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Heterotrophic Activity and Biodegradation of Labile and Refractory Compounds by Groundwater and Stream Microbial Populations

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The bacteriology and heterotrophic activity of a stream and of nearby groundwater in Marmot Basin, Alberta, Canada, were studied. Acidine orange direct counts indicated that bacterial populations in the groundwater were greater than in the stream. Bacteria that were isolated from the groundwater were similar to species associated with soils. Utilization of labile dissolved organic material as measured by the heterotrophic potential technique with glutamic acid, phenylalanine, and glycolic acid as substrates was generally greater in the groundwater. In addition, specific activity indices for the populations suggested greater metabolic activity per bacterium in the groundwater. 14C-labeled lignocellulose, preferentially labeled in the lignin fraction by feeding Picea engelmannii [14C]phenylalanine, was mineralized by microorganisms in both the groundwater and the stream, but no more than 4% of the added radioactivity was lost as 14CO2 within 960 h. Up to 20% of [3′-14C]cinnamic acid was mineralized by microorganisms in both environments within 500 h. Both microbial populations appear to influence the levels of labile and recalcitrant dissolved organic material in mountain streams.

The microbial utilization of dissolved organic matter (DOM) is an important component of the total carbon flux in aquatic ecosystems. The uptake kinetics of various compounds by microbial populations in flowing waters has been estimated by using radioactively labeled substrates (1, 2, 33). There are very few data available, however, on heterotrophic activity in mountain streams and the groundwater associated with stream flow (20, 24).

In Marmot Basin near Calgary, Alberta, Canada, the groundwater in the saturated zone contains an average concentration of 5.9 mg of DOM liter$^{-1}$ (39), and stream waters in the area contain 4.0 mg of carbon liter$^{-1}$ (35). Of the compounds identified, the recalcitrant humic and fulvic acids, tannins, and lignins were the most abundant, followed by carbohydrates, amino acids, phenolic compounds, fatty acids, and hydrocarbons. The DOM of the Marmot Basin system is therefore composed of both labile and refractory carbon compounds (39, 40).

This paper reports the heterotrophic activity of planktonic microbial populations in the groundwater and an adjacent stream in Marmot Basin. The heterotrophic potential technique used to measure the utilization of labile components was modified to assure that all experimentation was done immediately after sample collection. In addition, the utilization of refractory compounds was investigated. As these data were not amenable to a short-term kinetic analysis, they are reported here as long-term biodegradation rates.

We also report the first studies of the biodegradation of 14C-labeled lignin by groundwater and stream bacteria in a subalpine woodland ecosystem.

MATERIALS AND METHODS

Study area. The area chosen for study was Marmot Basin (Canadian topographic series map 82 J/14, 115°10' W, 50°57' N) in a subalpine forest region occupying an area of 9.2 km$^2$. The soil types, geology, and vegetation of the region have been discussed by Telang et al. (34, 35) and Wallis et al. (39).

Groundwater installations. The basin was instrumented with 13 piezometers in 1976 and 1977, 3 piezometers in 1978, and 1 piezometer in 1979. Piezometers constructed of 4.1-cm-bore polyvinyl chloride pipe and modified with a central glass column for aseptic bacteriological sampling were used to collect water from the saturated groundwater zone (38). Due to the underlying layer of bedrock, the maximum depth of the installations was 1.5 m. Activity and bacterial counts were measured from six different piezometers in the same sampling quadrant during this study.

Sampling procedure. Samples for bacterial enumeration were collected 1 month after the initial installation of the piezometers. Groundwater was sampled by evacuating the central glass column and collecting the
water in a sterile 500-ml Erlenmeyer flask fitted with 10 18-gauge needles which served as collection and evacuation ports (38).

Groundwater samples used in determining dissolved oxygen levels were collected by evaporation into 60-ml stoppered serum vials in which the air had been displaced with N2 gas. Dissolved oxygen determinations were made on the second and third 60-ml samples by methods previously described (4).

Stream samples were collected at a single site in a sterile 1-liter glass bottle for bacterial counts and uptake analysis and in a sterile 60-ml serum vial flushed with N2 for dissolved oxygen determinations.

**Bacteriology.** Bacteria were enumerated primarily by epifluorescence microscopy. Two 10-ml samples of groundwater or stream water were filtered through 0.2-μm Nuclepore membranes and fixed in an atmosphere of 0.5% glutaraldehyde as described by Geese et al. (16). Back in the laboratory, the cells were stained with acridine orange according to the procedure outlined by Zimmermann and Meyer-Reil (46). The bacteria on the filters were observed under a Zeiss standard microscope (magnification, ×1,000) with epifluorescence illumination from an HBO 50-W mercury arc burner (filter combination: excitation, LP 490, LP 455; beam splitter, LP 510; barrier filter, LP 520). Bacterial counts were calculated from the mean number of fluorescing cells in 10 to 20 different grid areas of 0.0025 mm2 each. Counts which showed a negative binomial distribution were transformed using log10 (x + 1) (14).

In addition to the acridine orange direct counts (AODC), colony-forming units were enumerated by a spread-plating technique on agar plates containing 0.01 and 0.2% brain heart infusion (Difco Laboratories, Detroit, Mich.). All plating was done in a mobile laboratory in the field. Replicate plates were incubated aerobically at 10 and 23°C. Isolated colonies were picked and restreaked twice on 0.2% brain heart infusion agar. Gram-positive isolates were characterized from the results of the catalase, glucose utilization, amylase, and nitrate reduction biochemical tests. Gram-negative isolates were characterized with a Corning/NF kit (Corning Glass Works, Corning, N.Y.).

**Uptake of labeled substrates.** Kinetic analyses were performed by the method of Parsons and Stickland (30), as refined by Wright and Hobbie (43) and Hobbie and Crawford (22), in which both 14CO2 evolution and 14C assimilation are measured. 1-[(U-14C)glutamic acid (New England Nuclear Corp., Boston, Mass.; specific activity, 296 μCi/mmol), 1-[(U-14C)phenylalanine (Amersham Corp., Arlington Heights, Ill.; specific activity, 531 mCi/mmole], and [1-14C]glucolic acid (Amersham; specific activity, 8.4 mCi/mmol) were used as substrates. Three replicate samples and a control were incubated in the dark at each of five or six substrate concentrations, ranging from 0.16 to 16.0 μg liter−1, at 4°C for 8 to 12 h. Assimilated uptake reactions were terminated by filtration onto a 0.2-μm Nuclepore filter under a light vacuum (<100 mmHg [13,330 Pa]), and respired uptake was measured in three parallel vials at each concentration (after acidification with 0.2 ml of 2 N H2SO4) by collecting the 14CO2 in a center well containing a fluted-filter vial soaked with 0.2 ml of phenethylamine (J. T. Baker Chemical Co., Phillipsburg, N.J.). The evolved 14CO2 was collected over a period of 3 h at an efficiency of 96% as determined by using NaH13CO3. Air-dried Nuclepore filters and the saturated wicks were transferred to 12 ml of Omnifluor in toluene (New England Nuclear Corp.) and counted with a Packard Tri-Carb scintillation counter. Radioactive counts were corrected for quenching by the channels ratio method, for machine efficiency, and for nonspecific binding in acid-killed or 5% HgCl2-killed controls.

All water samples were processed and incubated at in situ temperatures in the field of foil-lined coolers modified to exclude light and allow continual water circulation into and out of the incubation chamber. Stream flow through the chamber gently agitated the vials placed in a partially submerged rack within the cooler. The number of uptake analyses run on the groundwater samples were restricted by inadequate piezometer flow and slow recharging time. Collection of water for dissolved oxygen measurements precluded performing a second uptake experiment from the same piezometer until several days had passed.

To obtain the specific activity index (SAI) expressed as 10−2 μg cell−1 h−1, the Vmax was divided by the AODC (11).

**Preparation and analysis of labeled lignocellulose.** Labeled lignocellulose was prepared essentially by the method of Crawford and Crawford (8, 11) and Crawford et al. (9, 10, 13). The lignin component of lignocellulose from *Picea engelmannii* (Krumholz spruce) was selectively labeled by feeding plants 1-[1-14C]phenylalanine (50 μCi ml−1) through their cut stems. Samples were incubated for 48 to 72 h at room temperature in a lighted fume hood. Care was taken that the buffered solution of filter-sterilized stream water and phenylalanine did not become dry. After the incubation period, the cambial wood was removed, dried for 12 h at 60°C, and cut into 5-mm pieces. The wood was then heated with a series of extractions as outlined by Crawford and Crawford (12). The resultant material was dried overnight at 60°C, ground into a coarse powder with a mortar and pestle, and autoclaved. Specific activities of the preparations were determined by oxidizing triplicate 10-μg samples in a Packard 306 Autooxidizer. The distribution of 14C in the lignocellulose was analyzed by a modified Klasen procedure (12). Table 1 shows the specific activity and distribution of 14C between the acid-soluble and acid-insoluble fractions of replicate Klasen-extracted lignocelluloses.

Radioactivity of the acid-soluble material was determined by placing samples of acid extract into Formula 947 (New England Nuclear Corp.). Radioactivity of the acid-insoluble fraction was determined by digestion in Protosol tissue solubilizer (New England Nuclear Corp.), by direct oxidation, or by both methods. Between 52.4 and 68.7% of the total radioactivity was located in the acid-insoluble (lignin) fraction, indicating that [14C]phenylalanine preferentially labels the lignin fraction of the lignocellulose molecule (9). Crawford et al. (13) have suggested that degradation studies in which plants labeled with phenylalanine are used may introduce an error due to the breakdown of phenylalanine-labeled protein compounds in the wood. Pronase (0.1 mg ml−1) digestion steps have been incorporated into the extraction procedure of recently labeled *Picea* samples. The specific activities of enzyme-treated and control samples were within 2 to 3% of one another, and duplicate uptake experiments with the pronase-treated and control extracted
VOL above procedure (data not shown). Protein degradation was free air, by titration to 4 to 14 with a degradation. Wood sugars in the supernatant obtained by the above procedure were separated after neutralization with a saturated barium hydroxide solution by descending paper chromatography with a butanol-pyridine-water (10:3:3, vol/vol/vol) solvent system (12). The supernatant was concentrated by rotary evaporation to 4 to 14 ml before spotting 0.1 ml onto the chromatography paper. Chromatography samples were run in duplicate for 24 to 30 h with glucose, mannose, xylose, arabinose, and galactose as standards. One set of the air-dried chromatographic strips was developed in AgNO₃ as described by Trerelyan et al. (36), and the other set was cut into strips for scintillation counting without the development procedure. Radioactivity in the wood sugars from P. engelmannii appeared to be equally distributed among glucose, mannose, xylose, and three unidentified wood sugars. Only 0.58% of the radioactivity added to the paper was recovered in the six sugars.

Biodegradation of ¹⁴C-labeled lignocellulose in the groundwater and stream. The rate of biodegradation of the lignocellulose was determined by adding 5 to 10 mg of the ¹⁴C-labeled lignocellulose at a specific activity of 4.2 × 10⁴ dpm mg⁻¹ to 60-ml sterile serum vials containing 10 ml of undiluted groundwater or stream water. Duplicate samples from each date were incubated at 4°C for 6 weeks. The evolution of ¹⁴CO₂ was followed by two methods. (i) The serum vials were continuously flushed with sterile, humidified, CO₂-free air, and the ¹⁴CO₂ was collected in percolation towers containing glass beads and 1.0 ml of ethanolamine (Eastman Organic Chemicals, Rochester, N.Y.). Carbon dioxide was removed from the air stream by passing the air through concentrated NaOH, and the air was humidified by bubbling through water. An alternating series of prefilters (Bio-Rad Laboratories, Richmond, Calif.) and 0.45-µm Nuclepore filters (three sets) was placed after an air trap to sterilize the air. The trapped ¹⁴CO₂ was washed from the percolation towers by using three 1-ml portions of methanol to remove the ethanolamine. (ii) The samples were incubated for up to 10 weeks in sealed serum bottles, and the ¹⁴CO₂ evolution was measured by stopping the reaction of individual replicates during weekly intervals with the addition of 0.5 ml of 5 N H₂SO₄. The ¹⁴CO₂ was flushed from the serum vials with a closed-loop circulation system (45) and was then collected in 10 ml of ethanolamine-methanol (1:3, vol/vol). Flushing was completed in 6 ml with a 95% collection efficiency. The methods gave comparable results for up to 10 weeks; however, the latter method required less bench space, glassware, and equipment, and it increased the number of samples and replicates which could be run simultaneously.

Trapped ¹⁴CO₂ was counted by liquid scintillation spectrometry in Omniflour, and data were corrected for counting efficiency and for acid-killed controls as described above. Biodegradation was recorded as the percentage of total radioactivity recovered as ¹⁴CO₂.

Biodegradation of cinnamic acid. [Side chain-³⁴C]Cinnamic acid (Amersham; specific activity, 55 mCi/mmol), redissolved in ethanol after removing the benzene by evaporation, was adjusted to the original concentration and added to vials containing 10 ml of undiluted stream water or groundwater (final concentration, 50 µg liter⁻¹). All samples were incubated for 3 weeks at 4°C under aerobic conditions. Twice each week, replicate samples were terminated by injection of 0.2 ml of 5 N H₂SO₄ and flushed with the closed-loop circulation system described above.

Enumeration of lignin-degrading microorganisms. A radiorepimetric most-probable-number technique (5, 25) was used to enumerate microorganisms able to degrade lignocellulose. Dilutions of groundwater and stream water were added to 60-ml sterile serum vials containing 9 ml of Bushnell-Haas medium (45) and 5 to 10 mg of the ¹³C-labeled lignocellulose. Three- tube most-probable-number duplicates were incubated for 8 weeks at 4°C under aerobic conditions. At the end of the incubation period, the reaction was terminated with acid and flushed to collect ¹⁴CO₂, which was counted by liquid scintillation counting. Samples that gave counts twice as great as those from the killed controls were scored as positive, and the number of lignin or hydrocarbon degraders was determined from the appropriate most-probable-number tables.

Additional analysis. Amino acid analysis of the groundwater and stream water was performed by gas-liquid chromatography by the method of Roach and Gehrke (31), and the dissolved organic carbon was determined with a Beckman 915 carbon analyzer at the Kananaskis Centre for Environmental Research at the University of Calgary.

### RESULTS

**Bacteriology.** Bacterial concentrations in the groundwater and stream as determined by epifluorescence microscopy (AODC) are shown in Fig. 1. The population density was generally one log higher in the groundwater, and fluctuations in cell density with time were also greater in the groundwater. In both 1977 and 1978, the population in the groundwater increased one order of magnitude after complete thawing of the surrounding soil. It is interesting to note that the soil was still frozen during the June 1978 sampling period. The increase observed in the groundwater population in September 1979 oc-

<table>
<thead>
<tr>
<th>Fine lignocellulose sample</th>
<th>Sp act of ¹⁴C (dpm/mg)</th>
<th>Acid insoluble ¹⁴C (%)</th>
<th>Acid soluble ¹⁴C (%)</th>
<th>Total recovered* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.782</td>
<td>63.1</td>
<td>22.0</td>
<td>85.1</td>
</tr>
<tr>
<td>B</td>
<td>4.792</td>
<td>59.3</td>
<td>13.5</td>
<td>72.8</td>
</tr>
<tr>
<td>C</td>
<td>3.826</td>
<td>52.4</td>
<td>21.0</td>
<td>73.4</td>
</tr>
<tr>
<td>D</td>
<td>4.213</td>
<td>68.7</td>
<td>13.2</td>
<td>81.9</td>
</tr>
</tbody>
</table>

* (Total acid-soluble and acid-insoluble disintegrations per minute divided by total disintegrations per minute of lignocellulose) × 100.
curred during a period of more rapid leaf fall, gentle rains, and increased stream flow (T. I. Ladd, personal observation). Enumeration of bacteria by the plate count methods followed similar trends. However, the counts calculated from colony-forming units which developed on either medium were always two to three logs lower than the AODC (data not shown); thus, they were used primarily to obtain isolates.

Five gram-negative and four gram-positive species could be isolated from the groundwater on either diluted brain heart infusion medium (Table 2). Approximately 40% of the colonies picked from the original isolation plates were gram-positive bacteria. All the isolates except the Flavobacterium sp. grew at 23 °C. Bacillus subtilis and Bacillus licheniformis grew under anaerobic conditions; however, no strict anaerobes were isolated. Dissolved oxygen levels in the shallow groundwater samples ranged from 0.8 to 3.9 mg liter⁻¹, with a mean value of 2.3 mg liter⁻¹ (n = 15), and were within the limits reported by Baker et al. (6) for deep groundwater samples.

Kinetic analyses. Kinetic analyses for glutamate uptake for July 1978 by the groundwater bacteria gave a $V_{max}$ of 114.7 ng liter⁻¹ h⁻¹, with a turnover time of 22.74 h. The $V_{max}$ and turnover time for stream bacteria were 1.5 ng liter⁻¹ h⁻¹ and 250.04 h, respectively (Table 3). When the natural substrate level of glutamate in the groundwater was determined (0.28 µg liter⁻¹), the real velocity of uptake ($V_r$ = substrate level divided by turnover time) for glutamate was 12.31 ng liter⁻¹ h⁻¹. The $V_r$ value for bacteria in the stream (substrate level, 0.28 µg liter⁻¹) was 0.8 ng liter⁻¹ h⁻¹.

Additional $V_{max}$ and SAI data for glutamate, phenylalanine, and glycolic acid are summarized in Table 3. With the exception of the June 1977 sample, the $V_{max}$ and SAI values for the three substrates were greater for the groundwater bacterial populations than for the stream plankton. Generally, the glutamate $V_{max}$ for the stream population was less variable (Table 3). In addition, the turnover time for the three substrates was always lower in the groundwater.

Although the most rapid uptake and turnover of glutamate occurred in the groundwater in July 1977, the SAI was only 1.2 times greater than that of any other groundwater sample. The general trend for the SAI data is a higher variation in the index for the stream bacteria, the opposite of the trend seen for the $V_{max}$ data. After a late complete thawing at the sampling site in July 1978, the SAI in the groundwater was 974.7, the highest noted in this study.

When the rates of uptake for the three substrates were compared in both the stream water and the groundwater samples from the August 1978 sampling period, glycolic acid appeared to be utilized more rapidly than the two amino acids. The apparent difference of two orders of magnitude in the rate of uptake for glycolic acid between the groundwater and the stream is reduced to only a twofold difference when one compares the SAI data (Table 3). In these experiments, the magnitude of the differences in activity between the groundwater and the stream water was reduced for each substrate when activity per cell was considered.

Biodegradation of 14C-labeled lignocellulose and [14C]cinnamic acid. Figure 2 shows the rates of mineralization of [14C]cinnamic acid and 14C-lignin-labeled lignocellulose by microflora from groundwater and the stream. It is evident that the stream and groundwater microflora showed comparable rates of removal of the lignocellulose and cinnamic acid. Cinnamic acid mineralization, however, was approximately eight times greater than lignin degradation over the same period of time.

### TABLE 2. Characterization of bacterial isolates from groundwater in Marmot Basin

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Growth at 10°C</th>
<th>Growth at 23°C</th>
<th>Anaerobic growth at 23°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aeromonas sp.</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Pseudomonas diminutia</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>P. fluorescens and</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. putida</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Flavobacterium sp.</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Acinetobacter lwaffii</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Gram positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. cereus</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>B. licheniformis</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. megaterium</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*Incubation period, 2 weeks.

Incubation period, 48 to 72 h.
TABLE 3. Kinetic analysis for glutamic acid, phenylalanine, and glycolic acid utilization by bacterial populations in the groundwater and a stream in Marmot Basin

<table>
<thead>
<tr>
<th>Sample site and date</th>
<th>Substrate</th>
<th>Cell no. (×10^6 ml^-1)</th>
<th>V_max (ng liter^-1 h^-1)</th>
<th>Turnover time (h)</th>
<th>SAI (10^-12 µg cell^-1 h^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marmot Creek</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/77</td>
<td>Glutamic acid</td>
<td>0.065</td>
<td>50.00</td>
<td>198.00</td>
<td>769.00</td>
</tr>
<tr>
<td>7/77</td>
<td>Glutamic acid</td>
<td>0.042</td>
<td>4.42</td>
<td>426.70</td>
<td>105.00</td>
</tr>
<tr>
<td>7/78</td>
<td>Glutamic acid</td>
<td>0.059</td>
<td>1.15</td>
<td>250.00</td>
<td>19.50</td>
</tr>
<tr>
<td>8/78</td>
<td>Glutamic acid</td>
<td>0.043</td>
<td>2.20</td>
<td>228.00</td>
<td>51.00</td>
</tr>
<tr>
<td>8/78</td>
<td>Phenylalanine</td>
<td>0.049</td>
<td>1.63</td>
<td>551.00</td>
<td>33.27</td>
</tr>
<tr>
<td>8/78</td>
<td>Glycolate</td>
<td>0.049</td>
<td>8.07</td>
<td>2,523.72</td>
<td>164.70</td>
</tr>
<tr>
<td>Groundwater</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/77</td>
<td>Glutamic acid</td>
<td>0.210</td>
<td>99.60</td>
<td>138.00</td>
<td>474.20</td>
</tr>
<tr>
<td>7/77</td>
<td>Glutamic acid</td>
<td>1.990</td>
<td>1,129.18</td>
<td>7.34</td>
<td>567.00</td>
</tr>
<tr>
<td>7/78</td>
<td>Glutamic acid</td>
<td>0.158</td>
<td>154.00</td>
<td>62.15</td>
<td>974.70</td>
</tr>
<tr>
<td>8/78</td>
<td>Phenylalanine</td>
<td>0.135</td>
<td>114.70</td>
<td>22.74</td>
<td>850.00</td>
</tr>
<tr>
<td>8/78</td>
<td>Glycolate</td>
<td>2.83</td>
<td>368.00</td>
<td>5.14</td>
<td>130.00</td>
</tr>
<tr>
<td>8/78</td>
<td>Glycolate</td>
<td>2.83</td>
<td>608.35</td>
<td>155.06</td>
<td>214.96</td>
</tr>
</tbody>
</table>

Each sample represents a triplicate analysis at six concentrations at a single sampling site.

Correlation coefficient for regression analysis of kinetic data.

A new piezometer installed in the sampling quadrant was used each successive year.

Enumeration of microorganisms capable of degrading lignocellulose. The most probable numbers of lignocellulose degraders were 45, 75, and 0 ml^-1 for three piezometers analyzed in August 1980. The average for stream water was 45 ml^-1 during the same period.

DISCUSSION

The gram-negative bacterial isolates characterized in the groundwater were similar to those reported for the stream in Marmot Basin by Ladd et al. (24) but showed less diversity than the gram-negative bacteria isolated from the water column in Canmore Creek, a small, subalpine woodland stream (T. I. Ladd, unpublished data). Although few gram-positive bacteria were isolated from the Marmot Basin stream, the relative distribution of gram-positive and gram-negative isolates in the groundwater was similar to that seen in the Canmore Creek system. Overall, the bacteria isolated from the groundwater were similar to those commonly associated with soils (3).

Although the AODC in the stream varied very little, fluctuations were evident in the groundwater. These fluctuations appeared to occur after events that could alter the moisture content of the soil (thawing, rainfall, reduced evapotranspiration); however, hydrological data supporting changes in moisture infiltration or groundwater flow in Marmot Basin after these events are lacking. The greater number of cells in the subsurface water undoubtedly reflected the intimate association with the soil and may also have been related to higher levels of DOM in the groundwater (5.9 mg liter^-1) than in the stream (2.1 mg liter^-1).

The V_max of uptake for glutamic acid by the stream bacteria was generally lower than that reported by Crawford et al. (7) for estuarine microorganisms and by Griffiths et al. (19) for Beaufort sea samples. It was, however, within the limits of values for glutamic acid in an oligotrophic mountain stream (24) and for glucose within the Essex estuary (46) and in three

![FIG. 2. Mineralization of 14C-lignin-labeled lignocellulose from P. engelmannii and (side chain-3-14C]cinnamic acid by groundwater and stream microbial populations, August 1980. Symbols: O, 14C-labeled lignocellulose from groundwater; ●, 14C-labeled lignocellulose from stream; △, [14C]cinnamic acid from groundwater; ▲, [14C]cinnamic acid from stream. Each point represents the mean of at least two replicates.](http://aem.asm.org/Downloaded)
New Zealand rivers (33). The \( V_r \) of glutamate in the stream at the natural substrate level agreed well with that reported by Ladd et al. (24) and was approximately 53% of the \( V_{\text{max}} \) value, indicating that the \( V_{\text{max}} \) value was a reasonable estimate of actual activity.

Even though the estimated potential uptake for glutamic acid by the groundwater bacteria was greater than that for the stream bacteria, the \( V_{\text{max}} \) values for the groundwater bacteria were frequently less than those reported for glutamate utilization by estuarine (7) and freshwater microorganisms (41), but greater than the \( V_{\text{max}} \) values of Griffiths et al. (19). Nevertheless, in Marmot Basin the \( V_{\text{max}} \) data and turnover times indicated a faster rate of flux for glutamate in the groundwater. In addition, the \( V_r \) value for the groundwater bacteria was greater than that for Marmot Creek or the previously reported \( V_r \) for Luscar Creek (24). The \( V_r \) value was only 11% of the calculated \( V_{\text{max}} \), however, indicating that the \( V_{\text{max}} \) value may overestimate the utilization of glutamate. However, even if the \( V_r \) values were 1% of the calculated \( V_{\text{max}} \), the groundwater microflora would be more active.

Although the apparent differences in the rate of uptake undoubtedly reflect differences in the total numbers of cells in both ecosystems, the variations in rates may indicate changes in the number of actively metabolizing cells (23) or be due to apparent fluctuations in bacterial activity in the presence of changing levels of DOM in the aquatic ecosystem (1, 2, 17). The positive correlation \( (r = 0.977) \) between the \( V_{\text{max}} \) for glutamate and the total epifluorescence count in the groundwater suggests that a constant proportion of active bacteria was using the substrate; i.e., the percentage of active cells in the groundwater did not appear to vary greatly. This was even more evident in the glutamate SAI of the groundwater microflora, which showed no more than a twofold difference between samples. Direct experimental results demonstrating the number of active bacteria in the groundwater are lacking. However, seasonal variations in the percentage of active bacteria, as determined by nalidixic acid sensitivity, formazan crystal deposition, and radioreispirometric techniques, in the Marmot Basin stream are not significant (Ladd, unpublished data). These results indicate that a change in the percentage of actively metabolizing bacteria is not the only mechanism which may function in controlling the apparent rate of carbon utilization in lotic ecosystems.

Wright (42) and Goulde (18) have recently demonstrated that the specific activity per bacterium may vary as much as 32-fold between aquatic sampling sites without significant changes in the total count. This large variation suggested to the authors that there was an increase in the activity per bacterium and not simply an increase in the percentage of active cells in the ecosystem. During the present investigation, the large variation in the activity per bacterium in the stream tended to support this contention. The reason(s) for an apparent change in activity per bacterium is not clear. Wallis et al. (39) reported that the DOM level in Marmot Creek remained nearly constant between May and July 1977. Levels of specific organic compounds in Marmot Creek, however, including carbohydrates and total amino acids, were reported to have reached peaks in June of that year, with carbohydrates showing another peak in August and September (35). Interestingly, the SAI for the stream bacteria declined after the June 1977 carbohydrate and total amino acid peaks and increased during the August 1978 carbohydrate peak. Whether the fluctuations in these compounds played an important role in controlling the activity of the bacteria in Marmot Creek is not known. Multivariant regression analysis of the SAI versus numerous organic parameters measured in the Red Deer River has shown no significant correlations (6).

Glutamate, of course, is not the only available labile organic substrate and cannot be taken as representative of the overall heterotrophic activity of the groundwater or stream bacteria. For this reason, two additional substrates were used to measure the kinetic uptake of the two bacterial populations. The value for \( V_{\text{max}} \) in the groundwater for phenylalanine was greater than that in the stream and was as high as values reported for winter estuarine samples (7) and samples from Lake Ontario (41). The rate of uptake of glycolic acid by groundwater bacteria was more rapid than in the stream and was greater than the summer values reported in the Gulf of Maine, but less than the \( V_{\text{max}} \) reported for the Essex River estuary (44). The rates of uptake of both phenylalanine and glycolic acid in the stream were comparable to the lower values previously reported (7, 44). The results indicate that the bacteria in both ecosystems actively transport and metabolize additional organic compounds, including a more stable amino acid such as phenylalanine (35).

Clearly, the removal of labile substrates from the water column can only account for a small portion of the dissolved organic carbon flux. Wallis et al. (39) have demonstrated that 88.0% of the total organic matter identified in the groundwater in Marmot Basin is composed of refractory compounds, primarily humic and fulvic acids, tannins, and lignins. Streams in Marmot Basin have been reported to contain 4 mg of carbon liter\(^{-1}\) only 2% of which was identified as labile compounds (35). Thus, any significant decreases in the level of total organic matter
would require degradation of the refractory components in the aquatic ecosystems.

Lignocelluloses are very complex polycyclic aromatic compounds of plant origin. Since much of the allochthonous input in streams and groundwater is vegetation, the utilization of these recalcitrant components was of great interest. The mineralization of the 14C-lignin-labeled lignocellulose by the microflora of the two aquatic systems was very slow, the maximum value noted being 4% after 40 days. The rate of degradation of the Picea lignin, underestimated since assimilated uptake and lignin incorporation into fulvic and humic material were ignored, showed that the microorganisms in the two aquatic ecosystems mineralize lignin. It is not known whether the rates are rapid enough to contribute to the seasonal fluctuations in the levels of lignin reported in Marmot Creek (35) or in the groundwater (39). Changes in the levels of tannins and lignins may simply reflect the rate of leaching of newly deposited wood in the stream or forest floor.

These data appear to be significantly lower than data reported for lignin utilization in other aquatic ecosystems. When data reported by Federle and Vestal (15) are extrapolated to 40 days of incubation time, 8.5% of pine and 32.4% of Carex 14C-lignin-labeled lignocellulose were mineralized by arctic lake sediments. Mineralization of oak and maple 14C-lignin-labeled lignocellulose reported for other lotic environments was 10-fold higher (12). Only the mineralization of Picea lignin in salt marsh sediments, which averaged 1.6% in 40 days, was comparable with our data. The lower rates observed for the mineralization of 14C-lignin-labeled lignocellulose from pine and spruce (Fig. 2) (11, 15, 26) may be indicative of their greater recalcitrance. This is substantiated by the report that 14C-lignin-labeled lignocellulose from Virginia pine was the least susceptible of six wood lignins to microbial decomposition (11).

As has been previously noted (15), it may be difficult to directly compare rates of utilization of 14C-labeled lignocellulose from different systems. The differences could reflect the chemical makeup of the specific lignins, the nature of the chemical bonds involved, or the method of labeling and preparation of the substrates. Ball milling would increase the surface area of the substrate and possibly produce greater utilization than our larger mortar-ground particles. Further study of factors affecting lignin decomposition will be required to assess the validity of datum comparisons.

The number and species of the microbial populations in the environment would most certainly affect mineralization rates. The number of microorganisms capable of mineralizing the lignocellulose in the groundwater and stream was within the range of lignin degraders found in other aquatic systems (12). Although no effort was made to identify specific bacteria and fungi capable of degrading lignocellulose in this investigation, several genera identified in the groundwater (Table 2) and the stream (24) included species with this capability (28, 29, 32). The bacterial species which were identified as being common to both the groundwater and the stream may have been responsible for the comparable rates of lignocellulose degradation observed in the two ecosystems, but the contribution of unidentified actinomycetes, fungi, and bacteria to this degradation cannot be excluded.

The mineralization of side-chain-labeled cinnamic acid, a potential intermediate involved in the biosynthesis of lignin alcohols in plants (21), was also assessed. Similar rates were noted for both the groundwater and the stream and resembled those of 14C-side-chain 14C-labeled maize and p-OH-[1-14C]cinnamic acid, reported for a Norcardia species (37). The results of this investigation suggest that three carbon segments, perhaps resembling an exposed phenylpropane building block of the lignin polymer, are readily cleaved by the microflora in both the groundwater and the stream.

Allochthonous input from vegetation and wildlife and autochthonous input from primary production are both sources of DOM in stream ecosystems. Groundwater flux into the stream may greatly affect DOM concentrations as well (27, 28, 39). This study has shown that microflora in both the stream and the groundwater influences the quantity and quality of stream DOM. Labile components are more readily utilized, particularly by the groundwater, whereas the mineralization of recalcitrant components occurs at a slower rate and is similar in both systems. The rates of utilization of individual labile and refractory compounds reflect the differential utilization of DOM fractions noted by Wetzel and Manny (40). Future studies of the heterotrophic activity of the diverse microflora in the stream and groundwater should help better define the role of bacteria in the utilization of DOM in lotic environments.

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LITERATURE CITED


