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Effects of augmentation of coarse particulate organic matter on metabolism and nutrient retention in hyporheic sediments

C. L. CRENSHAW, H. M. VALETT and J. R. WEBSTER
Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA, U.S.A.

SUMMARY

1. 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 first-order Appalachian stream.
2. Sixteen plots were established on a tributary of Hugh White Creek, NC, U.S.A. Sediment was extracted and treated with leaves, wood, plastic strips or remained unamended. Following treatment, sediment was returned to the stream and, approximately 3 months later, samples were removed from each plot.
3. 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 nutrient concentrations. Aerobic metabolism was low in all treatments and respiratory quotients calculated for all treatments indicated that metabolism was dominated by anaerobic processes.
4. Rates of anaerobic respiration and total (combined aerobic and anaerobic) respiration were significantly greater ($P < 0.05$) in plots treated with leaf organic matter compared to controls.
5. Addition of leaves, which had a low C:N ratio, stimulated respiration in hyporheic sediments. Anaerobic processes dominated metabolism 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.

Keywords: anaerobic contact information, CPOM, hyporheic, metabolism, nutrients

Introduction

Recent investigations have addressed the importance of organic matter (OM) in hyporheic zones (Herbst, 1980; Metzler & Smock, 1989; Boulton & Foster, 1998). Because groundwater systems are heterotrophic and detritus based (Culver *et al.*, 1994; Jones, 1995), metabolic processes in groundwater may be limited by OM availability (Culver *et al.*, 1994; Jones, 1995). Consequently, allochthonous materials are crucial for subsurface metabolism, and OM must be imported from the surface (Herbst, 1980; Metzler & Smock, 1989; Gibert, Danielopol & Stanford, 1994; Pusch & Schwoerbel, 1994; Jones, 1995; Jones, Fisher & Grimm, 1995; Boulton & Foster, 1998).

Rates of community respiration (CR) and microbial processes in hyporheic sediments can be high in areas with high standing stocks of coarse particulate organic matter (CPOM) or higher concentrations of dissolved organic carbon (DOC). For example, Sobczak, Hedin & Klug (1998) found that bacterial productivity and biomass at the soil–stream interface of a headwater

Correspondence: Chelsea Crenshaw, Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, U.S.A. E-mail: chelseacrenshaw@hotmail.com

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stream in southwest Michigan (U.S.A.) were related to small-scale spatial variations in sediment POC. The rate of CR in the hyporheic zone of the Steina, a boulder dominated montane stream in Germany, was shown to be similar to that of benthic respiration, and interstitial CR was highly correlated with hyporheic POM (Pusch & Schwoerbel, 1994). On the other hand, 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 probably supported by labile DOC released from algae. 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 ^{3}H thymidine incorporation) was positively related to streambed DOC retention efficiency.

Crocker & Meyer (1987) argued for a linkage between interstitial POC and DOC and contended that the contribution of DOC and POC to microbial production varies temporally and spatially. They found that supplementing POM increased both the quantity of interstitial DOC and bacterial biomass.

Metabolism within the hyporheic zone may be a substantial component of whole-system energy flow (Grimm & Fisher, 1984; Jones, 1995; Jones *et al.*, 1995). Hyporheic respiration can create areas of interstitial hypoxia or anoxia (Dahm, Carr & Coleman, 1991; Findlay, 1995; Baker *et al.*, 1999). Baker *et al.* (1999) found that metabolic processes ranging from aerobic respiration to methanogenesis co-occur in the hyporheic zone along short flowpaths. They showed that during summer base flow 20–100% of acetate, a labile low molecular weight DOC compound, was retained along subsurface flow paths. Thus, biota associated with hyporheic sediments has the potential to immobilise, transform and consume dissolved solutes via metabolic processes.

Our research focused on how the quality of OM affects metabolism and nutrient retention in the hyporheic zone of a first-order Appalachian stream. Using field manipulation of sediment OM, coupled with laboratory assays of microbial metabolism and nutrient cycling, we addressed experimentally how the quantity and quality of carbon alters the rate of metabolism and associated terminal electron accepting processes (TEAPs, *sensu* Vroblesky & Chapelle, 1994). Our objectives were to find how the type and amount of OM affects hyporheic metabolism and nutrient retention. We hypothesised that more labile sources of OM should support greater hyporheic metabolism and result in increased nutrient retention.

Methods

Study site

Our field site was a 95-m reach of a first-order headwater stream that drains into Hugh White Creek at Coweeta Hydrologic Laboratory (Macon County, North Carolina, U.S.A.: −83.43 longitude, 35.05 latitude, 800 m) in the southern Appalachian Mountains. The climate is wet and cool and surrounding vegetation is mixed hardwood. The stream is heavily shaded with a rhododendron (*Rhododendron maximum* L) understory, which leads to low primary productivity (Webster *et al.*, 1995). The substratum consists mainly of cobbles and small boulders (Table 1). Discharge ranged from 0.5 to 4.5 L s^{-1} over the 3 months of the study and dissolved nutrient concentrations were low (Table 1).

Organic matter incubation and dynamics

On 15 June 1999, sediment from the stream was experimentally manipulated with one of four treatments: leaves, wood, plastic or no amendment. Sixteen plots of streambed spanning the width of the stream (0.5 m × 0.25 m × 0.25 m) were excavated at random (determined by random number generator) locations along the study reach. Sediment from each

Table 1 Base flow characteristics of the study stream. Data are mean ± SE (*n*) for average particle size, temperature, vertical hydraulic gradient, DO, all nutrients, N : P ratio and percentage OM. All means are the average of samples over the 3-month study except percentage OM, which was determined at the beginning

Discharge (L s^{-1})	1–4.5
Surface temperature (°C)	14.85 ± 0.05
Subsurface temperature (°C)	14.55 ± 0.03
Average particle size (ϕ)	−3.5 (95)
Vertical hydraulic gradient (cm cm^{-1})	−0.1 ± 0.03 (32)
NO_3-N (μg L^{-1})	12 ± 1 (80)
NH_4-N (μg L^{-1})	8 ± 1 (80)
PO_4-N (μg L^{-1})	6 ± 1 (80)
DOC (mg L^{-1})	2 ± 1 (80)
Dissolved oxygen (mg L^{-1})	8 ± 1 (80)
Atomic N : P	12.0 ± 2.2 (80)
Sediment percentage OM	1.5 ± 1 (28)

plot was removed with a shovel, manipulated with one of the four treatments and placed back in the respective pit. Thus, there were four replicates of each treatment. As a result of difficulty in extracting sediment from the streambed only four replicates of each treatment were used. The leaf treatment consisted of crushed (approximately 3 × 1 cm) white oak (*Quercus alba* L) leaves that were added approximately to double the proportions of OM in the sediment. Similar amounts of white oak wood veneer were cut into 3 × 1 cm strips and added to the wood plots. Plastic was cut into 3 × 1 cm strips and mixed with sediment to produce the plastic treatment in order to evaluate the potential physical effects of OM additions (e.g. a surface area for microorganism colonisation or alteration of interstitial hydrologic conditions). In the fourth treatment, sediment from the non-amended plots was mixed, returned to the plot and used to control for effect of sediment extraction.

Sediment was collected from each plot 3, 4 and 6 months after the experiment began. Particulate OM content of sediment was determined as ash-free dry mass (AFDM) after the sediment was dried at 60 °C for 72 h, weighed, combusted at 550 °C for 2 h and reweighed. Percentage OM was determined as the percentage of total sediment dry weight represented as AFDM. In order to determine breakdown rates specific to the OM introduced experimentally, average percentage OM for controls (unamended) was subtracted from the average percentage OM in leaf-amended and wood-amended plots for each date and breakdown rates calculated. Exponential rates of OM breakdown (e.g. Petersen & Cummins, 1974) were calculated by regressing the natural log of percentage OM remaining against exposure time.

Carbon (C) to nitrogen (N) ratio was determined for sediment samples from each plot (collected in October 1999) using high-temperature combustion (Carlo-Erba NA2100 soil analyser, Thermo Quest Italia S.P.A., MI, Italy) of oven-dried samples. Three replicates were analysed from each sediment sample.

Physical and chemical characteristics

After experimental sediment plots were established along the study reach, they were equipped with monitoring wells, which were sampled every 2 weeks during the first 3 months of the study. Wells were constructed of 5-cm diameter polyvinyl chloride (PVC) with a screen (slotted PVC to allow for flow through) length of 10 cm and were inserted 20 cm into the sediment plots. Interstitial water from within plots was collected using a 60-mL plastic syringe placed on the end of Tygon tubing to slowly draw 50–100 mL of interstitial water from each well. Surface water samples were collected at the same time. Samples were filtered in the field with 0.7-μm glass fibre filters (Whatman GF/F), stored in acid-washed polyethylene bottles and placed on ice until they were taken to the laboratory. We analysed chloride (Cl), nitrate-nitrogen (NO_3-N) and nitrite-nitrogen (NO_2-N) by ion chromatography with a Dionex DX-500 (Sunnyvale, CA, U.S.A.). Ammonium-nitrogen (NH_4-N) and phosphate-phosphorus (PO_4-P; measured as soluble reactive phosphorus), were analysed on a Technicon AutoAnalyser (Saskatoon, SK, U.S.A.) following the techniques of Solorzano (1969) and Murphy & Riley (1962), respectively. Total inorganic nitrogen (TIN) was calculated as the sum of NO_3-N, NO_2-N and NH_4-N. Atomic N : P ratios were calculated as molar ratios of TIN and PO_4-P (Grimm & Fisher, 1984). Samples were analysed for DOC by wet persulphate digestion using an Oceanography International 700 Total Carbon Analyzer (College Station, TX, U.S.A., Menzel & Vaccaro, 1964). All chemical analyses were completed within 48 h of collection. Dissolved oxygen (DO) was measured using an YSI DO probe (model 55, YSI Inc., Yellow Springs, OH, U.S.A.) before water was extracted from the monitoring wells.

Mean substrate particle size (MPS) was determined using standard granulometry techniques on 95 randomly selected rocks and a USGS gravelometer (FISP US SA-97). The vertical hydraulic gradient was determined by measuring the pressure differential in mini-piezometers installed in the streambed (Lee & Cherry, 1978). Mini-piezometers were made of 1.27 cm diameter PVC pipe, cut 1 m in length, placed in the active channel and inserted to a depth of 20 cm. Mini-piezometers were placed every 10 m, with additional ones placed inside and directly outside each sediment plot.

Sediment extraction

Sediment plots were sampled on 10 October 1999, after 3 months. The top layer (0–5 cm) of each plot was scrapped off to remove surficial influences, after

which four samples of sediment (each about 200 g) were removed (5–20 cm depth) and placed into a plastic bag. The samples were placed on ice and taken to the laboratory where metabolism assays were run within 48 h of extraction.

Microcosm assays: heterotrophic metabolism and solute dynamics

Heterotrophic metabolism was measured using recirculating microcosms following methods modified from Pusch & Schwoerbel (1994), Jones (1995) and Baker, Dahm & Valett (2000). Microcosms were constructed of clear plexiglass tubes (20 cm long, 7 cm diameter) in which the inside walls were roughened with sandpaper to prevent preferential flow along the walls (Baker *et al.*, 2000). Sediment (approximately 800 g wet weight) from each plot was placed into each microcosm. Water used for circulation during metabolism assays was both groundwater, collected from the respective well, and surface water (1 : 1 ratio), collected near the plot. Water was collected in the field on 10 October 1999 before sediment extraction, and surface and groundwater were combined in order to mimic downwelling conditions characteristic of the study reach. Before circulation in the microcosms, the water was bubbled with helium until the DO was reduced to ambient concentrations, determined for each respective well on the day of extraction. Microcosms then were purged with the water for 2 h before sampling began in order to rid microcosms of residual gases.

Water was recirculated through the microcosms at an estimated subsurface pore velocity of 1.6 cm s^{-1} using a peristaltic pump. The chambers were run for 6 h in the dark at ambient stream temperature (10 °C), during which time samples for dissolved gases and nutrients were taken every 2 h. A 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. This sample was later analysed for DO using a modified Winkler technique (Wetzel & Likens, 1991) within 15 min of sampling. Immediately after obtaining the DO sample, another 5-mL water sample was extracted in a sampling syringe, equilibrated (shaken vigorously for 1 min) with 5 mL of air, and headspace gas was transferred to a clean evacuated 3-mL Vacutainer®. We measured pCO_2 in the equilibrated headspace by gas chromatography with a thermal conductivity detector. Dissolved CO_2 concentration was estimated from pCO_2, pressure, and temperature; calculations were based on Henry's Law (Kling, Kipphut & Miller, 1992).

A final 10-mL sample of water was extracted and used for analysis of dissolved nutrients and methane (CH_4). Methane samples were processed by placing approximately 1.5 mL of sample into evacuated Vacutainers® vials. Methane then was released from the water by agitation of the sample and analysed on a gas chromatograph fitted with a flame ionisation detector (SRI 8610, Torrance, CA, U.S.A., De Angelis & Lilley, 1987; Dahm *et al.*, 1991). The remaining water was used in analysis of dissolved nutrients (NO_3-N, NH_4-N, SO_4 and DOC) as described earlier.

Rates of aerobic respiration were calculated as the change in DO over time. Metabolic rate was estimated from the rate of CO_2 liberation, and total metabolism was estimated as the increase in CO_2 + CH_4 (combining C only) 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 determine the use of potential TEAPs. Regression of solute concentration versus time was used to quantify rates of consumption/production for NO_3-N, CH_4 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 was used to determine the rate of denitrification quantified using the acetylene block technique (Duff & Triska, 1990). Sediment (approximately 250 mg wet weight) was placed in microcosms (clear 5-cm diameter PVC capped on both ends) with 250 mL of stream water. Microcosms were made anaerobic by sparging with helium for 10 min before capping and 10% by volume of acetylene was added to each microcosm. Gas samples were taken 0, 1.5, 3, 6 and 12 h after capping and were analysed for N_2O using a gas chromatograph (SRI 8610) fitted with an electron capture detector.

Residence time ($year^{-1}$) for OM in recirculating core sediments was calculated by dividing standing stocks of OM by respiration (total metabolism rate quantified in each sediment microcosm). Rate of turnover ($year^{-1}$) was calculated as the inverse of residence time. The proportional contribution of DOC to respi-

ration was calculated by dividing the loss of DOC by total metabolism rate for each sediment microcosm.

Statistical analysis

All ratios and percentages were arcsin square-root transformed before analysis, and all data presented are back-transformed. Concentration of OM was normalised by logarithm transformation prior to statistical analysis. Analysis of covariance (ANCOVA) was used to compare breakdown rate (k) among treatments (i.e. OM manipulations) using the natural logarithm of percentage OM as the dependant variable and time as the covariate (Webster & Benfield, 1986). One-way analysis of variance (ANOVA) was used to compare differences among treatments for a number of dependent variables including changes in concentration of CO_2, O_2, N_2O, CH_4, NO_3-N, NH_4-N and DOC. Sediment C : N, OM, residence time and turnover ratios were also analysed with a one-way ANOVA. Tukey *post-hoc* tests following significant ANOVAS ($P < 0.05$) were used to differentiate among treatments. Repeated-measures ANOVA 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 3-month study (Table 1). Dissolved oxygen concentration in the stream varied from 8 to 10 mg L^{-1} and remained near saturation. Interstitial DO concentration was significantly lower, ranging from 1 to 4 mg L^{-1}, with no significant differences among any of the subsurface treatments (Fig. 1a). Dissoved organic carbon concentration in surface water ranged from 1.5 to 5 mg L^{-1} over the incubation and typically remained lower than interstitial water concentration, but interstitial DOC concentration was more variable. For the first 4 weeks of the experiment, DOC content in leaf litter plots was higher than in other treatments, with a maximal concentration greater then 50 mg L^{-1} (Fig. 1b). By the end of the study, wood plots had the highest DOC concentration (Fig. 1b). Dissolved organic carbon concentration was relatively low (2–5 ppm) and less variable in control and plastic plots.

Subsurface NO_3-N concentration was low (generally less than 20 μg L^{-1}) in all treatments but, over the course of the study, increased from less than 10 to about 20 μg L^{-1} in control, leaf and plastic plots (Fig. 2a). In contrast, NO_3-N concentration remained low in the wood plots. Ammonium in the two control plots increased by 40 μg L^{-1}. Concentration of NH_4-N in wood treatments was very low and rather constant (Fig. 2b). By contrast, NH_4-N increased in other treatments, with the greatest increase observed for leaf plots where concentration increased from about 20 to over 90 μg L^{-1} (Fig. 2b). During the first 2 weeks of incubation, PO_4-P concentration was raised in all treatments ranging from 10 to over 30 μg L^{-1}. Subsequently, concentration dropped and remained low (< 5 μg L^{-1}) throughout the experiment. Statistical analyses indi-

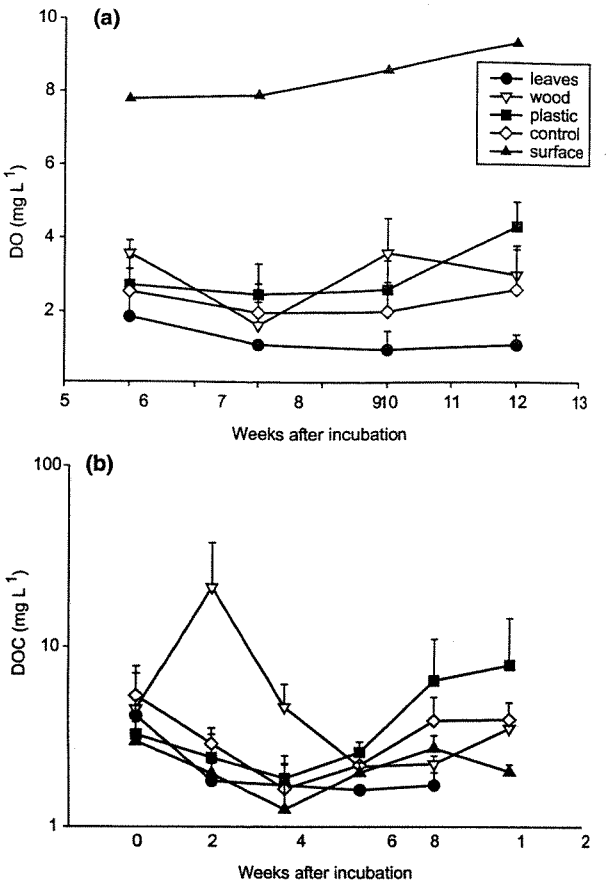

Fig. 1 Dissolved oxygen (a) and DOC (b) concentrations (all means ± SE) for surface and subsurface water taken over a 12-week period during organic matter incubation. DO and DOC are presented as mg L^{-1}. OM manipulation occurred on 13 June 1999, and samples were collected at 2-week intervals. Dissolved oxygen measurements began on 29 July 1999. Concentrations are represented by ▲ for surface, leaves ●, wood ▽, plastic ■ and control ◇.

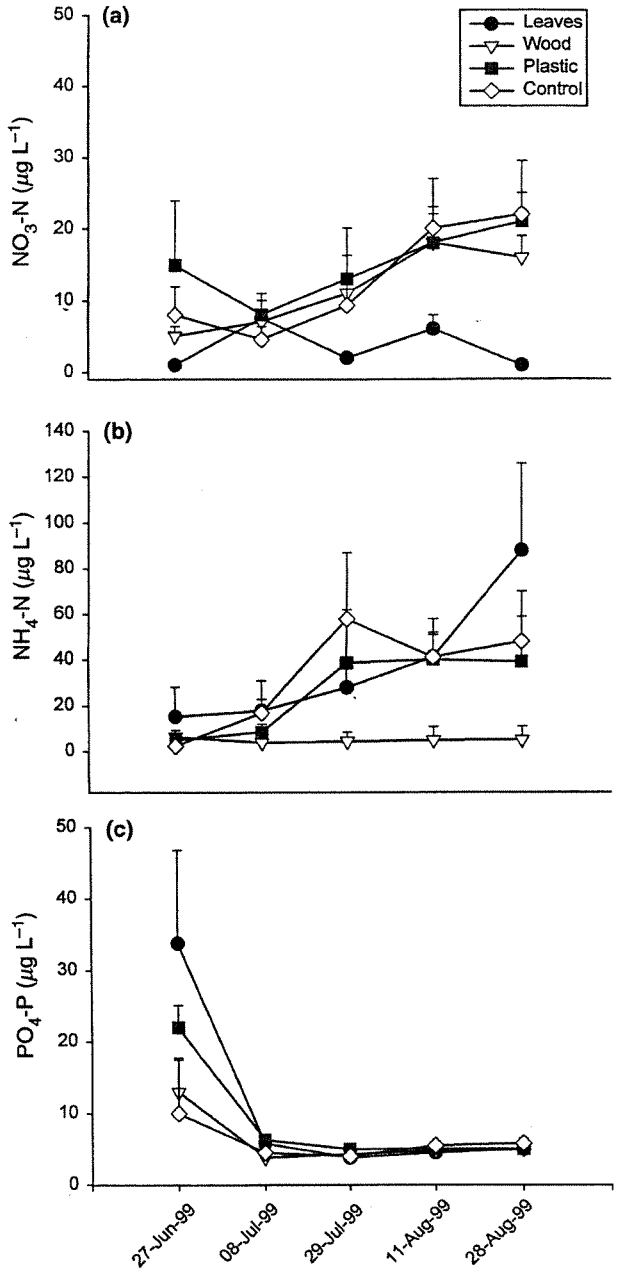

Fig. 2 Subsurface nutrient concentrations (mean ± SE) for sediment plots receiving different OM treatments. Concentrations are represented by ● for leaf, wood ▽, plastic ■ and control ◇.

cated that mean solute concentration did not differ significantly among treatments over the course of the study.

Breakdown rates and sediment characterisation

Organic matter content of hyporheic sediment prior to treatment averaged 1.5 ± 0.1% (Table 1) and after 3 months of the study ranged from a minimum of 1.4 ± 0.3% in the control plots to a maximum of 2.9 ± 0.4% in leaf plots (Table 2). Percentage OM was significantly greater in the leaf and wood amendments than in the other two treatments ($P = 0.0005$). Carbon to nitrogen ratio ranged between 29.5 and 32.5 and did not vary significantly among treatments (Table 2).

Table 2 Sediment characteristics for each treatment. For each treatment data are mean ± SE

	Percentage OM	C : N (molar ratio)
Leaf	2.9 ± 0.4 ^{A}	32.5 ± 6.0
Wood	2.4 ± 0.2 ^{A}	32.1 ± 0.4
Plastic	1.4 ± 0.1^{B}	29.5 ± 0.2
Control	1.6 ± 0.3^{B}	29.8 ± 0.5

Within a column, means with like superscripts are not significantly different.

Rate of breakdown of the added OM was 0.0039 day^{-1} in the leaf plots and 0.0022 day^{-1} in the wood plots (Table 3) and the difference was not significant ($P = 0.13$).

Heterotrophic metabolism and biogeochemical processes

Aerobic respiration rate ranged from 0.02 ± 0.06 mg (L sed)$^{-1}$ h^{-1} in the wood treatments to a maximum 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 measured as CO_2 production was significantly greater ($P < 0.05$) in the leaf-enriched treatments than in other treatments (Fig. 3a). Respiratory quotients were greater than 1.0 in all treatments and highest in the carbon amended treatments. The leaf treatment plot had the highest respiratory quotient (RQ) (over 45) of all carbon-amended plots (Fig. 3b). Nevertheless, no statistical differences ($P > 0.05$) in RQs were observed

Table 3 Breakdown rates (day^{-1}) for sediment organic matter. Leaves–control and wood–control show breakdown rates of the introduced organic matter only

Leaves	0.0039
Wood	0.0035
Plastic	0.00061
Control	0.00068
Leaves–control	0.0039
Wood–control	0.0022

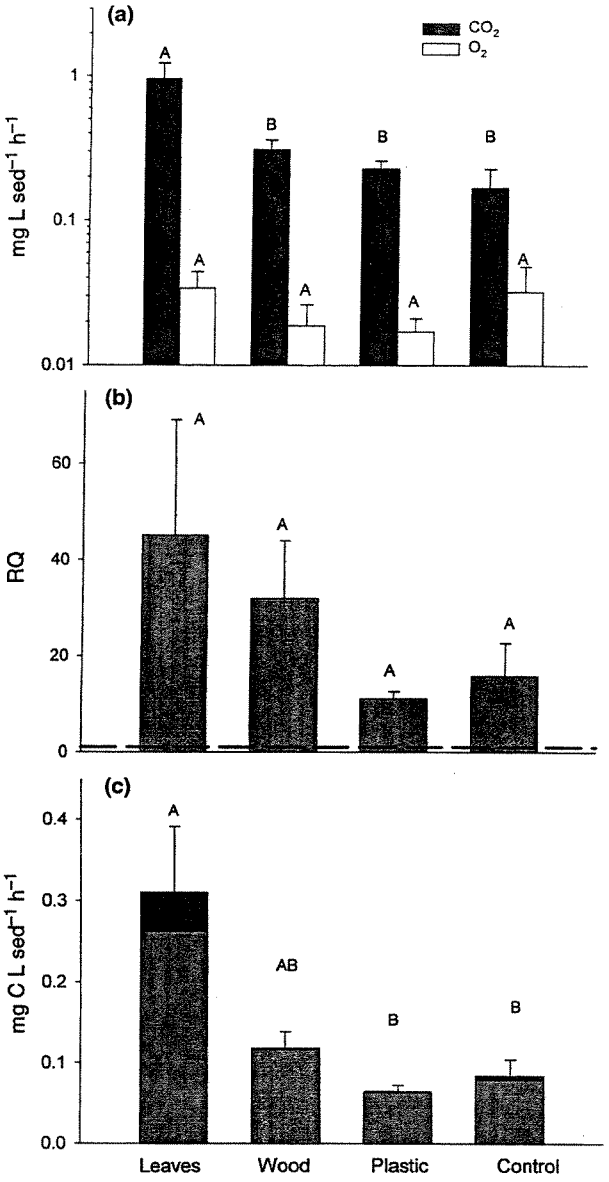

Fig. 3 Metabolic responses to organic matter augmentation treatments. (a) Aerobic (open bars) metabolic rates and respiration based on CO_2 liberated (black bars) for each treatment. (b) Respiratory quotient (RQ: molar ratio of CO_2 : O_2) for each treatment, dashed line represents one (i.e. strictly aerobic conditions). (c) Respiration rates based on total carbon liberated (CO_2-C + CH_4-C) for different treatments. Black portions of each bar in panel (c) represent the amount of CH_4 production for each treatment. Rates and RQs are presented as mean ± SE. For a given response variable, significant differences among treatments are indicated by differing superscripts ($P < 0.05$).

among treatments. The rate of methanogenesis was significantly greater ($P = 0.007$) in leaf treatments [60.0 ± 20.0 μg (L sed)$^{-1}$ h^{-1}] than in the other three treatments, where the rate ranged from 2 to 3 μg (L sed)$^{-1}$ h^{-1} (Fig. 3; Table 4). Total carbon 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 (Fig. 3c). The mean for plots amended with wood was not significantly different from the mean for the other three treatments (Fig. 3c). Methane-C contributed from 1.7% of total carbon produced in plastic treatments to 27% in leaf treatments (Fig. 3c).

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

	O_2 [μg (L sed)$^{-1}$ h^{-1}]	N_2O [μg (L sed)$^{-1}$ h^{-1}]	CH_4 [μg (L sed)$^{-1}$ h^{-1}]
Leaf	−30.0 ± 10.0	0.02 ± 0.005	60.0 ± 20.0^{A}
Wood	−20.0 ± 40.0	0.05 ± 0.02	3.0 ± 2.0^{B}
Plastic	−20.0 ± 4.0	0.04 ± 0.03	2.0 ± 1.0^{B}
Control	−32.0 ± 16.0	0.04 ± 0.01	2.0 ± 7.0^{B}

Addition of OM to the hyporheic zone influenced biogeochemical transformations, as indicated by altered rates of microbial TEAPs (Table 4). The rate of denitrification (i.e. accumulation of N_2O) was greatest in the wood treatment, whereas the lowest rate of denitrification occurred in leaf treatments (Table 4). Over the course of the experiment changes in NO_3-N concentration decreased in all recirculating microcosms (Fig. 4a). The highest and lowest rate of NO_3-N consumption occurred in treatments amended with wood and leaves, respectively. Although, there were no significant differences ($P > 0.05$) in the rate of N_2O production or NO_3-N consumption among treatments there was evidence of other TEAPs (specifically Fe^{2+} production and SO_4 consumption, data not shown).

Consumption and production of DOC and NH_4-N in recirculating microcosms differed among treatments (Fig. 4). Consumption of DOC was greatest in wood and leaf treatments and was not different from zero in the control and plastic plots (Fig. 4b). DOC consumption in plots amended with leaves and wood represented about 50% of total carbon liberated by respiration during microcosm experiments (Table 5), whereas consumption of DOC in the plastic treatment and control was between 3 and 7% of observed respiration. 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).

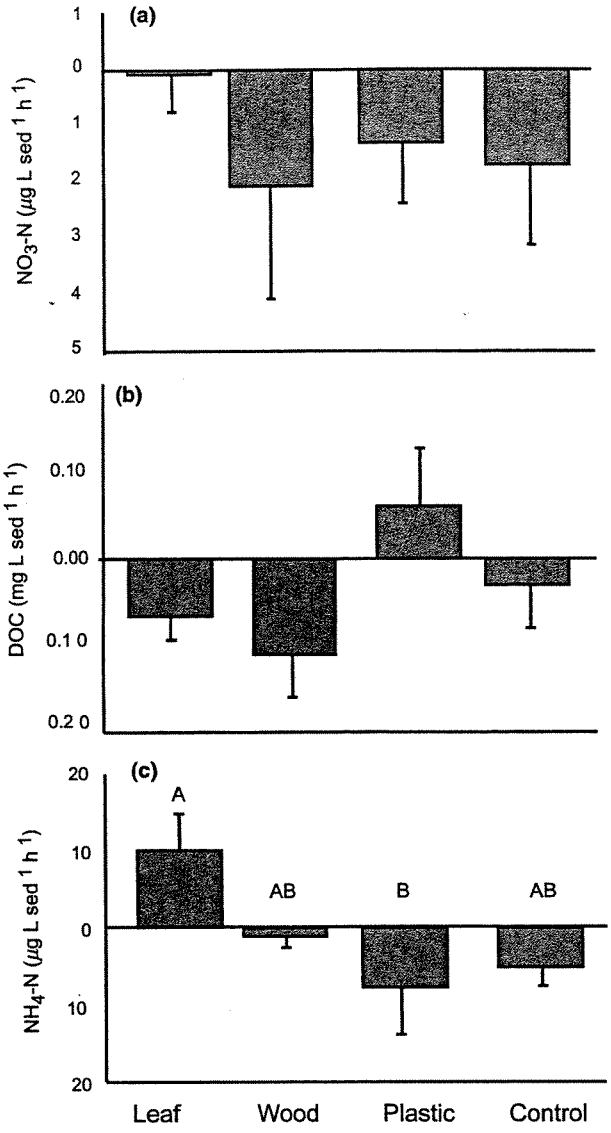

Fig. 4 Nutrient consumed (–) and produced (+) of (a) NO_3-N, (b) DOC and (c) NH_4-N for OM augmentation treatments. Values are presented as mean ± SE. Significant differences are represented by different capital letters ($P < 0.05$).

Retention of POM and DOM in hyporheic sediments

Based on OM standing stocks measured in sediment microcosms, the OM residence times differed among treatments (Table 5). The residence time of POM treatments ranged from about 4 to 10 years (Table 5). Thus, turnover ratios for POM were on the order of 16–25% loss per year (Table 5). In general, residence times for POM were shorter for plots augmented with OM compared with control plots, but the differences were not statistically significant ($P > 0.05$).

Table 5 Summary of OM residence time, turnover rate, and percentage respiration attributed to DOC consumption for each treatment (means ± SE, $n = 4$)

	POM (year^{-1})		
	Residence time	Turnover rate	Percentage R as DOC consumption
Leaves	4.3 ± 2.4	0.24 ± 0.2	50.0 ± 40.0
Wood	4.4 ± 0.74	0.25 ± 0.05	60.0 ± 40.0
Plastic	9.4 ± 4.1	0.16 ± 0.04	3.0 ± 7.0
Control	5.9 ± 1.3	0.2 ± 0.04	7.0 ± 7.0

Discussion

The effect of adding organic matter

Despite doubling the content of OM in augmented plots, C : N ratio varied little among treatments (C : N: 29–33, Table 2). The C : N ratio of subsurface leaves and wood was lower than that of benthic leaves and wood in Upper Ball Creek, Coweeta, which had C : N ratios of 50 and 45, respectively (Tank *et al.* 2000). While we did not determine the C : N ratio of OM before amendment, we observed a lack of variation in C : N among treatments after a relatively short (i.e. 3 months) period, which has also been seen in other studies (Baker *et al.*, 1983). This lack of difference may be due: (a) to the initial added OM (leaves and wood) having a similar C : N ratio as the native sediment; or (b) to a rapid uptake of nitrogen in amended plots. Except for NH_4-N in the leaf plots, nitrogen concentration in the subsurface water decreased over the 3-month period. These results also illustrate that native sediment (i.e. unmanipulated controls) contained OM of similar stoichiometric composition as did the sediment augmented with leaves and wood and exposed to interstitial microbial processes for 3 months. These results are consistent with perspectives that biogeochemical processes in groundwater environments are dependant on imported OM (Gibert *et al.*, 1994) and that leaves and wood may be primary sources of OM to the subsurface of headwater streams.

Percentage OM reported in sediments of various lotic ecosystems ranged around those reported here (1.4–2.9%). Pusch & Schwoerbel (1994) found 0.8% OM in sediments of the Steina River, Germany, while Bott & Kaplan (1985) recorded a similar value (0.6% OM) for streambed sediments in White Clay Creek, PA, U.S.A. Crocker & Meyer (1987) showed that OM ranged from 1.5 to 4.6% in the sediments of Dryman Fork, Coweeta Hydrologic Laboratory, NC, U.S.A. In

this study, the proportion of OM in sediments was greater (approximately twofold) in the amended than the control plots, but these values were similar to naturally occurring conditions in Appalachian streams (Crocker & Meyer, 1987).

Rates of OM breakdown in our leaf and wood enriched sediments were similar (0.0039 and 0.0022 day^{-1}, respectively) to published rates (e.g. Peterson & Cummins, 1974; Findlay, Smith & Meyer, 1986; Webster & Benfield, 1986; Metzler & Smock, 1989; Meyack, Thorp & Cothran, 1989; Webster *et al.*, 1995; Tank & Webster, 1998). Our rates for wood amended plots are generally faster than most reported values. Because of the high lignin and cellulose content of wood, breakdown rates should be slower for wood than leaves (Wallace, Ross & Meyer, 1982; Melillo *et al.*, 1983). Burial of OM also is thought to decrease its breakdown rate (Harmon, 1986), though Smith & Lake (1993) reported breakdown of introduced subsurface leaves at rates similar to those on the surface. We suggest that the more rapid wood decomposition observed here was because of the high surface area of the pieces of wood veneer compared to natural substrates.

It has been reported that mean DOC concentration in stream water is directly related to streambed leaf-litter standing stock (Meyer, Wallace & Eggert, 1998). They found that about 30% of DOC export is because of instream generation of DOC produced by leaf litter. In our study, leaf plots had a very high subsurface concentration of DOC after 4 weeks of burial. We suggest this is because of the leaching of DOC from the leaves and the slow flow rate of subsurface water. Despite this spike of DOC in the leaf plots during our study, stream water showed no response to DOC released from subsurface sediments, and DOC concentration in surface water remained low. This may be because DOC was removed rapidly by sediment and benthic biofilms. McDowell & Fisher (1976) found that stream DOC concentration did not change after autumnal leaf fall, suggesting that DOC is removed rapidly. Similarly, Crocker & Meyer (1987) reported no response of stream water DOC concentration to experimentally increased subsurface POM.

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; Jones, 1995). Pusch & Schwoerbel (1994) found that POM in hyporheic sediment was highly correlated with hyporheic CR and suggested that this respiration may in part be because of DOC leached from buried POM.

Particulate OM that leaches DOC may increase microbial biomass by acting as an energy source for interstitial biofilms (Crocker & Meyer, 1987). Crocker & Meyer (1987) suggest that benthic POM may be a source of hyporheic DOC, depending on the molecular weight of the DOC, sediment OM content and hydrologic exchange. DOC may be rapidly taken up either by microbes or by sorption to sediments (Findlay & Sobczak, 1996). Enriched leaf plots in our study did leach DOC with a subsequent reduction in O_2, suggesting high aerobic metabolic activity within the first 5 weeks. In our study, consumption of DOC in the recirculating cores accounted for less then 50% of the total carbon metabolised, suggesting that DOC is not the sole carbon source for microbial respiration and that DOC is present in short supply compared to microbial respiratory demand. These data suggest that both DOC and POC are important components of subsurface energetics in this headwater stream.

Metabolic rates in our study suggest that increased POM lowered DO concentration and limited aerobic respiration. At the same time, RQs in the control treatments indicated that unmanipulated sediment supported considerable anaerobiosis. The occurrence and importance of anoxia in the hyporheic zone is becoming more appreciated as a greater variety of streams are studied (Baker *et al.*, 1999). For example, Morrice (1997) found that hyporheic water moves much slower than surface water and may have areas of anoxia due to microbial uptake of O_2 (Dahm *et al.*, 1991; Baker *et al.*, 1999).

In areas where O_2 supply is diminished, alternative electron acceptors are used in OM catabolism. The loss of DO and changes of solutes and terminal electron acceptors in our system indicated that, besides increasing aerobic respiration, increased OM can increase other electron accepting microbial processes (Table 4). Baker *et al.* (1999) found that, with added labile organic carbon (acetate) to hyporheic flow paths of a headwater stream, the rate of O_2 consumption and anaerobic processes increased and organic carbon retention was affected mainly by

anaerobic metabolism. This also occurred in our study: higher quality OM (leaf treatment) generated the highest rate of respiration and greatest CH_4 production. The low rate of aerobic respiration and the high rate of CH_4 production in the leaf treatments suggest that the supply of terminal electron acceptors other than CO_2 was diminished.

The effect of increased OM on nutrient dynamics

Retention of nutrients in streams occurs mainly at or within the streambed (Meyer *et al.*, 1988; Grimm & Fisher, 1984). Holmes *et al.* (1998) found that denitrifiers retained from 5 to 40% of nitrate produced by nitrifiers in a desert stream. In our study, NO_3-N and DO concentration decreased in the leaf treatments while NH_4-N increased over time. These results, coupled with the results of our denitrification assays, suggest that microbial ammonification was occurring along with denitrification. Depletion of O_2 creates hypoxic conditions and allows denitrification to occur. 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 removal of NO_3-N and N_2O were limited by oxidisable carbon availability in short hyporheic flowpaths of Smith Creek, MI, U.S.A. This suggests that, in our leaf plots, denitrification occurred early on with high levels of DOC and POM and later decreased because of lack of NO_3-N.

Effects of OM on system energetics

Organic matter in POM-enriched treatments had a similar residence time to natural sediments along our study reach. This indicates that processing of OM is occurring at a similar rate (Table 5) in enriched and control treatments and demonstrates a proportional increase in POM and hyporheic respiration. In our study, turnover rates for hyporheic POM ranged from 0.16 to 0.25 $year^{-1}$ and were similar to rates (0.04–0.641 $year^{-1}$) found by Pusch & Schwoerbel (1994).

While it is well known that CPOM is broken down to FPOM and DOM in the benthos, and each of these categories of OM has associated microbial respiration, little is known about the relative importance of these forms of OM to subsurface respiration. The spatial variation of OM and nutrients in hyporheic sediments has been fairly well established, but there is evidence in this study (even with its low statistical power) that CPOM is a major component in subsurface metabolism in a headwater stream during baseflow, and this is supported by other studies (Hedin, 1990; Pusch & Schwoerbel, 1994; Sobczak *et al.*, 1998; Chafiq, Gibert & Claret, 1999). Our work has shown that increased OM in the subsurface can increase respiration rate and increase other TEAPs. Given this scenario, traditional oxygen-based measurements may greatly underestimate whole-stream metabolism.

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