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Biogeochemical hotspots: temporal and spatial scaling of the impact of freshwater mussels on ecosystem function

CARLA L. ATKINSON*,[1] AND CARYN C. VAUGHN*

Oklahoma Biological Survey, Department of Biology & Ecology and Evolutionary Biology Graduate Program, University of Oklahoma, Norman, OK, U.S.A.

SUMMARY

1. In streams, the creation of nutrient-transformation hotspots by aggregated organisms may have heterogeneous and strong cumulative influences on stream nutrient dynamics. Here, we examine the potential for aggregations of freshwater mussels to create such hotspots.
2. We measured nitrogen (N) and phosphorus (P) excretion rates of six mussel species and body tissue composition of eight. We combined these data with population densities of surveyed mussel beds in the Kiamichi River, OK, to estimate reach-scale and stream-scale nutrient recycling and storage. Additionally, we estimated the temporal variability in the magnitude of mussel nutrient recycling combining volumetric excretion at a reach scale with discharge and temperature data.
3. Mussel beds constituted 1.45% of the area of the Kiamichi River. Mussel nutrient remineralisation varied greatly across beds (11.1–699.5 μmol N m^{-2} h^{-1} and 0.8–53.0 μmol P m^{-2} h^{-1}), because of varying mussel densities. The community-wide average excretion N:P (molar) of the mussel communities was 29.57, with higher excretion N:P significantly associated with higher abundances of *Actinonaias ligamentina*. Total nutrient storage per bed varied two orders of magnitude (6.3–631.7 kg N and 2.3–227.5 kg P) between mussel beds. Moreover, areal nutrient storage varied among the beds (11.2–133.7 mg N m^{-2}, 4.1–48.9 mg P m^{-2}) with the majority of nutrient storage in a long-term store, shell (*c.* 87% of total N storage, *c.* 95% of total P storage).
4. Freshwater mussels can be important to nutrient dynamics through nutrient regeneration and the creation of storage hotspots. However, the importance of nutrient remineralisation varies dramatically in response to organism patchiness, flow conditions and background nutrient concentrations.

Keywords: consumer nutrient recycling, ecosystem functioning, nitrogen, phosphorus, spatial heterogeneity

Introduction

It is increasingly recognised that animals play a large role in nutrient cycling and storage within aquatic ecosystems. Biotic and abiotic environmental characteristics interact to control the potential influence of consumer excretion as a significant flux in ecosystems (Hall *et al.*, 2007; Benstead *et al.*, 2010). Moreover, organismal traits, behaviour and distribution can also affect the role consumers play in nutrient dynamics (Vanni, 2002; Capps & Flecker, 2013b). For example, aquatic organisms frequently have heterogeneous or patchy distributions (Pickett & Cadenasso, 1995) and can produce biogeochemical hotspots in streams and rivers (McIntyre *et al.*, 2008; Atkinson *et al.*, 2013; Capps & Flecker, 2013b).

Much of the work integrating the spatial distribution of species with consumer-driven nutrient dynamics have investigated species distributions across a given stretch of stream (McIntyre *et al.*, 2008) or across distinct sample sites (Benstead *et al.*, 2010); however, studies that include broader spatial and temporal contexts are needed to provide insight on how the distributions of species and their traits vary across space. This will allow for better predictions regarding how biogeochemical processes vary within and across ecosystems. For example, McIntyre *et al.* (2008) showed that aggregating fish could create biogeochemical hotspots and meet a large

Correspondence: Carla L. Atkinson, Oklahoma Biological Survey, Department of Biology & Ecology and Evolutionary Biology Graduate Program, University of Oklahoma, 111 E. Chesapeake Street, Norman, OK 73019, U.S.A. E-mail: carlalatkinson@gmail.com

[1] Present address: Department of Biological Sciences, University of Alabama, Tuscaloosa, AL 35487, U.S.A.

proportion of ecosystem nutrient demand. Similarly, variation in invertebrate densities between tide pools created differences in nutrient recycling rates, thereby affecting algal primary productivity (Bracken & Nielsen, 2004; Pfister, 2007). Furthermore, patches of organisms such as aquatic plants (Caraco & Cole, 2002; Forshay & Dodson, 2011; Tall, Caraco & Maranger, 2011) and molluscs (Bruesewitz, Tank & Hamilton, 2009; Kellogg *et al.*, 2013) can facilitate denitrification through alteration of water movement, substrate and light penetration. Scaled up to the landscape level, aggregations can have a large overall impact on nutrient recycling rates and bottom-up processes.

Within ecosystems, there are feedbacks between the physicochemical environment and the role of species in biogeochemical processes (Benstead *et al.*, 2010; Small *et al.*, 2011; Wilson & Xenopoulos, 2011). Hence, there is a great potential for strong interactions to exist between the spatial relationship of organisms and ecosystem-level nutrient dynamics, yet little has been performed to investigate these relationships. The influence of animal aggregations on nutrient dynamics is also strongly controlled by the temporal variability within and among systems. Temporal variation in discharge within and among ecosystems presents a great challenge to studying the influence of organismal distribution on ecosystem processes. The relative impact of organisms on stream processes is highly dependent on stream discharge (Benstead *et al.*, 2010). Thus, incorporation of the role of biogeochemical hotspots in stream function should be viewed in the appropriate temporal and spatial context. Floods and droughts can lead to tremendous variation in flow, and consequentially ecosystem size and water residence time (Junk, Bayley & Sparks, 1989; Fisher *et al.*, 1998). Understanding the importance of consumers in nutrient recycling at the ecosystem scale and the mechanisms and patterns driving these processes require understanding the variables that influence these impacts (i.e. stream temperature, discharge).

Consumer-driven nutrient dynamics are thought to be taxon specific and influenced by both abiotic and biotic variables (Benstead *et al.*, 2010; El-Sabaawi *et al.*, 2012). Ecological stoichiometry (ES) predicts that elemental biogeochemical cycles are interdependent because organisms that drive these cycles require fixed ratios to maintain homeostasis (Sterner & Elser, 2002; Elser *et al.*, 2007). ES predicts that organisms with high dietary requirements for an element will selectively retain and store that element (Elser & Urabe, 1999; Elser *et al.*, 2000). Therefore, an animal with low tissue nitrogen: phosphorus (N:P) requirements should excrete less P (with higher N:P) than an animal with high tissue N:P. Freshwater ecosystems are often limited by N and P. Consequently, the nutritional requirements of animals, especially animals at high densities and/or with unusual body stoichiometries, are important determinants regulating the availability of nutrients across ecosystems (Small, Helton & Kazanci, 2009; Capps & Flecker, 2013b; Atkinson, Julian & Vaughn, 2014b).

Freshwater mussels (Bivalvia: Unionidae) are large, long-lived (6–100 years), filter-feeding molluscs (Strayer, 2008). Molluscs are well known as structural engineers (Gutierrez *et al.*, 2003; Allen & Vaughn, 2011), and understanding the influence of both native (Atkinson *et al.*, 2013) and invasive mussels (Goedkoop, Naddafi & Grandin, 2011) on nutrient dynamics continues to develop. Mussels link the pelagic and benthic compartments by filtering suspended material and excreting and biodepositing nutrients near the benthos. Nutrients excreted by mussels enter stream food webs (Atkinson, Kelly & Vaughn, 2014c) and may alter benthic species composition (Allen *et al.*, 2012; Atkinson *et al.*, 2013). In rivers, mussels occur in dense, speciose aggregations (mussel beds) that are patchily distributed. Previous studies indicate that mussel beds may constitute hotspots of ecosystem productivity in many river ecosystems (Strayer, 2014). In addition, they vary greatly in their spatial distributions (Strayer, 2008; Haag, 2012), and species compositions (Atkinson, Julian & Vaughn, 2012), which should promote resource heterogeneity. The combination of enhanced nutrient availability and substrate provided by mussel shells make mussel beds important ecosystem patches within rivers, especially in otherwise nutrient-limited systems (Atkinson *et al.*, 2013). In addition, freshwater mussels are North America's most imperilled faunal group (Bogan, 2008), thus research on this group is critical for understanding how freshwater systems have and are changing as a result of mussel decline.

Here, we assess the significance and spatial distribution of nutrient cycling and storage hotspots created by freshwater mussels and examine the temporal variability of their effects. We mapped the distribution and abundance of mussel beds across the Kiamichi River, Oklahoma, U.S.A. Previous research in this system showed that mussels occur in high density, diverse (up to 20 species per aggregation) beds and increase primary and secondary production (Vaughn & Spooner, 2006; Vaughn, Spooner & Galbraith, 2007; Spooner, Vaughn & Galbraith, 2012). Benthic primary productivity in this system is N-limited, but N limitation is alleviated by N excretion by mussels (Atkinson *et al.*, 2013). Building

upon those results, our goal was to assess the spatial heterogeneity in mussel distribution and species composition and their role in system-wide N and P dynamics. The results of extensive mussel surveys were used to calculate aggregate (community) excretion rates and nutrients stored in mussel tissue and shell in mussel beds throughout the river.

Methods

Study area and mussel beds

The Kiamichi River (Fig. 1) is a small (drainage area 4600 km^2), relatively pristine river that flows through the Ouachita highlands and Gulf Coastal Plains region of the southern U.S. and is known for its high fish and mussel biodiversity (Matthews *et al.*, 2005). The study area is dominated by forest cover (*c.* 70%), and agriculture is primarily cattle and chicken raising (Atkinson *et al.*, 2014a). The river is influenced by two reservoirs. Sardis Reservoir impounds a major tributary, Jackfork Creek, which provides *c.* 25% of the inflow to the river. Hugo Reservoir impounds the lower Kiamichi before it flows into the Red River. The river is typically shallow, with warm water temperatures (often exceeding 30° C) in the summer and more moderate temperatures and flow during the remainder of the year (Galbraith, Spooner & Vaughn, 2010).

We used survey data from 2005 to 2013 (Galbraith, Spooner & Vaughn, 2008; Spooner & Vaughn, 2008; Atkinson *et al.*, 2012) to map (see methods below) the spatial location of 47 mussel beds in the Kiamichi River. For each bed, we determined species composition and densities of individual species. Each bed was quantitatively surveyed during this time period by sampling 10 or more 0.25 m^2 randomly placed quadrats at each site to determine densities (Vaughn, Taylor & Eberhard, 1997), and the lengths of all individual mussels sampled were recorded. We used previously determined length–biomass relationships (Vaughn, Gido & Spooner, 2004) to estimate the biomass of the mussel species within each of the beds. The area of each mussel bed was determined in the field in one of two ways: manually with a tape measure or by marking waypoints with a GPS unit (±4 m accuracy; Garmin GPSmap 60Cx, Olathe, KS, U.S.A.).

Spatial extent of mussel beds

We mapped the spatial extent of mussel beds within the Kiamichi River. We obtained the National Agricultural Inventory Program (NAIP) 2010 aerial photographs (http://gis.apfo.usda.gov/gisviewer/) for all the counties within the Kiamichi River basin. Using the NAIP photographs, we digitised the entire area of the Kiamichi stream channel between the Big Cedar and Antlers USGS stream gauges (Fig. 1) in ArcGIS 10.0 (Environmental System Research Institute, Redlands, CA, U.S.A). We digitised and created a shape file for the 47 mussel beds that included information on density and species composition. Individual GIS polygons were created for each mussel bed, and area was calculated for each mussel bed. We assessed whether there was a relationship between mussel bed area and mussel densities within beds using ordinary least squares regression in R v2.15.1 (R Development Core Team, 2012).

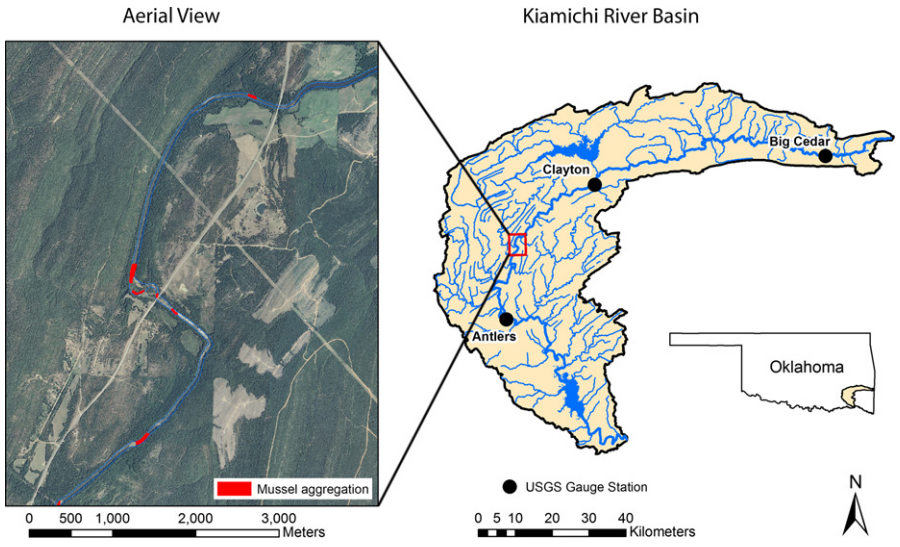

Fig. 1 Map of the study area. The aerial view depicts an example of the NAIP photography with the stream (blue) and mussel beds (red) digitised. The catchment map shows the Kiamichi basin and the three USGS gauges in the basin.

Nutrient storage, remineralisation and volumetric excretion

We used the spatially explicit mussel species composition and abundance data to estimate the magnitude of variation in aggregate nutrient excretion and storage among mussel beds. We estimated the N and P areal remineralisation rates and areal storage for each mussel bed based on field-measured excretion rates and mussel biomass and stoichiometry. Excretion rates for the six most common species at five representative sites (comprised 83 ± 17% of the total assemblage across all of these sites) were measured in June–September 2010 (Atkinson *et al.*, 2013) and 2012 (Table S1). Excretion rates were calculated based on the difference in dissolved nutrient concentrations between the control and mussel containers following a 1-h incubation. Excretion measurements were conducted in containers filled with 1000 mL of filtered river water (GF/F; 0.7 μm pore size; Whatman, Buckinghamshire, U.K.) following Atkinson *et al.* (2013). Mussels were gently scrubbed under water to remove biofilm, and one individual mussel was used in each excretion chamber. Empty, scrubbed 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 bacteria passing through the filter. Scrubbed mussels and shells were removed from containers after an hour, and the water from each container was filtered through a GF/F filter (0.7 μm pore size; Whatman) to separate egestion products (i.e. biodeposits), collected on the filter, from soluble nutrients (i.e. the filtrate-excreta). Samples for total dissolved N and P were collected, acidified and analysed (following persulfate digestion) within 28 days of collection using a Lachat QuikChem FIA +8000 Series flow injection analyser (Hach Company, Loveland, CO, U.S.A.). We also sampled ambient nutrient concentrations in the river five times between June 2010 and August 2012 at six sites within the stream (Table S2).

Following the excretion measurements, a subset of mussels (both used in the experiments and other species, for a total of eight species) were placed on ice and returned to the laboratory (n = 138). 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 soft tissue from the shell of each individual and drying the soft tissue at 50 °C until mass remained constant. Total tissue biomass is the sum of the dry soft tissue and shell mass. We determined tissue nutrient composition (%C, %N and %P) to estimate nutrient storage by mussels. Mussel soft tissue and shell tissue samples were analysed on a Finnigan Delta Plus mass spectrophotometer in the University of Georgia's Analytical Laboratory for the determination of total %C and %N. For %P, samples were weighed, combusted at 550 °C for 2 h, and analysed with H_2SO_4 digestion followed by soluble reactive P analysis (Solorzano & Sharp, 1980). For each bed, we determined nutrient excretion of each species as the product of population density and per capita excretion rates (μmol nutrient h^{-1} g^{-1} mussel) and areal excretion (E_A; μmol nutrient m^{-2} h^{-1}) by summing the nutrient excretion rates per m^2. Areal excretion during the summer was multiplied by bed area to yield aggregate excretion rates of N and P for each bed (μmol nutrient h^{-1}). Areal storage (g nutrient m^{-2}) by each species was calculated as the product of biomass and % nutrient composition of the tissue (both shell and soft tissue) for individual species and then summed across species for an entire bed. For these estimates, we used the species specific, biomass-corrected excretion rates for the six most common species as described above; for the rest of the mussel community in each bed, we used an average of these rates. Areal excretion, total excretion per bed, areal storage and total storage per bed (N and P) were calculated for each of the 47 mussel beds.

To understand how temporal variability of mussel excretion may contribute to ambient nutrient concentrations, we calculated volumetric excretion and turnover distance as in McIntyre *et al.* (2008) and Benstead *et al.* (2010). Volumetric excretion was calculated as:

$$E_v = (E_A \times A)/Q$$

where E_V is expressed in moles of nutrient per litre, E_A is the areal excretion rate of the community (mol nutrient m^{-2} h^{-1}), A is area of the study reach (A = length × width) and Q is water discharge (Q = volume/travel time through the reach). Temperature-dependent excretion rates of five of the species were used (average was used for all other species) to calculate a temperature-dependent E_A for the community. E_V allows comparison of mussel excretion to ambient nutrient concentrations in the river and expresses the average addition of dissolved nutrients by mussel excretion as water flows through the study reach. Excretion turnover distance (m) is the distance required for excretion to turn over the entire ambient nutrient pool and is calculated by dividing the ambient nutrient concentration by E_V and then multiplying by the reach length for which E_V was calculated. Both E_V and excretion turnover distance were calculated for 8 June 2011 through 8 June 2012 for a well-studied mussel bed that is approximately 100 m long

near the USGS gauge at Clayton, Oklahoma (where the *Q* values were obtained) to assess the temporal variability on the effect of mussels on nutrient availability. Temperature-dependent mass-specific (soft tissue only) excretion rates were recalculated from original data in Spooner & Vaughn (2008) for five species (average was used for all other species) to calculate a temperature-dependent E_A regression relationship for the community (Fig. S1). The temperature-dependent E_A relationship was used to calculate E_V over the time period. Temperature data were obtained from a HOBO U20 logger (Onset, Bourne, MA, U.S.A.) placed at the site 8 June 2011.

Stoichiometric constraints and community structure

To assess the factors responsible for spatial patterns in nutrient recycling, we fit a linear regression relationship between tissue stoichiometry (areal N:P tissue storage) of the community and areal excretion N:P (molar) of the community. We also tested the relationship between aggregate excretion estimates and mussel community structure. We determined whether the presence of two dominant species, *Actinonaias ligamentina* and *Amblema plicata*, significantly influenced the N:P of areal excretion and storage of each individual bed using Pearson correlations. We chose these two species because they dominate Kiamichi River mussel biomass and are similarly sizes, but have different nutrient excretion rates (Vaughn *et al*., 2004; Spooner *et al*., 2012). All statistical analyses were performed in R v2.15.1 (R Development Core Team, 2012).

Results

Spatial extent

We mapped 651 km^2 of the Kiamichi River between the Big Cedar and Antlers USGS gauge stations. Mussel beds covered approximately 95.8 km^2 or 1.4% of the area in the Kiamichi River. Mussel density was greater in larger area mussel beds ($r^2 = 0.21$, $P = 0.001$).

Nutrient storage, remineralisation and volumetric excretion

Areal storage (g nutrient m^{-2}) of mussel beds varied across beds with the majority of both N and P stores contained in the shell (Fig. 2a,b). Shell made up 86% of N and 91% of P stores on average, although soft tissue was more nutrient-rich per g. Living mussels stored a total of 4690 kg (335 kmol) of N and 69 kg (2.2 kmol) of P across the 47 mussel beds. Individual beds stored a considerable amount of both N (6.3–632 kg bed^{-1}; 449–45 110 mol bed^{-1}) and P (0.65–67 kg bed^{-1}; 21–2161 mol bed^{-1}), which tended to peak in the middle and lower river reaches (Fig. 2c,d). Storage by spent shells was not measured.

Mussels excreted a large amount of both N and P with areal excretion varying as a function of densities and biomass within mussel beds. Areal excretion of both N and P was higher in the downstream sections of the river below the Clayton stream gauge (Fig. 1). Both N and P areal excretion rates varied with the biomass of the beds (11–700 μmol N m^{-2} h^{-1} and 0.8–53 μmol P m^{-2} h^{-1}; Fig. 3a,b), while total site excretion varied as a function of both mussel biomass and the areal coverage of the bed (0.21–136 mol N day^{-1} and 0.01–9.2 mol P day^{-1}, Fig. 3c). Excretion N:P varied across the beds (22-43) with some of the highest values in the mid-reaches near Clayton.

Expressing nutrient excretion in volumetric units shows that mussels are a large source of N and P, but more so during low flows (Fig. 4a,b). Volumetric excretion in a 100-m reach of the Kiamichi at mean flow was 213 nM total dissolved N and 3.1 nM total dissolved P. Volumetric excretion was highly variable across the year due to the high variability in discharge, but was greatest and exceeded background nutrient concentrations during base flow. Volumetric excretion by mussels exceeded ambient N concentrations more frequently than ambient P concentrations (Fig. 4a,b). These data combined with ambient nutrient concentrations showed that excretion turnover distances at mean flow were 11 km for total dissolved N and 89 km for total dissolved P. However, these turnover distances are reduced at low and base flow with N turnover <100 m 11% of the year and P turnover <100 m 9.5% of the year.

Community structure

Mussel bed areal excretion N:P was predicted by the N:P of soft tissue storage of the community ($r^2 = 0.80$, $P < 0.001$, $y = 65.94–2.06x$; Fig. 5a). Mussel beds dominated by *Actinonaias ligamentina* had lower storage N:P ($r^2 = 0.95$, $P < 0.001$, $y = 21.1–8.1x$; Fig. 5b), and greater abundances of *Amblema plicata* led to higher N:P stored in mussel tissue ($r^2 = 0.63$, $P < 0.001$, $y = 7.2x + 12.4$). Conversely, mussel beds dominated by *A. ligamentina* had higher areal excretion N:P ($r^2 = 0.73$, $P < 0.001$; $y = 16.3x + 22.7$; Fig. 5b), while higher proportions of *A. plicata* were significantly related to mussel beds with lower areal excretion N:P ($r^2 = 0.40$, $P < 0.001$, $y = 39.3–13.1x$).

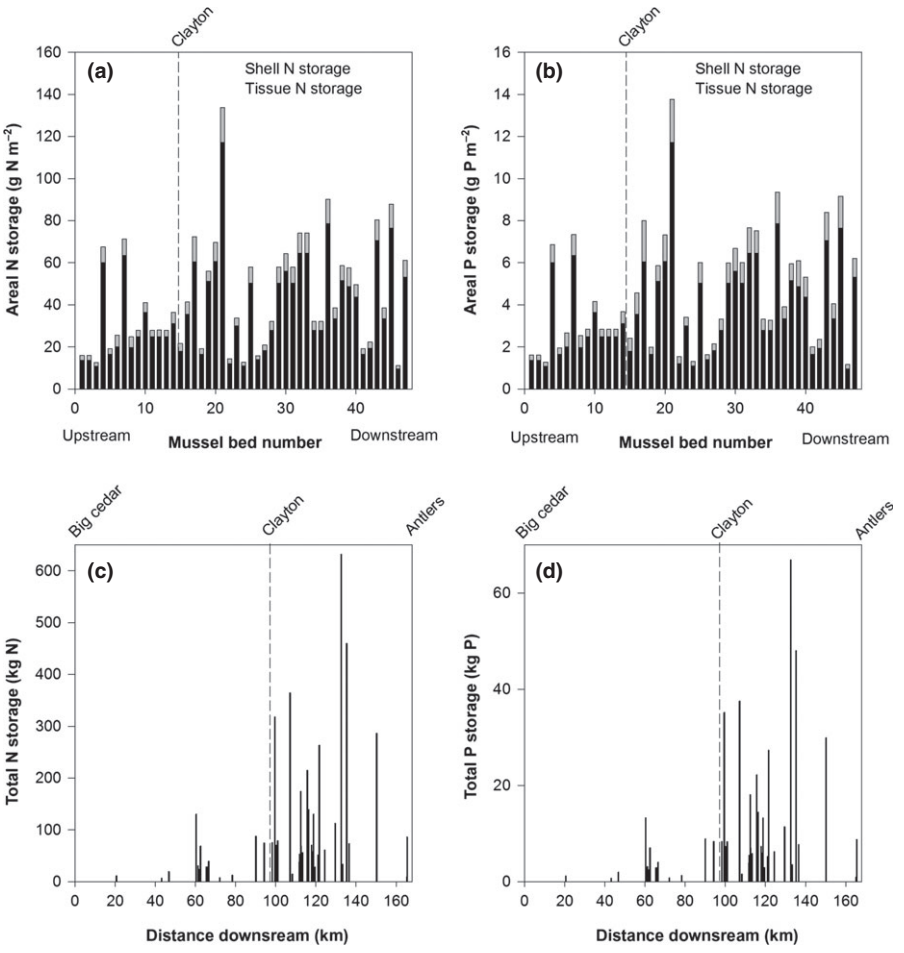

Fig. 2 (a) Areal N storage and (b) areal P storage in both soft tissue and shells of living mussels across the 47 mussel beds used in this study, from upstream to downstream. (c) Total N and (d) total P storage in mussel tissue in the Kiamichi River between the Big Cedar and Antlers USGS gauging stations.

Discussion

This work demonstrates that the heterogeneous distribution of aggregates of long-lived, sedentary mussels create hotspots of nutrient recycling and storage in streams that are strongly influenced by mussel biomass and species composition. Spatial heterogeneity influences population dynamics, community structure and ecosystem function (Pickett & Cadenasso, 1995; McIntyre *et al*., 2008; Winemiller, Flecker & Hoeinghaus, 2010). Our results add to the growing body of knowledge that show how spatial heterogeneity governs the contribution of organisms to stream nutrient recycling and storage, and how species composition influences these effects. Mussel beds were patchily distributed within the river; there was variation in species composition between beds and beds increased in biomass and abundance downstream as the river increased in size.

The contribution of organisms to nutrient dynamics is influenced by the relative biomass of the focal taxa within a given community, taxon-specific nutrient remineralisation and storage rates, ambient nutrient concentrations and other sources of nutrient regeneration (Vanni *et al*., 2002; Benstead *et al*., 2010; Capps & Flecker, 2013a). Mussel beds are spatially heterogeneous and community composition of mussel beds and biomass often vary as a function of stream size or catchment area (Atkinson *et al*., 2012; Haag, 2012). Ecological stoichiometry predicts that excretion rates and the ratio of excreted nutrients are a function of both the nutrient content of food and the body tissue composition of the organism (Elser & Urabe, 1999; Sterner & Elser, 2002). Our results show a strong relationship between tissue N:P and excretion N:P. Although mussels typically have been considered to belong to one functional group (filter-feeding bivalves), recent work shows that species have unique traits ranging from overall size and shell morphology to growth rates and gill structure (Vaughn, 2010; Haag, 2012). We found interspecific differences in tissue stoichiometry that resulted in differences in excretion stoichiometry across species. When these differences across species were scaled up to whole mussel beds, both mussel storage N:P and excretion N:P were highly variable and strongly correlated with mussel bed species

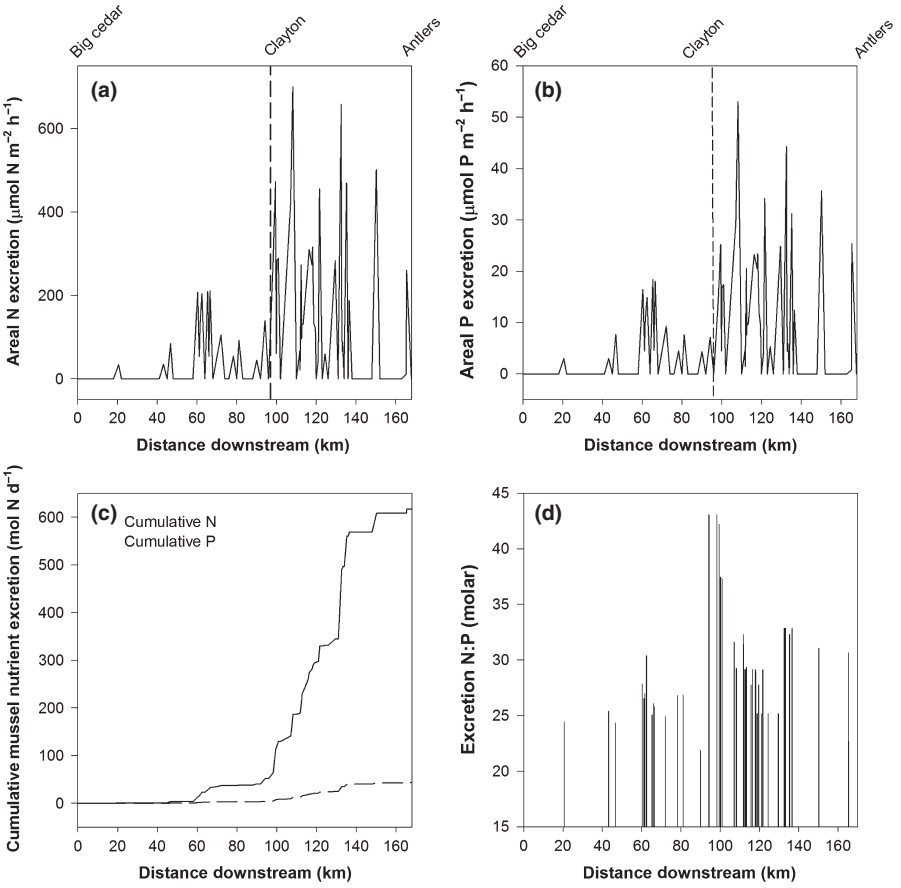

Fig. 3 (a) Nitrogen areal excretion and (b) P areal excretion at each bed between the Big Cedar and Antlers USGS gauges. (c) Cumulative mussel N and P excretion per day. (d) The excretion N:P of each of the 47 mussel beds across the length of the river.

composition. In particular, the presence of a species that excretes at high N:P, *Actinonaias ligamentina*, resulted in higher excretion N:P (also found by Spooner & Vaughn, 2008) of the bed and greater storage of P as indicated by the low N:P of their tissue. *Actinonaias ligamentina* is a thermally sensitive species (Spooner & Vaughn, 2008). This species was once co-dominant in the Kiamichi River, but has declined significantly in the last two decades due to drought and higher water temperatures (Galbraith *et al.*, 2010). Drought and warmer temperatures are predicted to become more frequent and more severe in this region with climate change (Seager & Vecchi, 2010). Thus, we anticipate a change in the relative availability of N and P in this system as mussel community composition changes. This will likely have repercussions for the rest of the food web because this system is N-limited (Atkinson *et al.*, 2013). Greater understanding of the role of specific species in ecosystem processes such as nutrient cycling and storage will lend to our overall understanding of the repercussions of species loss on ecosystems.

Mussels stored a large amount of both N and P, and may be acting as nutrient sinks in the Kiamichi River (Kitchell, Koonce & Tennis, 1975; Vanni, Boros & McIntyre, 2013). At the ecosystem-level, animals can act as nutrient sinks by removing nutrients from a system permanently or for a 'long' period. Animals can do this in three ways: biomass increase via population growth, when emigration exceeds immigration, and if the tissues of dead individuals are sequestered in a recalcitrant form (Vanni *et al.*, 2013). In the case of mussels, a large quantity of N and P is stored in shell tissue, mainly because of the high biomass of mussel shell. The areal biomass of N stored in the shell within a mussel bed is greater than the quantity of N stored in periphyton and aquatic insects combined in a nearby stream (P biomass not estimated; Atkinson *et al.*, 2014c). Shell tissue is largely recalcitrant, taking a long time to decompose (Strayer & Malcom, 2007), so large quantities of nutrients are retained in shell material even after mussel death. Additionally, mussels are relatively long-lived (typically 10–25 years, but up to 190 years; Haag & Rypel, 2011), and our data suggest differences in nutrient ratios across stores with different decomposition rates (shell versus soft tissue). During the growth phase, a large amount of P is sequestered, and in stable, high

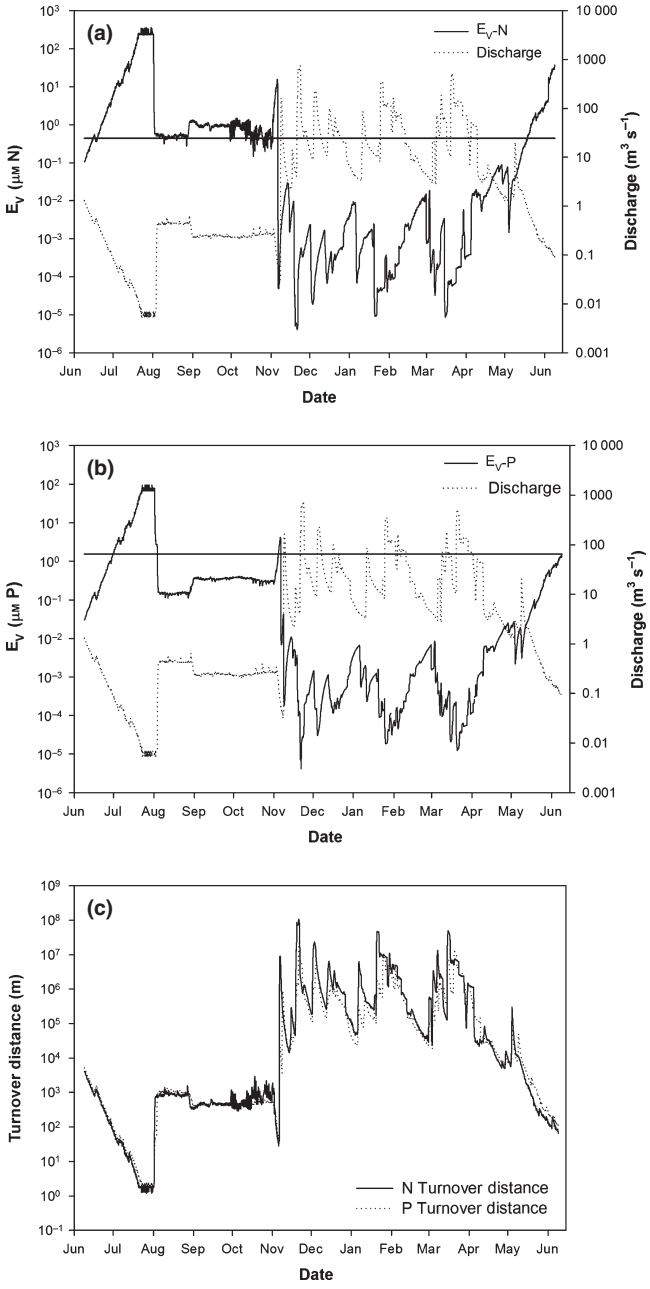

Fig. 4 (a) Volumetric excretion (E_V) of N and (b) P over a year as it changes with discharge (shown on right *x*-axis). The horizontal line in both panels (a) and (b) depicts the average background N and P concentrations at this location in the Kiamichi River to reflect when E_V exceeds background nutrients. (c) Turnover distance of both N and P across the year.

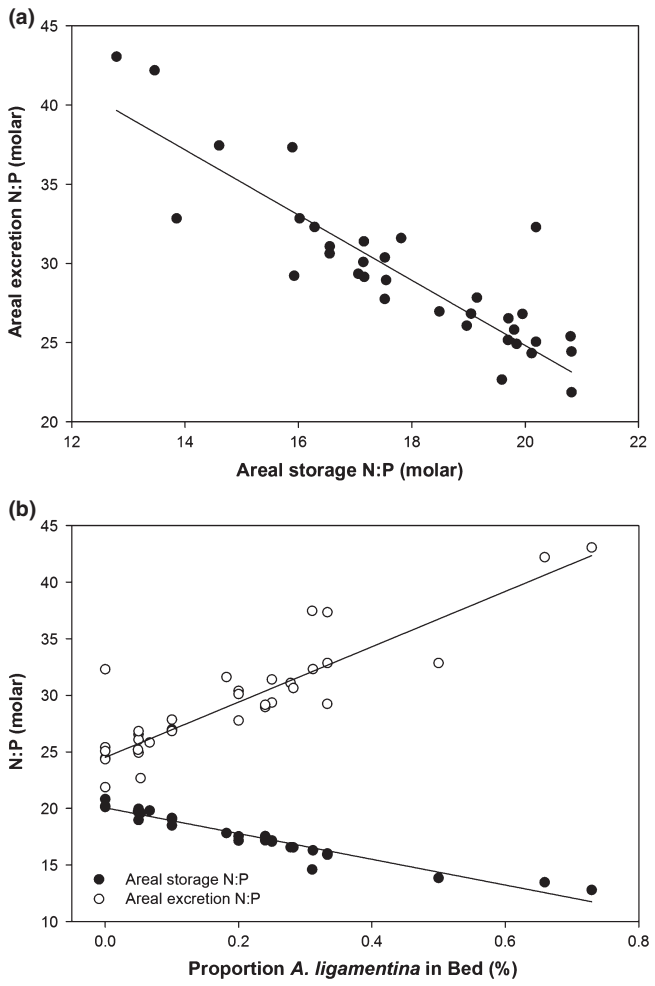

Fig. 5 (a) Mussel bed soft tissue storage N:P predicts mussel bed areal excretion N:P ($R^2 = 0.80$, $P < 0.001$, $y = 65.94–2.06x$). (b) The abundance of a dominant species in the Kiamichi, *A. ligamentina*, across beds strongly influences the N:P of both storage ($R^2 = 0.95$, $P < 0.001$, $y = 21.1–8.1x$) and excretion ($R^2 = 0.73$, $P < 0.001$, $y = 7.2x + 12.4$).

biomass mussel beds N transformation is high. This suggests that transient nutrient capacitance (storage and release) by unionids may be important in regulating ecosystem structure and function (Strayer, 2014).

The variability in mussel-generated biogeochemical hotspots has landscape-level consequences for river ecosystem function and biodiversity. As discussed above, stoichiometric regulation leads to high excretion rates and overall high N:P of mussel excretion, which probably affects the supply of nutrients to primary producers (Elser *et al.*, 1988). Previous work in the Kiamichi and nearby rivers shows differences in nutrient limitation patterns with N limitation occurring in areas without mussels and co-limitation within mussel beds (Atkinson *et al.*, 2013). These differences in nutrient availability have led to spatial differences in periphyton assemblages with mussel beds dominated by diatoms, and cyanobacteria more prevalent in reaches without mussels (Atkinson *et al.*, 2013). Further, mesocosm experiments have shown that different algal assemblages resulting from variation in mussel excretion leads to differences in benthic invertebrate production and export of adult insects to the terrestrial environment (Allen *et al.*, 2012).

The importance of spatial variation in nutrient recycling for ecosystem functioning depends upon the temporal stability of aggregations (Hall *et al.*, 2007) and the magnitude of their effect (Small *et al.*, 2009). Adult mussels are highly sedentary; they rarely move further than a few metres laterally per year (Kappes & Haase, 2012) and less than a half metre in a week (Allen & Vaughn, 2009), so these aggregations are usually stable at the ecological time scale of stream food webs and ecosystem function. For example, many of the mussel beds studied here have occupied the same general areas in the Kiamichi River for at least 100 years (Isley, 1924). These stable aggregations of enhanced nutrient and material translocation (Strayer, 2014) have led to more and higher quality benthic algal food resources (Vaughn *et al.*, 2007; Allen *et al.*, 2012; Atkinson *et al.*, 2013), increased abundance and richness of macroinvertebrates (Vaughn & Spooner, 2006) and emergent insect subsidies to riparian areas (Allen *et al.*, 2012). While in some cases these effects may be partially due to biogenic habitat provided by the mussels and their spent shells (Vaughn & Hakenkamp, 2001; Spooner & Vaughn, 2006), a field experiment in an adjacent river and mesocosm experiments show that mussel-regenerated N is assimilated into the food web and has a bottom-up effect on productivity (Vaughn *et al.*, 2004; Allen *et al.*, 2012; Atkinson *et al.*, 2014c). In short, mussel-mediated biogeochemical hotspots have cascading effects in stream food webs, supporting and enhancing production across all trophic levels studied to date.

Abiotic factors, primarily related to patterns in stream discharge and ambient nutrient concentrations, strongly affected the significance of mussels to ecosystem nutrient dynamics. The temporal variability of water flow through the study system varied by orders of magnitude across the year and led to large differences in E_v across time. This variation affected the magnitude of nutrients excreted by mussels relative to the ambient nutrient pool. Our estimates of turnover distance were sensitive to changes in hydrology as found in Benstead *et al.* (2010), yet turnover distance for both N and P was <100 m for more than 9% of the year. Excretion turnover distance varied orders of magnitude in response to stream flow with a mean of 11 km for N at mean stream flow, which is within the range reported by previous studies (McIntyre *et al.*, 2008; Benstead *et al.*, 2010). Our E_V values exceeded ambient nutrient concentrations during base-flow periods, which is when N is limiting (Atkinson *et al.*, 2013), but when stream temperatures are warmer and biological activity is highest. Thus, mussels appear to have their largest impact on nutrient dynamics when their excretion rates are the highest (Spooner & Vaughn, 2008) and during the time of the year when biological activity in the river is highest.

There is increased recognition of the importance of animals in shaping the structure and function of ecosystems (Covich *et al.*, 2004; Polis, Power & Huxel, 2004; Moore, 2006). The decline or addition of large suites of species from an ecosystem may possibly have a larger effect on consumer-driven nutrient dynamics than the change in abundance of a single species. Many studies have documented the pivotal role of a single species in mediating biogeochemical processes in streams (e.g. McIntyre *et al.*, 2007; Moslemi *et al.*, 2012; Capps & Flecker, 2013b) and questioned the idea of functional redundancy among freshwater organisms. Our study of freshwater mussels demonstrates how a distinct group of organisms can fundamentally alter nutrient dynamics that may have cascading impacts on community composition and food-web dynamics. As our work demonstrates, the widespread loss of unionid populations (Bogan, 2008; Strayer, 2008; Vaughn, 2010) has the potential to fundamentally alter nutrient dynamics and other ecosystem functions (McIntyre *et al.*, 2007; Vaughn, 2010; Atkinson *et al.*, 2014b) in rivers where mussels were once abundant.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Relationship between temperature and areal excretion (E_A; μmol nutrient m^{-2} h^{-1}) for the mussel bed at Clayton.

Table S1. Average soft tissue nutrient composition and excretion rates (± standard deviation) of eight common species that occur in the Kiamichi River.

Table S2. Mean background nutrient concentrations measured five times across six sites within the Kiamichi River basin.

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