The effects of augmentation of coarse particulate organic matter in hyporheic sediments

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Abstract

Metabolic and biogeochemical processes in hyporheic zones may depend on inputs of coarse particulate organic matter. Our research focused on how differing quantity and quality of organic matter affects metabolism and nutrient retention in the hyporheic zone of a low-order Appalachian stream. We hypothesized that hyporheic metabolic rates should increase with organic matter additions because the biotic activity is limited by organic matter availability. Four sets (n=4 amendments/set) of plots were established on a tributary of Hugh White Creek, NC. Sediment was extracted and was either supplemented with leaves, wood, or plastic strips, or sediments remained unamended sediments. Following augmentation sediments were reimbedded and approximately three months later sediment was removed from each plot. Aerobic and anaerobic metabolism were measured as the change in O_2 , and CO_2 in recirculating microcosms. At the same time, we monitored other possible terminal electron accepting processes and changes in nutrients. Aerobic metabolism was low in all treatments and respiratory quotients calculated for all treatments indicated that metabolism was dominated by anaerobic processes. Anaerobic and total (combined aerobic and anaerobic) respiration rates were significantly greater (P < 0.05) in plots treated with leaf organic matter compared to controls. Nutrient retention (NO_3 -N, NH_4 -N, and DOC) was enhanced in augmented treatments. Measured losses of dissolved organic carbon accounted for 50% total carbon liberated by respiration in amended sediments, whereas in unamended sediments loss of DOC represented only 7% of measured respiration. Augmentation with greater quality organic matter stimulated respiration in hyporheic sediments. Anaerobic processes dominated metabolic rates in both control and amended sediments. Enhanced metabolic rates increased retention of many solutes indicating that energy flow and nutrient

dynamics in the subsurface of streams may depend upon the quantity and quality of imported carbon.

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Dissolved oxygen measurements were initiated on July 29, 1999

Introduction

The interstitial environment of streams and rivers where conditions are influenced by both surface water and ground water is known as the hyporheic zone (Orghidan, 1959; Schwoerbel, 1961). Recent investigations have addressed the importance of organic matter in hyporheic zone (Herbst, 1980; Rounick & Winterbourne, 1983; Metzler & Smock, 1990; Boulton & Foster, 1998). Because energy flow in groundwater systems is heterotrophic and detritus-based (Culver, 1994; Jones, 1995), groundwater systems are almost completely dependent on allochthonous inputs and many ecosystems processes may be limited by organic matter availability (Culver, 1994; Jones, 1995). Consequently, allochthonous materials are crucial for subsurface metabolism and must be imported from the surface (Gibert, 1994; Pusch & Schwoerbel, 1994; Jones, Fisher & Grimm 1995; Jones, 1995).

An estimated 4 X 10¹⁴ g organic matter is being transported in the world's rivers (Meybeck, 1981; Schlesinger & Melack, 1981) and nearly half is particulate organic matter (POM) (Meybeck, 1981). Some of this organic matter may mix with entrained sediment and become buried by storms. Studying a blackwater stream in Virginia Metzler & Smock (1989) found, the hyporheic zone received up to 21% of autumnal leaf input as a result of spates. During their study, buried leaves were processed more slowly than leaves on the surface (25% of buried leaves remained after one year). Similarly, Herbst (1980) reported that 50% of autumnal leaf fall in a Wisconsin stream may be buried during floods. He found that buried leaves were processed more slowly than surface-placed leaves and emphasized the importance of leaf burial as a control on POM processing. Boulton & Foster (1998) found that leaves buried in a sandy bottom headwater stream broke down at the same rate as those on the surface.

When subsurface POM includes woody debris, standing stocks may be large. Metzler & Smock (1989) found that hyporheic sediments stored 4.8 kg m⁻² organic matter, an order of magnitude more than surface standing stock (Smock, Metzler & Gladden, 1989). Forty percent of this organic matter was wood greater then 8 mm diameter. Evans, Townsend & Crowl (1993) found that wood incorporated into the substrate of a New Zealand stream influenced bed stability,

and it was suggested that wood may both provide a colonizable surface and be used as a carbon source for microbial processing in the subsurface. Tank & Winterbourne (1996) found that buried wood lost about 39% dry weight over 250 days. This wood was colonized by fungi, bacteria and some species of macro- and micro-invertebrates. In a study conducted on headwater stream in New Mexico (Crenshaw, Valett & Tank in review), which examined fungal growth on woody debris placed in hyporheic sediments, showed fungal growth was associated with greater invertebrate density. These studies (Evans *et al.*,1993; Tank & Winterbourne, 1996; Crenshaw *et al.*, in review) indicated that woody debris in the subsurface may play a role in stability of the stream bed, as well as a role in supporting stream community metabolism.

Community metabolism rates in hyporheic sediments can be high in areas with coarse particulate organic matter (CPOM). Community respiration in the hyporheic zone of a mountain stream in Germany were shown to be similar to rates of benthic respiration (Pusch & Schwoerbel, 1994). In the case of the Stiena, a boulder dominated montane stream, interstitial POM was highly correlated with hyporheic community respiration (Pusch & Schwoerbel, 1994). Crocker & Meyer (1987) found that experimentally increased quantities of sediment POM resulted in increased amounts of interstitial dissolved organic carbon (DOC) and increased bacterial biomass. Sobczak, Hedin & Klug (1998) found that bacterial productivity and biomass at the soil-stream interface of a headwater stream in southwest Michigan were related to small scale spatial variations in sediment POC and not linked to DOC. Crocker & Meyer (1987) and Sobczak et al. (1998) argued for a linkage between interstitial POC and DOC and contended that contribution of DOC and POC to microbial production may vary temporally and spatially. Jones et al. (1995) showed that rates of metabolism in the hyporheic zone were directly correlated to surface algal production in a desert stream and that interstitial respiration was most likely supported by labile organic matter inputs in the form of DOC. Similarly Baker, Dahm & Valett (1999) found that respiration in the hyporheic zone of a headwater stream was limited by the availability of labile DOM. Battin (2000) found that biofilm activity (esterase activity and ³H thymidine incorporation) was positively related to streambed DOC retention efficiency, indicating the role of microbes in solute retention. Therefore, when supplied with labile organic carbon, metabolism of the hyporheic zone may be very important to the system as a whole (Grimm & Fisher, 1984; Jones et

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al.,1995; Jones, 1995).

Hyporheic respiration rates can decrease oxygen levels and can create areas of hypoxia or anoxia (Dahm, Carr & Coleman, 1991; Findlay, 1995; Baker *et al.*, 1999) with a potential for organic carbon mineralization by anaerobic microorganisms (Vroblesky & Chapelle, 1994). Hyporheic sediments can support strong gradients of redox potential and varying concentrations of many different solutes (Dahm *et al.*, 1998). Potential terminal electron acceptors (e.g. oxygen, nitrate, ferrous iron, sulfate, and carbon dioxide) are distributed heterogeneously both spatially and temporally. Baker *et al.* (1999) found that metabolic processes ranging from aerobic respiration to methanogenesis co-occur in the hyporheic zone along flowpaths of short lengths. Baker *et al.* (1999) showed that during summer base flow subsurface flow paths retained 20 % to 100 % of acetate which was added to represent labile low molecular weight DOC. Thus, interstitial sediments have the potential to transform and consume dissolved solutes.

Relatively little is known about how differing sources of POM may affect rates of metabolism once introduced into hyporheic sediments. Our research focused on how different quality of organic matter affects metabolism and nutrient retention in the hyporheic zone of a loworder Appalachian stream. With field augmentation and manipulation of sediment organic matter coupled to laboratory assays of microbial metabolism and nutrient cycling, we experimentally addressed how the quantity and quality of carbon supply alters rates of metabolism and associated terminal electron accepting processes (TEAPs, sensu Vroblesky and Chapelle, 1994). The objectives of this study were to address the impact of type and amount of organic matter on hyporheic metabolism and nutrient retention. Some sources of organic matter (OM) are more labile than others and should support greater hyporheic metabolism, resulting in increased nutrient retention.

Materials and Methods

Study Site

Field activities were carried out along a 95-m reach of a 1st-order headwater stream that drains into Hugh White Creek located in Coweeta Hydrologic Laboratory (Macon County, North

Carolina, USA) in the southern Appalachian Mountains. At the study site, the stream has an elevation of 800 m (Table 1). The climate is wet and cool and surrounding vegetation is mixed hardwood stands. The stream is heavily shaded with a rhododendron understory, which leads to low primary productivity (Webster *et al.*, 1997). The substrata consist mainly of cobbles and small boulders (Table 1). Discharge was estimated to range from 0.5- 4.5 L sec⁻¹ over the course of the study and dissolved nutrient concentrations were low (Table 1).

Organic matter incubation and dynamics

On June 15th, 1999, sediment from the stream was experimentally augmented with one of 4 treatments: leaves, wood, plastic, or no amendment. Sixteen blocks of streambed (0.5 m X 0.25 m X 0.25 m) were excavated at random locations along the study reach. Sediment from each plot was removed, manipulated with one of the four treatments and replaced to the respective pit. Thus, there were four independent replications per treatment group. Crushed white oak (*Quercus alba* L.) leaves were added to approximately double sediment percent organic matter (% OM). Similar amounts of white oak wood veneer were cut into 3 X 1 cm strips and added to the wood plots. Plastic was cut into 3 X 1 cm strips and mixed in with sediment and added to plastic plots in order to account for the possibility that organic matter may act as a site for microorganism colonization or alter hydrologic conditions. Sediment from the non-amended plots were mixed and returned to the plot and used to control for the effects of the extraction.

Sediment samples were taken from each plot after 3, 4, and 6 months. Organic matter (OM) content of sediment was determined as ash-free dry mass (AFDM) after sediment was dried at 60° C for 72 hours, weighed, and combusted at 550° C for 2 hours, and reweighed. Percent OM was determined as the percentage of total sediment dry weight represented as AFDM. Organic matter breakdown rates were calculated by regressing the natural log of percent organic matter over exposure time (three dates: October and November 1999, and March 2000). In order to determine breakdown rates only for organic matter experimentally introduced, average control (unamended) % OM was subtracted from average % OM in leaf and wood amended plots for each date and breakdown rates for added organic matter were determined as described above.

Carbon (C) to nitrogen (N) ratios were determined on sediment samples collected on

October 10, 1999 using dry combustion with a Carlo-Erba NA2100 soil analyzer with a hightemperature combustion furnace on oven-dried samples that were ground to a fine dust. Three replicates were run for each sediment sample from each plot and C:N in each plot was represented by the mean.

Physical and chemical characteristics

After sediment plots were established along the study reach, plots were equipped with monitoring wells, which were sampled every two weeks during the 3 month study. Wells were constructed of 5-cm diameter poly vinyl chloride (PVC) with a screen length of 10 cm and were inserted 20 cm into the streambed. Interstitial water from within plots was collected using a 60ml plastic syringe placed on the end of Tygon tubing (approximately 50 cm) to slowly draw 50 -100 ml of interstitial water from each well. Surface water samples were collected from the thalweg using similar techniques. Samples were filtered in the field with 47-mm glass fiber filters (Whatman GF/F), stored in acid-washed polyethylene bottles, and placed on ice until they were returned to the lab. Chemical analyses included chloride (Cl), nitrate-nitrogen (NO₃-N) and nitrite-nitrogen (NO₂-N) by ion chromatography with a Dionex DX-500. Ammonium-nitrogen (NH₄-N) and phosphate-phosphorus (PO₄-P), measured as soluble reactive phosphorus, were analyzed on a Technicon AutoAnalyzer following techniques of Solorzano (1969) and Murphy & Riley (1962), respectively. Total inorganic nitrogen (TIN) was calculated as the sum of NO₃-N and NH₄-N plus NO₂-N. Atomic N:P ratios were calculated as molar ratios of TIN and PO₄-P (sensu Grimm & Fisher, 1986). Samples were analyzed for DOC using an Oceanography International 700 Total Carbon Analyzer by wet persulfate digestion (Menzel & Vaccaro 1964). All chemical analysis occurred within 48 hours of collection. Dissolved oxygen was measured using a YSI DO (model 55, YSI Inc.) probe before water was extracted from the monitoring wells.

Mean substrate particle size (MPS) was determined using standard granulometry techniques (Wolman, 1954) on 95 randomly selected rocks and a USGS gravelometer (FISP US SA-97). Vertical hydrological gradient was determined by measuring the pressure differential in mini-piezometers (Lee & Cherry, 1978). Mini-piezometers were made of 1.27 cm diameter PVC

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pipe cut 1 meter in length, placed in the active channel, and inserted to a depth of 20 cm. Minipiezometers were placed every ten meters, additionally one was placed inside each plot and another directly outside each plot.

Sediment extraction

After 3 months, sediment plots were sampled on October 10, 1999. The top layer (0-5 cm) of each plot was scrapped off to remove surficial influences after which approximately 200 grams (wet weight) sediment was removed (5 - 20 cm depth) and placed into a whirl-pak. This was done in four different locations in each plot to account for spatial heterogeneity within each plot. Sediment was placed on ice and taken to the lab after which metabolism assays were run within 48 hours of extraction. Water samples for nutrients and for microcosm circulation (see below) were taken before sediment was extracted from plots.

Microcosm assays: heterotrophic metabolism and solute dynamics

Heterotrophic metabolism was measured using recirculating microcosms following modified methods from Pusch & Schwoerbel (1994), Jones (1995) and Baker *et al.* (2000). Microcosms were constructed of clear plexiglass tubes (20 cm long, 7 cm diameter) in which the inside of the tubes were roughened with sandpaper to prevent preferential flow along the inner walls. Sediment (approximately 800 g wet wt.) from each plot was placed into a microcosm. Water used for circulation during metabolism assays was groundwater from the respective well and from surface water near the plot in order to mimic the downwelling conditions characteristic of the study reach. Before circulation, the water was deoxygenated by bubbling helium until the DO was at ambient concentrations determined from each respective well on the day of extraction. Microcosms were purged with the deoxygenated water for two hours before sampling began in order to rid microcosms of residual air gases.

Water was recirculated through the microcosms at an estimated subsurface pore velocity of 1.6 cm sec⁻¹ using a peristaltic pump. The chambers were run for 6 hours in the dark at ambient stream temperature (10 °C) during which time dissolved gases and nutrient samples were taken every two hours. A total of 15 mls were extracted during each sampling period.

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An initial 5-ml sample of microcosm water was obtained by routing the circulating loop to a sample syringe and allowing the pump to displace the syringe to avoid degassing the water. This sample was analyzed for dissolved oxygen measured using a modified Winkler technique (Wetzel & Likens, 1984). Another 5-ml water sample was extracted in a sampling syringe and equilibrated (shaken vigorously for 1 minute) with 5 mls of air and headspace gas was transferred to clean evacuated 3-ml Vacutainers[®]. We measured pCO₂ using a syringe headspace equilibration technique (Kling *et al.*, 1992). The pCO₂ sample were analyzed on a gas chromatograph with a thermal conductivity detector (SRI 8610). Dissolved CO₂ concentrations were calculated from Henry's Law using pCO₂ pressure, and temperature (Kling *et al.* 1992).

A final 5-ml sample of water was used for analysis of dissolved nutrients, and CH_4 and Fe^{++} were extracted from microcosms as described above. Methane samples were processed by placing 1.5 mls of the water sample into evacuated Vacutainers[®] vials. Methane was then released from the water by agitation of the sample and analyzed on a gas chromatograph fitted with a flame ionization detector (deAngeles & Lilley, 1987; Dahm *et al.*, 1991). Dissolved ferrous iron (Fe⁺⁺) was measured colorimetrically on 1.5-ml samples, which were passed through a 0.2-mm filter and reacted with 40 mls of Ferrozine[®] (Stookey, 1970). The remaining water was used in analysis of dissolved nutrients (Cl, NO₃-N, NH₄-N, SRP, DOC) as described earlier.

Aerobic respiration rates were calculated as the change in DO over time. Metabolic rates were also determined by the rates of CO_2 liberation, and total metabolism was determined as the increase in $CO_2 + CH_4$ over time (Baker *et al.*, 1999). Respiratory quotients were calculated as the molar ratio of CO_2 produced to O_2 consumed.

Net production or consumption of solutes was used to address the fate of potential terminal electron acceptors. Regression of solute concentration changing with time was used to quantify rates of consumption/production for NO_3 -N, Fe⁺⁺, SO₄, CH₄ and DOC. Potential for DOC to serve as an electron donor was addressed by comparing rates of DOC consumption to total metabolic rates.

A second set of sediment microcosms were taken to determine denitrification rates quantified using the acetylene block technique (Duff & Triska, 1990). Sediment (approximately 250 mg wet wt) was placed in microcosms (clear 5-cm diameter PVC capped on both ends) with

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250 mls of stream water. Microcosms were made anaerobic by sparging with helium for 10 minutes before capping. Gas samples were taken 0, 1.5, 3, 6 and 12 h after capping and were analyzed for N_2O using a GC (SRI 8610) fitted with an electron capture detector.

Residence times for POM and DOM in recirculating core sediment were calculated by dividing the OM standing stock by respiration (total metabolism rate quantified in each sediment microcosm) and represent the average amount of time a molecule of OM would remain before being oxidized by microbial activity. Turnover time represents the fraction of OM oxidized per unit time and is the inverse of the residence time.

Statistical analysis

All ratios and percentages were arcsin square-root transformed before analysis, and all data presented are back transformed. Organic matter data were normalized by log transformation prior to statistical analysis. Analysis of covariance (ANCOVA) was used to compare breakdown rates (k) among treatments (i.e. OM manipulations) using the natural log of %OM as the dependant variable and time as the covariate (Webster & Benfield, 1986). One-way analysis of variance (1-way ANOVA) was used to compare differences among treatments for a number of dependant variables including changes in concentration of CO_2 , O_2 , N_2O , Fe^{++} , SO_4 , CH_4 , NO_3 -N, NH_4 -N and DOC. Sediment C:N, OM, residence time, and and turnover ratios were also analyzed with a one-way analysis of variance. A Tukey post-hoc test followed significant ANOVA (P < 0.05) was used to compare nutrient concentrations in wells and surface water during the experiment.

Results

Concentrations of NO_3 -N, NH_4 -N, SRP, DOC and N:P of the surface water were low and relatively constant during the three month study. Dissolved oxygen concentrations in the stream varied from 8 -10 mg L⁻¹ and remained near saturation (Table 1). Dissolved oxygen concentrations, which

were much lower, ranging from 1 - 4 mg L⁻¹ with no significant differences among any of the treatments (Fig. 1A). Dissolved organic carbon concentrations in the surface water ranged from 1.5 to 5 mg L⁻¹ over the incubation and typically remained lower than interstitial water concentrations. Interstitial DOC concentrations varied during the study. For the first 4 weeks of the experiment DOC content in leaf litter plots was elevated compared to other treatments with a high of greater then 50 mg L⁻¹ (Fig. 1B). By the end of the study, wood plots had the highest DOC concentrations (Fig. 1B). Dissolved organic carbon concentrations were relatively low (2-5 ppm) and invariant in control and plastic plots.

Over the course of the study, subsurface NO₃-N increased in all treatments from less then $10 \ \mu g \ L^{-1}$ to about 20 $\ \mu g \ L^{-1}$ except in plastic plots (Fig. 2A). Ammonium in the two control plots increased by 40 $\ \mu g \ L^{-1}$. Concentrations of NH₄-N in wood treatments were very low and did not fluctuate (Fig. 2B). By contrast, NH₄-N increased steadily in leaf treatments from about 20 $\ \mu g \ L^{-1}$ to over 90 $\ \mu g \ L^{-1}$ (Fig. 2B). During the first two weeks of incubation PO₄-P concentrations were elevated in all treatments ranging from over 30 $\ \mu g \ L^{-1}$ to 10 $\ \mu g \ L^{-1}$. Concentrations then dropped and remained low (< 5 $\ \mu g \ L^{-1}$) throughout the experiment.

Breakdown rates and sediment characterization

Percent organic matter of hyporheic sediment prior to treatment was 1.5 ± 0.1 (Table 1) and after the 3mo. study ranging from a low of 1.4 ± 0.3 in the control plots to a high of 2.9 ± 0.4 in leaf plots (Table 2). Percent OM was significantly greater in the leaf and wood amendments than in the other two treatments (P = 0.0005). Carbon to nitrogen ratios varied between 29.5 to 32.5 and did not vary significantly among treatments (Table 2).

Breakdown rates did not differ significantly from each other (P = 0.06) and ranged from 0.0039 d⁻¹ in leaf plots to 0.00061 d⁻¹ in the plastic plots (Table 3), although the breakdown rates for leaf and wood treatments were six times greater then the control plots. Breakdown rates for added OM were 0.0039 d⁻¹ in the leaf plots and 0.0022 d⁻¹ in wood plots (Table 3) and were not significantly different (P = 0.13).

Heterotrophic metabolism and biogeochemical processes

Aerobic respiration rates ranged from 0.0170 ± 0.06 mg• L sed ⁻¹• h⁻¹ in the wood treatments to a high of 0.03 ± 0.04 mg• L sed ⁻¹• h⁻¹ in the leaf treatments (Fig. 3A) and did not differ significantly among treatments (P > 0.05). Metabolism as measured by CO₂ production was significantly greater (P < 0.05) in the leaf enriched treatments (0.95 ± 0.2) than in other treatments (Fig. 3A). Respiratory quotients were greater in the carbon amended treatments with an RQ over 45 in leaf treatments. RQ for control and plastic treatments averaged 15 and 8 respectively (Fig. 4B). Total carbon (TC) liberated was significantly greater (P < 0.05) in leaf treatments (0.21 ±0.08 mg• L sed ⁻¹• h⁻¹) than in plastic and control (0.06 ± 0.009 mg• L sed⁻¹• h⁻¹ and 0.08 ± 0.02 mg• L sed ⁻¹• h⁻¹ respectively) (Fig. 3C). Wood treatment mean was not significantly different from the other three treatments (Fig. 3C). Methane - C contributed from 1.7 % of total C produced in plastic treatments to 27 % in leaf treatments (Fig. 3C).

Addition of OM to the hyporheic zone stimulated biogeochemical transformations as indicating by altered rates of microbial TEAPs (Table 4). Rates of denitrification (i.e. accumulation of N₂O) were greatest in the wood treatment $(0.05 \pm 0.02 \,\mu\text{g} \cdot \text{L} \text{ sed}^{-1} \cdot \text{h}^{-1})$, whereas lowest rates of denitrification occurred in leaf treatments $(0.02 \pm 0.005 \,\mu\text{g} \cdot \text{L} \text{ sed}^{-1} \cdot \text{h}^{-1})$ (Table 4). Changes in NO₃-N concentrations in recirculating microcosms, showed the same trend (Fig. 4B) with all microcosms showing NO₃-N consumption. Highest and lowest rates occurred in treatments amended with wood and leaves, respectively. There were no significant differences in the rates of NO₃-N consumption among treatments. Rates of Fe⁺⁺ accumulation were greatest in the leaf treatments (70.0 ± 30.0 $\mu\text{g} \cdot \text{L} \text{ sed}^{-1} \cdot \text{h}^{-1}$) (Table 4). While rates of change in Fe⁺⁺ concentration were essentially zero for wood and control treatments (Table 4), some Fe⁺⁺ accumulation was noted for the plastic treatment but rates were highly variable (CV = 120%). Sulfate consumption was evident in the wood and plastic treatments, while sulfate remained relatively unchanged in leaf and control treatments (Table 4). Rates of methanogenesis were significantly greater (P = 0.007) in leaf treatments (60.0 ± 20.0 $\mu\text{g} \cdot \text{L} \text{ sed}^{-1} \cdot \text{h}^{-1}$) than in the other three treatments where rates ranged from 2 to 3 $\mu\text{g} \cdot \text{L} \text{ sed}^{-1} \cdot \text{h}^{-1}$ (Table 4).

Consumption and production of other solutes in recirculating microcosms differed among treatments (Fig. 4) On average, DOC was consumed in all treatments except plastic, but rates of consumption were not significantly different among treatments. Consumption of DOC was

greatest in wood and leaf treatments and was not different from zero in the control and plastic (Fig. 4A). Ammonium accumulated in leaf treatments at a rate significantly greater (P < 0.05) than in plastic treatments where, as was the case with control and wood treatments, NH_4 -N was consumed (Fig. 4C). Dissolved organic matter consumption by leaves and wood treatments represented about 50 % total carbon liberated by respiration during microcosm circulation (Table 5), whereas plastic and control consumption of DOC was between 3% and 7 % of observed respiration.

Retention of POM and DOM in hyporheic sediments

Based on OM standing stocks and DOC concentrations measured in sediment microcosms, the residence time of organic matter in the subsurface varied greatly among treatments (Table 5). The residence time of POM was higher in the control treatments (plastic = $9.4 \pm 4.1 \text{ y}^{-1}$ and control = $5.9 \pm 1.3 \text{ y}^{-1}$) then in the enriched treatments where residence time was about 4.4 y⁻¹ (Table 5). The same pattern was seen for DOM in the treatments where residence times were approximately 20 d⁻¹ for control and plastic and approximately one week for OM augmented treatments (Table 5). Turnover rates were short for POM and rapid for DOM. Turnover rates for POM ranged from 0.16 y^{-1} in plastic treatments to 0.25 y^{-1} in leaf treatments. Dissolved organic matter turnover rates were much more faster ranging from 0.07 d^{-1} in control

Discussion

Impacts of OM addition on OM dynamics

Percent organic matter in sediments of various aquatic systems bracket those reported in this study (1.4 to 2.9). Pusch & Schwoerbel (1994) reported 0.8 % OM in sediments of the Steina River, Germany. Bott & Kaplan (1985) described 0.6% OM for streambed sediments in White Clay Creek, PA. Crocker & Meyer (1987) showed % OM ranging from 4.6% to 1.5% on native sediments for Dry Man's run in Coweeta. Rublee (1982) reported 0.6% for a salt marsh system. Schallenberg & Kalff (1993) contended that %OM in lake sediments in Quebec and Ontario ranged from 0.9% to 20.3% OM with a mean of 5.2%. Miller & Reed (1975) studied 2 ponds in Alaska and found % OM to be high, between 70% and 85%. Sediment in our study had greater (approximately 2-fold) % OM in the amended plots but these values are similar to naturally occurring conditions in Appalachian streams while they are not as high as in many lake or pond sediments (Miller & Reed, 1975; Crocker & Meyer, 1987; Scallenberg & Kalff, 1993).

Despite doubling OM content, C:N ratio varied little among treatments and ranged from a low of 29 to a high of 33. Meyack et al. (1989) found that leaves buried in the subsurface of an upper coastal plain stream had almost double our C:N ratios after similar burial time. Benthic leaves and wood of Upper Ball Creek in Coweeta had C:N ratios of 50 and 45 respectively (Findlay et al., in review), whereas in Bear Brook, New Hampshire, the C:N ratios were similar to ours with leaves at 21 and wood at 37 (Findlay et al., in review). Baker et al. (1983) manipulated sediments by adding different types of wood or bark to the sediment of a 2nd- order stream in Oregon. They reported that C:N ratios of red alder (Alnus rubra Bong.) wood and bark before adding it to the sediment were 424 and 61 respectively. After four weeks of burial, control sediments (nothing added) had a C:N of 25.3 and sediments amended with wood and bark were 32.9 and 24.9. After 28 weeks, control sediment C:N ratios were 21 and wood and bark were 29 and 23. Despite adding leaves and wood, C:N ratios remained essentially unchanged in sediment plots. This suggests that native sediment contained OM of similar stoichiometric composition as the added material. These results are consistent with perspectives that ground water environments are import dependant (Gibert et al., 1999) and that leaves and wood may be primary sources of OM to the subsurface of headwater streams.

Organic matter breakdown rates in our sediments are similar to other work done on CPOM decomposition (e.g. Peterson & Cummins, 1974; Webster & Benfield, 1986; Meyack *et al.*, 1987; Metzler & Smock, 1990). Webster *et al.* (1995 and authors therein) reported various published breakdown rates for white oak leaves ranging from .0021 to .0105 d⁻¹. When k values are not corrected for background % OM, our breakdown rates are similar to studies on the benthos (Webster *et al.*, 1995) and studies in the subsurface. For example, Meyack, Thorp & Cothran (1987) found that breakdown rates of sweetgum (*Liquidamber styraciflua* L.) litter in the subsurface ranged from 0.00196 to 0.0052 d⁻¹ whereas our values for leaf plots were 0.0039

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d⁻¹. Metzler & Smock (1990) found blackgum (*Nyssa sylvatica*, Marsh.) (0.005 d⁻¹) and red maple (*Acer rubrum*, L.) and white ash (*Fraxinus americana*, L.) (0.003 d⁻¹) broke down at similar rates to ours. As for the wood enriched sediment, Tank & Webster (1998) found that white oak had decomposition rates of 0.0015 d⁻¹ and poplar sticks broke down at a rate of 0.004 d⁻¹. Meillo *et al.* (1983) reported breakdown rates for a variety wood (0.0007 to 0.003 d⁻¹), and Golladay & Sinsabaugh (1991) had similar breakdown rates on birch ice cream sticks (0.0016 - 0.0019 d⁻¹). Webster *et al.* (1999) report breakdown rates of sticks (< 3 cm diameter) ranging from 0.00017 to 0.00103 d^{-1.} Our rates were within the range of other studies though generally some what faster. We suggest that rapid decomposition are due to the high surface area of the pieces of wood veneer. As compared to other studies (Meillo *et al.*, 1983; Golladay & Sinsabaugh, 1991; Webster *et al.*, 1999) and faster then those calculated for small woody debris in Coweeta (0.0004 - 0.0008 d⁻¹, Golladay & Webster, 1988).

When corrected for background %OM, breakdown rates for the leaf and wood were fast. Decomposition of % OM of leaf treatments were 0.0039 d⁻¹ and rates for wood were 0.0022 d⁻¹. Findlay, Smith & Meyer (1986) found breakdown rates of 0.0023 d⁻¹ for oak leaves in the Ogeechee River. Peterson & Cummins (1974) report that white oak leaves usually have slow breakdown rates (0.005 d⁻¹) and because of the lignin and cellulose content of wood, results should show even slower breakdown rates (Melillo *et al.*, 1983; Wallace, Ross & Meyer, 1982). Burial of OM should also decrease breakdown rates (Harmon *et al.*, 1986), but Smith & Lake (1993) reported breakdown in the subsurface at rates similar to those on the surface.

Leaves deposited on the streambed usually leach carbohydrates and polyphenols (Suberkropp *et al.*, 1976) within a few days (Webster & Benfield, 1986). Meyer, Wallace & Eggert (1998) reported that mean DOC concentrations in stream water was directly related to streambed leaf-litter standing stock. They found that about 30% of DOC export is due to instream generation of DOC produced by leaf litter. However, McDowell & Fisher (1976) found that DOC concentrations did not change in the stream after autumnal leaf fall, suggesting that DOC is removed rapidly. Webster & Meyer (1997) found that streams dominated by allochthonous inputs are inefficient at processing organic matter and export more material (mainly refractory) then is metabolized. During our study, stream water showed no response to the DOC

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release of the subsurface sediments, and DOC concentrations in surface water remained low. This is not surprising given the downwelling characteristics within our study reach. Similarly, Crocker & Meyer (1987) reported no response of stream water DOC concentrations to experimentally increased subsurface POM. In our study, leaf plots had very high concentrations of DOC after 4 weeks of burial. Thereafter, DOC in leaf plots decreased and at the time of metabolism assays there were no differences in DOC concentrations among treatments.

Effects of organic matter on heterotrophic metabolism

Significantly greater rates of heterotrophic metabolism in the leaf enriched sediments indicate that POM may increase respiration in hyporheic sediments as suggested in other studies (Pusch & Schwoerbel, 1994; Pusch, 1996). Jones (1995) also suggested that POM supported hyporheic metabolism in a headwater stream, and Crocker & Meyer (1987) found that with increased POM there was higher bacterial biomass in the sediment of a 2nd-order stream. Findlay, Smith & Meyer (1986) added oak leaves and alligatorweed to the sediment of a 6th-order blackwater stream and showed a higher rate of respiration in sediment with alligatorweed after 4 d⁻¹. They also found that rates of respiration did not change over 14 d⁻¹ following augmentation with oak leaves. Pusch & Schwoerbel (1994) found that POM in hyporheic sediment was highly correlated with hyporheic community respiration and suggested that the production of DOC from this buried POM may be a major factor in hyporheic metabolism.

Particulate organic matter that leaches DOC may increase microbial biomass by acting as an energy source to interstitial biofilms (Crocker & Meyer, 1987). Feibig & Lock (1991) showed that groundwater may contribute DOC to stream systems and that this DOC was immobilized by the streambed at rates that ranged from 62.2 to 205.9 g \cdot m⁻² \cdot h⁻¹. Wallis et al (1981) showed that DOM concentrations from groundwater were not manifested in increased stream water concentrations during storm runoff because of the ability of streambed bacteria to rapidly use imported DOC, but Hendricks (1996) reported that increased DOC enhanced bacterial production and metabolic activities in the downwelling zone of a 3rd-order sandy bottom Michigan stream. Similarly, Lush & Hynes (1978) showed rapid uptake of leaf leachate and Jones (1995) found higher rates of metabolism in downwelling areas of a desert stream, which he attributed to DOC transport from benthic algae. Enriched leaf plots in our study did leach DOC and have a reduction in O_2 , although the concentrations of O_2 and DOC among treatments were not significantly different from each other when metabolism was measured. Crocker & Meyer, 1987 suggests that benthic POM may be a source of hyporheic DOC, depending on the molecular weight of the DOC, sediment organic matter content, and hydrologic exchange.

In our study, the consumption of DOC in the recirculating cores accounted for less then 50% of total C metabolized, suggesting that DOC is not be the sole carbon source for microbial respiration. These data suggest that both DOC and POC are important components of subsurface energetics in this headwater stream.

Metabolism rates in our study suggest that increased POM lowered dissolved oxygen levels and limited aerobic respiration. At the same time, RQs in the control treatments indicated that the unmanipulated system supported considerable anaerobiosis as well. The occurrence and importance of anoxia in hyporheic zones is becoming more well known as a greater variety of conditions is addressed (Dahm *et al.*, 1987). Baker *et al.* (1999) reported RQs in the subsurface of a headwater stream that ranged from <1 to over 999. Morrice *et al.* (1997) found that hyporheic water moves slower than surface water and may have areas of anoxia due to microbial uptake of O₂ (Dahm *et al.*, 1991; Baker *et al.*, 1999).

In areas where O_2 supplies are diminished, alternative electron acceptors are used in organic matter catabolism. Organic matter decomposition can be carried out via the reduction of O_2 , NO_3 -N, Fe^{+++} and $SO_4^{=}$, or via CH_4 production depending on the availability of the different terminal electron-accepting compounds in a system (Vroblesky & Chapelle, 1994; Strumm & Morgan, 1996). The loss of DO and changes of solutes and terminal electron acceptors in our system indicated that besides altering aerobic respiration, increased organic matter can increase other electron accepting microbial processes (Table 4). Legendre *et al.* (1994) showed a significant correlation between POM and the production of CO_2 and CH_4 in Hamilton Harbor (Lake Ontario, Canada). Baker *et al.* (1999) found that with added labile organic carbon (acetate) rates of O_2 consumption and anaerobic processes increased and organic carbon retention was caused mainly by anaerobic metabolism. This occurred in our study as well: higher quality OM (leaf treatment) generated highest rates of respiration and highest rates of Fe⁺⁺

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accumulation and greatest CH_4 production. Low aerobic respiration rates and highest CH_4 production in the leaf treatments suggest that the supply of other terminal electron acceptors was diminished (as seen in well data).

Effects of increased OM on nutrient dynamics

Retention of nutrients in streams occurs mainly at or within the streambed (Meyer *et al.* 1988), thus hydrologic processes that favor exchange between surface and interstitial water will enhance the retention and recycling of nutrients (Jones, 1995; Valett *et al.*, 1996). In a comparison of three streams with different parent lithology, Valett *et al.* (1996, 1997) showed that the three streams displayed different storage sizes, which resulted in a correlation between the extent of interaction of groundwater and surface water and nitrate retention by the stream ecosystem.

Retention of nutrients depends upon the interaction of hydrologic, chemical and biologic processes. In an Appalachian headwater stream with up to 10 cm of gravel sediments overlying bedrock, chloride tracer infiltrated rapidly, especially in coarser sediments, reached the deepest sediments within minutes and equilibrating within hours (Munn & Meyer, 1988). Grimm & Fisher (1984) compared cores of living and killed hyporheic sediments and found that rates of nitrate retention were higher in the live cores and that the killed sediments released inorganic nitrogen. Holmes *et al.* (1998) found that denitrifiers retained from 5 to 40% of nitrate produced by nitrifiers. Munn & Meyer (1990) compared two headwater streams, one in North Carolina and one in Oregon, and found that the Oregon stream was most retentive of NO_3 -N and retention of SRP was greater in the North Carolina stream. They also showed that sites with downed timber, which retained both fine and coarse particulate organic matter, displayed higher rates of NO_3 -N retention.

In our study, NO_3 -N and DO concentrations decreased in the leaf treatments while NH_4 -N increased over time. This, coupled to the results of our denitrification assays suggests that ammonification was occurring along with denitrification. A gradual depletion of O_2 that creates semianaerobic conditions appears to allow normal denitrification processes to occur (Payne *et al.*, 1971). At the same time, nitrification was probably limited by the lack of DO and NO_3 -N was depleted by denitrifiers. Hedin *et al.* (1998) reported that the removal of NO_3 -N and N_2O were limited by oxidizable carbon availability in short hyporheic flowpaths of Smith Creek, Michigan. This suggests that in our leaf plots, denitrification occurred early on with high levels of DOC and POM and later decreased due to lack of NO_3 -N.

Effects of OM on system energetics

Organic matter in POM-enriched treatments had similar residence times to natural sediments along our study reach. This indicates that processing of OM is occurring at similar rates (Table 5) in enriched and control treatments and demonstrates that with increased POM that the respiration will increase as well, but all the POM will be gone at similar rates. Turnover rates for POM ranged from 0.16 to 0.25 y^{-1} and is similar to rates (0.04-0.641 y^{-1}) found by Pusch & Schwoerbel (1994). Pusch (1996) reported turnover rates for POM as 0.245 y^{-1} to 0.51 y^{-1} for pools and riffles in the Stiena River during baseflow conditions.

Compared to POM, residence times for DOM were much shorter and rates of DOM consumption were high. As a result, turnover rates for DOM were all less than one, ranging from 0.07 d⁻¹ in the control treatments to 0.32 d⁻¹ in the leaf treatments. This is an indication that DOM is present in short supply compared to microbial respiratory demand. Dissolved organic matter may be rapidly taken up either by microbes or by sorption to sediments (Findlay & Sobczak, 1996). Battin (2000) found that DOC residence times were lower than ours (0.05 d⁻¹) and these differences may relate to the size and quality of compounds comprising the DOC pool (Crocker & Meyer, 1987). In any case, consumption of DOC in our study accounted for only 50% of the observed respiration rates, emphasizing that DOC is not the sole source of energy for respiratory processes.

In our system, anaerobic metabolism occurred at very high rates and may play an important role in whole stream metabolism. In order to test this we assumed a hyporheic depth of 20 cm and an RQ of 0.85 (Kaplan & Bott, 1985) and calculated areal respiration rates (g C•m⁻²• d⁻¹) for the study stream. This calculation was based on total carbon liberation from recirculating cores representing unamended (i.e. control) conditions (Fig. 6). Whole stream metabolism was run on HWC during August of 1999 (Fellows 2000) indicating a whole stream respiration rate of

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0.79 g C• m⁻²•d⁻¹. At the same time, Fellows (2000) determined rates for benthic respiration using the chamber method (Bott *et al.*, 1978; Dodds & Brock, 1998) and derived a value of 0.184 g C• m⁻²•d⁻¹. By difference, hyporheic respiration was 0.61 g C• m⁻²•d⁻¹. Extrapolation from aerobic respiration rates determined in our experimental microcosms yielded a value 10 times lower (0.05 ± 0.02 g C• m⁻²•d⁻¹), but application of anaerobic rates determined from total C liberation resulted in a rate (1.33 ± 0.4 g C• m⁻²•d⁻¹) over double that determined by Fellows (2000).

Differences between hyporheic aerobic respiration rates determined from microcosms and whole system approaches may result from at least two factors: methodological artifacts or errors in estimation of hyporheic sediment depth. Microcosm aerobic respiration represents sediment from 10- 20 cm run at ambient O_2 concentrations (i.e. ca. 1- 2 ppm for initial oxygen concentrations). Because recirculating cores isolate the system from continued oxygen input, volumetric rates of aerobic respiration may decreased within microzones due to oxygen limitation. In addition, determining the appropriate size (i.e. depth) of the hyporheic zone is difficult (White, 1993; Harvey, 1997). If hyporheic sediments are deeper than 20 cm, then our values underestimate aerobic respiration.

Fellows (2000) suggested that hyporheic respiration represents approximately 77 % of whole stream respiration in HWC. Summing her aerobic and our anaerobic values results in a hyporheic respiration rate that equals 245 % of total aerobic respiration as measured by upstream-downstream methods. This indicates that our failure to include anaerobic respiration in total metabolism underestimates total metabolism, and we contend that this may be true for a great number of metabolic studies with implications for organic matter budgets.

To better understand the magnitude and nature of respiration in the sediments of HWC, we compared our aerobic and anaerobic respiration rates to a number of representatives taken from the literature (Table 6). In general, measurements of total respiration (i.e. aerobic and anaerobic) are rare. In the systems for which it was calculated, reported anaerobic rates are equal or greater than associated aerobic metabolism. This is an indication that CO_2 liberation and methanogenesis may be an important component in whole system metabolism. We observed similar trends in metabolic rates reported for a low-order stream in New Mexico (Baker *et al.*,

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1999) and our study with high rates of anaerobic metabolism and low aerobic respiration characteristic of hyporheic sediments. At the same time, our rates of aerobic respiration are low in comparison with other studies of metabolism in the subsurface of streams (Table 6). In conceptual models developed to predict the functional significance of the hyporheic zone, Findlay (1995) and Valett *et al.* (1996) emphasized that hydrogeologic features of stream alluvium will dictate subsurface rates and biological activity in systems with longer residence times and may render hyporheic zones anoxic. More work is needed to broadly characterize the nature of metabolism in hyporheic sediments.

While it is well known that POM is broken down to FPOM and DOM and each of these categories of OM has associated microbial respiration rates, less is known about the relative importance of these forms of organic matter to the subsurface. There is evidence in this study that POM is a major component in the subsurface metabolism in a headwater stream during baseflow. This is supported by other studies as well (Hedin, 1990; Pusch & Schwoerbel, 1994; Pusch, 1996; Sobczak *et al.*, 1998; Chafiq *et al.* 1999). Our work has shown that increased organic matter in the subsurface can increase respiration rates and increase the potential for other TEAPs to occur. Given this scenario, traditional oxygen-based measures may greatly underestimate whole-stream metabolism.

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Discharge (L s ⁻¹)	1 - 4.5		
Surface temperature (°C)	14.85 ± 0.05		
Subsurface temperature (°C)	14.55 ± 0.03		
Average particle size (ö)	-3.5 (n = 95)		
Vertical hydrologic gradient	$-0.1 \pm 0.03 \ (n = 32)$		
NO ₃ -N (μg L ⁻¹)	$12 \pm 1(n = 80)$		
NH ₄ -N (μg L ⁻¹)	$8 \pm 0.2 \ (n = 80)$		
PO ₄ -N (μg L ⁻¹)	$6 \pm 0.3 \ (n = 80)$		
DOC (mg L^{-1})	$2 \pm 0.4 \ (n = 80)$		
Dissolved oxygen (mg L ⁻¹)	$8 \pm 0.1 \ (n = 80)$		
Atomic N:P	$12.0 \pm 2.2 \ (n = 80)$		
Sediment %OM	$1.5 \pm 0.2 \ (n = 28)$		

Table 1. Base flow characteristics of the study stream. Data are mean \pm SE, for average paritcle size, vertical hydrolaulic gradient (VHG), all nutrients, N:P ratio, and % OM parameters. All means are the average of samples over the 3-mo study, except % OM, which was determined once prior to incubation.

	% OM	C:N
Leaves	$2.9\pm0.4^{\rm A}$	32.5 ± 6.0
Wood	$2.4\pm0.2^{\rm A}$	32.1 ± 0.4
Plastic	$1.4\pm0.1^{\text{B}}$	29.5 ± 0.2
Control	$1.6\pm0.3^{\text{B}}$	29.8 ± 0.5

Table 2. Sediment characteristics for each treatment. For each treatment data are mean \pm SE. Means with like superscripts are not significantly different.

Leaves	0.0039 ± 0.2
Wood	0.0035 ± 0.1
Plastic	0.00061 ± 0.2
Control	0.00068 ± 0.16
Leaves - Control	0.0039 ± 0.3
Wood - Control	0.0022 ± 0.7

Table 3. Breakdown rates (k, d^{-1}) for sediment organic matter (± 95 % C.I). Leaves - Control and Wood - Control show breakdown rates of the introduced organic matter only (see text for details).

	O_2	N ₂ O	Fe ⁺⁺	SO_4	CH ₄
	μ g L sed ⁻¹ hr ⁻¹	μ g L sed ⁻¹ hr ⁻¹	μg L sed ⁻¹ hr ⁻¹	μg L sed ⁻¹ hr ⁻¹	μ g L sed ⁻¹ hr ⁻¹
Leaves	-30.0 ± 10.0	0.02 ± 0.005	70.0 ± 30.0	6.8 ± 21.2	$60.0\pm20.0^{\rm A}$
Wood	-20.0 ± 40.0	0.05 ± 0.02	-3.0 ± 4.0	-63.0 ± 38.0	$3.0\pm2.0^{\text{B}}$
Plastic	-20.0 ± 4.0	0.04 ± 0.03	50.0 ± 30.0	-53.0 ± 95.0	$2.0 \pm 1.0^{\text{B}}$
Control	-32.0 ± 16.0	0.04 ± 0.01	-0.4 ± 20.0	-0.09 ± 3.0	$2.0\pm7.0^{\text{B}}$

Table 4. Rates of terminal electron accepting processing estimated from changes in solute concentrations during core recirculation. Data are average change in concentration \pm SE (n = 4 per treatment). Negative values indicate uptake and positive values represent accumulation of the solute.

-	Residence time		Turnov	Turnover rate		
-	POM (y)	DOM (d)	POM (y)	DOM (d)		
Leaves	4.3 ± 2.4	7.4 ± 3.5	0.24 ± 0.2	0.32 ± 0.14	50.0 ± 40.0	
Wood	4.4 ± 0.74	7.0 ± 1.0	0.25 ± 0.05	0.15 ± 0.02	60.0 ± 40.0	
Plastic	9.4 ± 4.1	22 ± 9.6	0.16 ± 0.04	0.07 ± 0.02	3.0 ± 7.0	
Control	5.9 ± 1.3	17.0 ± 3.1	0.2 ± 0.04	0.07 ± 0.02	7.0 ± 7.0	

Table 5. Summary of OM residence time, turnover rate, and % respiration attributed to DOC consumption for each treatment. For each parameter data are means \pm SE.

	aerobic g C m ⁻² d ⁻¹	anaerobic g C m ⁻² d ⁻¹	Author
2 Alaskan ponds	N/C	0.53 to 0.21*	Miller and Reed 1975
Steina River	0.545	N/C	Pusch 1996
Steina River	0.25	N/C	Pusch and Schwoerbel 1994
Subsurface of 1 st -order stream	0.00	0.24	Baker et al. 2000
Floodplain of 1 st -order stream	0.11	1.12	Baker et al. 2000
Sycamore Creek	1.3	N/C	Grimm and Fisher 1984
Sycamore Creek	N/C	0.6 - 2.6	Jones et al. 1995
Ogeechee River	1.17 to 3.7	N/C	Edwards and Meyer 1987
New River	0.58 to 1.08	N/C	Hill and Webster 1982
anoxic lake sediment (Netherlands)	0.0	0.0079#	Sweerts et al. 1991
Floodplain tiaga forest Alaska	1.57 to 3.44	1.3 to 3.44	Gulledge and Schimel 2000
control	0.05	1 22	This study
leaves	0.13	2 30	inio budy
wood	0.10	1.34	
plastic	0.08	0.50	

Table 6. Aerobic and anaerobic respiration rates calculated from other studies.* = CO_2 , # = CH_4 only.





Fig. 2



Fig. 3



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- Research technician, University of New Hampshire, LINX project, Luquillo, PR. Responsibilities: coordination, execution of field work, laboratory work and data collection. January March, 1998
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- Crenshaw, C.L., H.M. Valett and J.L. Tank. Fungal Colonization and effects of buried wood in hyporheic and parafluvial environments. Student travel award for American Society of Limnology and Oceanography Meeting, January 1999; \$400.00
- Crenshaw, C.L. Metabolism and nutrient retention in the hyporheic zone in a headwater stream. Sigma Xi Grant-in-Aid of Research, October, 1999, \$600.00
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- Crenshaw, C.L. The effects of augmentation of coarse particulate organic matter on metabollism and nutrient retention in hyporheic sediments. Student travel award Graduate research committee at VPI&SU. May, 2000. \$200.00

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