

Hydrodynamics is a major determinant of streambed biofilm activity: From the sediment to the reach scale

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Abstract

The effects of hydrodynamics and organic matter on sediment biofilm esterase activity and [³H]thymidine incorporation were investigated at several depths in the streambed and riparian zone of the alpine stream Oberer Seebach (Austria). On the sediment scale, microbial activity increased with both upwelling and downwelling Darcian velocities of interstitial water and was minimal when the surface/subsurface water exchange decreased. As revealed by stepwise multiple regression analyses, sediment carbohydrates and chlorophyll *a* explained a considerable percentage (up to 97% in summer) of the variance of biofilm esterase activity and [³H]thymidine incorporation, whereas pore-water temperature and concentrations of dissolved organic carbon (DOC) and dissolved oxygen (DO) were poor predictors of biofilm activity. DOC and DO fluxes, however, accounted for a considerable percentage of the variance in [³H]thymidine incorporation, which points toward the importance of flow in biofilm functioning. On the reach scale, the ratio of surface water travel length to the subsurface travel length, which is an index of the surface/subsurface water exchange frequency, determined average reach biofilm activity. Downstream routing along predominantly subsurface flow paths increased streambed esterase activity, whereas elevated surface travel lengths reduced esterase activity. Esterase activity and [³H]thymidine incorporation were also positively related to the streambed DOC retention efficiency, which underscores the role of biofilms in solute retention. These results highlight the coupling between streambed hydrodynamics and microbial activity and link sediment-scale and reach-scale processes.

While it is commonly agreed that organic matter is a major control of the heterotrophic metabolism in streambeds (e.g., Bott and Kaplan 1985; Jones et al. 1995; Findlay and Sobczak 2000), the role of hydrodynamics in streambed biofilm functioning remains poorly investigated (Boulton et al. 1998). This is surprising given that hydrodynamics control key factors such as macroscale solute transport through the streambed (e.g., Stream Solute Workshop 1990) or microscale mass transfer into, and drag on, biofilms (e.g., De Beer et al. 1994). Heterogeneous patterns of particulate organic matter (POM) and water flow can induce substantial diversification of streambed microbial processes. Where hydrologic exchange is low, anoxic and hypoxic pockets can develop, and alternative electron acceptors result in a variety of anaerobic respiratory pathways that may account for a significant proportion of streambed organic matter mineralization (Storey et al. 1999; Baker et al. 2000). Reach-scale hydrologic exchange between the streambed and stream water determines the relative contribution of these biogeochemical processes to the overall stream ecosystem functioning (Findlay 1995; Kaplan and Newbold 2000). Herein, particular significance is attributed to the hyporheic zone (i.e., the streambed where shallow groundwater and stream water mix; sensu Triska et al. 1989).

Despite considerable advances in hydrodynamic modeling

and novel tracer approaches (Harvey and Wagner 2000), research that integrates streambed hydrodynamics, biogeochemistry, and microbial ecology still remains largely comparative and qualitative rather than quantitative. For instance, nitrogen biogeochemistry has received much attention in relation to streambed hydrologic retention (Triska et al. 1989; Valett et al. 1997) and surface/subsurface exchange (Valett et al. 1994; Jones et al. 1995). Furthermore, Vervier and Naiman (1992) and Findlay and Sobczak (1996) investigated dissolved organic carbon (DOC) dynamics and metabolism along flow paths through gravel bars. Stream microbial ecologists have investigated the effects of downwelling (i.e., stream water entering the streambed) and upwelling (i.e., subsurface water returning to the stream) on microbial biofilms (e.g., Hendricks 1996). Battin and Sengschmitt (1999) related the hydrolytic activity of riverbed biofilms to surface water inflow velocity and presented field evidence for microbial participation in river-bottom clogging (i.e., microbial control on hydrodynamics).

In previous work (Battin 1999), reach-scale reactive uptake of streambed DOC and dissolved oxygen (DO) was related to hydrologic exchange processes in the alpine stream Oberer Seebach (OSB) in Austria. The degree of downstream routing via subsurface flow paths increased the reach-scale streambed DOC metabolism. The central hypothesis of the present study is that streambed hydrodynamics also affect biofilm activity on the sediment and the reach scales. I predict that surface/subsurface exchange rates control biofilm processes. To test this prediction, hydrologic exchange and streambed solute fluxes were computed from hydrometric data, and their effects on biofilm thymidine incorporation and esterase activity were explored. Sediment-associated organic matter was also considered in order to partition among

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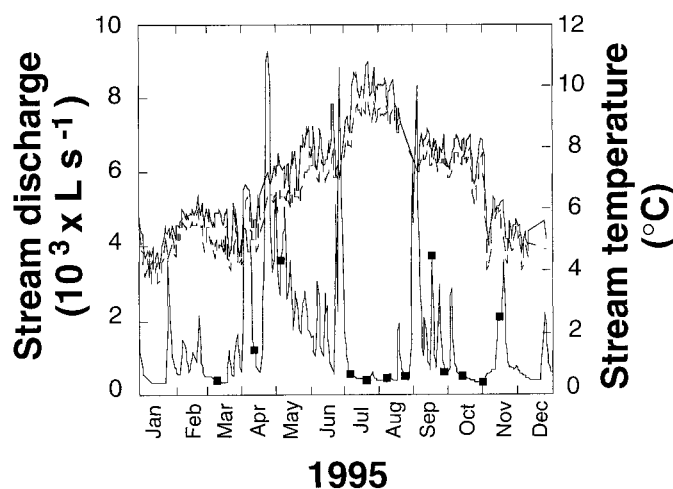


Fig. 1. Annual stream discharge and stream water temperature (minimum–maximum) in the OSB experimental reach. Squares refer to sampling dates.

effects between hydrodynamics and substrate supply on microbial activities.

Site description

OSB (47°15'N, 15°04'E; 600 m above sea level) is a pristine, second-order stream that drains a karstic, forested watershed (20 km²) in Austria. The 100-m-long study reach (see Bretschko 1991 for detailed description) is located in an approximately 3-m-deep glacial alluvial deposit. The predominantly calcareous sediment has a median grain size of 23.1 mm, and porosity averages 29%; the average streambed gradient is 0.41%. The annual stream water temperature ranged from 1.9 to 11.1°C and averaged 6.8°C during the study period. Average stream water DOC was 1.36 ± 0.08 mg C L⁻¹, DO was 11.1 ± 0.2 mg O₂ L⁻¹, and pH was 8.1. The catchment is seasonally snow-covered, which partially shapes the flashy hydrograph with a distinct snow-melt peak in spring (Fig. 1). OSB is a losing stream, and the downward flux of stream water constitutes the major DOC source to the streambed. The topography adjacent to the stream channel largely determines subsurface water flow paths, which results in shallow hill-slope groundwater that flows from the left bank beneath the stream and enters the alluvial groundwater at the opposite bank. For further details on the hydrologic setting of the study reach, see Battin (1999).

Sampling

The OSB reach was outfitted with eight nests of polyvinyl-chloride observation wells that were installed in the streambed and in the nearstream riparian zone at 30, 60, and 90 cm below the sediment surface. Wells were closed at the bottom and perforated (1-cm diameter), with no screening in a defined depth. Interstitial water, sediment, and organic particles were pumped from the respective depth layer with teflon tubing, collected in precombusted (450°C, 5 h) borosilicate bottles, and chilled in the dark pending laboratory

processing within 3 h. The supernatant water was used for DOC analyses, whereas the sediment—along with organic particles—was collected for analyses of microbial activity and organic matter. The sediment collected had a median grain size of 480 μm and was characterized by high organic matter content (8.2–10.6% as ash-free dry mass) and bacterial abundances (2.83 ± 0.28 to $3.21 \pm 0.97 \times 10^9$ cells g⁻¹ dry mass), as determined by epifluorescence microscopy (Porter and Feig 1980) on 3 October 1995 and 5 May 1995. Pump sampling methods can bias toward smaller sediment grain sizes and can overestimate organic matter content, yet they are widely used in hyporheic research (Fraser and Williams 1997). Furthermore, most organic matter is associated with a grain size of <1 mm in OSB (e.g., Leichtfried 1996), which is considered the most significant fraction for microbial colonization. DO was measured with a WTW OXI 196 probe that was deployed in the well. In order to estimate hydraulic gradients in the OSB study reach, 10 nested pairs of piezometers were installed 25–30 and 65–60 cm below the sediment surface. Piezometers were constructed from metal pipes (3.8 mm in diameter) with 10-cm-long screens (perforation, 0.4 mm). Streambed and nearstream riparian wells were sampled on 12 dates (Fig. 1) for sediment and pore-water parameters; low riparian groundwater tables prevented regular sampling of the shallow wells (30 cm).

Methods

Biofilm activity—The incorporation of [³H]thymidine was used to estimate bacterial activity and was measured according to Findlay et al. (1984). Approximately 2–3 g (wet mass) sediment was incubated with [³H]thymidine (specific activity, 20 Ci mmol⁻¹, American Radiochemicals) at a saturation concentration of 300 nM for 1 to 2 h at in situ temperature in the dark. Triplicate [³H]thymidine assays and duplicate formaldehyde-killed (2.5% final concentration, 30 min prior to [³H]thymidine addition) controls were run per well and date. [³H]thymidine incorporation was stopped, and samples were repeatedly washed with 5 ml formaldehyde (2.5% final concentration) and frozen (–20°C) pending alkaline (0.3 N NaOH, 0.1% sodium dodecyl sulfate, and 25 mM ethylenediaminetetraacetic acid) extraction. After centrifugation (2,000 × g, 50 min), the supernatant was acidified with 0.5 ml 3 N HCl, and 50% trichloroacetic acid (TCA) along with hering-sperm DNA solution (Sigma-Aldrich) were added to foster precipitation of labeled DNA. After cooling on ice for 30 min, [³H]DNA was cleaned up by repeated centrifugation (20,000 × g, 15 min) and resuspension in 5% TCA. [³H]DNA was hydrolyzed (100°C, 30 min) in 5% TCA, and a 1-ml aliquot was radioassayed with a liquid scintillation counter (Canberra Packard Tricarb 2000).

Esterase activity was used to estimate total biofilm hydrolytic activity and was assayed with fluorescein diacetate (FDA, Sigma-Aldrich) according to Battin (1997). Triplicate trials were incubated for 30 min at the saturation concentration of 200 μM FDA. Hydrolysis was stopped with acetone, and samples were put on ice. Fluorescein was further extracted by sonicating (45 s, 30-W output) the suspensions, and particles were subsequently removed by centrifugation.

The absorbance of the supernatant was measured at 490 nm, and Na₂-fluorescein (Serva, analytical grade) was used as a standard. Duplicate control blanks were inactivated with 50% (vol/vol) acetone 30 min prior to the addition of the FDA solution.

Sediment organic matter—Bulk sediment carbohydrates (CHO) were measured with the sulfuric acid–phenolic assay according to the method of Liu et al. (1973) and are considered to be at least partially of microbial origin; glucose was used as a standard, and values are given as carbon units. The sediment total organic matter was determined as ash-free dry mass (450°C, 5 h). Sediment chlorophyll *a* (Chl *a*) was extracted with analytical-grade acetone from 3–5 g (wet mass) sediment for 12 h in the dark (4°C); sediment was then centrifuged and the absorbance of the supernatant measured. After the addition of two drops of 10% HCl, the absorbance was measured again. Concentrations of Chl *a* and pheophytin were estimated according to the method of Parsons et al. (1984).

DOC and gross aromaticity—Water samples for DOC measurements were glass-microfiber filtered (Whatman GF/F) and analyzed with the Pt-catalyzed high-temperature combustion method (Shimadzu TOC 5000). Standards were prepared with potassium hydrogen phthalate in deionized and doubly distilled MilliQ water. The absorbance A ($\lambda = 250$ nm) was measured with a Hitachi U-2000 spectrophotometer on aliquot samples and was converted to the specific absorption $a^*_{250} = 2.303A/Cr$, where r is the cell path length in meters and C is the DOC concentration in milligrams of carbon per liter. Measurements of absorption in the ultraviolet range normalized to the DOC concentration have been shown to be an adequate estimator for gross aromaticity of DOC (e.g., Chin et al. 1994).

Hydrologic exchange and DOC fluxes—The specific discharge (q), or flux, of water through the streambed was computed from the sediment hydraulic conductivity (K), the hydraulic head (dh), and the piezometer installation depth (dl), according to the Darcy equation $q = -Kdh/dl$. Using a field permeameter, the hydraulic conductivity was measured with a falling head test in each piezometer. Hydraulic heads were measured with a graduated rod outfitted with an electrical sensor. Positive heads indicate upwelling, and negative heads indicate downwelling. Small-scale DOC and DO fluxes were calculated from the water flux as calculated from paired piezometers and the pore-water DOC and DO concentrations in the respective well. Reach-scale fluxes were computed by averaging the specific discharge from all piezometers and relating them to the wetted streambed area for vertical exchange. For reach-scale horizontal fluxes, the throughflow surface area was estimated from a 1-m streambed depth and the channel length of the study reach and was further corrected for the stream water level to account for the wetted surface of overtopped banks. See Battin (1999) for a discussion on the reliability of Darcian flux estimates and the averaging scheme employed. The reach-scale streambed DOC retention efficiency (sensu Mulholland 1981) was computed from (input – output)/input DOC streambed fluxes

accounting for vertical stream and horizontal riparian exchange. The DOC retention efficiency is a dimensionless number, and negative values indicate outputs that exceed inputs, and hence, streambed DOC production. The dominant downstream routing of water (e.g., stream versus subsurface flow paths) was determined from the ratio $S_{sw}:S_{sb}$, where S_{sw} is the travel length of a given stream water parcel before it enters the streambed (i.e., downwelling) and where S_{sb} is the travel length of a given subsurface water parcel before it returns to the stream water (i.e., upwelling). S was calculated from $S = Q/qW$, where Q refers to the respective downstream discharge (i.e., surface versus subsurface), q refers to the respective vertical upwelling and downwelling fluxes, and W refers to the streambed width.

Statistical analyses—One-way analysis of variance (ANOVA) was used to test for seasonal effects on microbial activity; depending on the well installation depth, three to four field replicates per date entered the analysis. Comparison between sites (e.g., riparian zone versus streambed) and depths was also accomplished with one-way ANOVA. The variation of [³H]thymidine incorporation and esterase activity was explored with stepwise multiple regression analysis. Step order was forward, P to enter was 0.15, and minimum tolerance for entry into the model was 0.01. Independent variables considered were the sediment ash-free dry mass, Chl *a*, the Chl *a*:pheophytin ratio, pore-water temperature, DO and DOC concentrations, the vertical Darcian velocity, and DO and DOC fluxes. All tests were considered significant at the level $\alpha = 0.05$, and values are given as mean \pm standard error (SE). Analyses were performed with SYSTAT (SYSTAT, Inc.). All biofilm parameters are expressed per gram dry mass (DM) sediment.

Results

Temporal and spatial variability of biofilm activity—The biofilm activity increased with the onset of the snow melt and rising stream water temperature in March (Fig. 2). Shallow streambed [³H]thymidine incorporation and esterase activity were elevated during low summer flow and high stream water temperature and gradually declined during the dormant season. The average streambed biofilm activity did not change significantly with depth (Table 1). However, esterase activity was significantly higher in the streambed than in the riparian zone (60 cm: $P = 0.009$; 90 cm: $P < 0.001$). No such spatial patterns were found for [³H]thymidine incorporation. Furthermore, the 90-cm esterase activity ($P = 0.003$) and pore-water DOC gross aromaticity ($P = 0.032$) were significantly higher in the right riparian zone, where hill-slope groundwater entered the streambed, than in the opposite riparian zone. Spatial differences in [³H]thymidine incorporation were not significant.

Sediment scale—To investigate the effects of sediment organic matter and pore-water temperature and DO and DOC concentrations on biofilm activity, I computed stepwise multiple regressions for each depth in the streambed and the riparian zone (Table 2). From 37 to 95% of the variance in biofilm activity was explained by an array of six independent

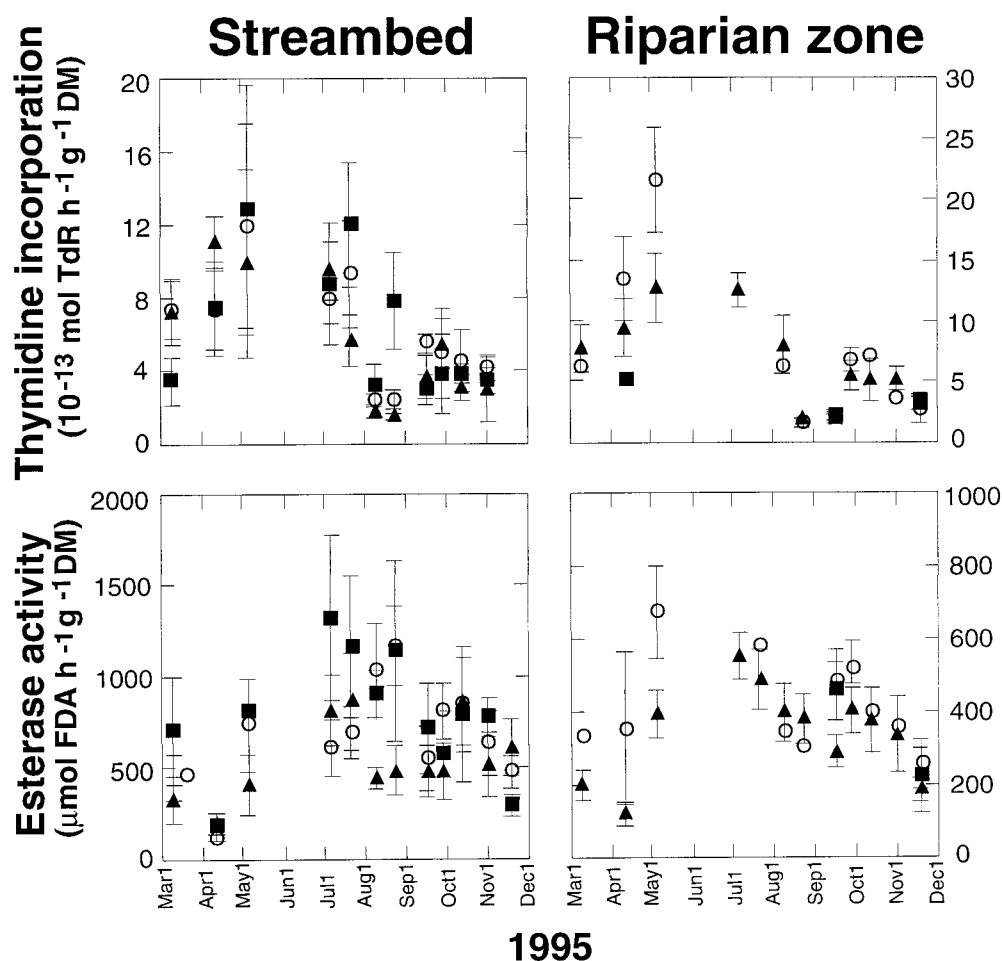


Fig. 2. Seasonal variation of biofilm $[^3\text{H}]$ thymidine incorporation and esterase activity in the streambed and the riparian zone. Squares refer to 30-cm, circles to 60-cm, and triangles to 90-cm sediment depth. ANOVA detected significant temporal variation of $[^3\text{H}]$ thymidine incorporation (30 cm: $P = 0.014$; 60 cm: $P < 0.001$; 90 cm: $P < 0.001$) and of esterase activity (30 cm: $P = 0.043$; 60 cm: $P = 0.048$) in the streambed; in the riparian zone, variation was significant for $[^3\text{H}]$ thymidine incorporation (60 cm: $P = 0.012$; 90 cm: $P < 0.001$) and esterase activity (60 cm: $P = 0.001$; 90 cm: $P = 0.394$).

variables. Sediment CHO and Chl *a* correlated highly with esterase activity in the streambed and the riparian zone. Beside Chl *a*, the ratio of Chl *a* to pheophytin was a good predictor for $[^3\text{H}]$ thymidine incorporation. DO only entered the regression model to explain variance in riparian $[^3\text{H}]$ thymidine incorporation, where minimal concentration was occasionally $< 1 \text{ mg O}_2 \text{ L}^{-1}$. DOC concentration correlated only with the 30-cm streambed esterase activity. Furthermore, streambed esterase activity was inversely related to pore-water DOC gross aromaticity, and the relationship was best described by a power model (Fig. 3). DOC gross aromaticity was therefore excluded from the multiple regression analyses.

The effect of seasonality was obvious in summer, when stream flow was low ($Q = 436 \pm 19 \text{ L s}^{-1}$) and when streambed water temperature was elevated ($9.7 \pm 0.2^\circ\text{C}$). Shallow Chl *a* and CHO explained 97% ($P < 0.001$) and 95% ($P = 0.001$) of the variance of $[^3\text{H}]$ thymidine incorporation and esterase activity, respectively. However, pore-

water temperature did not affect $[^3\text{H}]$ thymidine incorporation ($r^2 = 0.002$, $P = 0.913$) or esterase activity ($r^2 = 0.023$, $P = 0.654$).

In order to investigate the effect of hydrodynamics on the sediment-scale microbial activities, $[^3\text{H}]$ thymidine incorporation and esterase activity were plotted against the vertical streambed Darcian velocity (Fig. 4). Because hydrometric data were predominantly collected from a mid-streambed depth, the 60-cm $[^3\text{H}]$ thymidine incorporation and esterase activity data were considered for analyses. The relationship between biofilm activity and the Darcian velocity was best described by a quadratic regression model. Minimal activity was related to low Darcian velocities (i.e., moderate hydrologic exchange between the stream and the streambed) and increased with both upwelling and downwelling velocities. Less scattering of esterase activity and $[^3\text{H}]$ thymidine incorporation was associated with downwelling than with upwelling Darcian velocities. DOC and DO fluxes averaged $0.56 \pm 0.06 \text{ mg DOC m}^{-2} \text{ h}^{-1}$ and $4.61 \pm 0.54 \text{ mg O}_2 \text{ m}^{-2}$

Table 1. Summary statistics for streambed and riparian biofilm activity, organic matter, and pore-water chemistry in Oberer Seebach.

| Streambed | ^{13}C incorporation ($10^{13} \times \text{mol TdR h}^{-1} \text{g}^{-1} \text{DM}$) | Esterase activity ($\mu\text{mol FDA h}^{-1} \text{g}^{-1} \text{DM}$) | Ash-free dry mass ($\text{mg g}^{-1} \text{DM}$) | Carbohydrates ($\text{mg g}^{-1} \text{DM}$) | Chlorophyll <i>a</i> ($\mu\text{g g}^{-1} \text{DM}$) | Chlorophyll <i>a</i> :phycocyanin ($\mu\text{g g}^{-1} \text{DM}$) | DOC (mg C L^{-1}) | a^{*250} ($\text{L mg}^{-1} \text{m}^{-1}$) | DO ($\text{mg O}_2 \text{L}^{-1}$) | pH | Temperature ($^{\circ}\text{C}$) |
|---------------------|---|---|---|---|--|---|---------------------------------|--|---|-------------|---------------------------------------|
| | | | | | | | | | | | |
| 30 cm (36)* | 5.48 ± 0.85 | 729 ± 97.9 | 95 ± 9.5 | 7.27 ± 0.65 | 10.5 ± 0.28 | 1.09 ± 0.28 | 1.81 ± 0.12 | 11.3 ± 1.27 | 11.0 ± 0.52 | 7.50 ± 0.05 | 6.98 ± 0.31 |
| 60 cm (45) | 6.32 ± 0.76 | 658 ± 60.2 | 116 ± 6.4 | 8.08 ± 1.42 | 8.81 ± 0.51 | 0.75 ± 0.09 | 1.73 ± 0.18 | 11.8 ± 1.17 | 10.9 ± 0.49 | 7.51 ± 0.04 | 6.86 ± 0.37 |
| 90 cm (47) | 5.82 ± 0.66 | 589 ± 65.2 | 101 ± 6.3 | 7.12 ± 0.45 | 6.07 ± 0.92 | 0.79 ± 0.11 | 1.47 ± 0.07 | 10.7 ± 0.87 | 10.7 ± 0.49 | 7.57 ± 0.04 | 7.08 ± 0.28 |
| Left riparian zone | | | | | | | | | | | |
| 30 cm (4) | 2.93 ± 0.29 | 414 ± 89.4 | — | 10.1 ± 0.02 | 1.14 ± 0.01 | 0.72 ± 0.07 | 2.70 ± 0.60 | 8.52 ± 1.21 | 4.66 ± 1.13 | 6.98 ± 0.09 | 6.98 ± 1.96 |
| 60 cm (14) | 6.32 ± 1.86 | 499 ± 54.2 | 133 ± 5.7 | 8.34 ± 0.32 | 2.74 ± 0.41 | 0.73 ± 0.15 | 2.25 ± 0.15 | 16.5 ± 2.18 | 4.37 ± 0.69 | 6.94 ± 0.08 | 7.20 ± 0.73 |
| 90 cm (24) | 5.63 ± 0.94 | 273 ± 30.5 | 102 ± 7.8 | 7.01 ± 0.59 | 1.44 ± 0.20 | 0.82 ± 0.17 | 2.10 ± 0.12 | 13.9 ± 1.71 | 4.23 ± 0.62 | 7.18 ± 0.07 | 7.58 ± 0.54 |
| Right riparian zone | | | | | | | | | | | |
| 30 cm (2) | 2.19 ± 0.99 | 288 ± 167 | — | 7.95 ± 2.78 | — | — | 2.67 ± 0.32 | 7.85 ± 0.87 | 9.07 ± 3.16 | 6.81 ± 0.03 | 8.90 ± 0.96 |
| 60 cm (8) | 7.18 ± 2.75 | 394 ± 63.9 | 122 ± 19 | 9.60 ± 0.94 | 1.78 ± 0.31 | 0.76 ± 0.14 | 2.16 ± 0.32 | 9.16 ± 1.19 | 10.5 ± 1.45 | 7.11 ± 0.21 | 6.32 ± 0.57 |
| 90 cm (24) | 8.95 ± 1.54 | 414 ± 32.7 | 151 ± 8.7 | 10.2 ± 0.26 | 1.24 ± 0.21 | 0.69 ± 0.10 | 1.63 ± 0.09 | 9.66 ± 0.10 | 9.75 ± 0.74 | 7.35 ± 0.06 | 7.38 ± 0.57 |

* Number of samples (*n*).Table 2. Results of stepwise multiple regression analyses (partial correlations) of streambed and riparian zone esterase activity and TdR incorporation. Variables for which partial correlations are not listed were excluded from the model because of low *F* ratios. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

| Independent variable | Streambed | | | | | | Riparian zone* | | | | | |
|------------------------------------|-------------------|----------|----------|-------------------|---------|--------|-------------------|--------|----------|-------------------|----------|--------|
| | Esterase activity | | | TdR incorporation | | | Esterase activity | | | TdR incorporation | | |
| | 30 cm | 60 cm | 90 cm | 30 cm | 60 cm | 90 cm | 30 cm | 60 cm | 90 cm | 30 cm | 60 cm | 90 cm |
| AFDM | — | — | — | — | — | — | — | — | — | — | — | — |
| CHO | 0.959*** | 0.822*** | 0.597*** | 0.671** | — | — | — | — | — | — | — | — |
| Chl <i>a</i> | — | 0.401* | 0.385* | 0.372* | 0.473* | — | 0.645*** | 0.660* | 0.376* | — | 0.929*** | 0.296* |
| Chl <i>a</i> : pheophytin ratio | 0.463* | — | — | 0.661** | 0.414* | — | — | — | — | — | — | 0.336* |
| DOC | 0.343* | — | — | — | — | — | — | — | — | — | — | — |
| DO | -0.549* | — | — | — | 0.572** | — | — | — | — | 0.814** | — | — |
| Temperature | — | 0.557** | — | — | — | 0.304* | — | — | 0.639*** | — | — | — |
| Multiple <i>R</i> ² | 0.954 | 0.816 | 0.564 | 0.419 | 0.568 | 0.625 | 0.524 | 0.703 | 0.726 | 0.726 | 0.372 | 0.372 |
| <i>N</i> | 31 | 34 | 39 | 27 | 30 | 33 | 15 | 15 | 14 | 14 | 35 | 35 |
| <i>F</i> ratio | 138.94 | 44.41 | 15.07 | 8.675 | 17.72 | 16.09 | 6.609 | 27.665 | 14.609 | 14.609 | 6.128 | 6.128 |
| <i>P</i> | <0.001 | <0.001 | <0.001 | 0.002 | <0.001 | <0.001 | 0.011 | <0.001 | <0.001 | <0.001 | 0.002 | 0.002 |

* The 30-cm riparian layer was excluded from the analyses because of low degrees of freedom.

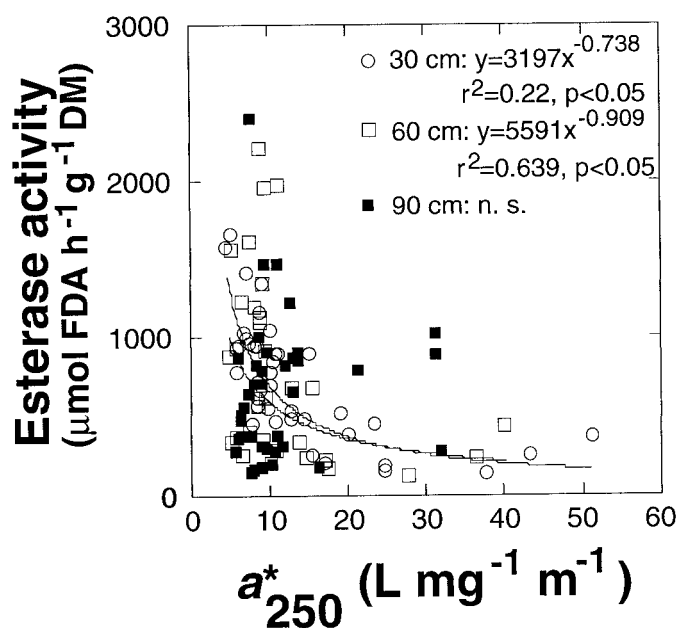


Fig. 3. Relationship between streambed esterase activity and DOC gross aromaticity as a^*_{250} .

h^{-1} and correlated with biofilm activity (Fig. 4). Entering the absolute Darcian velocity as an extra independent variable in the multiple stepwise regression model lowered the model R^2 for esterase activity but increased the model R^2 for the $[^3H]$ thymidine incorporation (Table 3). Furthermore, substituting DOC and DO concentrations (Table 2) by their relative fluxes obviously increased the variance explained in $[^3H]$ thymidine incorporation (Table 3). Streambed esterase activity, however, remained largely unaffected by DOC and, notably, by DO fluxes. Considering the temperature trace and the biofilm response variables (Figs. 1, 2), thermal effects on microbial activity appear likely. Thus, in order to test for possible covariance between pore-water temperature and the Darcian velocity, I plotted both variables in Fig. 5. Obviously, no relationship exists between temperature and Darcian velocity, and the possibility that covariance led to a misinterpretation of the regression results can be ruled out.

Reach-scale hydrodynamic control—Streambed biofilm activity was inversely related to the relative proportion of surface to subsurface downstream routing ($S_{sw}:S_{sb}$; Fig. 6). This was particularly pronounced for the 30-cm esterase activity and became less clear with depth. A similar depth-related trend was not evident in $[^3H]$ thymidine incorporation. However, in the shallow streambed, the highest $S_{sw}:S_{sb}$ value

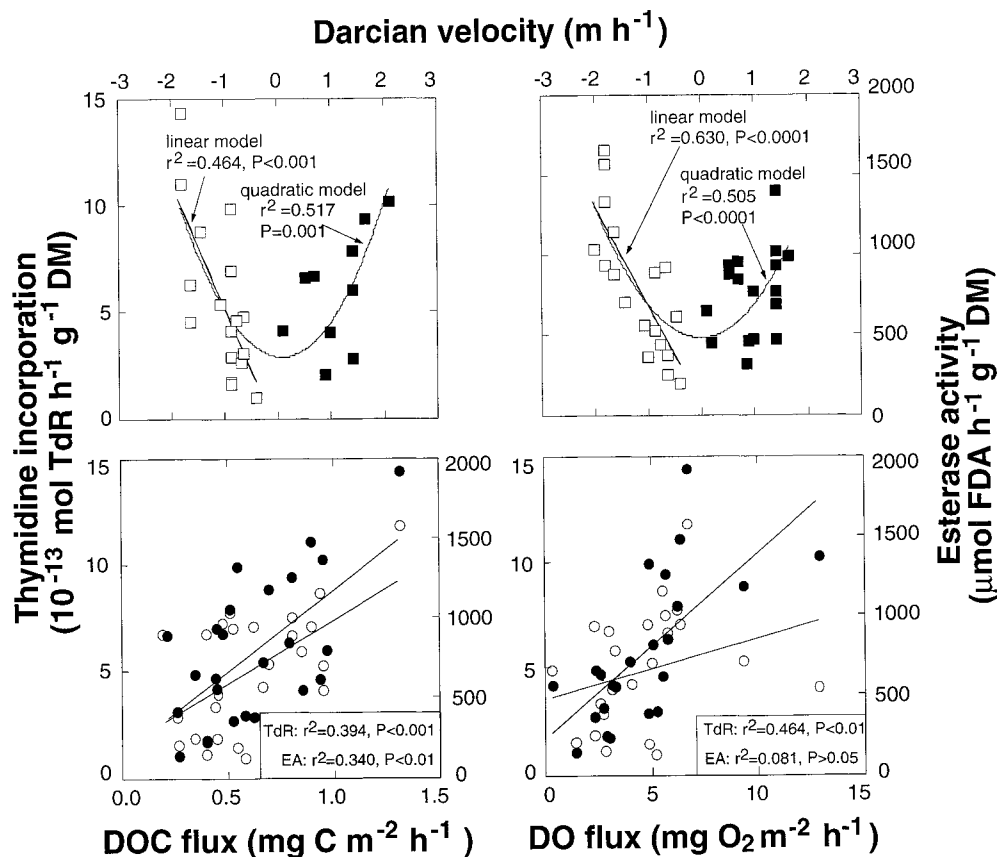


Fig. 4. Relationships between Darcian velocities, DOC and DO fluxes, and $[^3H]$ thymidine (TdR) incorporation and esterase activity (EA) in the OSB streambed (60-cm depth). Open and closed squares denote downwelling and upwelling, respectively; open and closed circles denote esterase activity and $[^3H]$ thymidine incorporation, respectively.

Table 3. Results (partial correlations) of stepwise multiple regression on [³H]thymidine incorporation (TdR) and esterase activity (EA) from the 60-cm streambed with the vertical Darcian velocity, DOC flux, and DO flux as extra independent variables (partial correlation coefficients in bold). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

| | AFDM | | | CHO | | Chl α | Chl α :pheo- phytin | | DOC | DO | Temperature | Darcian velocity | DOC flux | DO flux | Multiple R^2 | N | F ratio | P | |
|-------------------|------|--|--|-----|--|--------------|-------------------------------|--|-----|----|-------------|------------------|----------|---------|----------------|---|---------|---|--|
| | | | | | | | | | | | | | | | | | | | |
| Darcian velocity* | | | | | | | | | | | | | | | | | | | |
| TdR incorporation | | | | | | | | | | | | | | | | | | | |
| EA | | | | | | | | | | | | | | | | | | | |
| DOC flux | | | | | | | | | | | | | | | | | | | |
| TdR incorporation | | | | | | | | | | | | | | | | | | | |
| EA | | | | | | | | | | | | | | | | | | | |
| DO flux | | | | | | | | | | | | | | | | | | | |
| TdR incorporation | | | | | | | | | | | | | | | | | | | |
| EA | | | | | | | | | | | | | | | | | | | |

* Independent variable entered additionally to the basic array from Table 2.

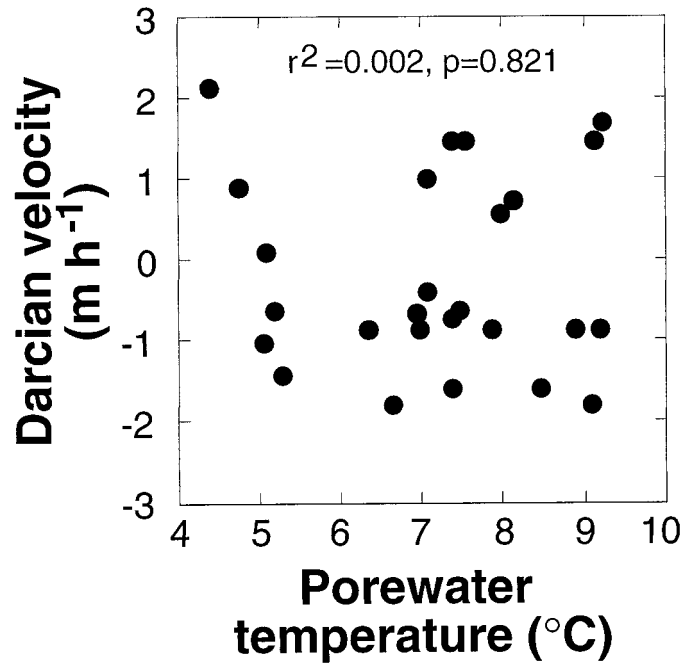


Fig. 5. Relationship between pore-water temperature and Darcian velocity in the OSB streambed.

coincided with the lowest [³H]thymidine incorporation rates. Again, no relationship was found between average 30-cm pore-water ($r^2 = 0.05$, $P = 0.51$) or stream water temperature ($r^2 = 0.06$, $P = 0.46$), respectively, and $S_{sw}:S_{sb}$. Therefore, covariance between $S_{sw}:S_{sb}$ and temperature can also be excluded on the reach scale.

On the reach scale, the vertical flux of stream DOC explained 78% of the variance of the average [³H]thymidine incorporation in the 30-cm depth layer (Fig. 7). This relationship did not exist in deeper sediment layers. In the left-bank riparian zone, where hill-slope groundwater enters the streambed, the 60-cm [³H]thymidine incorporation correlated weakly with the horizontal DOC flux. No such relationships were found between reach-scale DOC fluxes and average esterase activity. Average [³H]thymidine incorporation ($2.92\text{--}3.76 \times 10^{-13}$ mol [³H]TdR h⁻¹ g⁻¹ DM) and esterase activity ($570\text{--}898$ $\mu\text{mol FDA h}^{-1}$ g⁻¹ DM) were lowest in the shallow streambed when streambed DOC outputs exceeded DOC inputs (i.e., during instream DOC production; Fig. 8). Instream DOC production co-occurred with lower Chl α :pheophytin ratios. During streambed DOC removal, average Chl α :pheophytin ratios increased by a factor of 3.1 ($P = 0.043$), 1.7 ($P = 0.036$), and 2.1 ($P = 0.038$) at the 30-, 60-, and 90-cm depths, respectively. [³H]thymidine incorporation and esterase activity increased 3.6 and 1.5 times, respectively, with increasing streambed DOC retention efficiency. No such trend was observed in deeper sediment layers.

Discussion

The quantitative relationships between Darcian velocity, streambed solute fluxes, [³H]thymidine incorporation, and

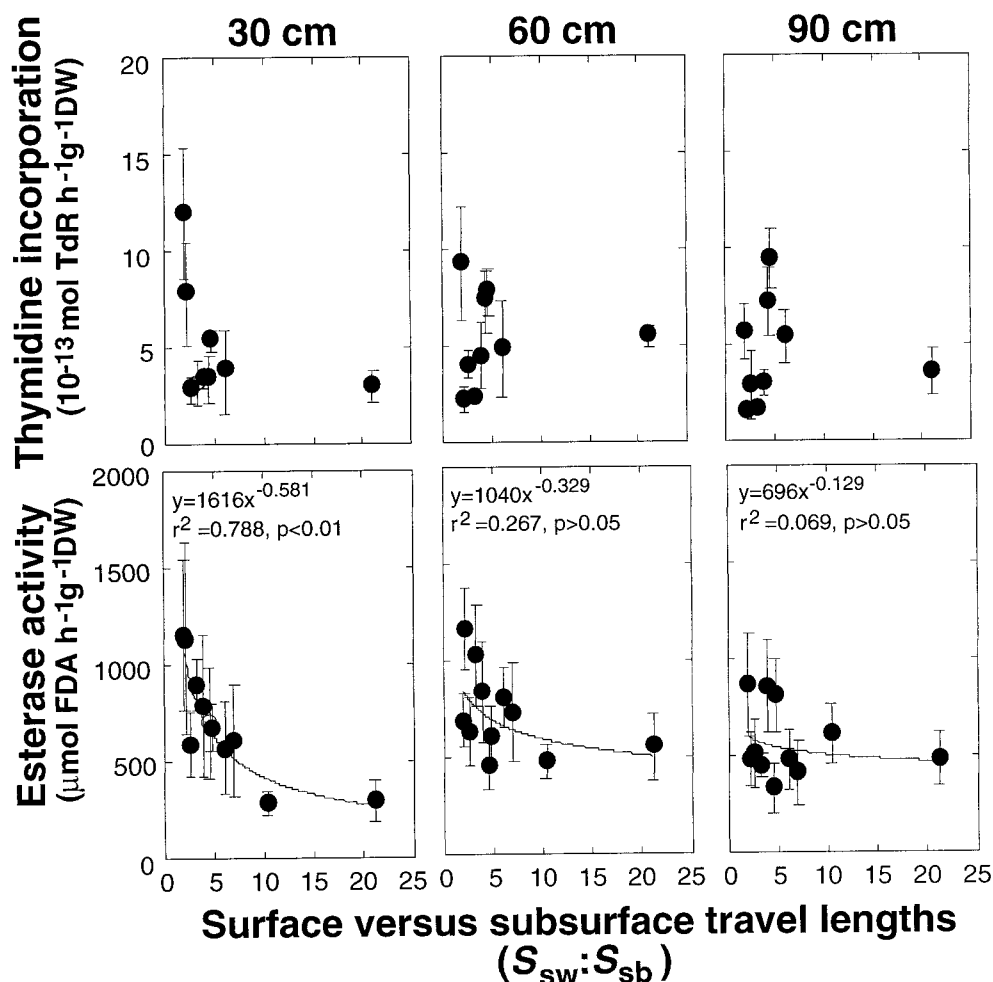


Fig. 6. Relationships between [^3H]thymidine incorporation, esterase activity, and the ratio of surface (S_{sw}) to subsurface (S_{sb}) water travel lengths in the OSB streambed.

esterase activity support the initial hypothesis that hydrodynamics significantly affect biofilm activity. Hydrodynamic effects on microbial activities, as revealed in this study, largely agree with the reach-scale patterns of streambed metabolism in OSB (Battin 1999) and thus highlight hydrodynamics as a template that drives microbial and biogeochemical processes on the sediment and reach scales. The reach-scale results also provide field evidence to support the conceptual models of Findlay (1995) and Kaplan and Newbold (2000) that postulate the contribution of the hyporheic zone to the stream ecosystem functioning is a function of the hyporheic processing rate, hydrologic exchange, nutrient supply, and temperature.

DOC fluxes and concentration versus sediment POM—Although stream scientists have previously recognized the need for integrating hydrology and biology (Hakenkamp et al. 1993), field results from the OSB streambed are among the first to document the quantitative relationship between subsurface flow velocity, solute fluxes, and biofilm activity. Elevated Darcian velocities—either upwelling or downwelling—related to higher microbial activity point at the key role

of water flow in the delivery of solutes to biofilms and mass transfer within biofilms. There is emerging evidence that biofilms grown under oligotrophic conditions develop a complex matrix structure with cell clusters, pores, and conduits (Wimpenny and Colasanti 1997), by which advective solute transport becomes increasingly important relative to diffusion (De Beer et al. 1994). Furthermore, the mass transfer in biofilms is positively related to the bulk liquid flow velocity (Stoodley et al. 1997), which would, of course, explain the fact that DOC and DO fluxes, rather than their relative concentrations, accounted for 39 and 46%, respectively, of the variance of the mid-depth streambed [^3H]thymidine incorporation. This result also agrees with experimental work by Lock and John (1979) that demonstrated elevated flow velocity to stimulate ^{32}P uptake by periphyton. In OSB, DO fluxes clearly improved the multiple regression model of [^3H]thymidine incorporation (Table 3). OSB sediment biofilms were in fact shown (Battin et al. 1999) to have an immediate and pronounced respiratory response to substrate amendments, whereas immobilized DOC molecules are subjected to considerable transient storage within the ma-

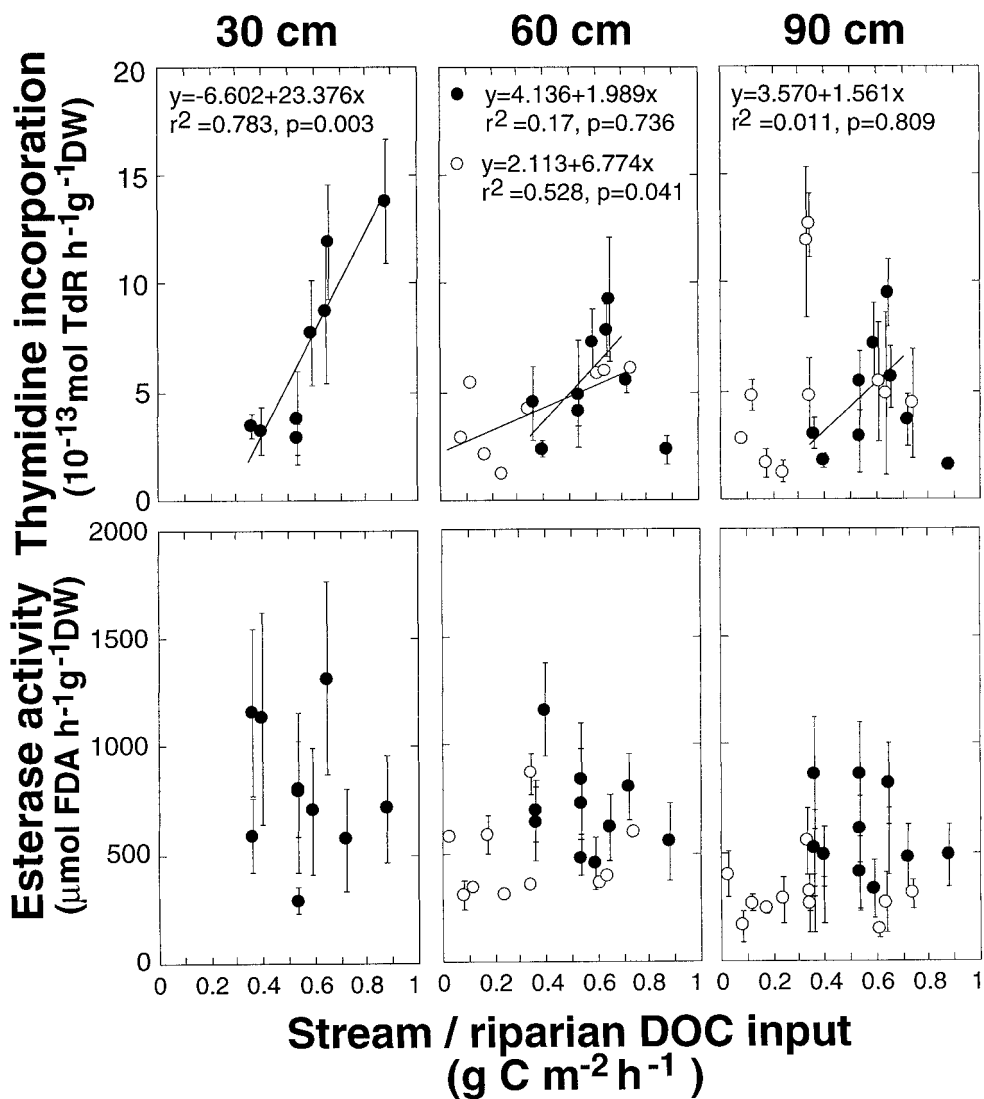


Fig. 7. Relationships between [^3H]thymidine incorporation, esterase activity, and the DOC input flux from the stream (closed circles) and the left riparian zone (open circles) into the OSB streambed.

trix. The sensitive respiratory response is thus most likely facilitated by advective DO transport through biofilm voids.

The relationship between downwelling velocities and microbial activity underscores the role of stream water in entraining solutes into the OSB streambed. This finding agrees with the mass-balance calculations that revealed stream water as the major DOC source to the OSB streambed (Battin 1999). Jones et al. (1995) also reported elevated stream DOC from benthic algal extracellular release and POM solubilization that downwells into the Sycamore Creek hyporheic zone, where it fuels the heterotrophic metabolism. In OSB, average DOC concentration was lower in the stream than in the streambed. Thus, more relevant for the streambed metabolism are the DOC fluxes and bioavailability rather than the concentration. Algal exudates can in fact influence DOC availability (Kaplan and Bott 1989), which became particularly evident during summer in OSB, when sediment Chl *a* and CHO explained more than 90% of the variance of shal-

low streambed microbial activity. This also agrees with the seasonally differing respiratory DOC turnover times (summer: 113 ± 30 d; fall and winter: 352 ± 89 d) in the OSB streambed (Battin 1999). The coexistence of algae and heterotrophs within biofilms ensures immediate availability of exudates to heterotrophic cells and thus reduces substrate spiraling length and downstream transport of labile DOC molecules. This would explain the consistent correlations between Chl *a*, CHO, and microbial activity. Notably, the tight relationship between the Chl *a*:pheophytin ratio, which is indicative for the senescence of algae, and thymidine incorporation pinpoints the metabolic role of algae in the OSB streambed. The linkage between sediment CHO and microbial activity can have multiple causes. The polymeric fraction of the CHO pool may constitute the major structural element of the biofilm matrix, whereas oligomers might serve as a carbon source to the microbial heterotrophs. Furthermore, algae and, notably, diatoms, which are abundant

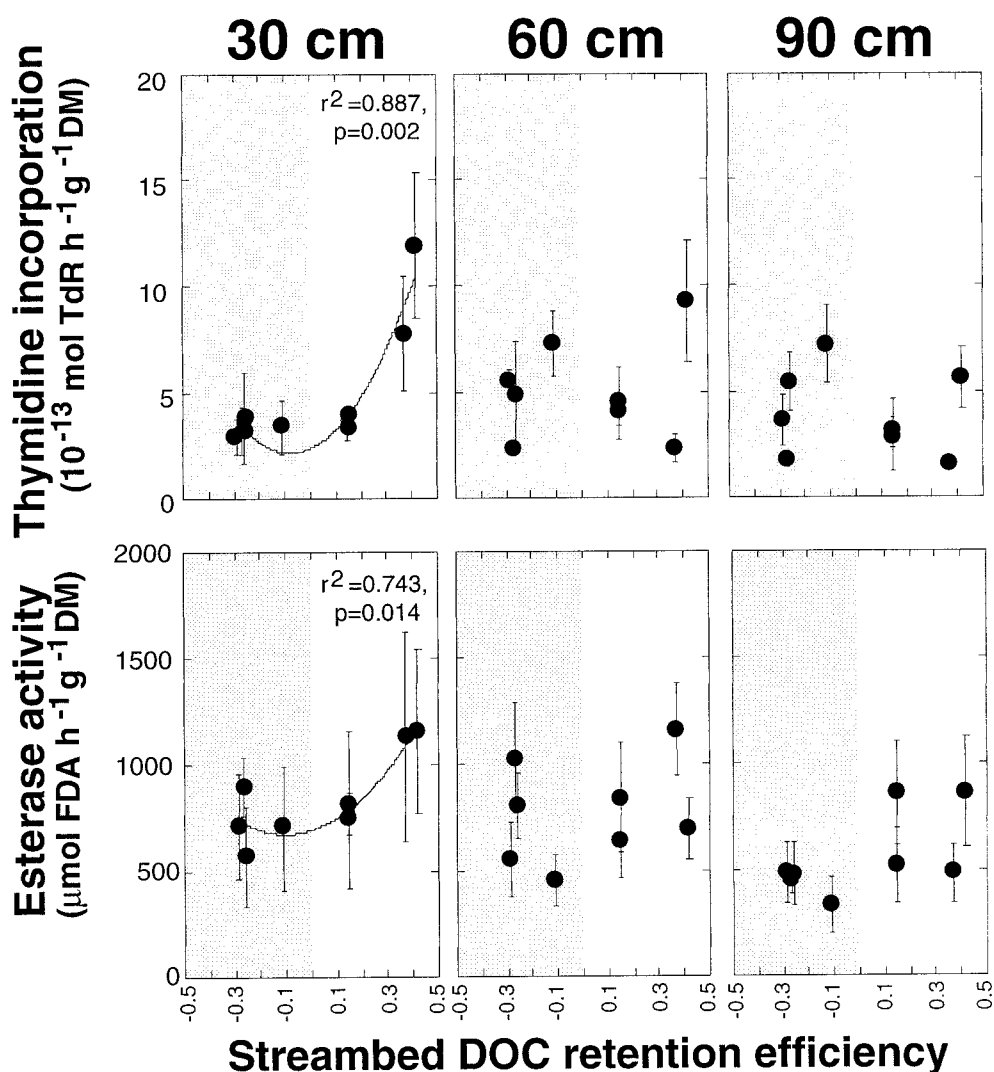


Fig. 8. Relationships between [^3H]thymidine incorporation, esterase activity, and the streambed DOC retention efficiency (sensu Mulholland 1981). The shaded area refers to DOC production within the streambed.

in OSB, contribute to the CHO pool via either leakage or (when decaying) release of molecules that are normally bound to the intact cell wall (Underwood et al. 1995). Finally, algae also express esterases, which helps explain the apparent relationship between Chl *a* and esterase activity.

The higher scattering of microbial activity associated with upwelling Darcian velocities (Fig. 4) is likely due to the temporal variation in the chemistry of the hill-slope groundwater entering the streambed. For instance, as indicated by the specific DOC absorption, a^*_{250} , hill-slope groundwater DOC had significantly more humic moieties than did pore-water DOC in the opposite riparian zone. Humic and fulvic acids are in fact known to inhibit biofilm activity via occlusion of sorption sites on the matrix (Freeman and Lock 1992). Inverse relationships between gross DOC aromaticity and microbial activity in the streambed and riparian zones support this finding. The apparent sensitivity of esterase activity to gross DOC aromaticity is likely attributable to the complexation of enzymes with humic compounds (Wetzel

1993). Alternatively, a shift of enzyme allocation in response to changing DOC quality might generate this pattern. Certainly, lower DO concentration in the left riparian zone (Table 3) could also induce lower microbial activity, yet no significant difference was found in [^3H]thymidine incorporation.

There is evidence that the hill-slope groundwater DOC entering the OSB supports the streambed heterotrophs during snow melt (Fig. 2), which would explain the positive relationship between riparian DOC input flux and [^3H]thymidine incorporation. This supports work by Hornberger et al. (1994), who reported flushing of soil DOC during snow melt as a major contribution to the stream DOC pool in Snake River, Colorado.

Reach-scale hydrodynamics—Findlay (1995) proposed a conceptual model in which rates of hyporheic biogeochemical processes and the surface/subsurface hydrologic exchange are the two variables that determine the functional

significance of the hyporheic zone in terms of the stream ecosystem. Kaplan and Newbold (2000) recently expanded this model to DOC dynamics and included in the model such variables as temperature, supply rate of nutrients and oxygen, and the size of the hyporheic zone. They expect the total utilization of DOC to be limited by the size of the hyporheic zone and even more so by hydrologic exchange. The reach-scale relationships between water travel lengths, DOC fluxes, and microbial activity, as presented in this study, as well as the DO removal rate derived from mass-balance calculations (Battin 1999) provide evidence supporting these conceptual models. Extended subsurface travel length of water (i.e., $S_{sw} : S_{sb}$ is low) was related to increased biofilm esterase activity and [^3H]thymidine incorporation, whereas predominantly surface downstream routing of water (i.e., $S_{sw} : S_{sb}$ is high) reduced microbial activity. This pattern was particularly clear for shallow streambed esterase activity, whereas it became blurred with depth and was less evident for [^3H]thymidine incorporation. Reach-scale downwelling supplies the streambed biota with solutes that, depending on the size of the hyporheic zone and the hydrologic exchange rate, experience different residence time in the subsurface. Thus, low $S_{sw} : S_{sb}$ values translate into higher residence time of water and solutes within the streambed, which in turn enhances the immobilization of solutes by biofilms and their subsequent processing. Kaplan and Newbold (2000) describe hydrologic exchange as the effective vertical velocity at which water masses are transferred from the stream into the streambed. As shown above, the Darcian velocity has, in fact, a significant effect on the streambed microbial activity. Concomitantly, the supply rates of substrate and oxygen, as DOC and DO fluxes, also influence streambed biofilm activity. Interestingly, both biofilm activity and streambed DO removal (Battin 1999) were not strongly affected by the pore-water temperature, which points to a possible adaptation of the microbial community to average low temperature.

Although neither sediment CHO and Chl *a* nor pore-water DOC and DO concentrations changed with depth, a depth-related gradient of functional biofilm response to hydrodynamics was apparent (Figs. 6–8). This behavior is likely attributable to the dominating subsurface flow patterns. Microbial processes at the stream/streambed interface are driven by vertical exchange, whereas horizontal inflow from the hill-slope groundwater becomes increasingly significant with depth. Battin and Sengschmitt (1999) observed a similar congruence between shallow vertical and deeper horizontal flow paths and sediment-associated esterase activity in a large river bottom. The disparities between esterase activity and [^3H]thymidine incorporation patterns are probably due to their different roles in biofilm functioning. Many organic substrates need enzymatic processing prior to being taken up and metabolized. This indeed requires transient storage within the biofilm and implies time-lagged processes along the metabolic pathway (e.g., enzymatic processing versus uptake).

The positive relationship evidenced by the DOC retention efficiency of the shallow streambed microbial activity underscores the role of biofilms in carbon retention in alpine streams with large subsurface hydrologic fluxes. This role is

also supported by the experimental findings of Battin et al. (1999), which showed that OSB sediment biofilms transiently store, on average, $0.16\text{--}0.22 \mu\text{g DOC g}^{-1} \text{EPS-C h}^{-1}$, where EPS are extracellular polymeric substances. This corresponds to $\sim 74\text{--}83\%$ of the DOC immobilized, with the residual (i.e., $17\text{--}26\%$) being respired shortly after immobilization.

In conclusion, there is ample evidence that the hyporheic zone is a major retention site that drives stream nutrient uptake, remineralization, and metabolism (e.g., Grimm and Fisher 1984; Findlay 1995; Mulholland et al. 1997; Battin 1999). Results of the present study now expand this view by factoring sediment-scale and reach-scale hydrodynamics into biofilm processes and hyporheic functioning. Furthermore, the consistency between sediment and reach-scale microbial and biogeochemical patterns, as generated by hydrodynamics, provides evidence that small-scale processes can be extrapolated to the reach scale (cf. Boulton et al. 1998). More work needs to be done to comprehend microscale hydrodynamic effects on the functioning of heterogeneous lotic biofilms.

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