

Acetate retention and metabolism in the hyporheic zone of a mountain stream

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Abstract

An in situ acetate injection was used to determine the influence of labile dissolved organic carbon (DOC) availability on microbial respiration in the hyporheic zone of a headwater stream. We added bromide as a conservative tracer and acetate as an organic substrate to the hyporheic zone of Rio Calaveras, New Mexico, via an injection well. Tracer was observed in four of eight capture wells. Three of the four wells showed increases in bromide without concurrent increases in acetate concentration, suggesting 100% acetate retention. One well had 38% acetate retention. Pore velocity and acetate retention were negatively correlated, suggesting hydrologic control of acetate retention. Acetate did not significantly sorb to the sandy hyporheic sediments at this site, indicating biological consumption of acetate. Acetate addition stimulated total CO₂ production along monitored flowpaths and led to changes in solutes associated with microbial terminal electron-accepting processes (TEAPs). Dissolved oxygen (DO), nitrate, and sulfate significantly decreased, and ferrous iron and methane significantly increased compared to background concentrations in most wells. These results support the hypothesis that microbial respiration in the hyporheic zone is limited by labile DOC availability. Furthermore, we have shown that a suite of metabolic processes, from aerobic respiration to methanogenesis, cooccur and that anaerobic processes dominate heterotrophic metabolism in the hyporheic zone of Rio Calaveras.

In the past 10–15 yr, the importance of the hyporheic zone (i.e., subsurface water containing at least 10% surface water, *sensu* Triska et al. 1989) to lotic biogeochemistry and ecology has been documented for streams and rivers worldwide (e.g., Jones and Holmes 1996; Brunke and Gonser 1997; Gibert et al. 1997). Hyporheic sediments are metabolically active, are an important site for organic matter decomposition, and can have a large impact on respiration (i.e., decrease P:R ratios) in measures of whole-stream ecosystem metabolism (Grimm and Fisher 1984; Mulholland et al. 1997; Nageli and Uehlinger 1997). Microbial metabolism in the hyporheic zone depends on transport of organic substrates and electron acceptors from the surface stream and/or nearby groundwater (e.g., Findlay 1995; Jones et al. 1995a). Since subsurface organisms are mostly heterotrophic (Jones et al. 1995b) and require allochthonous organic matter sources, it is commonly hypothesized that microbial metabolism in the hyporheic zone is limited by organic matter availability (e.g., Jones 1995). The relationship between hyporheic zone aerobic respiration and organic matter availability has been examined using sediment microcosms (e.g., Pusch and Schwoerbel 1994; Jones 1995; Fuss and Smock

1996) but not by direct in situ manipulation of organic matter availability.

Because water moves more slowly in the hyporheic zone than in the surface stream (Morrice et al. 1997) and because the hyporheic zone is isolated from the atmosphere, DO can become depleted during microbial respiration, creating locations of hypoxia or anoxia (Dahm et al. 1991; Findlay 1995; Jones and Holmes 1996; Baker et al. 1999). Under anoxic conditions, organic carbon mineralization by microorganisms proceeds via alternative TEAPs (Vrobesky and Chapelle 1994). These include denitrification, metal (e.g., iron) reduction, sulfate reduction, and methanogenesis (Table 1). Most knowledge about anaerobic TEAPs comes from studies of lake and ocean sediments (e.g., Carlton et al. 1989; Sweerts et al. 1991; Thamdrup and Canfield 1996) and regional aquifers (e.g., Champ et al. 1979; Vrobesky and Chapelle 1994). These microbial processes are not yet well understood in stream ecosystems (but *see* Morrice 1997; Hedin et al. 1998; Baker et al. 1999).

TEAPs in lakes, oceans, and aquifers are spatially segregated according to thermodynamic (Champ et al. 1979; Vrobesky and Chapelle 1994), geochemical (Potsma and Jakobsen 1996), or ecological (Lovley and Klug 1983) constraints. Often, TEAPs are separated in order of decreasing free energy as in Table 1. In lake and ocean sediments, where diffusion dominates water and solute transport, segregation of TEAPs occurs over small (millimeter to centimeter) vertical scales (Carlton et al. 1989). Advective transport is important in aquifers where TEAPs are distributed longitudinally over much larger (meter to kilometer) scales, and discrete zones often are dominated by a single TEAP (Champ et al. 1979; Vrobesky and Chapelle 1994). Because studies of hyporheic zone microbial metabolism have generally focused on aerobic processes (*see* Jones and Holmes 1996), the spatial distribution and ecological importance of anaerobic TEAPs in this environment are poorly understood (Morrice 1997; Baker et al. 1999).

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Table 1. Stoichiometry and Gibbs free energy change of microbial TEAPs with acetate (CH_3COO^-) as electron donor (Stumm and Morgan 1981; Lovely and Phillips 1988). Solutes and dissolved gases monitored during the injection experiment are indicated in bold font.

TEAP	Stoichiometry	ΔG° (kJ/mol)*
Aerobic respiration	$\text{CH}_3\text{COO}^- + 2\text{O}_2 = \text{HCO}_3^- + \text{CO}_2 + \text{H}_2\text{O}$	-881.6
Denitrification	$\text{CH}_3\text{COO}^- + 1.6\text{NO}_3^- + 1.6\text{H}^+ = 0.8\text{N}_2 + \text{HCO}_3^- + \text{CO}_2 + 1.8\text{H}_2\text{O}$	-860.6
Iron reduction	$\text{CH}_3\text{COO}^- + 8\text{Fe}^{3+} + 3\text{H}_2\text{O} = 8\text{Fe}^{2+} + \text{HCO}_3^- + \text{CO}_2 + 8\text{H}^+$	-494.4
Sulfate reduction	$\text{CH}_3\text{COO}^- + \text{SO}_4^{2-} + \text{H}^+ = \text{HS}^- + \text{HCO}_3^- + \text{CO}_2 + \text{H}_2\text{O}$	-92.3
Methanogenesis	$\text{CH}_3\text{COO}^- + \text{H}_2\text{O} = \text{CH}_4 + \text{HCO}_3^-$	-14.6

* Assumes 25°C and pH = 7.

There is a long history of study of DOC in stream ecology (e.g., Fisher and Likens 1973; Lush and Hynes 1977; Dahm 1981; McDowell 1985; Fiebig and Lock 1990), and research has shown that DOC is composed mostly of complex organic (e.g., humic and fulvic) acids. Far less is known about how low-molecular-weight (LMW) compounds like acetate and other volatile fatty acids are cycled in lotic ecosystems (Thurman 1985). Acetate is both produced and consumed by microbial metabolism in many aquatic ecosystems, especially in hypoxic or anoxic sediments (Chapelle and Bradley 1996). While many forms of DOC are not directly available, acetate is readily consumed by microorganisms (Drake 1994), and experimental acetate addition may stimulate microbial respiration in the hyporheic zone.

We used an in situ acetate injection experiment to determine (1) how the availability of DOC as acetate influences microbial respiration in the hyporheic zone, and (2) how aerobic and anaerobic TEAPs are distributed in the hyporheic zone. We hypothesized that acetate addition would stimulate aerobic and anaerobic microbial respiration and that TEAPs would be segregated longitudinally along flowpaths

in order of the thermodynamic energy yields given in Table 1.

Study site

Rio Calaveras is a first-order, spring-fed, perennial stream at 2,475-m elevation in the Jemez Mountains of New Mexico (35°56'N, 106°42'W). Catchment area is 3,760 ha, and average stream gradient is 1.3%. Alluvium is poorly sorted, coarse, rhyolitic sand derived from weathered Bandelier tuff (Wroblicky et al. 1998). Porosity ranges between 39 and 60%, and mean hydraulic conductivity is $1.2 \times 10^{-3} \text{ cm s}^{-1}$ (Morrice et al. 1997; Wroblicky et al. 1998). Sediment organic matter content is 1% by weight (Baker 1998).

Climate at the study site is semiarid, with a mean annual temperature of 8.8°C (range = 2.2–15.4) and precipitation of 450 mm (National Weather Service). Most of the precipitation occurs as rain during monsoon thunderstorms from July to September and as snow from November to March. Stream discharge and groundwater table elevation respond to precipitation inputs from rain and snowmelt. Groundwater table elevation may increase by as much as 50 cm during spring snowmelt, and it averages 1 m below ground surface during autumn base flow (Baker 1998). Stream discharge ranges from ca. 1 liter s^{-1} during autumn base flow to >100 liters s^{-1} during spring snowmelt. Surface-water discharge during this study (July 1997) was 4.27 liters s^{-1} .

Well network—Beginning at the perennial spring source, a 120-m reach at Rio Calaveras was equipped with 73 polyvinyl chloride (PVC) wells for monitoring subsurface hydrology and biogeochemistry (Fig. 1A; Valett et al. 1996; Morrice et al. 1997). To better understand microbial processes in the hyporheic zone, a network of nested 1.9-cm-diameter PVC wells (screen length = 10 cm) was installed along the streambank and floodplain adjacent to Rio Calaveras (Fig. 1B). Each nest consisted of three wells at depths of 25, 60, and 100 cm below the base flow water table (Morrice 1997). Wells at 60-cm depth were used to construct a water table map for the smaller lateral hyporheic study area (Fig. 1B). Using a conservative tracer injected into the surface stream, Morrice (1997) showed that these wells contained >10% surface water (i.e., they were in the hyporheic zone, *sensu* Triska et al. 1989). Furthermore, hydrologic modeling showed they were in lateral hyporheic flowpaths where stream water enters the subsurface then returns to the surface some distance downstream (Wroblicky et al. 1998).

For the purpose of conducting subsurface injection exper-

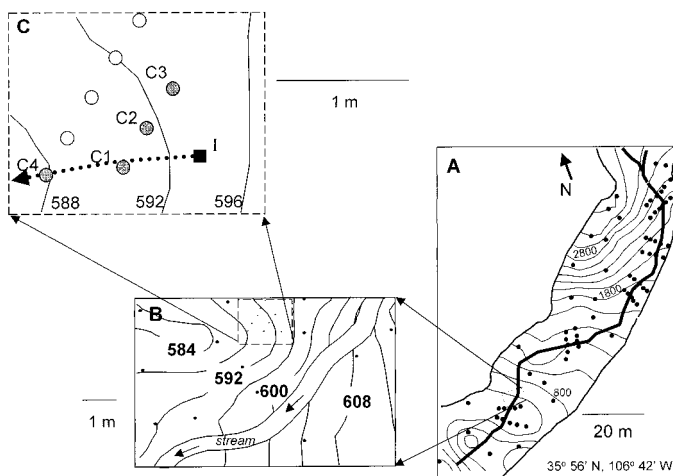


Fig. 1. (A) Map of the Rio Calaveras study site. Circles indicate wells, and the bold line indicates the stream. Water table elevation (centimeters above datum) contours are shown in the thin lines. (B) Map of the hyporheic study section showing locations of nested wells (dots) and piezometric surface at Rio Calaveras in July 1997. Contours represent water level in centimeters above datum. (C) Expanded view of hyporheic injection plot. Dotted arrow indicates estimated flowpath between injection (I, square) and capture wells (C, circles). Filled circles indicate capture wells that received tracer during the injection monitoring period.

iments, a single injection well (screened for 10 cm at a depth of 43 cm below the water table) was installed 1.5 m down-gradient from the stream (Fig. 1C, upper panel). Eight capture wells (fully screened, depth = 80 cm) were installed in a 60° arc at distances of 50 and 100 cm downgradient from the injection well (Fig. 1C). Well installation details are provided in Morrice (1997). Based on prior estimates of pore velocity (Morrice 1997), the travel time for stream water to reach these wells is between 0.5 and 2 d.

Methods

Solute injection—Injection solution was prepared using groundwater pumped from a well located ca. 50 m up-gradient of the injection site. We maintained anoxic conditions within the injection solution vessel by continuous bubbling with N₂ gas (Smith et al. 1991). Dry solutes were thoroughly mixed with the deoxygenated groundwater prior to injection, creating a solution containing 900 μM Br⁻ (as NaBr), a conservative tracer, and 4,193 μM acetate (as NaCH₃COO), a biologically active tracer. The mixed solution was added to the injection well at a rate of 8.5 ml min⁻¹ for 2 h using a metering pump (Fluid Metering). The water level in the injection well increased 0.3 cm during the injection. Background Br⁻ and acetate concentrations in wells averaged 0.64 and 39 μM, respectively.

Sample collection and analysis—Water table elevation, temperature, solutes, and dissolved gases in the eight capture wells were monitored every 3–4 h for 48 h following the initiation of the injection. The time frame was chosen based on prior experiments at this site (Morrice 1997), which showed that concentrations of injected tracers return to background conditions after 48 h. Water table elevation was measured using a battery-operated water level meter that detects specific conductance changes, and temperature was measured using a standard thermocouple inserted to the mid-depth of the well screen.

Water for dissolved solutes was obtained using a peristaltic pump (GeoTech) with in-line Whatman GF/F glass-microfiber filters (pore size = 0.7 μm) to remove suspended particles. To minimize pumping effects, we limited our withdrawal of water to a maximum of 200 ml per well per sample. Observed changes in water table elevations were <0.5 cm after sample withdrawal, and pumping from one well did not influence water levels in adjacent wells. Filtered samples were stored in acid-washed polyethylene bottles on ice until they were returned to the lab for analysis. We measured Br⁻, acetate, nitrate (NO₃⁻), and sulfate (SO₄²⁻) using a DIONEX-500 ion chromatograph with suppressed conductivity detection (Chapelle and Bradley 1996). Ferrous iron (Fe²⁺) was measured colorimetrically on 1.5-ml samples that were passed through a 0.2-μm filter and reacted in the field with 40 μl of Ferrozine® (Stookey 1970). DOC was determined using wet persulfate oxidation (100°C) on an Oceanography International 700 total organic carbon analyzer (Menzel and Vaccaro 1964).

Water for dissolved gas analysis was obtained using the peristaltic pump with no filter attachment. DO was measured on 30-ml samples using Winkler titration in the field (Wetzel

and Likens 1991). Free carbon dioxide (PCO₂) was obtained using a syringe headspace equilibration technique (Kling et al. 1992). To minimize degassing, the water was pumped directly into the syringe, allowing the force of the water to move the syringe plunger. Gas from the headspace was transferred to clean, evacuated 15-ml serum vials until transport to the lab for analysis. PCO₂ was determined using a gas chromatograph with a thermal conductivity detector (Buck Scientific). The amount of CO₂ dissolved in water was calculated from PCO₂ and temperature using Henry's Law (Kling et al. 1992). Dissolved methane (CH₄) samples were obtained by pumping 1–2 ml of water directly into clean, evacuated Vacutainer® vials. CH₄ was released into the headspace by shaking and was measured using a Shimadzu gas chromatograph with a flame ionization detector (deAngelis and Lilley 1987; Dahm et al. 1991). Internal calibration of analytical instruments and techniques showed that precision varied from ca. 2% for DO, DOC, PCO₂, and CH₄ to ca. 7% for Br⁻, NO₃⁻, and SO₄²⁻, and 12% for acetate.

Acetate transport and retention—Previous work at Rio Calaveras has shown that Br⁻ is transported conservatively (Valett et al. 1996; Morrice et al. 1997), and pore velocity (centimeters per minute) was calculated from the time required for half the Br⁻ mass in the pulse to be transported between the injection well and the sampling point.

To estimate acetate transport and retention, we compared observed Br⁻ and acetate concentrations in capture wells following solute injection initiation. Without biological or chemical retention, acetate and Br⁻ transport should be nearly identical. We calculated predicted acetate concentration based on observed Br⁻ response in wells using the following formula (Morrice 1997):

$$\text{acetate}_{p,w,T} = \left\{ \frac{(\text{Br}_{w,T} - \text{Br}_{bkg})}{(\text{Br}_{inj} - \text{Br}_{bkg})} \times (\text{acetate}_{inj}) \right\} + \left\{ 1 - \frac{(\text{Br}_{w,T} - \text{Br}_{bkg})}{(\text{Br}_{inj} - \text{Br}_{bkg})} \right\} \times \text{acetate}_{bkg} \quad (1)$$

where subscripts are defined as *p* = predicted, *w* = well, *T* = time, *inj* = injection solution concentration, and *bkg* = background concentration. Acetate retention is indicated when observed acetate is less than predicted acetate.

We calculated the difference between observed and predicted acetate concentrations in each well for all samples taken when Br⁻ concentrations were elevated above background. The null hypothesis that median observed minus predicted acetate = 0 for each connected well was tested statistically using the Wilcoxon Sign Rank test or a Sign test if the data distributions were not symmetric (Sokal and Rohlf 1981). The null hypothesis was rejected at *p* values ≤ 0.05. We also calculated relative acetate retention for each well by dividing the total concentration of acetate retained by the total concentration of bromide that reached the well. In this way, acetate retention is expressed relative to the amount of injection solution that reached the well.

Freundlich adsorption isotherm—To assess the potential for acetate sorption on sediments, we equilibrated five 50-g

Table 2. Temperature (mean of eight wells over diel cycle \pm SD) and biogeochemistry of hyporheic zone (mean of eight wells before injection \pm SD). Background temperature and biogeochemistry (one observation before injection \pm analytical error) of connected capture wells.

	Hyporheic average	Well C1	Well C2	Well C3	Well C4
Temperature ($^{\circ}$ C)	14.0 \pm 0.5	13.4 \pm 0.7	13.4 \pm 0.5	13.6 \pm 0.5	12.2 \pm 0.7
Acetate (μ M)	39 \pm 0.8	41 \pm 4.9	37 \pm 4.5	27 \pm 3.2	41 \pm 4.9
Bromide (μ M)	0.64 \pm 1.0	0.36 \pm 0.02	0.25*	0.26 \pm 0.02	0.38 \pm 0.02
Dissolved oxygen (μ M)	54 \pm 75	55 \pm 1.0	63 \pm 1.3	19 \pm 0.4	48 \pm 1.0
Nitrate (μ M)	33 \pm 23	30 \pm 1.8	24 \pm 1.4	2.4 \pm 0.14	23 \pm 1.4
Ferrous iron (μ M)	35 \pm 23	27 \pm 1.4	6.6 \pm 0.33	21 \pm 1.1	50 \pm 2.5
Sulfate (μ M)	52 \pm 18	40 \pm 3.2	68 \pm 5.4	51 \pm 4.1	46 \pm 3.7
Methane (μ M)	14 \pm 11	8.0 \pm 0.20	7.4 \pm 0.19	5.8 \pm 0.15	18 \pm 0.45
Carbon dioxide (μ M)	424 \pm 121	319 \pm 8.0	400 \pm 10.0	450 \pm 11.3	309 \pm 7.7
DOC (μ M)	154 \pm 20	238 \pm 9.5	164 \pm 6.6	160 \pm 6.4	175 \pm 7.0

* Below detection limit of 0.25 μ M.

(wet mass) Rio Calaveras aquifer sediment samples in 100-ml solutions containing 1, 10, 25, 50, or 100 mg acetate C liter⁻¹ for 30 min while shaking at 22 $^{\circ}$ C (Domenico and Schwartz 1990). After allowing the sediment to settle, supernatant was centrifuged at 3,000 rpm for 15 min. Subsamples of the supernatant were filtered (Whatman GF/F glass-microfiber filters) and analyzed for acetate content as described above. K_d , the distribution coefficient for the sediment, was calculated from the slope of the line relating C vs. C^* , where C is the equilibrium concentration of acetate in contact with the sediment (milligrams per liter), and C^* is the mass of acetate adsorbed per gram of sediment (milligrams per gram). C^* was calculated as (Domenico and Schwartz 1990)

$$C^* = (C_i - C)(\text{solution volume})/(\text{sediment mass}) \quad (2)$$

where C_i is the initial solute concentration. A greater tendency for adsorption is indicated by higher values of K_d (Domenico and Schwartz 1990).

Influence of acetate retention on microbial TEAPs—To quantify changes in TEAP solutes, we calculated differences between observed and predicted concentrations of CO₂, DO, NO₃⁻, SO₄²⁻, Fe²⁺, and CH₄ for all samples after Br⁻ from the injection solution appeared in the well. We determined the degree to which samples were diluted (D) by the injection solution according to the following formula (Hedin et al. 1998):

$$D = \frac{(\text{Br}_{w,T} - \text{Br}_{bkg})}{(\text{Br}_{inj})} \quad (3)$$

Predicted TEAP solute concentrations were calculated from the following formula (after Hedin et al. 1998):

$$[X_{p,w,T}] = [X_{bkg}] \times (1 - D) \quad (4)$$

where X represents the solute of interest (CO₂, O₂, NO₃⁻, Fe²⁺, SO₄²⁻, or CH₄). If acetate retention occurs via microbial respiration, then observed minus predicted CO₂ concentration should be >0 , regardless of which TEAPs are involved in microbial respiration (Table 1). If acetate retention occurs via aerobic respiration, denitrification, or sulfate reduction, then observed minus predicted concentrations for DO, NO₃⁻, and SO₄²⁻ should be <0 (Table 1). If acetate retention

occurs via iron reduction or methanogenesis, then observed minus predicted concentrations for Fe²⁺ and CH₄ should be >0 (Table 1).

For each connected well, we statistically tested the null hypothesis that median observed minus predicted concentrations = 0 using Wilcoxon Sign Rank tests or Sign tests if the data were not distributed symmetrically (Sokal and Rohlf 1981). The null hypothesis was rejected at p values \leq 0.05.

Finally, we determined the proportion of acetate retention occurring via each TEAP from the median solute concentration change and stoichiometry presented in Table 1. This approximation makes the assumptions that the system is closed and that the generation or consumption of TEAP-indicative solutes in the tracer plume was due only to microbial mineralization of added acetate according to the stoichiometries presented in Table 1 (implications of these assumptions are detailed in the Discussion section).

Results

Background hydrology and chemistry—Acetate concentration in the hyporheic zone averaged 39 μ M (range = 27–78 μ M), while mean DOC concentration was 154 \pm 20 μ M (Table 2). On average, background acetate concentration represented nearly 50% of measurable DOC and ranged between 27 and 70% of measurable DOC (Table 2). Hyporheic zone CO₂ was supersaturated relative to the atmosphere (atmospheric equilibrium concentrations ranging from 12.5 to 13.5 μ M), averaging 424 \pm 121 μ M, whereas DO was $<30\%$ of atmospheric saturation, ranging from 19 to 63 μ M (Table 2). NO₃⁻ concentration averaged 33 \pm 23 μ M, and SO₄²⁻ ranged from 40 to 68 μ M (Table 2). Products of anaerobic metabolism (Fe²⁺ and CH₄) were also present; Fe²⁺ averaged 35 \pm 23 μ M, and CH₄ values averaged 14 \pm 11 μ M (Table 2).

Acetate transport and retention—Based on water table elevations (Fig. 1A,B), we expected water to move along the identified flowpath from the injection well to the capture wells (Fig. 1C, dotted line). Four of eight capture wells were hydrologically connected to the injection well (Fig. 1C), as indicated by a Br⁻ spike during the 48 h of the experiment (shown as predicted line in Fig. 2). Solutes were transported

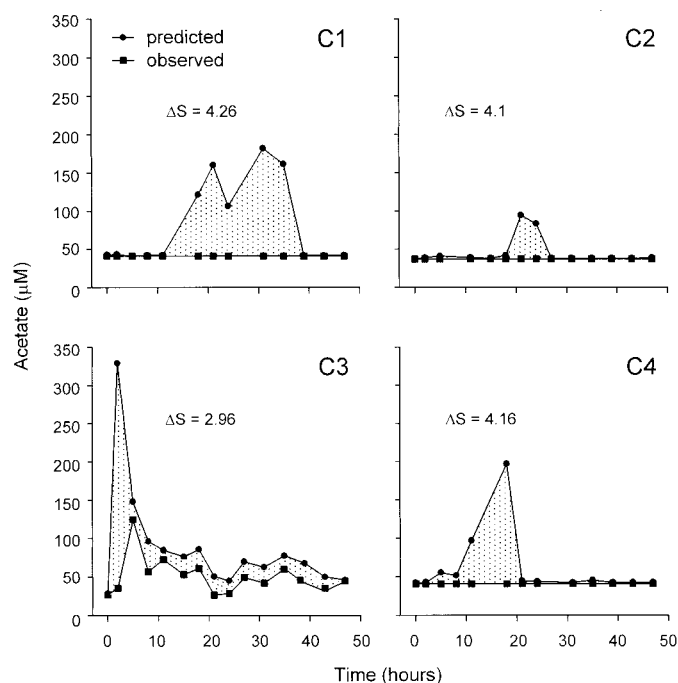


Fig. 2. Observed and predicted acetate concentration in wells with slow transport and 100% acetate retention (C1, C2, and C4) and with fast transport and 38% acetate retention (C3). Acetate retention is indicated by the shaded area. ΔS indicates the amount of acetate retained.

differentially to the four connected capture wells (Fig. 2). Three of the four wells showed increased Br^- levels 11–15 h following the initiation of the injection, with calculated pore velocities ranging from 0.031 to 0.096 cm min^{-1} (Fig. 2). One well (well C3) had more rapid transport (0.18 cm min^{-1}) and exhibited increased Br^- within 2 h following the start of the injection (Fig. 2). Complete acetate retention (100%) was indicated in the three wells (C1, C2, and C4) that had lower transport rates (Fig. 2), whereas the well with more rapid transport (C3) showed less acetate retention (ca. 38%, Fig. 2). Relative acetate retention was negatively correlated to pore velocity ($y = 4.32 - 41.7x^2$, $r^2 = 0.967$, $n = 4$, $p = 0.017$, Table 3).

Median acetate retention was determined as observed minus predicted concentration varied from 23 to 93 μM (Fig. 2). The difference between observed and predicted acetate concentration for each well was significantly <0 ($p \leq 0.05$).

Freundlich adsorption isotherm—The concentration of acetate remaining in solution following incubation with sediments was essentially equal (within analytical error) to the concentration measured before incubation. Regression of C vs. C^* was not statistically significant ($r^2 = 0.02$, $n = 5$, $p > 0.05$). The slope (K_d) of the line relating C to C^* was 0, indicating no net acetate sorption by the sediments.

Influence of acetate retention on microbial TEAPs—Acetate addition to the hyporheic zone at Rio Calaveras stimulated microbial metabolism, as evidenced by changes in dilution-corrected concentrations of solutes associated with

Table 3. Relative acetate retention, transport rate, and distance traveled during the injection.

Well	Distance (cm)	Transport rate (cm min^{-1})	Relative retention ($\mu\text{M acetate}/\mu\text{M bromide}$)
C1	50	0.0306	4.26
C2	50	0.039	4.1
C3	50	0.18	2.96
C4	100	0.18	4.16

microbial TEAPs (Fig. 3, $p < 0.05$). Following acetate addition, DO decreased between 8 and 28 μM (17–51%) below background in three wells (Fig. 3A). Nitrate concentration also decreased between 22 and 28 μM (93–100%) below background in three wells (Fig. 3B). In a single well, Fe^{2+} levels significantly increased 11 μM (52%) above background (Fig. 3C). Sulfate reduction was indicated in three wells, with SO_4^{2-} decreases ranging from 4 to 11 μM (10–24%) below background (Fig. 3D). Methane concentration increased by 1.5 and 31 μM (26 and 388%) above background in two wells (Fig. 3E). Observed minus predicted CO_2 concentration was >0 in three wells, with increases of 18–98 μM (6–30%) above background (Fig. 3F).

In two wells (C3 and C4), 100% of the retained acetate could be accounted for by mineralization to CO_2 (data not shown). Only 19% of the retained acetate could be accounted for by mineralization to CO_2 in well C1. In C2 and C4, wells with slow transport and 100% acetate retention, nitrate was the predominant terminal electron acceptor, accounting for 38 and 44% of acetate retention in each well, respectively (Fig. 4). Nitrate reduction also accounted for 19% of acetate retention in well C1 (Fig. 4). Sulfate reduction was an important TEAP in three of four wells, where it accounted for 4–44% of acetate retention (Fig. 4). Aerobic respiration accounted for 13–30% of acetate retention in three wells and was never the dominant metabolic process (Fig. 4). Methanogenesis accounted for the highest portion (33 and 6.5%) of acetate retention in wells C1 and C3. Iron reduction, indicated by Fe^{2+} production, was observed in only one well (C3) and accounted for 6% of acetate retention (Fig. 4).

The TEAPs stimulated by acetate addition did not appear to be spatially or temporally segregated (Fig. 4). The relative contribution of each TEAP to acetate retention did not change with distance from the injection well. Nitrate was generally the predominant terminal electron acceptor at both 50 and 100 cm from the injection well (Fig. 4). Similarly, the importance of the lower energy-yielding pathways, sulfate reduction and methanogenesis, did not increase with distance from the injection well (Fig. 4).

Discussion

Acetate retention in the hyporheic zone—Although acetate represented a substantial portion of total DOC, the hyporheic zone at Rio Calaveras had a high propensity for acetate retention. Nearly all of the injected acetate was consumed within the first 50 cm of transport. To our knowledge, this

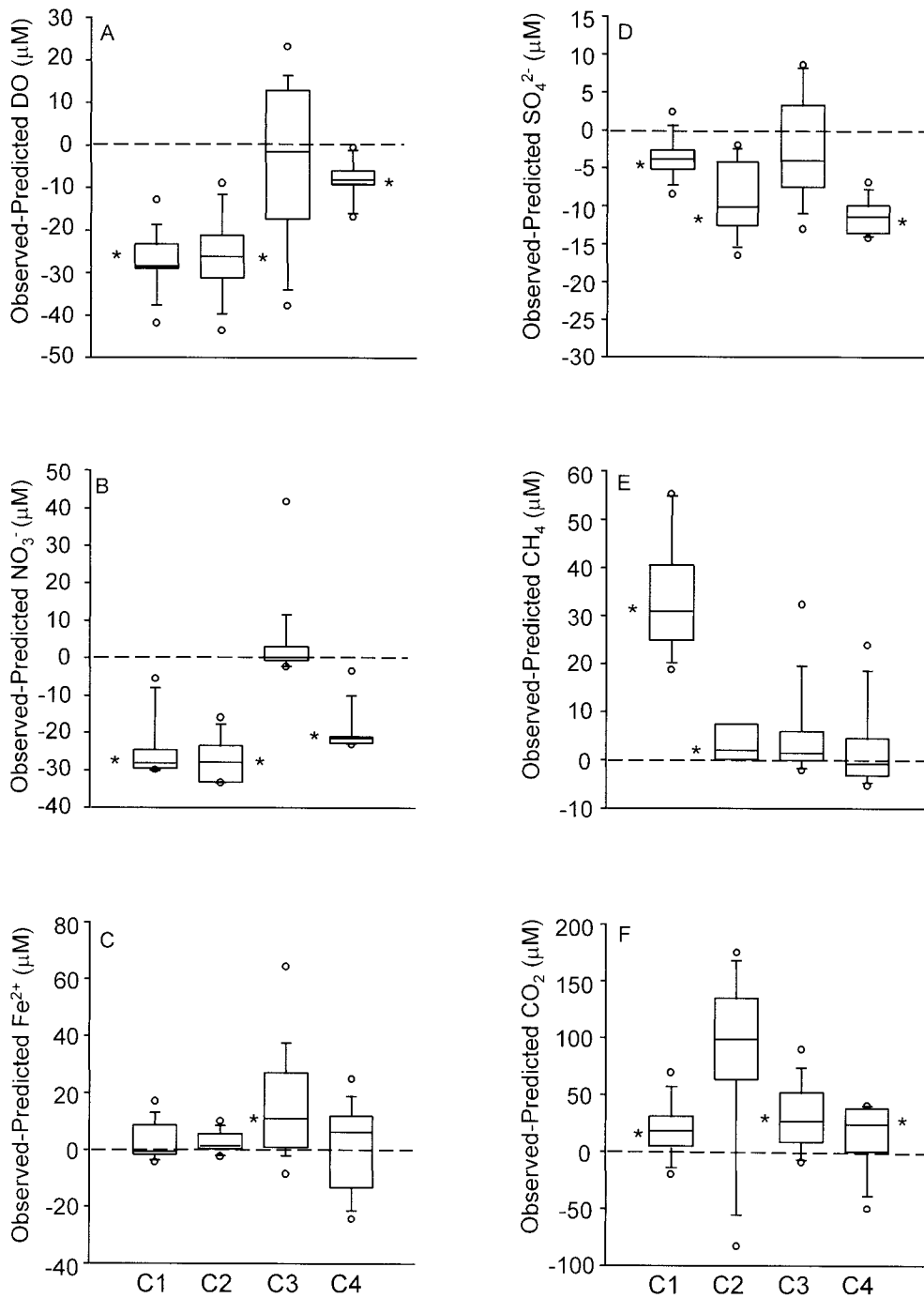


Fig. 3. Apparent changes in solutes affected by microbial TEAPs. Solutes that are affected by microbial metabolism include DO (A), nitrate (B), ferrous iron (C), sulfate (D), methane (E), and carbon dioxide (F). Boxes are drawn from the 25th to the 75th percentile. The horizontal line within each box is the median. Vertical lines extending above and below the box represent data within the 10th and 90th percentiles. Data lying outside this range are represented by open circles. Medians lying above zero (dashed line) indicate production, and medians lying below zero indicate consumption. Median differences between observed and predicted concentrations that are significantly different from zero ($p \leq 0.05$, Wilcoxon Sign test) are indicated by an asterisk.

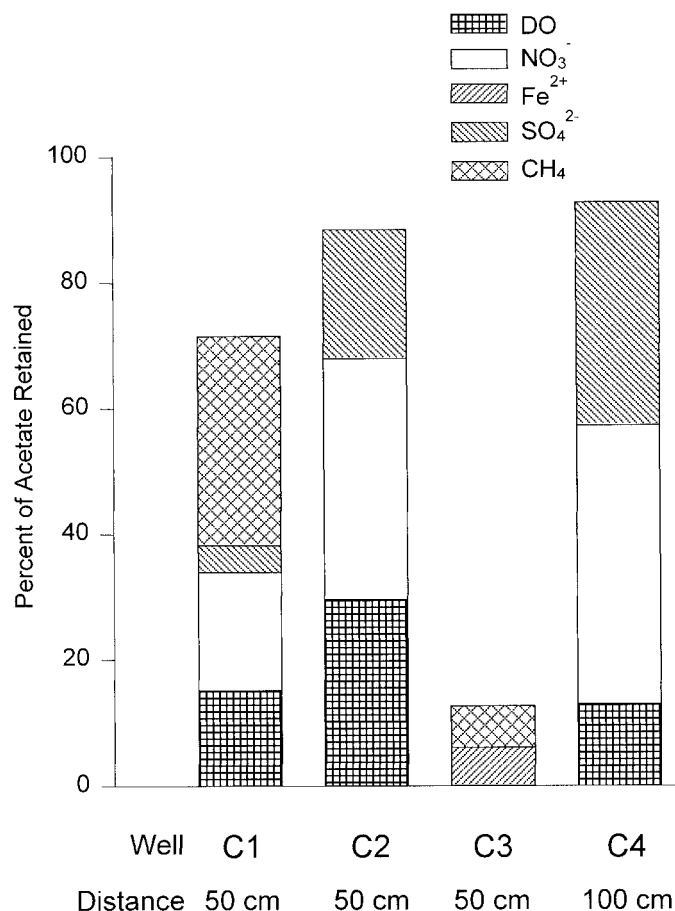


Fig. 4. Percent of acetate retention occurring via individual microbial TEAPs determined from the median change in concentration of solute indicative of TEAPs and stoichiometry presented in Table 1.

is the first study to report hyporheic zone DOC retention using direct injection techniques, but our results are similar to other studies using naturally occurring DOC gradients along hyporheic flowpaths. Comparing concentrations of stream water DOC to hyporheic water DOC, Findlay et al. (1993) and Findlay and Sobczak (1996) estimated that at least 50% of stream water DOC was retained in the hyporheic zone in the East Branch of Wappinger Creek, New York. Similar values of DOC retention (ca. 49–60%) were observed in gravel bars in the River Rhône, France (Claret et al. 1997). Rutherford and Hynes (1987) discussed two alternative roles for the groundwater–surface water interface in DOC retention in lotic ecosystems: DOC may be stripped as it is transported across the interface, or stream water DOC may be diluted by infiltration of DOC-poor groundwater. The two perspectives are not mutually exclusive, but in general, the interface between surface water and groundwater appears to be a DOC sink (e.g., Fiebig and Lock 1991; Vervier and Naiman 1992; Findlay et al. 1993; Vervier et al. 1993; Fiebig 1995), and our results are consistent with this trend.

Retention of DOC, including acetate, may occur via abiotic and biotic mechanisms. Abiotic sorption can play a significant role in DOC retention by stream sediments (e.g.,

Dahm 1981; McDowell 1985), but we did not observe acetate sorption by hyporheic sediments in our batch tests.

Material retention in lotic ecosystems is influenced by intensity of biotic processing and rates of transport (Elwood et al. 1983; Mulholland et al. 1985; Valett et al. 1996). This is true for DOC retention in the hyporheic zone as well (Findlay et al. 1993; Findlay and Sobczak 1996). Because solutes that move more slowly have longer contact time with biologically and chemically reactive sediments, flowpaths with longer residence time should exhibit more DOC retention. We found a negative relationship between pore velocity (travel time) and acetate retention, suggesting hydrological controls of retention.

Influence of acetate retention on microbial TEAPs—Changes in solute concentrations indicative of microbial metabolism (e.g., DO, NO₃⁻, Fe²⁺, SO₄²⁻, CH₄, and CO₂) show that respiration was an important factor in acetate retention in the hyporheic zone of Rio Calaveras. Assuming a closed system, acetate mineralization to CO₂ accounted for a minimum of 19–100% of total acetate retention.

Our observation that acetate addition stimulates respiration supports the hypothesis that microbial metabolism in the hyporheic zone is limited by labile DOC availability. Several studies have documented labile organic matter (dissolved and particulate) limitation of hyporheic zone metabolism. Jones (1995) showed that DOC and particulate organic matter (POM) addition stimulated respiration in sediments from the hyporheic zone at Sycamore Creek, Arizona. Other studies have correlated sediment POM content to hyporheic zone respiration (Pusch and Schwoerbel 1994; Fuss and Smock 1996). Our study has applied a field-scale approach (sensu Carpenter 1989) to show stimulation of hyporheic zone respiration by in situ organic matter addition.

Both aerobic and anaerobic respiration were stimulated by acetate addition to the hyporheic zone. DO decreased 13–30% below background; however, aerobic respiration, as indicated by decreased DO levels, contributed only a small percentage to overall acetate retention. Most acetate retention occurred via anaerobic pathways. In order of decreasing importance, the predominant anaerobic TEAPs appearing to contribute to acetate retention were denitrification, sulfate reduction, methanogenesis, and iron reduction.

In estimating the contribution of each TEAP to acetate oxidation, we assumed the hyporheic zone was a closed system and that TEAP-sensitive solutes were affected only by acetate oxidation according to stoichiometry given in Table 1. Thus, we can only estimate the minimum contribution of each TEAP to acetate mineralization. These assumptions are least likely to be met for dissolved gases (CO₂, DO, and CH₄) because (1) degassing could have occurred during pumping and sampling, (2) gases may be exchanged between the saturated and unsaturated zones, (3) CO₂ may be used chemoautotrophically in processes such as acetogenesis and methanogenesis, and (4) CH₄ may be oxidized microbially, forming biomass and CO₂. Degassing during sampling is probably the greatest error for CO₂ and CH₄. Concentrations of these gases in the hyporheic zone are much higher than those due to atmospheric equilibration, and bubble formation during sampling was inevitable. Diffusion of atmospheric

Table 4. DO ranges from several hyporheic zones in various climatic regions.

Study site reference	Substrate type	DO (μM) mean \pm SE or range
Rio Calaveras, New Mexico (this study)	Silty sand	54 ± 75
Sycamore Creek, Arizona (Valett et al. 1990)	Cobble, gravel, clay	31–250
River Glatt, Switzerland (von Gunten et al. 1991)	Gravel, sand, clay	16 ± 11
Wappanger Creek, New York (Findlay et al. 1993)	Fine sand to cobble	16 to 188
Rhône River, France (Claret et al. 1997)	Sand and gravel	63 to 280
Speed River, Ontario, Canada (Fraser and Williams 1998)	Gravel, silt, sand	38 ± 56 to 50 ± 97

oxygen into the hyporheic zone would not be a significant source of DO. Even assuming a DO concentration of 8 mg liter^{-1} (near atmospheric saturation) at the top of the water table, a maximum of $7 \mu\text{mol DO m}^{-2} \text{ d}^{-1}$ would diffuse the 0.5-m distance to the location of the well screen. More than $800 \mu\text{mol DO m}^{-3}$ was consumed during the 2-d experiment.

Denitrification may be overestimated if NO_3^- loss is due to assimilation and underestimated if NO_3^- is produced via nitrification. The influence of assimilation on nitrate retention in stream systems is poorly known. Hedin et al. (1998) estimated that 30–60% of nitrate retention in the surface water–groundwater interface was due to denitrification. Hyporheic zones can be important sites of nitrification (Holmes et al. 1994) if both DO and ammonium (NH_4^+) are available to nitrifying bacteria. In our study, nitrification may have occurred because DO was present, and $\text{NH}_4\text{-N}$ concentration averaged $5.0 \pm 2.6 \mu\text{M N}$.

Assuming that Fe^{2+} is not changed after it is produced may not be realistic. At circumneutral pH, Fe^{2+} can be rapidly oxidized abiotically or biotically if DO is present (Stumm and Morgan 1981; Emerson and Moyer 1997). Furthermore, Fe^{2+} can precipitate out of solution if sulfate reduction has produced sulfide levels that yield supersaturation relative to iron sulfide mineral solubility (Stumm and Morgan 1981). Using a geochemical speciation model (PHREEQC, Parkhurst 1995), we simulated decomposition of $100 \mu\text{M}$ acetate with initial water chemistry similar to that of the hyporheic zone at Rio Calaveras. According to the simulation, acetate oxidation results in Fe^{2+} and HS^- levels that exceed solubility (saturation indices ranging from 0.08 to 10) of iron sulfide minerals (FeS [ppt], pyrite [FeS_2], and mackinawite [FeS]). Given the amount of Fe^{2+} that we measured in the hyporheic zone, even small amounts of sulfide ($5 \times 10^{-12} - 1 \times 10^{-4} \mu\text{M HS}^-$) produced by sulfate reduction could cause iron sulfide precipitation. Based on this equilibrium calculation and our observation that sulfate reduction occurred (producing on the order of 4–11 $\mu\text{M HS}^-$), it is likely that our experimental injection resulted in iron sulfide precipitation, which led to an underestimation of the contribution of iron reduction (Fe^{2+} production) to acetate mineralization.

Finally, manganese reduction is a TEAP that we did not consider in this study and may account for some portion of acetate retention. In some wells, we were able to account for >90% of acetate retention via microbial TEAPs. This is within analytical errors associated with the solute analyses. Wells in which we were unable to account for all of acetate

retention via microbial TEAPs show that the closed-system assumptions described above were violated.

Contrary to our hypothesis, acetate addition stimulated a suite of microbial TEAPs that did not appear to be thermodynamically segregated. Nitrate was generally the predominant terminal electron acceptor. Similarly, the importance of the lower energy-yielding pathways, sulfate reduction and methanogenesis, did not increase with travel time or distance from the injection well. Our results are consistent with those of Morrice (1997), who did not observe distinct spatial segregation of TEAPs in the subsurface of Rio Calaveras at spatial scales similar to those used in our study. Together, these results do not support the findings of Hedin et al. (1998), who showed that microbial processes were longitudinally distributed along a thermodynamic gradient in control and DOC-fertilized flowpaths of riparian groundwater discharging into Smith Creek, Michigan. Unlike groundwater discharge zones, hyporheic zones may be in a state of thermodynamic disequilibrium due to greater spatial and temporal hydrologic variation.

Implications for stream ecosystem metabolism—Our observation that a suite of aerobic and anaerobic microbial TEAPs can cooccur in the hyporheic zone has important implications for measures of whole-stream metabolism. Several studies have shown that a majority (50–96%) of stream ecosystem respiration occurs in the hyporheic zone (e.g., Grimm and Fisher 1984; Fuss and Smock 1996; Mulholland et al. 1997; Nageli and Uehleringer 1997). These studies have relied on measurements of aerobic respiration (DO depletion).

Including total respiration in metabolic studies of stream ecosystems may alter perspectives of lotic ecosystem energetics. If our estimate that aerobic respiration accounts for only a small portion of acetate retention is similar for other organic substrates, whole-stream respiration based on measures of changes in DO concentration could substantially underestimate total respiration. Subsurface hypoxia and anoxia are common at Rio Calaveras during base flow (Baker et al. 1994, 1999; Valett et al. 1996, 1997), and it is unlikely that anaerobic respiration occurred as an artifact of excessive organic matter loading during our experiment. If acetate had been transported conservatively to a given capture well, a maximum acetate concentration of $329 \mu\text{M}$ ($7.9 \text{ mg C liter}^{-1}$) would have been observed, based on bromide response. This is within the seasonal range of DOC concentrations observed in the hyporheic zone at Rio Calaveras.

Furthermore, DO levels at the location of our experiment are similar to other regions of the hyporheic zone (Valett et al. 1996), where solutes indicative of microbial TEAPs (e.g., Fe^{2+}) are observed along flowpaths (Baker et al. 1999). Our results suggest that anaerobic processes dominate heterotrophic metabolism in the hyporheic zone at Rio Calaveras under summer base flow conditions.

Anaerobic metabolism is likely to occur in many hyporheic systems. A number of studies in desert and temperate streams have measured levels of hyporheic DO that are similar to those at Rio Calaveras (Table 4). Sediment particle size is one parameter that will strongly influence the ecosystem level importance of anaerobic metabolism. The residence time of stream water in the hyporheic zone and the spatial extent of surface water-groundwater interaction is controlled by sediment properties (e.g., Findlay 1995; Morrice et al. 1997), and sediment porosity controls the rate of oxygen transport (e.g., Dodds et al. 1996). Anaerobic metabolism likely occurs in microsites or patches of fine-grained sediments (e.g., Vervier et al. 1993; Holmes et al. 1994), where transport rates are slow, and there are often high levels of organic matter and a greater density of microorganisms (Jones 1995). The significance of hyporheic anaerobic metabolism to whole-stream metabolism will depend on how these patches are hydrologically linked within the system.

The influence of the hyporheic zone on stream ecosystem energetics is controlled by hyporheic zone size, subsurface residence time, and labile organic matter availability. Our results have shown that biological processes responsible for DOC retention in the hyporheic zone at Rio Calaveras are mostly anaerobic, and LMW organic acids like acetate may be key compounds involved in microbial metabolism in this environment.

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