

**Macroinvertebrate Excretion Rates and their Contribution to Nutrient
Cycling in a Rocky Mountain Stream**

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Abstract

Nutrient supply into an ecosystem is an important factor that can influence its productivity. While some nutrients enter the ecosystem from external sources, cycling of nutrients internally can also be an important process in determining nutrient supply. In this study I examined the role of benthic stream macroinvertebrates in supplying nutrients back into the water column. I measured excretion rates of $\text{NH}_4\text{-N}$ for all readily identifiable taxa in the upper East River in Gothic, CO and examined how these rates varied by body size and across taxa. I also measured nutrient uptake rates in order to determine what percent of ecosystem demand is met by the macroinvertebrate community. I found that invertebrate N excretion rates were positively correlated with individual body mass. Additionally both mean N excretion rate per individual and per unit of biomass varied by nearly three orders of magnitude across invertebrate taxa sampled. The invertebrate community supplied about 13% of nutrient demand. Nutrient supply by macroinvertebrates was dominated by the mayfly *Baetis bicaudatus*, Chironimidae, and the caddisfly *Arctopsyche grandis*. I also found that taxa with high mass-specific excretion rates tend to be most tolerant to organic pollution. This result could indicate the presence of a positive feedback chain where an initial disturbance leads to continued change in the invertebrate community. I determined that macroinvertebrates do play an important role in nutrient cycling in this Rocky Mountain stream and that changes in the macroinvertebrate community could have potentially large effects on stream nutrient cycling.

Introduction

The supply of nutrients to an ecosystem is one of among many factors that can affect an ecosystem's productivity (Leith and Whittaker 1975). Although some nutrients

are supplied by allochthonous inputs, the recycling of nutrients within an ecosystem via mineralization by heterotrophs can be of even greater importance in regulating the availability of nutrients. Animals can have both direct (through excretion and egestion) and indirect (through effects on prey and the physical environment) effects on nutrient cycling (Vanni 2002). Of these processes, excretion is particularly important to nutrient cycling in aquatic systems because most nutrients are excreted as inorganic forms that are readily used by primary producers (Vanni 2002). In aquatic ecosystems, nitrogen (N) is an important nutrient that can potentially limit primary production. Thus the supply of N in its inorganic form, ammonium, is important in controlling aquatic algal and plant growth and is potentially regulated by animal excretion (Vanni 2002).

In streams, invertebrates are widely known to have various functional roles such as leaf shredders, plant and algae grazers, and particle collector/gatherers. Regardless of this classification, they all contribute N to nutrient cycling through excretion, though they do so at different rates. Previous research has found a significant (15%-70%) contribution of invertebrate excretion to nitrogen cycling in streams (Grimm 1988, Hall et al. 2003). Because of the influential role of excretion in regulating stream nutrient dynamics, it is important to understand the factors affecting invertebrate excretion rates such as body size (Vanni 2002). Larger body size is related to decreased mass-specific excretion of N (Hall et al. 2007, Vanni 2002). While the influence of body size of other animals on nutrient recycling is relatively well understood, the contribution of stream invertebrate body size to nutrient cycling is not (Hall et al. 2007). Additionally the degree to which body size and invertebrate taxonomy differentially influence nutrient cycling is not well known (Hall et al. 2007).

Scaling up to the ecosystem level, it is important to understand how invertebrate excretion rates factor into stream nutrient dynamics. One factor that could affect stream nutrient dynamics is a change in the composition of the macroinvertebrates. The well-documented changes that occur in an impacted stream have led to the development of many different biomonitoring techniques (see Bonada 2006, Barbour et al. 1999). Different invertebrate taxa, such as Ephemeroptera, Plecoptera, and Trichoptera (EPT) are usually more sensitive to impacts than others and their proportional abundance is often used as an indicator of stream health (Lenat 1988). Previous research has also developed tolerance rankings to different types of disturbance or pollution for many other invertebrate taxa (Hilsenhoff 1988, Lenat 1983, 1988). When a stream is disturbed, the invertebrate community becomes dominated by high tolerance taxa. The changes in the stream invertebrate community caused by disturbances could impact the rate at which nutrients are recycled.

I explored the influence of invertebrate body size and taxonomy on excretion rates. I hypothesized that, like vertebrates, smaller-bodied invertebrates would have a higher mass-specific excretion rate of N (Hall et al. 2007). I then used excretion rate data to determine what proportion of nutrient demand is met by invertebrate excretion, and lastly to address how changes in the community composition of stream macroinvertebrates might translate into changes in nutrient recycling.

Methods (adapted from Koch 2005)

Field methods - I measured the excretion rate of NH_4^+ -N for all readily identifiable invertebrate taxa in a section of the East River located adjacent to the dining hall and Snowmass cabin. Invertebrates were collected with a kick-net and placed into individual 500 mL Whirl-Pak ® bags containing 120 mL filtered stream water for 50-80 minutes.

Each bag contained 1-20 individuals of similar size and of the same invertebrate taxa (identified to species or at least genera). The exact number of individuals per bag and the incubation time was dependent on the particular species of invertebrate. Excretion estimates were replicated 8-10 times (or bags) per species. Areal density and biomass of invertebrates were estimated from ten 0.09 m² samples as part of another study. Body mass of each individual in a bag was measured after drying for 24 hours in at 60 °C and weighing on a Mettler AP-6 microbalance.

The concentration of N as NH₄ in the water before incubation (ambient stream water concentration) was subtracted from the concentration after incubation to calculate the concentration excreted by the invertebrate. The per individual rate of excretion was calculated by dividing total N excreted by both incubation time and total individuals per bag; the mass-specific excretion rate was calculated similarly except that total N excreted was also divided by the individual or mean biomass of the invertebrates in each bag. I measured the concentration of NH₄-N using a fluourometric method (Holmes et al. 1999, Taylor et al. 2007). I also measured water temperature, which may be an important covariate for excretion rates, with a Thermchron iButton temperature logger recording every 10minutes that was placed in the cooler water containing the Whirlpaks. I used least squares linear regression to test how excretion rates varied with body mass of each invertebrate species. To compute the amount of nutrients recycled by the entire invertebrate community, I multiplied mass-specific rates by the areal biomass of each taxon in the East River and summed across all taxa.

I measured uptake rates of N using short-term, whole-stream additions of NH₄ following standard methods (Stream Solute Workshop 1990, Mulholland et al. 2002). I added low levels of NH₄Cl and a conservative tracer dye (Rhodamine WT) into the

stream and monitored the concentration of the dye 600 m downstream every 2 minutes with a submersible fluorometer. When the concentration of Rhodamine dye reached an equilibrium concentration (~1 hour), I sampled nutrient concentrations at 50-75 m intervals along the stream section. Per meter nutrient uptake rate was estimated from the longitudinal decline in nutrient concentrations along the 600 m reach. I used the per meter nutrient uptake estimates to calculate areal uptake rates, or ecosystem demand, for N following the equations in the Stream Solute Workshop (1990). All statistical analyses were done in JMP v. 8 (SAS Institute 2009).

Results

Across all taxa the $\log_{10}$ of invertebrate N excretion rate ($\mu\text{g}\cdot\text{ind}^{-1}\cdot\text{hr}^{-1}$) is positively correlated to $\log_{10}$ of mean individual invertebrate biomass (Figure 1; $r^2 = 0.24$, $F_{1,122} = 38.45$, $P < 0.001$). Both the mean $\log_{10}$ of N excretion rate per individual (ANOVA: $F_{29,121} = 4.52$, $P < 0.001$) and mean $\log_{10}$ mass-specific excretion rate varied by three orders of magnitude among invertebrate species (Figure 2; ANOVA: $F_{28,94} = 5.55$, $P < 0.001$).

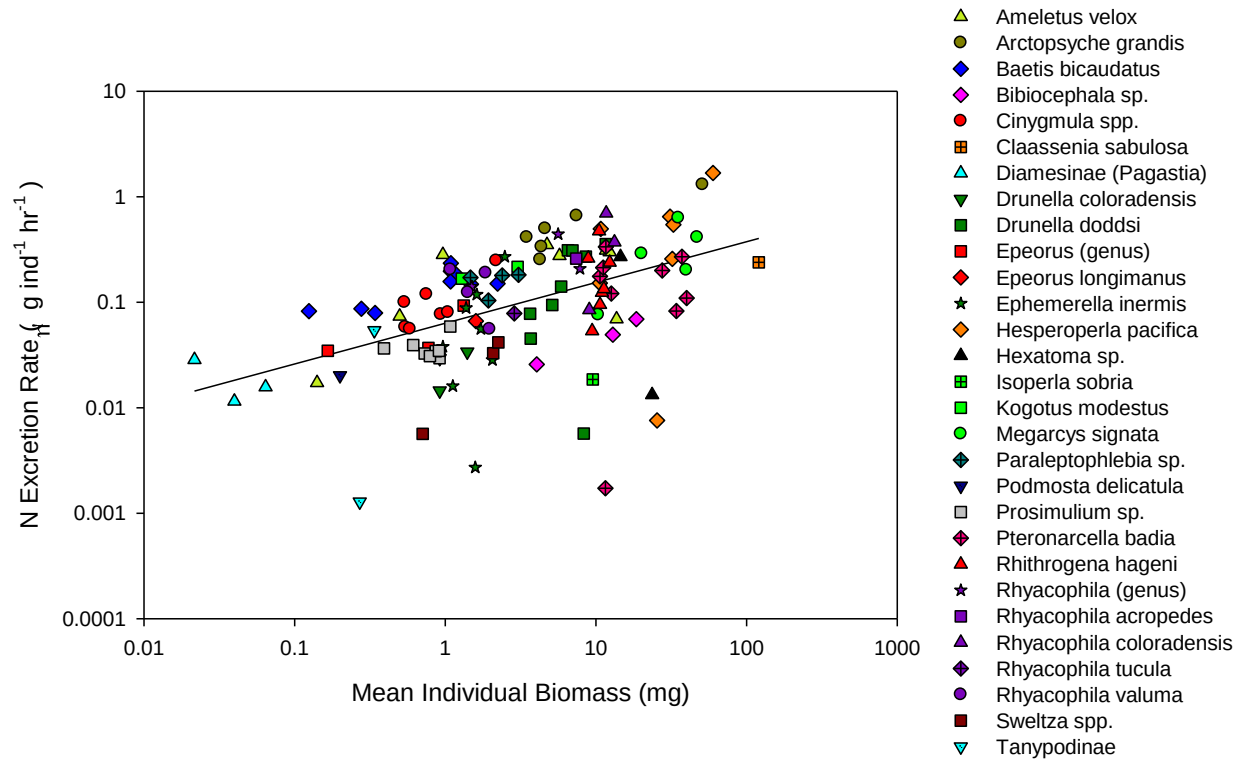


Figure 1. Excretion rate of $\text{NH}_4 - \text{N}$ increases less than proportionally with average invertebrate biomass ($\log(\text{excretion rate}) = -2.76 + 0.386 \times \log(\text{mean invertebrate biomass})$). Each species sharing a color is a member of the same taxonomic family. Chironomids are an exception and could only be identified to subfamily by eye in the field.

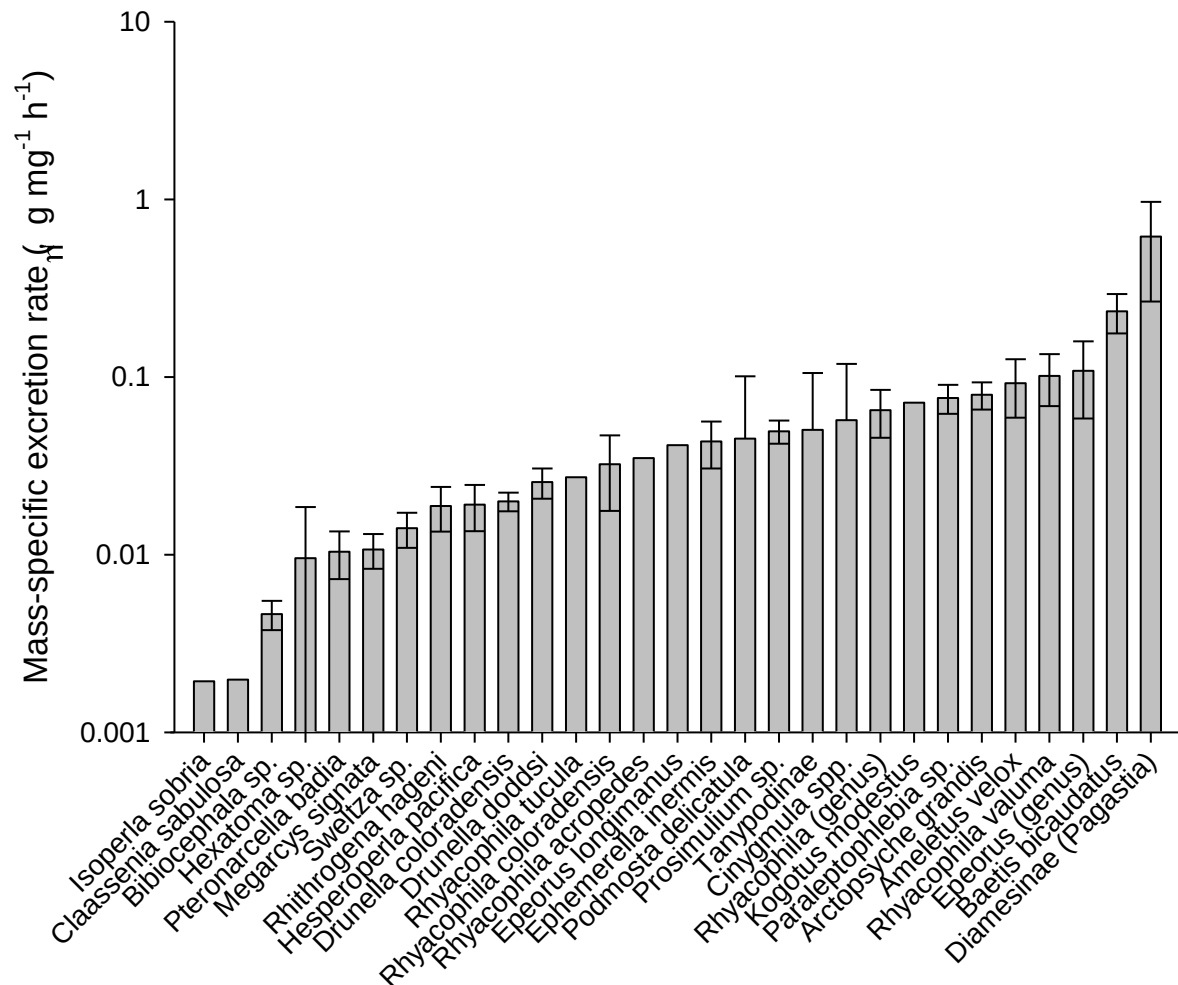


Figure 2. Mass specific excretion rate of $\text{NH}_4 - \text{N}$ varies by nearly three orders of magnitude among invertebrate taxa. Error bars indicate 1 standard error of the mean. Values without error bars indicate only one measurement of excretion rate.

Stream N demand ranged from $11.2 - 12.4 \text{ mg m}^{-2} \text{ d}^{-1}$ while estimated nutrient supply by stream invertebrates ranged from $1.6 - 1.3 \text{ mg m}^{-2} \text{ d}^{-1}$ from streams with and without *Didymosphenia geminata* respectively. Thus stream invertebrates supplied 11-14% of N demand. However, using two standard deviations of community biomass to calculate a range of nutrient supply rates, it is possible for the community to supply up to 36% of N demand in this Rocky Mountain stream. Total N supply was dominated by a

few taxa: the mayfly *Baetis bicaudatus* (36%), Chironimidae (26%), and the caddisfly *Arctopsyche grandis* (26%).

Based on preliminary examination using the Hilsenhoff (1988) Family Biotic Index for organic pollution, it appears that more tolerant taxa have higher mass-specific excretion rates (Table 1).

Table 1. The mean mass-specific N excretion rate was calculated with taxa grouped by FBI tolerance value. The mean mass specific excretion rate is higher among more tolerant taxa. No taxa had a tolerance value of 5.

Hilsenhoff FBI Tolerance Value	Mean Mass- specific Excretion Rate of Taxa
0	0.02622699
1	0.02117629
2	0.05106006
3	0.00954108
4	0.12142455
6	0.19148535
7	0.09240456

Discussion

Although macroinvertebrates were found to supply a lower proportion of stream N demand when compared to other studies (Grimm 1988, Hall et al. 2003), they are important in ecosystem functioning nonetheless. They fill many functional roles in the stream while also serving as prey for fish and other macroinvertebrate species. Although all taxa contribute to some degree to total N supply, that only three taxa dominated supply rates is important when understanding the contribution of the invertebrate community to N supply. Loss or increase in biomass of any of these three taxa (*B. bicaudatus*, Chironimidae, and *A. grandis*) could have large impacts on nutrient supply

due to the relatively high mass-specific excretion rates of these taxa and large body mass and high density in the case of *A. grandis*.

I found a large variation in mean N excretion rates among taxa present in the East River. This finding shows that each taxa plays a unique role in the way it supplies N back into the water column, i.e. there is little functional redundancy with respect to excretion rate. This uniqueness may be important when considering internal nutrient supply in streams under scenarios of community change. For example, when a stream experiences organic pollution, such as that from a sewage treatment plant or agriculture, the community will change such that the taxa present will be tolerant to this type of pollution. Considering a situation where overall biomass remains constant, the remaining taxa, whose mass-specific rates of excretion are higher, will excrete a greater amount of N into the water than the unimpacted community. This could potentially lead to a positive feedback where an initial change favors a community that excretes more, which increases nutrient concentrations, thus leading to further community change

(Figure 3).

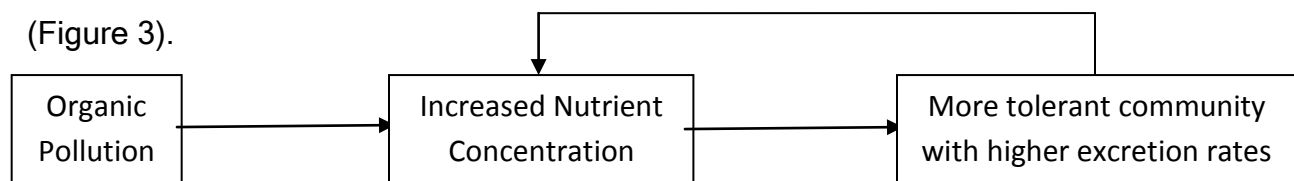


Figure 3. A positive feedback loop between community change and nutrient concentration.

This is merely a preliminary analysis and a more robust model will be developed to test the strength of this prediction.

The influence of macroinvertebrates on stream nutrient recycling has been studied very little. Continued research into this area is needed to fully understand stream nutrient dynamics.

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