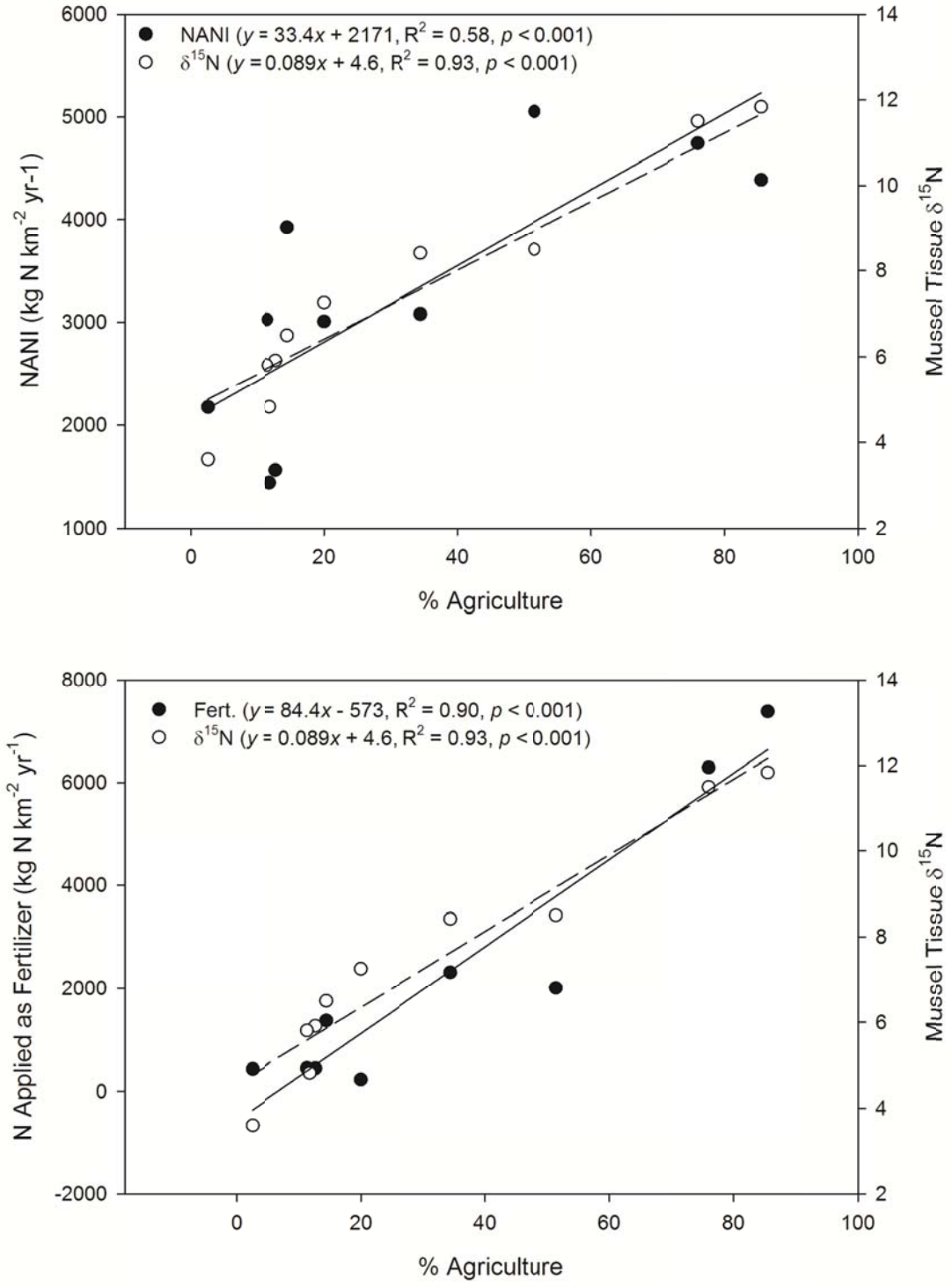
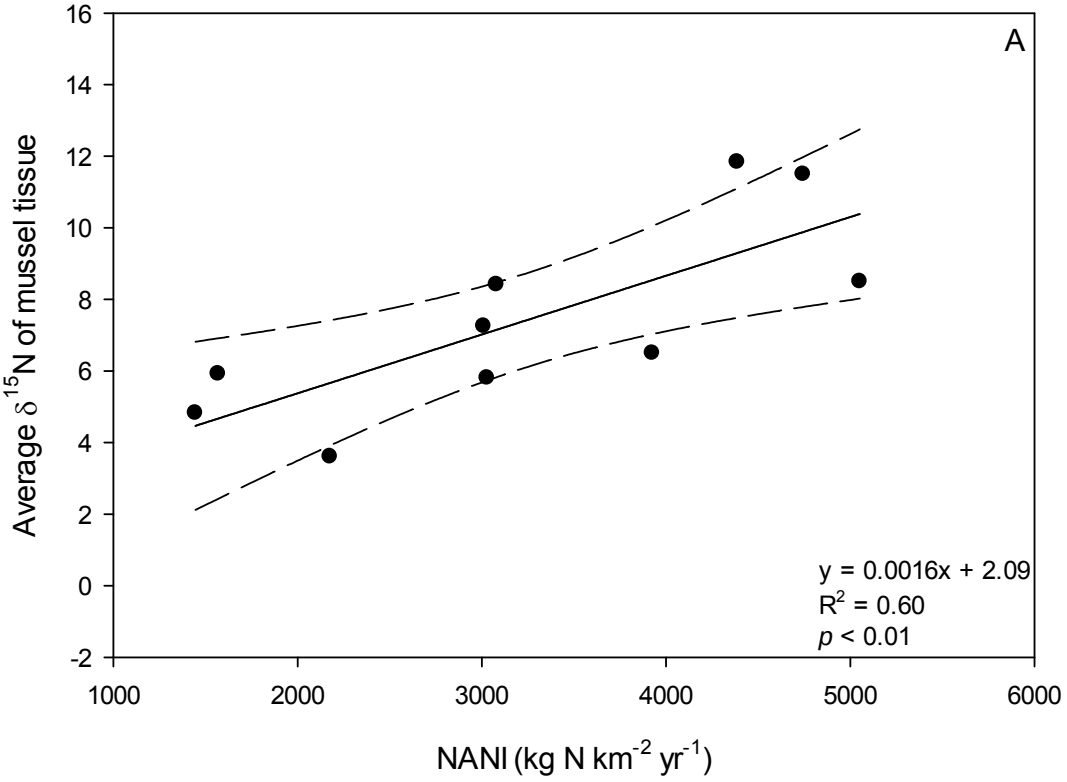
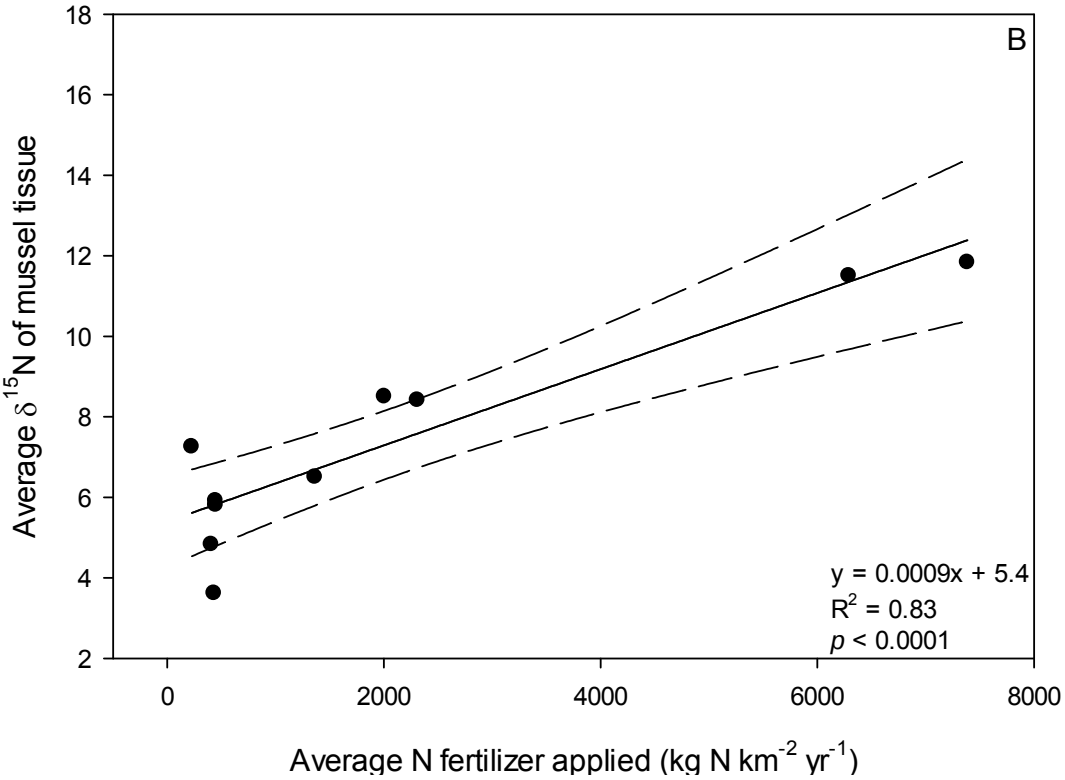

NANI (y = 33.4x + 2171, R2 = 0.58, p < 0.001)
δ15N (y = 0.089x + 4.6, R2 = 0.93, p < 0.001)
NANI (kg N km-2 yr-1)
Mussel Tissue δ15N
% Agriculture
Fert. (y = 84.4x - 573, R2 = 0.90, p < 0.001)
δ15N (y = 0.089x + 4.6, R2 = 0.93, p < 0.001)
N Applied as Fertilizer (kg N km-2 yr-1)
Mussel Tissue δ15N
% Agriculture

FIG. 5.


A
Average δ15N of mussel tissue
NANI (kg N km-2 yr-1)
y = 0.0016x + 2.09
R2 = 0.60
p < 0.01
16
14
12
10
8
6
4
2
0
-2
1000
2000
3000
4000
5000
6000


B
Average δ15N of mussel tissue
Average N fertilizer applied (kg N km-2 yr-1)
y = 0.0009x + 5.4
R2 = 0.83
p < 0.0001
18
16
14
12
10
8
6
4
2
0
2000
4000
6000
8000

SUPPLEMENTAL MATERIAL

**Appendix A**. Unpublished data used in the study.

**Appendix B.** Water chemistry of the Oklahoma sites was not significantly related to the % agriculture in the watershed, while the $\delta^{15}N$ of mussels was significantly positively related.

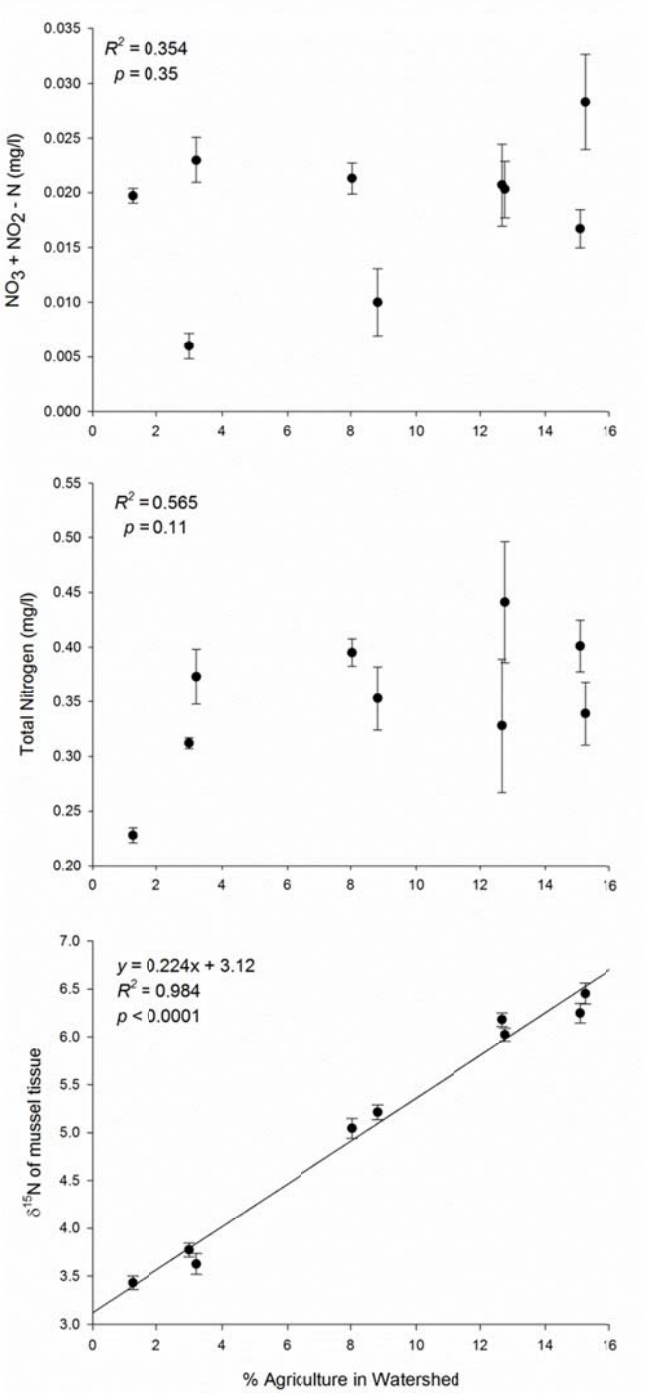
**Appendix C.** Relationship between NANI and the % of N flux that is NANI. Based on data from Howarth et al. (2012). The box shows within the figure indicates range of NANI observed in the U.S. sites of this study. We found that 80-94% of the total nitrogen loading in all stream reaches included in this study was anthropogenic in origin.

Appendix A. Unpublished mussel tissue $\delta^{15}N$ data used in the study. The data includes sites from the Buffalo River (Arkansas), Kiamichi (Oklahoma), Little Darby Creek (Ohio), Little River (Oklahoma), Mountain Fork River (Oklahoma), and Ouachita River (Arkansas).

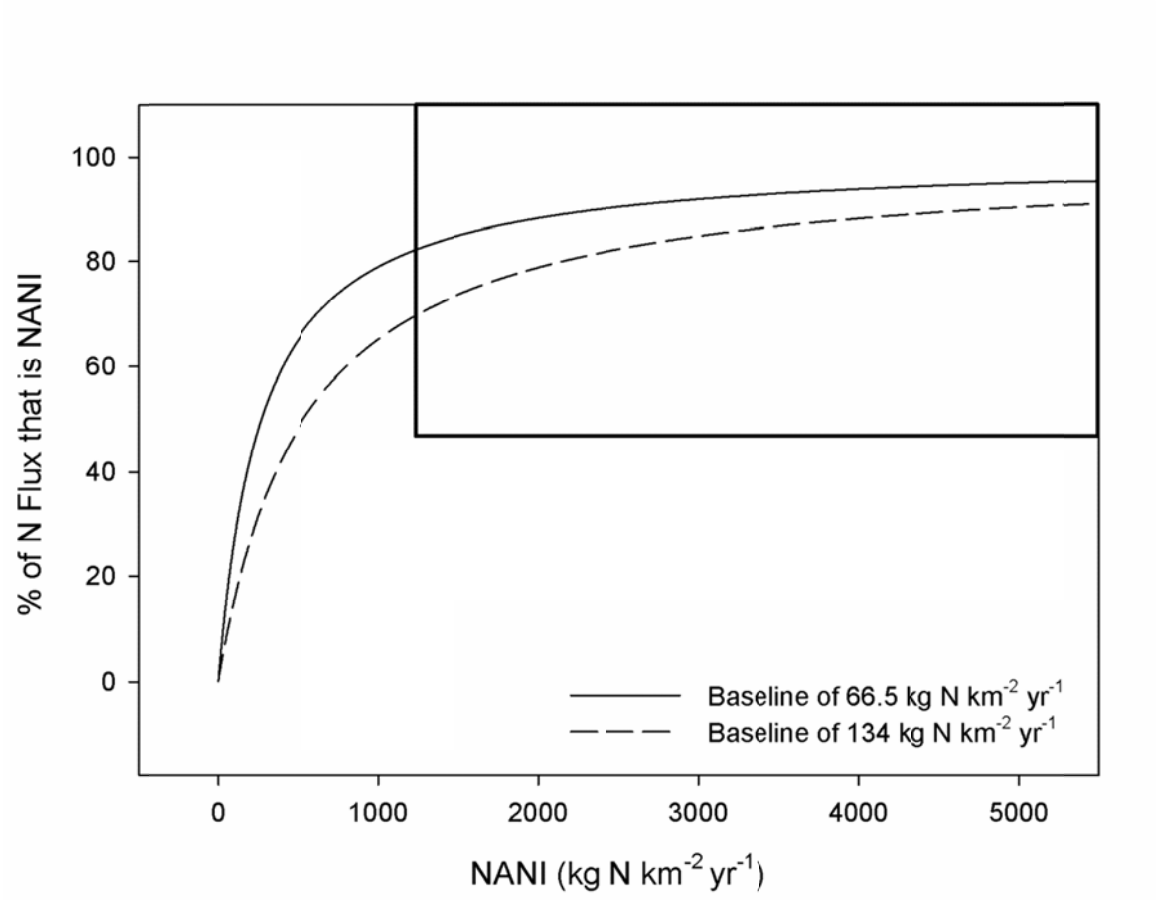
| Major Drainage Basin | Study Area | Sample Site | Species Used | N | Mean $\delta^{15}N$ | Standard error |
|---|---|---|---|---|---|---|
| White River | Buffalo River | BR1 | *Lampsilis reeviania* | 5 | 3.41 | 0.24 |
| | | | *Ptychobranchus occidentalis* | 5 | 3.42 | 0.16 |
| | | BR2 | *Lampsilis reeviania* | 5 | 5.68 | 0.18 |
| | | | *Ptychobranchus occidentalis* | 5 | 5.5 | 0.14 |
| | | BR3 | *Actinonaias ligamentina* | 5 | 5.85 | 0.04 |
| | | | *Lampsilis reeviania* | 5 | 4.53 | 0.22 |
| | | BR4 | *Actinonaias ligamentina* | 5 | 5.88 | 0.18 |
| | | | *Lampsilis reeviania* | 5 | 5.13 | 0.21 |
| | | BR5 | *Cyclonaias turbiculata* | 5 | 5.09 | 0.11 |
| | | | *Lampsilis reeviania* | 5 | 5.02 | 0.07 |
| | | BR6 | *Cyclonaias turbiculata* | 6 | 4.87 | 0.14 |
| | | | *Lampsilis reeviania* | 5 | 5.11 | 0.07 |
| Scioto River | Little Darby Creek | LD2 | *Elliptio dilatata* | 5 | 10.96 | 0.25 |
| | | | *Ptychobranchus fasciolaris* | 6 | 10.9 | 0.18 |
| Ouachita River | Ouachita | OR1 | *Actinonaias ligamentina* | 3 | 7.03 | 0.18 |
| | | | *Elliptio dilatata* | 5 | 7.09 | 0.21 |
| | | | *Ptychobranchus fasciolaris* | 5 | 7.01 | 0.22 |
| | | OR2 | *Actinonaias ligamentina* | 5 | 7.59 | 0.16 |
| | | | *Elliptio dilatata* | 4 | 7.23 | 0.28 |
| | | | *Ptychobranchus fasciolaris* | 5 | 7.46 | 0.25 |
| Red River | Kiamichi River | KM1 | *Amblema plicata* | 5 | 5.04 | 0.1 |
| | | KM2 | *Actinonaias ligamentina* | 5 | 6.43 | 0.05 |
| | | | *Amblema plicata* | 5 | 6.33 | 0.18 |
| | | KM3 | *Actinonaias ligamentina* | 5 | 6.35 | 0.11 |
| | | | *Amblema plicata* | 3 | 6.17 | 0.17 |
| | | | *Megalonaias nervosa* | 2 | 6.55 | 0.11 |
| | Little River | LM1 | *Lampsilis spB* | 5 | 3.43 | 0.07 |
| | | LM2 | *Amblema plicata* | 5 | 3.85 | 0.12 |
| | | | *Fusconaia flava* | 5 | 3.57 | 0.1 |
| | | | *Quadrula verrucosa* | 5 | 3.94 | 0.04 |
| | | LM3 | *Amblema plicata* | 5 | 3.66 | 0.2 |

| | | | | | |
|---|---|---|---|---|---|
| | | *Quadrula pustulosa* | 5 | 3.53 | 0.1 |
| | | *Quadrula verrucosa* | 4 | 3.68 | 0.26 |
| Mt. Fork River | MFM1 | *Amblema plicata* | 5 | 6.03 | 0.05 |
| | | *Fusconaia flava* | 4 | 6.44 | 0.06 |
| | | *Lampsilis spB* | 4 | 6.01 | 0.09 |
| | | *Ptychobranchus occidentalis* | 5 | 5.81 | 0.08 |
| | MFM2 | *Ptychobranchus occidentalis* | 5 | 5.96 | 0.07 |
| | | *Quadrula verrucosa* | 5 | 6.43 | 0.06 |
| | MFM3 | *Amblema plicata* | 5 | 5.5 | 0.11 |
| | | *Lampsilis spB* | 2 | 4.93 | 0.06 |
| | | *Ptychobranchus occidentalis* | 5 | 5.34 | 0.08 |
| | | *Quadrula pustulosa* | 4 | 4.95 | 0.07 |

Appendix B. Water chemistry of the Oklahoma sites was not significantly related to the % agriculture in the watershed, while the $\delta^{15}N$ of mussels was significantly positively related.



Appendix C. Relationship between NANI and the % of N flux that is NANI. Based on data from Howarth et al. (2012). The box shows within the figure indicates range of NANI observed in the U.S. sites of this study. We found that 80-94% of the total nitrogen loading in all stream reaches included in this study was anthropogenic in origin.



# CHAPTER 3

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

Carla L. Atkinson, Caryn C. Vaughn, Kenneth J. Forshay, Joshua T. Cooper

Keywords:

stoichiometry, nutrient translocation, algae, non-metric multidimensional scaling, spatial heterogeneity, nitrogen, unionid, mussel, nutrient limitation

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**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 3 rivers (Kiamichi, Little, and Mt. Fork rivers, south-central U.S.). 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 are 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.

## 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 control rates of primary productivity and

decomposition in streams (Meyer et al. 1998), and nutrient concentrations in streams can vary substantially across short distances (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 U.S. (Kiamichi - K, Little - L, and Mountain Fork - M) 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/$m^2$ 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): 9 sites with dense mussel aggregations and 9 sites with no or few mussels. All sites were approximately 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 1).

*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 M $NaNO^{3-}$), phosphate (P, 0.25 M $KH_2PO^{4-}$), a combined treatment containing

0.25 M 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 (6/22/10-7/6/10). We attached the NDS randomly to a plastic L-bar (3 replicates of each treatment per L-bar) and secured 4 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 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, CO, USA). Turbidity was measured with a Turner Designs Aquafluor Handheld fluorometer (Turner Designs, Sunnyvale, CA, 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, CO, USA) for

determination of water column N:P. Total dissolved carbon was determined from filtered (GF/F) samples collected in 40 ml VOA vials using a Phoenix 8000 Carbon analyzer (Teledyne Tekmar, Mason, OH, USA). We estimated stream shading using a spherical densiometer to quantify riparian forest canopy cover over the stream (Appendix 1).

*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 5 fields of view at 200x magnification (>150 cells identified for each sample). Further observation of cells was done at 400x 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 1 species at each site; Appendix 2). 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 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 hours, and analyzed with $HSO_4$ digestion followed by SRP analysis (Solorzano and Sharp 1980). Excretion samples (filtrate) were analyzed for total dissolved N (TN), P (TP), and dissolved organic carbon (DOC) as described above 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 6 different species (Appendix 2).

*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 Core Development Team).

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 3) using R version 2.14.0 with package sem version 2.0-1. We treated a path model as ''valid'' only if the model's $X^2$ was non-significant, 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 non-metric 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 1) 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 4 of the sites). In contrast, densities at mussel sites were 6.8 – 20.2 mussels/$m^2$.

*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 (2-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 (approximately 1.2x 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 1.3x 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 (mean 4.45 ± 0.06, 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 (mean 8.12 ± 0.12, N = 85), but N:P (mean 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 $X^2$ of our path analysis was not significant ($X^2 = 3.72$, $AIC_c = 9.4$, $df = 3$, $p > 0.30$), indicating good model fit. Only one other hypothesized model (Appendix 3) had a non-significant $X^2$, 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 versus 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 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 % 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 non-native, 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, 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 heterogenous 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 (this study), 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 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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## FIGURE LEGENDS

**Figure 1.** Map depicting the study area. 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.

**Figure 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 + 1 SE are shown for all the sites combined (9 mussel sites and 9 no mussel sites).

**Figure 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.

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

**Figure 5.** 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 versus 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) Joint plot indicated how environmental drivers are correlated to the NMDS plot (minimum $R^2$ set at 0.15).

Fig. 1

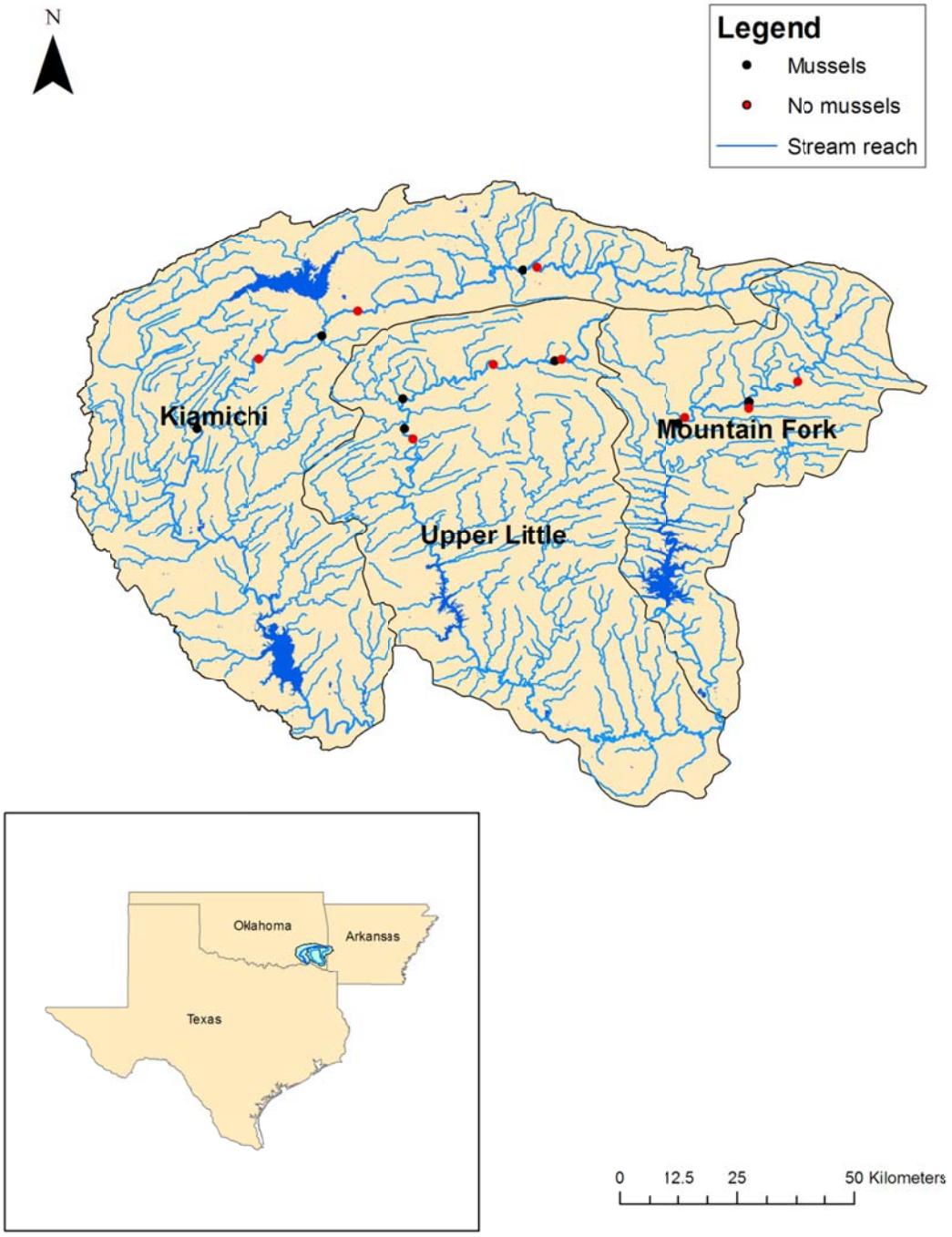

N
Legend
Mussels
No mussels
Stream reach
Kiamichi
Upper Little
Mountain Fork
Oklahoma
Arkansas
Texas
0
12.5
25
50 Kilometers

Fig. 2

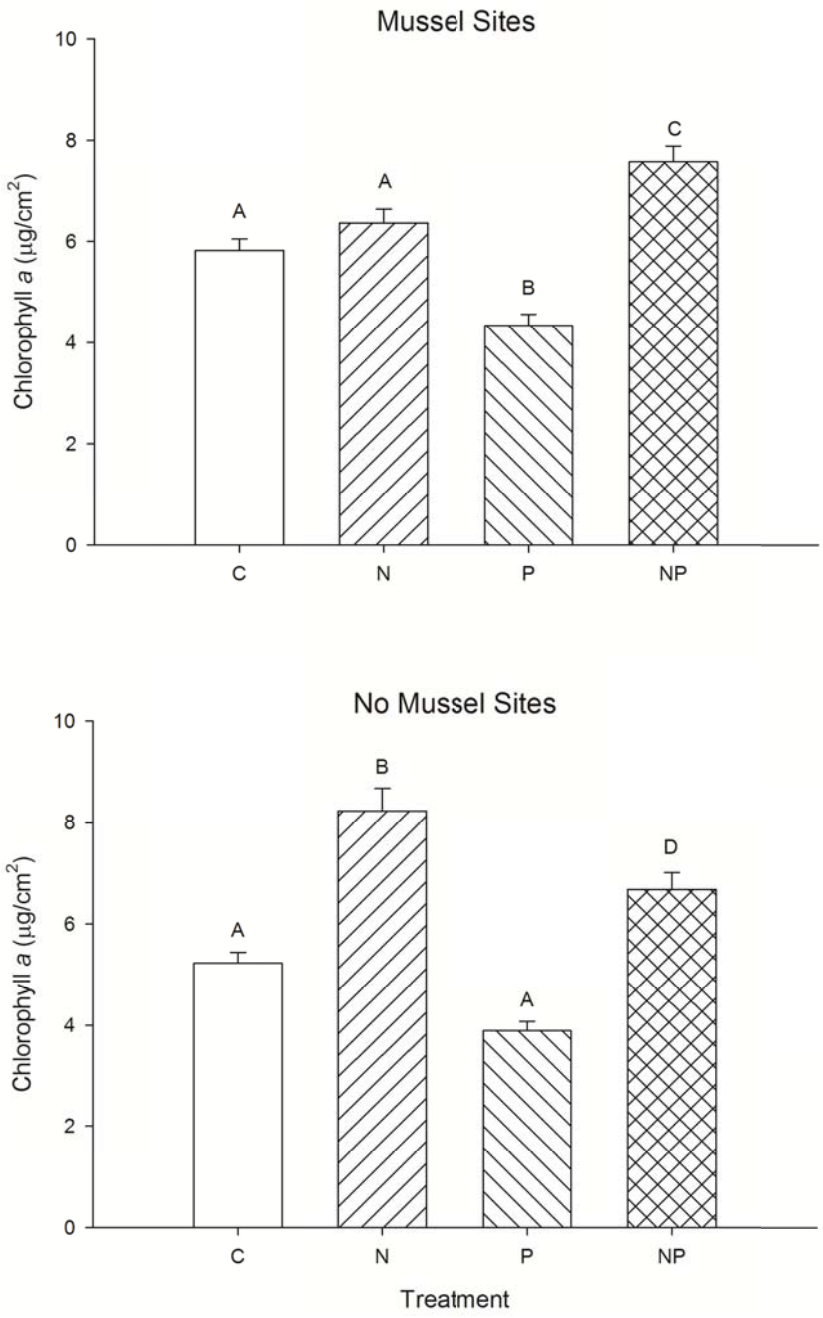

Mussel Sites
Chlorophyll a (µg/cm2)
10
8
6
4
2
0
A
A
B
C
C
N
P
NP
No Mussel Sites
Chlorophyll a (µg/cm2)
10
8
6
4
2
0
A
B
A
D
C
N
P
NP
Treatment

Fig. 3

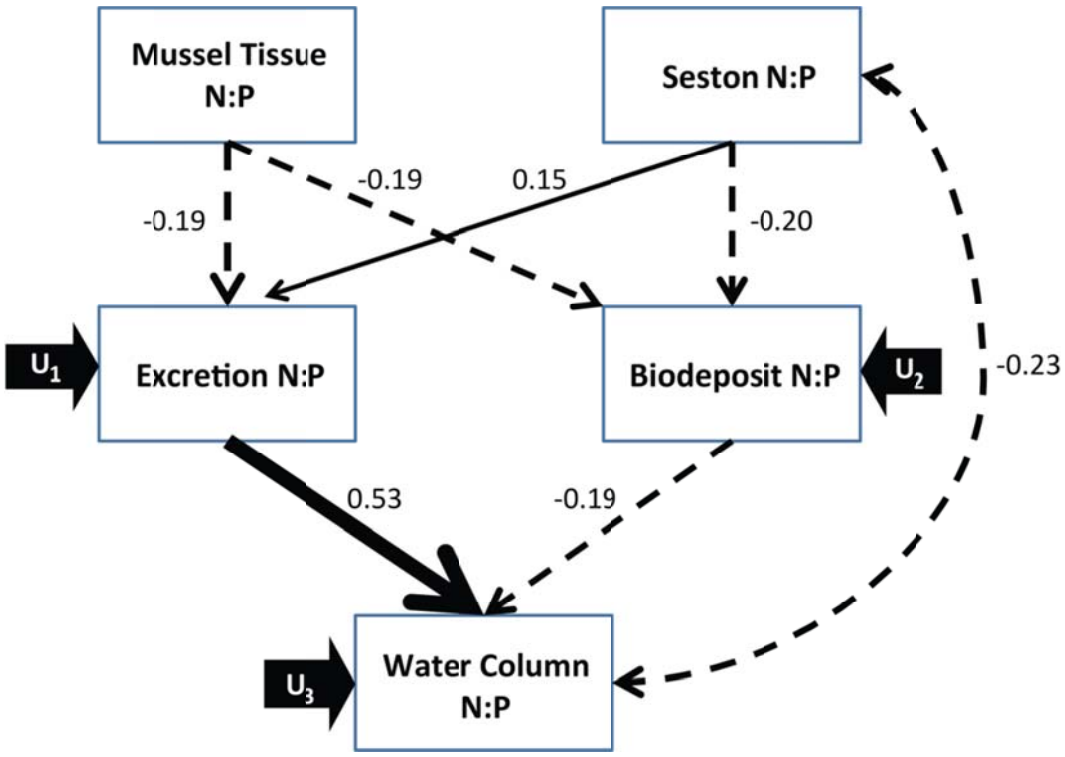

Mussel Tissue N:P
Seston N:P
-0.19
0.15
-0.19
-0.20
U1
Excretion N:P
Biodeposit N:P
U2
-0.23
0.53
-0.19
U3
Water Column N:P

Fig. 4

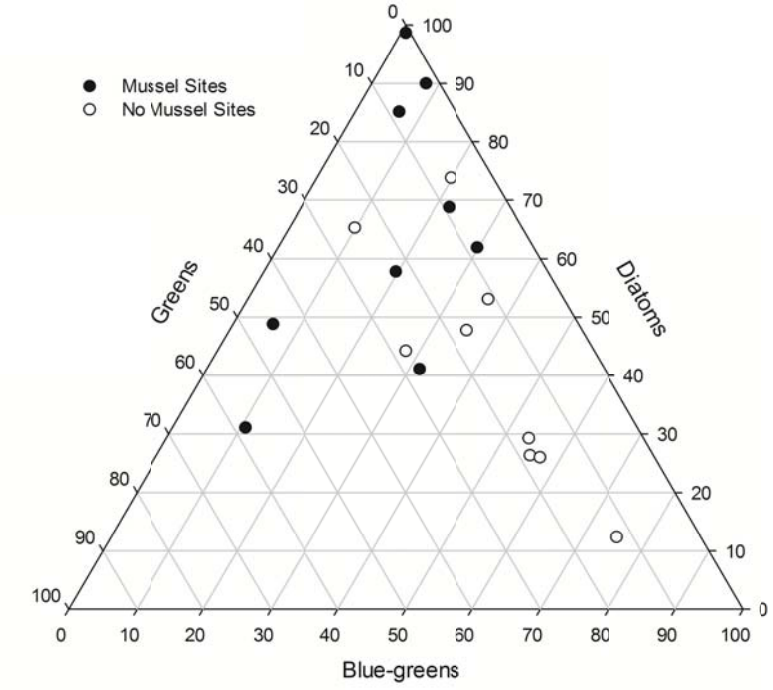
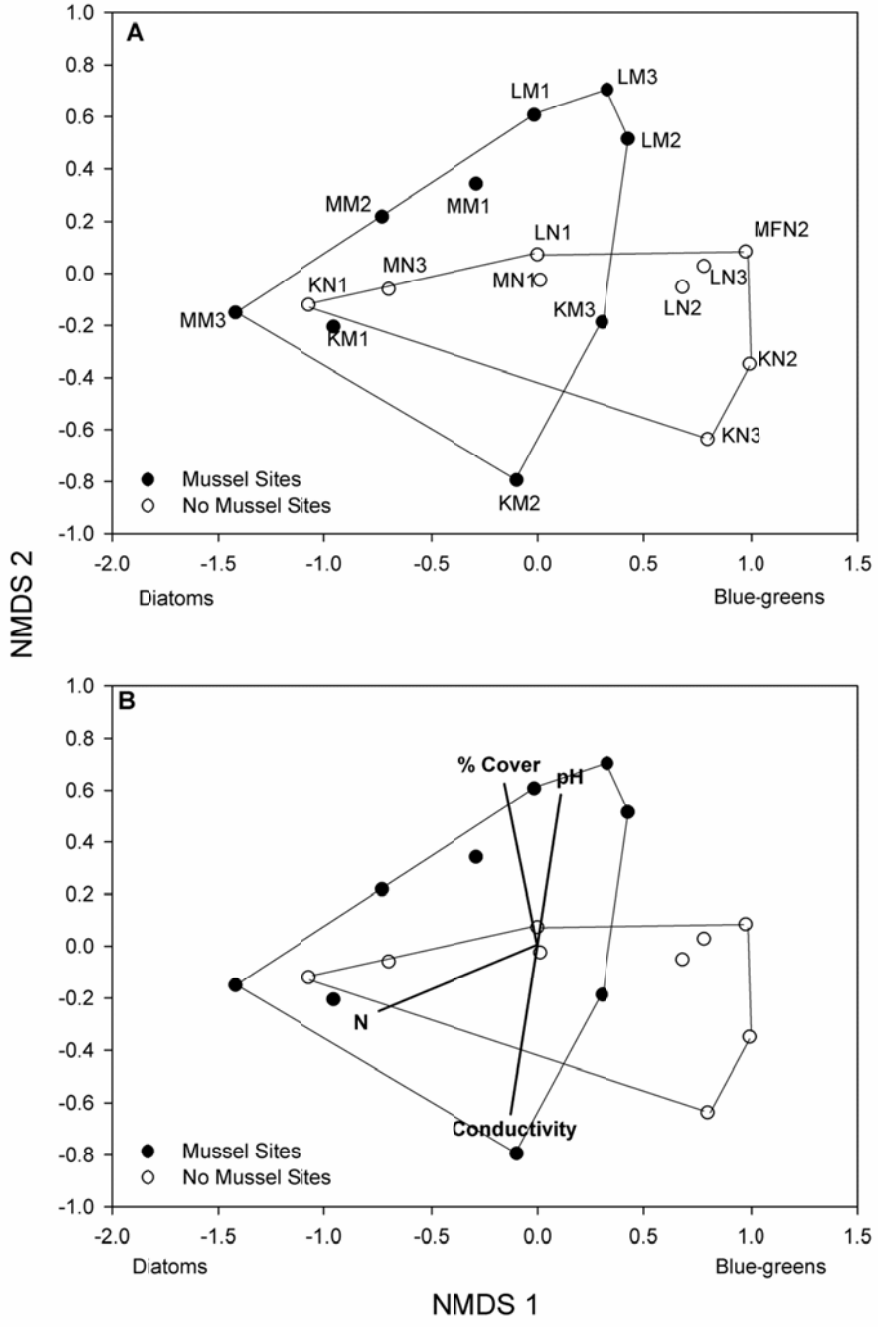

Mussel Sites
No Mussel Sites
Greens
Diatoms
Blue-greens
0
10
20
30
40
50
60
70
80
90
100

Fig. 5


A
LM1
LM3
LM2
MM2
MM1
LN1
MFN2
MN3
KN1
MN1
LN3
LN2
MM3
KM3
KM1
KN2
KN3
KM2
Mussel Sites
No Mussel Sites
Diatoms
Blue-greens
B
% Cover
pH
N
Conductivity
Mussel Sites
No Mussel Sites
Diatoms
Blue-greens
NMDS 1
NMDS 2

## SUPPLEMENTAL MATERIAL

**Appendix A.** Physiochemical parameters of all the sample sites used in the study.

**Appendix B.** Mussel species used in the excretion experiments.

**Appendix C.** The five candidate models used in the path analysis selection ordered by AICc scores.

**Appendix D.** The nutrient diffusing substrate (NDS) response at each study site.

**Appendix E.** Underwater photograph of the nutrient-diffusing substrates in the stream. Photo credit: Carla Atkinson

## SUPPLEMENTAL MATERIAL

Appendix A: Physiochemical parameters of all the sample sites used in the study.

| River | Site | Temp (˚C) | pH | Conductivity (µS) | Dissolved Oxygen (mg/l) | Turbidity (NTU) | Water N:P | % Cover |
|---|---|---|---|---|---|---|---|---|
| Kiamichi | KM1 | 30.41 ± 0.40 | 7.5 ± 0.21 | 52.75 ± 9.55 | 5.655 ± 0.60 | 34.83 ± 1.09 | 21.8 ± 0.78 | 6.96 |
| | KN1 | 28.93 ± 0.08 | 7.39 ± 0.20 | 49.75 ± 0.2 | 4.89 ± 0.01 | 33.94 ± 0.62 | 19.27 ± 1.81 | 8.85 |
| | KM2 | 29.13 ± 1.22 | 7.31 ± 0.10 | 60.8 ± 13.7 | 5.94 ± 0.15 | 29.32 ± 0.97 | 18.07 ± 1.5 | 0.00 |
| | KN2 | 29.45 ± 1.05 | 7.69 ± 0.26 | 41 ± 0.1 | 8.09 ± 0.03 | 30.46 ± 0.30 | 22.64 ± 1.76 | 22.33 |
| | KM3 | 30.74 ± 0.99 | 7.53 ± 0.10 | 36.95 ± 1.75 | 5.99 ± 0.46 | 30.73 ± 0.43 | 21.62 ± 2.65 | 1.03 |
| | KN3 | 30.40 ± 1.76 | 7.45 ± 0.01 | 40.4 ± 1.4 | 6.42 ± 1.00 | 27.78 ± 0.53 | 17.61 ± 2.31 | 14.97 |
| Little | LM1 | 29.43 ± 0.89 | 7.56 ± 0.07 | 37.97 ± 3.54 | 5.48 ± 0.31 | 20.46 ± 1.23 | 17.11 ± 2.61 | 52.18 |
| | LN1 | 27.13 ± 0.77 | 7.59 ± 0.04 | 34.03 ± 4.42 | 7.34 ± 0.39 | 20.5 ± 1.96 | 19.34 ± 2.14 | 38.04 |
| | LM2 | 30.73 ± 0.07 | 7.51 ± 0.10 | 26.97 ± 0.37 | 5.56 ± 0.21 | 24.20 ± 0.80 | 22.50 ± 3.71 | 3.76 |
| | LN2 | 28.66 ± 0.28 | 7.75 ± 0.12 | 26.4 ± 0.7 | 6.99 ± 2.14 | 23.28 ± 0.33 | 14.56 ± 4.01 | 18.62 |
| | LM3 | 28.41 ± 1.47 | 7.69 ± 0.21 | 31.35 ± 2.75 | 5.99 ± 0.04 | 26.33 ± 1.08 | 21.73 ± 4.42 | 21.29 |
| | LN3 | 31.52 ± 2.49 | 7.58 ± 0.05 | 30.85 ± 0.35 | 6.95 ± 0.01 | 25.23 ± 0.54 | 18.44 ± 0.77 | 2.72 |
| Mt. Fork | MM1 | 28.55 ± 1.47 | 7.54 ± 0.13 | 25.8 ± 3.3 | 6.07 ± 0.50 | 36.48 ± 2.81 | 17.75 ± 2.43 | 41.49 |
| | MN1 | 29.10 ± 2.34 | 7.59 ± 0.17 | 31.4 ± 7.1 | 6.89 ± 0.02 | 42.34 ± 1.43 | 23.51 ± 1.55 | 14.06 |
| | MM2 | 29.40 ± 0.70 | 7.71 ± 0.04 | 27.7 ± 2.40 | 6.17 ± 0.27 | 29.40 ± 4.48 | 22.47 ± 2.09 | 19.14 |
| | MN2 | 29.48 ± 0.48 | 7.43 ± 0.47 | 35.45 ± 4.55 | 6.58 ± 0.09 | 30.24 ± 3.21 | 15.87 ± 1.74 | 1.48 |
| | MM3 | 30.39 ± 0.52 | 7.45 ± 0.24 | 27.5 ± 2.7 | 6.73 ± 1.57 | 22.47 ± 1.96 | 16.19 ± 3.78 | 38.36 |
| | MN3 | 32.4 ± 0.53 | 7.76 ± 0.22 | 37.60 ± 12.5 | 7.15 ± 0.12 | 26.45 ± 1.09 | 17.20 ± 1.96 | 27.03 |

Appendix B: Mussel species used in the excretion experiments.

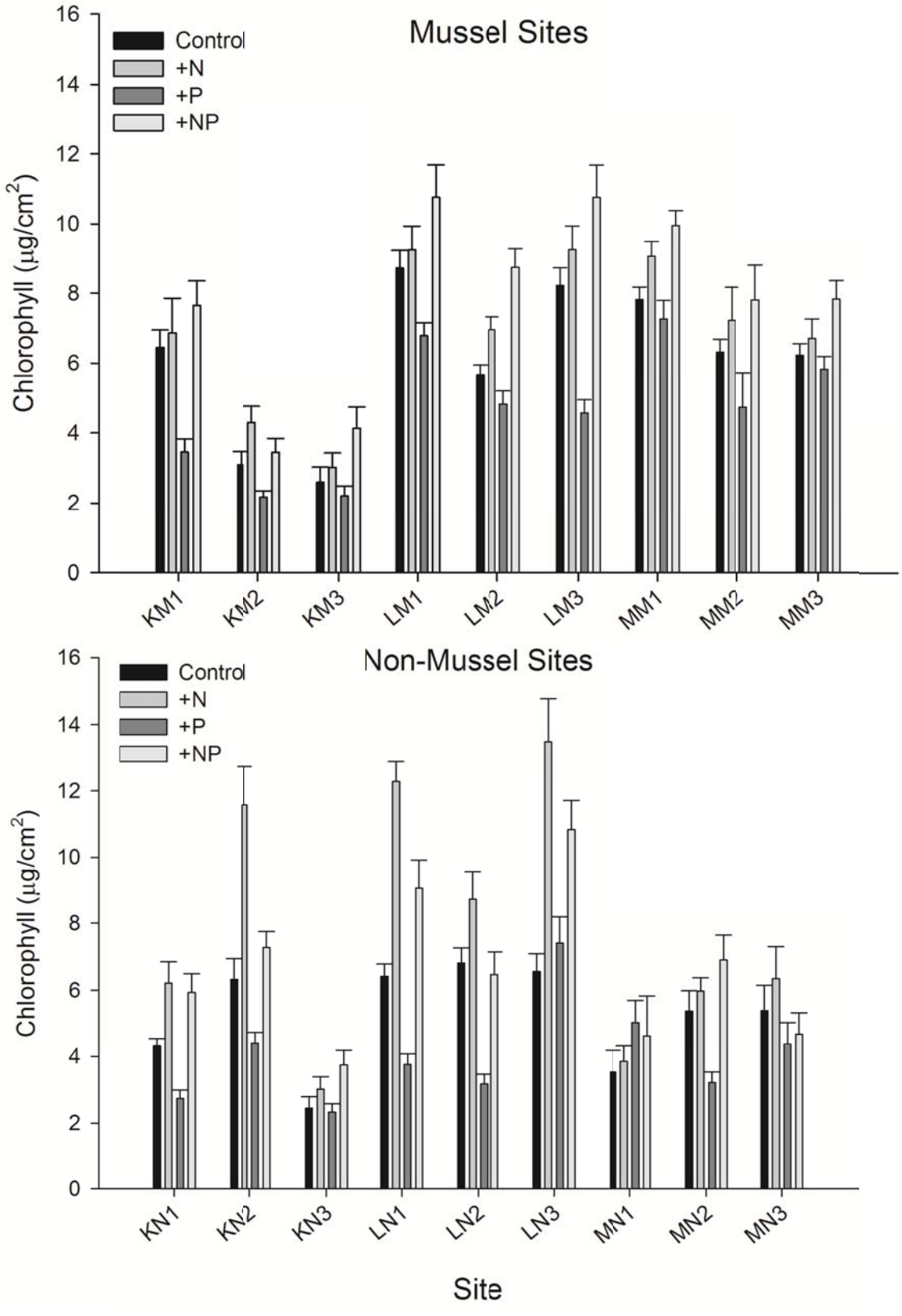
| Site | Species Used for Excretion Experiments | % of Site Species Composition | Mean Length (mm) | Mean Tissue N:P | Mean Excretion N:P | Mean Biodeposit N:P |
|---|---|---|---|---|---|---|
| KM1 | *Amblema plicata* | 85.0 | 103.8 ± 1.9 | 21.6 ± 0.8 | 21.9 ± 2.3 | 9.6 ± 2.7 |
| KM2 | *Actinonaias ligamentina* | 67.4 | 114.4 ± 7.0 | 18.5 ± 2.5 | 26.8 ± 1.5 | 9.2 ± 0.9 |
| | *Amblema plicata* | 11.6 | 88.4 ± 8.8 | 26.8 ± 2.6 | 27.6 ± 1.9 | 9.1 ± 1.6 |
| KM3 | *Actinonaias ligamentina* | 34.7 | 108.0 ± 16.1 | 13.3 ± 0.7 | 34.6 ± 1.5 | 10.6 ± 0.9 |
| | *Amblema plicata* | 20.4 | 90.2 ± 7.4 | 21.0 ± 3.2 | 27.8 ± 1.2 | 9.7 ± 1.8 |
| LM1 | *Lampsilis spB** | 25.0 | 59.7 ± 4.4 | 11.8 ± 1.1 | 18.9 ± 1.9 | 6.3 ± 1.1 |
| | *Villosa lienosa* | 37.8 | 45.7 ± 1.3 | 14.5 ± 2.3 | 16.1 ± 1.6 | 5.7 ± 2.3 |
| LM2 | *Amblema plicata* | 33.3 | 79.0 ± 14.1 | 30.5 ± 4.8 | 26.7 ± 0.3 | 3.5 ± 1.9 |
| | *Fusconaia flava* | 19.0 | 61.3 ± 4.9 | 24.6 ± 4.8 | 25.7 ± 0.6 | 6.0 ± 1.8 |
| | *Quadrula verrucosa* | 14.3 | 87.3 ± 9.5 | 23 ± 3.8 | 22.5 ± 1.9 | 7.8 ± 2.1 |
| LM3 | *Amblema plicata* | 37.0 | 76.3 ± 18.4 | 28.5 ± 5.6 | 36.4 ± 1.3 | 6.0 ± 0.9 |
| | *Quadrula pustulosa* | 26.1 | 52.9 ±3.2 | 27.3 ± 4.1 | 17.0 ± 2.1 | 8.0 ± 1.0 |
| MM1 | *Amblema plicata* | 32.0 | 85.2 ± 8.7 | 27 ± 3.2 | 13.0 ± 0.9 | 7.4 ± 0.9 |
| | *Fusconaia flava* | 36.8 | 65.4 ± 3.9 | 30.8 ± 3.0 | 19.4 ± 2.8 | 8.9 ± 3.3 |
| MM2 | *Ptychobranchus occidentalis* | 15.0 | 89.3 ± 10.2 | 18.7 ± 1.2 | 28.6 ± 4.9 | 6.3 ± 0.5 |
| | *Quadrula verrucosa* | 38.9 | 99.1 ± 13.0 | 25 ± 3.3 | 25.9 ± 2.1 | 7.8 ± 1.5 |
| MM3 | *Amblema plicata* | 27.3 | 77.6 ± 2.5 | 30.9 ± 4.0 | 12.7 ± 0.4 | 8.3 ± 1.1 |
| | *Ptychobranchus occidentalis* | 21.2 | 81.7 ± 9.0 | 27.6 ± 0.7 | 9.8 ± 0.5 | 7.5 ± 0.6 |

* This species is currently being described.

Appendix C: The five candidate models used in the path analysis selection ordered by AICc scores. A smaller $AIC_c$ score is represents the "best" model.

| Model | Correlation Matrices | Covariance Matrices | $AIC_c$ | Pr > Chi-sq | Adjusted Goodness of Fit |
|---|---|---|---|---|---|
| Top Model | Tissue N:P -> Excretion N:P<br>Tissue N:P -> Biodeposit N:P<br>Seston N:P -> Excretion N:P<br>Seston N:P -> Biodeposit N:P<br>Biodeposit N:P -> Water N:P<br>Tissue N:P -> Excretion N:P | Seston N:P <-> Water N:P | 9.3 | 0.29 | 0.90 |
| Model 2 | Tissue N:P -> Biodeposit N:P<br>Tissue N:P -> Excretion N:P<br>Seston N:P -> Excretion N:P<br>Seston N:P -> Biodeposit N:P<br>Excretion N:P -> Water N:P | Seston N:P <-> Water N:P<br>Excretion N:P <-> Biodeposit N:P | 12.6 | 0.07 | 0.81 |
| Model 3 | Tissue N:P -> Biodeposit N:P<br>Tissue N:P -> Excretion N:P<br>Seston N:P -> Excretion N:P<br>Seston N:P -> Biodeposit N:P<br>Biodeposit N:P -> Water N:P | Seston N:P <-> Water N:P<br>Excretion N:P <-> Biodeposit N:P | 31.9 | < 0.001 | 0.81 |
| Model 4 | Tissue N:P -> Biodeposit N:P<br>Tissue N:P -> Excretion N:P<br>Seston N:P -> Excretion N:P<br>Seston N:P -> Biodeposit N:P<br>Excretion N:P -> Water N:P<br>Biodeposit N:P -> Water N:P | Excretion N:P <-> Biodeposit N:P | 32.4 | 0.04 | 0.76 |
| Model 5 | Water Column N:P -> Seston N:P<br>Tissue N:P -> Excretion N:P<br>Tissue N:P -> Biodeposit N:P<br>Seston N:P -> Excretion N:P<br>Seston N:P -> Biodeposit N:P | Excretion N:P <-> Biodeposit N:P | 34.3 | < 0.001 | 0.58 |

Appendix D: The nutrient diffusing substrate (NDS) response at each study site.



Appendix E: Underwater photograph of the nutrient-diffusing substrates in the stream.

CHAPTER 4

# Tracing consumer-derived nitrogen in riverine food webs

Keywords:

stable isotope, enrichment, unionid, mussel, nitrogen,nutrient uptake,

turnover, stream food web, nitrogen tracer

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ABSTRACT

The flux of consumer-derived nutrients is recognized as an important ecosystem process, yet few studies have quantified the impact of these fluxes on freshwater ecosystems. The high abundance of bivalves in both marine and freshwater suggests that bivalves can exert large effects on aquatic food webs. The objective of our study was to determine the importance of unionid mussel-derived nitrogen (MDN) to the food web. We used a stable isotope tracer approach in conjunction with nutrient uptake and excretion experiments. We fed mussels (*Lampsilis siliquiodea*, n=249) a $^{15}N$-enriched algal diet and placed them into a N-limited stream for 63 days. Mussel hemolymph was non-lethally sampled over the course of the experiment to measure tissue turnover of $\delta^{15}N$ and excretion experiments were done to model the amount of N mussels provided in comparison to stream N uptake demand. Multiple food web pools were sampled twice prior and five times following the mussel addition to trace the $^{15}N$ through the food web. Our mussel excretion rates in comparison to areal uptake demand suggested that mussel excretion can account for 40% of the total N demand in this stream. Our enrichment showed that MDN was entering the food web and supplied up to 19% of the N in specific compartments of the food web near the mussel bed. When scaled to a natural mussel aggregation, our results suggest up to 74% of N in the food web may be mussel-derived. Our results show that N supplied by mussels can be an important nutrient subsidy that provides food web support.

## INTRODUCTION

Consumer–mediated nutrient cycling has been increasingly recognized as an important category of functional processes in many ecosystems (Vanni 2002, Schmitz et al. 2010, Small et al. 2011). Consumers play an important role in nutrient cycling by remineralizing nutrients that would otherwise be unavailable to an ecosystem. Several studies have quantified the flux of consumer-derived nutrients into various ecosystems (McIntyre et al. 2008, Small et al. 2011, Allgeier et al. 2013, Whiles et al. 2013), and some studies have quantified the additional amount of primary producer biomass that may occur because of these fluxes (Flecker et al. 2002, Spooner et al. 2012, Allgeier et al. 2013). Despite the growing recognition that consumer nutrient recycling is important, no study we are aware of has directly traced and quantified the contribution of consumer nutrient remineralization to the food web.

Ecologists have long recognized how certain species can have large effects on ecosystems (ecological engineers, sensu Moore 2006). However, research in this area has focused primarily on engineering habitat and species' roles in trophic interactions rather than in recycling nutrients (but see, Molvar et al. 1993, Knapp et al. 1999). Species vary in their functional effects and those who have large effects are key in controlling ecosystem dynamics. Nutrient recycling by animals can constitute an important biogeochemical flux and supply nutrients that limit primary productivity especially within aquatic ecosystems (Grimm 1988, Vanni 2002, Vanni et al. 2002, McIntyre et al. 2008). Previous studies have shown that both marine and freshwater bivalves can be important in influencing N and P cycles (Bruesewitz et al. 2009, Dame 2011, Goedkoop et al. 2011, Jansen et al. 2011). While their effect may not be large

relative to their biomass, nutrient cycling by mussels has an impact on primary productivity and algae species composition (Allen et al. 2012, Atkinson et al. 2013), suggesting they are important ecological engineers within stream systems (Moore 2006). Yet, the importance of nutrient fluxes on food webs has not been well examined (except see, Helfield and Naiman 2001). Quantification would allow a better understanding of the importance of consumer-mediated nutrient fluxes.

The impact of a particular organism on the ecosystem depends on density and biomass of the organism, ecosystem size, and other abiotic factors (Moore 2006, McIntyre et al. 2008, Small et al. 2009, Benstead et al. 2010, Small et al. 2011). The high abundance of bivalves in both marine and freshwater systems and their high filtration rates suggest that bivalves can exert large effects on stream food webs (Wotton et al. 2003, Porter et al. 2004, Vaughn et al. 2008). Freshwater mussels (Bivalvia: Unionidae, hereafter "mussels") are a diverse group of long-lived (6-100 y), burrowing, filter-feeders that are often abundant, but are experiencing rapid biodiversity declines (Strayer et al. 2004). Freshwater mussels occur in large aggregations (known as beds, up to 100 mussels $m^{-2}$) in rivers. The ecological functions performed by mussels (e.g., filter-feeding, nutrient excretion, biodeposition, bioturbation) affect both primary producers and consumers through direct and indirect pathways. Recent studies have shown that mussels, by filtering the water column and releasing nutrients and biodeposits, stimulate both water column and benthic primary production (Vaughn et al. 2007, Vaughn et al. 2008, Atkinson et al. 2011, Spooner et al. 2012), which in turn is correlated with higher abundance and richness of benthic invertebrates (Howard and Cuffey 2006, Spooner and Vaughn 2006, Vaughn and Spooner 2006, Vaughn et al.

2008) and even secondary consumers (Allen et al. 2012). While mussels are not creating nutrients, they are transforming them through physiological activities and providing them in a readily available form that is like a nutrient subsidy (Atkinson et al. 2013, Spooner et al. 2013). The direct linkages that connect nutrient fluxes from mussels to other food web components need to be quantified.

Our goal was to determine the importance of mussel-derived nitrogen (MDN) to stream food webs. We used an experimental nitrogen (N) stable isotope tracer approach in conjunction with nutrient excretion assays. Mussels labeled with an algal food resource enriched in $^{15}N$ were used to trace the N leaving the mussels and entering the food web. Excretion rates were measured to model the flux of N from mussels. Nitrogen is a key element that often limits the productivity of streams (Dodds 1997). Previous results at our study site suggest N limitation, which is typical of streams of the Ouachita Mountains and Upper Gulf Coastal Plain (Atkinson et al. 2013), thus we predicted the ecosystem would respond to increased availability of N provided by mussels. We hypothesized that MDN would enter the food web and we would see a significant increase in tracer $^{15}N$ of primary producers and stream consumers. Additionally, we determined how much MDN was recovered, percentages of N in tissue biomass of the ecosystem compartments were MDN, and the uptake rate of N into the system, and estimated the amount of MDN that directly entered the food web.

## METHODS

*Mussel Enrichment and Addition:*

We used a stable isotope approach to track mussel-derived nutrients in a field-based experiment. Juveniles of *Lampsilis siliquoidea,* a freshwater mussel species commonly found in the upper Little River, Oklahoma, were obtained from Missouri State University's freshwater mussel propagation program. For 41 weeks mussels were fed a cultured algal mixture enriched in $^{15}N$ (~1000 ‰ relative to atmospheric $N_2$) in a Living Stream (Frigid Units Inc., Toledo, Ohio) at the Aquatic Research Facility at the University of Oklahoma every other day. Three days prior to placing the mussels in the river, mussels were cleaned of biofilm and were moved to a separate holding tank. During this time, mussels were individually tagged, measured (mean ± SE = 62.45 ± 0.55 mm), weighed (mean ± SE = 28.72 ± 0.75 g), and held without food to allow egestion of enriched algae. We changed the water in the holding tanks daily.

Mussels were placed in a small, forested reach of the upper Little River (see description below) approximately 15.6 km downstream from the headwaters (watershed area 73.5 km; Figure 1) on 14 May 2011. Mussels were added to a reach without mussels, but approximately 700 m upstream of a known mussel aggregation or bed. The experiment was done in an area without mussels because we wanted to work in a N-limited area, and previous work in this system has shown that areas with mussels are co-limited by both nitrogen and phosphorus (Atkinson et al. 2013). Quarter-meter square quadrats were placed in the middle 24 $m^2$ (leaving 4 m on each side of the stream margins without mussels) of the stream in a checkerboard pattern 48 times and 5-6 mussels (equivalent to 20-24 mussels $m^{-2}$) were placed in each quadrat. The initial area of the reach with mussels, including stream margins (which declined over the summer), was 72 $m^2$. A total of 249 mussels were added to the stream. The mussels were allowed to

move freely within the substrate following placement in the stream. Unfortunately, our study region experienced exceptional drought conditions in summer 2011. The study reach began to dry and mussels were at risk of dying, so they were moved to a downstream pool after 63 days to prevent mortality.

*Abiotic Variables and Food Web Pools*

We established transects up and downstream of the boundaries of the mussel release location (transect "0") (Figure 1A). Temperature was continuously recorded every 15 minutes throughout the experiment at the -5 meter transect using a Hobo U20 submergible logger (Appendix 1, Onset, Cape Cod, Massachusetts). Discharge was measured using a Marsh McBirney flow meter at the site twice in August 2010 before the addition (during the nutrient uptake experiments) and then three times following the addition throughout the experiment (May, June, and July 2011). We collected water samples at the -10, -5, 0, 5, 10, and 25 meter transects the summer prior to the experiment (9 Aug 2010), at the beginning of the experiment (14 May 2011), and at 41 days (23 June 2011), 61 days (12 June 2011), and 81 days into the experiment (1 August 2011). Water samples were analyzed spectrophotometrically for $NH_4^+$-N by the phenol hypochlorite method (APHA 1995).

Periphyton, water willow (*Justicia americana*), mayfly nymphs (Heptageniidae), stonefly nymphs (Perlidae), water pennies (Psephenidae), and limpets (*Laevapex* spp.) were collected the summer before the experiment (August 2010), in the spring just before the experiment (April 2011), seven days following the addition, and then approximately bi-weekly following the introduction of mussels to the site for up to 81

days for stable isotope analyses. Additionally, biomass of each of these food web pools was determined across the sample reach in the summer prior to the experiment (August 2010) and following the experiment (September 2011). Collection for determination of periphyton biomass was also done prior to the experiment (April 2011). For periphyton, we scraped the periphyton from a known area of a rock face and collected it on a glass-fiber filter (1.0 μm pore size). We determined dry mass for a total of 32 samples (4 samples per transect) per sample period. We haphazardly placed a 0.25 $m^2$ quadrat and collected all water willow within it and determined dry mass for a total of 16 samples per sampling period (2 samples per transect). A Surber sampler (500 μm mesh) was used to quantitatively sample for macroinvertebrates for a total of 24 samples (3 samples per transect) during each sampling period. Following collection, insects were sorted and dry mass was determined. Additionally, we determined percent composition of N of the ecosystem pools (described below) to estimate mass of N in each ecosystem pool.

*Mussels:*

We did field excretion experiments to estimate the amount of $NH_4$ flux from mussels to the stream. We focused on $NH_4$ because it is the mostly readily bioavailable form of N. Excretion experiments were performed with 10 individual mussels (*Lampsilis siliquoidea*) following Atkinson et al. (2013) on days 18, 30, and 60 for a total of 30 individuals. Five controls were done at the same time, and excretion rates were calculated as the difference in nutrient concentrations between mussel treatments and the average of the control containers. We sampled mussel hemolymph non-lethally (Gustafson et al. 2007) for $\delta^{15}N$ prior to (from the experimental mussels and mussels

near the site), during, and following the enrichment period during each field sampling period. The $\delta^{15}N$ of mussel hemolymph was paired with excretion rates to estimate $^{15}N$ release. Stable isotope analysis of mussel hemolymph showed that $\delta^{15}N$ enrichment of mussels declined throughout the experiment. We used this value to determine the amount of $^{15}N$ mussels were releasing into the ecosystem (in conjunction with excretion rates), to estimate water column $\delta^{15}N$ (see methods below), and tissue turnover. We estimated daily turnover rates from the time series of hemolymph tissue $^{15}N$ by a decay model adapted from Tieszen et al. (1983) and Hesslein et al. (1993):

$$^{15}N_t = {}^{15}N_{pre} + (^{15}N_{peak} - {}^{15}N_{post})e^{-kt}, \quad (1)$$

where $t$ is the number of days the mussels had access to non-enriched food (modeled up to 81 days), $^{15}N_t$ is the tissue atom % $^{15}N$ at time $t$, $^{15}N_{pre}$ is the tissue atom % $^{15}N$ prior to enrichment, $^{15}N_{peak}$ is the highest tissue atom % $^{15}N$ during enrichment, and $^{15}N_{post}$ is the atom % $^{15}N$ as it is returning to equilibrium, and $k$ is the absolute value of the $^{15}N$ depletion rate. We assumed that $^{15}N_{post} = {}^{15}N_{pre}$ in our experiments as in McIntyre and Flecker (2006). The term $k$ was estimated as the slope of the regression line of $\ln(^{15}N_{peak}/ {}^{15}N_{peak} - {}^{15}N_{post})$ versus time, as in Gustafson et al. (2007) and is the exponent describing the proportion of $^{15}N$ lost daily from the tissue due to growth and metabolic replacement. We also calculated the tissue turnover time (Hobson and Clark 1992, MacAvoy et al. 2001), or half-life, of hemolymph as:

$$T_{1/2} = (\ln 2)/k, \quad (2)$$

*Isotope and Elemental Composition Analyses:*

Total carbon and total nitrogen composition as well as the carbon and nitrogen stable isotopic signatures were determined for the each of the ecosystem pools. Isotope

ratios are expressed in the delta (δ) notation: $\delta^{15}N$ (units of ‰) = (($R_{sample}$ - $R_{standard}$ )/$R_{standard}$) x 1000, where R is the $^{15}N$:$^{14}N$ ratio. A bovine protein (peptone) lab standard was referenced against an international standard and precision averaged 0.1‰ or less. Stable isotope analyses were performed at the University of Georgia's Stable Isotope Facility using a Finnigan Delta Plus mass spectrometer or at the University of Oklahoma using a Costech elemental analyzer (Costech Analytical Technologies, Valencia, California, USA) interfaced through a Conflo III valve with a Thermo Delta V Plus isotope ratio mass spectrometer (Thermo Fisher Scientific, West Palm Beach, Florida, USA).

*Uptake and Uptake Rates:*

We measured areal $NH_4$ uptake twice (at discharges of 21 L $sec^{-1}$ and 49 L $sec^{-1}$) in the experimental stream reach in August 2010. Uptake rates were calculated from the longitudinal decline in N that was added to the stream to enhance ambient concentrations along a reach and were corrected for hydrological exchange following standard methods (Mulholland et al. 2002). The mean uptake of $NH_4$ is a minimum estimate of the demand for dissolved organic N (DIN) because it does not include both assimilatory and nonassimilatory (nitrification and denitrification) demand for DIN. However, the measurements of uptake rates are for comparison to $NH_4$ excretion rates. To measure $NH_4$ uptake lengths and rates, we conducted short-term (~3 h) additions of $NH_3$-N, in conjunction with a conservative tracer ($Br^-$ as KBr) (Tank et al. 2006). After collecting six background samples of stream solute concentrations along the study reach, a solution of $NH_4Cl$ and the conservative tracer was pumped steadily into the

stream. Target enrichments of dissolved ammonia were 50 μg $NH_3$-N $L^{-1}$. Target concentration of the conservative tracer was 570 μg Br $L^{-1}$. When the conservative tracer concentration was constant through time at the downstream end of the study reach, we collected water samples at each of five sites (every 20 m up to 100 m downstream) along the study reach. By increasing stream water concentration of N to measure uptake, it is possible that we underestimated uptake velocity relative to using isotope additions (Mulholland et al. 2002) because of saturation of microbial uptake. However, this effect was likely low because these streams are N-limited (Atkinson et al. 2013) and uptake is likely higher in mussel beds because of increased availability of N (Dodds et al. 2002). Samples were analyzed spectrophotometrically for $NH_4^+$-N by the phenol hypochlorite method (APHA 1995) and bromide was measured in the field using a bromide ion probe (Cole-Parmer, Court Vernon Hills, IL, USA) and in the lab using capillary electrophoresis via flow injection using a Lachat QuikChem FIA+ 8000 (Hach Company, Loveland, CO, USA). We calculated nutrient uptake lengths from the injection data using the linear form of an exponential model:

$$\ln N_x = \ln N_0 - ax, \quad (3)$$

where $N_0$ and $N_x$ are nitrogen concentrations at the addition site (0 m) and $x$ m downstream from the addition site, and $a$ is the per meter uptake rate (Newbold et al. 1981). Uptake length $Sw$ (m) equals $a^{-1}$. We used ordinary least squares regression to estimate parameters for Eq. 1 from the field data. We calculated nutrient uptake velocity ($V_f$), also referred to as a mass transfer coefficient, to account for the influence of depth and velocity on uptake length (Tank et al. 2006):

$$V_f (\text{m min}^{-1}) = Qa/w, \quad (4)$$

where $Q$ is stream discharge ($m^3$ $min^{-1}$), and $w$ is wetted channel width (m). Discharge was measured using a Marsh-McBirney flow meter and width was measured at 5 transects along the study reach. The nutrient uptake velocity can be interpreted as the velocity at which a nutrient moves through the water column toward the benthos and represents the biotic demand for nutrients relative to concentration in the water column. Areal uptake rate of N ($U$, mg N $m^{-2}$ $min^{-1}$) was calculated as:

$$U = V_f N_b , \qquad (5)$$

where $N_b$ equals the ambient N concentration in the stream based on the 14 pre-release measurements.

To estimate the uptake rates of MDN into ecosystem pools and the transfer between the pools, we used a box model approach similar to Dodds et al. (2000). In order to model the uptake rates of periphyton and water willow, we had to estimate the water column $\delta^{15}N$. We did this by quantifying the flux of $^{15}N$ and $^{14}N$ from mussel excretion and the flux of background $^{15}N$ and $^{14}N$ and quantifying the mass using the measured discharge values. From that we determined the background $\delta^{15}N$ by summing the total mass of $^{15}N$ and $^{14}N$ from mussels and the background and then calculated the $\delta^{15}N$. Using this estimated $\delta^{15}N$ of the water column, we estimated uptake for water willow and periphyton at the 0 and 5 meter transects. The estimated uptake rates for these primary producers may be too low because they ignore uptake of $NO_3$ and DON from the water column, and uptake of N from the sediments and interstitial water. Using the $\delta^{15}N$ values of periphyton, we modeled the uptake rates (in μmol N $m^{-2}$ $d^{-1}$) of both mayflies and stoneflies at the 0, 5, 10, and 25 meter transects. The estimates for uptake rates for mayflies and stoneflies assume non-selective and complete assimilation of

periphyton (and use of no other food resources). If these consumers use highly labeled fractions of the periphyton (as described by Dodds et al. 2000), estimated uptake rates could be too high, while use of other food sources could drive the estimates either too high or too low.

*Data Analyses*

To determine percent recovery of MDN over the course of the experiment, we derived best fit curves to each of the measured food web pools for each individual transect across time (corrected for background signatures of the pools) using the curve function trapz in MATLAB R2012a and then calculated the integral of the relationship to determine the areas under the curve. We also fit a curve to the mussel hemolymph values over time as a signature of the $^{15}N$ released into the environment and integrated that value to determine the area under the "source" curve. The area of the ecosystem pools (biomass corrected) were summed and then compared to the source curve to determine percent recovery at each transect for each time period. Following this, observed $\delta^{15}N$ values were converted to mussel-derived percentages using a two source mixing model (Helfield and Naiman 2001, Allen et al. 2012) to determine the amount of mussel-derived N (MDN) entering each ecosystem pool. The mixing model calculates MDN percentages as:

$$\%MDN = [(EP - EP_0)/(MUS - EP_0)] \times 100 \quad (6)$$

where %MDN is the percentage of MDN in a given sample, EP is the observed $\delta^{15}N$ of the sample, $EP_0$ is the ecosystem pool end member (i.e., $\delta^{15}N$ value representing 0% MDN), and MUS is the mussel derived N end member (i.e., $\delta^{15}N$ value representing

100% MUS). In this study, $EP_0$ was calculated as the mean $\delta^{15}N$ of each ecosystem pool prior to the mussel addition at the site, EP was the mean $\delta^{15}N$ of the ecosystem pool following addition, and MUS was the mean $\delta^{15}N$ of mussel hemolymph tissue during the addition. This is a static model that assumes isotopic fractionation associated with N uptake is negligible and does not take temporal variability into account. Using the average %MDN in each of the ecosystem pools over all the sampling periods post-mussel addition and the biomass of the pools, we estimated the mass of N in the ecosystem on a per square meter basis and estimated the total amount of N that was MDN in the 50 meter reach. To do this, we split the stream into 5 segments: -5 to 0 m, 0 to 5 m, 5 to 10 m, 10 to 25 m, and 25 to 50 m. Using the area of each of these segments, we used the %MDN value from the most downstream transect (the -5m transect in the case of -5 to 0 m) and multiplied by the mass of each of the ecosystem pools.

*Scaling to a Natural Mussel Bed*

We wanted to determine how the importance of MDN would scale to a natural mussel bed. The influence of MDN depends upon the biomass and excretion rates of mussels within a reach, N demand, and ecosystem size. Using previously collected data from a mussel bed in the Little River approximately 53 km downstream from our study reach, we scaled our results to a natural mussel community. This mussel bed was composed of multiple species, but biomass was dominated by *Amblema plicata* (37% of biomass), *Fusconaia flava* (13%), and *Quadrula pustulosa* (26%). We have data on areal excretion rates of the most common species (Atkinson et al. 2013), stream width and depth, nitrogen uptake rates from $NH_4^+$-N addition experiments (Atkinson, unpublished), background nutrient concentrations, and average summer discharge

(Appendix 2). From these data we calculated areal excretion rates (E*a*, μmol N $m^{-2}$ $d^{-1}$) for the study reach with the enriched mussels and the natural mussel bed. We calculated the percent $NH_4$ demand that mussels provided through excretion by dividing the $NH_4$-N areal excretion rates by $NH_4$-N uptake rates. Following this, we compared the supply to demand at the enriched mussel site and the natural mussel bed. To account for differences in the two stream reaches and ambient nutrient conditions, we calculated volumetric excretion following McIntyre et al. (2008) and Benstead et al. (2010) based on a 100 m stream length at both sites. Volumetric excretion ($E_v$, mol nutrient $L^{-1}$) is a useful metric because it describes the average addition of excreted nutrients by mussels to water as it flows along a given reach, assuming no uptake and perfect mixing. Volumetric excretion was calculated as:

$$E_v = (E_a \times A \times T)/V \quad (7)$$

Volumetric excretion integrates data on substrate area, A (length×width, $m^2$), volume, V (length × cross-sectional area, $m^3$) and travel time, T (length/water velocity, h) of each channel unit. Comparisons of these metrics allowed us to estimate the contribution of MDN by a natural mussel community.

## RESULTS

### *Ammonium:*

Mussels had a measurable effect on water column nitrogen availability and the tracer $^{15}N$ released from the mussels was assimilated by the food web pools. Ammonium concentrations increased in water around the mussels following their addition. As water levels dropped throughout the summer this effect became more

pronounced (Figure 2). Ammonium concentrations dropped following the removal of the mussels and returned to similar levels as found upstream and prior to the addition.

*Isotopes:*

Using the decline in $\delta^{15}N$ in mussel hemolymph, we calculated an N turnover rate of 0.009 $day^{-1}$ ($R^2 = 0.74$), which is equivalent to a 72 day half-life. Prior to enrichment, the average background $\delta^{15}N$ (± standard deviation) was 2.03‰ (± 0.22) for periphyton, 1.57‰ (± 0.35) for water willow, 2.15‰ (± 0.32) for mayflies, 2.13‰ (± 0.71) for stoneflies, 1.84‰ (± 0.28) for water pennies, and 3.64‰ (± 0.24) for limpets. We noted enrichment following the mussel addition in the periphyton (maximum enrichment of 5.62‰ at the 5 meter transect 33 days following addition), water willow (maximum enrichment of 5.14‰ at the 25 meter transect 55 days following addition), mayflies (maximum $\delta^{15}N$ of 7.21‰ at the 5 meter transect 33 days following addition), and stoneflies (maximum enrichment of 5.21‰ at the 5 meter transect 74 days following addition (Table 1, Figure 3), while we did not see any enrichment effects (not above the 95% confidence intervals of the pre-enrichment values) in water pennies or limpets. Additionally, we had upstream enrichment at the -5 m transect in periphyton, water willow, and mayflies presumably due to upstream movement of animals and low flows in the stream. Some food web pools responded faster to the enrichment, such as the periphyton and mayflies, while other pools such as the water willow and stoneflies, responded more slowly (Figure 3). On average, within 50 meters of the introduction area, approximately 3% of the $^{15}N$ released from the mussels was recovered across transects (Figure 4). Some of the N that was captured in

an upstream transect may have been recycled or remineralized and moved into the N pool of a downstream transect. Within the ecosystem pools measured, up to 19.3% (mayflies at the 5m transect) of the N was MDN. MDN entered the more upstream ecosystem pools early in the experiment and did not affect the lower reaches until later in the experiment (Figure 5). Based on our $^{15}N$ tracer results, there was ~58 mmol N on day 7, ~436 mmol N on Day 19, ~951 mmol N on day 41, ~1100 mmol N on day 61, and ~270 mmol on day 81 of MDN across the 50 m reach. Areal MDN in the downstream transects following the mussel addition ranged from 71 μmol N $m^{-2}$ at the 25 meter transect at day 19 to 1485 μmol N $m^{-2}$ at the 5 meter transect at day 19.

*Nitrogen Uptake and Demand*

The Little River is N-limited (Atkinson et al. 2013) and our nitrogen uptake experiments quantified the demand of ammonium. Nitrogen uptake length ($S_w$) measured during the summer of 2010 ranged from 32 m during the low flow to 161 m during higher flows. Both measurements resulted in a similar uptake velocity, averaging 2.42 (range: 1.5-3.3) mm $min^{-1}$, resulting in an uptake rate of 116.7 μmol N $m^{-2}$ $hr^{-1}$ (range: 79.9-153.5) μmol N $m^{-2}$ $hr^{-1}$. This uptake rate in comparison to the areal excretion rate of mussels (47.2 μmol N $m^{-2}$ $hr^{-1}$) in our experimental area suggests that these mussels could account for approximately 40% of the ammonium demand within the mussel bed in this reach and could account for some downstream demand. Uptake rate into the food web pools was similar in the primary producers (periphyton and water willow), with our model indicating a rate of 1.0-1.5 μmol N $m^{-2}$ $d^{-1}$. There was more variability in the primary consumers, with mayflies having an uptake rate of 4.0-108.0

μmol N $m^{-2}$ $d^{-1}$, and stoneflies having an uptake rate of 3.1-669.2 μmol N $m^{-2}$ $d^{-1}$ (Table 1). The highest uptake flux rates were observed at transects closest to the mussel addition (maximum rates at 5 m).

*Scaled to a Natural Mussel Bed*

Measured ammonium uptake rates at the natural mussel bed were 184.16 μmol N $m^{-2}$ $h^{-1}$ in 2012 and areal excretion by the mussel community at this site was 181.02 μmol N $m^{-2}$ $h^{-1}$, suggesting that mussels could provide up to 98% of the ammonium demand in this downstream reach. The mussel bed we created with enriched mussels accounted for approximately 40% of ammonium demand, such that natural mussel aggregations provided more N than our created single-species mussel bed. We found that $E_v$, which scales for stream size, at the created mussel bed was 6.03 μM N, while $E_v$ at the natural mussel bed was 23.35 μM N. Our data suggest that MDN from naturally occurring mussel beds may be 3.9x more available to the food web than in our experimental bed and represents a very large source of nutrient subsidies, particularly in the case of dense, species-rich mussel beds. If MDN is used proportionately to its availability, it could account for up to 74% of the N in the biomass of various components of the food web.

DISCUSSION

Our results directly link a well-studied process, nutrient remineralization, to its bottom-up contribution to stream food webs. Specifically, by creating a mussel bed and tracing the nitrogen remineralized by mussels into the stream, we demonstrated that

mussel derived nitrogen moves directly into the stream food web and likely is an important form of nutrient flux around natural mussel communities. Previous studies suggested that mussel remineralization alters algae species composition (more diatoms and less cyanobacteria compose the periphyton assemblage; Allen et al. 2012, Atkinson et al. 2013), so this enhanced availability of N may be increasing both the quantity and the quality of resources available to stream organisms. This study and others (Spooner and Vaughn 2006, Atkinson et al. 2011, Allen et al. 2012) show that nutrients released by mussels are an important regulating factor affecting nutrient availability and food web support. Further, our research contextualizes the role a once common group of organisms, unionid mussels, play in supporting nutrient cycling and food webs in streams. Our data underscore the essential ecosystem processes mussels provide in streams.

We determined the relative demand for N as ammonium in comparison to that made available by mussel excretion. While ammonium is not the only form of N that satisfies ecosystem demand (e.g., nitrate and organic N), $NH_4^+$ is the preferred form of N for both algae and microbes (Dortch 1990, Tank et al. 2006), and uptake of $NH_4^+$ can suppress nitrate uptake (Tank et al. 2008). Thus, mussels are providing a form of N with high demand. Previous studies have found increased nutrient concentrations near aggregated organisms (McIntyre et al. 2008, Jansen et al. 2011). In our study, we noted increased ammonium availability around the created mussel bed in a system that is N-limited (Atkinson et al. 2013), and that this N was assimilated by the food web. While we did not consider the microbial loop (Meyer 1994) or the uptake of N from stream sediments, our study indicated that periphyton, water willow, and aquatic insects were

assimilating mussel derived N. This assimilation of MDN suggests that mussels are ecosystem engineers through regenerating limiting nutrients.

The assimilation of MDN has important bottom-up repercussions for stream food webs. In a previous study in nearby streams, Vaughn and Spooner (2006) found increased abundance and richness of insect larvae in mussel aggregations which could have been in response to higher algal production due to enhanced bottom-up nutrients. Mussel bottom-up nutrient remineralization not only influences stream food webs, but also likely impacts nearby terrestrial food webs. In a mesocosm experiment, Allen et al. (2012) showed that nitrogen from mussels entered algae, which was utilized by insects consumers, which were in turn tracked by predatory, terrestrial spiders. Helfield and Naiman (2001) quantified the important roles of salmon in supplying nitrogen to Pacific Northwest streams of North America and that this N was exported to and assimilated by the nearby riparian forest. Therefore, the major remineralization pathway that mussels provide is not only important for stream food webs, but may also being exported from the stream to subsidize riparian zones.

Effects of freshwater mussels on ecosystem function and food web support are not continuous because mussel beds are spatially heterogenous in this system (Atkinson et al. 2012) and others (Haag 2012). This spatial heterogeneity is integral to system function and mussel beds may constitute hot spots of ecosystem productivity in many river ecosystems (Strayer 2013). Spatial heterogeneity influences population dynamics, community structure, and ecosystem function (Zerba and Collins 1992, McIntyre et al. 2008). Mollusks are well known as structural engineers (Gutierrez et al. 2003, Allen and Vaughn 2011), but the influence of native freshwater mussels (Atkinson et al. 2013),

invasive freshwater mussels (Goedkoop et al. 2011), and marine mussels (Aquilino et al. 2009) on nutrient dynamics is becoming better appreciated. These results underscore the importance of this bottom-up source of nutrients from consumers in river systems. The combination of enhanced nutrient availability and substrate may make mussel beds essential ecosystem patches within rivers.

We were unable to document the total N that left mussels. Some nutrient pathways, including coupled nitrification-denitrification, were not sampled during this study. Future studies that attempt to quantify total N budgets would be valuable. Additionally, certain food web pools that we sampled did not show evidence of connection to MDN. The reasons behind this lack of effect are not clear, but it could be that limpets and water pennies have slower tissue turnover than the other food web pools sampled. Therefore, these tissues may not incorporate short-term changes in N isotope ratios. Previous studies have shown that snails have relatively slow turnover relative to many other stream consumers, with half-lives ranging from 20-231 days (Kemp et al. 1990, Mulholland et al. 2000, McIntyre and Flecker 2006). More research is needed to understand this unexpected observation. Additionally, the recovery of MDN was low and some MDN assimilated in upstream areas may have been later released and picked up by downstream transects. The importance of increased nutrient availability depends on background nutrient conditions, stream size, and biomass and density of the consumer providing the nutrient subsidy (Small et al. 2009). However, when we scaled our results to a natural mussel bed within the same river, our calculations suggest that a natural mussel bed may account for much of the N demand

in the stream reach, potentially constituting mussels a primary bottom-up influence on stream food webs.

There has been increased recognition of the importance of animals in shaping ecosystems (Polis et al. 2004, Moore 2006). We provide evidence that nutrient inputs from freshwater mussels are substantial and released nutrients are moving directly into stream food webs. Our study of freshwater mussels demonstrates how a taxonomically distinct group of organisms can be an important bottom-up nutrient subsidy for food webs. 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). Both the loss of species (McIntyre et al. 2007, Hooper et al. 2012) and the invasion of species (Bruesewitz et al. 2009, Capps and Flecker 2013) have the potential to drastically alter nutrient recycling and other ecosystem functions. Our results suggest that bottom-up nutrient supply by freshwater mussels helps maintain food webs. The full ramifications of past and future losses of freshwater mussels are not known, but our results suggest that loss of species has contributed to a decreased efficiency of nutrient cycling and potential alteration of food web dynamics in streams. Our research highlights the importance of linkages between bottom-up nutrient supply and individual, population, and community ecology.

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Table 1. Various measurements of the food web pools that responded to the $^{15}N$-enriched mussels. The uptake rates were calculated using a box model approach as in Dodds et al. (2000).

| Food Web Pool | Biomass Range (g DM $m^{-2}$) | Average %N (SE) | Maximum $\delta^{15}N$ (Transect) | Average Uptake Rate μmol N $m^{-2}$ $d^{-1}$ (Range) |
|---|---|---|---|---|
| Periphyton | 18.92 - 27.88 | 0.83 (0.34) | 5.62 (5 m) | 1.0 - 1.5 |
| Water willow | 8.15 - 40.21 | 3.07 (0.22) | 5.14 (25 m) | 1.1 - 1.4 |
| Mayflies | 0.15 - 0.68 | 7.49 (0.18) | 7.21 (5 m) | 4.8 - 10.7 |
| Stoneflies | 0.1 - 0.42 | 9.76 (0.37) | 5.21 (5m) | 389.0 - 741.3 |

**Figure Legends**

Figure 1. Conceptual diagram depicting the experimental setup. The food web was sampled at each of the transect points depicted in the diagram. The water lines represent riffle areas.

Figure 2. Ammonia concentrations at the -10, -5, 0, 5, 10, and 25 meter transects during different sampling periods. Black symbols represent time periods when mussels were in the study reach, while clear symbols represent when they were not present. The highest concentrations (12 July 2011) coincided with some of the lowest water levels during the experiment. Mussels were removed from the sampling reach following the July sampling date. The final sampling date is following the mussels being removed from the sampling reach.

Figure 3. Depiction of the percent recovery model at the 0 meter transect. The black area shows the baseline signature of the ecosystem pool prior to the mussel addition. The points (±SE) and line show the ecosystem pool following enrichment. The best fit line was fit to the points after correcting for the baseline signature and the area under the curve was found. The grey shaded area depicts the $\delta^{15}N$ signature of mussel hemolymph. A best fit line was derived for the decay in $\delta^{15}N$ in mussel hemolymph to estimate the amount of tracer $^{15}N$ mussels were releasing to the ecosystem.

Figure 4. The average % recovery across all the post mussel addition sampling events of MDN in the ecosystem pools as found by comparing the mass corrected areas under the curve of the enriched ecosystem pools (periphyton, water willow, mayflies, and stoneflies) to the amount of MDN released.

Figure 5. (A) The average amount of MDN in each of the ecosystem pools across all sampling periods following the mussel addition at the 0 meter transect. (B) The average MDN across all the pools over time during each of the sampling dates.

Fig. 1.

Fig. 2.

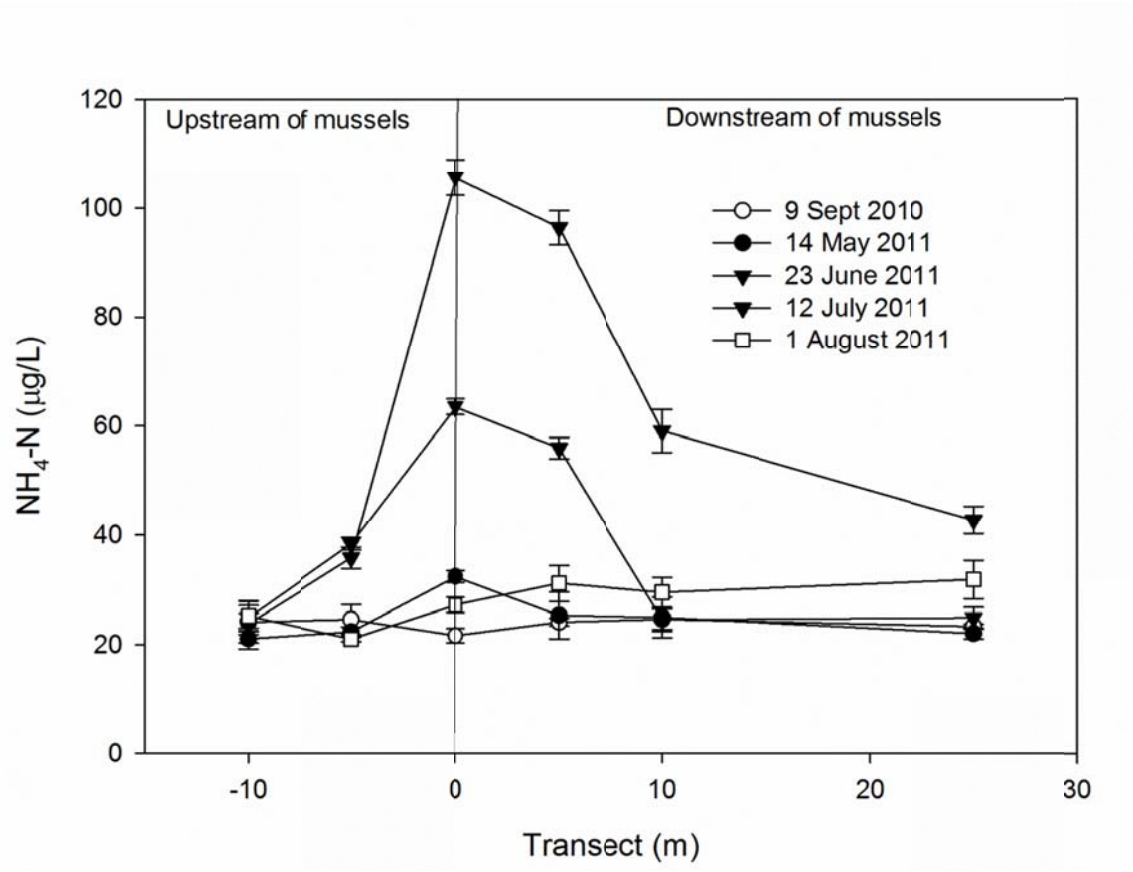

Upstream of mussels
Downstream of mussels
9 Sept 2010
14 May 2011
23 June 2011
12 July 2011
1 August 2011
NH4-N (µg/L)
Transect (m)
120
100
80
60
40
20
0
-10
0
10
20
30

Fig. 3.

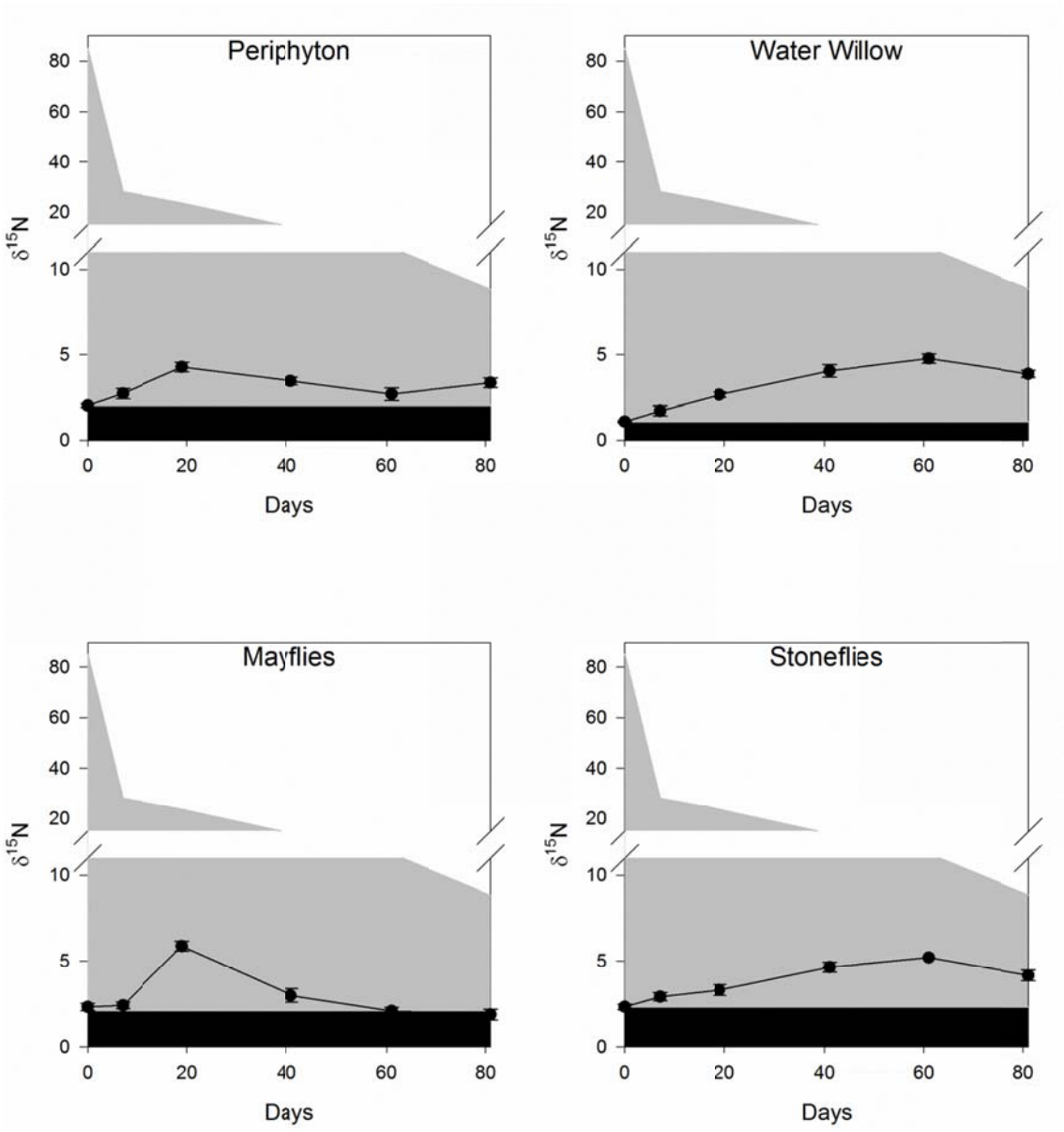

Periphyton
Water Willow
Mayflies
Stoneflies
δ15N
Days
0
5
10
20
40
60
80

Fig. 4.

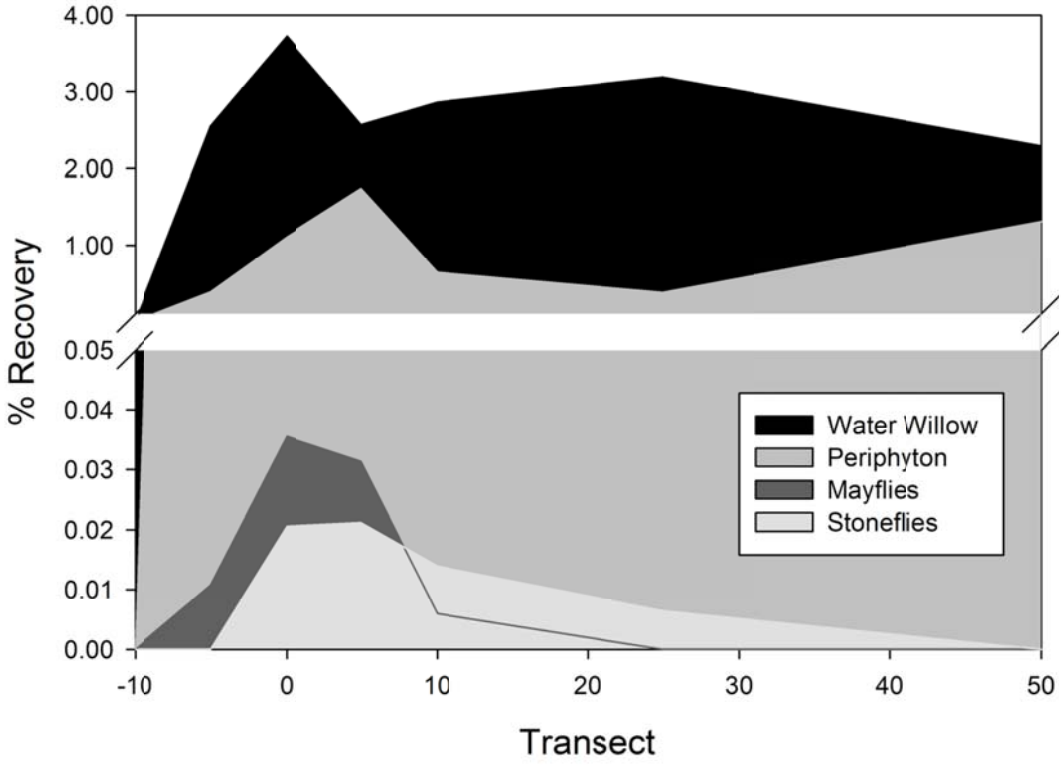
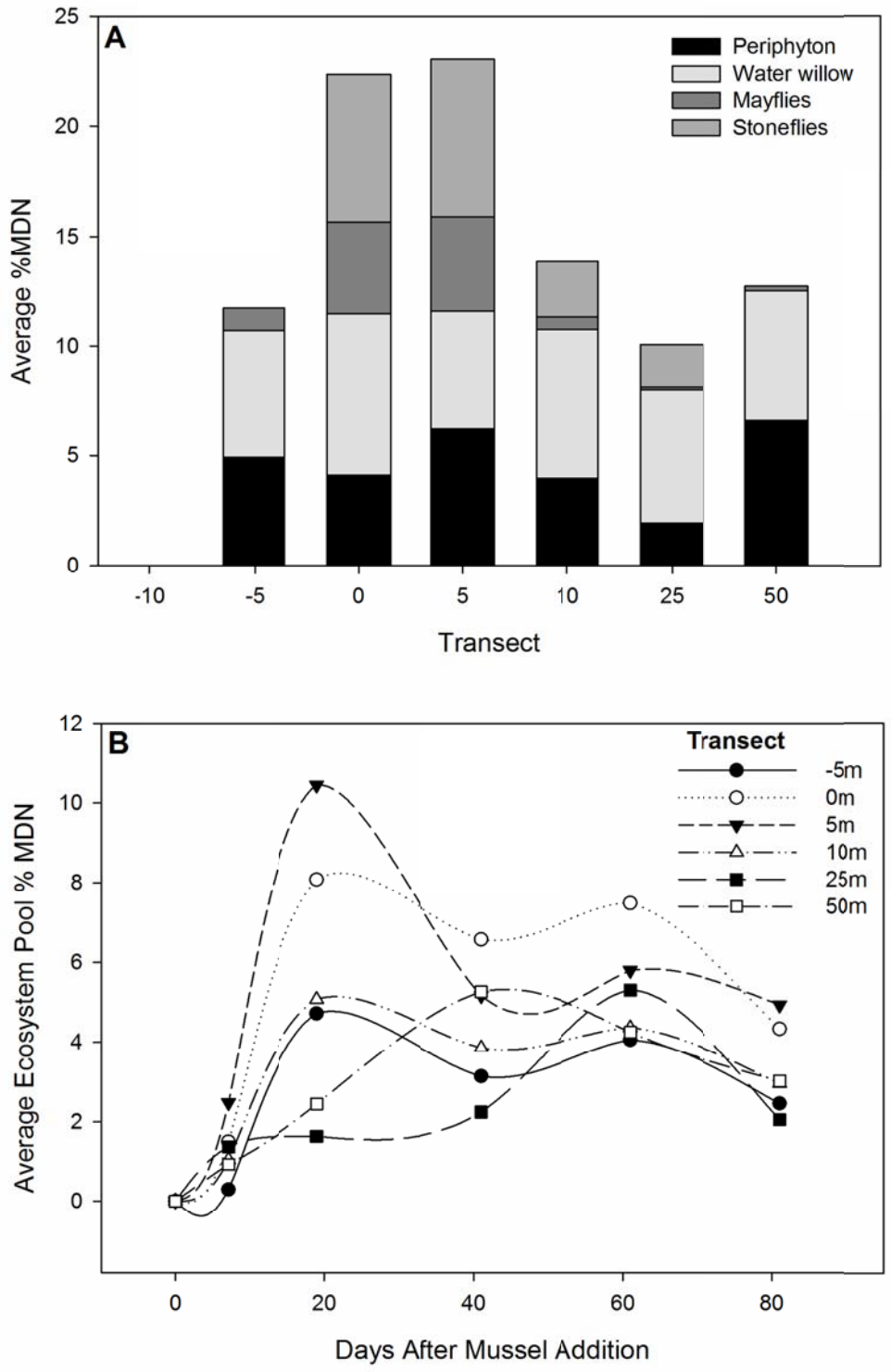

4.00
3.00
2.00
1.00
0.05
0.04
0.03
0.02
0.01
0.00
% Recovery
-10
0
10
20
30
40
50
Transect
Water Willow
Periphyton
Mayflies
Stoneflies

Fig. 5.


A
Periphyton
Water willow
Mayflies
Stoneflies
Average %MDN
0
5
10
15
20
25
-10
-5
0
5
10
25
50
Transect
B
Transect
-5m
0m
5m
10m
25m
50m
Average Ecosystem Pool % MDN
0
2
4
6
8
10
12
0
20
40
60
80
Days After Mussel Addition

Appendix 1. Physiochemical parameters at the mussel addition site. Temperature was monitored continuously every 15 minutes with a Hobo U20 submersible logger (Onset, Onset, Cape Cod, Massachusetts), and other parameters were measured as a point sample that day. Point samples were taken between 10:00 and 15:00.

| Date | Temperature | pH | Dissolved Oxygen (mg/L) | Discharge (L/s) |
|---|---|---|---|---|
| 8/2/2010 | 26.98 ± 0.38 | 7.29 | 6.88 | 31.3 |
| 8/8/2010 | 26.76 ± 1.07 | 7.28 | 7.92 | 45.9 |
| 4/29/2011 | 16.71 ± 0.30 | 7.35 | 9.74 | - |
| 5/13/2011 | 18.30 ± 0.63 | 7.30 | 9.61 | - |
| 6/1/2011 | 23.51 ± 1.55 | 7.26 | 10.04 | - |
| 6/23/2011 | 27.96 ± 1.81 | 7.39 | 5.59 | 17.2 |
| 7/12/2011 | 29.89 ± 1.64 | 7.34 | 5.24 | 9.3 |
| 8/1/2011 | 30.27 ± 1.31 | 7.35 | 4.82 | - |

Appendix 2. Physical, chemical, and ecological measured during summer base flow conditions characteristics at the mussel addition site and a natural mussel bed site in the Little River.

| Parameter | Created Mussel Bed | Natural Mussel Bed |
|---|---|---|
| Areal Excretion Rate of Mussels (μmol N $m^{-2}$ $h^{-1}$) | 47.2 | 181.0 |
| Average Stream Width (m) | 13.5 | 30.3 |
| Average Stream Depth (m) | 0.21 | 0.39 |
| Background N Concentration (μmol $L^{-1}$) | 15.7 | 25.0 |
| Background P Concentration (μmol P $L^{-1}$) | 1.1 | 1.1 |
| Nitrogen Uptake Rate (μmol N $m^{-2}$ $h^{-1}$) | 116.7 | 184.2 |
| Average Summer Discharge ($m^3$ $sec^{-1}$) | 25.9 | 66.2 |

CHAPTER 5

# Species and function lost:

# Role of drought in structuring stream communities

Keywords:

biodiversity, ecosystem function, unionid,

nitrogen, phosphorus, species traits, south-central US

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**ABSTRACT**

Drought is an important natural disturbance that influences community structure by altering species composition, abundance, and richness. Human-induced alterations of the hydrologic cycle and climate change can exasperate the impact of drought, potentially leading to extirpations and changes in community structure. These changes in community structure can lead to substantial alterations and losses of ecosystem functions. Nutrient recycling is an important ecosystem function that helps modify rates of production and food web structure. Animals are important in cycling and storing nutrients in aquatic ecosystems through feeding, growth, and excretion. Freshwater mussels are long-lived animals, often living more than 20 years, and perform important ecosystem functions such as nutrient storage and cycling. Mussels dominate benthic biomass in many aquatic systems, and thus can be an essential component affecting nutrient dynamics. Unfortunately, they are experiencing rapid declines. In this study, we surveyed freshwater mussel populations across nine sites in three rivers in southeastern Oklahoma during the summers of 2010 and 2012. An exceptional, regional drought in 2011 caused mass mortality of mussel populations. We characterized the hydrological severity of the drought in our study streams and estimated mussel biomass loss and the consequential losses of ecosystem functions such as nutrient cycling and storage. We determined if there were differences in functional groups that were lost and if they differed in their tissue and excretion stoichiometry. Additionally, we investigated whether losses caused by the drought were intensified by different land cover types. Our surveys indicated that there were declines in both density and biomass of mussels, and greater losses were associated with areas that had less forest cover. This die-off resulted in a lower availability of N and reduced P storage by freshwater mussels in these rivers,

potentially altering system nutrient availability. Additionally, our analyses showed that thermally sensitive declined in relative abundance and have lower tissue N:P. Thus, our results show that differences in species tolerance to drought may lead to varying storage and release of nutrients. Further studies incorporating net flux and storage will allow scientists to better understand the repercussions of species loss.

## 1. Introduction

On a global scale, freshwater biodiversity is declining precipitously, with extinction rates being more than five times higher in freshwater systems than in terrestrial systems (Dudgeon et al. 2006). Most of the factors underlying biodiversity loss in freshwater systems are human-derived and include water pollution, overexploitation of water resources, and habitat degradation. Climate change and human alterations to flows (e.g. water withdrawals, channelization) will potentially intensify these stressors (e.g. water temperatures, timing and magnitude of flows) (Palmer et al. 2008). Drought is an important natural disturbance that influences community structure (Boulton 2003; McCluney and Sabo 2012; Resh et al. 1988) and human induced alterations of the hydrologic cycle can exacerbate drought impacts (McCluney and Sabo 2012; Perry et al. 2012; Xenopoulos et al. 2005). Rivers around the world are drying with increasing frequency and severity (Cayan et al. 2010; Gleick 2003; Poff et al. 1997) and this has been a major cause of biodiversity loss (Postel and Richter 2003). There is evidence that declines in species richness and abundance alter ecosystem processes and reduce overall ecosystem function (Covich et al. 2004; Hooper et al. 2012; Hooper et al. 2005; Kirwan et al. 2009; Vaughn 2010), ultimately

compromising human well-being (Cardinale 2011). Understanding the consequences of biodiversity loss to ecosystem function is critical for predicting ecosystem change.

In both terrestrial and aquatic ecosystems, organisms directly affect nutrient dynamics by sequestering nutrients through growth and remineralizing nutrients via excretion and egestion (Vanni 2002). The relative magnitude of consumer excretion and its potential importance to ecosystem-level nutrient cycling depends on a number of biotic and abiotic factors. Characteristics of the consumer community are clearly important, including stoichiometric requirements, size, biomass, and aggregating behavior (Capps and Flecker 2013; McIntyre et al. 2008; Vanni 2002). Additionally, the importance of these consumer-mediated nutrient subsidies depends on the biomass and density of the organisms (Hall et al. 2003; McIntyre et al. 2008; Moore 2006; Small et al. 2009), ecosystem size (Benstead et al. 2010; McIntyre et al. 2008), and background nutrient conditions (Benstead et al. 2010; Wilson and Xenopoulos 2011). Although the linkages between biodiversity and ecosystem function are an area of intense research and debate (Duffy 2002; Schmid et al. 2009; Tilman 1999), there are significant gaps in our understanding of how species loss and declines affect ecosystem function, particularly in freshwater systems (Covich et al. 2004; Dudgeon et al. 2006). Many studies have documented the effects of organisms on nutrient dynamics, but few have documented the effects of biomass loss (except see, McIntyre et al. 2007) and species composition changes on this important ecosystem function.

Freshwater mussels (Bivalvia; Unionidae) are one of the most imperiled faunal groups globally. In North America, approximately 70% of the more than 300 recognized species are at risk of extinction (Bogan 2008). Mussels occur in many freshwater habitats, with the greatest abundance and diversity in medium to large rivers where they typically occur as dense, multi-species communities called mussel beds (Strayer 2008). Previous studies have shown the importance of mussels in nutrient cycling, community structure, and food web support (Allen et al. 2012; Atkinson et al. 2010; Atkinson et al. 2013; Vaughn et al. 2008). Mussels are thermo-conformers with different strategies to avoid physiological stress. More mobile species can move to deeper regions of a stream reach to survive high temperatures, while others become metabolically less active while catabolizing their energy reserves (McMahon & Bogan 2001). Regardless of their heat-avoiding strategy, no mussel can survive an extended amount of time in an isolated pool at high temperatures, low dissolved oxygen, and often high ammonia levels (Cherry et al. 2005; Gagnon et al. 2004; Golladay et al. 2004; Haag and Warren 2008). Losses due to drought conditions can drastically reduce mussel populations which will affect mussel-provided ecosystem functions such as filter-feeding and nutrient storage and cycling.

We studied an area in southeastern Oklahoma in which mussels and their influence on ecosystem functions have been well documented. Within this region, mussel densities have declined due to water management and regional drought, with a 65% decline between the early 1990s and 2000s including both rare and common species (Galbraith et al. 2008; Vaughn et al. 1996). Additionally, community

composition has shifted, with species more able to withstand warm water temperatures (thermally tolerant species) increasing in relative abundance compared to species less able to withstand warm temperatures (thermally sensitive species) (Galbraith et al. 2010; Spooner and Vaughn 2008). In this study, we assessed the impact of a severe drought on mussel density and biomass in this region and the associated impacts on mussel-provided nutrient cycling and storage. Additionally, we examined some of the underlying landscape factors that may lead to drought affecting some mussel populations more than others. We quantified the biomass and density loss of unionid mussels, determined the ecosystem functional consequences (nutrient dynamics), and determined if land use interacted with the drought potentially exacerbating the effects of the drought in certain locales.

## 2. Methods

### *2.1. Study Area*

We studied three mid-sized rivers in the south-central U.S. (Kiamichi - K, Little - L, and Mountain Fork – M; Fig 1), where previous work suggests mussels play an important role in supporting primary and secondary production (Spooner and Vaughn 2009; Vaughn and Spooner 2006). Here mussel beds are diverse, dense, and species composition changes longitudinally along the length of the rivers (Atkinson et al. 2012). Rivers in this region tend to be N-limited and nutrient-poor, with mussels often playing an important role in nutrient cycling and food web provisioning (Allen et al. 2012; Atkinson et al. 2013; Spooner et al. 2012).

*2.2. Drought assessment*

Whereas many drought indices use monthly hydrological measures, we used daily data in this assessment given the extreme daily flow variability (i.e. dry vs. flood) of rivers in this region and the sensitivity of mussels to extremely low flows over short periods (i.e. days). Given the highly variable response of streamflow to precipitation in this ecoregion (*personal observation*, Poff 1996), as well as private upstream water diversions/abstractions, we rely primarily on streamflow rather than precipitation data to characterize *hydrological drought*. Nevertheless, we use weekly drought indices from the Drought Monitor (Svoboda et al. 2002) to characterize drought for each of our three study watersheds separately, where severe drought (D2) represents the $< 10^{th}$ percentile of weekly flow. We assigned severe drought if a majority of the watershed had a D2 magnitude or higher. To be consistent with the Drought Monitor, we quantified the number of days where daily flow was below the $10^{th}$ percentile on the flow duration curve. Further, we quantified the number of "no flow" ($<0.01$ $m^3/s$) days because of their lethal effect on mussels.

Kiamichi River flow data were obtained from a gage (USGS 07336200) just downstream of KM2, which had continuous daily flow records for 1972 – present. Flow data for the Mountain Fork River were obtained from a gauge (USGS 07338750) just upstream of MF3, which had continuous daily flow data for 1991 – present. There was not a long-term flow gage on the Upper Little River, and thus we relied on the Drought Monitor data for this watershed. Because all three watersheds are in the same physiographic region and the Little River watershed is sandwiched between the Kiamichi and Mountain Fork watersheds, we assumed that Little River flow patterns

followed those of the other two rivers. Hydrological drought was assessed for the hydrological years (Oct 1 – Sep 30) of 2009 – 2012, which coincide with the two years before each mussel survey.

*2.3. Mussel Surveys*

To determine the influence of drought on mussel communities, nine mussel beds that were sampled during the summer of 2010 were resampled during the summer of 2012 (Fig 1). 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, identified to species, and measured to the nearest 0.1 mm. Length data were used to estimate tissue biomass based on previously determined length-weight regressions (Atkinson, unpublished data).

*2.4. Storage and Cycling*

Field excretion measurements were conducted in the summers of 2010 and 2012 on the 6 most common species in the study area (Appendix B). Five control containers filled with 1000 ml of filtered river water were used for all treatments and controls. 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 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). Samples for

total dissolved nitrogen and phosphorus were collected, acidified, and analyzed (following persulfate digestion) within 28 days of collection using a Lachat QuikChem FIA +8000 Series flow injection analyzer (Hach Company, Loveland, CO, USA).

Following the excretion experiments, a subset of mussels (those used in the experiments and other species) were placed on ice and returned to the laboratory ($n$ = 108). Length, total wet mass, and tissue dry mass (both soft tissue alone and soft tissue with shell) were determined for each individual. We determined soft tissue dry mass by separating the foot muscle tissue from each individual and drying it at 50˚ C until mass remained constant. Total tissue biomass is the sum of the dry soft tissue and shell mass. To estimate nutrient storage of mussels, tissue nutrient composition (%C, %N, and %P) was determined. Tissue samples were analyzed on a Finnigan Delta Plus mass spectrophotometer 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 hours, and analyzed with $HSO_4$ digestion followed by soluble reactive phosphorus analysis (Solorzano and Sharp 1980).

Excretion rates were calculated based on the difference in dissolved nutrient concentrations between the control and mussel containers following the 1-hr incubation. Species-level nutrient excretion was calculated as the product of population density and per capita excretion rates. Areal excretion was determined by summing the nutrient excretion rates per $m^2$ for each individual site. Summing across a site yielded aggregate excretion rates of N and P for the site. Areal storage by each species was calculated as

the product of biomass per $m^2$ and % nutrient composition of the tissue (both shell and soft tissue) and then summed for all species. We determined total storage by multiplying the areal storage by the total area of the mussel bed.

*2.5. Ecosystem Function*

To determine the impact of species loss on ecosystem function, we quantified the areal nutrient storage, areal nutrient excretion, and total nutrient storage provided by each mussel bed prior to and following the drought. Areal excretion is the excretion rate per unit area (μmol N $m^{-2}$ $d^{-1}$) and the areal storage is the amount of nutrients stored in mussels per unit area (μmol nutrient $m^{-2}$). We used the survey data from both sampling periods to estimate areal excretion, areal storage, total remineralization, and total storage of N and P for each site. We then compared the data from the two sampling periods to determine the change in these mussel-provided ecosystem functions. We also calculated areal storage N:P and excretion N:P (molar) for all mussel beds across the two sampling years. Additionally, we investigated the role of community composition, particularly the proportion of thermally sensitive species in determining the N:P of excretion and if tissue stoichiometry, specifically tissue N:P, was a good predictor of N:P both using ordinary-least squares regression.

*2.6. Temperature and land use*

Beginning 8 June 2011,we placed HOBO U20 water depth and temperature loggers (Onset, Bourne, MA) at twelve sites along the mainstem of the three rivers (Fig. 1). Seven out of nine of the mussel sample locations had a HOBO U20 logger at

the site or within 2 river kilometers. All sites were located upstream of in-channel reservoirs and had similar water chemistry (Appendix A). Watersheds for each sampling point were derived using ArcMap 10.0 (Environmental System Research Institute, Redlands, CA) with a 30-m digital elevation model (DEM) from the National Elevation Dataset. Land cover (30-m resolution) was obtained from the 2006 National Land Cover Database (Homer et al. 2004), which we used todetermine the percent of each cover type within the watershed of each sample site.

*2.7. Statistical analysis*

We used a Wilcoxon signed rank test to determine if there were significant changes in mussel density and biomass between the two sampling periods. To determine if ecosystem function (N and P areal excretion and storage) changed following the drought, we used paired t-tests. Additionally, we separated the mussel species into known thermal guilds (thermally sensitive and tolerant) based on Spooner and Vaughn (2008) and used paired t-tests to determine if there were significant differences in composition and density of the two thermal guilds over time. We used ordinary-least squares regression to investigate the role of community composition, particularly the proportion of thermally sensitive species, in determining the N:P of excretion and if tissue stoichiometry, specifically tissue N:P, was a good predictor of N:P. We also determined if areal storage N:P and excretion N:P varied across the two sampling years using paired t-tests. To determine if drought impacts were influenced by land cover, we first used Moran's *I* to see if there was spatial autocorrelation across sites, and then used Pearson correlation to examine the association between land use,

stream temperature, and mussel biomass loss. We examined the relationship between forest and agriculture land coverage in the watershed and stream temperature at all the sites with HOBO loggers, and then we examined the relationship between water temperature, forest coverage, and change in mussel biomass. Stream water temperature was calculated as the mean water temperature at these sites from 1 August 2011 until 30 Sept 2011. Proportion data were arcsine square root transformed to meet assumptions of normality (Gotelli and Ellison 2004). All statistical analyses were done in R v2.15.1 (R Development Core Team 2012).

## 3. RESULTS

### *3.1. Drought characteristics*

The drought of 2011 - 2012 reached the magnitude of exceptional (D4), the most severe category identified by the U.S. Drought Monitor. In the two years preceding the 2010 mussel surveys, none of the watersheds experienced severe drought (Table 1). Between the 2010 and 2012 mussel surveys, each watershed was in severe drought for approximately 40 weeks. The 2011-2012 drought caused reaches along the three rivers to change from continuously flowing to a series of shallow, isolated pools in which water temperatures sometimes exceeded 40 °C. Many sections also ceased flowing. The Kiamichi River had 84 days of no flow (< 0.01 $m^3 s^{-1}$) during the period of study, all occurring after the mussel surveys of 2010. Flow in the Mountain Fork River never ceased, but it dropped below 0.02 $m^3 s^{-1}$ on 26 days during 2011 - 2012. The lowest discharge on the Mountain Fork in 2009 and 2010 was 0.04 $m^3 s^{-1}$. While continuous flow data are not available for Little River, numerous field visits and HOBO logger

depth data revealed that flow was absent between mid-July and late August during 2011 and 2012. In sum, the mussel surveys of 2010 followed a relatively drought-free and temperate period, but the 2012 mussel surveys followed a period of extremely low flows and lethal water temperatures in the three rivers.

*3.2. Density and Biomass Changes*

Population densities at survey sites ranged from 4.8 to 19.6 individuals $m^{-2}$ across both years. Mean soft-tissue dry mass for the 6 common species ranged from 0.4 to 22.2 g $m^{-2}$ and estimated total biomass ranged from 102.5 to 4190 g dry tissue (shell+soft tissue) $m^{-2}$. Mussel abundance declined considerably between the two sampling intervals (Fig 2A, 2B). We measured a significant decline in density between 2010 and 2012 (W = -32.0, Z = -2.24, $p = 0.02$; Fig 2A), with an average decline of 3.03 ± 1.09 individuals $m^{-2}$ (mean ± SE). Accordingly, we found a significant decline in biomass (W = -45.0, Z = -2.67, $p = 0.004$; Fig 2B) with an average decline in 593.1 ± 171.0 g mussel $m^{-2}$, which was a 28.7 ± 6.1% reduction in soft tissue biomass across the sites between the two years.

*3.3. Ecosystem Function*

Due to mussel mortality, there was a reduction in mussel-provided ecosystem functions (Fig 3). Both N and P areal excretion were reduced following the drought. Nitrogen areal excretion declined significantly by 52.5 ± 18.4 μmol N $m^{-2}$ $h^{-1}$ ($t_8 = 2.86$, $p = 0.02$), which was a 22% average decline in mussel N excretion across the sites (Fig 2C). We found that P areal excretion rates declined by 3.1 ± 1.1 μmol P $m^{-2}$ $h^{-1}$, which

is equivalent to a 15% average decline in mussel P areal excretion across the sites, but this change was not statistically significant ($t_8 = 2.05$, $p = 0.07$; Fig 2D).

Mussel soft tissue ranged 10.1 - 13.9% (mean 11.93%) N and 0.7 – 2.7% (mean 1.4%) P. Shell tissue ranged 1.5 - 3.1% (mean 1.9%) N and 0.05 - 0.21% (mean 0.08%) P. We found a 30% average decline in N areal storage, equating to an average loss of 13.5 ± 4.2 g N $m^{-2}$ ($t_8 = 3.21$, $p = 0.01$). Phosphorus storage by mussels was also reduced by 30%, equivalent to a loss of 4.9 ± 1.5 g P $m^{-2}$ ($t_8 = 3.28$, $p = 0.01$). Total nutrient storage of these mussel beds ranged 1.1 - 682.3 kg N and 0.3 - 240.3 kg P, with significant declines between the two sampling periods.

*3.4. Species-specific changes*

Overall, densities of both thermally sensitive and tolerant mussel guilds declined between the two sampling periods and there were no significant differences in the absolute decline of the two guilds ($t_{14} = -0.14$, $p = 0.89$; Fig 3A). While the relative abundance of thermally sensitive species decreased across sites during the drought and the relative abundance of tolerant species increased slightly (Fig 3B), this trend was not significant ($t_{14} = -0.98$, $p = 0.34$). However, our data indicate that the loss of a higher proportion of thermally sensitive mussel individuals is affecting stream ecosystem function through changes in aerial N:P excretion. Mussel bed areal excretion N:P increased with the proportion of thermally sensitive species in a bed in both 2010 and 2012, although these patterns were not significant (Fig 3C; 2010: $r^2 = 0.38$, $y = 9.5x + 26.6$, $p = 0.08$; 2012: $r^2 = 0.20$, $y = 8.29x + 21.6$, $p = 0.22$). The N:P of mussel bed areal excretion declined significantly between 2010 and 2012 (W = -45.0, Z = -2.66, $p$ =

0.004; Fig 3D) and was strongly correlated to areal tissue N:P in both 2010 ($r^2 = 0.58$, $y = -1.6x + 61.32$, $p < 0.02$; Fig 3D) and 2012 ($r^2 = 0.53$, $y = -2.0x + 63.95$, $p < 0.03$; Fig 3D).

*3.5. Land use and temperature*

Our data did not exhibit spatial autocorrelation across the sites in the proportion of agriculture in the watershed ($I = 0.06$, $p = 0.37$). Mean stream water temperature decreased with increasing forest coverage in the watershed ($r = -0.70$, $p = 0.01$; Fig 4A). Biomass losses increased with increasing mean stream temperature (Fig 4B), although this relationship was not significant ($r = -0.58$, $p = 0.18$). Smaller reductions in biomass of mussels between the sampling periods were positively correlated to forest cover ($r = 0.67$, $p < 0.05$; Fig 4C), while greater losses in biomass were associated with higher agricultural land cover ($r = 0.77$, $p = 0.04$).

## 4. Discussion

Our study provided a quantitative assessment of how river ecosystem function has changed and is changing in response to the continued loss of freshwater mussels, North America's most imperiled faunal group (Bogan and Roe 2008). Drought caused a large reduction in freshwater mussel populations, and our results indicate that declining mussel abundance reduces both nutrient recycling and storage within stream systems. Some nutrient storage will be maintained because of the relatively slow dissolution of shell material (Gutierrez et al. 2003; Strayer and Malcom 2007) which may constitute a nutrient sink in the system (Vanni et al. 2013). However, the loss of living mussels

results in the immediate loss and decomposition of nutrient-rich, soft tissue (Atkinson et al. 2013) and declines in the ecosystem functions provided by the living mussels such as water filtration (Vaughn 2010) and nutrient remineralization (Atkinson et al. 2013). As an example, we saw a dramatic decline in nitrogen remineralization by mussels (average of 22%).

Rivers in our study region are N-limited (Atkinson et al. 2013) and N provided by mussel remineralization has been shown to move into primary consumers and support the food web (Allen et al. 2012). These mussel-derived nutrients influence community structure of benthic primary producers (Allen et al. 2012; Atkinson et al. 2013) and fuel primary and secondary productivity (Howard and Cuffey 2006; Spooner and Vaughn 2006; Vaughn and Spooner 2006). Thus, the declines we observed in mussel biomass and ecosystem processes could lead to significant changes in stream function. A recent meta-analysis showed that the impact of species loss on ecosystems could be as great as environmental change (Hooper et al. 2012). Our results suggest the loss of mussels would lead to large changes in nitrogen and phosphorus cycling in these systems. Undoubtedly, environmental change often leads to species loss, thus the total change in ecosystem function is a consequence of both. Further losses of mussels could have dire consequences and may lead to an altered stable state in these rivers.

Our results, in combination with those of previous studies (Golladay et al. 2004; Haag and Warren 2008), show that severe drought has a detrimental effect on mussel communities. We observed declines in the biomass of all species, regardless of how rare

or common they were, as has also been documented for a previous drought in our study region (Galbraith et al. 2010) and for droughts in other systems (Haag and Warren 2008). We also observed changes in community structure, with the relative abundance of thermally sensitive species declining more than thermally tolerant species. Although this pattern was not significant here, it was previously documented in our study region and found to be significant over longer periods (Galbraith et al. 2010). Comparisons across sites revealed repeated patterns in ecosystem function (i.e. reduced remineralization N:P between years), but also underscore the complexity of predicting ecosystem-level effects of extirpations from species-rich natural communities. For example, mussel densities did not change between the two sampling years at K2, but biomass decreased presumably due to larger individuals being more sensitive to low flow conditions. This decline in biomass led to declines in both nutrient recycling and storage.

Species loss is not random: as thermally sensitive species begin to constitute a smaller proportion of the overall community, changes in nutrient cycling and storage will likely occur (Spooner and Vaughn 2008). The thermal trait relationships established for many of the mussels in these streams could be used in the future to further predict trait-based vulnerability to climate change. These thermal traits in combination with stoichiometric traits may be used to assess and predict future changes in stream nutrient dynamics. Traits including heat tolerance, feeding, and life history have already been used to assess risk to both drought and climate change (Chessman 2013; Villnas et al. 2012; Wenger et al. 2011), and thermal tolerance may drive

community composition in a changing climate and hydrologic regime (Spooner and Vaughn 2008). For example, river size and flow permanence are key factors controlling aquatic food chain length, with shorter food chain lengths in smaller rivers that dried more frequently (Sabo et al. 2010). More research is necessary to understand how species composition may change in the future and the consequential impact on ecosystem function and structure.

Land cover change in combination with a changing climate and water management could significantly alter mussel community structure and lead to declines (Galbraith et al. 2010). In our study, stream temperature was correlated to land cover: sites with higher percentages of watershed forest coverage had lower stream temperatures. Temperature and the density and biomass loss of mussels were uncorrelated (likely due to low sample size, N = 7), but we did see a significant negative relationship between forest coverage and change in mussel biomass. Land cover influences stream temperature, with higher water temperatures typically associated with lower forest cover (Poole and Berman 2001; Quinn et al. 1997; Sponseller et al. 2001). Forest cover mitigates the effects of drought and heat waves by reducing ground/water surface insolation and moderating soil water temperatures (Poole and Berman 2001). Previous studies have linked long-term declines in mussel species richness to changing land use practices and increased nitrogen concentrations (Arbuckle and Downing 2002; Poole and Downing 2004). The losses seen in these previous studies may not only be linked to nutrient or sediment runoff but also to alterations in thermal regime resulting from land cover change.

Future droughts, likely intensified by a warmer climate and greater human water demand, could lead to further species losses, changes in community structure, and degraded ecosystem function (Palmer et al. 2008). Indeed, climate change threatens most ecosystems and is predicted to alter freshwater biogeochemical processes, primary and secondary productivity, food-web structure, species ranges, population dynamics and species interactions, and large-scale patterns of freshwater biodiversity (Carpenter et al. 1992; Heino et al. 2009; Perkins et al. 2010; Sabo et al. 2010). Because the rivers in this study are threatened by planned municipal water extractions (Oklahoma Water Resources Board 2011) and further dam construction (Galbraith et al. 2010; Vaughn and Taylor 1999), an understanding of factors influencing species loss is critical to future river management plans. Interactions between species loss and environmental changes are important for understanding net effects on ecosystem processes because both will often occur simultaneously (Dudgeon et al. 2006). The full ramifications of past losses of freshwater mussels are not known, but our results suggest that the loss of this faunal group would alter the storage and availability of nutrients in riverine ecosystems, which would lead to further losses in ecosystem function and changes in community structure.

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Table 1. Drought characteristics for the Kiamichi, Little, and Mountain Fork watersheds. The Hydrologic (Hydro) Year runs from October 1 to September 30. No flow days occur when discharge is less than 0.01 $m^3/s$. Drought flow days occur when discharge is less than the $10^{th}$ percentile on the flow duration curve, which was 0.18 $m^3/s$ for both the Kiamichi and Mountain Fork Rivers. There is not a long-term flow gage for the Upper Little River. Drought Monitor (DM) severe drought (in weeks) occurs when the drought magnitude category for a majority of the watershed was D2 or higher, which represents the $10^{th}$ percentile.

| | Kiamichi | | | Little | | | Mountain Fork | | |
|---|---|---|---|---|---|---|---|---|---|
| Hydro Year | No flow days | Drought flow days | DM severe drought weeks | No flow days | Drought flow days | DM severe drought weeks | No flow days | Drought flow days | DM severe drought weeks |
| 2009 | 0 | 10 | 0 | NA | NA | 0 | 0 | 0 | 0 |
| 2010 | 1 | 27 | 0 | NA | NA | 0 | 0 | 32 | 0 |
| 2011 | 52 | 146 | 17 | NA | NA | 17 | 0 | 123 | 18 |
| 2012 | 31 | 103 | 19 | NA | NA | 22 | 0 | 114 | 22 |

Fig 1. Map of mussel sample sites surveyed in both 2010 and 2012. HOBO logger (water depth and temperature) locations and land use in the three basins are also shown. Mussel sites are arranged in numerical order from up to downstream.

Fig 2. Changes in density (A) and total biomass (dry tissue + shell biomass) (B) observed between the 2010 and 2012 surveys. Percent change in mussel areal excretion (C) and storage (D) in living mussels over the two sampling periods.

Fig 3. (A) The density of the two mussel thermal guilds, thermally sensitive and thermally tolerant, in 2010 and 2012. There was a decline in the density in both the sensitive and tolerant thermal mussel guilds between 2010 and 2012 (B) The average relative abundance of thermally sensitive species at the sampling sites declined between 2010 and 2012, while tolerant species increased slightly in relative abundance. (C) The relationship between the proportion of thermally sensitive species in a mussel bed to the areal excretion N:P. We observed lower areal excretion N:P in 2012 in comparison to 2010, however this was not significant ($p = 0.10$). (D) Areal excretion N:P was significantly related to the areal tissue N:P of the bed. The dashed arrows indicate changes in 2-dimensional space of storage and excretion N:P at each site between 2010 and 2012.

Fig 4. (A) Mean stream temperature (1 August 2011 through 30 September 2011) was significantly negatively correlated to forest coverage in the watershed. (B) Mussel biomass declined with increasing stream temperature at our study sites, but this pattern was not significant. We had temperature data for seven of the nine sites. (C) Smaller changes in biomass of mussels were correlated to sites that had a higher percentage of forest coverage in the watershed.

Fig 1.

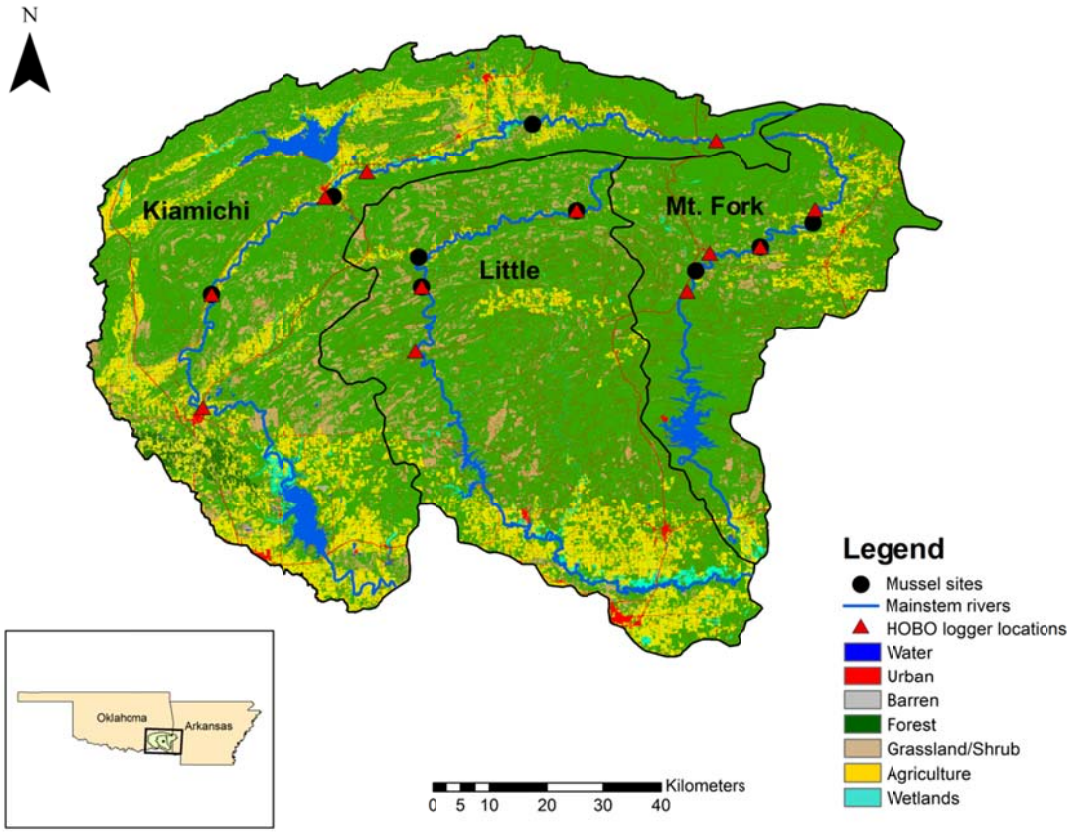

N
Kiamichi
Little
Mt. Fork
Oklahoma
Arkansas
Kilometers
0 5 10 20 30 40
Legend
Mussel sites
Mainstem rivers
HOBO logger locations
Water
Urban
Barren
Forest
Grassland/Shrub
Agriculture
Wetlands

Fig 2.

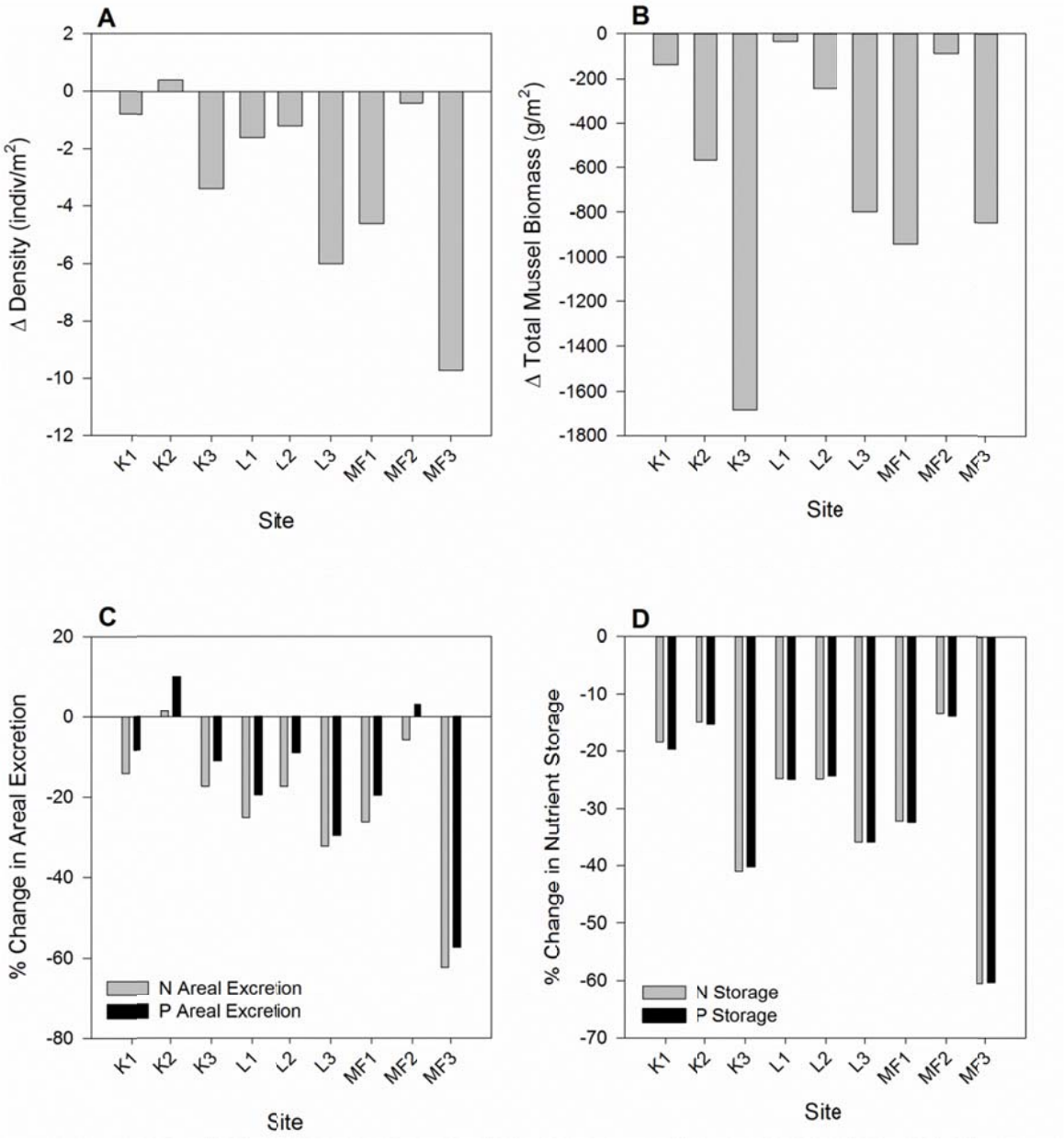

A
Δ Density (indiv/m2)
2
0
-2
-4
-6
-8
-10
-12
K1
K2
K3
L1
L2
L3
MF1
MF2
MF3
Site
B
Δ Total Mussel Biomass (g/m2)
0
-200
-400
-600
-800
-1000
-1200
-1400
-1600
-1800
Site
C
% Change in Areal Excretion
20
0
-20
-40
-60
-80
N Areal Excretion
P Areal Excretion
Site
D
% Change in Nutrient Storage
0
-10
-20
-30
-40
-50
-60
-70
N Storage
P Storage
Site

Fig 3.

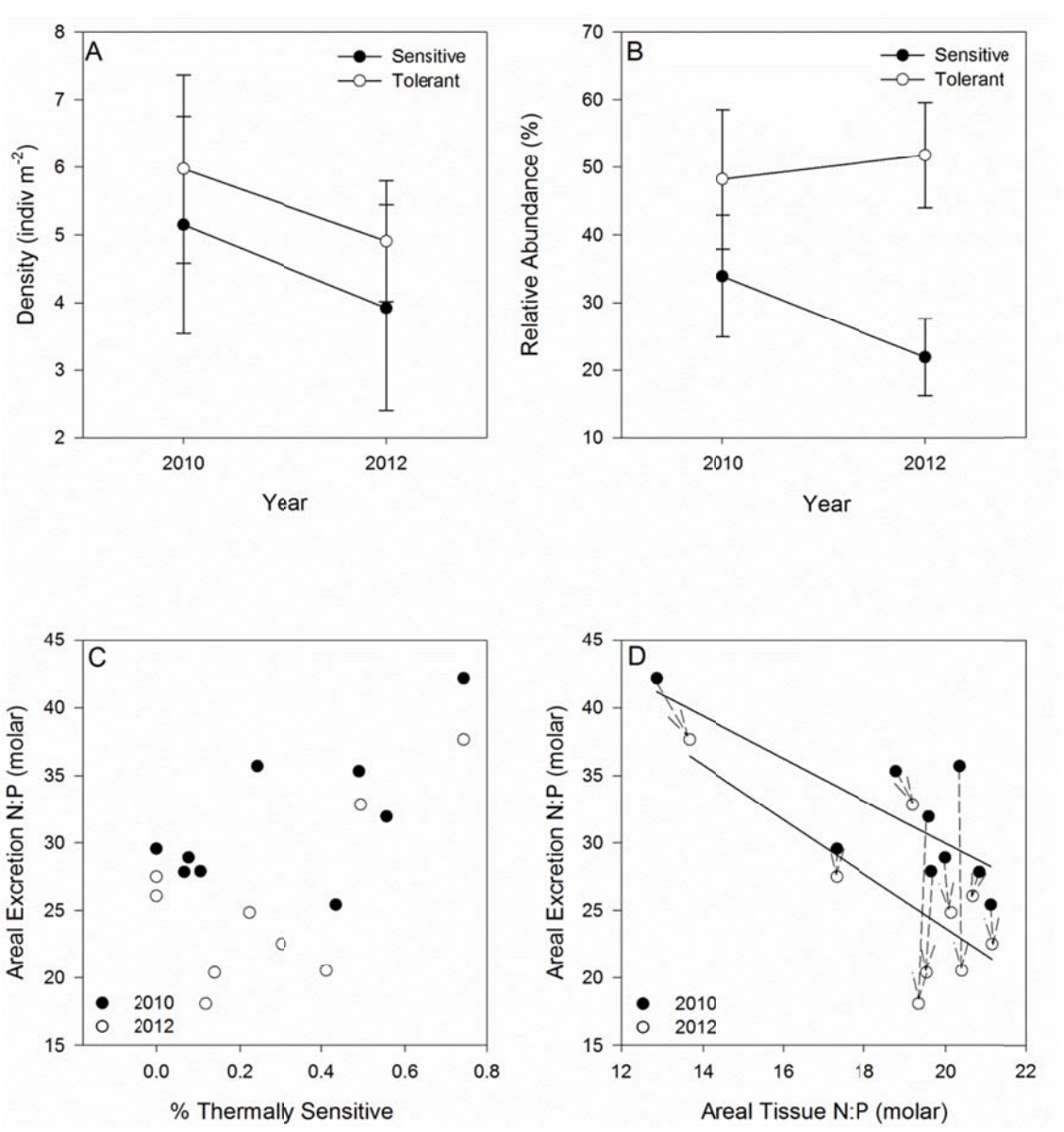
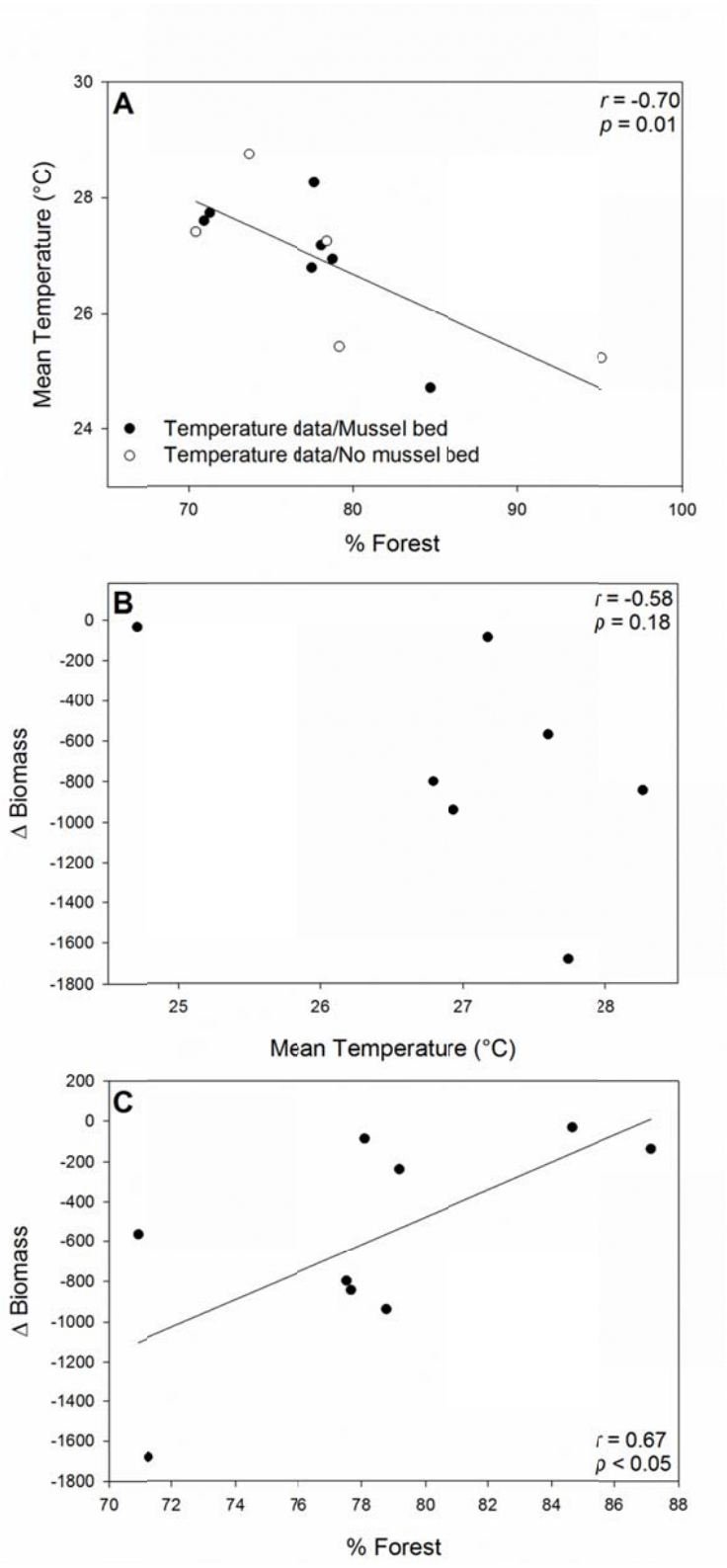

A
Sensitive
Tolerant
Density (indiv m-2)
Year
2010
2012
B
Relative Abundance (%)
C
Areal Excretion N:P (molar)
% Thermally Sensitive
D
Areal Tissue N:P (molar)

Fig 4.


A
r = -0.70
p = 0.01
Mean Temperature (°C)
% Forest
Temperature data/Mussel bed
Temperature data/No mussel bed
B
r = -0.58
p = 0.18
Δ Biomass
Mean Temperature (°C)
C
r = 0.67
p < 0.05
Δ Biomass
% Forest

Appendix A. Background water chemistry for the 9 sites used during the study.

| River | Site | pH | Conductivity (μS) | Dissolved Oxygen (mg/l) | Turbidity (NTU) | Water N:P |
|---|---|---|---|---|---|---|
| Kiamichi | K1 | 7.5 ± 0.21 | 52.75 ± 9.55 | 5.655 ± 0.60 | 34.83 ± 1.09 | 21.8 ± 0.78 |
| | K2 | 7.31 ± 0.10 | 60.8 ± 13.7 | 5.94 ± 0.15 | 29.32 ± 0.97 | 18.07 ± 1.5 |
| | K3 | 7.53 ± 0.10 | 36.95 ± 1.75 | 5.99 ± 0.46 | 30.73 ± 0.43 | 21.62 ± 2.65 |
| Little | L1 | 7.56 ± 0.07 | 37.97 ± 3.54 | 5.48 ± 0.31 | 20.46 ± 1.23 | 17.11 ± 2.61 |
| | L2 | 7.51 ± 0.10 | 26.97 ± 0.37 | 5.56 ± 0.21 | 24.20 ± 0.80 | 22.50 ± 3.71 |
| | L3 | 7.69 ± 0.21 | 31.35 ± 2.75 | 5.99 ± 0.04 | 26.33 ± 1.08 | 21.73 ± 4.42 |
| Mt. Fork | MF1 | 7.54 ± 0.13 | 25.8 ± 3.3 | 6.07 ± 0.50 | 36.48 ± 2.81 | 17.75 ± 2.43 |
| | MF2 | 7.71 ± 0.04 | 27.7 ± 2.40 | 6.17 ± 0.27 | 29.40 ± 4.48 | 22.47 ± 2.09 |
| | MF3 | 7.45 ± 0.24 | 27.5 ± 2.7 | 6.73 ± 1.57 | 22.47 ± 1.96 | 16.19 ± 3.78 |

Appendix B. Tissue nutrient concentration, excretion rates, and thermal guild placements for 6 of the most common species found across the 9 sampling sites. The thermal guild rating is based on Spooner and Vaughn 2008 and unpublished data.

| Species | Mean Tissue %N | Mean Tissue %P | N excretion rate (μmol N $h^{-1}$) | P excretion rate (μmol P $h^{-1}$) | Thermal Guild |
|---|---|---|---|---|---|
| *Actinonaias ligamentina* | 12.04 | 2.35 | 32.00 | 1.58 | Sensitive |
| *Amblema plicata* | 12.32 | 1.30 | 17.36 | 1.46 | Tolerant |
| *Fusconaia flava* | 12.30 | 1.40 | 13.53 | 1.46 | Tolerant |
| *Ptychobranchus occidentalis* | 11.52 | 1.14 | 17.65 | 1.59 | Unknown |
| *Quadrula pustulosa* | 11.85 | 1.03 | 8.93 | 1.24 | Sensitive |
| *Quadrula verrucosa* | 12.11 | 1.24 | 16.96 | 1.24 | Sensitive |
| *AVERAGE - Other mussels* | 11.81 | 1.51 | 17.74 | 1.43 | na |