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Metabolic and structural response of hyporheic microbial communities to variations in supply of dissolved organic matter

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Abstract

Hyporheic sediment bacterial communities were exposed to dissolved organic matter (DOM) from a variety of sources to assess the interdependence of bacterial metabolism and community composition. Experiments ranged from small-scale core perfusions with defined compounds (glucose, bovine serum albumin) to mesocosms receiving natural leaf leachate or water from different streams. Response variables included bacterial production, oxygen consumption, extracellular enzyme activity, and community similarity as manifest by changes in banding patterns of randomly amplified polymorphic DNA (RAPD). All DOM manipulations generated responses in at least one metabolic variable. Additions of both labile and recalcitrant materials increased either oxygen consumption, production, or both depending on background DOM. Enzyme activities were affected by both types of carbon addition with largest effects from the labile mixture. Cluster analysis of RAPD data showed strong divergence of communities exposed to labile versus recalcitrant DOM. Additions of leaf leachate to mesocosms representing hyporheic flowpaths caused increases in oxygen consumption and some enzyme activities with weaker effects on production. Community structure was strongly affected; samples from the leachate-amended mesocosms clustered separately from the control samples. In mesocosms receiving water from streams ranging in DOC (0.5–4.5 mg L^{-1}), there were significant differences in bacterial growth, oxygen consumption, and enzyme activities. RAPD analysis showed strongest clustering of samples by stream type with more subtle effects of position along the flowpaths. Responses in community metabolism were always accompanied by shifts in community composition, suggesting carbon supply affects both functional and structural attributes of hyporheic bacterial communities.

Bacterial communities inhabiting surface and subsurface streambed sediments catalyze a number of important ecosystem processes, including mineralization of organic matter (Findlay et al. 1993), serve as a source of food for some consumers (Hall and Meyer 1998), and assimilate inorganic nutrients (Hamilton et al. 2001). The abundance, metabolic activity, and taxonomic composition of these communities are subject to multiple controls. While the abundance of organic matter clearly influences the biomass and production of sediment bacteria (e.g., Schallenberg and Kalff 1993), much of the spatial and temporal variation in community composition and activity is linked to the delivery, size distribution, and composition of both particulate and dissolved organic carbon inputs (Sinsabaugh and Findlay 1995; Brunke and Fischer 1999; Battin 2000; Fischer et al. 2002; Sobczak and Findlay 2002). Several characteristics of dissolved organic matter (DOM) ranging from size distribution (Amon and Benner 1996) to bulk chemical composition (Sun et al. 1997) to abundance of more specific compounds (Benner 2003) have been identified as capable of generating differences in bacterial activity.

The availability of inorganic nutrients and terminal electron acceptors also affects bacterial abundance and activity. Dissolved inorganic phosphorous is frequently a limiting factor for bacterial growth in the plankton of freshwater systems (Brett et al. 1999; Kainanen et al. 2002) and ammonium is rapidly removed from stream water (Tank et al. 2000). The presence of energetically favorable terminal electron acceptors can exert strong control on the ability of bacteria to metabolize organic matter at redox interfaces in sediments and riparian soils (Hedin et al. 1998; Baker et al. 2000; Ito et al. 2002).

Although considerable information exists on the ecosystem-level regulation of hyporheic microbial activity (e.g., Baker et al. 2000; Findlay and Sobczak 2000), the mechanisms of microbial community response to environmental controls are less clear. The most rapid responses to changes in carbon supply would be phenotypic: the up- or down-regulation of gene expression and corresponding metabolic

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adjustments. Subpopulations might change their relative rates of carbon degradation, leading to changes in carbon removal or respiration. Alternatively, preservation of active extracellular enzymes in biofilms may buffer microbial communities against rapid environmental fluctuations (Freeman and Lock 1995). In the longer term, DOM-induced changes in growth rates may lead to shifts in community taxonomic composition. Whether functional shifts are paralleled by changes in community structure remains largely unaddressed in natural microbial systems, although laboratory experiments have documented effects of species richness on decomposition (Naem et al. 2000).

To address the scope and potential linkage of bacterial functional and structural response to variations in carbon inputs, we conducted a series of experiments in which the amount and nature of dissolved organic matter supplied to model hyporheic biofilms were manipulated. Our experiments ranged in realism from large additions of single compounds to additions of carbon from natural leaf leachates to comparisons using water collected from a diverse set of natural streams. We expected that addition of simple compounds would provide the best opportunity for parallel changes in function and structure, while water collected from natural streams would serve as the most realistic test of potential linkages between function and structure. We followed a consistent set of response variables, including bacterial growth, oxygen consumption, and a suite of extracellular enzyme activities considered important in releasing monomeric substrates from polymeric DOM. To assess compositional shifts in bacterial communities, we used the randomly amplified polymorphic DNA (RAPD) technique and compared amplicon distribution among treatments. All our experiments were ''grounded'' in prior field studies of hyporheic bacteria that have shown that these shallow subsurface bacterial communities are particularly sensitive to variation in DOM inputs (Findlay et al. 1993; Sobczak and Findlay 2002).

Methods

Experimental approach—For all experiments, we used a common substratum (0.6 cm [¼″] gravel from a local, alluvial mine) that was washed repeatedly to remove associated particulate organic matter. Experiments were conducted either in small perfusion cores (25-cm length, 5-cm diameter, 10 cm h^{-1} flow rate) or larger mesocosms (400 × 15 cm; 10 cm h^{-1}) intended to mimic natural hyporheic flowpaths. Flow rates approximated in situ velocities estimated from hydraulic heads and conductivities (cf. Sobczak and Findlay 2002). Flow through the cores and mesocosms was controlled by gravity feed from a head tank, and the mesocosms had four wells installed along their length to allow sampling of water and placement of removable sampling units. These sampling units were small mesh bags (1-mm opening) containing ~150 g of the same gravel as in the rest of the mesocosm. Bags were suspended in the sampling well such that they were exposed to water characteristics at a particular point along the flowpath. Further details and comparisons between mesocosm and field flowpaths were presented by Sobczak and Findlay (2002). Core experiments were conducted in the lab at room temperature; the mesocosms were outside and subject to ambient temperature variation (5–12°C for the leaf leachate experiment conducted in autumn, 20–25°C for the stream comparison conducted in summer).

Defined amendment experiment—The first experiment was a microcosm core perfusion using defined compounds representing labile carbon (a mixture of glucose and bovine serum albumin [BSA]) or relatively recalcitrant carbon (tannic acid). This experiment is intentionally an extreme manipulation of DOM supply to maximize the likelihood of generating functional and structural responses. Cores received either no amendment, a glucose/BSA mixture (GBSA) (0.9 mg L^{-1} of each), tannic acid (TA) (2 mg L^{-1}). One set of 12 cores (four replicates per treatment) used low-DOC spring water (<1 mg C L^{-1}); another set of 12 cores was perfused with water from the East Branch of the Wappinger Creek (EBWC) that has moderate concentrations of DOC (~2 mg L^{-1}) (Findlay and Sobczak 1996). Carbon additions to these waters therefore represent a 100 or 50% increase in background DOC, respectively. The microcosms were perfused for 6 d with unamended source water to allow for initial colonization of gravel by a common inoculum derived from the unfiltered, unsterilized source water prior to perfusing cores with source water plus amendment. Oxygen removal (influent minus effluent concentrations) was monitored to assess when cores had reached stable rates of metabolism following amendment, at which time (37 d in this case) cores were destructively sampled (details below).

Leachate amendment experiment—The second experiment used carbon derived from leaf leachates to represent a naturally occurring pulsed input of relatively labile DOM from a single source. Water (with or without leachate) was perfused through mesocosms under conditions intended to represent natural time scales of delivery, abundance, and composition of a labile type of dissolved organic matter. Mesocosms were conditioned with water from the East Branch of the Neversink River (Ulster County, New York) for 12 d prior to experimental manipulation. After these 12 d, all mesocosm head tanks and samples from the first and third wells had DOC concentrations between 0.4 and 0.5 mg C L^{-1}. DOC was leached from ~10 g of a mixture of freshly fallen red oak and sugar maple leaves overnight in 10 L of deionized water. Small volumes of the leachate, intended to increase background DOC by ~1 mg C L^{-1}, were added once/week to head tanks containing water from the East Branch of the Neversink river for three of seven mesocosms. Sampling of gravel bags from each well and head tank of mesocosms occurred 2 weeks after leachate addition.

Source comparison experiment—The third experiment examined response to ambient variability in source waters comparing three distinct source waters collected from regional streams: a groundwater spring, the East Branch of the Wappinger Creek near Millbrook, New York, and the Walkill River in New Paltz, New York, which vary in DOC concentration (0.5, 1.0, and 4.5 mg C L^{-1}, respectively). Three replicate mesocosms, containing the common gravel, re-

ceived unfiltered, unsterilized water from each source stream. Water from sampling wells was collected weekly to follow DOC and dissolved oxygen patterns. Removal and analysis of gravel samples occurred 30 d after initiation of the experiment.

Inoculum experiment—To examine the potential influence of initial inoculum on bacterial growth, enzyme activities, and oxygen removal, we inoculated triplicate cores with rock scrubbings from the East Branch of the Wappinger Creek, the Neversink River, and the Walkill River separately or with all inocula combined. Washed gravel was placed in tubs containing epilithic scrubbings from each stream or the three combined and ambient stream (matched to source of scrubbings) water for 5 d to allow colonization of gravel surfaces; then cores were filled and all 12 cores were perfused with water from the East Branch of the Wappinger Creek for 12 d. After that interval, oxygen removal during water passage through cores was determined (see below) and cores were destructively sampled for measurement of bacterial growth and enzyme activities.

Response variables—For all experiments, we assayed DOC, dissolved oxygen, bacterial growth, and extracellular enzyme activities. Community similarity was assessed for cases where a shift in functional attributes had occurred. All DOC analyses were conducted on glass-fiber filtered samples using a Shimadzu 5050 carbon analyzer (high temperature catalyzed). Dissolved oxygen in mesocosms was measured with a YSI Model 57 by dropping the probe into the access well and moving it gently to provide water movement across the membrane. For the cores, head tank and effluent O_2 were measured with the same meter. Effluent samples were collected in small-capped containers with an overflow tube to minimize contact with the atmosphere prior to measurement. Bacterial growth was estimated from the incorporation of tritiated thymidine into DNA (Findlay 1993). Briefly, ~10 g of gravel was incubated with 5 ml of water from the appropriate treatment for 1 h with 20 μCi of 20 Ci mmol^{-1} ^{3}H-TdR. Incubations were terminated with 2% formaldehyde and the gravel washed three times (5 ml 2% formaldehyde, centrifuge ~2,000 × g, aspirate supernatant). Samples were frozen until DNA was extracted overnight under alkaline conditions, precipitated, and washed in cold 5% trichloroacetic acid (TCA) prior to hydrolysis and scintillation counting. Extracellular enzymes were assayed on biofilm suspensions obtained by vortexing ~10 g of gravel for 2 min. Fluorescent-linked substrates were used to measure potential activities of β-glucosidase (βGLU), α-glucosidase (αGLU), N-acetylglucosaminidase (NAG), β-xylosidase (βXYL), phosphatase (PHOS), leucine aminopeptidase (LEU), esterase (ACE), and endopeptidase (GUAN) following Findlay et al. (2001). For the source-water comparison experiment, we also conducted colorimetric assays for phenol oxidase and peroxidase activities using L-3,4-dihydroxyphenylalanine as a substrate (Saiya-Cork et al. 2002).

Gravel samples reserved for DNA extraction were sealed in plastic bags and stored at −80°C. Community DNA was extracted using the MO-BIO Ultraclean Soil DNA Kit following the macro protocol (MO-BIO Laboratories; see Rowher et el. 2001). Sequences within the extracted DNA were amplified using the RAPD Analysis Primer Set (Amersham Pharmacia) following the protocol provided (see Welsh and McClelland 1990; Williams et al. 1990 for general description of RAPD analysis). The RAPD kit includes six arbitrary primers, each 10 bases in length. Each primer was added to an aliquot of community DNA and subjected to polymerase chain reaction amplification. Each primer binds to inverted repeats within the community DNA, thereby amplifying intervening sequences of varying length. The resulting amplicons were separated by agarose gel electrophoresis and stained with ethidium bromide. The gels were scanned with a densitometer. Linear regressions, based on the Promega 100-basepair DNA Ladder, were used to estimate fragment size. Fragments from the various samples were aligned, allowing for a ±5% error in size estimates. Because the primers differ in sequence, each produces a different distribution of amplicons. The size distribution of amplicons across the six primers represents a community ''signature.''

Data analyses—Differences among treatments were analyzed with a one-way ANOVA using replicate cores or sampling locations in mesocosms as observations. A two-way ANOVA was used to test for differences among treatments and position along mesocosm flowpaths. A principal components analysis (PCA) was used to simplify the 8 (or 10) enzyme activities into new linear variables (usually two) that encompassed the variability of the original data. The PCA uses the correlation among variables rather than absolute rates, so all variables have equal weight in the analysis. Similarity among RAPD samples was determined by cluster analysis (Ward's method, Euclidean distance), using presence/absence data. All statistical analyses were conducted with Statistica (Statsoft).

Results

Defined amendment experiment—Addition of GBSA or TA yielded significant increases in respiration and/or growth (Fig. 1). For overall amendment effects, combining across source waters, $F_{2,18} = 26.1$, $P < 0.0001$ for production; $F_{2,18} = 209.4$, $P < 0.0001$ for respiration. The responses in production were much greater than responses in respiration for both additions to low-DOC spring water. Production increased by four- to fivefold when GBSA or TA were added to stream water, while oxygen uptake increased by 58% following GBSA addition and declined by 70% after TA addition. The production responses for the spring water perfusions (380% increase for GBSA, 480% for TA) were greater than increases in production (14 and 13% for GBSA and TA, respectively) observed for additions to Wappinger Creek water (interaction term in two-way ANOVA, $P < 0.0001$, $F_{2,18} = 17.0$).

Six of eight extracellular enzyme activities showed significant increases with amendment; the exceptions were βGLU ($P = 0.08$) and GUAN ($P = 0.82$). In general, activity increases were larger for glucose/BSA amendments than for the tannic acid treatment. For spring water, tannic acid addition increased NAG and βXYL activities to values as high as those observed in the GBSA treatment. Because ac-

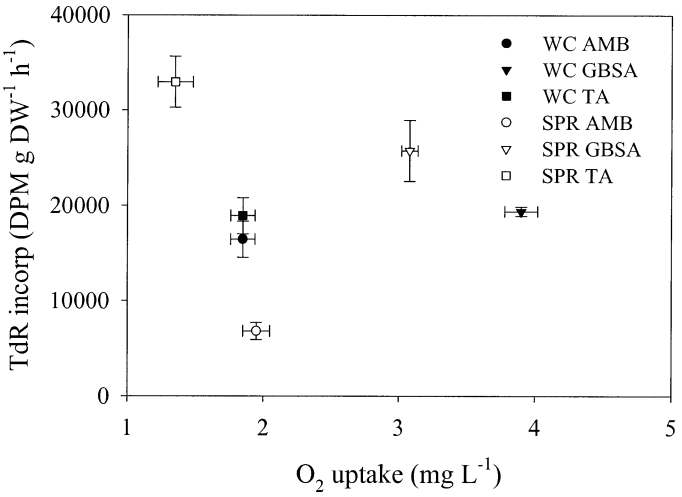

Fig. 1. Oxygen uptake and bacterial production in perfusion cores amended with either a glucose/BSA mixture (GBSA), tannic acid (TA), or no amendment (AMB). Cores were perfused with either spring water (SPR) or stream water (WC). Values are means of three cores ± 1 SE.

tivities were strongly correlated among many of the enzymes (e.g., βGLU was significantly positively correlated with five other enzymes), a principal components analysis was used to reduce the eight enzyme variables to a two-dimensional plot, accounting for 77% of total variance (Fig. 2). As was the case for respiration and growth, the GBSA treatment showed the largest displacement in extracellular enzyme activity; TA addition shifted EEA in the spring water microcosms but not for cores perfused with water from Wappinger Creek.

The RAPD data showed clear separation among amendments (Fig. 3), although some samples were lost due to technical problems. Consistent with the similarity in functional response to GBSA addition, the structural data indicate strong convergence for cores receiving labile carbon regardless of background source-water DOC. In contrast, TA addition generated stronger functional responses when added to low-DOC spring water than when added to water from Wappinger Creek. This functional divergence is also reflected in the RAPD data; the clusters for TA addition show greater separation between the two source waters, while GBSA addition resulted in a common cluster regardless of source water.

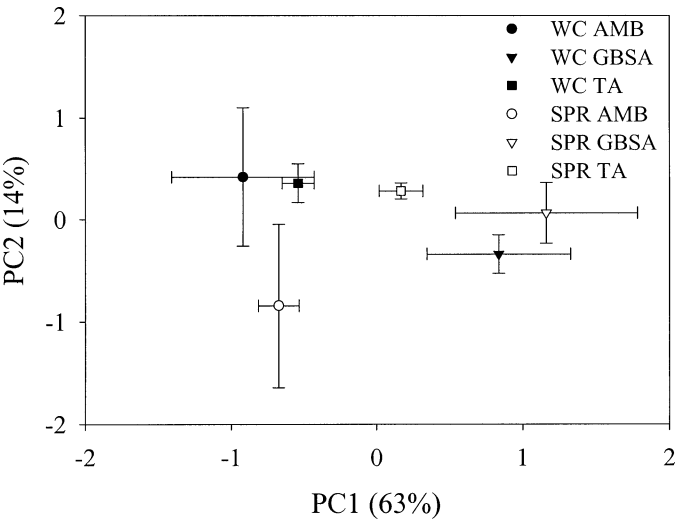

Fig. 2. Principal components (PC) analysis scores for extracellular enzyme activities from the perfusion cores. Values are mean scores for each treatment (±SE). Figures in parentheses show proportion of total variance explained by each PC. Treatment identifiers as in Fig. 1.

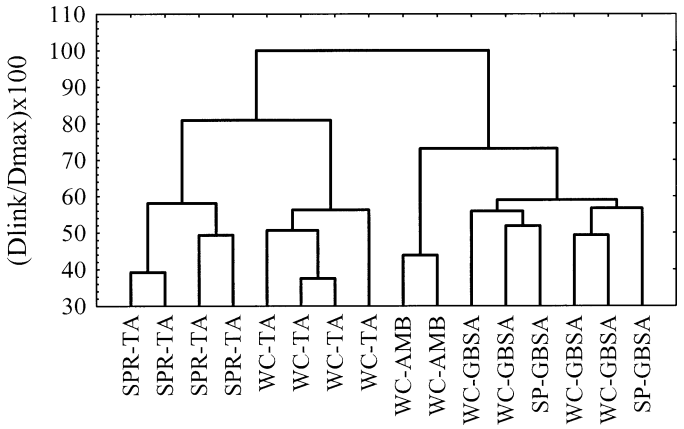

Fig. 3. Cluster diagram of results of RAPD analysis from perfusion cores. Treatment identifiers as in Fig. 1.

Leachate amendment experiment—Addition of leaf leachate increased DOC by 1.1 mg L^{-1} in the head tanks (mean addition = 1.6 ± 0.1 [SE]; mean control (CTL) = 0.5 ± 0.01), and there was a decline of 0.7 mg L^{-1} along the flowpath (Fig. 4). In parallel, dissolved oxygen showed a greater decline from head tank to mesocosm outlet (Δ = 2.4 mg L^{-1} ± 0.2) in the amended compared with control mesocosms (Δ = 0.6 mg L^{-1} ± 0.1). The molar difference in O_2 decline

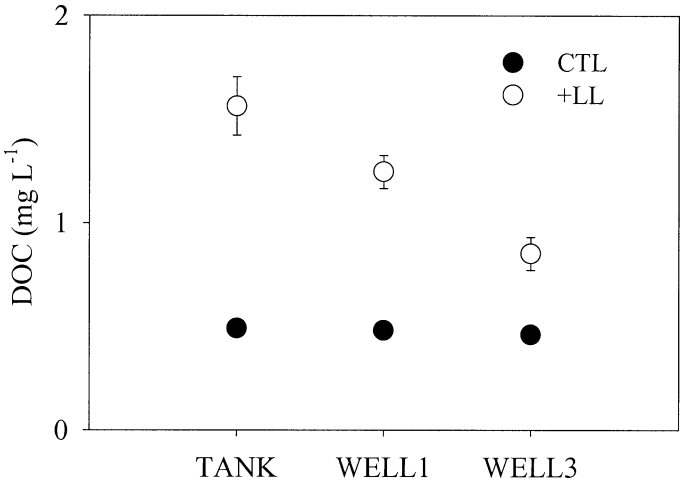

Fig. 4. Dissolved organic carbon concentration in mesocosms receiving leaf leachate (LL) or no addition (CTL). Values are means ± SE.

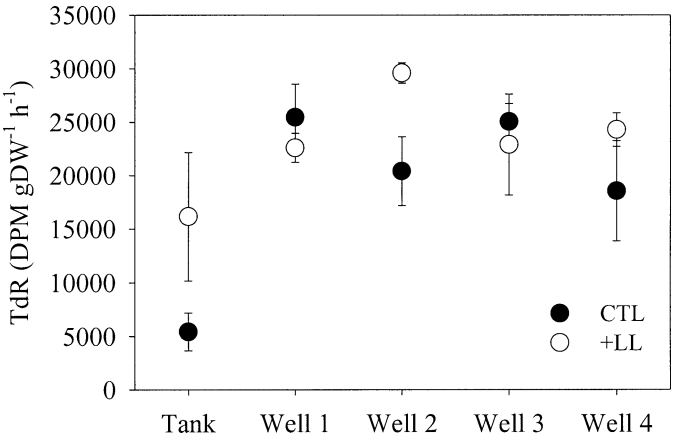

Fig. 5. Bacterial production (mean ± SE) in gravel biofilms for samples incubated at various points (well 1 is closest to head tank) along mesocosm flowpaths in LL or CTL mesocosms.

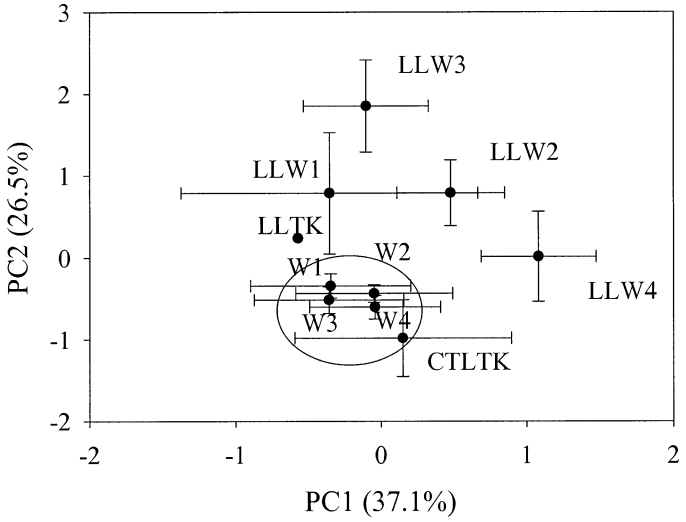

Fig. 6. PCA scores for enzyme activities in samples from different flowpath locations (well 1 to well 4) in LL or CTL mesocosms.

between treatments (56 μmol L^{-1}) is close to the decline in leachate DOC (60 μmol L^{-1}), suggesting much of the decline in DOC can be accounted for by metabolism along the flowpath rather than simple adsorption. Bacterial production differed only weakly between control and amendment mesocosms ($P = 0.06$), with grand means in plus-leachate mesocosms only 20% greater than in control mesocosms. The difference was driven largely by the differences in the head tanks (Fig. 5), where the maximum difference in leachate DOC occurred. Two of the eight enzyme activities (LEU and GUAN) differed significantly between treatments ($P < 0.001$ and $P = 0.003$, respectively), and two more differed weakly (βGLU $P = 0.11$; PHOS $P = 0.06$). A PCA of extracellular enzyme activity (Fig. 6) separated treatments, but data from individual wells did not show a consistent relationship with position along the flowpath.

There were parallels between the functional responses to leachate addition and results from the RAPD analysis. There were three distinct RAPD clusters: samples from the head tanks clustered together, although at a fairly low level of similarity. Samples from flowpath locations in the control mesocosms clustered together, also at a fairly low level of similarity. Samples from wells in the leachate-amended mesocosms clustered together, and samples joined the cluster in a down-gradient pattern (Fig. 7).

Stream comparison—The surface waters selected to provide a range in DOC concentration and composition varied substantially in initial concentration and magnitude of changes in DOC occurring along the flowpaths (Fig. 8A). The low-DOC spring water showed no declines along the mesocosm flowpath, values in Wappinger Creek mesocosms declined by 20% ($F_{4,10} = 3.2$, $P = 0.06$), while concentrations in the Walkill mesocosms declined by roughly 1 mg C L^{-1}. These changes in DOC were paralleled by modest declines in dissolved oxygen for the spring and Wappinger Creek mesocosms (minimum O_2 3.5–4 mg L^{-1}), while values in the Walkill mesocosms declined to 1 mg L^{-1} (Fig. 8B). Bacterial production differed significantly among sources ($F_{2,24} = 8.5$; $P = 0.002$), with marginally significant differences among flowpath locations ($F_{3,24} = 3.0$; $P = 0.05$) (Fig. 9). In contrast with the patterns in O_2 declines (greatest decline in Walkill mesocosms), Wappinger Creek and spring-fed mesocosms had higher rates of production than the Walkill mesocosms. For all source waters, there was some decline in production along the flowpaths, but these differences were small relative to overall source differences.

For 6 of the 10 extracellular enzymes assayed (8 fluorescent substrates plus peroxidase and phenoloxidase), there were significant differences among either source, flowpath location, or both (βXYL, PHOS, ACE, GUAN were not significant). Principal components analysis reveals reasonably coherent separation of sources and locations, with sources accounting for most of the separation (Fig. 10). There were patterns associated with flowpath locations, e.g., the spring water flowpath locations spread out in an upstream–downstream pattern from right to left. Also, there was a sharp break in both O_2 and production between sampling locations 1 and 2 (i.e., between the two most ''upstream'' points) in

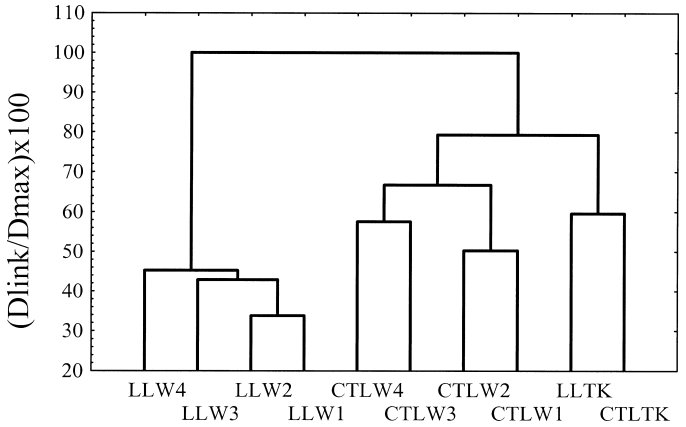

Fig. 7. Cluster diagram of RAPD results from samples incubated at different locations in LL or CTL mesocosms.

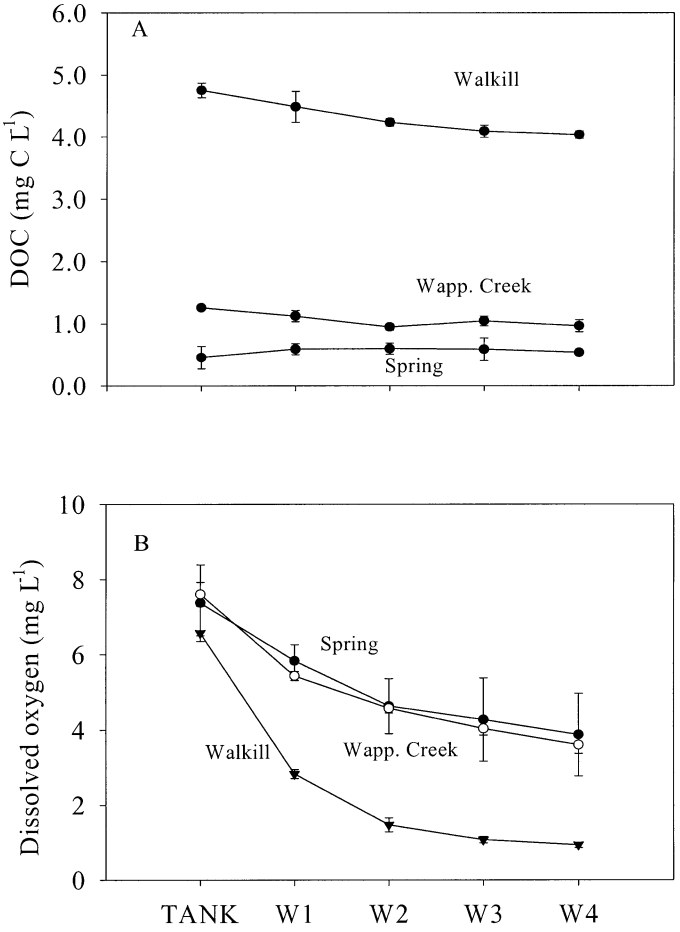

Fig. 8. (A) DOC and (B) dissolved oxygen concentrations in mesocosms supplied with water from three stream systems (spring water [SPR], east branch of the Wappinger Creek [WC], or the Walkill River [WK]). Values shown are means (±SE) for water samples collected on the same day gravel bag samples were retrieved.

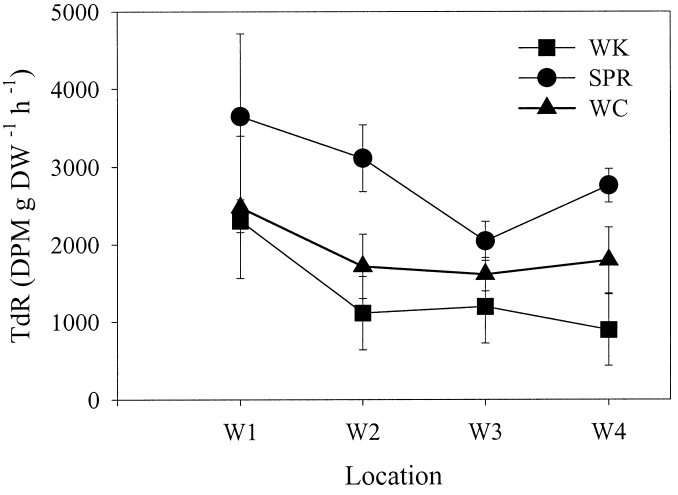

Fig. 9. Bacterial growth in gravel samples incubated at different flowpath locations in mesocosms receiving water from three different streams. Values are means (±SE). Treatment identifiers as in Fig. 8.

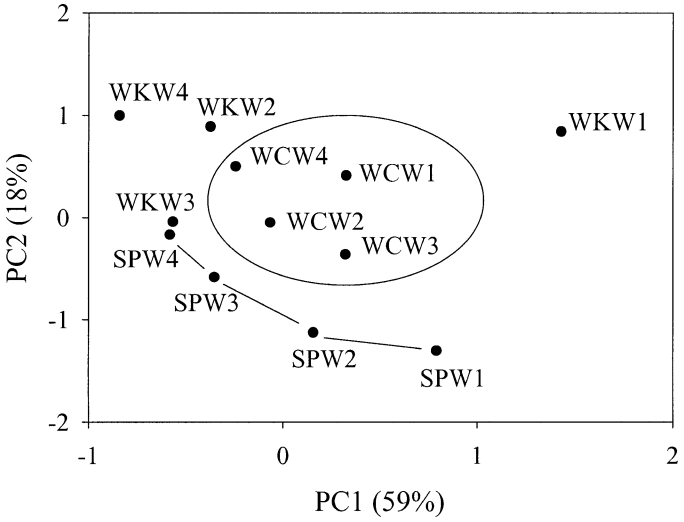

Fig. 10. PCA scores for enzyme activities from gravel samples described in Fig. 9. Treatment identifiers as in Fig. 8.

the Walkill mesocosms, and the score on PC1 for location 1 was markedly different from other locations along the Walkill flowpath.

RAPD analysis revealed clustering of samples primarily associated with differences in source waters (Fig. 11), with the Walkill samples showing the tightest clustering, although with no particular influence of location along the flowpath. The spring and Wappinger Creek samples show coherent clustering, with water source rather than position apparently accounting for most of the similarity.

Inoculum experiment—None of the functional variables (O_2 removal, production, or enzyme activity) differed among treatments inoculated with single-stream versus combined epilithon scrubbings (data not shown). *F* ratios from a one-way ANOVA ranged from 0.2 to 2.8, with corresponding *P* values ranging from 0.9 to 0.1. For the variable with the closest to a significant treatment effect (αGLU activity; $F_{1,8} = 2.8$, $P = 0.1$), the range in treatment means was only 21% of the grand mean. Due to the strong overlap in functional variables, RAPD analyses were not run on these samples.

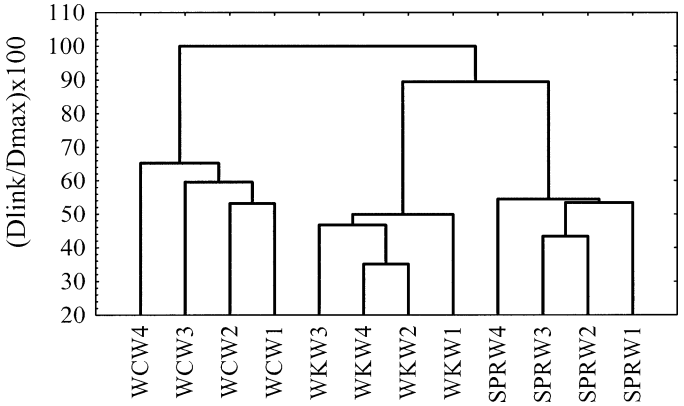

Fig. 11. Cluster diagram based on RAPD analysis of samples from stream mesocosms. Treatment identifiers as in Fig. 8.

Discussion

Our manipulations, ranging from single compounds to natural stream-water DOM, were intended to generate a range of functional shifts in bacterial communities allowing us to ask whether such shifts are accompanied by changes in community structure. An obvious prerequisite to examination of possible parallels in functional–structural shifts is consideration of the magnitude of functional shifts induced by our treatments. In general, responses were most distinctive when relatively high quantities of simple, defined compounds or fresh DOM were added. Even additions of tannic acid, presumably a relatively low-quality molecule due to its oxidation state and lack of associated nitrogen, was capable of inducing substantial increases in production when added to low-DOC spring water. The generally greater metabolic response to amendment by low-DOC spring water rather than water from the EBWC may suggest a difference in genetic or phenotypic scope for response between the microbial communities for the two background waters. Alternatively, the communities in the medium-DOC EBWC water may not have been carbon limited to the same extent as the low-DOC spring water and therefore do not respond as strongly to amendment.

Leaf leachates represent a more complex mix of growth substrates and have been shown to be readily metabolized by stream bacteria (Dahm 1981; Strauss and Lamberti 2002). In our experiments, realistic concentrations applied over a 2-week period yielded significant functional responses for a number of variables. Autumnal leaf fall obviously changes a host of stream characteristics (cf. Wallace et al. 1997) and, not surprisingly, microbes in our experiments were capable of degrading a significant proportion of leaf leachate DOC over realistic time scales.

For the natural stream-water experiment, the range in response in mesocosms was similar to variability observed among natural flowpaths. Mesocosm bacterial production, averaged across flowpath location, varied by 210% among streams, close to the twofold range in bacterial growth documented in a comparison of different in situ flowpaths (Sobczak and Findlay 2002). Most variables related to metabolic activity (bacterial growth, dissolved O_2, most enzymes) showed down-flowpath decreases in the mesocosms, confirming previous findings that some variable proportion of organic matter is removed during the 2-d transit along these subsurface flowpaths (Sobczak and Findlay 2002). Metabolic variables were differentially sensitive to manipulations both within and across experimental treatments (Fig. 12). For instance, bacterial growth and oxygen declines were generally less responsive than enzyme activities regardless of carbon manipulation imposed. Comparing among enzyme activities, esterase (ACE) and endopeptidase (GUAN) were typically the least variable, while the enzyme showing greatest variability varied by experiment. For the simple-compound amendments (GLU/BSA or TA), βXYL, PHOS, and LEU had the greatest range whereas the carbohydrases (αGLU, βGLU, NAG, βXYL) were more responsive in the leaf leachate experiment. Overall, response to differences among stream waters was less dramatic and αGLU and LEU showed greater variability than other enzymes. These patterns suggest the process of acquiring carbon from complex molecules has a broader scope for variability than the cellular level (more integrated?) processes of respiration and growth.

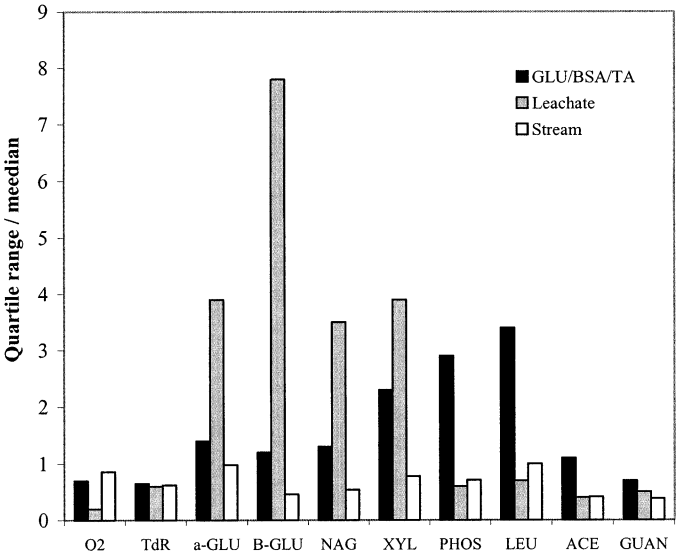

Fig. 12. Scope for variation (quartile range/median) in response variables across all experiments.

Microbial biofilms comprised of bacteria, protozoa, algae, and some metazoa appear to have the capacity to respond functionally to a wide array of carbon manipulations, even those such as tannic acid that might be considered relatively recalcitrant. The high population density, ''buffering'' by extracellular material, preservation of enzymes, and even cell-to-cell signals should all contribute to the ability of biofilms to metabolize novel compounds or respond quickly to pulsed inputs (Fischer 2003). Adsorption of compounds from overlying water and movement of water through macropores in the biofilm are physical mechanisms that should enhance the capacity of the biofilm to retain solutes. At the community level, high species diversity and the opportunity to persist at slow growth rates are biological mechanisms that increase the likelihood that the genetic capacity to respond is present within the biofilm. While it is clear from our experiments that DOM can be a strong control on bacterial function and structure, the relative contribution of differing compounds or classes of compounds also appears to vary considerably, with, as yet, no general link between variation in DOM composition and individual response variables. It seems likely that the multitude of sources of DOM (terrestrial, autochthonous, secondary diagenesis) combined with the importance of sporadic inputs generates such variability in DOM composition that components driving responses will vary considerably across space and time.

Paralleling the consistent functional response to the DOM manipulations we imposed, all treatments also exhibited demonstrable shifts in bacterial community similarity. The strength of community structure differentiation varied within and among experiments. For instance, in the simple compound amendments, addition of labile carbon to either back-

ground water type apparently caused a convergence of community composition such that all samples, regardless of background water source, clustered together. In contrast, the tannic acid addition had background-specific effects on both function and structure. The RAPD analyses separated spring water from Wappinger Creek samples amended with tannic acid, implying either differences in genetic potential in the two water types or, more likely, a difference in ''selective pressure'' exerted by tannic acid when added to low-DOC spring water versus higher DOC stream water. Selection of bacterial guilds by availability of labile versus refractory carbon has been documented in previous carbon amendments (Foreman and Covert 2003; Sinsabaugh and Foreman 2003).

Addition of naturally occurring concentrations of leaf leachate to mesocosms yielded large differences in enzyme activities and overall oxygen removal although only small effects on bacterial growth. The community similarity differences were clear and interpretable. Samples collected from head tanks were similar to each other regardless of DOM amendment but distinct from any of the flowpath locations. Presumably, the fact that these samples were suspended in tanks of water rather than surrounded by other gravel has some strong effect on bacterial taxa. Dissolved oxygen, presence of grazers, and turbulence are all potential (although untested) explanations for similarity in the head tank samples. Samples incubated at points along the flowpath (well 1 to well 4) showed clear separation between control and leachate treatments, with much tighter clustering in the leachate-amended treatments. Even though much of the added leachate had disappeared from solution by the third well (~1 d travel time), some of the functional differences persist (e.g., leucine aminopeptidase activity was marginally significantly lower in leachate vs. control even in the most downstream well; $F_{1,5} = 5.97$, $P = 0.06$), and it appears the residual leachate DOM can still shift community composition relative to communities not exposed to leachate. Leaching of DOM from stream particulate organic matter can represent a significant contribution to stream bulk DOM pools (Kaplan and Newbold 1993), and our data show it can have strong effects on biofilm metabolism and community composition as well.

Mesocosm experiments using different stream water to represent natural variability in DOM also generated substantial differences in dissolved oxygen concentrations and bacterial production among treatments in parallel with previous experimental and field observations of hyporheic response (Sobczak and Findlay 2002). There were distinct differences in allocation among extracellular enzyme activities, implying the bacterial communities were degrading different components of the DOM pool among treatments. Community composition also showed strong distinctions among treatments, with coherent clustering of samples from each water type. These surface waters were selected to span a broad range in DOC concentration and presumed composition, i.e., the Walkill is a high-DOC stream draining high organic soils while the Wappinger Creek drains glacial tills and the spring water represents relatively deep groundwater. The composition of bacteria growing in biofilms exposed to these water types was cleanly separated by clustering of the RAPD data, implying strong differences in taxa present. There were weak indications of compositional differences among flowpath locations with, e.g., adjacent wells clustering together or wells entering clusters in positional sequence such as those from the Wappinger Creek treatment.

Recent application of molecular techniques and other biomarkers to description of natural bacterial taxonomic composition has led to an explosion of studies on how composition varies over time and space (e.g., Brachvogel et al. 2001; Smoot and Findlay 2001; Gasol et al. 2002). Linking functional differences with compositional differences has been somewhat less common, although it is a prerequisite to the question of whether community compositional change is necessary to allow large changes in microbial function. DOM is a much more dynamic and variable component of aquatic ecosystems than envisioned a decade ago, with dramatic changes in concentration and composition due to high flow events (McKnight et al. 1993; Brooks et al. 1999), hydrologic conditions permitting flow through certain soil horizons (Neff and Asner 2001), and response to changing land use/land cover (Findlay et al. 2001). We have shown multiple responses of bacterial communities to shifts in carbon supply, suggesting in situ bacteria can respond via phenotypic and genotypic mechanisms to changes in resource supply.

Our experiments were intended to elicit functional changes, and they were successful in most instances. Our amendments and natural stream water comparisons were intended to be within the scope of natural variation but still represent fairly large differences in several DOM-related attributes such as absolute concentration or shifts in concentration of specific compounds. For instance, our leaf leachate addition increased bulk DOC by ~1 mg L^{-1}, well within the range of temporal variation in many stream systems. However, the glucose addition (12.5 μmol L^{-1}) represented a more extreme manipulation, being several to 10-fold higher than ambient dissolved free carbohydrate concentrations (Kaplan and Newbold 2003). Given the large magnitude of our simple compound additions, these results probably represent something close to the extremes in both functional and compositional responsiveness to variation in carbon supply. Presumably, comparisons of sites or types of DOM that yielded less distinct functional differences would have a lower likelihood of parallel shifts in composition. Our experiments have demonstrated that variation in carbon supply can be a strong control on biofilm bacterial characteristics, yet we recognize that other forcing functions (grazing pressure, redox conditions) may generate equal or greater variability in these attributes.

At least two previous studies have documented differences in bacterial taxa degrading distinct classes of DOM. Cottrell and Kirchman (2000) used fluorescent in situ hybridization to document a prominent role for β-proteobacteria in assimilation of low molecular weight compounds (amino acids) and a predominance of cytophaga/flavobacteria in degradation of protein and chitin. Covert and Moran (2001) separated estuarine DOM into high and low molecular weight classes and followed bacterial community composition over a several week period. They found clear differences in relative abundance of α, β, γ, and ε-proteobacteria and cytophaga/flavobacteria following a grow-out period in seawater

enriched with high or low molecular weight DOM. In a soil amendment study using a range of monomeric, polymeric, and natural substrates, Schutter and Dick (2001) found a significant correlation between substrate utilization diversity and fatty-acid methyl ester richness, again suggesting carbon supply has strong effects on both functional and structural characteristics of microbial communities.

These results are consistent with our finding that variations in DOM supply can yield compositional shifts in the bacterial community over relatively short time periods. One implication is that compositional shifts accompany the more widely documented functional response to DOM, but we cannot presently answer the question of whether compositional shifts are necessary to allow large changes in functional attributes. Function may be constrained by a number of trophic or edaphic factors, and the relative importance of these versus genetic diversity remains unknown. Much of the debate about diversity–ecosystem function deals with control of function by species richness (Loreau 2000; Cardinale et al. 2002). Our results show that trophic factors drive shifts in function that are paralleled by shifts in composition. The converse question, whether taxonomic composition affects function, was addressed by our inoculation of cores with single-source versus combined biofilm scrubbings to alter initial community composition. There was no significant inoculum effect on any of the variables, suggesting there was sufficient diversity in all the source communities to allow convergence in function following perfusion of cores with a common water type. Apparently the influence of resources (carbon supply) on functional capacity and community composition is stronger than effects of initial community composition on functional capacity. One potential explanation for this inequality in influence is that the high abundances of bacteria, many of which are not actively metabolizing and so are not engaged in resource competition, allow persistence of sufficient genetic diversity to respond to fluctuating conditions. If so, there may be a reservoir of diversity in natural biofilm communities providing the genetic capability to respond to a number of fluctuations in environmental conditions. Our experiments show that shifts in carbon supply can yield metabolic and compositional responses, but we found no evidence for the converse, i.e., that initial community composition constrains functional responses.

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