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Hyporheic invertebrates affect N cycling and respiration in stream sediment microcosms

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Abstract. The region of surface water–groundwater interaction in streams, the hyporheic zone, is important for biogeochemical processes and provides habitat for specialized microbial and invertebrate assemblages. Although hyporheic invertebrates contribute little biomass and respiration relative to microbes in stream sediments, invertebrate effects on biogeochemical processes may be disproportionately large. We tested how various interstitial invertebrate assemblages affected N cycling and respiration in flow-through microcosms filled with alluvial sediment in the laboratory. Average invertebrate biomasses in low and high invertebrate treatments were 0.20 and 19 mg dry mass/L sediment, respectively. Average net NO_3^- regeneration/uptake rate increased with increasing invertebrate biomass, showing invertebrates suppressed NO_3^- uptake or stimulated in situ NO_3^- production. Average respiration (normalized for sediment organic matter) and particulate organic matter (POM) increased 51% and 33%, respectively, with increasing invertebrate biomass, suggesting direct contribution to hyporheic metabolism and/or stimulation of microbial activity and an accumulation of POM driven by invertebrates. We suggest that interstitial invertebrates can substantially alter biogeochemical processes in hyporheic zones.

Key words: hyporheic, invertebrates, nitrogen cycling, nitrification, ammonium uptake, community respiration, ecosystem processes, sediments, Grand Teton National Park.

Recent studies demonstrate the importance of headwater streams in the transformation and export of N (Peterson et al. 2001, Bernhardt et al. 2002) and C (Webster and Meyer 1997, Jones and Mulholland 1998). Substantial amounts of this processing can be attributed to hydrologic exchanges between surface water and ground water (Valett et al. 1994, Brunke and Gonser 1997). The degree of exchange can correspondingly affect the rates of microbial processes in alluvial sediments (Findlay 1995). Because of these effects, the zone of mixing of surface and ground waters (i.e., the hyporheic zone) plays a critical role in biogeochemical cycling in streams (Jones and Holmes 1996a, Dahm et al. 1998).

The hyporheic zone also provides habitat for invertebrates that spend all (obligate) or part (occasional) of their life cycles in the subsurface (Danielopol et al. 1994). The occurrence and structure of these invertebrate assemblages are commonly treated as dependent variables, responding to a variety of parameters largely determined by physical, chemical, and/or microbial processes in the sediment and overlying surface water (Dole-Olivier and Marmonier 1992, Strayer et al. 1997, Brunke and Gonser 1999). But what effect might interstitial invertebrates have on hyporheic processes?

Recent reviews emphasize the importance of species and ecosystem functioning (sensu Jones and Lawton 1995) in freshwater sediment systems (Freckman et al. 1997, Palmer et al. 1997, Covich et al. 1999, Boulton 2000a, Hakenkamp and Morin 2000). These recent reviews suggest that certain taxa of sediment-dwelling invertebrates affect hyporheic ecosystem processes, but few empirical data support this hypothesis. Although the effects of 1 or 2 species may contribute significantly to ecosystem functioning, most systems include many more species and the interactions among these species may result in completely different contributions.

Many studies have shown correlative relationships between aquatic invertebrate assemblages and bacterial activity and abundance (Traunspurger et al. 1997), nutrient concentrations, and other physicochemical factors (Dole-Olivier and Marmonier 1992), but few experimental studies have been attempted in alluvial sediments (but see Hakenkamp and Palmer 1999, Mermillod-Blondin et al. 2000, 2003). Bärlocher and Murdoch (1989) described hyporheic biofilms as a potential food source for interstitial invertebrates, but few studies have detailed the effects of feeding in alluvial sediments. Benthic cope-

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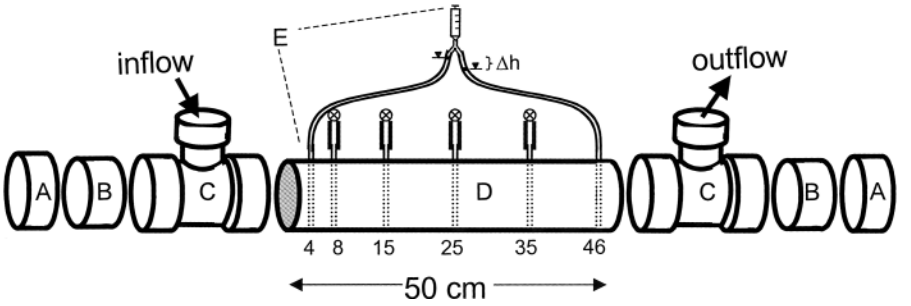

FIG 1. Microcosm components and manometer. A—caps, B—connectors, C—T-connectors, D—sediment-filled section with 6 sampling ports at 4, 8, 15, 25, 35, and 46 cm downstream and 100-μm mesh at both ends, E—removable manometer shown with decreasing head gradient (Δh, from left to right) and syringe at the top.

pods and chironomids preferentially assimilate bacterial C in streams (Hall and Meyer 1998), and stream-dwelling harpacticoid copepods can select bacteria of different sizes (Perlmutter and Meyer 1991). In addition, numerous studies have shown that ecosystem processes, such as solute transport (Aller and Aller 1992), nitrification (Lee and Welander 1994, Parent and Morin 1999), and P release rates (Gallepp 1979) can be affected by benthic invertebrates in wastewater, marine, and lake systems. Budgetary approaches have also shown how benthic invertebrates could be important nutrient recyclers in streams (Grimm 1988, Hall et al. 2003) and lakes (Devine and Vanni 2002). Extrapolating these findings to alluvial sediments suggests that these invertebrate-driven population- and community-level impacts could also substantially contribute to ecosystem-level processes.

We addressed the degree to which interstitial invertebrate assemblages affect hyporheic microbial processes, specifically N cycling and community respiration. Our research differed from previous studies in 2 ways: 1) we used intact hyporheic invertebrate assemblages as opposed to one or a few specific taxa, and 2) we measured rates of biogeochemical transformation, in addition to concentrations. We used flow-through microcosms filled with sediment and a range of interstitial invertebrate biomasses. This laboratory experiment allowed greater control of discharge and other physical and chemical variables than would be possible in the field and, thus, increased sensitivity to potentially small changes caused by invertebrates. We predicted that N cycling rates (NH_4^+ uptake and nitrification) and community respiration would increase with increasing invertebrate biomass, based on previous observations of hyporheic invertebrate assemblages and co-occurring N content and forms.

Methods

Microcosm design and assembly

Each microcosm consisted of a 50-cm length of 7.6-cm diameter PVC pipe (2.3 L), two 7.6-cm × 5.1-cm diameter PVC T-couplings, and caps at both ends connected to the T-couplings with 10-cm lengths of additional 7.6-cm diameter PVC pipe (Fig. 1). PVC joints were sealed with petroleum jelly. Perforated Teflon tubes (1-mm ID) wrapped in 100-μm mesh provided sampling ports at 4, 8, 15, 25, 35, and 46 cm along the pipe (Fig. 1). Each end of the 50-cm length of PVC was covered with 100-μm mesh netting to contain invertebrates and sediment, but to allow water to flow through the microcosm. The PVC T-couplings at both ends allowed water to overflow at a constant level and allowed access for upstream/downstream measurements. Sediment characteristics and flow regimes are described below.

Sediment and invertebrate sources

We elutriated interstitial invertebrates through a 100-μm mesh net (Pfannkuche and Thiel 1988) from fresh sediment (5–25 cm depth) of the active channel of Two Ocean Lake Creek, a 2nd-order, medium-gradient stream that drains Two Ocean Lake in Grand Teton National Park, Wyoming. Streambed substrate consists predominantly of gravel and sand. We aerated the remaining sediment with an aquarium pump and

maintained the sediment at room temperature until we constructed each experimental replicate (<48 h). We separated live invertebrates from dead invertebrates and associated particulate organic matter (POM >100 μm) under a 10× dissecting microscope. Stock invertebrate concentration varied between experimental replicates (742 to 1061 individuals/130 mL), depending on the number of invertebrates originally in the sediment and the number that survived elutriation for a given batch of sediment. We added 1 of 3 volumes (0, 10, or 120 mL) of live invertebrate stock solution and equal volumes of the remaining untreated POM to equal volumes of the remaining sediment. We reintegrated the invertebrates and POM into equal volumes of the elutriated sediment by gently pouring the mixture from one container to another and back 2 times with only interstitial water present (i.e., ~1 L water:2.3 L sediment). We allowed each block of 3 biomass treatments to run with flowing water for 6 to 9 d before any water was sampled. We set up 5 replicate blocks 3 to 4 d apart, from late July to early September 2001.

Microcosm monitoring and measurements

We positioned the sediment-filled microcosms horizontally with a slight declination to maintain a constant hydraulic head between the upstream and downstream ends (i.e., the microcosms were constant-head permeameters). We aerated unchlorinated well water with aquarium pumps to ~7 mg dissolved oxygen (DO)/L in a 4-L head tank and gravity fed the oxygenated water to the upstream T-coupling of each microcosm. We measured discharge by collecting downstream overflow. We measured the change in head as the difference in water levels between the sampling tubes at 4 and 46 cm using a manometer (Fig. 1, see Winter et al. 1988 for basic potentiomanometer design). We adjusted hydraulic head in each microcosm to maintain approximately the same discharge within each replicate block of 3 microcosms. Discharge at the time of sampling ranged from 5 to 16 mL/min for all 15 microcosms (mean 9.6 mL/min), but average discharge within each replicate block of 3 microcosms had relatively little variance (±1.7 mL/min pooled SD). We ran microcosms with flowing water for 6 to 9 d before sampling, long enough for the physical and respiration measurements to approach asymptotes (MCM, unpublished data).

At the end of the 6 to 9 d of run time, we sampled water with 10-mL syringes from each sampling port. We immediately filtered (25-mm diameter Gelman Ion Chromatograph Acrodisc 0.45-μm pore filters) and froze water samples at −4°C until analysis. We analyzed NH_4^+ by fluorometry (Holmes et al. 1999) using either a fluorometer (Turner Designs, Sunnyvale, California, Model 10-AU field, detection limit: 4.7 μg N/L, blocks 1 and 2) or a spectrofluorophotometer (Jobin Yvon-Spex, Edison, New Jersey, Model Fluorolog-3, detection limit: 2.5 μg N/L, blocks 3, 4, and 5). We analyzed NO_3^- using ion chromatography (DIONEX Ion Chromatograph, Sunnyvale, California, detection limit: 3.8 μg N/L). We measured DO with an oxygen probe (Quanta, Hydrolab, Austin, Texas).

Sediment, invertebrate, and organic matter processing

After the experiment, we again elutriated invertebrates and POM from the sediment. We preserved ~200 mL of the elutriated wet sediment in 95% ethanol for sediment-associated organic matter (SOM) assessment. We sorted, identified, and measured live and intact invertebrates for biomass estimations of macrofauna (Benke et al. 1999) and meiofauna, assuming a specific gravity of 1.13 and a dry mass:wet mass conversion of 0.25 (Feller and Warwick 1988). We preserved organic matter remaining in 95% ethanol after live-sorting. We dried, weighed, combusted POM and SOM (and associated preservative) at 500°C for at least 2 h and reweighed to estimate ash-free dry mass (AFDM) in each microcosm.

Hydraulic and N cycling calculations

We used Darcy's Law (Fetter 1994) to estimate the seepage velocity for each microcosm at the time of sampling. We estimated average sediment porosity by weighing a known volume of elutriated, saturated sediment before and after drying. We substituted this porosity for effective porosity in the above seepage velocity calculation (Fetter 1994). Average seepage velocity in microcosms ranged from 0.15 to 0.89 cm/min, which is comparable to field seepage velocities based on falling head tests (0.04–0.24

cm/min). To correct for slight differences in flow velocity within and among experimental replicates, we calculated the amount of time it took water to travel downstream to a given sampling tube for each microcosm using the above seepage velocity.

To estimate NH_4^+ uptake rate (k_1, /min) in each microcosm, we plotted the logarithm of the first 3 to 5 consistently decreasing NH_4^+ concentrations versus time traveled (t) downstream, where the slope of the regression is the uptake rate (Webster and Ehrman 1996). We estimated nitrification rate and NO_3^- uptake/regeneration by using a 2-compartment model that describes changes in NH_4^+ and NO_3^- concentrations (Mulholland et al. 2000). Travel time varied slightly with distance downstream between microcosms, so we used time (t) rather than distance (x) in the equation:

$$N_t = [k_N A_0/(k_2 - k_1) \times (e^{-k_1 t} - e^{-k_2 t})] + N_0 e^{-k_2 t} \quad [1]$$

where N_t is NO_3^- flux (μg N/min) at time t, k_N is the nitrification rate (/min), A_0 is the NH_4^+ flux at the upstream end (μg N/min), k_2 is the NO_3^- uptake/regeneration rate (/min), k_1 is the NH_4^+ uptake rate (/min), and N_0 is the flux of NO_3^- at the upstream end of the microcosm (μg N/min) (Mulholland et al. 2000). This model calculates k_N based on the upstream flux (i.e., allochthonous NH_4^+ supply), whereas k_2 represents changes in NO_3^- concentration not explained by k_N. For example, NH_4^+ that is produced by mineralization within the microcosms may in turn be nitrified, but this new NO_3^- would contribute only to the k_2 component of equation 1. We estimated these 2 unknowns in the above equation (k_N and k_2) using a least-squares fitting procedure in the Solver tool in Microsoft Excel (Bernhardt et al. 2002). We also calculated the fraction of allochthonous NH_4^+ that was actually nitrified (k_N/k_1). After solving for the unknowns, we multiplied k_2 by -1 so that positive values represented net NO_3^- production and negative values represented net NO_3^- uptake in the Results section. Henceforth, we refer to k_2 as the NO_3^- regeneration/uptake rate.

We calculated community respiration (CR) as the change in DO concentration from upstream to downstream ends of the microcosm divided by travel time. Most of the organic matter in microcosms was associated with the post-elutriation sediment (i.e., SOM, see Results section), so we assumed that sediment-associated bacteria accounted for most microcosm respiration (Hedin 1990). Therefore, we divided CR by SOM (CR/SOM), and used this quotient as the response variable to estimate the invertebrate-specific effects on CR.

We estimated egestion by invertebrates using the material flow equation:

$$F = (P \times (1 - AE))/(AE \times NPE) \quad [2]$$

where F is egestion (mg DM/L sediment), P is the consumer production (mg DM L sediment^{-1} d^{-1}), AE is the assimilation efficiency (assumed to be 0.2), and NPE is the net production efficiency (assumed to be 0.4) (Benke and Wallace 1980, Hall et al. 2000). We estimated consumer production (P) using the final invertebrate biomass × a growth rate of 0.014/d (Benke 1984) × the days invertebrates spent in microcosms.

Added POM to each replicate depended on original POM, so we used the lowest observed POM of each replicate as the baseline concentration. We subtracted this baseline POM value from the POM recovered from each microcosm within that replicate to estimate change in POM during the experiment. Initial invertebrate lengths were not measured, so we estimated initial invertebrate biomass using the initial number of invertebrates in each invertebrate slurry × the proportion of slurry added to a particular treatment × the average individual biomass for recovered invertebrates. We then used these estimated initial biomasses to calculate potential invertebrate biomass contributions to POM and SOM.

Data analysis

We used replicates as blocks because of high variation among replicates (i.e., 3 treatments in 5 groups). This grouping also allowed us to identify the variation among replicates. We regressed each functional (k_N, k_2, k_1, k_N/k_1, and CR/SOM) and structural (SOM and POM) response variable against log-transformed invertebrate biomasses grouped by replicate. We calculated the mean of these slopes for each of the 5 replicates and then used a 2-tailed t-test to assess the difference between the mean slope and 0 for each response variable at an $\alpha = 0.05$ level.

TABLE 1. Average % contributions and absolute values of abundances (A, individuals/L sediment) and biomasses (B, mg dry mass/L sediment) of invertebrate taxa in each treatment category.

		%					
		Treatment					
		Low		Medium		High	
Taxon		A	B	A	B	A	B
Diptera							
Chironomidae		18.5	0.5	13.6	0.1	15.6	0.1
Chironomidae	Tanypodinae	3.4	4.9	1.9	0.4	7.3	0.6
Ceratopogonidae		0.0	0.0	0.0	0.0	0.4	0.0
Tipulidae	*Dicranota*	0.0	0.0	0.0	0.0	0.5	1.4
Tipulidae	Limoniinae	0.0	0.0	0.0	0.0	0.1	0.4
Tipulidae	*Hexatoma*	1.5	74.7	1.4	94.8	1.7	91.6
Ephemeroptera							
Baetidae		24.4	3.4	44.3	0.5	11.6	0.2
Plecoptera							
Capniidae		0.0	0.0	0.2	0.1	0.1	0.0
Chloroperlidae		0.0	0.0	0.5	0.3	0.4	0.1
Leuctridae		1.0	5.5	1.2	1.2	5.9	4.1
Trichoptera		0.5	0.2	1.4	0.0	0.0	0.0
Ostracoda		2.0	8.3	9.8	0.5	20.6	0.6
Harpacticoida		6.3	0.2	6.5	0.0	5.5	0.0
Hydracarina		20.5	1.8	17.7	0.3	26.8	0.4
Nematoda		0.0	0.0	0.2	0.0	0.9	0.1
Oligochaeta		2.0	0.4	1.2	1.8	1.9	0.4
Nematomorpha		0.0	0.0	0.0	0.0	0.6	0.0
Tardigrada		0.0	0.0	0.0	0.0	0.1	0.0
Totals							

Results

Treatment effects

Invertebrate manipulations provided a wide range of density, biomass, and composition of taxa (Table 1). There were 18 invertebrate taxa in the assemblage from the source stream. The experiment was designed to estimate the effects of invertebrate *biomass* (i.e., not diversity) on response variables, so taxa richness increased with increasing biomass. Thus, the effects of taxa diversity cannot be tested independently. Dipteran larvae, especially *Hexatoma* sp., dominated average biomass across treatments (Table 1). Major invertebrate contributors to abundance included early instars of Baetidae, ostracods, Hydracarina, and chironomids. Fourteen to 84% (mean = 38% by abundance) of the invertebrates originally added to each replicate were recovered with elutriated POM at the end of the experiment. The remaining 16 to 86% of the invertebrates presumably were eaten, contributed to POM (see Discussion), or remained in the sediment, contributing to SOM. Mean invertebrate densities in experimental microcosms were 18, 36, and 95 individuals/L sediment within low, medium, and high treatments, respectively (Table 1). Among all 15 microcosms, invertebrate biomasses ranged over 4 orders of magnitude from 0.003 to 44 mg DM/L sediment, with averages among 5 replicate blocks increasing with the intended treatment (Table 1).

Effects on standing crops of organic matter

POM ranged from 0.05 to 0.38 g AFDM/L sediment, with an average of 0.22 g AFDM/L sediment. POM increased with increasing invertebrate biomass across all 5 replicates (Fig. 2A). The average slope of POM against invertebrate biomass was significantly greater than 0 (Table

TABLE 1. Extended.

Values					
Treatment					
Low		Medium		High	
A	B	A	B	A	B
3.3	0.001	5.0	0.002	14.8	0.010
0.6	0.010	0.7	0.013	6.9	0.108
0.0	0.000	0.0	0.000	0.3	0.002
0.0	0.000	0.0	0.000	0.4	0.274
0.0	0.000	0.0	0.000	0.1	0.078
0.3	0.151	0.5	2.985	1.6	17.431
4.3	0.007	16.1	0.017	11.0	0.039
0.0	0.000	0.1	0.002	0.1	0.003
0.0	0.000	0.2	0.009	0.3	0.026
0.2	0.011	0.4	0.036	5.6	0.774
0.1	0.000	0.5	0.001	0.0	0.000
3.9	0.017	3.6	0.014	19.5	0.110
1.1	0.000	2.3	0.001	5.2	0.003
3.7	0.004	6.4	0.010	25.3	0.079
0.0	0.000	0.1	0.000	0.9	0.012
0.3	0.001	0.4	0.057	1.8	0.080
0.0	0.000	0.0	0.000	0.5	0.008
0.0	0.000	0.0	0.000	0.1	0.000
17.8	0.202	36.3	3.147	94.5	19.037

2, $p = 0.03$). Calculated egestion could account for an average of 34.1% (SE ± 18.4%) of increased POM, whereas unaccounted invertebrate biomass accounted for an average of 49.4% (SE ± 19.6%). SOM was not significantly related to invertebrate biomass (Table 2). SOM averaged 95% of total organic matter retrieved from the microcosms and ranged from 5.8 to 14.7 g AFDM/L sediment, with no consistent trends among replicates.

Effects on respiration

CR ranged from 0.16 to 0.55 mg DO L sediment^{-1} h^{-1}, with an average of 0.25 mg DO L sediment^{-1} h^{-1}. Sediments always had >5 mg DO/L. CR/SOM ranged from 0.017 to 0.064 mg DO h^{-1} g AFDM^{-1} and increased with increasing invertebrate biomass in each replicate (Fig. 2B; Table 2, $p = 0.03$). CR/SOM increased an average of 51% from the lowest to highest invertebrate biomasses. Non-normalized CR was not significantly related to invertebrate biomass.

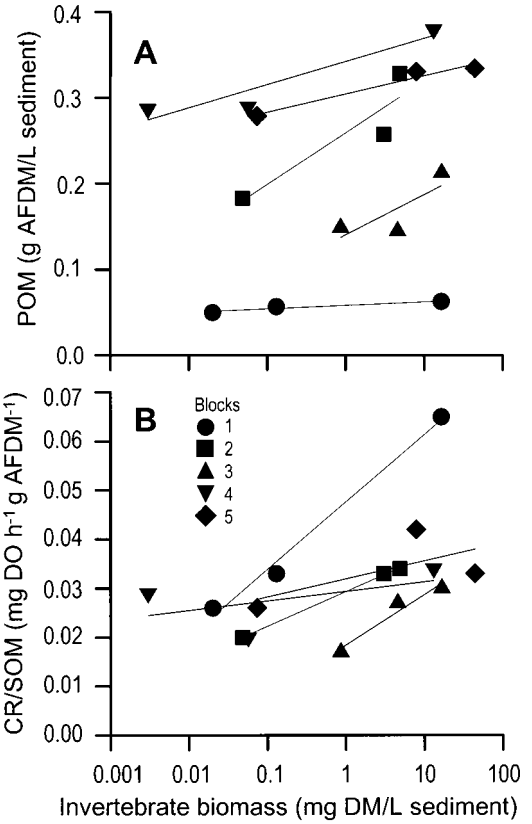

FIG 2. Relationship of particulate organic matter (POM) (A) and community respiration (CR, normalized by sediment organic matter, SOM) (B) to invertebrate biomass in hyporheic microcosms. Symbols are replicate blocks (not means) with 3 biomass treatments each. Lines are least-squares regressions for each replicate. AFDM = ash-free dry mass, DM = dry mass, DO = dissolved oxygen.

Effects on N cycling

Source ground water entering the microcosms contained naturally low, but measurable, concentrations of both NO_3^- and NH_4^+. Incoming concentrations ranged from 11 to 52 μg N/L as NH_4^+ and 4.9 to 13.6 μg N/L as NO_3^-. The NH_4^+ was quickly nitrified or assimilated by heterotrophic bacteria (see typical pattern in Fig. 3). As a result, NO_3^- concentration increased, although not as rapidly as NH_4^+ decreased in the upstream portions of the microcosms (Fig. 3). Occasionally, NH_4^+ peaks were

TABLE 2. Mean slopes and 95% confidence intervals (CI) for response variables regressed against the natural logarithm (ln) of invertebrate biomass. DM = dry mass, AFDM = ash-free dry mass, POM = particulate organic matter, SOM = sediment organic matter, CR = community respiration, DO = dissolved oxygen.

Response against ln invertebrate biomass (mg DM/L sediment)	Mean slope	95% CI		p[a]
		Lower	Upper	
POM (g AFDM/L sediment)	0.0139	0.0020	0.0258	0.032
SOM (g AFDM/L sediment)	−0.5430	−1.9117	0.8257	0.333
CR/SOM (mg DO h^{-1} g $AFDM^{-1}$)	0.0044	0.0007	0.082	0.030
NH_4^+ uptake rate (k_1,/min)	0.0038	−0.0017	0.092	0.126
NO_3^- regeneration/uptake (k_2,/min)	0.0015	0.0005	0.0025	0.015
Nitrification rate (k_N,/min)	−0.0007	−0.0015	0.0002	0.103
Fraction of NH_4^+ nitrified (k_N/k_1)	−0.0244	−0.0618	0.0130	0.144

[a] Probability value for slope not being equal to 0

observed mid-flowpath, indicating some NH_4^+ mineralization. Also, NO_3^- concentrations continually increased in most microcosms (i.e., little or no NO_3^- uptake) when NH_4^+ concentrations were low, suggesting that much of the NO_3^- production was from mineralization and nitrification of NH_4^+ within the microcosm. Modeled nitrification (using equation 1) fit the empirical data well for most microcosms ((model sums of squares)/(total sums of squares) ranged from 0.29 to 0.98; mean 0.82), suggesting that the rates derived from this model reflected the processes occurring in the microcosms (Fig. 3).

Nitrogen responses to invertebrate biomass treatments varied greatly among replicates (i.e., from one sediment batch to another) but, within replicates, invertebrate biomass was related to N cycling rates. Nitrate regeneration/uptake rate (k_2) ranged from −0.023 to +0.018/min, with an average of +0.005/min, and significantly increased with increasing invertebrate biomass (Fig. 4A; Table 2, $p = 0.02$). This result indicates that, as invertebrate biomass increased, sediments tended more toward producing than consuming NO_3^-. Nitrification (k_N) accounted for an average of ~35% of the NH_4^+ uptake rate (k_1) across all microcosms and treatments. Only 2 of

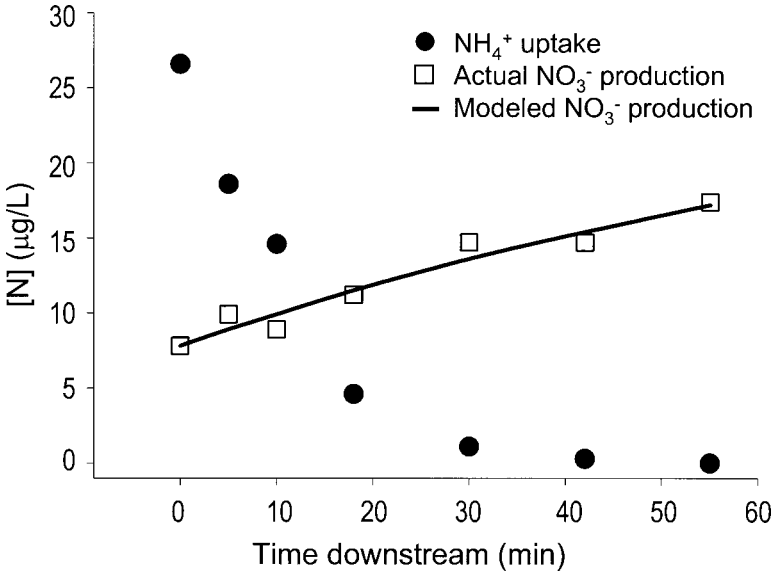

FIG 3. Example of typical NH_4^+ uptake, actual NO_3^- production, and modeled nitrification in one microcosm (not means).

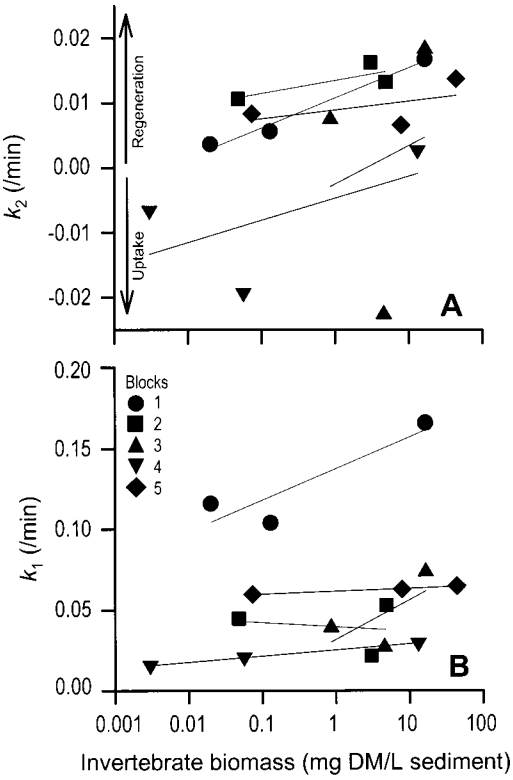

FIG 4. Relationships of NO_3^- regeneration/uptake (k_2) (A) and NH_4^+ uptake rate (k_1) (B) to invertebrate biomass in hyporheic microcosms. In panel A, positive values are NO_3^- regeneration and negative values are NO_3^- uptake. Symbols are replicate blocks (not means) with 3 biomass treatments each. Lines are least-squares regressions for each replicate. DM = dry mass.

15 k_N/k_1 (fraction of NH_4^+ nitrified) values approximated 100%, indicating all allochthonous NH_4^+ (see incoming concentrations above) was nitrified. All other k_N/k_1 values ranged from 1% to 48%, with an average of 19% of allochthonous or groundwater NH_4^+ being nitrified among remaining microcosms. k_1 (Fig. 4B), k_N, and k_N/k_1 were not significantly related to invertebrate biomass (Table 2).

Discussion

Invertebrates and hyporheic processes

It has been assumed that, because of their relatively small contribution to biomass and apparent respiration in hyporheic sediments (Pusch and Schwoerbel 1994), interstitial invertebrates contribute little to ecosystem processes (Jones and Holmes 1996b). More recently, researchers have hypothesized that invertebrates inhabiting freshwater sediments perform important roles within these ecosystems. Interstitial invertebrates in our study significantly affected both structural and functional ecosystem variables. POM and respiration increased with increasing invertebrate biomass (Fig. 2). For N-cycling measurements, invertebrate effects (within replicates) were small relative to overall (among replicate) variation (Fig. 4). The implications of these effects are that interstitial invertebrates play a significant role in hyporheic structure and function, but that these effects may be small relative to physical and chemical influences on hyporheic biogeochemistry.

Organic matter and respiration

In our microcosms, SOM ranged from 5.8 to 22 g AFDM/L sediment, with an average of 9.0 g AFDM/L sediment. Hedin (1990) found SOM ranged from 9.6 to 41.2 g AFDM/L sediment with an average of 18 g AFDM/L sediment (assuming a sediment depth of 5 cm) in benthic chambers, whereas Pusch and Schwoerbel (1994) found SOM (strongly associated particulate organic matter, SAPOM, in their terminology) ranged from 5.2 to 13.6 g AFDM/L sediment with an average of 10.2 g AFDM/L sediment in sediment microcosms. Unlike Hedin (1990), but like Pusch and Schwoerbel (1994), we found no significant relationship between SOM and CR among all replicates. This finding may be because 14 of our 15 SOM values were <10 g AFDM/L sediment, the low end of Hedin's range and, thus, less likely to show a significant relationship.

CR in our hyporheic microcosms (mean = 0.36 mg DO L sediment^{-1} h^{-1}) was comparable to other stream sediment studies, which ranged from 0.31 mg DO L sediment^{-1} h^{-1} in Steina, Germany (Pusch 1996) to 1.91 mg DO L sediment^{-1} h^{-1} in Sycamore Creek, Arizona (Jones 1995). Mermillod-Blondin et al. (2000) found higher DO consumption and dissolved organic C (DOC) uptake in the first few cm of sediment with benthic tubificid worms (Oligochaeta) than sediment without worms in flow-though columns. Mermillod-Blondin et al. (2000) attributed <10% of the actual respiration to oligo-

chaetes, but they suggested that additional respiration from slightly increased bacterial activity and biomass was most likely from worm-induced bioturbation. Bioturbation could also contribute to higher respiration in our microcosms with higher invertebrate biomasses. Although NO_3^- production was positively correlated with CR among replicates, nitrification could have accounted for only 15% of DO demand.

POM also increased with increasing invertebrate biomass (Fig. 2A). Low invertebrate recovery rates could explain some of the apparent increase in POM at the end of the experiment. About half (49.4%) of this POM increase corresponded to estimates of unaccounted invertebrate biomass initially added to microcosms. Part of the remaining increase in POM could be caused by invertebrate egestion (i.e., values on or under the 1:1 line in Fig. 5A). Egestion by consumers has long been recognized as an important component of pelagic marine (Turner and Ferrante 1979) and freshwater (Kitchell et al. 1979) ecosystems. In our microcosms, microbial biofilm could have been transformed to coarse fecal pellets by interstitial grazers such as harpacticoid copepods, ostracods, and midge larvae or prey material could have been egested as POM by interstitial predators such as *Hexatoma* sp. *Hexatoma*, an engulfing predator (Byers 1996), can account for up to 1.0 mg chironomid AFDM m^{-2} d^{-1} of organic matter flow between invertebrate predators and prey in benthic systems (Hall et al. 2000). Egested material from *Hexatoma* in our microcosms resembled the loose POM we collected at the end of the experiments, suggesting major POM generation.

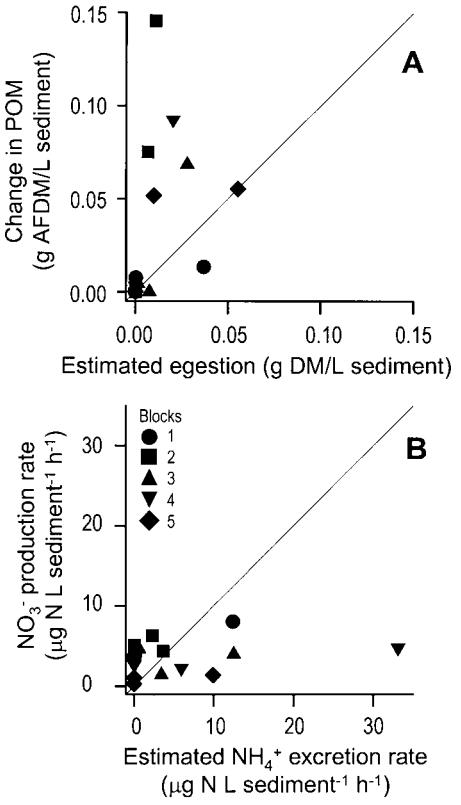

FIG 5. Effects of estimated invertebrate egestion relative to the particulate organic matter (POM) (A) and estimated invertebrate NH_4^+ excretion relative to the NO_3^- production (B). The x-axis (in panel A) is estimated invertebrate egestion in each microcosm and the y-axis is the observed POM found in each microcosm minus the lowest POM in each replicate. Values on or under the 1:1 line predict the same or less POM than potentially produced via egestion. In panel B, values on or under the 1:1 line indicate observed NO_3^- production rates equal to or less than those predicted by estimated excretion rates. Symbols are replicate blocks (not means) with 3 biomass treatments each. AFDM = ash-free dry mass, DM = dry mass.

Nitrogen cycling responses

The increase in NO_3^- regeneration or decrease in NO_3^- uptake rates with increasing invertebrate biomass (Fig. 4A) may have resulted from changes in the microbial assemblages or biofilm structure (Schramm et al. 1996), or perhaps from hydrodynamic changes mediated by invertebrate activities. Mermillod-Blondin et al. (2002, 2003) found that the redistribution of sediment particles differed among 3 different detritivorous invertebrate taxa, which changed the flow patterns and associated solute concentrations in their experimental microcosms. Mermillod-Blondin et al. (2001) observed lower concentrations of NO_3^- at the same depth (35 cm) in downwelling flow-though columns with tubificid worms than in columns without worms, suggesting suppression of nitrifier activity or abundance and/or enhancement of denitrification resulting from some invertebrate activity. Chatarpaul et al. (1980) found enhanced nitrification coupled to enhanced denitrification in the presence of oligochaetes. We similarly found an increase in NO_3^- production with increasing in-

vertebrate biomass, suggesting a nitrification-enhancing effect.

The simplest mechanism for this increase in nitrification is increased NH_4^+ availability via invertebrate excretion. NH_4^+ can be oxidized to NO_2^- and NO_3^- by nitrifying bacteria under oxic conditions (>1 mg DO/L) (Fenchel and Blackburn 1979). Assuming a moderate NH_4^+ excretion rate of 0.75 μg N mg DM^{-1} h^{-1} (Grimm 1988), and total nitrification of excreted NH_3, invertebrate excretion could explain <1 to >200% (mean = 52%) of NO_3^- production (Fig. 5B). Excretion from primary and secondary consumers contributes to the recycling of nutrients in both terrestrial and aquatic systems (Kitchell et al. 1979) and may have played a role in our study as well.

Invertebrate egestion may have indirectly increased nitrification in our experiment. Invertebrates in the stream sediments most likely consume detritus and associated biofilm (Bärlocher and Murdoch 1989). In addition, some benthic invertebrates obtain substantial amounts of their C from bacteria associated with detritus (Hall and Meyer 1998), relative to detrital C itself. Material egested by invertebrates generally contains less labile C than before ingestion (Maltby 1992, Boulton 2000b), presumably decreasing the overall labile C available for microbial assemblages. Strauss and Lamberti (2000) found that an increase in refractory C (or decrease in labile C) decreased competition for available NH_4^+ between heterotrophic bacteria and nitrifiers, leading to increased nitrification rates. This mechanism also seems plausible to explain the increased nitrification rates associated with increased invertebrate biomass in our study, given concomitant increases in POM (Figs 2A, 4A).

In conclusion, we found small, but significant, effects of intact hyporheic invertebrate assemblages on biogeochemical rates in flow-through, sediment-filled microcosms. We recognize that our laboratory study may not be easily extrapolated to field rates. However, the use of intact invertebrate and microbial assemblages in sediments from numerous stream locations likely reflects processes occurring in the field. A laboratory study was necessary because excessive environmental variability, particularly in flow rates, made relatively minor effects of invertebrates difficult to detect during experimental trials in the field (MCM, unpublished data).

Interstitial invertebrate assemblages are important to nutrient and organic matter processing in hyporheic microcosms. Nutrient processing in the hyporheic zone affects processes and biota in surface water (Dent et al. 2000), so the effects of hyporheic invertebrates becomes increasingly important as exchanges between surface and subsurface waters become more extreme. Land use and water regulation (diversion and storage) may not only temporarily change hyporheic processes, but also could disrupt the long-term functioning provided by hyporheic invertebrates, which may recover from disturbance more slowly than microbial assemblages.

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