VARIATION IN BIOAVAILABILITY OF DISSOLVED ORGANIC CARBON AMONG STREAM HYPORHEIC FLOWPATHS

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Abstract. Dissolved organic carbon (DOC) dominates the flux of organic matter in most stream ecosystems, but the proportion susceptible to microbial degradation is often presumed to be low. The fraction of bulk DOC contributing to microbial metabolism was assessed in five streams representing the regional range in surface-water DOC concentration in eastern New York State, USA (range 0.5–7.7 mg/L; $n = 82$). Transects of shallow wells along two hyporheic flowpaths (i.e., saturated sediments found below and lateral to the open-stream channel in active exchange with surface waters) in each of five streams were sampled monthly at baseflow to determine changes in subsurface DOC and dissolved oxygen concentrations. Hyporheic DOC concentrations ranged from 50% to 100% of surface-water concentrations and decreased along hyporheic flowpaths in four of five streams. Dissolved oxygen losses along hyporheic flowpaths paralleled DOC loss, and bacterial activity on tiles incubated at points along the flowpaths generally declined as hyporheic DOC was depleted. DOC losses along natural flowpaths exceeded the quantity of DOC lost during laboratory bottle incubations, even when samples were amended with inorganic nutrients. Hyporheic mesocosms were used to examine the fate of stream-derived DOC along replicated flowpaths under controlled hydrologic conditions. The overall patterns of DOC losses along mesocosm flowpaths supplied with water from previously studied streams were similar to DOC losses along natural flowpaths. DOC declines were paralleled by declines in bacterial activity and dissolved oxygen. Mesocosm results indicated that variation in percentage of DOC loss along natural flowpaths was not a function of dilution, residence time, or initial DOC concentration and that subsurface DOC dynamics were linked to variation in microbial metabolism.

The fraction of total DOC available for metabolism varied markedly among regional streams and was independent of initial DOC concentration. DOC near the end of hyporheic flowpaths was not subject to further degradation, regardless of the bioavailability of surface-water DOC entering these flowpaths. Hence, in streams with significant hyporheic exchange, the amount and bioavailability of DOC transported to downstream ecosystems may be affected by subsurface metabolism. DOC depletion during hyporheic transport may provide a general in situ measure of bioavailable DOC in surface water and be a powerful predictor of rates of heterotrophic activity in sediments at the reach level.

Key words: bacteria; bioavailable DOC; dissolved organic carbon; hyporheic zone; mesocosms; microbial metabolism; organic matter bioavailability; sediments; stream ecosystem.

INTRODUCTION

Dissolved organic carbon (DOC) influences, if not governs, many aspects of the biology and chemistry of aquatic ecosystems (Thurman 1985). Frequently, DOC regulates biotic processes such as bacterial productivity which in turn influences dissolved oxygen concentrations, food-web structure, and microbially mediated biogeochemical transformations (Wetzel 2001). Stream ecosystems integrate DOC fluxes from adjacent terrestrial, riparian, and groundwater environments (McDowell and Likens 1988, Hedin et al. 1998, Baker et al. 2000*a*) and downstream fluxes of DOC are typically

the largest component of stream organic matter budgets (Fisher and Likens 1973, Webster and Meyer 1997). Differences among streams in the proportion of DOC susceptible to metabolism over relevant time scales may influence stream heterotrophy and downstream organic carbon transport (Mulholland 1997, Sinsabaugh 1997), but there has not been an explicit comparative study of how the bioavailable fraction of total DOC might vary among streams.

Most of the metabolic activity in streams occurs in bed sediments either at the sediment–water interface or in hydrologically connected deeper sediments (i.e., hyporheic zone). Hyporheic zones influence the biogeochemistry of stream ecosystems by increasing solute residence times, and more specifically, solute contact with biofilms (Bencala 2000). Microbial processes in the hyporheic zone can be a significant component of whole-stream nutrient transformations (Triska et al.

1989, Baker et al. 2000*b*, Duff and Triska 2000) and organic carbon transformations (Grimm and Fisher 1984, Mulholland et al. 1997, Kaplan and Newbold 2000). Surface water perfusing hyporheic sediments provides a renewable dissolved organic carbon source to maintain hyporheic metabolism (Jones 1995, Findlay and Sobczak 1996, Battin 2000), although its flux may vary with season and discharge (Vervier and Naiman 1992, Harvey and Wagner 2000). Particulate organic carbon (POC) is physically removed as streamwater downwells into sediments (Marmonier et al. 1995), but large standing stocks of particulate detritus may occasionally be buried in hyporheic sediments and significantly contribute to hyporheic metabolism (Metzler and Smock 1990, Sobczak et al. 1998). In addition, the breakdown of existing biofilms may also contribute to hyporheic metabolism (Fiebig 1995, Findlay and Sobczak 1996). Regardless of existing standing stocks of sediment-bound POC, DOC in transit through hyporheic zones provides a large and renewable potential energy source for hyporheic microbes. The realized importance of DOC as an energy source is a function of its bioavailability and rate of supply (Findlay 1995, Findlay and Sobczak 2000).

Assessment of among-stream differences in the bioavailable fraction of DOC requires evidence that apparent DOC loss is due to biotic processes and ancillary factors such as inorganic nutrient availability are not major controls on DOC removal. We examined DOC dynamics and microbial metabolism along hyporheic flowpaths to address the linked questions of whether there was removal of DOC from surface waters moving through these subsurface sediments and whether these changes were associated with differences in metabolism. Appreciable quantities of surface water can move along hyporheic flowpaths and relatively slow water movement through hyporheic sediments allows ample opportunity for microbial metabolism of bioavailable soluble materials.

Here, we determined bioavailability of stream-derived DOC by examining DOC and dissolved oxygen concentrations along hyporheic flowpaths in five streams representing the regional range of surface-water DOC (Hudson River Valley and Catskill Mountain region, New York, USA). Measurements of biofilm bacterial activity were used to explore potential links between DOC removal and changes in bacterial growth among streams. These flowpath studies were supported by an array of field, laboratory, and mesocosm experiments that were used to systematically eliminate alternate explanations for observed differences in DOC bioavailability such as differences in hydrology, inorganic nutrient availability, and bulk-DOC concentration. Among-stream differences in DOC removal were related to variation in hyporheic-zone metabolism, which provided insight into how surface–subsurface interactions can be used to understand the energy flow and biogeochemistry of stream ecosystems.
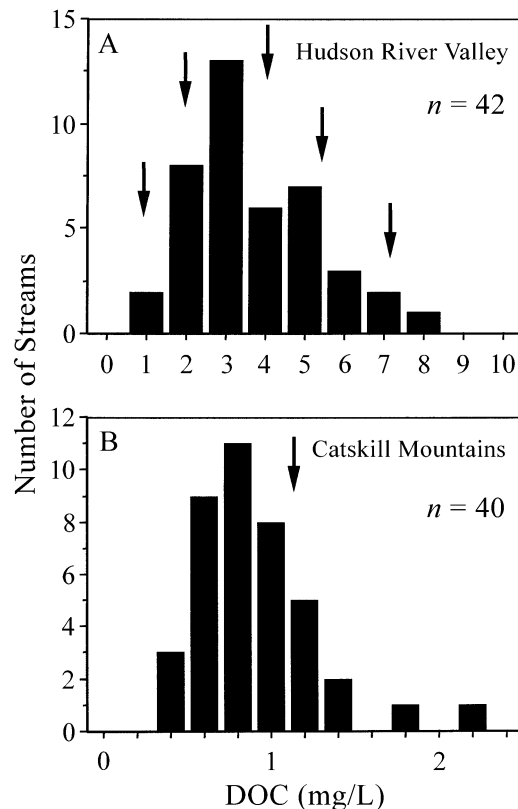

FIG. 1. DOC survey histograms: (A) Hudson River Valley; (B) Catskill Mountains. Arrows indicate DOC values of streams examined throughout the study.

METHODS

Stream survey and selection

We surveyed a wide array of streams ($n = 82$) in the Hudson River Valley region (New York, USA; $n = 42$) and in the Catskill Mountain region (New York, USA; $n = 40$) to select streams spanning the regional range in surface-water DOC concentration. Hudson River Valley streams were sampled on at least two dates at summer baseflow during June and July 1994. Catskill Mountain region streams were surveyed en masse on four dates representing summer, fall, winter, and spring flow. DOC concentration in the Hudson River Valley region ranged nearly an order of magnitude (range 0.9–7.7 mg/L at baseflow) and had a mean of 3.5 mg/L (Fig. 1A). Streams draining the primarily forested catchments of the Catskill Mountains exhibited a smaller range in DOC concentration (0.4–2.2 mg/L) with a mean of 0.9 mg/L (Fig. 1B). The five streams selected for study spanned the range in DOC concentrations found in the regional survey (see arrows in Fig. 1A): West Branch Neversink River (DOC = 1.1 ± 0.2 [mean ± 1 SE]; $n = 8$), Ham Creek (DOC = 1.9 ± 0.3), East Branch Wappinger Creek (DOC = 3.1 ± 0.2), Shawangunk River (DOC = 5.4 ± 0.6), and Wallkill River (DOC = 7.7 ± 0.5).

PLATE 1. (Left) The West Branch Neversink River is a low-nutrient, third-order stream that drains the forested catchment in the southern Catskill Mountains of New York State. The Neversink River is underlain by sandstone and conglomerate and overlain by till deposits and alluvium derived from the last glaciation; thus, the potential for exchange of surface water and subsurface water appears high. (Right) Mesocosm studies were conducted to examine dissolved organic carbon and microbial variables under conditions of controlled interstitial flow rates. Each of the nine mesocosms shown consisted of a 440-L tank connected to a 4-m sediment-packed PVC pipe and a series of sampling ports.

The five streams not only represented the regional DOC concentration, but also varied in catchment characteristics and inorganic water chemistry (Table 1). The West Branch Neversink River (Neversink; see Plate 1) is a low-nutrient, third-order stream that drains a forested catchment containing second-growth oak–hickory–maple in the southern Catskill Mountains. The Neversink watershed is underlain by sandstone and conglomerate and overlain by till deposits and alluvium derived from the last glaciation. The East Branch Wappinger Creek (Wappinger) is a high-nutrient, third-order stream that drains a heterogeneous watershed that contains low-density housing, second-growth forests, agricultural land, and some wetlands. Ham Creek (Ham) is a second-order tributary of the Wappinger and drains a watershed containing lowland forests and wetlands. The Wallkill River (Wallkill) is a high nutrient, fourth-order stream that drains a heterogeneous watershed that contains small towns, agricultural land, wetlands, and lowland forests. The Shawangunk River (Shawangunk) is a third-order tributary of the Wallkill and drains a watershed containing low-density housing, agricultural land, and up-

land forests. The Wappinger and Wallkill are tributaries of the Hudson River, and alluvial deposits for these low-elevation streams are derived from glacial till.

Transect installation

On each of the five streams, we located exposed gravel bars (i.e., alluvium not inundated at baseflow) in riffles where downwelling of surface waters was indicated by negative vertical hydraulic gradient (Fig. 2A). We established transects (two per stream) of shallow (0.5 m deep) wells made of PVC pipe (diameter = 1.91 cm) with 10-cm perforated sections between depths of 40 cm and 50 cm. Distances between transects varied among streams and ranged from several to ~100 m. Wells were fixed by inserting prepared PVC sections into removable steel sleeves driven into sediments with a post driver (Lee and Cherry 1978, Dahm and Valett 1996). Where coarse substrate was encountered, malleable conduit was driven into the sediments and PVC with smaller diameter (diameter = 0.95 cm) was used. One transect of wells in the Wappinger consisted of steel well-points (diameter = 2.54 cm). Transect length

TABLE 1. Chemical characteristics of the West Branch Neversink River, East Branch Wappinger Creek, and Wallkill River surface water throughout the study period (1994–1996).

Stream (n = dates)	Chemical characteristics						
	TOC (mg/L)	NO_3-N (mg/L)	NH_4-N (mg/L)	TN (mg/L)	TP (mg/L)	SpCond (μS/cm)	Temp. (°C)
Neversink (n = 73)	1.2	0.24	0.005	0.31	0.004	30	7.8
Wappinger (n = 34)	3.1†	5.93	0.065	6.35‡	0.138§	291	10.7
Wallkill (n = 25)	5.7	1.02	0.047	1.42	0.160	335	10.8

Notes: Wallkill River data span from March 1993 through March 1995. The data are presented as overall means. Sample size varies among streams. Abbreviations in column heads are: TOC, total organic carbon; TN, total nitrogen; TP, total phosphorus; SpCond, specific conductivity; Temp., temporature.
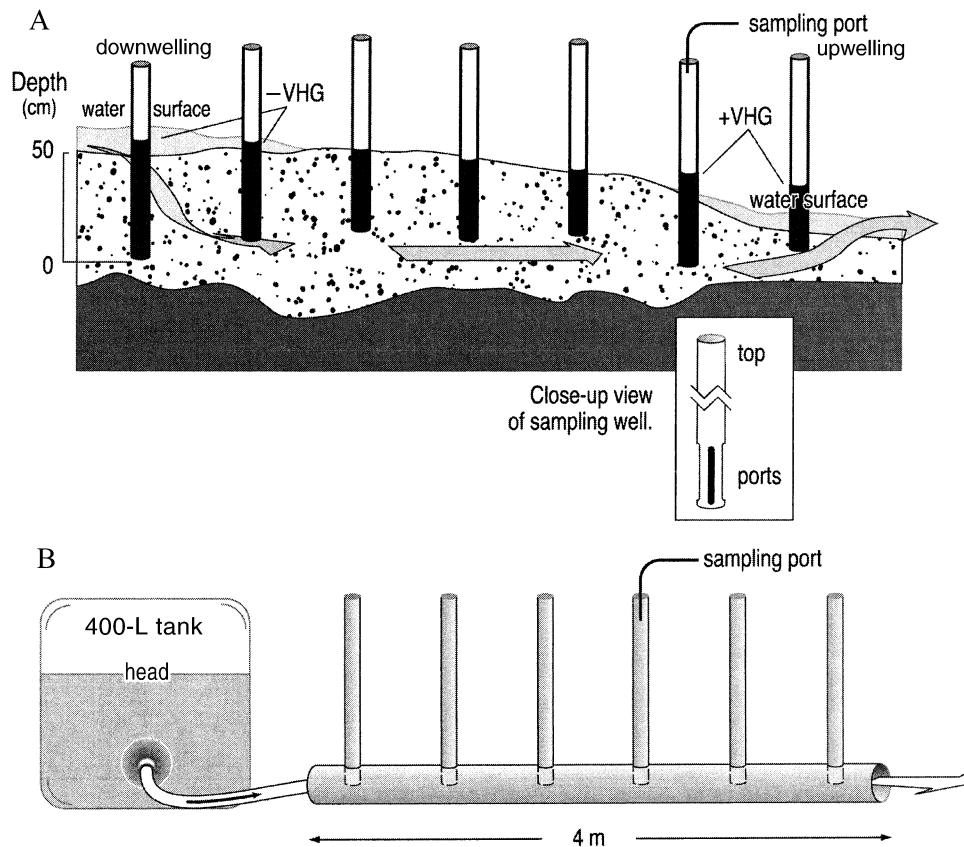
† n = 26.
‡ n = 15.
§ n = 5.

FIG. 2. (A) Cross-sectional view of exposed gravel bar, highlighting the hydrological characteristics of the hyporheic flowpaths that were studied. (B) Schematic of one of nine mesocosms that were constructed to simulate natural hyporheic flowpaths. VHG = vertical hydraulic gradient.

averaged 11 m (range 4–17 m). Distances between wells were 2 m along Neversink, Shawangunk, and Wallkill transects and ~1 m along Ham and Wappinger transects.

All transects were located along riffles that had hydraulic-head differences between transect heads (i.e., downwelling surface water) and tails (upwelling hyporheic water), as measured using clear plexiglass piezometers (Fig. 2A). In addition, sampling wells were used as piezometers: well heights were surveyed in relation to a fixed datum and depth to water estimated the slope of the water table along flowpaths (Table 2). Water temperature (White et al. 1987) and conductivity (Dahm and Valett 1996) measured along all flowpaths were indistinguishable from surface waters, suggesting that surface water was the principal water source to

TABLE 2. Hydrologic and sediment characteristics for hyporheic zones from the five streams examined.

Streams	Hydrologic and sediment characteristics						
	Slope (%)	Hydraulic conductivity (cm/s)	Hydraulic conductivity (slug test, s‡)	Velocity (cm/h)	Grain size, d_{10}	Porosity (% water)	POC (%)§
Neversink	4.4	>1.0†	8	>10	>2.0–3.35	>28	<0.5
Ham	1.1	0.8	17	3.3	0.5–1.0	33	1.0
Wappinger	1.0	0.8	16	2.8	0.5–1.0	29	0.9
Shawangunk	0.7	0.7	28	1.6	2.0–3.35	22	0.7
Wallkill	ND	0.7	36	ND	2.0–3.35	20	0.7

Note: ND = not determined.
† Large cobble were excluded from estimates in the Neversink.
‡ Denotes relative times.
§ POC = particulate organic carbon.

hyporheic flowpaths. For example, in 1995, specific conductivity of hyporheic water along Neversink (mean surface = 23 μS/cm; mean hyporheic = 22 μS/cm) and Wappinger (mean surface = 258 μS/cm; mean hyporheic = 248 μS/cm) transects was indistinguishable from surface water on all sampling dates (slopes of conductivity against transect distance did not deviate from zero). In the Shawangunk, conductivity of surface water (mean = 167 μS/cm) and hyporheic water (mean = 185 μS/cm) were significantly different on one of three sampling dates.

Sediment properties

Hydraulic conductivity was estimated in situ using slug tests and in the laboratory using a modification of the falling-head permeameter method (Freeze and Cherry 1979). In situ slug tests were conducted on all PVC wells by measuring the rate at which 5 L of water drained from a suspended funnel. Change in flux through saturated sediments with change in hydraulic head was measured in the laboratory and used to empirically derive hydraulic conductivity using Darcy's equation (Freeze and Cherry 1979). Porosity was determined by drying a known volume of saturated sediment. Particulate organic matter was estimated for 10 sediment cores from each of the five streams by measuring the difference in dry mass (24 h at 60°C) and ashed mass (6 h at 500°C).

Hyporheic water chemistry

Duplicate samples were taken monthly (July through November 1994, $n = 5$) from each well along both transects of Ham, Wappinger, and Shawangunk flowpaths. Wells were installed in the Neversink in early August, and sampled August through November ($n = 4$). Wells were installed in the Wallkill in late August, and sampled September through November ($n = 3$). Four transects of wells were installed in the Wallkill, but repeated sampling was only successful on one transect due to clogging of wells by fine sediment. We persisted in the Wallkill because it had the highest DOC concentrations in the Hudson River valley and is a significant DOC source to the Hudson River. Sampling was continued in a subset ($n = 3$) of these transects July through October 1995. This subset included Neversink, Wappinger, and Shawangunk transects. Routine sampling was conducted at baseflow. Wells were sampled with a 60-mL syringe connected to tygon tubing with a three-port connector. Wells were cleared and allowed to recharge prior to sampling. Dissolved oxygen (DO) was measured with the Winkler titration method. Porewater was slowly withdrawn to prevent degassing and injected through a short piece of tygon tubing into the bottom of a 60-mL Winkler bottle until water spilled from the top. Samples were considered anoxic when DO < 0.5 mg/L. DOC was measured by high-temperature combustion, following acidification and sparging, using a Shimadzu 5000 TIC/TOC ana-

lyzer (Shimadzu Scientific Instruments, Columbia, Maryland, USA; minimum detection limit is 0.1 mg/L). Each field sample consisted of 100 mL porewater and contained two 50-mL aliquots from the sampling syringe. DOC samples were field filtered through 1.0-μm GF/F filter (Whatman, Clifton, New Jersey USA). Since this pore size is not completely effective in removing all bacteria, samples were placed on ice, stored at 4°C, and routinely analyzed within 24 h of returning to the laboratory to minimize DOC loss. Soluble reactive phosphorus (SRP) and nitrate (NO_3^-) in surface and hyporheic water (from tail of flowpath) were analyzed with a Technicon autoanalyzer (Shimadzu Scientific Instruments, Columbia, Maryland, USA).

Biofilm sampling

In 1994, pairs of steel well points were placed at several distances along transect A in the Neversink, Ham, Wappinger, and Wallkill and ceramic tiles (size = 2.5 × 2.5 cm) were incubated to examine bacterial productivity and cell accrual. Ceramic tiles provided an artificial substrate for microbial colonization that normalized differences in natural substrate type among streams. Tiles were initially combusted at 500°C for 6 h and soaked in distilled water for 24 h before being enclosed in plastic mesh sleeves that were inserted into wells. Packets of tiles were removed after a 1-mo colonization period during October 1994. In 1995, larger PVC wells (diameter = 3.81 cm) were installed along flowpaths in the Wappinger and Shawangunk, while steel well points were reinserted into the Neversink. Ceramic tiles were sampled in August and October.

Bacterial abundance.—We measured bacterial abundance (i.e., cell accrual) with direct microscopic counts. Individual tiles were placed into whirl-pak bags ($n = 2$ per well) and fixed with 5% formalin. Tiles were scrubbed with autoclaved brushes and suspended material was sonicated for ~15 s. Subsamples were stained with acridine orange, filtered onto a stained 0.22-μm polycarbonate filter, and washed with nanopure water. Filters were mounted on slides and examined using epifluorescence microscopy at 1250× magnification. Ten fields with 10–50 bacteria were counted per slide (Kirchman et al. 1982). Direct counts were converted into bacterial abundance per tile.

Bacterial activity.—We determined bacterial activity by measuring the rate of incorporation of [^{3}H]thymidine into bacterial DNA (Findlay et al. 1984). Oxic environments were investigated throughout the experiment, hence sampling and laboratory protocols were designed for aerobic bacteria. Individual tiles ($n = 3$ or 4 tiles per well) were placed in Whirl-Paks (NASCO, Fort Atkinson, Wisconsin USA) and incubated in 10 mL of porewater. Samples were incubated at 15°C for 1 h on a mechanical shaker with 1 nmole (20 Ci/mmol) of [^{3}H]TdR. We used the washing and extraction procedure described by Findlay (1993).

Laboratory bottle experiments

Surface and hyporheic water samples were collected from the five study streams on two dates: October 1994 (bottle experiment I) and November 1994 (bottle experiment II) to examine the potential for longer term DOC loss. Surface water samples were collected immediately upstream of previously identified hyporheic flowpaths. Duplicate hyporheic water samples (2 L) were collected from wells located towards the end of flowpaths to ensure maximal microbial depletion of labile constituents. Water samples were transported to the laboratory on ice and analyzed for initial DOC (i.e., time = 0 d) as previously described. Bottles were subsequently incubated in the dark at room temperature with inoculums from individual streams. DOC concentration was measured weekly for 6 wk, and at 22 wk during bottle experiment I and weekly for 4 wk during bottle experiment II. Identical filtration procedures (as previously discussed) were used for all water samples (fresh samples and incubated samples). Our filtration procedure did not remove all free-floating bacteria, hence estimates of bioavailable DOC may have been slightly underestimated in theory. We also examined DOC loss following nitrogen (1 mg/L N-NaNO$_3$) and phosphorous (0.1 mg/L P-NaPO$_4$) additions to subsamples from bottle experiment I following the 28-d incubation period. In addition, we examined DOC loss following N and P additions relative to unamended controls for surface water sampled in June 1996 from each of the five streams. Surface water was sampled at three separate locations in each of the five streams, subdivided into amended and control batches, incubated for 7 d, and tested for differences in DOC concentration. Nutrient additions (molar equivalent of stream DOC assuming C:N:P = 106:15:1) were large enough to potentially allow complete metabolism of ambient DOC.

Mesocosm experiments

To systematically eliminate the confounding effects of velocity differences and mixing of multiple water sources during the field studies, we used mesocosm studies to examine dissolved oxygen, DOC and microbial variables under conditions of controlled interstitial flow rates. The mesocosms consisted of nine sediment-packed pipes (PVC pipe, inner diameter = 15 cm, length = 4 m) in which DOC supply was experimentally manipulated in a controlled, replicated manner (Fig. 2B; see also Plate 1). Local quarry sediments (washed crushed gravel) that provided a standard hydraulic conductivity and porosity were placed within each pipe. Prior to experimentation, sediments were incubated with unfiltered streamwater for 1 mo to ensure colonization by native microbial communities. Stream surface-water was collected in a 1500-L tank and transferred to mesocosm reservoirs (volume = 440 L) that were refilled weekly. Water was gravity fed through the sediment-packed pipe from an elevated reservoir. Discharge (~1 L/h) was regulated on each mesocosm by adjusting a ball valve and outlet hose height. This corresponded to an interstitial flow rate of ~10 cm/h. A series of sampling ports allowed water and ceramic tiles along the flowpaths to be sampled. Dissolved oxygen was routinely measured to ensure oxic conditions using a portable DO meter (YSI Model 57, YSI, Yellow Springs, Ohio, USA). Chemical and microbial measures were the same as for natural flowpaths.

Mesocosm experiments performed during November 1995 (stream comparison I) and May 1996 (stream comparison II) compared potential metabolism of surface water from the Neversink (n = 3 mesocosms), Wappinger (n = 3), and Shawangunk (n = 3). Tank water was replaced weekly unless surface water DOC was elevated (e.g., storm run-off). DOC and DO were sampled weekly during experiments in tanks and outlets. In addition, DOC, DO, SRP, and NO$_3^-$ were sampled at three intermediate locations (head, midpoint, and tail) at the end of the experiments. Bacterial productivity and abundance were measured on tiles after 3-wk incubations in the tanks, heads, and tails of all nine mesocosms. The mesocosm experiment in July 1996 (dilution experiment) compared potential metabolism of the Neversink, Wappinger, and Wappinger diluted 1:1 with deionized water. This dilution treatment made Wappinger DOC concentration approximate the Neversink DOC concentration, but maintained the DOC composition of the Wappinger.

Statistical analyses

Field 1994 DOC and DO data were analyzed by pooling sampling dates from individual flowpaths such that error was derived from independent sampling dates. Field 1995 data were analyzed separately. Bacterial activity and abundance were not replicated in time, hence we present mean values from tiles incubated within wells. Linear regression with distance as the independent variable was used to examine changes along flowpaths. Laboratory bottle experiments were analyzed using paired t tests. Mesocosm experiments provided replication of flowpaths in space and wells at fixed distances among treatments. Sources of water (i.e., water from streams) were considered treatments. Sources were selected because they vary in DOC concentration, hence DOC data was analyzed using linear regression. Bacterial activity and abundance data were analyzed with two-way ANOVAs in which source and distance were considered fixed factors. Significance was attributed to statistical values in which the probability of a type I error is $P \leq 0.05$. SYSTAT version 6.1 (SPSS, Chicago, Illinois, USA) was used for all statistical analyses.

RESULTS

Hyporheic flowpath studies

Physical conditions.—Sediments consisted of a mixed alluvium containing gravel and sand, although
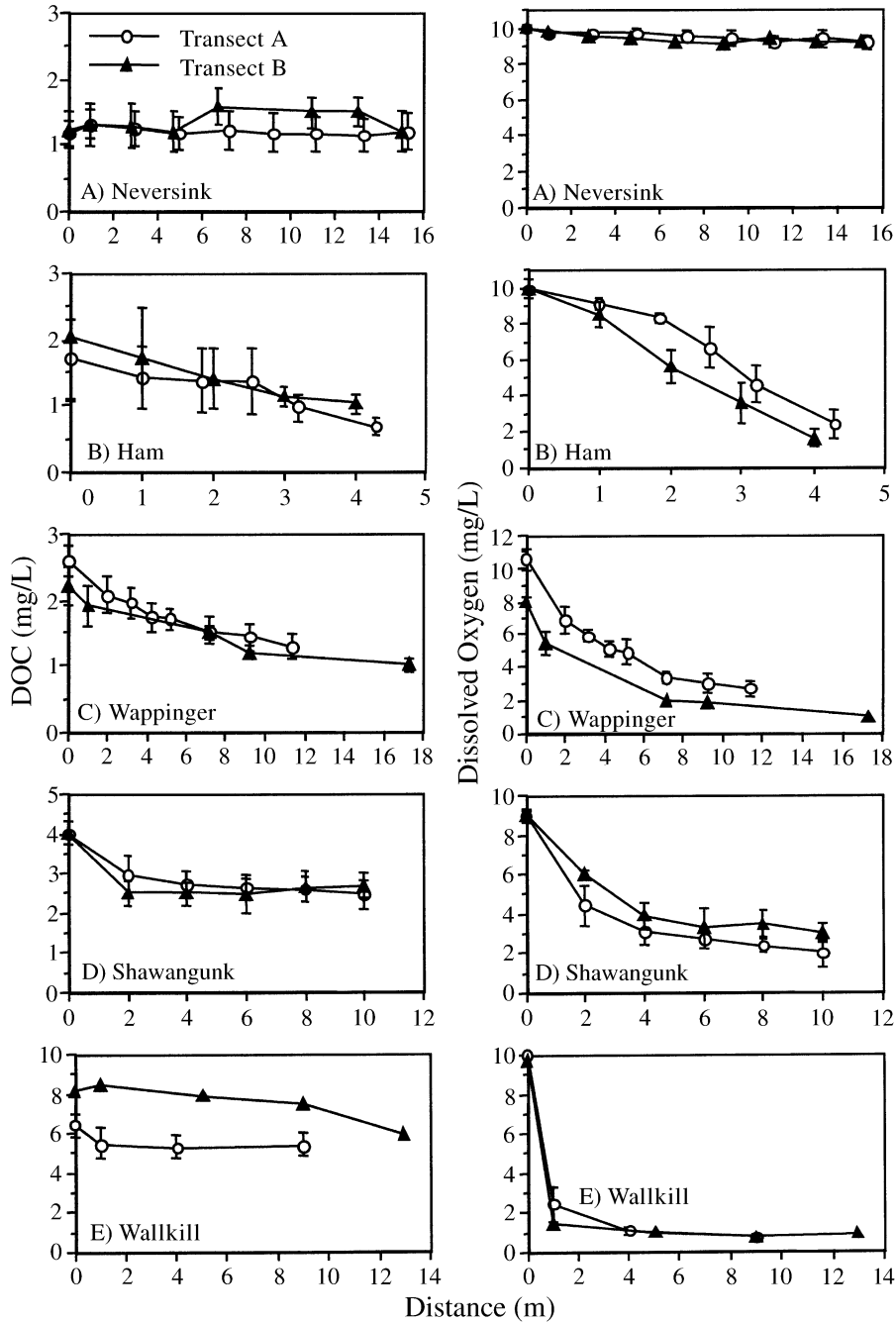
FIG. 3. DOC and dissolved oxygen along two hyporheic flowpaths in each of five streams: West Branch Neversink River, Ham Creek, East Branch Wappinger Creek, Shawangunk River, and Wallkill River in 1994. Points represent means (± 1 SE) for sampling dates (Neversink, $n = 4$; Ham, $n = 5$; Wappinger, $n = 5$; Shawangunk, $n = 5$; Wallkill, $n = 3, 1$; see *Methods* for details). Distance spans from downwelling surface water (i.e., distance $= 0$) to final well along flowpath. Open circles represent transect A, and filled triangles represent transect B.

sediment-size distribution varied among streams (Table 2). Hydraulic conductivity estimated from the falling-head permeameter was greatest for the Neversink (Table 2). Estimated interstitial water velocity was greatest along Neversink flowpaths due to relatively large hydraulic head and conductivity. Since large cobble in Neversink sediments were excluded, we probably underestimated Neversink velocity. In situ slug tests with Neversink wells required much less time than other streams (Table 2), strongly suggesting that Neversink flowpaths had a higher interstitial water velocity than flowpaths from other streams.

TABLE 3. Paired comparisons between surface water and hyporheic water NO_3-N and SRP during multiple years and experiments.

Stream	SRP†		NO_3-N	
	Surface	Hyporheic	Surface	Hyporheic
Field 1994				
Neversink	<0.002	<0.002	0.080 (0.010)	0.100 (0.005)
Ham	<0.002	<0.002	0.663 (0.017)	0.667 (0.028)
Wappinger	0.080 (0.023)	0.046 (0.022)	0.415 (0.045)	0.420 (0.135)
Shawangunk	0.019 (0.009)	0.026 (0.004)	0.375 (0.005)	0.163 (0.043)
Wallkill	0.575 (0.032)	ND	1.618 (0.005)	ND
Field 1995				
Neversink	0.004 (0.000)	0.005 (0.000)	0.254 (0.017)	0.260 (0.020)
Wappinger	0.189 (0.101)	0.055 (0.035)	0.560 (0.145)	0.550 (0.205)
Shawangunk	0.109 (0.061)	0.051 (0.010)	0.625 (0.275)	0.055 (0.014)
Stream comparison I, Nov 1995				
Neversink	0.004 (0.002)	0.005 (0.002)	0.173 (0.033)	0.123 (0.057)
Wappinger	0.145 (0.015)	0.063 (0.010)	0.923 (0.034)	0.587 (0.052)
Shawangunk	0.107 (0.020)	0.043 (0.020)	0.387 (0.035)	0.323 (0.035)
Stream comparison II, May 1996				
Neversink	0.010 (0.004)	0.011 (0.001)	0.270 (0.021)	0.183 (0.012)
Wappinger	0.079 (0.005)	0.022 (0.005)	0.516 (0.023)	0.453 (0.032)
Shawangunk	0.014 (0.010)	0.008 (0.002)	0.167 (0.026)	0.158 (0.007)
Dilution experiment, July 1996				
Neversink	<0.002	0.003 (0.001)	0.213 (0.041)	0.153 (0.020)
Wappinger	0.182 (0.009)	0.049 (0.014)	0.662 (0.037)	0.567 (0.037)
Wappinger/Diluted	0.077 (0.009)	0.029 (0.009)	0.374 (0.022)	0.305 (0.013)

Notes: Summary values are presented as mg/L (± 1 SE); $n = 2$ dates for field 1994 and 1995 data, $n = 3$ mesocosms for all mesocosm experiments. ND = not determined.
† Soluble reactive phosphorus.

Chemical conditions.—In 1994, we documented significant declines in porewater DOC concentrations along hyporheic flowpaths in the Ham, Wappinger, Shawangunk, and Wallkill, but no drop along Neversink flowpaths (Fig. 3) and these patterns were con-
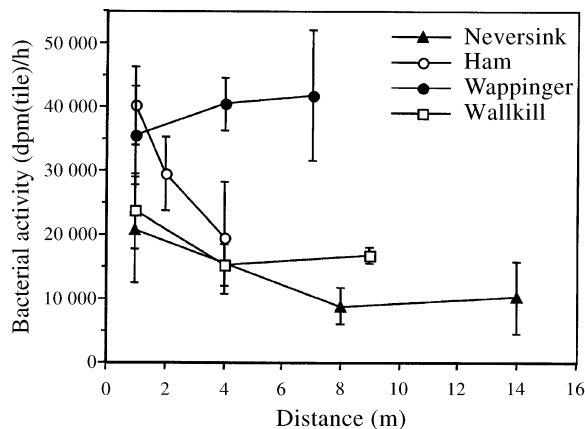

FIG. 4. Bacterial activity on tiles incubated along hyporheic flowpaths in four streams (Neversink River, Ham Creek, East Branch Wappinger Creek, and Wallkill River) in 1994. Bacterial activity is measured as disintegrations per minute (dpm) for biofilm removed from one sampling tile following a 1-h [3H]TdR incubation (shown as ''dpm(tile)/h''; see *Methods* for details). Points represent means (± 1 SE) of tiles ($n = 3$). Productivity was not measured along Shawangunk River flowpaths.

sistent across multiple sampling dates. DOC patterns varied considerably among streams, yet showed remarkable consistency between flowpaths (A and B) within streams (Fig. 3), so we discuss only transect A from each stream. In the Neversink (low-DOC river), hyporheic DOC remained similar to surface-water DOC along 15-m and 17-m flowpaths. In contrast, DOC consistently decreased along shorter hyporheic flowpaths in the Ham, Wappinger, Shawangunk, and Wallkill (Fig. 3). Hyporheic DOC concentrations never exceeded surface-water DOC in the Wappinger ($n = 55$ samples) or Shawangunk ($n = 50$ samples) and declined 50% along Wappinger flowpaths ($P < 0.001$) and 38% along Shawangunk flowpaths ($P < 0.001$). Hyporheic DOC declined 60% along Ham flowpaths ($P < 0.05$), but declines varied among dates (range 13–68%). Percentage of DOC loss was greatest when surface-water DOC was highest in July. Excluding this date, the mean decline in DOC was 39%. Porewater DOC declined 16% along Wallkill flowpaths ($P < 0.01$).

We found consistent DO declines of variable magnitude along hyporheic flowpaths in the Ham, Wappinger, Shawangunk, and Wallkill ($P < 0.001$), but no appreciable decline in hyporheic DO in the Neversink (Fig. 3). Hyporheic DO never exceeded surface-water DO in any stream ($n = 214$) and subsurface concentrations ranged from nearly saturated to anoxic <0.5
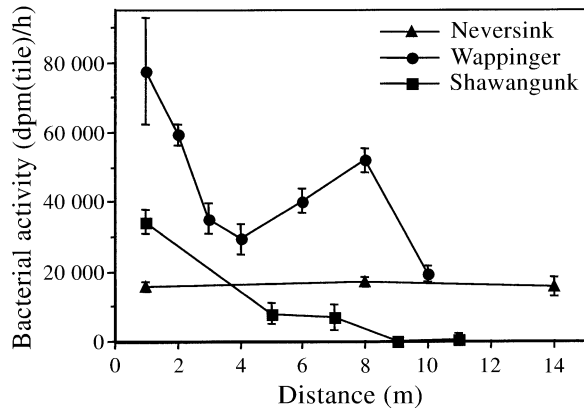
FIG. 5. Bacterial activity along hyporheic flowpaths in three streams (Neversink River, East Branch Wappinger Creek, and Shawangunk River) in August 1995. Bacterial activity was measured as disintegrations per minute (dpm) for biofilm removed from one sampling tile following a 1-h [3H]TdR incubation (shown as "dpm(tile)/h"; see *Methods* for details). Points represent means (± 1 SE) of tiles ($n = 3$).

mg/L. Wallkill porewater was consistently low in DO, regardless of flowpath location and frequently suboxic.

Field efforts in 1995 focused on flowpaths (two per stream) in three streams showing extreme patterns: Neversink, Wappinger, and Shawangunk. These flowpaths showed stable clines in DOC and DO during the 1994 field season and provided a range in DOC concentrations and relative losses. DOC patterns along these flowpaths were generally similar to those documented in 1994. However, drought conditions throughout summer 1995 resulted in low discharge and low surface-water DOC in all streams, and storm events in September variably impacted surface-water DOC concentrations (e.g., Neversink surface-water DOC increased to 2.6 mg/L). Excluding storm-flow data, the pattern of DOC declines were similar to 1994, but percentage losses were smaller: Wappinger (37%) and Shawangunk (21%). Similar to 1994, DOC declines were coupled to decreasing DO along Wappinger and Shawangunk flowpaths ($P < 0.001$).

Surface water and hyporheic NO_3^- and SRP were examined for field data on two dates in 1994 and two dates in 1995. NO_3^-, the predominant form of N in regional streams, remained unchanged along hyporheic flowpaths in the Neversink, Ham, and Wappinger in 1994, however NO_3^- decreased in hyporheic water relative to surface water in the Shawangunk in 1995 (Table 3). SRP was routinely less in hyporheic water relative to surface water in Wappinger and Shawangunk on most dates (Table 3). SRP was below detection in surface and hyporheic waters of the Neversink and Ham for both dates in 1994, and was low in the Neversink (0.005 and 0.012 mg/L) for two dates in 1995 (Table 3).

Biofilm activity

Bacterial activity (measured as thymidine incorporation into bacterial DNA) on ceramic tiles incubated

for 1 mo in 1994 was generally higher in the Wappinger relative to Neversink and Wallkill flowpaths (Fig. 4). Bacterial activity decreased sharply with distance in the Ham ($P < 0.001$), modestly in the Neversink and Wallkill ($P < 0.05$), and not at all in the Wappinger. Bacterial activity was not measured in the Shawangunk. Bacterial activity along flowpaths in August 1995 varied among streams and along flowpaths (Fig. 5). Bacterial activity was low in the Neversink and did not decrease along the hyporheic flowpath ($P > 0.05$). In contrast, activity was substantially higher in the Wappinger at the head of the flowpath and decreased ($P < 0.001$) by nearly 75% at the tail to rates as low as those found in the Neversink. Although the Shawangunk had higher surface and hyporheic DOC concentrations than the Wappinger, bacterial activity at the head of the Shawangunk hyporheic flowpath was much less than
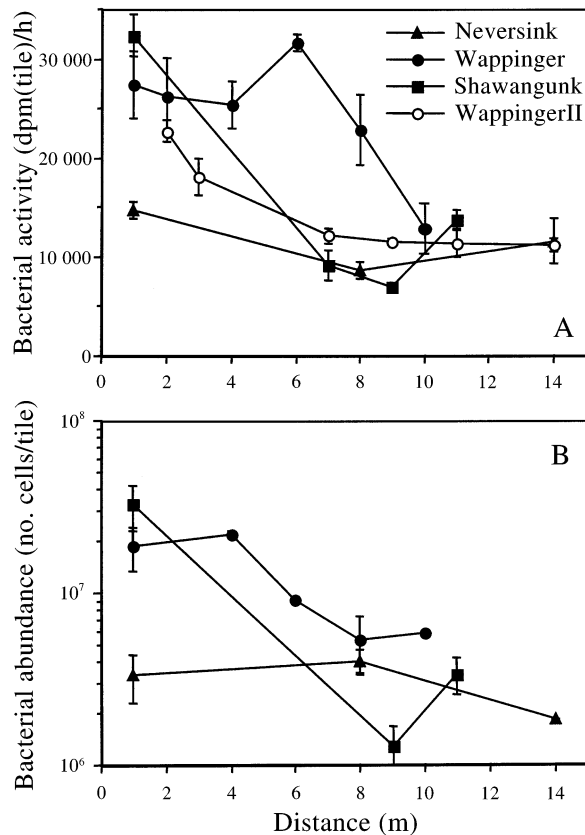

FIG. 6. (A) bacterial activity along hyporheic flowpaths in three streams (Neversink River, East Branch Wappinger Creek, and Shawangunk River) in October 1995. In addition, bacterial activity along an additional flowpath in the East Branch Wappinger Creek (i.e., WappingerII) is presented. Bacterial activity was measured as disintegrations per minute (dpm) for biofilm removed from one sampling tile following a 1-h [3H]TdR incubation (shown as "dpm(tile)/h"; see *Methods* for details). Points represent means (± 1 SE) of tiles ($n = 3$). (B) Bacterial abundance along hyporheic flowpaths in the three streams. Points represent means (± 1 SE) of tiles ($n = 2$).

TABLE 4. DOC concentrations from laboratory bottle experiments in which surface water and hyporheic water were incubated for 28 d to examine DOC loss over time.

| | DOC (mg/L) | | | | | | |
| | Surface | | | Hyporheic | | | |
Stream	$T = 0$	$T = 28$ d	Loss (%)	$T = 0$	Surface (%)	$T = 28$ d	Loss (%)
Experiment I							
Neversink	1.3	1.2	5	1.2	5	1.2	0
Ham	1.4	1.5	−4	1.1	19	1.3	−13
Wappinger	2.5	2.1	16	1.5	41	1.6	−7
Shawangunk	4.6	3.1	34	2.4	48	2.4	0
Wallkill	6.2	5.0	19	4.5	27	4.5	0
Experiment II							
Neversink	1.5	1.4	7	1.3	13	1.3	0
Ham	1.9	1.7	11	1.1	42	1.0	9
Wappinger	2.6	2.3	12	1.5	42	1.4	7
Shawangunk	3.3	2.9	13	2.1	37	2.0	5
Wallkill	5.3	4.5	15	4.3	19	3.7	14

Note: Values are the mean of two bottles.

at the head of Wappinger flowpaths and only modestly greater than the Neversink flowpath. Bacterial activity along the Shawangunk flowpath decreased to undetectable rates ($P < 0.001$). Bacterial activity patterns were similar in October 1995 (Fig. 6A), suggesting consistent response. Bacterial abundance was low and constant along the Neversink flowpath but showed marked declines along Wappinger and Shawangunk flowpaths (Fig. 6B). For the one date (October 1995) where we have concurrent abundance and activity data, these were strongly correlated ($P < 0.001$; $r^2 = 0.81$).

Laboratory bottle experiments

DOC from longer-term bottle incubations of surface water DOC from the Wappinger, Shawangunk, and Wallkill declined appreciably in both experiments with declines ranging from 0 to 1.5 mg C/L (Table 4) over the 4-wk span. In contrast, most hyporheic samples from the tail of sampling transects did not show further declines (paired t test, $n = 10$, $P > 0.05$). However, ''residual'' surface DOC concentrations following the bottle incubation (i.e., surface water at $T = 28$ d) were significantly greater than hyporheic DOC concentrations at the start of the incubations (i.e., hyporheic water at $T = 0$ d; paired t test, $n = 10$, $P < 0.001$; Table 4). DOC concentrations before and after the nutrient addition were indistinguishable (paired t test, $n = 10$, $P = $ NS; data not shown), suggesting that inorganic nutrients did not constrain DOC loss. Inorganic nutrient limitation was re-examined in all five streams in June 1996. Excluding the Wallkill, there was no difference between the amended and control treatments (paired t test, $n = 12$, $P = $ NS; data not shown).

Mesocosm experiments

Stream comparison I.—In general, patterns of DOC loss in mesocosms supplied with water from a particular stream paralleled field observations for that stream suggesting our in situ observations were not badly confounded by variable hydrology or mixing of multiple water masses. DOC significantly declined in both Wappinger (28%) and Shawangunk (28%) mesocosms ($P < 0.001$), but increased slightly in Neversink mesocosms (9% increase), probably by leaching of gravel-associated POC (Fig. 7A). Replicate mesocosms were very consistent in percentage of DOC loss (mean CV of the percentage of DOC loss = 8%). DO declined in all mesocosms ($P < 0.001$) during transport, even in the absence of DOC loss, suggesting POC mineralization in sediments. DO declined to 3.0 mg/L in Wappinger mesocosms and 3.8 mg/L in Shawangunk mesocosms, but also declined to 5.4 mg/L in Neversink mesocosms (Fig. 7B). Similar to field studies, DOC
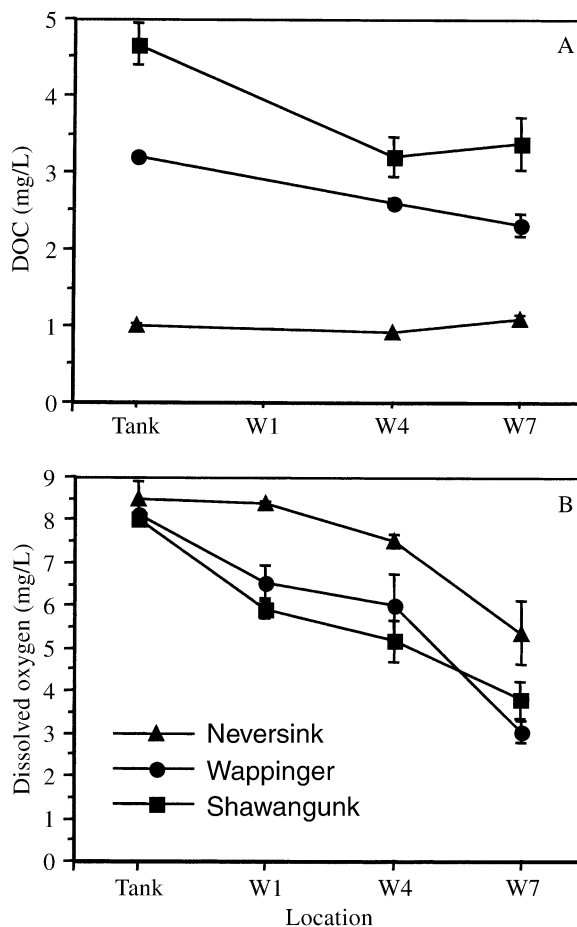

FIG. 7. (A) DOC and (B) dissolved oxygen along mesocosm flowpaths containing water from three streams (Neversink River, East Branch Wappinger Creek, and Shawangunk River) from mesocosms in stream comparison I. Points represent means (± 1 SE) for replicate mesocosms ($n = 3$; see Fig. 2).
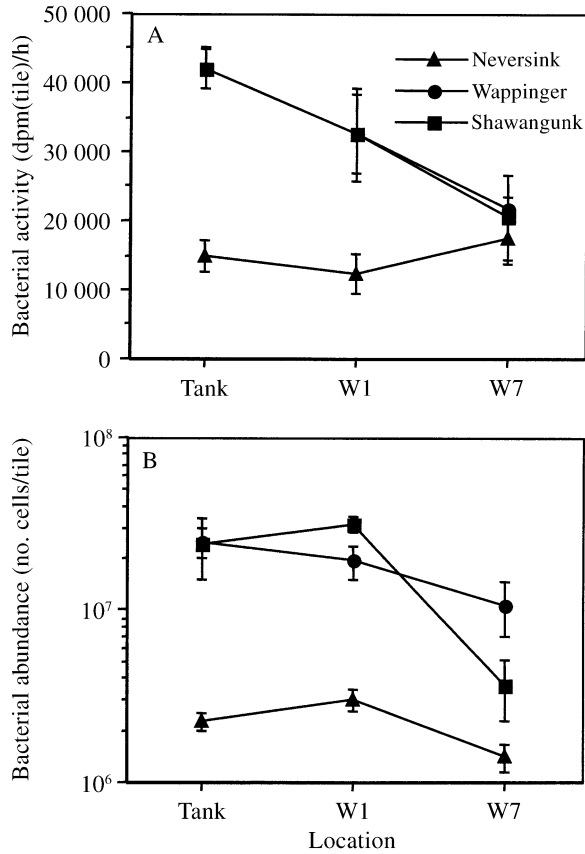
FIG. 8. (A) Bacterial activity and (B) bacterial abundance along mesocosm flowpaths containing water from three streams (Neversink River, East Branch Wappinger Creek, and Shawangunk River) in mesocosms in stream comparison I. Bacterial activity was measured as disintegrations per minute (dpm) for biofilm removed from one sampling tile following a 1-h [3H]TdR incubation (shown as ''dpm(tile)/h''; see *Methods* for details). Points represent means (± 1 SE) for replicate mesocosms ($n = 3$).

losses corresponded to declines in bacterial activity. Bacterial activity declined in both Wappinger (49% decline) and Shawangunk mesocosms (51% decline; $P < 0.001$; Fig. 8A). In contrast, bacterial activity was significantly lower in Neversink mesocosms compared to Wappinger and Shawangunk tank water ($P < 0.001$; Fig. 8A). Bacterial abundance showed similar patterns as bacterial activity ($P < 0.001$; $r^2 = 0.88$) with marked declines along Wappinger and Shawangunk flowpaths and low and static densities along Neversink flowpaths (Fig. 8B).

Stream comparison II.—The mesocosm experiment conducted in November 1995 was repeated in May 1996. DOC was higher in Neversink surface water (1.5 mg/L) than during the initial comparison and declined 20% (to 1.2 mg/L; Fig. 9A). DOC declines along Wappinger and Shawangunk flowpaths were 21% and 19% respectively, modest in comparison with losses during stream comparison I. The discrepancy between the two experiments may be associated with seasonal differ-

ences in DOC composition. DO significantly declined ($P < 0.001$) along all mesocosm flowpaths: Neversink declined to 5.8 mg/L, Wappinger declined to 4.3 mg/L, and Shawangunk declined to 5.4 mg/L. Bacterial activity significantly declined along Wappinger (42%) and Shawangunk (52%) mesocosms ($P < 0.001$), and was significantly different among streams ($P < 0.001$; Fig. 9B).

Dilution experiment.—The July 1996 mesocosm experiment was designed to examine the influence of initial DOC concentration on relative DOC loss along hyporheic flowpaths. DOC removal along Wappinger flowpaths was less pronounced compared to previous mesocosm experiments (mean = 13%), but loss still exceeded DOC removal along Neversink flowpaths. The 1:1 dilution of Wappinger water brought initial DOC roughly equal to Neversink total DOC concentrations. The percentage of removal of DOC in the
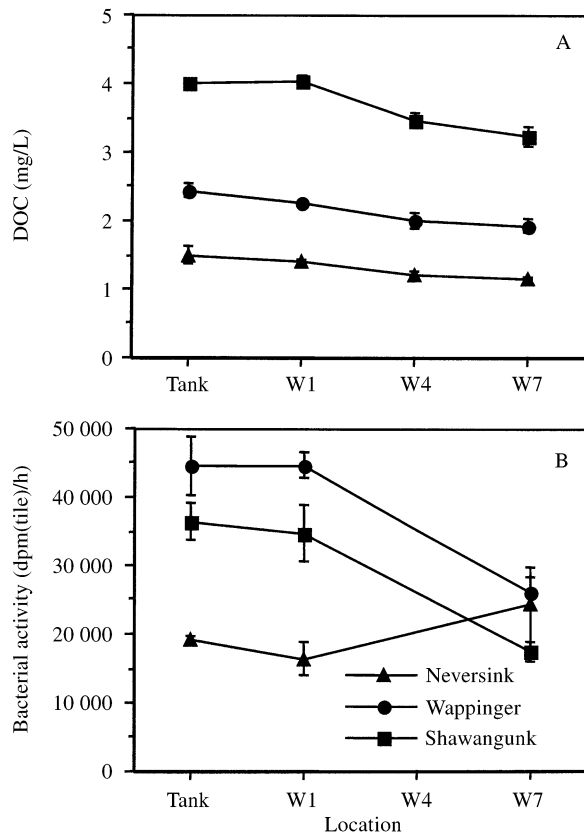

FIG. 9. (A) DOC along mesocosm flowpaths containing water from three streams (Neversink River, East Branch Wappinger Creek, and Shawangunk River) from mesocosms in stream comparison II. Points represent means (± 1 SE) for replicate mesocosms ($n = 3$). (B) Bacterial activity along mesocosm flowpaths containing water from the three streams above. Bacterial activity was measured as disintegrations per minute (dpm) for biofilm removed from one sampling tile following a 1-h [3H]TdR incubation (shown as ''dpm(tile)/h''; see *Methods* for details). Points represent means (± 1 SE) for replicate mesocosms ($n = 3$).

diluted treatment was not significantly affected by dilution (mean = 16% vs. 13%).

DISCUSSION

Hyporheic DOC removal

In four of five streams we investigated, surface-water DOC concentrations decreased after perfusing into hyporheic sediments and percentage of DOC loss varied among streams (e.g., Wappinger = 50% and Shawangunk = 38%). Only in the Neversink, a low-DOC stream in the Catskill Mountains, was DOC removal negligible and transport apparently conservative. If declines in DOC concentration were mediated by microbial metabolism a concomitant loss of DO is predicted. Our results showed that DO consistently decreased along flowpaths in which DOC declined and DOC never declined independently of DO, regardless of flowpath location or date. For example, the correlation between DOC and DO losses along flowpaths was highly significant and variation in DOC loss accounted for a large portion of the variance in DO declines (linear regression, $r^2 = 0.76$, $P < 0.001$, $n = 29$). DOC metabolism accounted for 52% of the DO demand assuming DOC and DO were consumed in a 1:1 molar ratio. The ''residual'' oxygen consumption (48%) could be due to metabolism of buried POC (i.e., detritus), previously adsorbed DOC, or DO consumption by reactions with other reduced species (e.g., $Fe++$, NH_4, CH_4). We cannot unambiguously resolve these alternatives but believe that metabolism of buried POC is probably the major contributor to the residual oxygen consumption because of consistent relationships between sediment respiration and POC content (Jones 1995, Fischer et al. 1996, Pusch 1996, Sinsabaugh 1997).

Bacterial activity and abundance declined in parallel with DOC during both incubation periods in 1995, however, a significant decline in production was not found in the Wappinger during 1994. Bacterial activity and abundance were lowest in the Neversink and never decreased significantly along hyporheic flow in any experiment. Concomitant decreases in bacterial activity and abundance along hyporheic flowpaths in which DOC loss was reported and the absence of such declines along Neversink flowpaths supports microbial metabolism as the mechanism for DOC removal along hyporheic flowpaths.

Differences in the hydrologic regimes among the five streams we studied (Table 2) may have confounded interpretation of in situ biogeochemical patterns (Findlay 1995, Jones and Holmes 1996, Morrice et al. 1997). For example, Neversink flowpaths had a steeper slope and coarser sediments than other flowpaths thus producing faster interstitial-water velocity. Estimated hyporheic water velocity in the Neversink was at least five-fold higher than in the Wappinger. The absence of DOC losses may have partially resulted from insuffi-cient contact time with hyporheic bacteria or the mixing of infiltrating surface water with additional sources of groundwater. In support of our metabolism explanation, consistent water temperature and conductivity along flowpaths made dilution a less likely mechanism and results from laboratory bottle experiments suggest hyporheic contact time was sufficient. Mesocosm experiments clearly delineated hydrological from biogeochemical controls on hyporheic DOC removal and the parallel results for DOC declines in mesocosm and field experiments make differential residence time or mixing unlikely explanations for our observations. In addition, the mesocosm studies provided spatial replication of hyporheic flowpaths, rather than replication in time.

In stream comparison I, DOC declined along Wappinger and Shawangunk flowpaths, yet remained unchanged along Neversink flowpaths as was observed in the field. DOC declines were always mirrored by declines in bacterial activity and abundance that were always lowest at the tail of hyporheic flowpaths. Similar patterns were found in the stream comparison II experiment, although DOC declines were less pronounced and may be the result of seasonal differences in DOC composition. The mesocosm experiments, like the field observations, provided strong evidence that DOC removal along hyporheic flowpaths in the Wappinger and Shawangunk resulted from microbial metabolism. The mesocosm experiments demonstrated that inadequate contact time could not explain the absence of DOC removal along Neversink flowpaths. Rather, Neversink DOC appears to be resistant to hyporheic metabolism on the scale of the 2-d travel time through the mesocosm flowpaths and on the scale of the 28-d laboratory incubations. In contrast, significant fractions of Wappinger (50%) and Shawangunk (38%) surface-water DOC were consumed by hyporheic metabolism within several days. Further, it appears from the long-term bottle experiments that DOC exiting hyporheic flowpaths was largely unavailable for additional metabolism, regardless of its initial bioavailability.

Carbon limitation in hyporheic bacteria

Variation in DOC loss along hyporheic flowpaths from different streams shows clearly that the proportion of DOC susceptible to metabolism under real world conditions can be both large and variable. Differences among streams were not simply related to initial concentration and the proportional removal of Wappinger DOC was unaffected by a 1:1 dilution with low-DOC water to reduce the concentration. Our mesocosm experiments refute the possibility that among-stream differences were simply due to hydrology or mixing. There are a number of biogeochemical explanations for variation in DOC removal including availability of thermodynamically favorable electron acceptors, inorganic nutrient limitation of metabolism, temperature, pH, and meiofaunal grazing.

Heterotrophic bacteria require organic carbon as

both a carbon and energy source (i.e., electron donor). In anoxic environments bacterial activity can be constrained by the absence of thermodynamically favorable electron acceptors. In this study, we limited our focus to aerobic respiration, hence electron acceptor limitation was not an issue.

Temperature and pH can also influence aerobic respiration. Sinsabaugh (1997) found that heterotrophic respiration among 22 streams correlated with temperature, although he acknowledges that this correlation is confounded by a latitudinal gradient. In another useful regional survey, Osgood and Boylen (1994) showed that decomposition rates correlated with pH in Adirondack streams. In our study, Neversink is at a higher elevation and has a lower annual water temperature than other streams, however, summer and fall temperatures would not limit bacterial activity and mesocosm studies controlled for temperature differences among streams. Streams varied in pH as well (e.g., Neversink = 6.9, Wappinger = 7.9), but again such neutral pH values were unlikely to constrain whole-community bacterial activity (Atlas and Bartha 1998).

Availability of inorganic N and P are known to influence bacterial productivity in some freshwater ecosystems (Pace and Cole 1996, Miettinen et al. 1997). Nitrogen and P concentrations in surface waters in this study were generally high, except in the Neversink River where P was relatively low (Table 1 and 3). Nitrate was always abundant even at flowpath tails and DOC loss in bottle experiments was not stimulated by addition of inorganic nutrients. SRP concentrations were lower in hyporheic than surface waters of the Wappinger and Shawangunk on most dates (Table 3) and Neversink SRP was below detection (<0.002 mg/L) for both dates in 1994 and was low (0.005 and 0.012 mg/L) for two dates in 1995. SRP declined along Wappinger and Shawangunk flowpaths in mesocosm experiments (Table 3), but SRP removal was not related with DOC removal implying abiotic removal. Overall, results from field, laboratory, and mesocosm experiments do not support inorganic nutrient limitation, thus differences in DOC losses among flowpaths and experiments do not appear to be a function of variation in inorganic nutrient availability.

Grazing has long been considered a potential mechanism for the removal of bacterial biomass in aquatic ecosystems (Fenchel 1978, Pratt and Cairns 1985; see reviews by Bott 1995, 1996) although there is scant evidence that grazing is severe enough to limit bacterial metabolism in sediments (Bott 1995). Observations that few bacteria in stream sediments are viable (e.g., usually <10%; Fischer et al. 1996, Hendricks 1996) suggests that rapid division of cells is not necessary to sustain reported densities. We conclude (cautiously) that among-stream differences in grazing are unlikely to be the proximate cause of differences in DOC loss or hyporheic metabolism.

Overall, our results suggest sediment-bound bacteria in aquatic ecosystems are often carbon limited (Findlay and Sobczak 2000, Kaplan and Newbold 2000) and variation in DOC quality can be an important control on their metabolism.

Hyporheic metabolism

Metabolism of DOC varied markedly among hyporheic zones, and, in some streams, the differences in downwelling vs. upwelling DOC concentrations was large relative to our documented distribution of DOC in regional streams. For example, the grand mean DOC concentration entering Wappinger flowpaths was 3.1 mg/L, corresponding to the median concentration for the region, however the DOC concentration after hyporheic processing was on average 50% less corresponding to the sixteenth percentile for surface-water concentrations in Hudson River valley streams. Thus, the among-stream differences in hyporheic metabolism can result in variation in DOC of the same magnitude as regional differences in surface water concentrations. For streams where there is substantial exchange between surface waters and the hyporheic zone, hyporheic metabolism has the potential to alter DOC export to downstream reaches. The ecosystem-level importance of hyporheic metabolism in material budgets has been documented in several cases and our results show that among stream variation in metabolic potential is large. The other requisite for quantifying the budgetary implications of hyporheic metabolism is the variation in proportion of water exchange (Findlay 1995). Few individual studies have documented that the rate of infiltration influences small-scale metabolism (Battin 2000) and hydrologic and metabolic variability have not been linked at the cross-system scale necessary to arrive at a general description of conditions necessary for hyporheic processes to affect ecosystem budgets.

The wide range in DOC consumption among hyporheic flowpaths corresponded to a surprisingly large range in bacterial activity (approximately four orders of magnitude) on tiles incubated along these flowpaths. Variation in bacterial activity on these tiles was a function of DOC quality or bioavailability, as opposed to concentration, and was not associated with variation in sediment-bound POC. The range in bacterial activity we observed in the field and mesocosms, and believe to be primarily driven by differences in DOC bioavailability, is large relative to variation in the accumulation of bacterial-activity measures collected from diverse stream ecosystems. Based on available, yet sparse, literature data, sediment-associated hyporheic bacterial production ranged only three orders of magnitude (Findlay and Sobczak 2000), surprisingly less than the range for bacterial activity we observed. Therefore, we believe that among stream differences in DOC bioavailability represent a significant source of variability for sediment communities.

The discrepancy between molar losses of DOC and DO suggests that additional metabolic processes (other

than the metabolism of DOC) were occurring along the hyporheic flowpaths. Metabolism of buried POC (i.e., detritus; Sobczak et al. 1998) or previously adsorbed DOC (Findlay and Sobczak 1996) may have contributed to the additional oxygen demand. Even assuming high rates of external DOC supply, sediment-bound POC could still factor prominently in hyporheic metabolism even if only a small percentage (e.g., $<0.1\%$ per day) were consumed (see Findlay and Sobczak 2000). In many hyporheic zones, sediment-bound POC may be largely derived from DOC that fluxes through the sediments, hence hyporheic DOC and POC are not independent organic matter pools. A component of hyporheic oxygen may have resulted from reactions with reduced inorganic compounds (Baker et al. 2000*b*, Duff and Triska 2000). Since this study focused on oxic hyporheic zones, aerobic respiration was likely the dominant metabolic process.

Hyporheic zone influence on stream organic matter dynamics

There has been long-standing (Fisher and Likens 1973) and continued interest in quantifying organic inputs, standing stocks, and exports from stream ecosystems (Webster and Meyer 1997). In some streams, hyporheic zones may account for a large fraction of stream heterotrophy (e.g., Grimm and Fisher 1984) and may be poorly represented in organic matter budgets (Jones 1997). The importance of hyporheic flow on respiration most likely varies markedly among streams (Findlay 1995, Jones and Holmes 1996), and is difficult to quantify empirically due to uncertainty in the extent of hyporheic storage and exchange (Bencala 2000, Harvey and Wagner 2000). Efforts to quantify hyporheic respiration at the reach scale frequently require extrapolation of isolated measures (Mulholland et al. 1997), and experimental verification of causal mechanisms underlying variance in heterotrophy is a daunting task (Sinsabaugh 1997). In addition to respiration supported by downwelling DOC, hyporheic metabolism may also influence the quality of DOC eventually transported downstream. This study showed that transport along some hyporheic pathways could remove as much as 50% of the DOC leaving a fraction of DOC relatively resistant to further metabolism. Whether this material is truly refractory or might be susceptible to further degradation by exposure to sunlight or novel microbial consortia is unknown, but streams with significant hyporheic water flow and substantial DOC consumption may act as a filter altering organic material availability to downstream communities. It is now well established that streams are not simply passive conduits that transport terrestrial-derived organic matter to downstream ecosystems, but organic-matter dynamics vary tremendously among streams (Webster and Meyer 1997). Land use and land cover certainly have an impact on the variation in DOC concentrations among streams (Gergel et al. 1999), but few studies have explicitly examined the effects of land use on the quality of DOC delivered to streams (Findlay et al. 2001).

Comparison and synthesis of existing hyporheic studies on the microbial metabolism of organic matter is difficult due to the paucity of studies and inherent uncertainties in the hydrologic regimes among streams in which hyporheic DOC and microbial measures have been described. Estimates of hyporheic bacterial activity are surprisingly uncommon and only rudimentary efforts have been made at uncovering broadly applicable controlling factors (see Findlay and Sobczak 2000). Declines in bacterial activity have been related to declines in DOC along hyporheic flowpaths in several streams (Findlay et al. 1993, Jones 1995, Hendricks 1996, Ellis et al. 1998) and bacterial production (Marxsen 1996), abundance (Fischer et al. 1996), and respiration (Pusch 1996) are known to decrease with sediment depth and correlate with sediment-bound POC in stream reaches in which surface-water DOC exchange is unknown. The importance of a hyporheic microbial process on the stream ecosystem's energy or mass budgets is a function of the rate of the process and a function of the proportion of stream discharge that exchanges with the hyporheic zone (Findlay 1995). This study documented a wide range in DOC metabolism among hyporheic zones suggesting variable importance on stream mass balances, but quantifying hydrologic exchange becomes increasingly difficult beyond the reach scale (see Harvey and Wagner 2000).

Conclusions

Overall, we systematically controlled, removed, or examined hydrological (e.g., dilution, water velocity), physical (e.g., temperature, sediment type), and chemical (e.g., dissolved oxygen, inorganic nutrients) variables that may confound comparisons of DOC loss among hyporheic flowpaths from multiple streams. We documented significant differences among streams in the magnitude of DOC loss along these flowpaths and showed these changes were associated with significant variability in hyporheic metabolism. In many streams, hyporheic metabolism of stream-derived DOC may be an important process in regulating the flux of organic matter to downstream ecosystems. Regardless of the hyporheic zone's extent of influence in individual streams, DOC removal along hyporheic flowpaths may be a powerful predictor of rates of heterotrophic activity at the reach scale. Large variation in DOC bioavailability and hyporheic metabolism among regional streams highlights the functional diversity of hyporheic zones. The role of surface-water–groundwater interfaces in the retention and transport of organic matter appears highly variable and translation of findings at the reach scale to the regional and global scales (sensu Alexander et al. 2001) remains a major challenge (Harvey and Wagner 2000).

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