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# Tracing Consumer-Derived Nitrogen in Riverine Food Webs

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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 siliquiodera*,  $n = 249$ ) a  $^{15}\text{N}$ -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  $\delta^{15}\text{N}$  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  $^{15}\text{N}$  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.

**Key words:** stable isotope; enrichment; unionid; mussel; nitrogen; nutrient uptake; turnover; stream food web; nitrogen tracer.

## INTRODUCTION

Consumer-mediated nutrient cycling has been increasingly recognized as an important category of functional processes in many ecosystems (Vanni

2002; Schmitz and others 2010; Small and others 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 and others 2008; Small and others 2011; Allgeier and others 2013; Whiles and others 2013), and some studies have quantified the additional amount of primary producer biomass that may occur because of these fluxes (Flecker and others 2002; Spooner and others 2012; Allgeier and others 2013). Despite the growing recognition that consumer nutrient recycling is important, no study we are aware of has directly traced and quantified the

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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 and others 1993; Knapp and others 1999). Species vary in their functional effects and those that 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 and others 2002; McIntyre and others 2008). Previous studies have shown that both marine and freshwater bivalves can be important in influencing N and P cycles (Bruesewitz and others 2009; Dame 2011; Goedkoop and others 2011; Jansen and others 2011). Although 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 and others 2012, 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 and others 2008; Small and others 2009; Benstead and others 2010; Small and others 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 and others 2003; Porter and others 2004; Vaughn and others 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 and others 2004). Freshwater mussels occur in large aggregations (known as beds, up to 100 mussels  $m^{-2}$ ) in rivers. The ecological functions performed by mussels (for example, 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 and others 2007; Vaughn and others 2008; Atkinson and others 2011; Spooner and others 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 and others 2008) and even secondary consumers (Allen and others 2012). Although 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 and others 2013; Spooner and others 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 and others 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 that 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  $^{15}N$  ( $\sim 1,000$  ‰ relative to atmospheric  $N_2$ ) in a Living Stream (Frigid Units Inc., Toledo, OH) 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  $\pm$  SE = 62.45  $\pm$  0.55 mm), weighed (mean  $\pm$  SE = 28.72  $\pm$  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<sup>2</sup>; 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 and others 2013). Quarter-meter square quadrats were placed in the middle 24 m<sup>2</sup> (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<sup>-2</sup>) were placed in each quadrat. The initial area of the reach with mussels, including stream margins (which declined over the summer), was 72 m<sup>2</sup>. 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 1). Temperature was continuously recorded every 15 min throughout the experiment at the –5 m transect using a Hobo U20 submersible logger (Appendix 1 in supplementary material,

Onset, Cape Cod, MA). 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-m transects the summer prior to the experiment (9 August 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<sub>4</sub><sup>+</sup>-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. In addition, 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- $\mu$ m 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<sup>2</sup> 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- $\mu$ m 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. In addition, we determined percent composition of N



**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.

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  $\text{NH}_4$  flux from mussels to the stream. We focused on  $\text{NH}_4$  because it is the most readily bioavailable form of N. Excretion experiments were performed with ten individual mussels (*Lampsilis siliquoidea*) following Atkinson and others (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 and others 2007) for  $\delta^{15}\text{N}$  prior to (from the experimental mussels and mussels near the site), during, and following the enrichment period during each field sampling period. The  $\delta^{15}\text{N}$  of mussel hemolymph was paired with excretion rates to estimate  $^{15}\text{N}$  release. Stable isotope analysis of mussel hemolymph showed that  $\delta^{15}\text{N}$  enrichment of mussels declined throughout the experiment. We used this value to determine the amount of  $^{15}\text{N}$  mussels were releasing into the ecosystem (in conjunction with excretion rates), to estimate water column  $\delta^{15}\text{N}$  (see “Methods” section), and tissue turnover. We estimated daily turnover rates from the time series of hemolymph tissue  $^{15}\text{N}$  by a decay model adapted from Tieszen and others (1983) and Hesslein and others (1993):

$$^{15}\text{N}_t = ^{15}\text{N}_{\text{pre}} + (^{15}\text{N}_{\text{peak}} - ^{15}\text{N}_{\text{post}})e^{-kt}, \quad (1)$$

where  $t$  is the number of days the mussels had access to non-enriched food (modeled up to 81 days),  $^{15}\text{N}_t$  is the tissue atom%  $^{15}\text{N}$  at time  $t$ ,  $^{15}\text{N}_{\text{pre}}$  is the tissue atom%  $^{15}\text{N}$  prior to enrichment,  $^{15}\text{N}_{\text{peak}}$  is the highest tissue atom%  $^{15}\text{N}$  during enrichment, and  $^{15}\text{N}_{\text{post}}$  is the atom%  $^{15}\text{N}$  as it is returning to equilibrium, and  $k$  is the absolute value of the  $^{15}\text{N}$  depletion rate. We assumed that  $^{15}\text{N}_{\text{post}} = ^{15}\text{N}_{\text{pre}}$  in our experiments as in McIntyre and Flecker (2006). The term  $k$  was estimated as the slope of the regression line of  $\ln(^{15}\text{N}_{\text{peak}} / ^{15}\text{N}_{\text{peak}} - ^{15}\text{N}_{\text{post}})$  versus time, as in Gustafson and others (2007) and is the exponent describing the proportion of  $^{15}\text{N}$  lost daily from the tissue due to growth and metabolic replacement. We also calculated the tissue turnover time (Hobson and Clark 1992; MacAvoy and others 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 each of the ecosystem pools. Isotope ratios are expressed in the delta ( $\delta$ ) notation:  $\delta^{15}\text{N}$  (units of ‰) =  $[(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 1,000$ , where  $R$  is the  $^{15}\text{N}:^{14}\text{N}$  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, FL, USA).

## Uptake and Uptake Rates

We measured areal  $\text{NH}_4$  uptake twice (at discharges of 21 and 49  $\text{l s}^{-1}$ ) 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 and others 2002). The mean uptake of  $\text{NH}_4$  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  $\text{NH}_4$  excretion rates. To measure  $\text{NH}_4$  uptake lengths and rates, we conducted short-term ( $\sim 3$  h) additions of  $\text{NH}_3\text{-N}$ , in conjunction with a conservative tracer ( $\text{Br}^-$  as  $\text{KBr}$ ) (Tank and others 2006). After collecting six background samples of stream solute concentrations along the study reach, a solution of  $\text{NH}_4\text{Cl}$  and the conservative tracer was pumped steadily into the stream. Target enrichments of dissolved ammonia were  $50 \mu\text{g NH}_3\text{-N L}^{-1}$ . The target concentration of the conservative tracer was  $570 \mu\text{g Br L}^{-1}$ . 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 and others 2002) because of saturation of microbial uptake. However, this effect was likely low because these streams are N-limited (Atkinson and others 2013) and uptake is likely higher in mussel beds because of increased availability of N (Dodds and others 2002). Samples were analyzed spectrophotometrically for  $\text{NH}_4^+$ -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 and others 1981). Uptake length  $Sw$  (m) equals  $a^{-1}$ . We used ordinary least squares regression to estimate parameters for equation 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 2006):

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

where  $Q$  is stream discharge ( $\text{m}^3 \text{ min}^{-1}$ ), and  $w$  is wetted channel width (m). Discharge was measured using a Marsh-McBirney flow meter and width was measured at five 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 ( $U$ ,  $\text{mg N m}^{-2} \text{ min}^{-1}$ ) was calculated as:

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

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 and others (2000). To model the uptake rates of periphyton and water willow, we had to estimate the water column  $\delta^{15}\text{N}$ . We did this by quantifying the flux of  $^{15}\text{N}$  and  $^{14}\text{N}$  from mussel excretion and the flux of background  $^{15}\text{N}$  and  $^{14}\text{N}$  and quantifying the mass

using the measured discharge values. From that we determined the background  $\delta^{15}\text{N}$  by summing the total mass of  $^{15}\text{N}$  and  $^{14}\text{N}$  from mussels and the background and then calculated the  $\delta^{15}\text{N}$ . Using this estimated  $\delta^{15}\text{N}$  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  $\text{NO}_3$  and DON from the water column, and uptake of N from the sediments and interstitial water. Using the  $\delta^{15}\text{N}$  values of periphyton, we modeled the uptake rates (in  $\mu\text{mol N m}^{-2} \text{ d}^{-1}$ ) of both mayflies and stoneflies at the 0, 5, 10, and 25-m 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 and others 2000), estimated uptake rates could be too high, whereas 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  $^{15}\text{N}$  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  $\delta^{15}\text{N}$  values were converted to mussel-derived percentages using a two source mixing model (Helfield and Naiman 2001; Allen and others 2012) to determine the amount of mussel-derived N (MDN) entering each ecosystem pool. The mixing model calculates MDN percentages as:

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

where %MDN is the percentage of MDN in a given sample, EP is the observed  $\delta^{15}\text{N}$  of the sample,  $EP_0$  is the ecosystem pool end member (that is,  $\delta^{15}\text{N}$  value representing 0% MDN), and MUS is the mussel-derived N end member (that is,  $\delta^{15}\text{N}$  value representing 100% MUS). In this study,  $EP_0$  was calculated as the mean  $\delta^{15}\text{N}$  of each ecosystem pool



prior to the mussel addition at the site, EP was the mean  $\delta^{15}\text{N}$  of the ecosystem pool following addition, and MUS was the mean  $\delta^{15}\text{N}$  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  $-5$  m 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 and others 2013), stream width and depth, nitrogen uptake rates from  $\text{NH}_4^+\text{-N}$  addition experiments (Atkinson, unpublished), background nutrient concentrations, and average summer discharge (Appendix 2 in supplementary material). From these data we calculated areal excretion rates ( $E_a$ ,  $\mu\text{mol N m}^{-2} \text{d}^{-1}$ ) for the study reach with the enriched mussels and the natural mussel bed. We calculated the percent  $\text{NH}_4$  demand that mussels provided through excretion by dividing the  $\text{NH}_4\text{-N}$  areal excretion rates by  $\text{NH}_4\text{-N}$  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 and others (2008) and Benstead and others (2010) based on a 100-m stream length at both sites. Volumetric excretion ( $E_v$ ,  $\text{mol nutrient L}^{-1}$ ) 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:

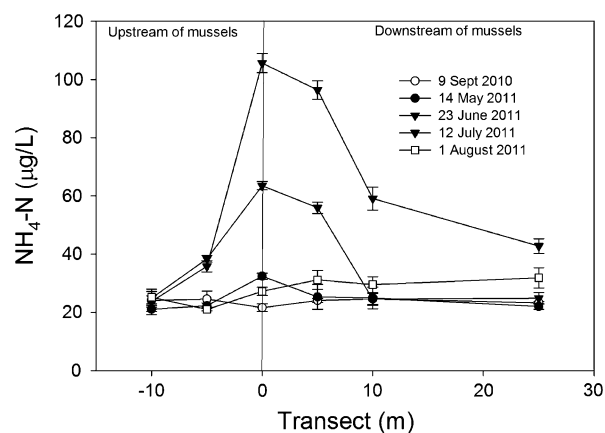
$$E_v = (E_a \times A \times T)/V \quad (7)$$

volumetric excretion integrates data on substrate area,  $A$  (length  $\times$  width,  $\text{m}^2$ ), volume,  $V$  (length  $\times$  cross-sectional area,  $\text{m}^3$ ) 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  $^{15}\text{N}$  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.



**Figure 2.** Ammonia concentrations at the  $-10$ ,  $-5$ ,  $0$ ,  $5$ ,  $10$ , and  $25$ -m transects during different sampling periods. *Black symbols* represent time periods when mussels were in the study reach, whereas *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.



## Isotopes

Using the decline in  $\delta^{15}\text{N}$  in mussel hemolymph, we calculated an N turnover rate of  $0.009 \text{ day}^{-1}$  ( $R^2 = 0.74$ ), which is equivalent to a 72-day half-life. Prior to enrichment, the average background  $\delta^{15}\text{N}$  ( $\pm$ standard deviation) was  $2.03\text{‰}$  ( $\pm 0.22$ ) for periphyton,  $1.57\text{‰}$  ( $\pm 0.35$ ) for water willow,  $2.15\text{‰}$  ( $\pm 0.32$ ) for mayflies,  $2.13\text{‰}$  ( $\pm 0.71$ ) for stoneflies,  $1.84\text{‰}$  ( $\pm 0.28$ ) for water pennies, and  $3.64\text{‰}$  ( $\pm 0.24$ ) for limpets. We noted enrichment following the mussel addition in the periphyton (maximum enrichment of  $5.62\text{‰}$  at the 5-m transect 33 days following addition), water willow (maximum enrichment of  $5.14\text{‰}$  at the 25-m transect 55 days following addition), mayflies (maximum  $\delta^{15}\text{N}$  of  $7.21\text{‰}$  at the 5-m transect 33 days following addition), and stoneflies (maximum enrichment of  $5.21\text{‰}$  at the 5-m transect 74 days following addition) (Table 1; Figure 3), whereas 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  $-5\text{-m}$  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, whereas other pools such as the water willow and stoneflies, responded more slowly (Figure 3). On average, within 50 m of the introduction area, approximately 3% of the  $^{15}\text{N}$  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 5-m 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  $^{15}\text{N}$  tracer results,

there were approximate values of 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 N on day 81 of MDN across the 50 m reach. Areal MDN in the downstream transects following the mussel addition ranged from  $71 \mu\text{mol N m}^{-2}$  at the 25-m transect at day 19 to  $1485 \mu\text{mol N m}^{-2}$  at the 5-m transect at day 19.

## Nitrogen Uptake and Demand

The little river is N-limited (Atkinson and others 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:  $1.5\text{--}3.3$ )  $\text{mm min}^{-1}$ , resulting in an uptake rate of  $116.7 \mu\text{mol N m}^{-2} \text{ h}^{-1}$  (range:  $79.9\text{--}153.5$ )  $\mu\text{mol N m}^{-2} \text{ h}^{-1}$ . This uptake rate in comparison to the areal excretion rate of mussels ( $47.2 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ ) 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  $1.0\text{--}1.5 \mu\text{mol N m}^{-2} \text{ d}^{-1}$ . There was more variability in the primary consumers, with mayflies having an uptake rate of  $4.0\text{--}108.0 \mu\text{mol N m}^{-2} \text{ d}^{-1}$ , and stoneflies having an uptake rate of  $3.1\text{--}669.2 \mu\text{mol N m}^{-2} \text{ d}^{-1}$  (Table 1). The highest uptake flux rates were observed at transects closest to the mussel addition (maximum rates at 5 m).

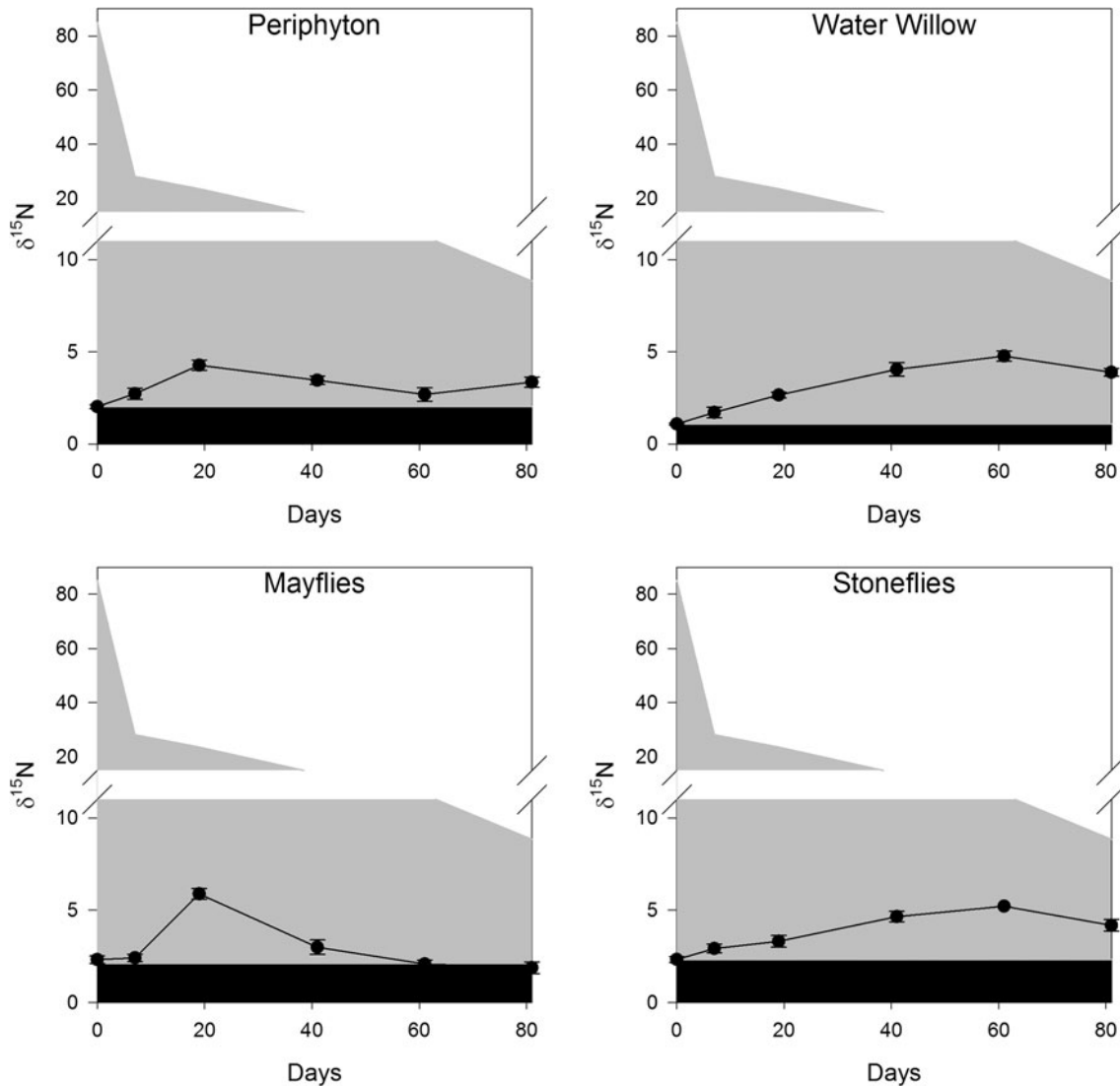
## Scaled to a Natural Mussel Bed

Measured ammonium uptake rates at the natural mussel bed were  $184.16 \mu\text{mol N m}^{-2} \text{ h}^{-1}$  in 2012 and areal excretion by the mussel community at this site was  $181.02 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ , suggesting

**Table 1.** Various Measurements of the Food Web Pools that Responded to the  $^{15}\text{N}$ -Enriched Mussels

Food web pool	Biomass range ( $\text{g DM m}^{-2}$ )	Average %N (SE)	Maximum $\delta^{15}\text{N}$ (transect)	Average uptake rate $\mu\text{mol N m}^{-2} \text{ d}^{-1}$ (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

The uptake rates were calculated using a box model approach as in Dodds and others (2000).



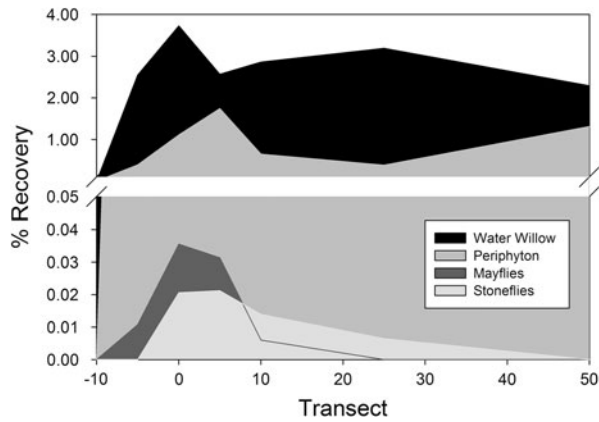
**Figure 3.** Depiction of the percent recovery model at the 0-m transect. The *black area* shows the baseline signature of the ecosystem pool prior to the mussel addition. The *points* and *line* show the ecosystem pool following enrichment. The best fit line was fit to the points after correcting for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

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  $6.03 \mu\text{M N}$ , whereas  $E_v$  at the natural mussel bed was  $23.35 \mu\text{M N}$ . Our data suggest that MDN from naturally occurring mussel beds may be  $3.9\times$  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

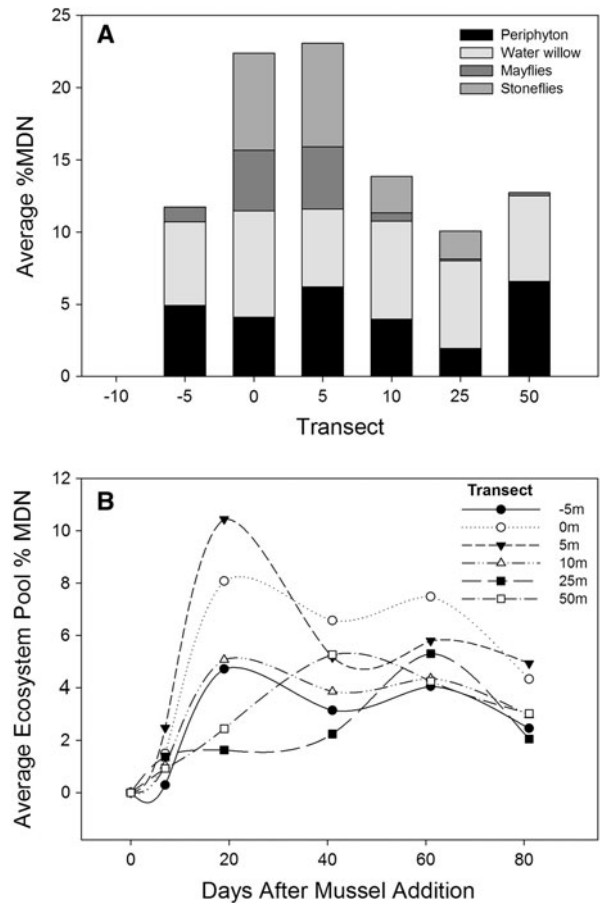
Our results directly link a well-studied process, nutrient remineralization, to its bottom-up contribution to stream food webs. Specifically, by creating a mussel bed and tracing the nitrogen remineralized by mussels into the stream, we demonstrated that mussel-derived nitrogen moves directly into the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



**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.

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 and others 2012; Atkinson and others 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 and others 2011; Allen and others 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. Although ammonium is not the only form of N that satisfies ecosystem demand (for example, nitrate and organic N),  $\text{NH}_4^+$  is the preferred form of N for both algae and microbes (Dortch 1990; Tank 2006), and uptake of  $\text{NH}_4^+$  can suppress nitrate uptake (Tank and others 2008). Thus, mussels are providing a form of N with high demand. Previous studies have found increased nutrient concentrations near aggregated organisms (McIntyre and others 2008; Jansen and others 2011). In our study, we noted increased ammonium availability around the created mussel bed in a system that is N-limited (Atkinson and others



**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-m transect. **B** The average MDN across all the pools over time during each of the sampling dates.

2013), and that this N was assimilated by the food web. Although 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 and others (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 be 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 heterogeneous in this system (Atkinson and others 2012) and (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 and others 2008). Mollusks are well known as structural engineers (Gutierrez and others 2003; Allen and Vaughn 2011), but the influence of native freshwater mussels (Atkinson and others 2013), invasive freshwater mussels (Goedkoop and others 2011), and marine mussels (Aquilino and others 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 to 231 days (Kemp and others 1990; Mulholland and others 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 in-

creased nutrient availability depends on background nutrient conditions, stream size, and biomass and density of the consumer providing the nutrient subsidy (Small and others 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 and others 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 (for example, dams, dredging, sedimentation; Strayer 2008; Vaughn 2010). Both the loss of species (McIntyre and others 2007; Hooper and others 2012) and the invasion of species (Bruesewitz and others 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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